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A U S T R A L I A

DEVELOPING SUGARCANE - LEGUME COMPANION CROPPING SYSTEMS TO MINIMISE NITROUS OXIDE EMISSIONS

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Abstract

Global efforts are underway to reduce greenhouse gas emissions (GHG) from anthropogenic activities. Nitrous oxide (N_2O) emissions accounted for 13% of Australia's National GHG inventory over the period 2016-2017 (NGGI, 2017) with most N_2O derived from agricultural soils. Sugarcane soils are high emitters of N_2O, and this thesis explores whether legumes, grown as a companion crop with biological N_2 fixation (BNF) capacity, can partially replace N fertiliser to lower the emissions of N_2O from sugarcane soil.

Chapter 2 synthesises published literature on sugarcane intercropping. Most research has focussed on the productivity of sugarcane with intercrops, including legumes. Intercropping can benefit sugarcane yield, have neutral or negative effects. This practice is common in subsistence agriculture, and farm income benefits, but environmental benefits intercropping have not been a research focus.

Chapters 3 and 4 explore sugarcane-legume intercropping at three commercial farms in Australia. N_2O emissions, soil and crop variables were quantified with different N fertiliser applications and in the presence or absence of legumes. The farms, two Rain-fed, one Irrigated, were located in the dry and wet tropics, and in the subtropics, representing different climate and agronomic settings. Industry-recommended (full) N fertiliser rates were compared with up to 50% reduced N fertiliser rates in the presence or absence of legume and benchmarked against a zero N fertiliser control. We hypothesised that reduced application of N fertiliser limits sugarcane growth and that legumes can alleviate N limitation. However, full and reduced N fertiliser treatments mostly generated similar sugarcane yields, confirming that industry-recommended full N fertiliser rates exceed sugarcane needs. In line with this notion, the reduced N+legume treatments did not improve sugarcane growth. N_2O emissions in reduced N+legume were either similar to reduced N fertiliser sugarcane monoculture or higher and similar to the emissions observed with full N fertiliser rates. Soybean strongly benefitted sugarcane yield under N limiting conditions (zero N fertiliser) at one farm, increasing soluble soil N levels and nearly doubling sugarcane yield compared to zero-N sugarcane monoculture, and generating 6-times lower N_2O emissions than the full N rate. At the Rain-fed sites, soil nitrate levels explained 81 and 64% of N_2O emissions; at the Irrigated site, the interaction of soil nitrate and soil moisture explained 63% of N_2O emissions. High N_2O emissions factors at the subtropical site were associated with wet, low drainage soil (>70% water filled pore space over summer), conditions that promote denitrification. High N fertiliser rates in Irrigated, well-

draining soils had lower N_2O emissions, possibly shifting N losses from gaseous to leaching. The promising findings observed with soybean under N limitation require further investigation to explore N_2O mitigation options with a view of optimising legume facilitation and the 'tipping point' for N fertiliser applications.

Chapter 5 presents a glasshouse experiment investigating the effect of N fertiliser rate on competition vs facilitation up to peak N accumulation of soybean. No or low N fertiliser rates enhanced soybean BNF but reduced growth of sugarcane. With moderate to high N fertiliser rates, soybean BNF and growth diminished, while sugarcane growth increased which indicatives increased competitive ability. We conclude that N fertiliser rates substantially impact upon the relative performance of sugarcane and legume intercrop, with a trade-off between N fertiliser application and legume BNF. Under the experimental condition, direct N transfer from soybean to sugarcane was negligible. Rather, decomposition and mineralisation of legume biomass are likely to be the main pathway for increased N accumulation in intercropped sugarcane observed in some instances.

Chapter 6 synthesises the findings from literature, field and glasshouse experimentation and discusses future directions for sugarcane-legume intercropping research.

<u>Declaration by author</u>

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly- authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, financial support and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my higher degree by research candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Contributions by others to the thesis

Professor Susanne Schmidt, Dr Richard Brackin and Dr Nicole Robinson designed the field experiments, and contributed to the design of glasshouse experiments. Prof Susanne Schmidt, Dr Richard Brackin, Dr Nicole Robinson and Dr Ryo Fujinuma assisted with data interpretation chapter editing. Further people contributed to this thesis as listed below.

Chapter 2

Dr Henrique Coutinho Junqueira Franco (Centro Nacional de Pesquisa em Energia e Materiais, CNPEM, Brazil) and Dr Ovidio Perez (Sugarcane Research Centre Guatemala, CENGICANA) provided reports and papers from Brazil, Guatemala and Mexico on sugarcane intercropping.

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Chapter 5

Dr Nicole Robinson assisted with the experimental design, planning and set-up of the glasshouse trial. Dr Richard Brackin, Dr Nicole Robinson and Scott Buckley assisted with the harvest.

Statement of parts of the thesis submitted to qualify for the award of another degree

"No works submitted towards another degree have been included in this thesis".

Research Involving Human or Animal Subjects

"No animal or human subjects were involved in this research".

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Dedication

To my wonderful parents Ana and Miguel, and my beloved brothers and sister who always were with me. To my father who taught me nothing is easy in life but with the perseverance and determination, I can achieve my dreams.

In your memory Daddy

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List of Abbreviations

°C	Degree Celsius
%	Percent
µl	Microlitre
µS	Microsiemens
ANOVA	Analysis of variance
C	Carbon
CH_4	Methane
CO_2	Carbon dioxide
cm	Centimetre
d	Day
EC	Electric conductivity
EF	Emission factor
g	Gram
GWP	Global warming potential
h	Hour
ha	Hectare
IPCC	Intergovernmental Panel on Climate Change
K	Potassium
KCl	Potassium chloride
kg	Kilogram
L	Litre
LSD	Least significant difference
log	natural logarithm
m	Metre
mg	Milligram
ml	Millilitre
min	Minute
mm	Millimetre
n	Sample size
N	Nitrogen

N_2	Dinitrogen
Na	Sodium
N_2O	Nitrous oxide
NH_3	Ammonia
NH_4^+-N	Ammonium
NH_4^+-N	Ammonium nitrogen
NO	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NO_3^--N	Nitrate nitrogen
NOAA	National Oceanic and Atmospheric Administration
O_2	Dioxygen
P	Phosphorus
P	p-value
R^2	Coefficient of determination
SD	Standard deviation
sec	Second
SEM	Standard error of the mean
t	Ton
Tg	Teragram
WFPS	Water-filled pore space

Chapter 1 – Introduction

1.1 Climate Change and Agriculture

Global climate change is one of the grand challenges of our time (UN, 2017). Anthropogenic activities have been accelerating the increases in three main greenhouse gases (GHG): carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). Together, these gases account for up to 80% of total radiative forcing of all GHG (IPCC, 2014). The current concentrations of 409 ppm CO_2 (NOAA, 2018), 1853 ppb CH_4 and 328 ppb N_2O (WMO, 2016) in the atmosphere surpass those in the past 800,000 years (NOAA, 2018). The increase of GHG is caused predominantly by anthropogenic activities including the burning of fossil fuels, land use change (i.e. the conversion of natural ecosystems to agricultural land), and agriculture (IPCC, 2014, Schultz et al., 2010). Overall, GHG emissions from agriculture represent 13% of Australia's National GHG inventory over 2016-2017.

The focus of the research presented here is on nitrous oxide (N_2O), a trace gas with a global warming potential 265–298 times that of CO_2 for a 100-year timescale. N_2O emissions can be direct or indirect in agricultural soils (IPCC, 2006). The direct pathways increase N_2O emissions as a result of over-use of synthetic N fertilisers and manures, driven by biological nitrification and denitrification processes; the indirect pathways of N_2O emissions are the result of N volatilization, leaching, runoff or harvest of crop biomass (IPCC, 2013). It is estimated that indirect N_2O emissions released from these processes constitute about one third of total agricultural N_2O emissions (IPCC, 2006), with leaching and surface runoff as the major contributors (Tian et al., 2018, Xia et al., 2013b). For example, high ammonia (NH_3) volatilisation occurs with surface-applied urea or manures, and subsequent NH_3 oxidation can generate indirect N_2O emissions (Redding et al., 2016). In our study, urea was applied subsurface (as is industry practice in Australia) which minimises NH_3 emissions (Freney et al., 1994). The focus was therefore on direct emissions as main source of N_2O emissions from N fertilised agricultural soils. It is estimated that approximately 4.1 Tg N_2O-N y^{-1} (1.7 to 4.8 Tg N_2O-N y^{-1}) of the total anthropogenic sources of 6.9 Tg N_2O-N y^{-1} (2.7 to 11.1 Tg N_2O-N y^{-1}) are the results of N inefficiencies in agriculture (IPCC, 2013). The uncertainties for N_2O emissions are high, and improved understanding of N_2O emissions is therefore essential. Importantly, mitigation options are needed that do not compromise crop yields.

1.2 Pathways of nitrous oxide (N₂O) formation of in soils

The emissions of N_2O from soils are the result of biological and physio-chemical processes. Soil can be a sink or a source of GHG, including N_2O, depending on the dynamic equilibrium between microorganisms generating or metabolising GHG. This equilibrium is influenced by environmental factors including those imposed by the management of crop systems (Schlesinger and Bernhardt, 2013, Mosier et al., 2004, Dalal et al., 2003). Figure 1.1 illustrates the emissions from soil to the atmosphere (solid lines) with focus on the N transformations that are the focus here.
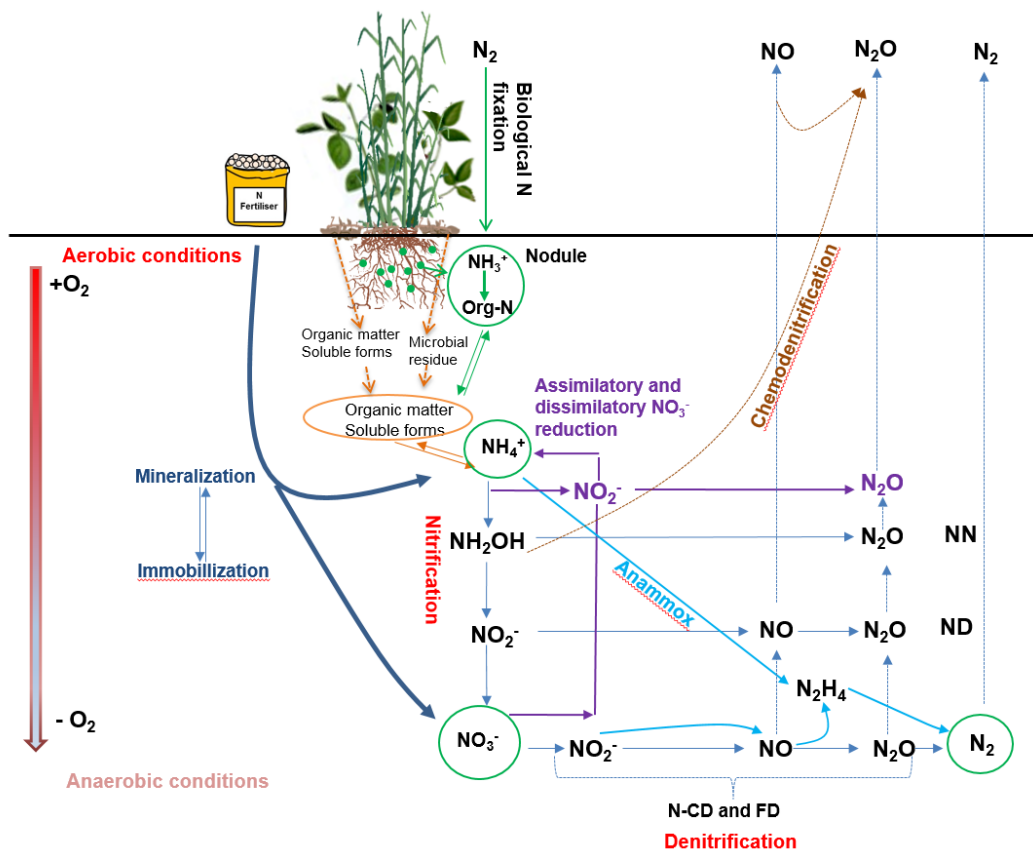
Figure 1.1 Conceptual diagram of the formation of nitrous oxide (N_2O) in the soil of a legume-sugarcane intercropping system (adapted from Dalal et al., 2003, Bodelier, 2011; Kool et al., 2011, Schlesinger and Bernhardt 2013, Itakura et al., 2012; Nazaries et al., 2013, Signor and Cerri , 2013, Meyer et al., 2008) . NN (nitrifier nitrification); ND (nitrifier denitrification); N-CD and FD (nitrification-coupled denitrification and reduction of the N fertiliser applied). Dotted lines show the contribution of the different pathways to form N gas compounds.

1.2.1 Nitrogen transformation in soils and N₂O emissions

The transformations of N in soils are mediated by soil microbial communities which perform the processes that result *inter alia* in the production of N_2O and other NO_x (Nazaries et al.,

2013, Xia et al., 2013c, Thomson et al., 2012, Bodelier, 2011). Four processes are implicated in the production of N_2O emissions; nitrification, denitrification, dissimilatory nitrate reduction to ammonium (DNRA) and assimilatory nitrate reduction; the latter pathway is considered to be less important as it is inhibited at low concentrations of NH_4^+ or soluble organic N. Nitrification (autotrophic nitrification) and denitrification (heterotrophic denitrification) are considered the main source of N_2O emissions (Müller et al., 2014, Venterea and Rolston, 2000), possessing the genes and enzymes responsible for N_2O formation (Figure 1.1; Table 1.1).

To devise mitigation strategies, understanding of the enzymatic pathways and microbes involved in N_2O emissions is important, which in turn are driven by agronomic management and environmental variables. For example, analysis of the molecular pathways suggest that the enzyme nitrous oxide reductase (N_2OR) could play an important role in mitigating N_2O emissions as it converts N_2O to dinitrogen (N_2) (Muller et al., 2014, Thomson et al., 2012, Kool et al., 2011). N_2O is the final product of the nitrifier denitrification pathway because ammonia oxidising bacteria (AOB) do not contain N_2OR (Cantera and Stain, 2007).

Table 1. 1 Processes, genes and enzymes responsible for N_2O emissions; modified from Thomson et al. (2012).

Transformation			Process	Genes	Encoded enzyme
N_2	$\rightarrow$	NH_3	*Nitrogen fixation*	nifHDK	nitrogenase
			Mineralisation		
NH_4^+	$\rightarrow$	NH_2OH	Ammonium	Amo	ammonia monooxygenase
NH_2OH	$\rightarrow$	NO_2^-	oxidation	Hao	hydroxylamine oxidoreductase
NO_2^-	$\rightarrow$	NO_3^-	Nitrite oxidation	NOR/NXR	nitrite oxidereductase
NO_3^-	$\rightarrow$	NO_2^-		narG	dissimilatory nitrate reductase
				napEDABC	periplasmatic nitrate reductase
NO_2^-	$\rightarrow$	NO	*Denitrification*	nirK, nirS	copper nitrite reductase, nitrite reductase haem cd1
NO	$\rightarrow$	N_2O		norCB	nitric oxide reductase
N_2O	$\rightarrow$	N_2		nosZ	nitrous oxide reductase N_2OR

With research on soil microbial communities and N transformations ongoing, this thesis focusses on agronomic practices and the net effect on N_2O emissions with the aim to mitigate N_2O emissions. Building on existing knowledge of N_2O emissions from Australian sugarcane soils and research globally, the aim was to quantify how recommended (full N rate) and reduced N fertiliser in combination with legumes affect N_2O emissions. The various pathways

are outlined below as they will be considered as potential contributors to N_2O in the research here.

1.2.2 Autotrophic nitrification

Autotrophic nitrification is an aerobic pathway that involves the oxidation of ammonium (NH_4^+) or ammonia (NH_3) to nitrate (NO_3^-) and is catalysed by two dominant bacterial groups and archaea (Hink et al., 2016, Bowatte et al., 2009). Ammonia oxidising archaea (AOA) and bacteria (AOB) in the *Nitrosomonas* genus (e.g. *Nitrosomonas europaea*) participate in the oxidation of NH_4^+ to NO_2^-. The oxidation of ammonium involves a three-step process termed nitrification (Thomson et al., 2012). Firstly, NH_4^+ is converted to hydroxylamine (NH_2OH) by ammonia mono-oxygenase (amo) that requires molecular oxygen (O_2). Then, NH_2OH is oxidised to NO_2^- by hydroxylamine oxidoreductase (hao) (Kool et al., 2009, Andersson and Hooper, 1983). The second bacterial group belong to *Nitrobacter* (e.g. *Nitrobacter winogradskyi*) and oxidise NO_2^- to NO_3^- by nitrite oxidoreductase (nor) (Thomson et al., 2012). Nitrifier nitrification (NN) and nitrifier denitrification (ND) produce N_2O by reduction of NH_2OH and NO_2^- respectively. In the latter pathway, copper nitrite reductase (nirK) reduces NO_2^- to NO, and nitric oxide reductase (norCB) reduces NO to N_2O (Thomson et al., 2012, Kool et al., 2011).

1.2.3 Heterotrophic nitrification

Microorganisms such as fungi can produce N_2O emissions under aerobic conditions through the heterotrophic nitrification process (Granli and Bøckman, 1995, Robertson et al., 1989). Heterotrophic and autotrophic nitrification processes involve similar N oxidation processes; however, the former has different enzymes responsible for N_2O emissions (Wrage et al., 2001). The magnitude of N_2O emission production during the heterotrophic nitrification process is dependent on low pH, high availability of O_2 and organic C supply (Wrage et al., 2001).

1.2.4 Heterotrophic denitrification

Denitrification is the main pathway of N_2O production performed by non-specialist heterotrophic bacteria (organotrophs, chemo- and photo-lithotrophs, and N_2 fixers among others) (Robertson and Thorburn, 2007). Denitrifiers use NO_3^- instead of O_2 as an electron acceptor during respiration (CHAPUIS-LARDY et al., 2007, Mosier et al., 2004). In this process, NO_3^- is reduced via two pathways, from both nitrification–coupled denitrification (NCD) and fertiliser denitrification (FD) as fertiliser NO_3^- (Kool et al., 2011, Kool et al., 2009). Nitrification coupled-denitrification relates to the existing proximity between nitrification and

denitrification process that frequently occurs in the soil. In this stage, a substantial part of the NO_3^- from nitrification diffuses to the anaerobic denitrification zone where NO_3^- is reduced to N_2; however, during this stepwise reduction, N_2O is one of the intermediate product that can be released to the atmosphere (Nielsen et al., 1996).

1.2.5 Other N transformation in soil

There are further microbial processes that currently become more important in the production of N_2O from soils (van Groenigen et al., 2014). Processes such as dissimilatory nitrate reduction to ammonium (DNRA), non-respiratory denitrification and anaerobic ammonium oxidation (anammox) are considered to play significant role in N gaseous cycle (Muller et al., 2014, Thomson et al., 2012, Robertson and Groffman, 2007). Specific soil conditions (aerobic or anaerobic) and microorganisms (e.g. facultative and obligate bacteria, fungi and/or yeast) are involved in the oxido-reduction reactions of these processes. However, the ecology of these processes is less well understood than nitrification or denitrification (Robertson and Groffman, 2007), and some of the processes that generate N_2O also are able to consume N_2O. For example, the DNRA can enhance N_2O consumption by the presence of typical enzymes like denitrifier nosZ I and atypical non denitriyfier nosZ II or by the production of N_2O as a by-product (van Groenigen et al., 2014). Therefore, N_2O emissions from the different N transformation pathways are complex due to the numerous soil biological, physical and chemical reactions. Substantial research has been directed at identifying the pathways of N_2O. However, in the applied context of agronomic practices aimed at reducing N_2O emissions from soil, research mostly centres on reducing soluble soil inorganic N pools as main substrate for N_2O emissions (Müller et al., 2014, Kool et al., 2011).

1.3 Drivers for the production of nitrous oxide from soils

Management practices combined with environmental factors in agricultural systems influence the rate of N_2O emissions (Zhu et al., 2013, Thomson et al., 2012). The rates of N_2O emissions from soil are determined *inter alia* by abiotic factors such as soil moisture (i.e. irrigation or rainfall) and N application rate and fertiliser type, e.g. manures, urea, nitrate or ammonium-based synthetic fertilisers (Vargas et al., 2014). We discuss the various factors in the following section.

1.3.1 Nitrogen sources

Available N in soil is the precursor for N_2O emissions, with emissions increasing under conditions of high N substrate availability (NH_4^+, NO_3^-, and organic N) (Yu et al., 2001). The

response of N₂O emissions to fertiliser applications depends on the composition and quantity of N fertiliser (Hut et al., 2010, Dalal et al., 2008). The highest risk of N₂O emissions occurs with large pools of soil inorganic N and anoxic conditions due to high rainfall or irrigation (Oertel et al., 2016, Signor and Cerri, 2013). Strategies to manage N₂O emissions are frequently based on N fertiliser reductions (Wang et al., 2016, Luo et al., 2016), splitting the application of fertiliser to reduce the size of soil inorganic N pools (Allen et al., 2010) or managing the pool size of inorganic N with slowed-release N fertilisers or using inhibitors that target some of the enzymes involved in N₂O generation (Wang et al., 2016, Subbarao et al., 2006).
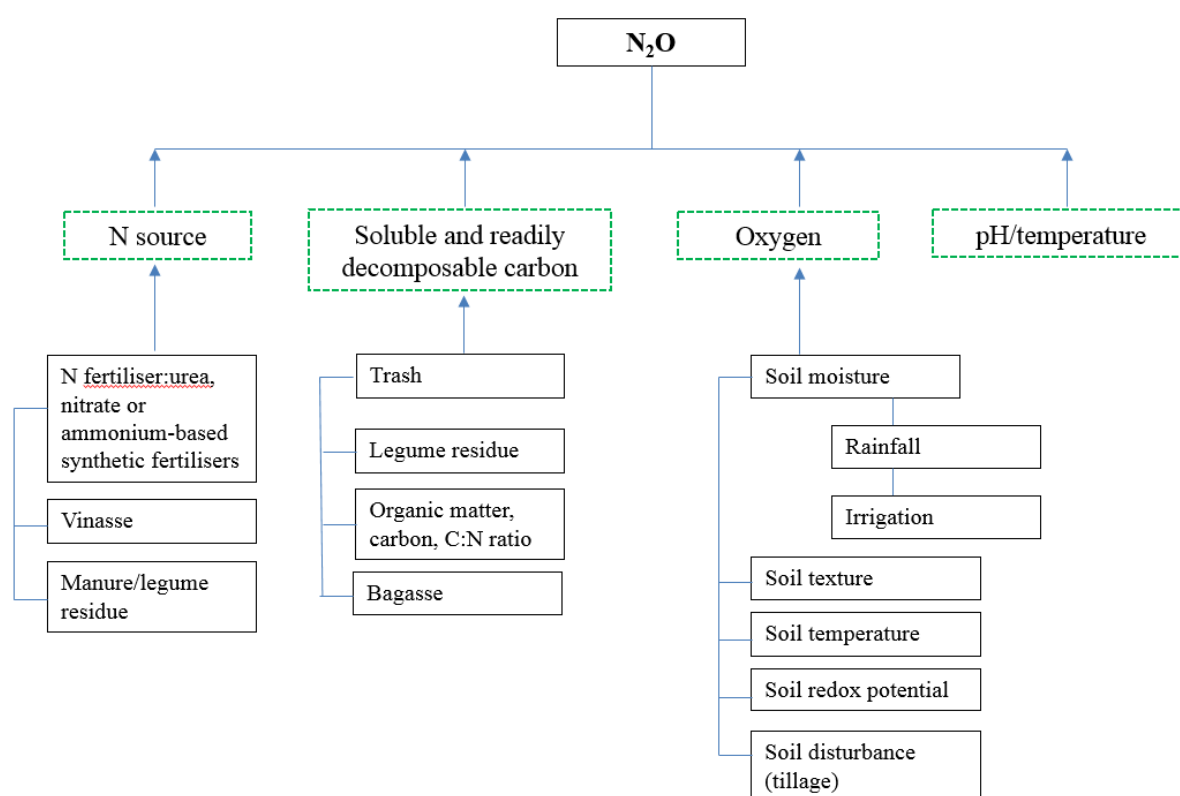
Figure 1. 2 Factors controlling N₂O emissions (adapted from Mosier et al., 2004, Dalal et al., 2003, 2008, Denmead et al., 2008, Vargas et al., 2014) in sugarcane crop systems.

1.3.2 Soil moisture, aeration and texture

Soil moisture greatly influences N₂O emissions as it regulates soil microbial activity, gas diffusion and aeration status (Dalal et al., 2008). The rate of N₂O production varies with the different N transformation processes in line with changes in water filled pore space (WFPS) and associated air diffusion rate. Very low fluxes of N₂O derived from nitrification occurred at levels of <40% WFPS which doubled at 55-65% WFPS (Dalal et al., 2003). Oxygen diffusion becomes limiting >60% of WFPS, and N₂O flux from denitrification sharply

increases. At very high WFPS (>90%), N_2O flux is lower as N_2 becomes the dominant form of gaseous N released from soils (Dalal et al., 2003, Granli and Bøckman, 1995). Beyond these broad categories of N_2O flux from soil, peak N_2O emissions can occur at different WFPS for various soil types under different soil management and weather conditions (Redding et al., 2016).

Soil texture interacts with soil moisture and influences the release of N_2O. Clay soils tend to release more N_2O than sandy soils (Redding et al., 2016) as water is retained in the soil pores and gas diffusion is lower than in course-textured soils (Signor and Cerri, 2013, Granli and Bøckman, 1995). Soil management can increase N_2O emissions. For example, in compacted soils, tillage decreases N_2O emissions by increasing soil aeration and facilitating soil O_2 and water exchange (Zhu et al., 2013). Figure 1.2 illustrates the factors that influence the production and consumption of N_2O. The interactions among these factors (i.e. soil moisture, soil mulch, N and C availability) are complex (Vargas et al., 2014); these factors (in the dashed rectangle) can affect microbial communities rapidly, and therefore immediate change the rates of N_2O production when conditions are altered, e.g. by management or weather.

1.3.2 Redox Potential, Temperature, pH

Nitrous oxide production is strongly affected by soil redox potential (*Eh*). In agricultural soils, highest N_2O emissions were observed in anaerobic conditions with the denitrification pathway active at redox potential of +120 to +250 mV (Yu et al., 2001). However, N_2O emissions also depend on interactions of soil nitrate availability, soil pH, soil organic matter content, soil temperature and soil moisture content as contributors (Vargas et al., 2014, Swamy et al., 2012, Dalal et al., 2008, Yu et al., 2001). For example, larger N_2O emissions were reported during the crop season of mung bean (*Vigna radiata*) with high redox potential (+100 to +150 mV). However, during the postharvest fallow, N_2O emissions were higher despite redox potential decreasing to +40 mV, high soil temperatures (36 to 45 ^{o}C), as rapid degradation and mineralisation of the N-rich legume residue and soil moisture contributed to N_2O emissions (Swamy et al., 2012).

Temperature affects general microbial activities including nitrification and denitrification (Oertel et al., 2016, Signor and Cerri, 2013). Denitrification is associated with high temperatures (up to 50°C) which increase soil respiration and decreases soil O_2 levels to form and distribute N_2O to the atmosphere (Signor and Cerri, 2013, Knowles, 1982). Additionally,

increasing temperatures are able to increase mineralisation and nitrification processes resulting in substrate availability for denitrification (Butterbach-Bahl et al., 2013).

Soil pH also influences N_2O emissions (Oertel et al., 2016, Signor and Cerri, 2013). During the denitrification process, N_2O emissions are produced with a high concentration of NO_3^- and low pH; at low pH, nitrous oxide reductase enzyme (N_2OR) is inhibited, and only a small amount of N_2O would be reduced to N_2 (CHAPUIS‑LARDY et al., 2007). While the denitrification (anaerobic) process increases soil pH (Khalil et al., 2004), the application of N fertiliser reduces soil pH (Thomson et al., 2012). During denitrification, highest N_2O emissions were reported in a pH range between 4.0 and 5.5 (Knowles, 1982); while, during nitrification, N_2O emissions increased with a pH range of 5.9 to 8.3 (Bremner and Blackmer, 1981).

1.4 Sugarcane management practices and N_2O production

1.4.1 Sugarcane agriculture in Australia

Sugarcane (*Saccharum officinarum x spontaneum*) is grown globally on approximately 26.8 million hectares in 108 countries in the tropics and subtropics (FAOSTAT, 2016). In 2016, Brazil was the largest producer of sugarcane globally, with 10.3 million ha of harvested area and average productivity of 75.2 t ha^{-1}. Australia has ≈0.37 million ha of sugarcane but higher production per hectare (91.2 t ha^{-1}) than Brazil (Australian Sugarcane, 2016). High sugarcane yields are enabled by breeding, disease/pest control and fertiliser application and depend on climate conditions, agronomic management and crop variety (Hartemink, 2008). Australian sugarcane is grown predominantly in intensive monocultures with high inputs of fertiliser, pesticides and herbicides. While efforts to transition the industry to improve farming systems with wider row spacings, GPS guidance, rotations to legumes between ratoon cycles, and reduced inputs are under way, progress has been relatively slow (Bell et al., 2007), with only 40% of growers currently using best practice management (Canegrowers, 2017).

1.4.2 N_2O emissions from sugarcane soils

Emissions of carbon dioxide, methane and nitrous oxide (CO_2, CH_4, N_2O) occur between sugarcane soils and the atmosphere and depend on climate, soil type, and management practices (Carmo et al., 2013, Allen et al., 2010, Denmead et al., 2010b, Hartemink, 2008, Weier et al., 1998, 1996, Macedo et al., 2008). Sugarcane soils often generate larger N_2O emissions than the 1% default emission factor of the IPCC, with N_2O emission factors up to 21% (de Oliveira et al. 2013, Denmead et al., 2010, Weier et al., 1998). Controlling factors such as high rates of fertiliser (100 to 300 kg N ha^{-1}), high rainfall, high retention of sugarcane

residues, fertiliser management (form and timing of N application), poorly drained soils, and dunder (vinasse) application contribute to high N_2O emissions (Carmo et al., 2013, de Oliveira et al., 2013, Denmead et al., 2010b, Weier et al., 1998, 1996).

In sugarcane systems, productivity is generally the priority; however, environmental compliance with a view of pollution linked to the inefficient use of N fertilisers is under scrutiny (Thorburn et al., 2013, Dalal et al., 2003). Sugarcane management strategies aim to improve crop yield and soil fertility (da Silva Paredes et al., 2014, Carmo et al., 2013, Weier, 1996). As sugarcane management differs regionally, N_2O emissions differ with location and climate (da Silva Paredes et al., 2014, de Oliveira et al., 2013, Dalal et al., 2008). Most N_2O emission rates in Australia detected so far surpass the default emission factor (1%) of the IPCC, and the global emission factor from sugarcane soils of 3.87% (Lisboa et al., 2011). The addition of either synthetic N fertiliser or organic amendments (vinasse, filter cake or unburnt sugarcane residues left on the field) increases N_2O emissions (Vargas et al., 2014, de Oliveira et al., 2013, Carmo et al., 2013, da Silva Paredes et al., 2014). Many factors impact on N_2O emissions: (i) high levels of N fertilisation, (ii) additional N supplied from organic amendments, which can also increase dissolved organic carbon availability and increase microbial activity and subsequent reduction in redox potential, (iii) low soil pH (acidic soils) that can limit completion of the denitrification process, (iv) soil tillage (especially in plant cane). Research has shown high variability of N_2O emissions from sugarcane soil and has demonstrated that emissions cannot be extrapolated to other sites if they vary in soil type and texture, temperature, carbon content, water filled pore space and soil soluble N (Vargas et al., 2014, Carmo et al., 2013, Denmead et al., 2010b, 2008).

Agricultural practices have been developed in sugarcane production to reduce N_2O emissions; however, the intricate interaction among climate, physical, chemical and biological soil conditions and anthropogenic activities complicate mitigation strategies as discussed above. In sugarcane production, the practice of retaining green crop residues after harvest (rather than burning residues) is termed 'trash blanketing'. Sugarcane trash can act as a source of carbon and increase soil organic carbon levels (De Figueiredo and La Scala Jr, 2011, Pretty et al., 2002). Sugarcane trash left on the soil surface had a range of effects on the production and consumption of N_2O (da Silva Paredes et al., 2014, Carmo et al., 2013, da Silva et al., 2013, de Oliveira et al., 2013, Weier, 1996). Efforts to reduce N_2O emissions and N leaching from sugarcane agriculture centre around tailoring N application rates to closely match crop demand

(Schroeder et al., 2014), and ongoing research is investigating fertiliser coatings and nitrification inhibitors as an avenue for reducing pool size of nitrate, and therefore the risk of denitrification events. These current technologies have variable success in different locations and years (Wang et al., 2016, Verburg et al., 2014), and investigations into alternative practices to reduce N_2O emissions are necessary.

In this thesis, the focus is on legumes as an important source of biologically fixed N, which can improve soil fertility and stimulate productivity of the crop following a legume rotation (Jensen et al., 2012, Park et al., 2010). The use of legumes could be instrumental in reducing N_2O emissions to the atmosphere by partially replacing synthetic N with biologically fixed N (Jensen et al., 2012). In Australia, rotations of legumes into sugarcane systems, by interrupting the sugarcane ratoon cycle with legume crops, are practiced by a small (~12.5% in 2004, Bell et al., 2007) but increasing number of growers, to reduce soil borne disease, pest and weed cycles. Soil physical and chemical characteristics improvement and N input into plant cane can be reduced (Thorburn et al., 2010, Park et al., 2010, Stirling, 2008). There are some indications that this practice has benefits for decreasing N_2O production from sugarcane cropping systems, although the timing of N release from some legume rotations means that legume-N is at risk of loss before the next sugarcane crop (Huth et al., 2010).

1.5 Sugarcane-legume companion cropping

This project investigates sugarcane-legume companion cropping as an alternative N supply system for sugarcane agriculture. Intercropping is generally an alternative row system in which two or more species are planted in separate alternate rows, and grow simultaneously (Arshad et al., 2014, Keating and Carberry, 1993). The slow growth rate of sugarcane during the first three months of its cycle and the wide space between sugarcane rows (75 to 183 cm) provides excellent conditions to integrate crops of short duration. Sugarcane is frequently intercropped with a variety of crops in developing countries, where both crops are harvested for yield by hand. This approach optimises use of space, natural resources and is used to stabilise profitability by minimising risk (Chogatapur, 2017, Shiming and Gliessman, 2016, Luo et al., 2016, Geetha et al., 2015, Ramesh et al., 2003), and allows high crop production for a specific area of land compared to the monoculture system (Duchene et al., 2017, Masasso, 2007). In mechanised agriculture and developed countries, there is little capacity to harvest multiple crops growing simultaneously in the same field, but there is interest in using legumes as sacrificial green manure crops. Growing legumes simultaneously with sugarcane may reduce

the temporal mismatch between the N release from legumes and N uptake by sugarcane, and the loss of N from legume rotations (Brackin et al., 2015, Huth et al., 2010, Park et al., 2010). In companion cropping systems where a legume is used as green manure, the non-legume crop benefits primarily from N released during the decomposition and breakdown of legume residues (Ong, 1995). Additionally, legumes contribute to other processes that increase N and other nutrient availability such as root exudates and rhizo-deposits (Jensen and Hauggaard-Nielsen, 2003, Jensen, 1996). Midmore (1993) pointed out that the benefit of legumes is not only limited to the current intercrop system, as residual nutrients may supply the succeeding crops. Sugarcane-legume intercropping can be classified as sustainable agricultural intensification because it reduces the use of external inputs such as N fertiliser, and connects biological nitrogen fixation to the nutrient cycling (Robertson, 2006). However, despite the potential benefits of this system, the use of legume as intercrop has received very little study under Australian commercial field conditions.

1.5.1 N_2O emissions from legume cropping

While the use of legumes in rotations or intercrops can reduce the requirements for inorganic fertiliser application (Park et al., 2010, Robertson, 2006), legumes themselves can contribute to N_2O emissions. Legumes, unless grown in soils with high-N fertiliser addition, derive N mainly from the atmosphere through biologically nitrogen fixation (BNF) by hosting symbiotic bacteria and/or archea (diazotrophs) in root nodules (Inaba et al., 2009). N_2O emissions from the process of BNF itself are negligible as recently recognised by removal of the emission factor by the IPCC (2014); however, N_2O emissions are produced from the decomposition of legume crop residue (Rochette and Janzen, 2005).

Within nodules, the activity of the nitrogenase enzyme breaks the triple bond of N_2 to produce ammonia (NH_3) (Thomson et al., 2012). The ammonia becomes part of the organic nitrogen pool that is transported throughout the plant. The decomposition of root nodules, roots and shoot residues generates soluble organic N, which soil microbes convert through the processes of ammonification, followed by nitrification and denitrification which produce N_2O emissions (Figure 1.1) (Itakura et al., 2013, Inaba et al., 2009).

A comparison of N_2O data from 171 sites from 1994 to 2011 showed estimated N_2O emissions were large and variable during the growing season of legumes, across different legume management and pasture systems (Jensen et al., 2012). At 77 sites without N fertiliser, annual

N_2O-N emissions or per growing season ranged from 0.29-7.09 kg ha^{-1}; however, the origin of these emissions was not clear because in some experiments the background emissions from soil were not quantified. On the other hand, where N_2O emissions from bare soil without N fertiliser or legume were measured, no difference in emissions occurred in comparison with non-N fertiliser legume crops (Jensen et al., 2012). The chemical composition and quantity of crop residues can have impacts on N_2O emissions. While sugarcane trash has a high C/N ratio (>100) producing immobilisation of N in the soil (Carmo et al., 2013, De Figueiredo and La Scala Jr, 2011), legumes by contrast have low C/N ratios and can increase N_2O emissions (Frimpong et al., 2012, Millar and Baggs, 2004). For example a 30 day incubation study showed the effects of using residue mixtures at different C:N ratio on N_2O production; mixing cowpea-maize residues in a sandy clay loam at ratios of 100:0, 50:50 or 0:100 resulted in cumulative N_2O emissions of 26, 23 and 13 mg N_2O m^{-2} d^{-1}, respectively (Frimpong et al., 2012). It is currently unclear how mixed sugarcane and legume residues in intercropping systems will influence overall system N_2O production.

1.6 Objectives of this thesis

The main objective was to evaluate legumes as companion crops of sugarcane to mitigate nitrous oxide (N_2O) emissions from wet and dry sub/tropical agricultural soils. The strong and positive relationship between N fertiliser application rate and N_2O emissions confirms the need for reducing emissions through improved N management (Müller et al., 2014, Allen et al., 2010, Denmead et al., 2010b, Huth et al., 2010). The research of this thesis has potential to discover if legumes with strong capacity for biological N_2 fixation (BNF), grown as a green manure (sacrificial crop) companion crop, can reduce off-site N losses, and N_2O emissions were quantified in context of environmental factors and sugarcane yield. Most studies on sugarcane-legume intercropping systems have aimed to improve productivity and profitability for smallholders by harvesting both crops. Sugarcane-legume companion cropping with sacrificial legumes requires research to evaluate the potential of such systems for increasing environmental compliance without jeopardising profitability of sugarcane production. We hypothesised that legume companion crops can reduce N fertiliser needs, concentrations of soluble N in soil and associated N_2O emissions.

Chapter 2 is a review of sugarcane intercropping. For this purpose, data from 32 studies published in English, Spanish and Portuguese were summarised to bring together current

research and knowledge in sugarcane intercropping systems worldwide. To the best of our knowledge, this is the first comprehensive review of intercropping in sugarcane.

Chapters 3 and 4 aimed to (1) quantify N_2O emissions with recommended (full N rate) and reduced N rates in the presence and absence of legumes, (2) identify drivers of N_2O emissions, and (3) evaluate interactions between N fertiliser rate, legumes and sugarcane yield in a commercial production setting. Our field studies quantified the effects of legume companion crops on sugarcane production over one to three growing seasons. The experiments were carried out on three farms in Australia's wet tropics, dry tropics and subtropics. Soybeans were used at all sites and mung beans were included at the wet tropics site. Field trial designs and treatments varied at each site due to local constraints and agronomic recommendations, but all had a fully fertilised treatment, a reduced N treatment (with and without legume), and a treatment without N fertiliser. In two trials, we were able to include a treatment without N fertiliser+legume. We hypothesised that reduced N fertiliser rates would result in decreased sugarcane yield and N_2O emissions compared to the fully N fertilised treatments, and that the addition of a legume would increase sugarcane yield by alleviating N limitation while decreasing N_2O emissions.

Chapter 5 focused on identifying the effects of N fertiliser rates on the performance of sugarcane and soybean in controlled glasshouse conditions. The use of a nodulating soybean and non-nodulating mutant soybean allowed to partially untangle the effects of interspecific competition and facilitative interactions derived from biological N fixation. We hypothesised that soybean will outcompete sugarcane under very low N fertiliser supply while the opposite will occur with high N fertiliser supply, and that a 'golden middle' N supply can be identified where soybean uses primarily N from biological N_2 fixation and sugarcane uses N fertiliser to foster facilitation between both crops.

Chapter 2 – Sugarcane intercropping systems.
A review

2.1 Introduction

Intercropping entails two or more crop species grown together with the broad aim of improving productivity, optimising natural resource use and steadying yield crops (Chogatapur, 2017, Dantata, 2014, Masasso, 2007, Keating and Carberry, 1993). Combining crops with different canopy structure, root properties, patterns of resource acquisition and requirements allows the complementary use of solar radiation, water, and nutrients, and reduces the presence of specific pests and pathogens (Duchene et al., 2017, Stagnari et al., 2017).

Sugarcane holds potential for intercropping as has been found in developing countries (Figure 2.1) where sugarcane is an important agricultural commodity and cash crop. Especially in subsistence farming, sugarcane is commonly intercropped (Kaur et al., 2016, Li et al., 2013). In these systems, intercropping facilitates food production to meet growing demand for diverse food and feed crops, achieves extra revenue and minimises risk (Chogatapur, 2017, Shiming and Gliessman, 2016, Parsons and Be, 2003). Sugarcane has a long growth cycle, is comparatively small early in the growing season, and is grown at wide row spacing so that intercrops can be accommodated temporally and spatially (Shiming and Gliessman, 2016, Geetha et al., 2015, Cadersa, 2001, Goviden, 1991). In South Africa, small-scale farmers grow sugarcane with food crops such as legumes and achieve greater food security and income and reduce the risk of crop failure (Parsons and Khubone, 1999). In India, intercropping is considered an alternative to crop rotations with a view of improving soil fertility, and increasing sugarcane production (Chogatapur, 2017, Geetha et al., 2015, Singh et al., 2003). In China, sugarcane-legume intercropping has been shown to alleviate pollution by reducing N fertiliser needs without affecting sugarcane yield (Luo et al., 2016, Shiming and Gliessman, 2016). In Mauritius, intercropping sugarcane addresses land shortages and the need for crop diversification and intensification (Goviden, 1991).

Research on sugarcane intercropping has concentrated on the compatibility of companion crops, agronomic performance and profitability relative to monoculture. Planting density, spatial arrangement, planting time and development of companion crops as food or green manure have been considered, while fertiliser management has received less attention (Brooker et al., 2015, Ijoyah, 2012, Kwong et al., 1996). In this review, we find that the outcomes of

intercropped sugarcane systems are highly variable and specific situation. This is unsurprising given the diversity of environmental settings and agronomies and prevents extrapolation of outcomes. However, key factors that determine the success of intercropped sugarcane include climate, soil type and crop management. Here, we synthesise the current state of knowledge from peer-reviewed published literature and propose next steps.

Figure 2. 1 Number of published sugarcane intercropping studies worldwide (1985-2016) compiled in this review (7 Brazil., 1 Mexico., 1 Guatemala., 5 Africa., 1 Philippines., 7 India., 6 China., 1 Bangladesh., 3 Mauritius)

2.2 Quantifying competition and facilitation

Interactions between crops in intercropping systems are characterised by competition and facilitation (Masasso, 2007). Competition occurs when one crop species exerts a negative effect on another, while facilitation results in positive effects that increase crop performance and yield (Lithourgidis et al., 2011). Interspecific competition for resources such as space, light, water, and nutrients (Li et al., 2001, Wallace, 1995) is modulated by agronomic measures such as irrigation or fertiliser application, planting time and others (Iijima et al., 2005).

To quantify interspecific competition, indices such as 'aggressivity' (AG) and 'competitive ratio' (CR) are useful. The former compares the yield of intercropped and monoculture systems about the area occupied by each crop, the latter measures the level of competition that one crop exerts on another. Values greater than 0 (AG) or 1 (CR), indicate that the competitive ability of one crop exceeds that of another crop (Yang et al., 2013, Billore et al., 2000).

The field experiment in China, a 3-year sugarcane-soybean intercropping, AG was -0.79 to -0.09 and CR were 0.6 to 0.9, indicating that sugarcane had a low competitive ability compared to soybean (Yang et al., 2013). At flowering stage, soybean had overgrown sugarcane, reducing light interception, N uptake and growth of sugarcane. In contrast, a 2-year sugarcane-soybean trial in India showed that sugarcane was more competitive than soybean with AG of -0.64 to -0.70 and CR of 0.18 to 0.25 because soybean was planted when sugarcane was three months old (Billore et al., 2000). In the Chinese study, soybeans were planted simultaneously with sugarcane. The authors of both studies concluded that the planting time of soybean is crucial for the success of intercropping.

2.3 Agronomic performance of sugarcane in intercropping

2.3.1 Compatibility of companion crops, spatial assessment and economic outcomes

Intercropping systems differ in their spatial and temporal design with various row intervals and spacing, or intermixing crops (Lithourgidis et al., 2011). In sugarcane, intercropping involves an alternative-row system with two or more species planted in separate and alternate rows (Arshad et al., 2014, Lithourgidis et al., 2011).

Table 2.1 summarises field studies with various spatial and temporal arrangements. The yield performance of sugarcane differed with planting arrangement, intercropped species and time of planting. Although most of the intercropped species were planted from zero to three days after the sugarcane planting, increase or decrease of the intercropped relative sugarcane yield in plant cane or ratoon were inconsistent. For example, common beans, potatoes and maize, planted in paired or triple rows decreased sugarcane yield by 2 to 36%, while soybeans marginally increased yield by up to 2% (Roodagi et al., 2001b). Maize and common beans planted at 1:1 spatial arrangement decreased sugarcane yield by 20 and 6%, while potatoes increased relative cane yield by 3% (Parsons and Khubone, 1999). Common beans planted in paired rows 15 days after sugarcane sowing (DASS) increased by 8 to 14% the sugarcane yield compared to monoculture sugarcane. While common beans planted simultaneously with sugarcane or planted >20 DASS reduced (4%) or increased (12%) sugarcane yield respectively (Souza Filho and Andrade, 1985).

Table 2. 1 Crop spatial arrangement of intercropped crops over cane plant and ratoon cane under intercropping systems. DASS (Days after the sugarcane sowing, it is related to plant cane), DASH (Days after the sugarcane harvest. It is related to ratoon cane). Economic advantage or disadvantages of the intercropping system related to the sugarcane monoculture is identified as + and - symbols, respectively.

Intercrop	Row arrangement	Intercrop planting day	Sugarcane row distance (m)	Trial years	Plant cane	Ratoon cane	Economic advantage	Author	Country
					Relative sugarcane cane yield (%) relative to monoculture (100%)				
Potato+mung bean	1:1				105		+		
Potato+mung bean	1:2				98		+		
Potato+mung bean	1:2	0 DASS	1.2	2	102	n/a	+	Islam et al., 2009	Bangladesh
Chilli	1:2				81		+		
Garlic	1:2				92		+		
Common bean					88	101	-		
Cabbage					88	95	+		
Maize					64	83	+		
Cowpea	1:1	3 DASS-DASH	1.2	3	93	n/a	-	Parson and Be, 2003	South Africa
Sweet potato					74	104	+		
Soybean					n/a	72	-		
Sweet corn					125	97	+		
Temperate potato	1:1	0 DASH	1.6	1	n/a	105	+	Cardesa et al., 2001	Mauritius
Tropical potato						104	+		
Brown hemp/ Soybean	1:1/2:3				103-102		+		
Cowpea	1:2/2:3				100		+		
Peanut	1:2/2:2	0 DASS	1.2	2	94	n/a	-	Roodagi et al., 2001b	India
Common bean	1:2/2:3				98		-		
Potato	1:2/2:4				66		-		
Maize	1:2/2:2				97		-		
Soybean	1:1/1:2	60 DASH	1.2	2	n/a	83	-	Billore et al., 2000	India
Maize					87	80	+		
Cabbage					77	88	+	Parson and Khubone, 1999	South Africa
Potato	1:1	3 DASS-DASH	1.2	2	82	103	-		
Peanut					65	94	-		
Common bean					91	94	-		
Green gram						102	+		
Black gram						104	+		
Peanut	1:1/1:2	0 DASH	0.9	3	n/a	99	+	Malavia et al., 1992	India
Peanut+irrigation*						97	+		
Black gram in strip						106	+		
Common bean						98	+		
Common bean						95	+		
Common bean+maize	1:1	0 DASH	1.6	1	n/a	91	-	Goviden, 1991	Mauritius
Alternate inter-row maize	1:2					99	+		
Each inter-row maize	1:1:1					90	+		
Common bean	1:1, 1:2 (0.5m) 1:2 (0.25m)	0 15/20 30/40 45/60 DASS	1.4	2	97-110 108-114 96-113 96-109	n/a	+	Souza and Andrade, 1985	Brazil

1:1 one row of legume is planted in the middle of the sugarcane inter-row; 1:2 two rows of legumes planted in the inter-row of two cane rows. 2:2 two rows of legumes planted every two-cane rows. 1:2 (0.5m) two rows of legumes planted at 50 cm far of two cane rows; 1:2 (0.25m) two rows of legumes planted at 25 cm far of two cane rows. 2:3 three rows of legumes planted every two-cane rows, 2:4 four rows of legumes planted every two-cane rows. DASS: days of the intercropped planting after sugarcane sowing; DASH: days of the intercropped planting after sugarcane harvest; 0: legumes were planted same day with sugarcane. Common bean *(Phaseolus vulgaris)*, cabbage (*Brassica oleracea var. capitata*), green gram *(Vigna radiata),* peanut (*Arachis hypogaea*), maize (*Zea mays*), brown hemp (*Crotalaria juncea*), cowpea (*Vigna unguiculata*); soybean (*Glicine max*), black gram (*Vigna mungo*), green gram *(Vigna radiata),* garlic *(Allium sativum),* chilli (*Capsicum annuum L*), sweet potato (*Ipomoea batatas*), sweet corn (*Zea mays L. var. rugose*), temperate and tropical potato *(Solanum tuberosum.)* *peanuts were planted first and then sugarcane at 1st irrigation.

In several studies, sugarcane intercropped with legumes or food crops had low yields and unfavourable sugarcane economies (Parsons and Be, 2003, Gana and Busari, 2003, Roodagi et al., 2001a, Billore et al., 2000, Parsons and Khubone, 1999). However, advantages of legumes in intercropping include soil improvement, which addresses the need for sustainable agriculture

to increase farm profits (Islam et al., 2009, Malavia et al., 1992, Goviden, 1991, Souza Filho and Andrade, 1985). In the studies examined here, intercropping with legumes as cash crops outweighed the economic disadvantages of reduced sugarcane yield. However, the variable outcomes of soybean, common beans, mung beans (Table 2.1) highlight that successful intercropping sugarcane requires knowledge about legume species and cultivars, spatial arrangement, the timing of sowing, competition for water and nutrients, and facilitation.

2.4 Fertiliser use

2.4.1 Soil quality, nutrient relations and microbial activity

Intensive sugarcane production can profit from intercropping, but few studies so far have assessed the interaction between nitrogen fertilisation and intercropping. The yield response of sugarcane to N fertiliser is variable and depends on a range of factors, including the type of intercrop and agronomic management (Table 2.2).

Plant cane was unresponsive to synthetic N fertiliser rates (applications from 0 to 100 % of the full N rate) when intercropped with cowpea (*Vigna unguiculata*), white lupin (*Lupinus albus*) and soybean (*Glycine max*). Whereas in the first ratoon crop, high N fertiliser rates (from 67 to 100% of the full N rate) sugarcane yield increased by 4 to 9% yield compared to monoculture (Ramouthar et al., 2014). In China, soybean intercropping had twin benefits of reducing N fertiliser needs from 525 to 300 kg ha^{-1} and increasing sugarcane yield by 14% (Luo et al., 2016). In Ethiopia, although intercropped soybean received 18 kg N ha^{-1} at planting time with sugarcane, the late application of N fertiliser (74 kg N ha^{-1}) to intercropped sugarcane (2.5 months after the sugarcane planting) reduced sugarcane yield by 2%. Conversely, a low fertiliser application (18 kg N ha^{-1}) to soybean improved sugarcane yield by 21% compared to sugarcane monoculture, showing the benefits of soybean at low N application (Teshome et al., 2015). Regarding sugarcane juice quality parameters, application of synthetically N fertiliser and presence of soybean as companion crop did not affect the percentage of sucrose in the raw sugar (Teshome et al., 2015, Yang et al., 2013).

The substitution of N fertiliser with organic sugar mill waste (termed filter cake, mill mud or press mud) with lentil (*Lens culinaris*) intercrop resulted in similar sugarcane yield as the sugarcane monoculture (Srivastava et al., 2009) indicating that alternative fertilisers and intercropping can be successful. Another study showed that soil physical properties improved during a two-year trial with the sugarcane+lentil intercropping system as soil bulk density of

the furrow in the first 15 cm depth decreased and soil infiltration rate increased compared to sugarcane only (Chogatapur, 2017)

Table 2.2 shows a consistent trend for increasing yield in intercropped sugarcane compared to sugarcane monoculture with non-legume crops is seen at rates >100 kg N ha^{-1} (Kaur et al., 2016, Vashishtha and Sinha, 2004, Singh et al., 2003). In India, intercropping with *Brassica* species and application of N fertiliser (112 to 275 kg N ha^{-1}) increased sugarcane yield by up to 38% (Kaur et al., 2016). In Mauritius, with 220 kg N ha^{-1} (100 and 120 kg N ha^{-1} applied to maize and sugarcane, respectively), intercropping with maize (*Zea mays*) was associated with a 29% increases in sugarcane yield, compared to the monoculture (Kwong et al., 1996). In this study, intercrops did not affect the uptake of N by sugarcane. For instance, the pure stand sugarcane, as well as the intercropped sugarcane with maize, showed similar N uptake despite that maize was fertilised with 100 kg N ha^{-1}. Intercropped sugarcane showed a low N recover ~10% (^{15}N-labelled fertiliser) of the N rates applied to maize. The low acquisition of N applied to the intercrop was related to the high rate of biological immobilisation and limited lateral movement of fertiliser.

From an economical viewpoint, the studies summarised in Tables 2.1 and 2.2 showed that sugarcane intercropping with food crops including legumes were viable economically in most of the studies. In some instances, the net margin (% of revenue remaining after operating expenses) with soybean as green manure or soybean grain was 17 and 4% lower respectively, compared to sugarcane monoculture. These reductions in profitability were due to the costs of soybean planting and harvesting. In contrast, Bolonhezi et al. (2010) showed that although sugarcane yield intercropped with common bean at N application rate of 80 kg N ha^{-1} had 23% lower yield than monoculture sugarcane; profitability increased with the bean harvest. Different organic amendments influenced the benefit/cost ratio in a sugarcane-lentils intercropping system. For example, application of 20 t ha^{-1} of farmyard manure had an economic advantage, while 10 t ha^{-1} press mud had a lower economic advantage because of its high cost (Srivastava et al., 2009). The economic benefits of intercropping with two food crops depend on the profitability of both individual cropping operations while intercropping with green manure (or companion cropping) depends on the green manure crop enhancing the yield of sugarcane.

Table 2.2 Crop spatial arrangement, N fertilisation rates and sugarcane performance in intercropping systems. Sugarcane monoculture is 100% relative yield of each study. Economic advantage or disadvantages of the intercropping system related to the sugarcane monoculture is identified as + and - symbols, respectively.

Intercropped crop	Row arrangement	Fertilizer N kg ha^{-1}	Trial years	Row distance (m)	Plant cane	Ratoon Cane	Economic advantage	Authors	Country
					Relative cane yield (%)				
Rapeseed	1:1/1:2	275				118		Kaur et al., 2016	India
Mustard		275	2	0.9		138	+		
Sugarcane monoculture		225				100			
Soybean	1:1	300			94			Shiming and Gliessman, 2016	China
	1:2	300	5	1.2	98	n/a	+		
	1:1	525			94				
	1:2	525			94				
Soybean	1:1	18	1	1.5	121	n/a	+	Teshome et al., 2015	Ethiopia
		18+74[†]			98				
		% of full N rate							
Cow pea		0		0.9	98	87		Ramouthar et al., 2013	South Africa
White lupin	1:1/1:2	33	2	1.6	97	95	-		
Soybean		67		1.5	89	104			
		100			98	109			
Common bean	1:2	0,40,80,120,160	1	1.6	n/a	77	+	Bolonhezi et al., 2010	Brazil
		t/ha							
Lentil+ manure+ T. viride	1:2	20	4	0.9	95	95	+	Srivastava et al., 2009	India
Lentil + filter cake		10			91	97	-/+		
Mustard	2:2	75	3	0.9	n/a	95	+	Vashistha and Sinha, 2004	India
		100				103			
		125				108			
Mustard	1:2	112	4	0.9	122	100	+	Pandey and Shukla, 2003	India
Autumn cane		150			100	100			
Spring cane		187			96	105			
Summer cane					67	n/a			
Maize	1:1	100	1	1.6	99		+	Kwong et al., 1996	Mauritius
		220				n/a			
		220			129				
Potato	1:1	100	1	1.6	103		+		
		220			141	n/a			
		220			142				

1:1 one row of legume is planted in the middle of the sugarcane interrow; 1:2 two rows of legumes planted in the interrow of two cane rows. 2:2 two rows of legumes planted every two-cane rows. [†] 18 kg N ha^{-1} applied to intercropped soybean at planting seeds of soybean and sugarcane sets and 75 kg N ha^{-1} applied after 2.5 months of the planting time. Common beans *(Phaseolus vulgaris)*, green gram *(Vigna radiata)*, peanut *(Arachis hypogaea)*, cowpea *(Vigna unguiculata)*; soybean *(Glicine max)*, rapeseed *(Brassica napus)*, mustard *(Brassica juncea)*, white lupin *(Lupinus albus)*, lentil *(Lens culinaris)*.

In addition to easily quantifiable agronomic and economic outcomes, intercropping systems influence microbial populations and soil enzymes as important components of agro-ecosystems (Li et al., 2013, Stigter and Baldy, 1995). Soil microbial composition and biomass are an important soil quality indicators, affected by plant community composition, soil organic matter

content and composition, soil moisture and temperature (Wardle, 1992, Staben et al., 1997), and contributing much to nutrient cycling and energy flow (Li et al., 2013).

A glasshouse study that was continued until the soybean crop flowered showed that sugarcane-soybean intercropping enhanced soil quality and promoted crop growth and yield (Li et al., 2013). Reasons included beneficial effects of soil microorganisms and soil enzymes that boosted the availability of inorganic N (66%) and P (311%) and increased soil organic matter (22%) (Li et al., 2013). An Indian study showed that intercrops increased soil microbial biomass C and N over monoculture sugarcane. Legume intercrops nearly doubled soil microbial biomass N compared to non-legume crops, while food crops (maize, wheat, mustard, potatoes) increased soil microbial biomass-C by 14% above that of legume intercrops (Suman et al., 2006). The low C/N ratio of legume residues (<14) increased total soil N by 24%, and allowed rapid decomposition and stimulated N mineralisation, which in turn increased the available N in the sugarcane root zone by 9%.

We conclude that the positive effects of intercropping on soil quality and nutrient relations demand further investigation. Soil quality, nutrient relations and the ongoing dynamics of intercropped systems should be assessed to identify suitable agronomic practices for particular settings and with a view of environment concerns. The potential of intercropping to reduce N pollution and increase soil carbon levels should be explored in more detail as these are principle concerns for the industry. It should be estimated how much BNF is performed by legumes in different environments to obtain a basis for reducing N fertiliser rates. Necessary next steps are field trials performed over several years, possibly accompanied by controlled experiments, and with a detailed analysis of processes to advance mechanistic understanding of intercropped systems to inform future design.

2.4.2 Legumes as green manure

Legume N-fixation provides obvious benefits for the intercrop and additional flow-on benefits through the breakdown of legume residues, root exudates and rhizo-deposits (Singh et al., 2003, Jensen, 1996, Ong, 1995, Lemaire, 1995). Few studies have considered legumes as green manure crops to supply N although legumes have been planted as intercrop alongside sugarcane row, cut and left to decompose to improve soil fertility and to enhance physical, chemical and biological characteristics through greater soil organic matter content and mineralisation rates (Trento Filho, 2010). It has been proposed that legume intercropping could

extend the sugarcane ratoon cycle and thereby reduce the cost of establishing sugarcane plant crops (Ambrosano et al., 2013, Trento Filho, 2010). Midmore (1993) cited that the benefits of legumes go beyond the immediate intercropping system, but that residual nutrients supply to subsequent crop. Sugarcane-legume intercropping is considered as a sustainable agricultural intensification because it reduces the input of N fertiliser and integrates BNF into the agronomic nutrient cycle (Robertson and Groffman, 2007, Robertson and Thorburn, 2007).

A wide range of tropical legumes species and genotypes has been assessed for compatibility and performance in sugarcane intercropping as green manure crops. Legumes within the sugarcane row cut or incorporated to the soil can be favourable or unfavourable to sugarcane yield (Córdova-Gamas et al., 2016, Ambrosano et al., 2013, Prellwitz and Coelho, 2011, Pérez et al., 2009, de Resende et al., 2003, Singh et al., 2003, Roodagi et al., 2001b, Yadav and Yaduvanshi, 2001). To account for the large range of yield responses, we converted absolute yield to relative yield with sugarcane monoculture set at 100% (Table 2.3).

In India, studies from 1997 to 2001 showed that the use of different legumes as green manure crops affected sugarcane yield widely from minor yield reduction (-2%) to a considerable increase (18%). Daincha (*Sesbania aculeata)*, brown hemp (*Crotalaria juncea*), cowpea (*Vigna unguiculata*) and manila agathi (*Sesbania rostrata*) benefited sugarcane productivity and soil fertility when they were incorporated at 45 days after the sowing (Ramesh et al., 2003). Species in the genus *Crotalaria* are the most studied green manure legumes followed by horse bean (*Canavalia ensiformis*) and pigeon pea (*Cajanus cajan*). Ambrosano et al. (2013) showed that slender leaf rattlebox (*Crotolaria ochroleuca*), increased the yield of sugarcane ratoons by up to 9%, while brown hemp decreased sugarcane yield by half. Legume management, including planting density and spatial arrangement, time of planting and harvesting allowed to overcome the suppressive effects of brown hemp and increased sugarcane yield by 24 to 46% (Prellwitz and Coelho, 2011, Pérez et al., 2009). Brown hemp, showy rattlebox (*Crotalaria spectabilis*), horse beans, pigeon pea and sesbania (*Sesbania cannabina*) up to doubled sugarcane yield compared to unfertilised monoculture, indicating that legumes benefit the N budget of sugarcane (Córdova-Gamas et al., 2016, Ambrosano et al., 2013, Prellwitz and Coelho, 2011, Pérez et al., 2009, Singh et al., 2003). However, in those studies, unfertilised sugarcane-legume intercrops yielded less than N-fertilised sugarcane. Application of 25 to 250 kg N ha^{-1} to sugarcane-legume intercrops boosted yield by 2 to 36% compared to non-N fertilised intercrops (Yadav and Yaduvanshi, 2001, Singh et al., 2003, Roodagi et al., 2001b).

Yadav and Yaduvanshi (2001) found that application of 75 and 150 kg N ha^{-1} to prickly sesban or daincha intercropping improved sugarcane yield from 21 to 40% following a wheat crop, and 16 to 26% following mustard. This effect continued in the 1st ratoon crop, where the residual effect of green manure and application of N resulted in significant increase in sugarcane yield of 12 to 40%. Similar outcomes were found when prickly sesban was incorporated as green manure to both plant and 1st ratoon sugarcane, increasing yield 12 to 36% with applications of 75 and 225 kg N ha^{-1}, with greater tiller number, sugarcane biomass and leaf area index (Singh et al., 2003).

Green manure intercropping (or companion cropping) has focused on legume planting methods, time of planting, cutting or incorporating legume biomass into the soil. Spatial arrangements (1:1, 1:2 and 2:3 sugarcane-legume) overall did not affect sugarcane yield (Singh et al., 2003, Yadav and Yaduvanshi, 2001, Roodagi et al., 2001a). However, in plant cane, paired rows of legumes caused competition and decreased sugarcane yield, reducing tiller numbers by 9-13% (Yadav and Yaduvanshi, 2001) and up to 30% (Singh et al., 2003). Advantages of two-rows of prickly sesban, horse bean and pigeon pea manifested themselves in the subsequent crop when sugarcane yield increased from 6 to 50%, due to residual effects of green manure (Córdova-Gamas et al., 2016, Singh et al., 2003, Yadav and Yaduvanshi, 2001). Soil properties improved with rising soil organic carbon levels (Singh et al., 2003, Roodagi et al., 2001b, Yadav and Yaduvanshi, 2001), increasing N availability and dropping in pH on alkaline soil after two years of evaluation (Singh et al., 2003, Yadav and Yaduvanshi, 2001). Similarly, Roodagi et al. (2001b) found that incorporation of brown hemp and cowpea biomass benefitted sugarcane yield (up by 3.5 and 1.6%, respectively) due to higher levels of soil organic matter and available N.

The effect of planting times has been explored at different planting days of legumes and sugarcane (Table 2.3). Planting horse bean and brown hemp 1, 10 or 20 days after planting or harvesting sugarcane did not affect sugarcane yield (Pérez et al., 2009) but the timing of cutting or incorporating legume biomass is important. In Brazil, sowing brown hemp by 45 and 51 days after the sugarcane harvest and incorporating legume biomass at 110 and 103 days after legume sowing resulted in highest sugarcane yield (up by 36 and 32% respectively) compared to uncut legumes (Prellwitz and Coelho, 2011). In order to limit competition, de Resende et al. (2003) reported that legumes had to be cut within 71 days when both sugarcane and legumes were planted together. Brown hemp covered over 55% of the soil by 35 days after planting

sugarcane, and 51 days after harvesting the 1st ratoon. The competition was even stronger with showy rattlebox, horse bean and velvet bean (*Mucuna deeringiana*) which exceeded 80% of soil cover by 51 after sugarcane planting and 71 days after harvest of the 1st ratoon (de Resende et al., 2003). In India, species in genus *Sesbania* reduced plant cane yield by 11% (*S. cannabina*) and 7% (*S. aculeata*) when they were cut 60 or 45 days after planting sugarcane, respectively (Singh et al., 2003, Yadav and Yaduvanshi 2001).

The time of sowing, cutting and incorporating legumes affected how much N legumes accumulated in vegetative biomass and seeds. Species or genotypes of *Crotalaria*, horse bean, pigeon pea, velvet contained between 114 and 350 kg N ha^{-1} when planted 60 days after sugarcane harvest and were harvested after 120 days (Ambrosano et al., 2013). A similar study in Mexico showed that horse bean and pigeon pea planted 20 days after planting sugarcane, accumulated 155 and 413 kg N ha^{-1}, respectively (Córdova-Gamas et al., 2016). Both studies considered whole biomass (roots, shoots, seeds) to determine the total amount of N contained in the legumes. Which combine N derived from soil and biological N fixation, brown hemp accumulated 250 kg N ha^{-1} when planted at 51 days after sugarcane harvest and incorporated into the soil at flowering stage (103 days post sowing), boosting sugarcane yield by 44% compared to uncut legumes (Prellwitz and Coelho 2011). Some studies detected lower N accumulation with 10 to 40 kg N ha^{-1} with pigeon pea, showy rattlebox, horse bean, velvet or sesbania cut or incorporated earlier at the vegetative stage 35 to 71 days post-planting (De Resende et al., 2003, Singh et al., 2003). Horse bean and pigeon pea grown for 65-70 days after sugarcane harvest and then cut and incorporated, accumulated 97 and 154 kg N ha^{-1} respectively. Fertile Andosol and other environmental factors (Pérez et al., 2009) may have enabled this large N accumulation over a comparatively short time span.

Two studies aimed to quantify the percentage of N derived from legume (biological N$_2$ fixation, BNF). De Resende et al. (2003) and Ambrosano et al. (2013) used ^{15}N natural abundance to show that tropical legumes acquired between 50 to 99% their N needs via BNF. We interpret these results with caution, as BNF is difficult to quantify in field grown plants using ^{15}N natural abundance.

Table 2. 3 Spatial arrangement, planting day of legumes (DASS, days after sugarcane sowing; DASH, days after sugarcane harvest), time of biomass cutting or incorporating into the soil of sugarcane-legume intercropping systems. Relative sugarcane yield in the intercropping systems is shown with monoculture sugarcane yield as 100%.

Intercropped crop	Row arrangement	Planting day of legumes	Days of legume treatment	Fertilizer kg N ha^{-1}	Trial years	Plant sugarcane	Ratoon sugarcane	Author	Country
						Relative sugarcane yield (%)			
Horse bean	1:2	21 DASS	120 (S)	0	3	72	122	Córdova-Gamas et al., 2016	Mexico
Pigeon pea						54	92		
Slender leaf rattlebox	1:2	60 DASS	120 (I)	0	3	n/a	109	Ambrosano et al., 2013	Brazil
Crotalaria pallida				0			92		
Shortflower rattlebox				0			91		
Pigeon Pea				0			96		
Brown hemp				0			59		
Horse bean				0			90		
Velvet bean				0			97		
Brown hemp	1:2	45 DASH	non- cut	0		n/a	47	Prellwitz and Coelho, 2011	Brazil
	1:2		110 (I)	0	2		83		
	1:1		110 (I)	0			72		
	1:2	51 DASH	non- cut	0			65		
	1:2		103 (I)	0	2		97		
	1:1		103 (I)	0			67		
Horse bean	1:2	1, 10, 20 DASS-DASH	PT: 65 (I)	0	5	112	107	Perez et al., 2009	Guatemala
Brown hemp			R: 60-70 (S)	0		101	105		
Pigeon pea	1:1	0 DASS	110 (S)	0	2	52	n/a	Trento Filho, 2010	Brazil
Brown hemp				0		101			
Pigeon pea				15*		39			
Brown hemp				15*		58			
Brown hemp	1:2	0 DASS /DASH	35-51 (S)	0	3	73	77	De Resende et al., 2003	Brazil
Showy rattlebox				0		91	97		
Horse bean			51-71 (S)	0		75	77		
Velvet bean				0		72	85		
Sesbania pea	1:1	30 DACS	45 (I)	0		99	105	Singh et al., 2003	India
			60 (I)	0		96	108		
	1:2		45 (I)	0	3	96	105		
			60 (I)	0		85	99		
Sesbania pea	1:1/1:2	30 DASS		75		114	112		
			45 (I)	150		121	123		
			60 (I)	225		136	132		
Prickly sesban	1:1	0 DASS /DASH	30 (I)	75	2	109	107	Yadav and Yaduvanshi, 2001	India
	1:2		45 (I)	150		101	113		
Brown hemp	1:2/2:3	0 DASS	? (I)	25	1	103	n/a	Roodagi et al., 2001a	India
Cowpea			? (I)	25		102			

570

1:1 (one row of legume was planted at 60, 65 and 75 cm far from the sugarcane row); 1:2 (two rows of legumes was planted at 20-25, 30, 40, 45 and 50 cm far from the sugarcane row); DACS: days after cane sowing; DACH: days after cane harvest; 0: legumes were planted same day with sugarcane; I: legume cut and incorporated into the soil; S: legume cut and left on the soil surface. PT: plant cane; R: ratoon cane. Sugarcane row distance in India was 0.90 m, in Mexico 1.35 m, in Guatemala 1.50 m and Brazil ranged between 1.20 to 1.40 m. *organic amendment composition: filter cake, ash and bagasse 3:1:5 proportion. Slender leaf rattlebox (*Crotalaria ochroleuca*), crotalaria pallida (*Crotalaria mucronata*), crotalaria brevifolia, pigeon pea (*Cajanus caja*n), brown hemp (*Crotalaria juncea*), horse bean (*Canavalia ensiformis*), velvet bean (*Mucuna deeringiana*), showy rattlebox (*Crotalaria spectabilis*), sesbania (*Sesbania cannabina*), prickly sesban (*Sesbania aculeate*), cow pea (*Vigna unguiculata*)

The method is inherently inaccurate due to different isotopic signals of soil N sources, impacts of rooting depth and mycorrhiza status, discrimination against the heavy N during uptake as well as internal N cycling, all of which affect the ^{15}N signatures of legumes and non-legume comparator species (Peoples et al., 2015). The finding showed that 99% of legume-N is derived

from BNF when legumes are grown in fertile agricultural soils is likely to be a substantial overestimate.

Taken together, the findings of these combined studies on sugarcane intercropping demonstrate that a range of outcomes is possible from this technique. Intercropping results in both positive and negative impacts on sugarcane yield, regardless of intercrop species (Figure 2.2a, b), country of study origin (Figure 2.2c), and row arrangement (Figure 2.2e). Some trends are evident: brown hemp and pigeon pea appear to produce predominantly negative yield responses in sugarcane, while *Sesbania* species produced largely positive yield responses (Figure 2.2b) and may be worthy of further investigation. The relative yield was highly variable at fertiliser application rates of zero kg N ha^{-1}. A trend of increased relative yield at N application rates of up to ~250 kg N ha^{-1}, and subsequent decrease up to N application rates of ~550 kg N ha^{-1} was apparent. However, this may be confounded by the relatively small number of fertilised studies and should be interpreted with caution. A slight trend towards higher relative yield at small (0.9m) and wide (1.6m) compared to moderate (1.2-1.3m) sugarcane row spacing was apparent. This could potentially suggest that competition is avoided at wide but not moderate row spacing; and that facilitation increases with decreased row spacing; however, other interpretations are possible. Only one dedicated trial assessed the impact of different row spacings and found similar results to the broad pattern seen here (Ramouthar et al., 2013). The unequal number of trials and unequal distribution of intercrop types across the different row spacing mean that further dedicated trials examining row spacing should be conducted in the future.

The studies above highlight that numerous factors have to be considered when deciding on legume species or genotypes as intercrops as they can be both competitor and facilitator crops under different circumstances. Key considerations from a N perspective include effective BNF and N acquisition from the soil, the ability to safeguard N and avoid losses, and allowing N to be available in synchrony with sugarcane needs. Other considerations include the effects of legumes on nutrient other than N, regarding both mobilisation and competition, water use, ability to outcompete weeds and tolerance to overall environmental conditions including waterlogging, climate, and tolerance to pests and diseases. In regions where, appropriate legume cultivars have been selected for grain, fodder or green manure production, it may be a small step to find suitable cultivars to test with sugarcane. It is clear that regional solutions have to be found to optimise sugarcane-legume intercropping.
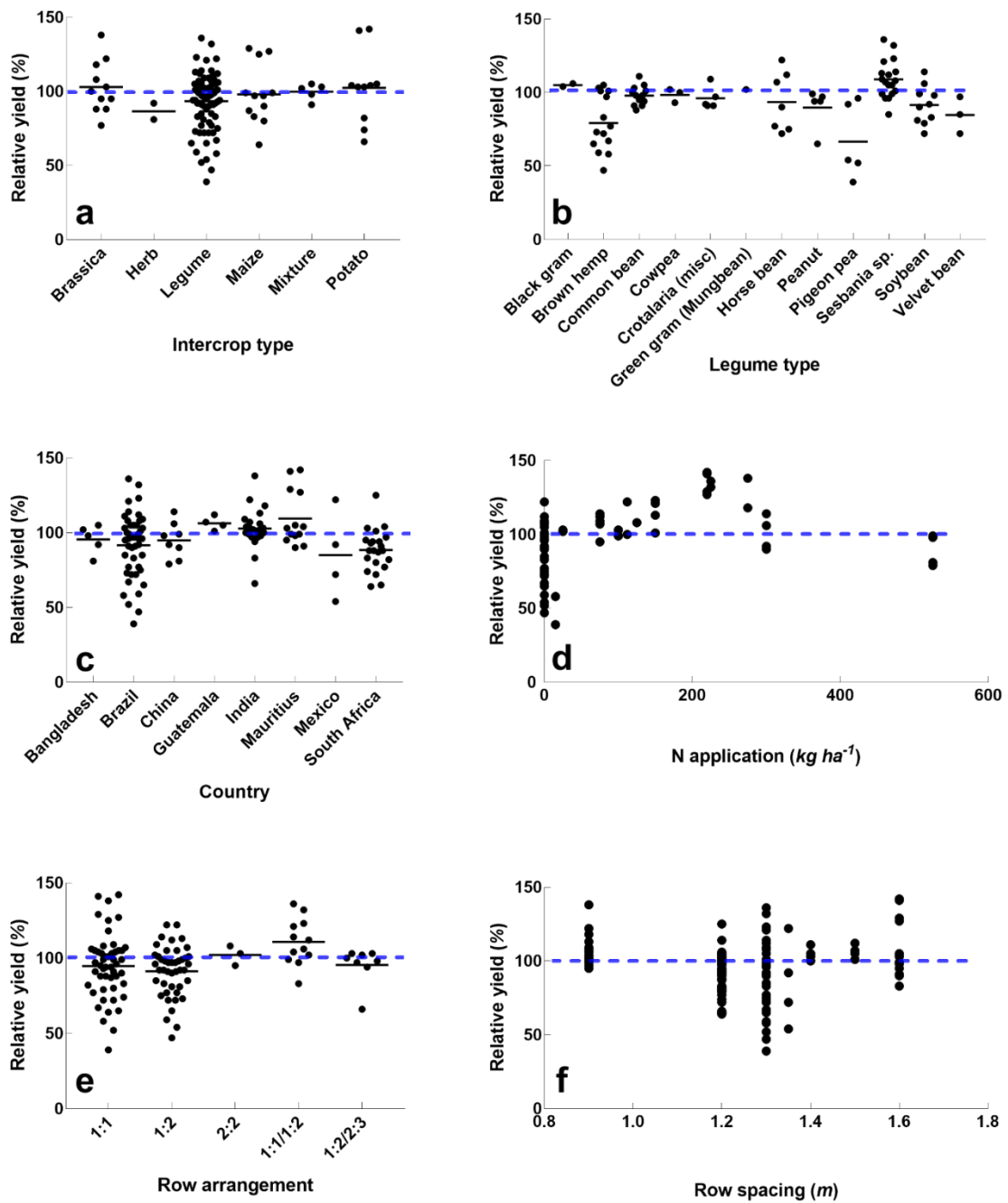
Figure 2. 2 Analysis of published sugarcane intercropping data. Relative yields of intercropping compared to equivalent monoculture are shown, grouped by a) broad botanical grouping of intercrop species b) only legumes, grouped by species, c) country of study and e) row arrangement. Each data point is plotted – horizontal lines indicate the average of each group. Relative yield is also shown versus d) N fertilizer application rate and f) sugarcane row spacing.

2.4.3 Legumes to control environmental pollution

Legumes as intercrops in sugarcane systems have not been studied in depth for environmental benefits. Most studies in sugarcane-legume intercropping systems have focussed on additional profit from legume grains or for soil improvement with legume residues. To the best of our knowledge, one study from China showed that reduction of N_2O emissions was associated to 40% reduction of N fertiliser in a sugarcane-soybean intercropping system and the presence of soybean did not affect sugarcane productivity negatively at the reduced N fertiliser rate (Luo et al., 2016).

2.5 Conclusions

This review summarised the impact of different crop species that have been used as intercropped in sugarcane monoculture systems. The studies provided a large range of sites, years and intercropping management. The productivity of the two components has been the main target in all studies followed by soil health improvement. Our results highlighted the effect of intercropped species (either as a cash crop or as green manure) on sugarcane yield. In general, intercropped sugarcane yields showed favoured, unfavoured or neutral response due to companion crops. However, the system was able to provide in most of the cases financial profits to subsistence farming. Additionally, when legumes used as green manure or in residue decomposition after harvest, sugarcane-legume intercropping systems can be considered as sustainable agricultural management because of enhancing physical and chemical soil properties, especially in the beneficial effect on N supply.

The lack of sugarcane-legume intercropping studies related to environment pollution was noticeable. Currently, sugarcane production is under examination and intercropped legumes within sugarcane systems might be adopted to as a sustainable and viable and practical agricultural management for farmers. Therefore, future research is needed under long term settings taking into account soil type, climatic conditions, legumes species and management to determine the real effect on environmental issues as intercropping systems. Thus, this review was the first outcome as part of a project that evaluated legumes (to be decomposed in the field as green manure) as intercrop in commercial sugarcane fields to reduce N losses as nitrous oxide (N_2O) and the partial dependency of synthetically N fertiliser.

Chapter 3 – Evaluating legume companion cropping to mitigate nitrous oxide emissions from sugarcane agriculture in tropical soils

Chapters 3 and 4 show the outcomes of cultivating ratoon sugarcane simultaneously with sacrifical legumes that decompose in the field as potential avenue to reduce nitrogen (N) fertiliser needs and mitigate nitrous oxide (N_2O) emissions.

In Chapter 3, we report 3-years of sugarcane yield measurements and 2-years of N_2O emissions quantification in field experimentation at a commercial sugarcane farm in Australia's Wet Tropics. In the first year, cane rats consumed most legume seedlings and prevented legume establishment. In following two years cane rats were controlled and soy and mung beans established well after sowing through the sugarcane trash blanket. Nitrous oxide emissions and sugarcane yields were mostly similar with recommended (full) and 38% reduced N rates (148 and 91 kg N ha^{-1}), irrespective of legumes present. Sugarcane yields were lower with a 56% reduced N fertiliser rate (66 kg N ha^{-1}) or no N fertiliser. N_2O emissions were influenced predominantly by N fertiliser rate and rainfall, with emission factors of 0.26 to 1.78. Soybean emerged as a more favourable companion crop than mung bean, which carried a 9% yield penalty in the third year.

3.1 Introduction

Soils in wet tropical climates that receive large doses of N fertilisers, especially as single applications of highly reactive N such as urea or ammonium nitrate, could generate large nitrous oxide emissions (N_2O) due to the temporal excess of N pool. This is under scrutiny because N_2O emissions from agriculture contribute at least 10-12% to anthropogenic greenhouse gas emissions (IPCC, 2014). Greenhouse gas (GHG) emissions (N_2O, CH_4, CO_2) from the agricultural sector account for 74% of anthropogenic N_2O emissions (Department of Environment, 2014) because of over- and inefficient use of N fertilisers and manures (Wang et al., 2016, 2011, Barton et al., 2008,).

Nitrous oxide is generated in soil by microbial conversion of N of ammonium and nitrate *via* nitrification and denitrification (Itakura et al., 2013, Bodelier, 2011, Kool et al., 2011, Robertson and Groffman, 2007, Mosier et al., 2004, Müller et al., 2004, 2014). Nitrous oxide emissions are greater under conditions of high soluble soil inorganic N, warm temperatures,

easily microbial accessible organic carbon, and micro-anaerobic conditions that generally coincide with high soil moisture and biological activity (da Silva et al., 2013, Nazaries et al., 2013, Schlesinger and Bernhardt, 2013, Thorburn et al., 2013, Macedo et al., 2008). These conditions characterise many tropical soils under high-production agriculture including sugarcane.

Particularly sugarcane cultivation often reported with large emission factor (the percentage of N fertiliser emitted as N_2O) of 3.87% in average (Lisboa et al., 2011), which is nearly 4-times of the general emission factor (1%) for crop production in a global scale (IPCC, 2006). Emissions factors from Australian sugarcane soils range widely from <1 to 21% (Kingston et al., 2016, Wang et al., 2016, 2014, 2008, Denmead et al., 2010b, Allen et al., 2010). Mitigation strategies include reducing N fertiliser rates, applying fertiliser in multiple smaller doses, using slow-release fertilisers and N-conversion inhibitors, and minimising waterlogging by avoiding flood irrigation (Kingston et al., 2016, da Silva Paredes et al., 2014, de Barros et al., 2010, Allen et al., 2010, Wang et al., 2008, Huth et al., 2010, Denmead et al., 2010b, 2008, Weier et al., 1996).

Another approach to mitigate N_2O emissions as yet unexplored in Australian sugarcane production is companion cropping with legumes to supply N *via* biological N-fixation (BNF). Companion legumes are a sacrificial crop that is not harvested but decomposes in the field. However, there are reports that legumes can increase N_2O emissions *via* root exudates and litter decomposition (Saggar et al., 2013, Jensen et al., 2012, Rochette and Janzen, 2005), and empirical research has to determine if legumes can reduce the N_2O footprint of sugarcane cropping.

In Australia, legumes, especially soybean (*Glycine max*) and peanut (*Arachis hypogaea*), are widely grown as rotation crops (i.e. between sugarcane ratoon cycles) and harvested for grain. The demonstrable benefits of legumes on the following sugarcane crop include reduced incidence of soil-borne diseases, weed suppression and N input (2003, Thorburn et al., 2010, Berry et al., 2009, Kaur et al., 2015). Legume companion cropping has not received much attention although individual farmers are experimenting with such systems. It is currently unknown whether companion cropping represents a viable alternative to current agronomic management, and how companion cropping should be optimised to maximise environmental and economic benefits.

Co-cultivation of sugarcane and legumes is documented in non-mechanised agriculture in Brazil, India, Guatemala and Mexico. Commonly used legumes are sunn hemp (*Crotalaria juncea*), canavalia (*Canavalia ensiforme*) and pigeon pea (*Cajanus cajan*). In these sugarcane-legume intercropping systems both crops are harvested for food and feed (Kaur et al., 2015, Arshad et al., 2014, Parsons and Be, 2003, Roodagi et al., 2001a, 2001b) although some legume residues may remain on the field. Documented benefits include improved soil physical, chemical and biological properties (Trento Filho, 2010, Ambrosano et al., 2013, Prellwitz and Coelho, 2011, Córdova-Gamas et al., 2016, Pérez et al., 2009, de Resende et al., 2003, Singh et al., 2003, Yadav and Yaduvanshi, 2001, Roodagi et al., 2001b).

There is little information on sugarcane-legume cropping in mechanised agriculture (Shiming and Gliessman, 2016), and this study represents an initial step assessing legumes as companion crops in commercial sugarcane production. We hypothesised that legume companion crops can reduce synthetic N fertiliser needs, and consequently result in lower N_2O emissions.

3.2 Materials and methods

3.2.1 Experimental site

The field trial was carried out on a commercial sugarcane farm located in the Herbert River region near Ingham, Abergowrie (18º 27.9" S, 145°50.9" E) in Queensland, Australia from 2013 to 2016. The areas have a wet tropical climate with mean maximum summer and winter temperatures of 31.7 and 13.7 °C, and mean annual rainfall of 1407 mm (Bureau of Meteorology, Site 032174, Abergowrie Alert).

The trial commenced in the third ratoon (*Saccharum officinarum* L, cultivar KQ 288) in August 2013. The first year was designed to test legume sowing rate and establishment, N fertiliser rates, and sugarcane yield. In the first year (2013-14 crop season), legumes germinated well, but the establishment was hampered by strong herbivory by cane rats (*Rattus sordidus*) in the first year. Poison baiting successfully controlled rats in the following years. In next crop seasons (2014-15 and 2015-16), we tested N rate and legume interactions by quantifying soil variables (soluble inorganic N, temperature, water filled pore space) and N_2O emissions, as well as sugarcane yield. Nitrogen fertiliser rates ranged from no N fertiliser, 56 % reduced N fertiliser rate (66 kg N ha^{-1}), 38% reduced rate (91 kg N ha^{-1}), to recommended (full) rate (148 kg N ha^{-1}, Table 3.1).

An overview of the trial is shown in Table 3.1. Over the three years of experimentation, we adjusted treatments according to outcomes in the previous year and other sites to modify N fertiliser and legume planting. Sugarcane yield was quantified with three N fertiliser rates; our budget constrained GHG measurements to full and reduced N rates and unfertilised control in years 2 and 3 (2014-15; 2015-16).

Table 3. 1 Nitrogen and legume treatments implemented over three seasons at a commercial sugarcane farm at the Abergowrie, North Queensland. In the first season, legumes were decimated by cane rats. GHG and soil variables were quantified over two seasons from 2014 to 2016.

Treatments	2013-2014	2014-2015	2015-2016
	N kg ha^{-1}		
Full N	148	148	148
38% Reduced N	n/a	91	91
	91+soybean	91+soybean	91+soybean
	91+mung bean	91+mung bean	91+mung bean
56% Reduced N	66+soybean	66+soybean	n/a
	66+mung bean	n/a	n/a
No N +legume	n/a	n/a	0+soybean
No N (control)	0	0	0

n/a not available

3.2.2 Experimental design

The trial was a randomised block design with three replicates. Each experimental plot had six sugarcane rows with 1.65 m row spacing and 210 m row length. All measurements were taken from the two central rows and all six rows were harvested. Soybean (*Glycine max* (L.) Merr), cultivar Leichardt and mung bean (*Vigna radiata* (L.) R. Wilczek), cultivar Krystal were sown with a legume planter and coulter that deposited the legumes through the sugarcane trash blanket into the soil. Legumes were sown 106 days after harvesting the 3rd ratoon crop (2014-15), and in the following season (2015-16) 122 days after harvesting the 4th sugarcane ratoon crop. The planting distance for soybean and mung bean was at 0.15 m on both sides of the sugarcane row with 60 and 30 kg seed ha^{-1}, respectively. Soybean was inoculated with Nodule NTM rhizobia bacteria to ensure maximum N fixation potential, mung bean was not inoculated as no commercial inoculants for the species are available. All plots received a basal fertilisation (448 kg ha^{-1}) of a commercial fertiliser (CK140 Incitec Pivot) contains 22 kg ha^{-1} of

phosphorus, 96 kg ha^{-1} of potassium and 28 kg ha^{-1} of sulphur at the time suggested by the management guidelines (Sugar Research Australia 2013). Nitrogen was applied as prilled urea at the same day of planting legumes (2013-14), 30 days (2014-15), and 42 days (2015-16) prior to planting legumes. All fertiliser was applied as banding at 10 cm soil depth.

3.2.3 N$_2$O sampling and analysis
We used manual GHG chambers to quantify emissions of N$_2$O from the soil surface. Two chambers were installed in each plot to cover the within-field variability introduced by field topography, fertiliser and legume placement. One chamber covered the row and part of shoulder directly over the fertiliser band, and the second chamber the inter-row space. Chambers consisted of a square stainless-steel base chamber of 0.25 m^2 introduced approximately 5 cm deep into the soil and a cubic top chamber placed over the base with a total volume of 140 litres. Top chambers were equipped with a valve for gas sampling. Joints between base and top chambers were sealed with air-tight door seals.

The frequency of gas sampling depended on local weather conditions. Fifty-one sampling dates were spread across 279 days after N fertiliser application in the 2014-2015 season, and 46 sampling dates across 288 days in the 2015-2016 season. Quantification of GHG emissions commenced two- and ten-days post N fertilisation in the 2014-15 and 2015-16 seasons, respectively.

More frequent sampling occurred during spring and summer when soil had high soluble N concentrations, moisture and temperature and conditions were most conducive for N$_2$O emissions. Gas sampling was conducted every 3 to 7 days during spring and summer, every 7 days in autumn and during the final two months every 14 days. Gas was sampled between 9:00 and 11:00 am, considered the most representative time of day for sampling with static chambers (Allen et al., 2010, Reeves and Wang, 2015). Chambers were closed for 60 minutes, and 30 ml of gas was taken using a gas-tight syringe and transferred into pre-evacuated Exetainer vials (Labco Limited, Lampeter, UK). The gas samples were analysed via gas chromatography (Varian CP-3800, Varian Inc., Middelburg, The Netherlands). Greenhouse gas emissions per hour were converted into daily emissions (multiplying by 24) and the sum of the estimated emission rates by linear interpolation between the days of measurement resulted in a cumulative emission total. Total cumulative N$_2$O-N emissions were calculated by weighting emissions from sugarcane row and inter-row relative to the area occupied with one third of

area occupied by row and two thirds of the field area occupied by inter-row. N_2O emission factors were calculated with the IPCC (2007) Tier 1 method:

[(cumulative N_2O-$N_{treatment}$ − cumulative N_2O-$N_{control}$)]/[N application] x 100

3.2.4 Sugarcane biomass and analysis

Sugarcane was harvested with a commercial harvester on 12/08/2014, 04/09/2015 and 15/08/2016. Harvest bin numbers were recorded from each treatment, and bin weights and sugar contents provided by the sugar mill to quantify biomass and sugar yield. In the 2015-16 season, sugarcane nitrogen content was quantified by separating stalks, top leaves (leaf cabbages) and dead leaves from 10 whole stalks. Then, each component of biomass was cut, sub-sampled and fresh weighed to be dried at 60 °C for > 48 hours to determine dry weight. Afterwards, the sub-samples were ground to 2mm Retsch ZM 2000; Ultra Centrifugal Mill, Haan, Germany) and analysed for total N by combustion (LECO TruSpec analyser, see above). Dry matter weight of each biomass component was expressed in kg ha^{-1} and multiplied by the respective N concentration to calculate N accumulation into shoots with

Total N uptake = [N% in stalks * dry matter of stalks (kg ha^{-1})]/ [100].

The response of sugarcane yield to N fertiliser rate was quantified by calculating Agronomic Efficiency of fertiliser N (AgroEff$_{Fert}$) (Schroeder et al., 2014).

AgroEff$_{Fert}$ = (t sugarcane yield $_{(N\ fertilised)}$ − t sugarcane yield $_{(unfertilised)}$]/[kg N fertiliser applied]

3.2.5 Soil sampling and analysis

Two soil samples were collected from each plot at every second GHG sampling event; one sample was taken from the sugarcane row and another from the inter-row within one meter of each chamber and at 0-10 cm depth. The soil samples were stored and transported at 4°C, and processed within one week of collection. Soil and air temperatures were measured in the field at the time of gas sampling by a hand-held thermometer. Gravimetric soil water content was determined by oven-drying of the soil at 105ºC to constant weight. Soil nitrate (NO_3^--N) and ammonium (NH_4^+-N) concentrations were quantified with standard 1M KCl extraction (1:2 ratio of soil: solution) and colorimetric analyses (Kandeler and Gerber, 1988, Miranda et al., 2001) (Table 3.2).

Table 3. 2 Soil properties of the upper 0-10 cm of the brown dermosol at the commercial farm and location of this study in Abergowrie, North Queensland, Australia.

Soil texture			Soil physic-chemical properties						
Clay (%)	Silt (%)	Sand (%)	TOC %	TC %	TN %	P mg/kg	CEC cmol(+)/kg	pH (water)	EC (uS/m)
20	10	70	1.72	1.74	0.15	138	5.22	5.55	57.5

TOC: Total organic carbon; TC: Total carbon; TN: total nitrogen, Colwell-P: phosphorus, CEC: cation exchange capacity, EC: electrical conductivity

Soil texture was determined by the hydrometer method (Gee and Or, 2002) and physiochemical properties determined in the top 10 cm (Table 3.2). The soil was classified as a brown dermosol soil with a dark clay loam texture and moderate soil drain. Soil pH and electric conductivity (EC) were assessed with a solution in a 1:5 soil to distilled water (Rayment and Lyons, 2011). Soil organic carbon and nitrogen and organic carbon were analysed by combustion-Dumas (after pre-treated with acid to remove inorganic carbonates; LECO CHN analyser, LECO ltd., St Joseph, MI, USA). Cation exchange capacity (CEC) was determined by silver thiourea (AgTU+) (1:50 soil solution extracts were prepared in 0.01 M silver thiourea and mixed for 24 h). Colwell P was quantified in a 1:50 soil solution extracts of 0.5 M sodium bicarbonate mixed for 16 h (Rayment and Lyons, 2011) (Table 3.2). Soil bulk density was determined for sugarcane-row and inter-row to calculate water filled pore space (%WFPS) as (volumetric water/total pore) x (100) (Linn and Doran, 1984).

3.2.6 Data Analysis

Statistical analyses were performed using Statistica (Dell-Inc., 2015) and Minitab (Minitab 17 Statistical Software 2010) software. Normal distribution of dependent and independent variables was tested using Shapiro-Wilk Normality test at $P<0.05$ level of significance. N_2O-N emissions were log-normally transformed before statistical analysis. Differences and interactions between treatments were calculated by GLM-ANOVA and LSD all-pairwise comparison test at $P <0.05$. An initial multiple regression (lmer) model fitted with linear mixed effect (Lme4) and non-linear mixed effect (nlme) models were performed using R Studio (R Development Core Team, 2011) to analyse the effect of water filled porous space (WFPS), soil NO_3^--N and NH_4^+-N content and their interactions on the N_2O-N emissions per each treatment and year. A maximum likelihood method was used to assess the relative contribution of independent parameters (WFPS, soil NO_3^--N and NH_4^+-N) and their possible interactions in a

multiple regression models. A comparison between one model that contains all defined predictors in the regression model against a second similar model without the predictor of interest assesses the percentage of the variance that is explained for the predictor in the study. The use of a known likelihood ratio to estimate a Chi-square distribution can identify whether a first model is significantly different from a second model that does not contain one of the parameters.

3.3 Results

3.3.1 Climate, N_2O emissions and soluble soil nitrogen concentrations

Annual rainfall in the year 1 was 1482 mm (5% above the 12-year average of 1408 mm), in the year 2 was 802 mm, 43% less than the 12-year average and in the year 3 was 1211 mm (14% below the 12-year average). In the first two crop seasons respectively, 40 and 53% of total annual rainfall occurred across spring/summer, and 53 and 39% in autumn. The third season (2015-16) had a rainfall distribution of 21% in spring/summer and 59% of the total annual rainfall in autumn (Figure 3.1h).

All results of WFPS were below 60% that indicated the soil was not waterlogged. In the year 2, WFPS ranged from 15 to 51% and from 30 to 60% in year 3. Soil temperature during GHG measurements decreased from a maximum of 31°C in spring and summer to 16.9-19.0 °C in winter (Figure 3.1g, h).

Nitrous oxide emissions and soluble soil nitrate (NO_3^--N) and ammonium (NH_4^+-N) levels had seasonal patterns with largest N_2O emission and N concentrations during spring and summer, and lowest values in autumn and winter (Figure 3.1c,d,e,f). Concentrations of soil NO_3^- and NH_4^+ within sugarcane rows were related to soil moisture and N fertiliser rate. The highest concentrations of NH_4^+-N and NO_3^--N occurred in spring and summer and resulted in significant ($P<0.05$) between treatments. Concentrations of both N forms were lower in autumn and winter and similar between treatments (Figure 3.1c, d, e, f). Nitrate concentrations were significantly ($P<0.05$) higher in the 2015-16 crop season than the previous crop season, while NH_4^+-N concentrations were similar in both years. Soil NH_4^+-N concentrations were ~5- and 3-times higher than NO_3^- in spring in 2014-15 and 2015-16, respectively (Figure 3.1c, d, e, f).
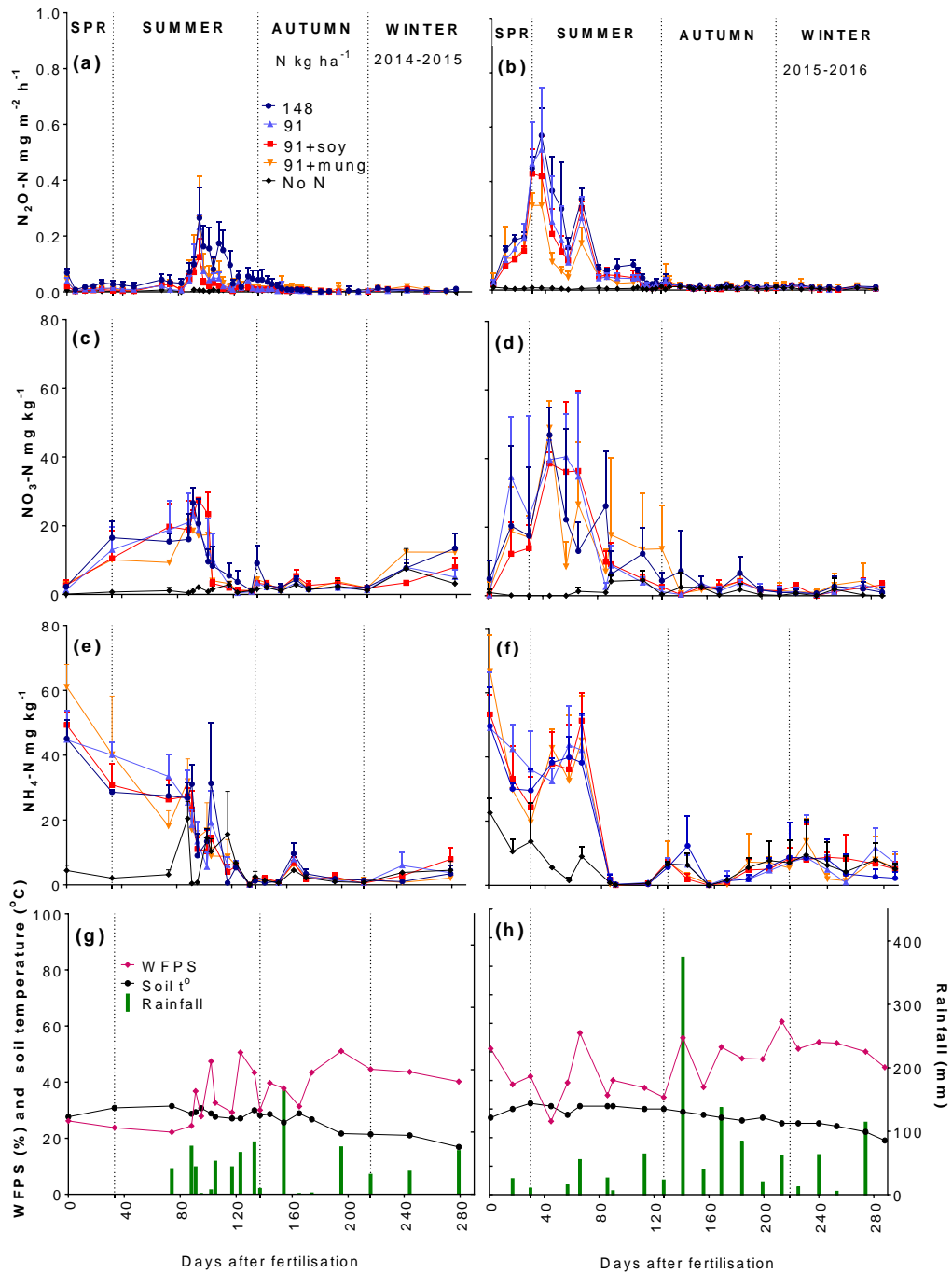
Figure 3. 1 Seasonal patterns of N$_2$O production (a, b), soluble soil NO$_3^-$ N and NH$_4^+$ N concentrations (c, d, e, f) and weather patterns (g, h) (S.E, n=3) with different N fertiliser rates in sugarcane monoculture and sugarcane-legume companion cropping in 2014-15 season (left column) and 2015-16 season (right column). Seasonal patterns of rainfall (mm), soil temperature (^{o}C) and water filled pore space (WFPS %) at in the Australian Wet Tropics (Abergowrie, Herbert River Catchment). The control treatments were a sugarcane monoculture without N fertilisation (No N). SPR: spring, S.E: standard error

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Table 3. 3 Cumulative N_2O emissions, emission factors (EF), Yield scaled emissions & Agronomic Efficiency) ($\pm$ S.D, n=3) from sugarcane rows and inter-rows averaged with 33% of area as row and 67% as inter-row, with different N fertiliser rates in sugarcane monoculture and sugarcane–legume companion cropping in the Australian Wet Tropics (Abergowrie, Herbert River Catchment).

2014-2015	kg Cumulative N_2O-N ha^{-1}					
N kg ha^{-1}	sugarcane row	sugarcane inter-row	Total	EF*	Yield scaled emissions (g N_2O-N ha^{-1} TCH^{-1})	AgEff (t cane increase in cane yield per kg applied N)
148	2.2^a $\pm$ 1.3	0.7^a $\pm$ 0.2	0.9^a $\pm$ 0.4	0.52	8.7	0.15
91	1.0^a $\pm$ 0.4	0.5^a $\pm$ 0.2	0.5^a $\pm$ 0.2	0.39	5.1	0.17
91+soy	0.7^a $\pm$ 0.3	0.5^{ab} $\pm$ 0.1	0.4^a $\pm$ 0.1	0.26	4.0	0.16
91+ mung	1.3^a $\pm$ 0.7	0.6^a $\pm$ 0.2	0.6^a $\pm$ 0.2	0.50	6.2	0.15
0	0.2^b $\pm$ 0.1	0.3^b $\pm$ 0.2	0.2^b $\pm$ 0.1	n/a	1.9	n/a
2015-2016						
148	6.3^a $\pm$ 0.5	1.3^a $\pm$ 0.3	2.5^a $\pm$ 0.2	1.50	22.3^a	0.29
91	5.0^a $\pm$ 2.9	0.8^{ab} $\pm$ 0.2	1.9^a $\pm$ 1.0	1.72	17.3^a	0.43
91+soy	4.5$^{a\ \pm}$ 1.7	1.4^a $\pm$ 0.7	2.0^a $\pm$ 0.8	1.78	18.3^a	0.38
91+mung	3.5^a $\pm$ 1.2	1.1^a $\pm$ 0.1	1.5^a $\pm$ 0.4	1.31	15.1^{ab}	0.35
0	0.3^b $\pm$ 0.1	0.6^b $\pm$ 0.4	0.3^b $\pm$ 0.1	n/a	4.8^b	n/a

Differences at *P<0.05* level of significance between N fertilizer treatments in cane row and inter-row and total cumulative emissions are highlighted by letters. *N_2O-N EF (emission factor) was calculated using the original data of the total cumulative emissions.

During spring and summer, the soil in the rows of the unfertilised control had lowest NO_3^--N and NH_4^+-N concentrations (0.3 to 1.6 mg NO_3^--N kg^{-1} soil; 3.3 to 15.6 mg NH_4^+-N kg^{-1}). Full and 38% reduced N treatments had significantly ($P<0.05$) higher soil NO_3^--N and NH_4^+-N concentrations compared to the unfertilised control, and were unaffected by the presence of legumes, (8.7 to 19.7 mg NO_3^--N kg^{-1}; 12.3 to 50.7 mg NH_4^+-N kg^{-1}). In autumn and winter, all treatments had similar NO_3^--N and NH_4^+-N concentrations. Across all seasons and treatments, inter-rows had similar soil N concentrations (1.2 to 5.8 mg NO_3^--N kg^{-1}; 2.4 to 6.8 mg NH_4^+-N kg^{-1} (Table S3.1).

3.3.2 Relationship between N_2O emissions, climate and soil properties
Nitrous oxide emissions from sugarcane rows varied seasonally, with all treatments having highest N_2O emissions in summer. Nitrous oxide emissions were statistically similar across N fertilised treatments with considerable variability among the three replicates.

We observed a statistically non-significant trend of the full N fertiliser treatment having the highest N_2O emissions. Emissions peaked in the 2014-15 season at 0.27 and 0.17 mg N_2O-N m^{-2} h^{-1} at 95 and 109 days after fertiliser application, respectively (Figure 4.1a). In the 2015-16 season, N_2O emissions peaked at 0.57 and 0.34 mg N_2O-N m^{-2} h^{-1} at 37 and 67 days after fertiliser application (Figure 3.1.b). In the 2014-15 season during these peak events, the reduced N treatments irrespective of legume presence, had significantly ($P<0.05$) lower emissions with up to 76% less N_2O emitted than the full N rate. In the 2015-16 season, the reduced N treatment with sugarcane monoculture had 21% lower N_2O emissions than the full N rate, while the presence of legumes decreased peak N_2O emissions by 30% compared to the full N rate ($P<0.05$). (Figure 3.1a, b).
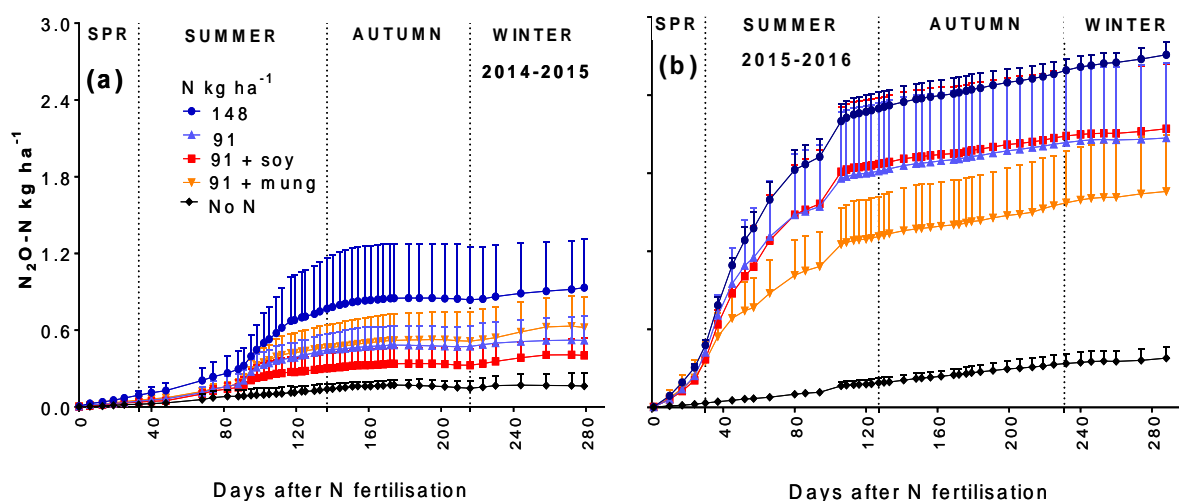
Figure 3. 2 Cumulative N_2O emissions (S.E, n=3) from sugarcane soil in the 2014-15 (a) and 2015-16 (b) seasons with different N fertiliser rates in sugarcane monoculture and sugarcane-legume companion cropping at in the Australian Wet Tropics (Abergowrie, Herbert River Catchment). The control treatment was a sugarcane monoculture without N fertilisation (No N). SPR: spring, S.E: standard error

The highest N_2O emissions coincided with major rainfall events, resultant high soil moisture and warmer soil temperatures. In 2014-15, N_2O emissions peaked 95 days after N fertiliser application after dry conditions during the initial 75 days. Cumulative rainfall of 163 mm occurred between days 75 and 95, resulting in 28% WFPS and accompanied by a soil temperature of 31°C. The second N_2O flux peak occurred after 61 mm of rainfall, a WFPS of 33% and soil temperature of 28°C. In 2015-16, the first N_2O peak occurred after 35 mm of rainfall during the first 37 days after fertiliser application, resulting in elevated WFPS (41.4%) and in conjunction with a soil temperature of 32°C. The second N_2O flux peak occurred 67

days after fertiliser application after 70 mm cumulative rainfall over 30 days elevated WFPS (56.7%) and soil temperature (31°C) (Figure 3.1g, h).

Over the four seasons of 2015-16, N_2O emissions were 2 to 12-fold higher than in the corresponding seasons in 2014-15 (Figure 3.1a, b). During spring and summer, statistically significant differences occurred only between the no N fertiliser control and N fertilised treatments. In contrast, N_2O emissions from the inter-rows were similar in no-N control and fertilised treatments.

Table 3. 4 Two-year multiple linear regression analysis (n=615) fitted in mixed-effect models of N_2O emissions versus soil soluble WFPS, NO_3^--N and NH_4^+-N in sugarcane monoculture and sugarcane-legume companion cropping system in the Australian Wet Tropics (Abergowrie, Herbert River Catchment).

Variable	Parameter estimate (Coefficients)	Proportion of variance explained (%)
Intercept	0.0028	
WFPS	-0.0357^{ns}	1.5^{ns}
NO_3^--N	0.4283 ***	81.4***
NH_4^+-N	0.1647**	5.7**
WFPS* NO_3^--N	-0.0859^{ns}	2.5^{ns}
WFPS* NH_4^+-N	0.1612***	7.1**
NO_3^--N * NH_4^+-N	0.0149^{ns}	0.1^{ns}
WFPS* NO_3^--N * NH_4^+-N	0.0721*	1.7*
Multiple R^2	0.3318	
Adjusted R^2	0.3241	
P-value	2.2e-16	

Significant codes: $P< 0.001$ '***'; $P< 0.01$ '**'; $P< 0.05$ '*'; $P> 0.05$ 'ns' ns: non-significant

Total cumulative N_2O emissions in 2015-16 were 3.2 times higher than in 2014-15 (Figure 3.2). N_2O emissions at the end of the measurements at 279 days (2014-15) and 288 days (2015-16) after N fertiliser application were highest for the full N rate with 0.93 and 2.54 kg N_2O-N ha^{-1}, respectively (Table 3.3, Figure 3.2). Over trial years, companion cropped and monoculture sugarcane at the reduced N rate had 23 to 57% lower N_2O emissions than the full N rate. Within the reduced fertiliser treatments, total cumulative N_2O emissions from companion cropping treatments varied, but these were not statistically significantly different. In 2014-15, N_2O emissions from the mung bean treatment were 16% higher than from the sugarcane monoculture, while emissions from the soybean treatment were 23% lower. In 2015-16, N_2O

emissions were 20% lower with mung bean than sugarcane monoculture, and 3% higher with soybean (Table 3.3).

Cumulative N_2O emissions from sugarcane rows were 2.3- to 4.2-fold higher than from inter-rows in the 2014-15 and 2015-16 seasons, respectively. The full N treatment resulted in the highest N_2O emissions from the sugarcane row (2.2 to 6.3 g N_2O-N ha^{-1}). Reduced N treatments (with or without legumes) were statistically similar to the full N treatment ($P<0.05$). Only the zero N treatment produced significantly lower emissions from 0.16 to 0.57 kg N_2O-N ha^{-1} (Table 3.3).

The N_2O emission factors (EF) obtained in 2014-15 were below the 1% N_2O EF of the IPCC standard emissions from crop soils, and below the global average for sugarcane soils (3.87%; Lisboa et al. 2013) in 2015-16 (Table 3.3). The presence of a legume companion crop did not produce consistent responses when compared to the sugarcane monoculture at the same N fertiliser rate. For example, in 2014-15, soybean companion cropped treatment had the lowest EF of 0.26%, while in 2015-16 the soybean treatment has the highest EF of 1.78%.

The multiple regression fitted in mixed-effect models provided seven models; three as single factors (WFPS, NO_3^--N and NH_4^+-N) and four based on WFPS and the interaction with soil NO_3^--N and NH_4^+-N (WFPS* NO_3-N, WFPS* NH_4^+-N, NO_3^--N * NH_4^+-N and WFPS* NO_3^--N * NH_4^+-N). The outcomes represent the coefficients or slope coefficients of each predictor obtained from the ANOVA and the proportion of each contributor to total emission of N_2O (Table 3.4). The 2-year multiple linear regression using the single predictors (WFPS, NO_3^--N, NH_4^+-N) and their interactions were highly significant (P<0.05) despite a relatively low R^2 of 0.33), indicating 33% of the variations in N_2O emissions can be explained by the independent variables (Table 3.4). Additionally, the coefficients of NO_3^--N, NH_4^+-N, WFPS* NH_4^+-N and WFPS* NO_3^--N* NH_4+-N were significant ranged between *P<0.001* and *P<0.05*

The mathematical model confirmed NO_3^--N as a single factor was significant (*P<0.001*) and explained 81.4% of the variance in N_2O emissions over the two crop seasons (2014-16). In contrast, NH_4^+-N and WFPS contributed less than 5.7% to explaining N_2O emissions. The contribution of WFPS alone was not significantly (*P>0.05*) linked to N_2O emissions, but in interaction with NO_3^--N, NH_4^+-N and the interaction NO_3^--N*NH_4^+-N predicted 11.3% of the soil N_2O flux (Table 3.4).

3.3.3 Sugarcane production and nitrogen uptake

Legumes did not establish well in the first season (2013-14) due to damage by cane rats soon after germination. Because of that, we observed no effect of legumes on sugarcane yield (Figure 3.3a). In contrast, legumes established well in the following years, but sugarcane yield remained similar with full and 38% reduced N fertiliser rates and was unaffected by legumes.

In 2014-15 and 2015-16, significantly differences in yield occurred only between the no-N control and full or 38% reduced N fertiliser treatments, with yields 21 to 40% higher in full N treatments, and 7 to 15% higher in reduced N treatments (Figure 3.3b, c). In the 58% reduced N fertiliser treatment (2014-15), soybean did not significantly improve yield when compared to the no-N control (*P>0.05*) although yield was 6% higher. Mung bean was associated with significantly (P<*0.05*) lower sugarcane yield in the 2015-16 season with a 6.3 and 2.5% compared to sugarcane monoculture and soybean at the reduced N level (Figure 3.3c). No benefit was derived from soybean when sugarcane was grown without N fertiliser and yield was 5.5% low with soybean although this was not statistically significant (*P>0.05*) (Figure 3.3c).

In 2015-16, we quantified the N accumulation of sugarcane shoots. The no-N treatment acquired ~90 kg N ha^{-1} irrespective of soybean presence, the reduced N treatment with or without legumes ~128 kg N ha^{-1}, and ~153 kg N ha^{-1} in the full N treatment. Nitrogen acquisition in the non-N treatments was significantly lower (*P<0.05*) than all N-fertilised treatments (Figure 3.3c). We observed a positive and significant linear regression (R^2=0.55, *P<0.001*) between total N$_2$O emissions and sugarcane N uptake. For every 1 kg of N accumulated in aboveground biomass per hectare, 0.0203 kg ha^{-1} of N$_2$O-N was emitted to the atmosphere (Figure 3.5).
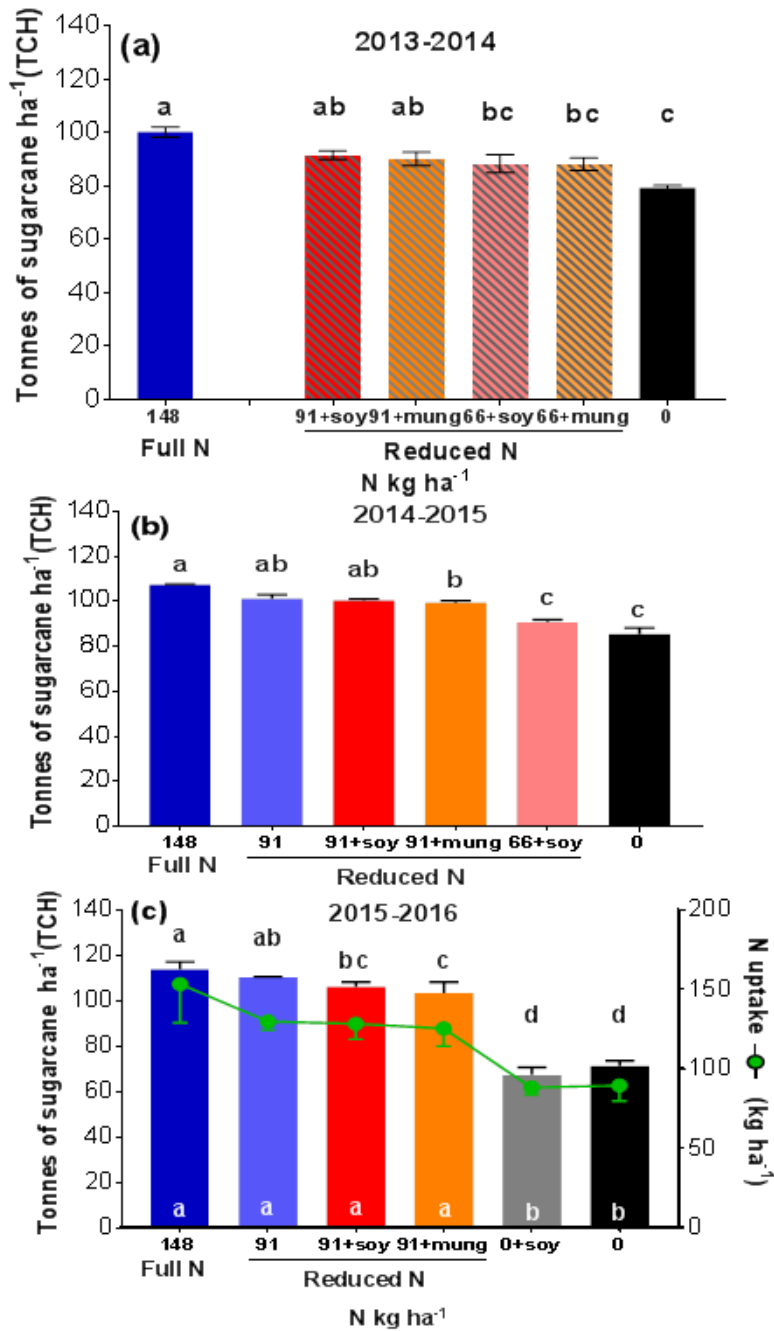
Figure 3. 3 Tonnes of sugarcane per hectare (TCH) at different N rates as monoculture and grown with legumes in the 2014-15 season (a), 2015-16 season (b) and sugarcane N uptake in 2015-16 (c). Data are means (n=3) at Abergowrie, North Queensland. different lowercase letters above the error bars indicate significant differences between treatments at *P<0.05* (LSD, Fisher test) in sugarcane yield and different lowercase letters within bars indicate significant differences between treatments at *P<0.05* (LSD, Fisher test) in sugarcane N uptake. Shade columns in 2013-14 where legumes did not grow.
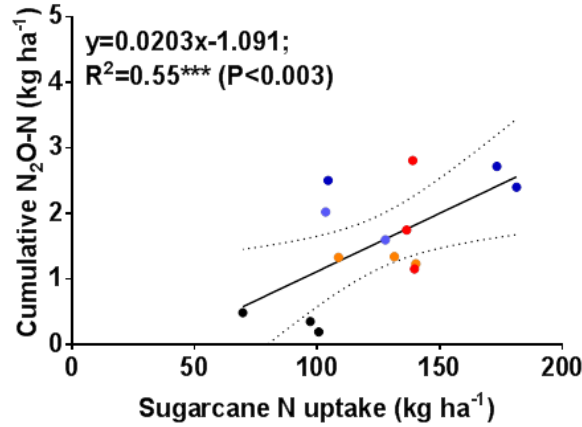
Figure 3. 4 Sugarcane N uptake (kg ha^{-1}) vs. cumulative N$_2$O-N emissions in the 2015-2016 season at different N rates as monoculture and grown with legumes. Data are means (n=3) at Abergowrie.
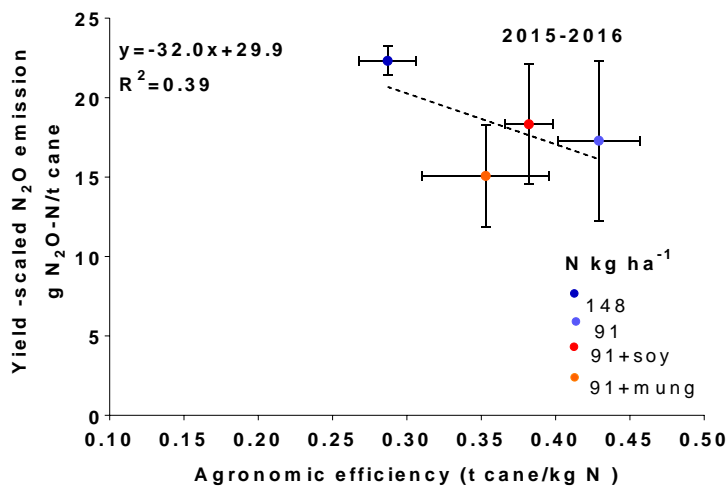
Figure 3. 5 Agronomic efficiency of the N fertiliser based on cane yield (t cane ha^{-1}/ kg N ha^{-1} after subtracting the contribution of the soil N background) vs. yield-scaled N$_2$O emissions (g N$_2$O-N/ t cane) in the 2015-2016 season, at different N rates as monoculture and grown with legumes. Data are means (S.E, n=3) at Abergowrie.

We observed an inverse relationship between agronomic N use efficiency (kg N in fertilised crop-kg N in the unfertilised crop)/kg N applied) and yield–scaled N$_2$O-N emissions (kg N$_2$O-N emitted/t sugarcane produced per hectare) over the 2-year experiment (Figure 3.5). On average, the 2014-15 season had a 68% lower agronomic efficiency of fertiliser-N use than the 2015-16 season, and there were no significant differences between treatments (Figure 3.5). The

reduced N treatments, irrespective of the presence of legumes, had 1.2- to 1.5 times greater agronomic N use efficiency than the full N rate in the 2015-2016 season. Mung bean companion cropping at the reduced N rate significantly decreased yield-scaled N_2O emissions by 32% compared to the full N treatment (Table 3.3, Figure 3.5).

3.4 Discussion

It is largely unexplored how sacrificial companion crops such as N_2 fixing legumes that decompose in sugarcane fields impact on soil N status and N_2O emissions in conventional agriculture. This study explored if this approach is an avenue to improve N efficiency in sugarcane cropping. We hypothesised that if legumes contribute biologically-fixed N and have facilitative rather than competitive interactions with sugarcane, benefits should arise. This study showed that legumes had only minor effects on sugarcane yield and total N_2O emissions although the presence of legumes was associated with lower N_2O emissions during peak emission periods. A caveat that restricted our ability to quantify the benefits of legumes as an N source was that sugarcane yields were relatively similar with full (148 kg N ha^{-1}) and reduced (91 kg N ha^{-1}) fertiliser rates over the three years of experimentation. Important observations were that legumes did not negatively affect sugarcane yield when rainfall was 48% below the annual average, and a trend suggesting that soybeans are as a more favourable companion crops than mung bean in the tested conditions.

3.4.1 N_2O emissions and sugarcane production from sugarcane–legume companion cropping
The potential of legume intercropping to mitigate N_2O production has been studied in herbaceous perennial pasture grass and grain cropping systems (Huang et al., 2014, Dyer et al., 2012, Hauggaard-Nielsen et al., 2016). Most research on sugarcane-legume intercropping systems has focussed on evaluating profitability and the effects on soil improvement (Córdova-Gamas et al., 2016, Shiming and Gliessman, 2016, Edmilson José et al., 2013, Trento Filho, 2010, Gana and Busari, 2003, Roodagi et al., 2001a). One study to date has assessed N_2O emissions from sugarcane-soybean intercropping (Luo et al. 2016).

Many studies under temperate climate conditions that have examined legumes as an N fertiliser replacement for N_2O mitigation showed advantages over monocultures, especially when intercropping systems received zero N fertiliser. For example, wheat-faba bean intercropping without N fertiliser significantly reduced N_2O losses (by 35%) compared to a wheat stand crop fertilised with 80 kg N ha^{-1} (Senbayram et al 2015). By contrast, in Scotland, after a 3-year fallow regime, an unfertilised barley-pea and unfertilised barley sole crop produced similar

N$_2$O emissions (Papa et al., 2011). On the other hand, application of N in addition to legume companion crops resulted in different N$_2$O emissions responses. Legume forages (Red clover, alfalfa, White clover) as companion with ryegrass (*Lolium perenne*) or with a grass mixture (cockfoot , and tall fescue,) decreased N$_2$O emissions and dry matter production up to 13% relative to the grass monoculture or mixed grasses at high inputs of N (325 kg ha^{-1}). In the same trial, reducing the full N rate by 72% (to 90 kg N ha^{-1}) reduced biomass production of grass sole crop by 33%, while mixed grasses intercropping had yield reduced by only 7%. N$_2$O emissions were mitigated by 43% regardless of presence or absence of the intercropping treatment (Haugagard-Nielsen et al., 2016).

Like our study, Luo et al. (2016) found that 43% reduction in N fertiliser application from 525 to 300 kg N ha^{-1}, irrespective of soybeans presence, N$_2$O emissions were reduced by 30 to 50% without affecting sugarcane yield. These results are somewhat unsurprising, as even the reduced fertiliser treatment (300 kg N ha^{-1}) had N additions likely to be well in excess of both crop's N requirements. By contrast in our study, the 56% N reduced rate (66 kg N ha^{-1}) significantly decreased sugarcane yield suggesting that sugarcane was N limited. Both studies showed that the presence of soybean did not significantly increase sugarcane yield compared to monoculture sugarcane at the same N application rate.

The impact of legume as intercropping on N$_2$O emissions varies with climate (temperature, dry and wet conditions), location, agricultural management, type of legume, N inputs from biological N fixation and the rate of mineralisation and immobilisation process (Senbayram et al., 2015, Luo et al., 2016, Dyer et al., 2012, Huang et al., 2014, Epie et al., 2015). N$_2$O emissions from intercropping systems can differ considerably between legume species and cultivars as well as from year-to-year (Pappa et al., 2011, Huang et al., 2014). In our study, N$_2$O production of soybean and mung bean companion cropping treatments differed with emissions of 0.40 and 0.62 kg N$_2$O-N ha^{-1} respectively in 2014-15 and 1.96 and 1.53 kg N$_2$O-N ha^{-1} in the following season. Luo et al. (2016) found that N$_2$O emissions of the same intercropping sugarcane-soybean cultivar varied from 1 to 8 kg N$_2$O ha^{-1} over several years with major drivers being edaphic conditions, soil water content, soil temperature, soil soluble N and rainfall. Emissions from monoculture sugarcane varied similarly from 2 to 8 kg N$_2$O ha^{-1} per season (Luo et al 2016), indicating that this year-to-year variability was not specific to legume treatments. Pappa et al. (2011) showed that N$_2$O emissions from a barley cultivar intercropped with pea cultivar Zero 4 were 80% smaller than barley-pea cultivar Nitouche. The

authors associated the difference in N$_2$O emissions between the pea cultivars with increases of nitrification in response to high temperatures, N supply by the fixed N of legumes and plant residues from the different cultivar and subsequent mineralisation and immobilization of legumes plants. Possible reasons for the lack of legume effects on in our study include (i) increased competition for water due to lower than average and N$_2$O emissions by nitrification process mainly, (ii) reduced mineralisation process due high limited water and less microbial activity, (iii) slower decomposition rate of legume biomass and N transfer from legumes to sugarcane.

In general, the magnitude of N$_2$O production is closely linked to the rate of N fertiliser applications; thus, smaller N$_2$O emissions are derived from small quantities of N fertiliser. However, Van Groenigen et al. (2010) argued that N$_2$O efficiency of a cropping system should be expressed per unit crop N uptake rather than per area of cropping, in order to capture an element of crop productivity in the measure. They found lowest yield-scaled emissions at moderate N applications rates, where N application facilitated optimum yields. Both under-and over-application of N resulted in higher yield-scaled N$_2$O emissions, due to poor crop performance and soil N surpluses respectively. By contrast, in this study yield-scaled N$_2$O emissions were lowest in zero N treatments, and increased with fertiliser application, indicating that yield limitation at zero N was less severe than that found by Van Groenigen et al (2010) in their meta-analysis. In 2015-16, yield-scaled N$_2$O emissions were significantly lower in the reduced N with Mung bean treatment than in the full N treatment, indicating a greater efficiency of production. Similarly, the treatments with 38% reduced N applications show higher agronomic efficiency than full N treatments, especially in the 2015-16 season where sugarcane N fertiliser efficiency improved by 22 to 33%. Treatments with lower yield-scaled N$_2$O emissions consistently had greater agronomic efficiency within each season, suggesting that a relationship exists between these agricultural efficiency metrics, despite one being calculated on yield and inputs, and the other being calculated using yield and losses. Substantial variation in both metrics from season to season occurred due to differences in magnitudes of both N$_2$O, and the decreased yield of the zero N fertiliser treatment in 2015-16, indicating that a consistent relationship between these metrics does not occur across sites and seasons.

3.4.2 Key drivers, magnitude and effects of N fertiliser of N$_2$O emissions

Nitrous oxide emissions vary with different N fertiliser application rates over the two years of experimentation with total estimated emissions of 0.93 and 2.54 kg N$_2$O-N ha^{-1} in the drier and

wetter year, respectively from the full N rate treatment. These emissions are substantially lower than previously published ones from sugarcane grown with similar N fertiliser rates that ranged from 3.6 to 45.9 kg ha^{-1} of N_2O-N (Allen et al., 2010, Wang et al., 2016, Denmead et al., 2010b) but are comparable to emissions previously measured in Brazil (de Oliveira et al., 2013).

The comparatively low N_2O emissions detected here were likely due to dry conditions with rainfall 48 and 14% below the 12 year-average of 1408 mm, especially during the first 3-4 months after N application when soil inorganic N and N_2O emissions are generally highest (Robinson et al., 2011, Holst et al., 2012, Allen et al., 2010). With the exceptions of few time points when high rainfall, soluble soil N concentrations and soil temperatures coincided and resulted in peak N_2O emissions, the studied soil did not have the high soil moisture levels (WPSP from 15 to 60%) that are not conducive to extreme N_2O emissions. Denitrification is the main process to release N_2O-N emissions under anaerobic conditions (Linn and Doran, 1984, Dalal et al., 2008, Robertson and Groffman, 2007, Dalal et al., 2003).Wet soils (by rainfall or irrigation) are the trigger for denitrification to occur and are directly associated with WFPS > 60% that decrease air filled pore space and restrict O_2 diffusion to microorganisms. Thus, WFPS influences the distinct N transformation pathways and is been highly correlated with N_2O production. Soil texture influences the onset of denitrification, and the sandy loam texture was a likely factor preventing high emissions, facilitating soil O_2 and water exchange, rapid water drainage and low water retention as has been found in other studies (Barton et al., 2008, Barton et al., 2011, Aguilera et al., 2013). Similar to our study, N_2O emissions from a sandy loam Kandosol were 88% smaller (3.6 kg N_2O-N ha^{-1}) than a silty clay Hydrosol (28.2 kg N_2O-N ha^{-1}) at similar N fertiliser rates (150 and 160 kg N ha^{-1}) and annual rainfall (1585 and 1665 mm) (Wang et al., 2016).

Contrary to research that has shown that N_2O emissions peaked with WFPS of >80 to <91% (Huang et al., 2017), we found that the largest N_2O emission peaks coincided with high levels of soil NO_3^-N (23.1 to 46.9 mg N kg^{-1} soil) and relatively low soil moisture contents (34.0 to 56% WFPS). Soil NO_3^- was the largest single factor explaining ~81% of the variance in N_2O emissions. In contrast, WFPS and NH_4^+ predicted ~6% of the soil N_2O flux, possibly due to the lack of waterlogging and denitrification events during both seasons. If higher WFPS had occurred during this trial, it is likely that emissions would have been greater.

Our study showed that N fertiliser input was the main driver of N_2O production with the highest hourly emissions detected in the full N treatment. Similarly, other Australian studies showed

that reducing N fertiliser by half also reduced N_2O emissions up to 50% (Allen 2010; Wang et al. 2016). In spring and summer, N_2O emissions and fluctuations are generally large in the presence of high levels soluble inorganic N levels after fertiliser application, high rainfall and associated to high WFPS as well as high soil temperature (Wang et al., 2011, Wang et al., 2016, Barton et al., 2010). In our study, high rainfall and associated soil moisture (WFPS) in autumn and winter did not promote N_2O emissions (<0.02 mg N_2O-N m^{-2} h^{-1}) likely because soil mineral N concentration was lower (<10.0 mg N kg^{-1}) in autumn and winter compare with >20 and <60 mg N kg^{-1} during spring and summer) and soil temperature (average 19 °C compared to 30 °C in spring and summer). Similarly, soils from an Australian subtropical cropland (wheat) released the lowest N_2O-N emissions (<0.004 mg N_2O-N m^{-2} h^{-1}) during autumn and winter because of low soil N concentrations (from 10 to 20 mg of N kg) and temperature ($<$ 10°C) in conjunction with low rainfall (Wang et al., 2011).

After the N fertiliser (urea) was applied, soil NH_4^+-N dominated the soil extractable N during spring and summer. In these seasons, while the concentration of NH_4^+-N decreased, the available NO_3^--N increased proportionally because of the nitrification of NH_4^+-N to NO_3^--N, coinciding with higher N_2O emissions. Even though this study did not aim to identify the biological processes influencing N_2O-N production, the fluctuations of N_2O-N in relation to NO_3^--N and WFPS ($<60\%$ WFPS) suggested that nitrification was the main pathway for N_2O emissions. Similarly, studies under low rainfall, soil temperature and light textured soils at cropland systems from Australian subtropical and semi-arid regions (Wang et al., 2015, Barton et al., 2008, Barton et al., 2011), temperate Chinese North plain (Huang et al., 2014) and Mediterranean climate (Aguilera et al., 2013) found that nitrification is an important pathway for N_2O emissions.

3.4.3 Sugarcane is grown with legumes

While intercropping can increase crop yield and profitability where both crops are harvested (Brooker et al., 2015, Kaur et al., 2016, Yang et al., 2013, Parsons and Be, 2003, Kwong et al., 1996), legumes in our study were examined solely as a potential source of N for sugarcane to address environmental concerns. It has been argued that the success of sugarcane-legume systems is largely determined by factors that include the capacity of legumes for BNF, water availability and planting time of legumes (Gana and Busari, 2003, Roodagi et al., 2001b). For example, legumes did not establish well in sugarcane older than three old months at the time of legume planting due to competition (Billore et al., 2000). Previous experimentation by

Australian sugarcane growers demonstrated good outcomes with soybean sown into ratoon sugarcane ~50 cm tall (Bryan Granshaw, *pers. comm.*). At this site, legumes were planted when sugarcane was ~80 cm tall to minimise the risk of competition from legumes during early sugarcane growth. As anticipated, canopy closure of sugarcane occurred within four months of legume planting and out-shaded the legumes. We also aimed to minimise competition for nutrients other than N with the application of basal fertiliser nutrients but did not investigate if nutrient competition occurred between both crops. For example, a glasshouse experiment showed that sugarcane-soybean intercropping enhanced facilitation processes by improving soil inorganic N and P availability by 66 and 117%, respectively relative to the monoculture, increasing sugarcane biomass by 35% (Li et al., 2013). Contrary, a field experiment showed no significant differences in soil N and P availability or sugarcane yield between sugarcane-soybean intercropping and pure stands of sugarcane (Luo et al., 2016).

In our study, competition for nutrients other than N was minimised with the application of basal fertiliser nutrients. Mung bean, but not soybean, was associated with lower sugarcane yield. It is possible that inefficient N-fixation by mung bean and competition for water or slow decomposition of mung bean biomass impacted on sugarcane productivity. Soybean is more effective than mung bean in N-fixation as soybean seeds were inoculated with efficient rhizobia, while mung bean relied on native soil microbes. Peoples et al. (2009) shown that mung bean has a low reliance on N fixation (28% Ndfa) than soybean (53% Ndfa) and that inoculation can improve legumes' symbiotic performance, growth and grain yield. In Australia, soybean and mung bean yield responded significantly to inoculation about 64 and 67%, respectively (Peoples et al., 2009). N_2 fixation - the proportion of N derived from the atmosphere (% Ndfa) is strongly regulated by legume growth. At Breeza, Australia an increase of 2.6 times shoot dry matter in soybean resulted in six and 28 times greater the %Ndfa and the amount of N fixed by soybean over mung bean (Peoples et al., 2009).

We did not aim to quantify N-fixation but or if legume-N was successfully acquired by sugarcane. Nitrogen fertiliser and, to a lesser degree, residual soil N may have negatively affected BNF. In the 2015-16 season soil from the zero N treatment (with or without legume) supplied N to sugarcane up to 89 kg ha^{-1}. This value doubled to other findings from Wet tropics (Ingham) in ratoon cane (42-55 kg N ha^{-1}) with a wide cane yield from 55 to 89 t cane per ha^{-1} that were similar to our zero N treatments (from 61to 88 t cane per ha^{-1}) (Bell et al. 2010, Wang et al., 2014, Schroeder et al., 2003). The high crop N content derived from soil reserves

in Abergowrie were similar to southern regions (Bundaberg and Rocky Point) where the rainfall was less than wet tropics and therefore fewer N losses (Kingston et al., 2008, Schroeder et al., 2003, Thorburn et al., 2003.). In the current experiment, the high N uptake from the background soils might be (i) the scarcity of rainfall was evident during the seasons 2014-2016 compared to 12-year average record, and probably less losses of N and more accumulation of background N mineralisation (ii) potential to release N because of a moderate soil organic carbon concentration (1.72%) and an adequate soil N index (iii) site to site variation.

There is much evidence that N fertiliser application and concomitant increases in soil inorganic N levels supresses BNF (Unkovich et al., 2008, van Kessel and Hartley, 2000, Salvagiotti et al., 2008, Streeter and Wong, 1988). Salvagiotti et al. (2008) demonstrated that the proportion of the fixed N decreased with increases of N fertiliser. Soybean stand crop fixed 57% of its N content at applications of less than 10 kg N ha^{-1}, while at applications between 85 to 160 kg N ha^{-1}, soybean nitrogen fixation supplied only 35% of crop N. In our experiment, we applied 66 and 92 kg N ha^{-1} that together with residual N may have reduced BNF. We did not detect an effect of legumes on the pool of soluble NO_3^- and NH_4^+ levels in the top 10 cm of soil, despite regular monitoring, suggesting that legume inputs were insufficient to substantially alter soil inorganic N levels, or that increased sugarcane uptake balanced any increases in N inputs. There are potentially great complexities in the N interactions between the two crops, as while legumes may be a source of soil N; both crops are also a sink for soil N. We did not comprehensively evaluate legume nodulation; however, spot-checks in all treatments found the presence of nodules.

3.5 Conclusions

Nitrous oxide emissions were primarily influenced by N fertiliser rates, and legumes only reduced peak emissions at certain time of evaluation but not in the total cumulative emissions. Other studies have shown that legumes as intercrop can facilitate reductions in N fertiliser requirements and N_2O emissions, and further research has to examine the potential of legumes as companion crops, and potentially intercrops. A promising outcome was legumes did not negatively affect sugarcane yield (the exception being mung bean in one crop season) which suggests that facilitative and competitive interactions were approximately equal. As these results are site-specific, research in different locations and under different agronomic managements has to establish context and generalities. For practitioners is attractive that

legumes can provide slow-release N, soil health benefits as well as potential income from grains.

Chapter 4 –Legume companion cropping to mitigate nitrous oxide emissions from Rain-fed and Irrigated sugarcane soils

Throughout this chapter, we emphasized the results of one-year sugarcane yield and N_2O emissions from field experiments at two farms with contrasting soil, climate and agronomy, located in dry tropic (irrigated site) and subtropic (rainfed site) of Australia. Rain-fed, low-drainage hydrosol soil had annual emissions of 14.4, 7.6 and 3.9 kg N_2O-N ha^{-1} with full N fertiliser rate (160 kg N ha^{-1}), with 50% N fertiliser rate+soybean, and with 50% N rate, respectively. Irrigated, well-drained vertisol soil had emissions of 1.7 to 2.6 kg N_2O-N ha^{-1}, unaffected by N fertiliser rate (full rate 250 kg N ha^{-1} or 28% reduced rate) or soybean. Sugarcane yield was similar with reduced and full N fertiliser rates irrespective of soybean present, indicating that reduced N rates provided sufficient N. In N limiting conditions (no N fertiliser applied), soybean increased sugarcane yield by 41% to match the full N fertiliser treatment but with 6-fold low N_2O emissions. Our study affords early insight into sugarcane-soybean systems, and next-step research has to evaluate a broad range of environmental and agronomic settings to examine soybean, and potentially other legumes, as an avenue for N_2O mitigation.

4.1 Introduction

Sugarcane is a food, bio-energy and fibre crop cultivated on ~27 million hectares in the tropics and subtropics (FAOSTAT, 2016), and 385,000 ha in Australia (Australia Sugarcane, 2016). Sustainable N use is an important consideration as sugarcane crops use on average only 50% of the applied N fertiliser (Robinson et al., 2011). In Australia, sugarcane farming is considered a main source of N pollution that affects the Great Barrier Reef (Kroon et al., 2012) and a considerable source of the potent greenhouse gas nitrous oxide (N_2O). Avenues are sought to reduce the N footprint of sugarcane.

Globally, N_2O emissions from agricultural soils contribute ~12% of anthropogenic greenhouse gas (GHG) emissions (IPCC, 2014), and the average N_2O emissions factor of sugarcane soils is 3.87 % (% of N fertiliser emitted as N_2O) (Lisboa et al., 2011), nearly 4-fold the estimated 1% for managed soils globally (IPCC, 2006). Australian sugarcane receives average N fertiliser rates of 160 kg N ha^{-1} y^{-1} (Fraser et al., 2017, Thorburn et al., 2017), mostly as a single dose of urea during early crop establishment. Whole-season studies of N_2O emissions from Australian

sugarcane soils show that typical N fertiliser rates (100-200 kg N ha^{-1}) generate 2.0 to 72.1 kg N_2O-N ha^{-1}, which translates to N_2O emissions factors of 0.3 to 21% (Table 1). Noticeable is that these emissions factors generally exceed the default factor of 1 for crops in general (IPCC 2006) and 1.25% for sugarcane specifically (Department of Environment 2014).

In a two-step system, legumes generate N *via* BNF and acquire soil N, including fertiliser N, in the early growing season when the N demand of sugarcane is low and N losses from soil are the highest (Robison et al., 2011). In the later season, decomposing legumes cam supply N to sugarcane which accumulates N over six months or more. Lowering the concentrations of soluble soil N, especially inorganic nitrate and ammonium, can reduce the rate of microbial nitrification and denitrification and reduce N_2O formation (Mosier et al., 2004, Bodelier, 2011, Kool et al., 2011, Itakura et al., 2013, Müller et al., 2004, 2014, Robertson and Groffman, 2007). Abiotic factors, including soil moisture, aeration, texture, temperature and pH affect N_2O production (Oertel et al., 2016, Signor and Cerri, 2013, Müller et al., 2014), and the numerous interactions between abiotic and biotic factors require empirical quantification of N_2O emissions. Measures to reduce N_2O emissions include lowering N fertiliser rates (Wang et al., 2016), split application of fertiliser (Allen et al., 2010), slow-release fertilisers and nitrification inhibitors (Wang et al., 2012), organic fertilisers and soil amendments (Westermann, 2017, Kingston et al., 2016), trash blanketing (Wang et al., 2016, Denmead et al., 2010b) and irrigation that avoids waterlogging (Allen et al., 2010). An untested strategy to abate N_2O emissions is legume companion cropping, which currently only very few sugarcane farmers practise in Australia.

This study follows on from our previous research on sugarcane-legume companion cropping in Australia's wet tropics (Table 4.1) to expand investigations to the Irrigated-Dry Tropics and Rain-fed Subtropics.

Table 4. 1 Summary of N_2O emissions and N_2O emission factors (EF) across sugarcane growing seasons in Australian studies.

Reference	Location/crop	N Fertiliser type/crop	N fertiliser kg ha^{-1}	N_2O kg ha^{-1}	N_2O EF (%)	Rainfall (mm)	Soil type
IPCC, 2006	Global	varied	100	n/a	1.00	n/a	Varied
Department of Environment, 2014	Australia	varied	90-200	n/a	1.25	n/a	Varied
Lisboa et al., 2011	Global sugarcane	urea, ammonium, nitrate	20-200	n/a	3.87	n/a	Varied
Allen et al., 2010	ratoon (trash)	urea (liquid)	100	3.9	1.09	850	Hydrosol*/ Inceptisol**
			200	9.6	6.70		
		urea (liquid)+nitram	50-50	3.9	1.00		
			100-100	5.8	2.90		
Denmead et al., 2010	ratoon (burned trash)‡	urea	160	72.1	21.00††	1879	Hydrosol/ Entisol
	Mackay ratoon (trash)	urea	150	7.4	2.80†	2142	Chromosol/ Alfisol
Wang et al., 2012	plant cane	bare fallow+ sugarcane+urea	150	12.3	4.5	1672	Chromosol/ Entisol
		bare fallow+ sugarcane+urea+ N inhibitors⁺	150	8.1	1.2		
		soybean fallow sugarcane+urea	75	20.9	6.6		
Wang et al., 2014	ratoon (trash)	urea	140	17.3-28.3	3.0†	2010	Dermosol/ Mollisol
Wang et al., 2016	ratoon (burned trash)	urea	160	44	10.0	1585	Hydrosol/ Entisol
	Mackay ratoon (trash)	urea	150	5.7	1.3	1665	Kandosol/ Aridisol
Kingston et al., 2016	ratoon (trash)	urea	100-150	7.5	1.9	1227-1707	Hydrosol / Entisol
			147-197	12.0			
Westermann 2017	(trash)	urea	160	13.7	8.0	1467	Hydrosol/ Inceptisol
		poultry litter+compost	160-220	11.7-15.7	6.8		
		poultry litter+biochar	198	7.5	3.4		
		soybean fallow+ cane+urea	75	20.9	6.6		
Salazar et al., unpublished	ratoon (trash)	urea	91/148	0.5-0.9£ 1.9-2.5§	0.3-0.6£ 1.5-1.7§	1206	Dermosol/ Mollisol
		urea	91 (soy/ mung)	0.4-0.6£ 1.5-2.0§	0.3-0.5£ 1.3-1.8§		
Salazar et al. this study	ratoon (trash) rain-fed	urea	80/160	3.9-14.4	3.5-8.3	1457	Hydrosol/ Inceptisol
		urea+soybean	80	7.6	8.0		
		soybean	0	2.4	n/a		
	irrigated	urea	180/250	1.7-2.6	0.8-0.9	850	Vertosol/ Vertisol
		urea+soybean	180	2.5	1.2		

'Trash' refers to the practice of green sugarcane harvesting where stalk tips and youngest leaves are recycled to the soil surface at harvest. *Australian Soil Classification (ASC adapted from Isbell 1996); ** Australian sugarcane soils relate to Soil Taxonomy (Soil Survey Staff USDA 1998). £ N_2O EF from season 2014-2015, § N_2O EF from season 2015-2016, N inhibitors (DMPP), †N_2O emissions from automatic chambers; †† N_2O emissions from micrometeorology

4.2 Materials and methods

4.2.1 Experimental sites

The experiments were carried out at two commercial sugarcane farms located in south-east Queensland (Sunshine Coast, Rain-fed farm, 26°34'S, 153°00'E) and north Queensland (Burdekin region, Irrigated farm, 19°49.23' S, 147°09.63' E), Australia. The Rain-fed site has a subtropical climate with average (1994-2016) maximum and minimum summer temperatures of 31.1 and 27.3 °C and max-min winter temperatures of 19.8 and 12.9 °C respectively, and average annual rainfall of 1457 mm (Bureau of Meteorology, Site 040861, Sunshine Coast Airport). The site has potentially acid sulphate soil (sulfidic hydrosol) (Isbell, 2002) with silty light clay texture and poor internal drainage. No fertiliser was applied in the year prior to the commencement of the experiment. The sugarcane was Australian sugarcane cultivar Q138 from the 5th ratoon crop over three crop seasons from December 2013 to December 2016.

The Irrigated site has a dry tropical climate with average (1994-2016) summer and winter maximum-minimum temperatures of 32.3 and 25.2ºC, 22.1 and 11.7ºC, respectively, and average annual rainfall of 850 mm (Bureau of Meteorology, Site 033002, Ayr DPI Research Station). The soil is a Vertisol (Isbell, 2002) with clay texture and moderate internal drainage. The site was Irrigated in regular intervals (cooler months every three weeks and warm months every seven days) according to grower practices in the region (Figure 4.1h). The amount of water per irrigation event was 12 mm. The experiment was carried out during the 2015-2016 season as a 2nd ratoon crop of Australian cultivar Q183.

4.2.2 Experimental design

Three N treatments were (1) Full N fertiliser (sugarcane monoculture, sugarcane industry recommended rate, (2) Reduced N fertiliser (sugarcane monoculture or cultivated with soybean), and (3) No (zero) N fertiliser (sugarcane monoculture or cultivated with soybean) (Table 2). At the Rain-fed site, the 2013-14 season tested the performance of crops to decide on legume and N fertiliser regimes for the following seasons. The next two seasons had five treatments assessed for soil traits, N_2O emissions and yield over 280 days (2014-2015) and 313 days (2015-16). At the Irrigated site, the experiment ran during the 2015-2016 crop season with four treatments evaluated over 272 days (Table 4.2).

At both sites, treatments were arranged in a randomised block design with three replicate plots, except the 2015-16 season at the Rain-fed site that had four replicates. Each plot consisted of six sugarcane rows with 1.65 m spacing between rows. The size of individual plots was 3564 m^2 (1st year) and 100 m^2 (2nd and 3rd year) at Rain-fed site. At the Irrigated site, each

experimental plot was 300 m^2. At both sites, the outer rows of each plot were buffer strips. At the Rain-fed site, additional sugarcane buffer strips of 5 meters length were set up between plots. Measurements were performed in the two central sugarcane rows. At the Rain-fed site, a sugarcane trash blanket from the previous harvest covered the soil (~13 t dry matter ha^{-1}). In line with common practice in the Burdekin region, the Irrigated site was not trash blanketed. All rain-fed plots received phosphorus (40 kg P ha^{-1}), potassium (100 kg K ha^{-1}) and sulphur (25 kg S ha^{-1}), and all Irrigated plots received phosphorus (56 kg P ha^{-1}), potassium (50 kg K ha^{-1}) and sulphur (50 kg S ha^{-1}) as triple super phosphate, mono-potassium phosphate and sulphate potash. Nitrogen was applied as prilled urea as outlined in Table 4.2. Fertiliser was banded at 10 cm depth. Plots were hand harvested (25th November 2014, 19th October 2015, 12th October 2016 at the rain-fed site; 10th October 2016 at the irrigated site).

Table 4. 2 Nitrogen fertiliser regime and legume treatments implemented at subtropical (Rain-fed site) and dry tropics (Irrigated site) commercial sugarcane farms in Australia.

	Rain-fed site		Irrigated site
Treatments	2013-2014 season	2014 to 2016 seasons	2015-2016 season
	kg N ha^{-1}		
Full N	128	160	250
Reduced N	96	80	180
Reduced N+legume	96+soybean[1]	80+soybean[3,4]	180+soybean[4]
Reduced N+legume	96+soybean[2]	n/a	n/a
No N+legume	n/a	0+soybean	n/a
No N	0	0	0

[1]cv. Fernside, [2] cv. Leichardt, [3]cv. Leichardt (2014-15), [4]cv. Bunya (2015-16), n/a not applicable

4.2.3 Soybean planting

Industry recommended, locally available soybean cultivars were chosen for the experiments. In the first year at the Rain-fed place, soybean cultivars Fernside and Leichardt were sown on 23rd December 2013, in the following years cultivars Leichardt (16th December 2014) and Bunya (4th December 2015) were sown. At the Irrigated site, Bunya was sown (12th August 2015). Soybean successfully established, except in the last year at the Rain-fed site where herbivory by hares prevented establishment. Soybeans were inoculated with Nodule N^{TM} rhizobia. Seeds (70 to 78 kg soybean ha^{-1}) were sown using a mechanical legume planter with

coulter wheels cutting into the soil and through the trash blanket, depositing legume seeds at three cm depth and a distance of ~15 cm on either side of the sugarcane row.

4.2.4 N_2O sampling and analysis

Two manual greenhouse gas (GHG) chambers were set up in each plot. One chamber was placed along the sugarcane row directly over the fertiliser band covering the row space, and the second chamber was positioned between rows, the inter-row space. Stainless steel base chambers of 0.25 m^2 were introduced to approximately 5 cm deep into the soil to ensure a tight seal. Top chambers were placed over the base to obtain a total volume of 140 and 68 litres for Rain-fed and Irrigated sites, respectively. Top chambers were equipped with vent tubes connected to valves (Reeves and Wang, 2015). Gas sampling occurred twice a week during the peak emissions period over 9 weeks of the crop season when soil moisture, temperature and soil soluble N concentrations were high. Sampling frequency was reduced to weekly from week 10, and to fortnightly sampling during the final two months of the crop season when soil moisture, temperature and soluble N concentrations were lowest. Gas sampling was conducted between 9:00 and 11:00 am to capture daily mean fluxes (Allen et al., 2010, Reeves and Wang, 2015). Briefly, one hour after top chambers were placed over the base chambers, 30 ml gas was extracted from chambers using a gas-tight syringe and transferred into pre-evacuated Exetainer vials (Labco Limited, Lampeter, UK). A total of 34 and 31 sampling events occurred over 2014-15 and 2015-16 seasons at the Rain-fed site, respectively, and 40 sampling events at the Irrigated site. Gas samples were analysed with gas chromatography equipped with an electron capture detector (ECD) (GC 2010, Shimadzu Co., Kyoto, Japan). Hourly N_2O emissions were converted into daily emissions by multiplying by 24, and cumulative emissions were estimated with linear interpolation between measurement days (Reeves and Wang, 2015). N_2O emission factors were calculated using the IPCC (2006) Tier 1 method:

$$[(\text{cumulative } N_2O\text{-}N_{treatment} - \text{cumulative } N_2O\text{-}N_{control})]/[N \text{ application}] \times 100$$

4.2.5 Sugarcane yield and nitrogen uptake

Sugarcane was harvested manually by cutting the two central rows over the 10 m length of each plot, and fresh biomass was weighed with electronic balance. Sugarcane biomass separated into stalks and leaves (three stalks, Rain-fed site; 10 stalks, Irrigated site). Biomass sub-samples were used to determine fresh weights and dry weights after drying at 60 °C for > 48 hours. Sub-samples were ground to a fine powder to determine N content of leaf and stalk tissues (LECO TruSpec analyser, LECO ltd., St Joseph, MI, USA). Dry biomass of each stalk

component (kg dry matter ha^{-1}) was multiplied by the respective N concentration to calculate aboveground N uptake:

Total N uptake (kg N ha^{-1}) = [N% in stalks * dry matter of stalks (kg ha^{-1})] / [100]

The response of sugarcane yield (tonnes sugarcane ha^{-1}) to N fertiliser rates was quantified by using the agronomic efficiency of fertiliser N (Schroeder et al., 2015)

AgroEff$_{Fert}$ (t sugarcane kg^{-1} N) = (t cane yield $_{(N\ fertiliser)}$ − t cane yield $_{(no\ N\ fertiliser)}$]/[kg fertiliser N applied]

4.2.6 Soil sampling and analysis

Soil samples were taken at 0-10 cm soil depth in sugarcane row and inter-row in each plot at every GHG sampling event at the Rain-fed site and every second event at the Irrigated site. Soil temperature was measured at 10 cm soil depth at the time of gas sampling with a hand-held thermometer. Soil samples were stored at 4 ℃ until processing in the laboratory within 24 hours of sample collection. Gravimetric soil water content was determined by oven drying at 105 ℃ to a constant weight. Soil bulk density was determined once for sugarcane-row and inter-row to calculate water filled pore space (%WFPS) as (volumetric water/total pore) x (100) (Linn and Doran, 1984). Soluble inorganic N was determined by extracting 15 g soil with 30 ml 1M KCl and mixed for one hour. Soil pH and electric conductivity (EC) were assessed with a solution in a 1:5 soil:distilled water (Thomas, 1996). Soil total and organic carbon and total nitrogen were analysed by combustion-Dumas (samples were pre-treated with acid to remove inorganic carbonates) using a LECO CHN analyser (see above). Cations and cation exchange capacity (CEC) were determined by silver thiourea (AgTU+) (1:50 soil solution extracts prepared in 0.01 M silver thiourea and mixed for 24 h). Colwell P was quantified in 1:50 soil:0.5 M sodium bicarbonate mixed for 16 h (Rayment and Lyons, 2011) (Table 4.2).

Table 4. 3 Soil physio-chemical properties at 0-10 cm depth of sugarcane rows at the rain-fed site and irrigated site.

Location	Soil texture			Bulk density	Soil chemical properties					
	Clay	Silt (%)	Sand	g cm⁻³	TOC (%)	TC (%)	TN (%)	pH	EC (µS/m)	CEC cmol kg⁻¹
Rain-fed site (Sunshine Coast)	40	10	50.0	1.35	1.95	1.99	0.22	5.2	71.8	9.79
Irrigated site (Burdekin)	42	8	50.0	1.16	0.99	1.04	0.13	6.5	72.9	26.00

TOC, total organic carbon; TC, total carbon; TN, total nitrogen; EC, electrical conductivity; CEC, cation exchange capacity

4.2.7 Data Analysis

Statistical analyses were performed using Minitab Software (Minitab Pty Ltd, Sydney NSW Australia). Normal distribution of dependent and independent variables was tested using Shapiro-Wilk Normality test at $P<0.05$. N_2O emissions were log-normally (ln) transformed before statistical analysis. Differences and interactions between treatments were calculated by GLM-ANOVA and LSD all-pairwise comparison test at $P<0.05$. Linear multiple regression fitted with linear mixed effect (Lme4) was performed using R Studio (R Development Core Team, 2011) to analyse effects of water filled pore space (WFPS), soil nitrate (NO_3^--N) and ammonium (NH_4^+-N) content and their interactions on the N_2O emissions per year and site. A maximum likelihood method was used to assess the relative contribution of independent parameters (WFPS, soil NO_3^--N and NH_4^+-N) and their possible interactions in the multiple regression model. A comparison between models with and without the predictors of interest assessed the proportion of the variance that is explained for the predictor on study. A Chi-square distribution determined if the models were significant.

4.3 Results

4.3.1 N₂O emissions, soil and climate variables

We analysed the effect of reduced N fertiliser rates and soybean companion crops on N_2O emissions and sugarcane yield over the 2014-2015 (Rain-fed site) and 2015-2016 (Irrigated site) crop seasons, when soybeans established well at both sites. Soil N_2O fluxes and soluble soil nitrate and ammonium levels were highest during the weeks following N fertiliser application. At the Rain-fed site, N_2O emissions and soluble soil inorganic N concentrations peaked after N fertiliser application in early summer and were lowest in autumn and winter-spring. At the Irrigated site, N_2O emissions and soluble soil N concentrations were highest in

late winter and spring following N fertiliser application in late winter and were lowest in summer and autumn (Figure 4.1a, b).

At the Rain-fed site, N_2O emissions and soluble inorganic N (ammonium, nitrate) concentrations were highest in sugarcane rows in summer, coinciding with high soil moisture content and temperatures. N_2O emissions were 16- and 100-fold higher in summer than in autumn and winter-spring seasons, respectively (Figure 4.1a). Similarly, soil nitrate and ammonium concentrations in summer were up to ~6- and 10-fold higher than in autumn and ~ 10- and 20-times higher than in the others seasons (Figure 4.1c).

Total rainfall was 1359 mm over the 2014-2015 crop season, 7% below the 22-year average (1467 mm), with 55% (749 mm) of rainfall received in summer, 27% (370 mm) in autumn, and 18% (240 mm) in winter-spring, respectively (Figure 4.1g). Soil temperatures were fluctuated around 26 to 30 ℃ and decreased to 13.8 ℃ at the end of winter (Figure 4.1g). Soil moisture (water filled pore space, WFPS) ranged from 54 to 97% across sampling times with high WFPS of 70 to 94% maintained during the first 44 days after N fertilisation, which coincided with highest N_2O emissions. High WFPS was maintained throughout the early crop season with the combination of high soil clay content (40%) and associated poor drainage, and two high rainfall events (117 and 312 mm).

Soluble soil N concentrations in the sugarcane rows differed significantly ($P<0.05$) between plots receiving N fertiliser and the unfertilised control plots in the summer season. In this season, nitrogen fertilised sugarcane plots (full and 50% reduced N rate) had concentrations of 29 and 30 mg NH_4^+-N kg^{-1}, and 50% reduced N sugarcane+soybean had 25 mg NH_4^+-N kg^{-1} (Table 4.4). The 50% reduced N treatment with sugarcane only or with sugarcane+soybean had NO_3^--N concentrations of 6.3 and 4.5 mg NO_3^--N kg^{-1}, respectively, which were lower than the full N rate (10.9 mg NO_3^--N kg^{-1}, Table 4.4).
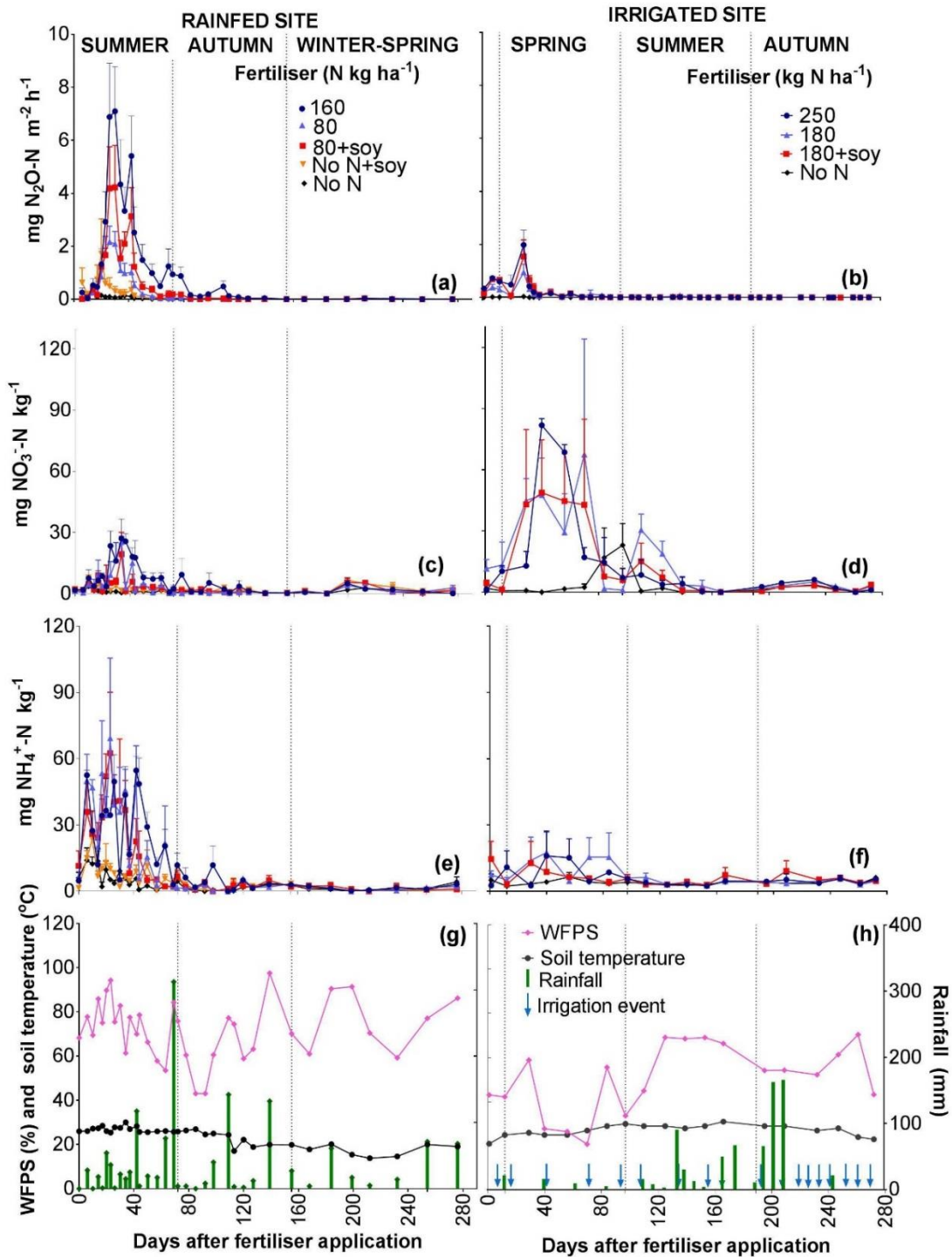
Figure 4. 1 Seasonal N_2O emissions (a, b), soluble soil nitrate (c, d), ammonium (e, f), water filled pore space from sugarcane row, soil temperature and rainfall (g, h) (S.E., n=3) at different N fertiliser rates in sugarcane monoculture and sugarcane-legume companion cropping at the Rain-fed site (2014-15) and Irrigated site (2015-16). Arrows represent irrigation events (Irrigated site).

Table 4. 4 Seasonal average concentrations of soluble soil ammonium and nitrate ($\pm$ S.D, n=96, Sunshine Coast, n=120, Burdekin), in the top 10 cm of soil profile. Details are listed for sugarcane rows and average for inter-row as no treatment differences were observed. Treatments included N fertiliser rates and sugarcane grown as monoculture or intercrop with soybean at Rain-fed site (2014-2015 season) and Irrigated site (2015-2016 season). Different letters in columns indicate significant differences (*P*<0.05) between N fertiliser and legume treatments

Rain-fed site	Summer	Autumn	Winter-Spring	Summer	Autumn	Winter-Spring
N fertiliser (kg N ha^{-1})	Soluble ammonium (mg NH$_4^+$-N kg^{-1})			Soluble soil nitrate (mg NO$_3^-$-N kg^{-1})		
sugarcane row						
160	29.0^a ± 3.2	3.7 ± 2.7	1.4 ± 0.6	10.9^a ± 2.9	2.4 ± 3.0	1.5 ± 1.2
80	29.8^a ±9.1	2.0 ± 0.3	1.2 ± 0.5	6.3^{ab} ± 3.4	0.5 ± 0.4	1.7 ± 1.7
80+soybean	24.8^a ± 3.5	2.2 ± 1.2	1.3 ± 1.5	4.5^b± 1.6	1.0 ± 0.3	2.1 ± 0.9
0+soybean	8.3^b± 1.1	2.1 ± 0.7	0.8 ± 0.3	2.1^c ± 0.2	0.3 ± 0.1	2.7 ± 0.6
0	3.3^c ± 1.3	1.3 ± 0.2	1.3 ± 0.6	1.0^d ± 0.2	0.6 ± 0.1	0.9 ± 0.3
sugarcane inter-row	3.6 ± 1.3	2.2 ± 0.7	1.1 ± 0.4	0.9 ± 0.2	0.7 ± 0.4	1.1 ± 0.8
Irrigated site	Winter	Spring	Sumer-Autumn	Winter	Spring	Sumer-Autumn
N fertiliser (kg N ha^{-1})	Soluble soil ammonium (mg NH$_4^+$-N kg^{-1})			Soluble soil nitrate (mg NO$_3^-$-N kg^{-1})		
sugarcane row						
250	6.8 ± 6.1	8.8 ± 6.0	4.1 ± 1.0	5.6^a ± 2.6	33.7 ± 6.3	3.0^{ab} ± 2.0
180	6.3 ± 0.6	12.2 ±12.1	4.2 ± 0.5	12.4^a ±12.0	32.0 ± 23.5	6.6^a ± 2.7
180+soybean	8.9 ± 7.9	7.2 ± 5.3	4.8 ± 0.8	2.9^{ab} ± 1.7	32.2 ± 25.1	3.2^{ab} ± 1.9
0	3.7 ± 2.6	4.3 ± 0.5	4.0 ± 04	1.2^b ± 2.2	7.5 ± 6.3	1.1^b ± 0.3
sugarcane inter-row	1.9 ± 0.6	3.7 ± 1.5	4.2 ± 0.8	1.7 ± 1.4	6.9 ± 4.5	1.7 ± 0.4

The presence of soybean in the zero N fertiliser treatment significantly (*P*<0.05) increased soluble N concentrations with 2-and 3-fold higher NO$_3^-$-N and NH$_4^+$-N concentrations detected relative to the zero N sugarcane monoculture. In the 50% reduced N fertiliser treatment, the presence of soybean was associated with significantly (*P*<0.05), 59 and 15% lower NO$_3^-$-N and NH$_4^+$-N concentrations relative to the full N rate (Table 3). Soil NO$_3^-$-N concentrations decreased from 9.3 to 1.6 mg kg^{-1} over autumn to winter-spring seasons (Figure 4.1c), and soil NH$_4^+$ concentrations declined gradually from 11.8 to 1.2 mg NH$_4^+$-N kg^{-1} over autumn to spring (Figure 1e). In contrast, similar concentrations of soluble N occurred inter-row soil across treatments and seasons with 1.1 to 3.6 mg NH$_4^+$-N kg^{-1} and 0.7 to 1.1 mg NO$_3^-$-N kg^{-1} (Table 4.4).

At the Irrigated site, sugarcane rows N_2O emissions and soluble N concentrations were highest in spring and decreased over summer and autumn (Figure 4.1b). N_2O emissions in winter were 2-fold higher than in spring, and N_2O emissions in spring were up to 27-fold higher than in summer and autumn (Table S4.1). The dry season corresponds to winter-spring (June to October) and the wet season occurs in summer-autumn (December to May). The annual rainfall during 2015-16 season was 13% lower than the 22-year annual average (850 mm), with 94% of the rainfall occurring in summer and autumn. As rainfall was low in winter and spring, five flood irrigation events were applied, and a further 14 irrigation events occurring during summer and autumn. Overall, water filled pore space was lower (24 to 69% WFPS) in the dry season and higher in the wet season (50 to 81% WFPS). Dry and wet season soil temperatures ranged from 21 to 28°C and 29 to 31°C (Figure 4.1h).

Soluble soil NO_3^--N and NH_4^+-N concentrations in sugarcane rows fluctuated considerably in spring, with NO_3^--N exceeding NH_4^+-N concentrations almost 4-fold (Figure 4.1d, f). During winter and summer-autumn seasons, soil NO_3^--N concentrations at the N fertilised plots, irrespective of legumes presence, were significantly ($P<0.05$) higher (2.9 to 12.4 mg NO_3^--N kg^{-1}) than zero N fertiliser plots (1.1 mg NO_3^--N kg^{-1}). Highest concentrations occurred in spring in all fertilised treatments (32 to 34 mg NO_3^--N kg^{-1}) while the control had the lowest $NO3^-$-N concentration (7.5 mg NO_3^--N kg^{-1}). Soil NH_4^+-N concentrations did not differ significantly between all treatments and seasons, ranging from 3.73 to 12.19 mg NH_4^+-N kg^{-1} (Table 4.4). In sugarcane inter-rows, NO_3^--N and NH_4^+-N concentrations ranged from 1.7 to 7.0 mg kg^{-1} without differences between treatments and seasons (Table 4.4).

4.3.2 Relationship between N_2O emissions, climate and soil properties
At the Rain-fed site, application of N fertiliser at the start of the field experiment resulted in highest N_2O emissions in summer. Emissions from sugarcane rows were statistically similar at the full N fertiliser rate (2.4 mg N_2O-N m^{-2} h^{-1}) and 50% reduced N fertiliser+soybean (1.3 mg N_2O-N m^{-2} h^{-1}), and significantly ($P<0.05$) lower in the reduced N fertiliser (0.7 mg N_2O-N m^{-2} h^{-1}). Zero N fertiliser+soybean had significantly ($P<0.05$) higher N_2O emissions (0.36 mg N_2O-N m^{-2} h^{-1}) than zero N sugarcane (0.08 mg of N_2O-N m^{-2} h^{-1}, Table S4.1).

Peak N_2O emissions from sugarcane rows were recorded in all N fertilised treatments during the first 30 days after N application and cumulative rainfall of 160 mm, and 157 mm cumulative rainfall 12 days after the first N_2O emissions peak. Both periods presented high N_2O emissions, high WFPS (70 to 82% (Figure 4.1a), high soluble soil N concentrations (9.0 to 23.0 mg NO_3^-

-N kg^{-1}; 23 to 54 mg NH$_4^+$-N kg^{-1}) and high soil temperatures 27.8 to 28.3°C (Figure 4.1g). N$_2$O emissions from all treatments gradually decreased 72 days after N fertiliser application and remained stable over autumn and winter-spring seasons (0.01 to 0.04 mg N$_2$O-N m^{-2} h^{-1}). In sugarcane inter-rows, N$_2$O emissions were similar across treatments and seasons (Table S4.2).

Total cumulative N$_2$O emissions were significantly higher ($P<0.05$) at the full N rate (14.4 kg N$_2$O-N ha^{-1}) than zero N (1.2 kg N$_2$O-N ha^{-1}). The presence of soybean in the zero N fertiliser treatment increased total cumulative N$_2$O emissions significantly ($P<0.05$) by 50% compared to the sugarcane monoculture (Table 4.5). Overall, sugarcane rows of all N fertilised treatments emitted from 10.7 to 41.3 kg N$_2$O-N ha^{-1}. Zero N fertiliser+soybean emitted significantly ($P<0.05$) more N$_2$O (5.4 kg N$_2$O-N ha^{-1}) than zero N fertiliser (0.8 kg N$_2$O-N ha^{-1}, Table 4.5). Full N and reduced N+soybean had similar N$_2$O emissions factors of 8.3 and 8.0%, respectively, while reduced N sugarcane monoculture had a lower factor of 3.5% (Table 4.5).

Table 4. 5 Cumulative N$_2$O emissions and N$_2$O emission factors (EF) (±S. D, n=3) from sugarcane rows and inter-rows, and total area calculated with the relative proportions of row and inter-row area. Sugarcane was grown as monoculture or with legume companion crops with different N fertiliser rates at Rain-fed site (2014-2015) and Irrigated site (2015-2016) sites. Different letters within columns denote significant differences at *P<0.05* level.

N fertiliser (kg N ha^{-1})	Cumulative N$_2$O emissions (kg N$_2$O-N ha^{-1})			
Rain-fed site	row	inter-row	total area	N$_2$O EF
160	41.3^a ± 20.8	1.9 ± 1.2	14.4^a ± 7.0	8.3
80	10.7^b ± 2.8	1.1 ± 0.6	3.9^{bc} ± 1.0	3.5
80+soybean	20.0^{ab} ± 9.5	2.6 ± 1.4	7.6^{ab} ± 3.5	8.0
0+soybean	5.4^c ± 5.6	1.6 ± 0.2	2.4^{cd} ± 2.0	n/a
0	0.8^d ± 0.2	2.0 ± 1.1	1.2^d ± 0.5	n/a
Irrigated site				
250	7.1^a ± 1.2	0.7 ± 0.1	2.6^a ± 0.4	0.9
180	4.7^a ± 2.9	0.5 ± 0.2	1.7^a ± 1.0	0.8
180+soybean	6.8^a ± 2.2	0.8 ± 0.7	2.5^a ± 0.6	1.2
0	0.3^b ± 0.2	0.4 ± 0.3	0.2^b ± 0.2	n/a

At the irrigated site, application of N fertiliser at the end of winter increased N$_2$O emissions and the widest range of emissions was recorded in spring. Following N fertiliser application, emissions from sugarcane rows during winter and spring were similar at full N rate (0.56 and 0.28 mg N$_2$O-N m^{-2} h^{-1}) and 28% reduced N fertiliser+soybean (0.50 and 0.25 mg N$_2$O-N m^{-2}

h^{-1}), respectively. In winter, the average hourly N_2O emissions rate of the reduced N treatment was significantly ($P<0.05$) lower than those of the full N fertiliser and reduced N sugarcane+soybean treatments. Lowest N_2O emissions occurred in summer and autumn in all treatments including control from sugarcane rows and inter-rows (0.002 to 0.01 mg N_2O-N m^{-2} h^{-1}) (Table S4.1).

N_2O emissions from sugarcane rows of N fertilised treatments peaked 29 days after N fertilisation (1.0 to 2.0 mg N_2O-N m^{-2} h^{-1}) with small fluctuations during late spring (0.03 to 0.16 mg N_2O-N m^{-2} h^{-1}), followed by low emissions over summer and autumn (0.04 mg N_2O-N m^{-2} h^{-1}) (Figure 4.1b). In the reduced N fertiliser treatments, irrespective of legume presence, spring N_2O emission peaked 29 days post fertiliser application which coincided with irrigation events, 59% WFPS, high concentrations of soil soluble N (44 mg NO_3^--N and 13 mg NH_4^+-N kg^{-1}) and soil temperatures of 26 ^oC (Figure 4.1b).

Full N and reduced N rates (with or without soybean) had similar total cumulative N_2O emissions ranging from 1.7 to 2.6 kg N_2O-N ha^{-1} (Table 4.5), and only the zero N fertiliser treatment had significant lower ($P<0.05$) N_2O emissions of 0.2 kg N_2O-N ha^{-1}. N_2O emission factors ranked from 1.3% (reduced N+soybean) > 0.9% (full N) > 0.8% (reduced N) (Table 4.5). Cumulative N_2O emissions from N fertilised sugarcane rows were ~6-times higher than from inter-rows which were similar across all treatments and seasons (0.4 to 0.7 kg N_2O-N ha^{-1}) (Table 4.5).

4.3.3 Drivers of N_2O emissions

N_2O emissions from the two sites fitted ($P<0.001$) a multi-regression model irrespective of a relatively low R^2 (0.35 and 0.27), with and 27% of the N_2O emissions explained by the predictor variables identified at the Rain-fed and Irrigated sites, respectively. At the Rain-fed site, the model indicates that soluble soil nitrate (*P<0.001*) is the main driver of N_2O emissions and explains 64% of variation, followed by single factors WFPS (17.7% of variation) and ammonium (15.5% of variation). At the Irrigated site, the interaction between WFPS and nitrate accounted for 63% of variation, and nitrate as single factor explained 17% (Table 4.6).

Table 4. 6 Multiple linear regression analysis fitted in mixed-effect models of N_2O fluxes and predictors as WFPS, soluble soil NO_3^- and NH_4^+ in sugarcane monoculture and with legume companion crops at wet Rain-fed site (n=510) and Irrigated site (n=480) sites. Multiple R^2: Proportion of the variation of N_2O emissions accounted for the predictors. Adjusted R^2: it is the adjusted for the number of predictors (independent variables) in the model. Coefficients: are known as slope coefficients and represent the average change of N_2O emissions per unit of change of each predictor (variable). *P*-value: Test the null hypothesis that the coefficient is equal to zero

	Parameter estimate (Coefficients)		Proportion of variance explained (%)	
Variable	Rain-fed site	Irrigated site	Rain-fed site	Irrigated site
Intercept	0.027^{ns}	0.093^{ns}		
WFPS	$0.075*$	0.110^{ns}	17.7^{**}	0.0^{ns}
NO_3^--N	0.422^{***}	0.590^{***}	64.0^{***}	17.0^{***}
NH_4^+-N	0.272^{**}	-0.031^{ns}	15.5^{**}	5.3^{ns}
WFPS* NO_3^--N	0.048^{ns}	0.402^{***}	0.0^{ns}	63.2^{***}
WFPS* NH_4^+-N	0.030^{ns}	-0.083^{ns}	0.0^{ns}	0.0^{ns}
NO_3^--N* NH_4^+-N	-0.052^{ns}	0.169^{**}	2.4^{ns}	2.5^{ns}
WFPS* NO_3^--N* NH_4^+-N	-0.027^{ns}	0.121^{**}	0.4^{ns}	12.0^{**}
Multiple R^2	0.35	0.27		
Adjusted R^2	0.34	0.25		
P-value	2.2e-16	1.18e-12		

Significances $P< 0.001$ ***; $P< 0.01$ **; $P< 0.05$ *; $P> 0.05$ ns, non-significant. WFPS: water filled pore space

4.3.4 Relationship between N_2O emissions and agronomic efficiency of fertiliser (AgronEff)

The agronomic N use efficiency of fertiliser calculates sugarcane yield relative to the rate of N applied and increased with decreasing N fertiliser at the studied sites (Figure 4.2). The reduction of N fertiliser by 50 or 28%, irrespective of soybean present, increased agronomic efficiency from 0.22 to 0.28 t sugarcane kg^{-1} N fertiliser at the Rain-fed site, and from 0.14 to 0.28 t sugarcane kg^{-1} N fertiliser at the Irrigated site. The full N rate had the lowest agronomic N use efficiency with up to 0.10 tonne sugarcane per kg N at the two locations (Figure 4.2). Relating N_2O emissions to sugarcane yield showed that yield-scaled N_2O emissions decreased at the two sites with decreasing N fertiliser application. Plot-to-plot variability was high, for example at the Rain-fed site, zero N+soybean reduced yield-scaled N_2O emissions by 28% in one plot and increased emissions by 13 to 148% at the other two plots compared to zero-N plots with sugarcane monoculture. On the other hand, at the Irrigated site, plot variability was low and yield-scaled N_2O emissions increased from 4.0 to 8.5% in all plots with reduced N fertiliser+soybean relative to the treatments with sugarcane at the same level of N fertiliser rate (Figure 4.2)
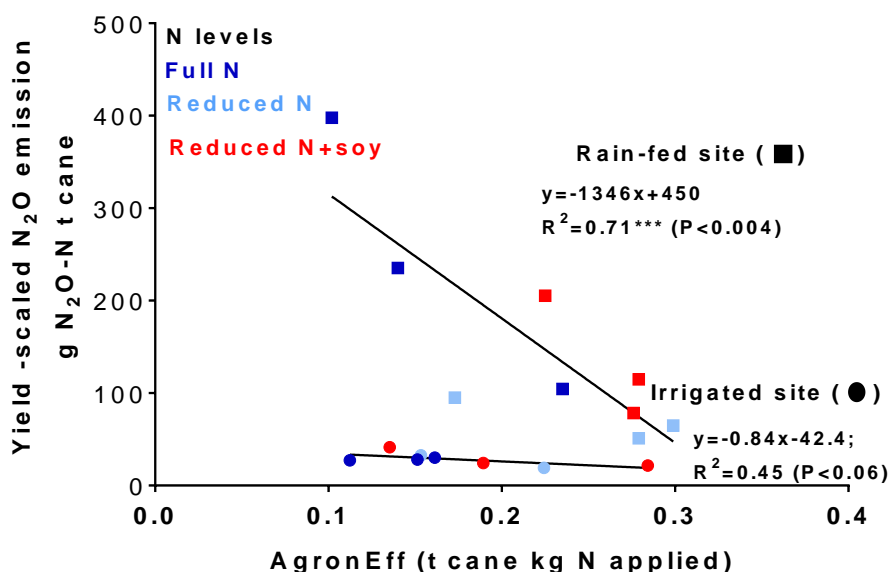
Figure 4. 2 Sugarcane agronomic efficiency of applied N (t cane/kg N applied after subtracting the contribution of the soil N background) *versus* yield –scaled N_2O emissions (g N_2O-N/t cane ha-1) at different N rates as monoculture and grown with soybean from Rain-fed site (Full N: 160 kg N ha^{-1}, Reduced N: 80 kg N ha^{-1}) and Irrigated site (Full N: 250 kg N ha^{-1}, Reduced N: 180 kg N ha^{-1})

4.3.5 Sugarcane yield and nitrogen accumulation into shoots

At the Rain-fed site, no significant yield differences were detected between treatments receiving N fertiliser and grown as sugarcane monoculture or with soybean; although sugarcane yields were 14% higher in the presence of soybean in the first season compared to the same rate of reduced N fertiliser with sugarcane only (2013-2014; Figure S4.2). In the 2014-15 season (the focus here), zero N fertiliser+soybean had a significant ($P<0.05$) 41% yield increase relative to monoculture (Figure 4.3a). In the third season (2015-2016), soybean did not establish due to herbivory by hares and sugarcane yields differed significantly ($P<0.05$) between full N fertiliser (70 t ha^{-1}), 50% reduced N (55 t ha^{-1}) and zero N (35 t ha^{-1}) (Figure S4.2). At the Irrigated site, full N or 28% reduced N treatments irrespective of soybean presence, had similar sugarcane yields (91 to 92 t ha^{-1}), while without N fertiliser, sugarcane yield was significantly ($P<0.05$) lower (81 t ha^{-1}; Figure 4.3b).

At the Rain-fed site, N accumulation into sugarcane shoots decreased by up to 10% in the 20% reduced N treatment, up to 16% in the reduced N+soybean treatment, and up to 20% in the zero N+soybean, but were statistically similar compared to the full N rate. Zero N+soybean increased ($P<0.05$) N uptake N 30% relative to zero N only (Figure 4.2c). Companion cropped

soybean in the zero N treatment accumulated 0.82 t of dry matter ha^{-1} and acquired 22 kg N ha^{-1}. With the reduced N rate (80 kg N ha^{-1}), soybean increased dry matter production and N uptake by 60% (1.35 t DM ha^{-1}; 36.2 kg N ha^{-1}, Table 4.7).
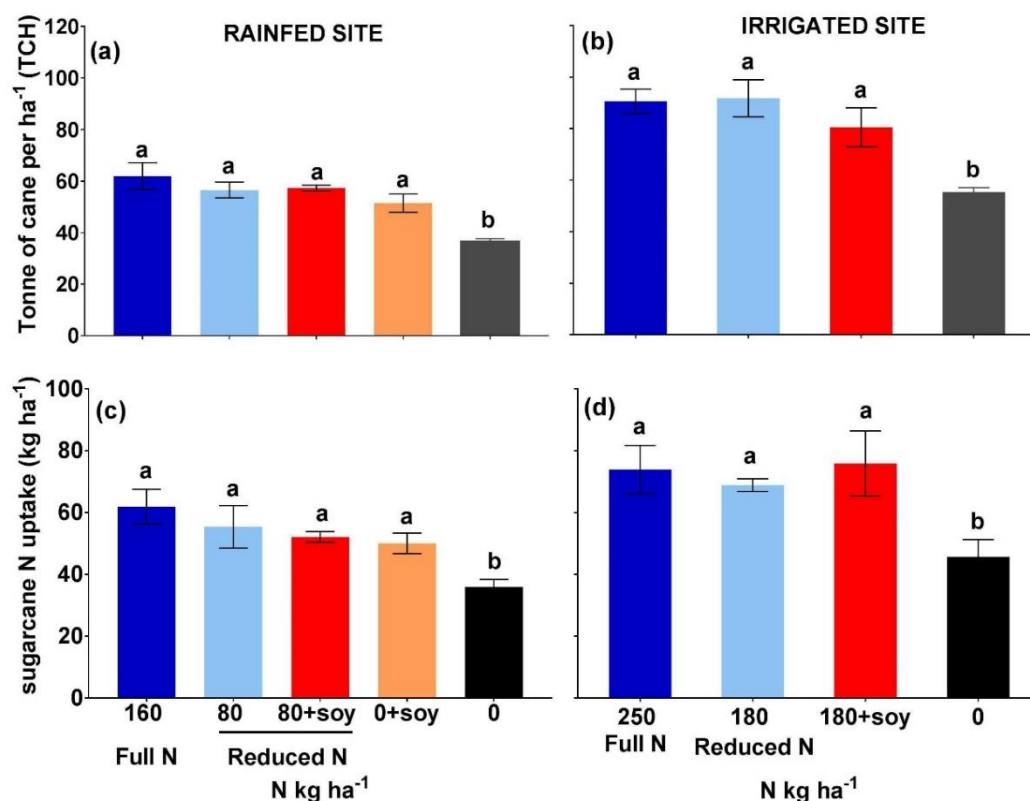
Figure 4. 3 Sugarcane grown at different N rates grown as monoculture or with soybean companion crop. Tonnes of sugarcane per hectare (TCH) (a,b), and N accumulation in aboveground biomass (c,d). Data are means S.E, n=3) from the Rain-fed site (2014-15) and Irrigated site (2015-16). Different letters above bars denote significant differences between treatments at $P< 0.05$ (LSD, Less significant difference test).

Table 4. 7 Companion cropped soybean dry matter yield (DM) and N uptake into shoots at two N rates (zero fertiliser and 80 kg N ha^{-1}). Data are means (n=3) from the Rain-fed site. 2014-15 season.

Treatment kg N ha^{-1}	DM t ha^{-1}	N uptake kg ha^{-1}
80+soybean	1.35	36.2
0N+soybean	0.82	22.0

At the irrigated site, N acquisition in the reduced N fertiliser+soybean was elevated (7 and 11% higher than full N and reduced N) but statistically similar, while a 37% reduced N uptake occurred in the zero N treatment (Figure 4.3d).

A significant positive relationship was observed between total cumulative N_2O emissions and sugarcane N uptake at the Rain-fed site (R^2=0.46, *P<0.001*) and the Irrigated site (R^2=0.62, *P<0.001*). Thus, for every 1 kg of N accumulated in sugarcane shoots above 33 kg N ha^{-1}, over 320 g N_2O-N ha^{-1} was released to the atmosphere in the Rain-fed site; and 53 g N_2O-N ha^{-1} at the Irrigated site (Figure 4.4).
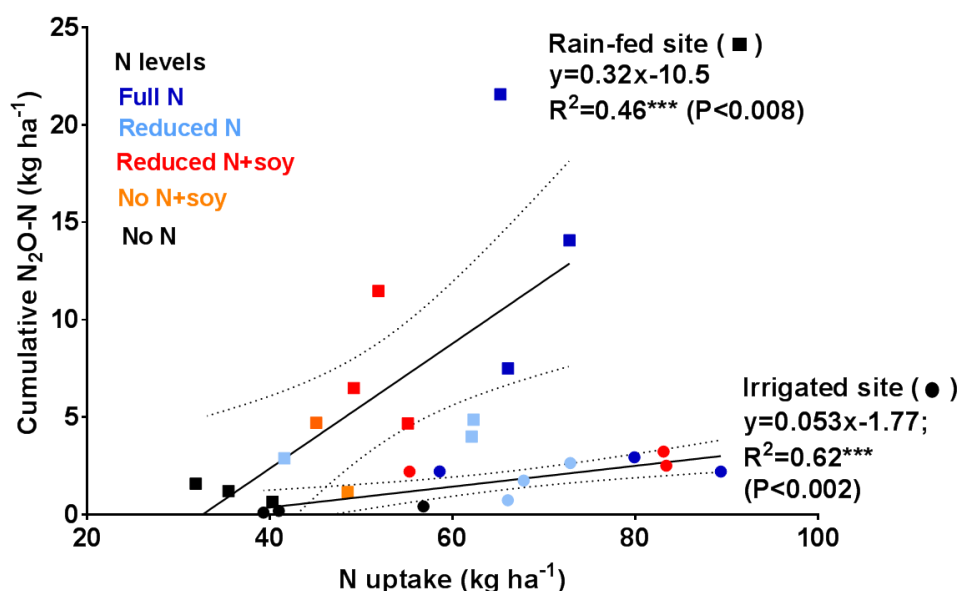
Figure 4. 4 Sugarcane N uptake (kg N ha^{-1}) *versus* cumulative N_2O-N (n=27) emissions at different N rates as monoculture and with soybean companion crop at Rain-fed site (Full N: 160 kg N ha^{-1}, reduced N: 80 kg N ha^{-1}) and Irrigated site (Full N: 250 kg N ha^{-1}, Reduced N: 180 kg N ha^{-1}).

4.4 Discussion

This study addresses the question whether soybean companion crops can reduce N_2O emissions from sugarcane soils by partially replacing N fertiliser with biologically fixed N. N_2O emissions increased in response to N fertiliser rate at one site but not the other, confirming that the environmental context is a strong determinant if N is lost from soil as gases or *via* leaching or run-off. Rain-fed, low-drainage hydrosol with high WFPS had high N_2O emissions in the order full N fertiliser > reduced N+soybean > reduced N > zero N+soybean > zero N. Irrigated, well-drained vertisol had lower N_2O emissions without an obvious relationship between N

fertiliser rate and soybean presence. At both sites, soybean did not affect sugarcane yield with full and reduced N fertiliser, most likely because full and reduced N fertiliser rates sufficiently supplied sugarcane and BNF had no benefit. At the Rain-fed site, a zero-N+soybean treatment was implemented which demonstrated that under N limitation, soybean can boost sugarcane yield and reduce N_2O emissions. The study advances understanding of sugarcane-legume companion cropping. It confirms that soybean as companion crop does not negatively affect sugarcane yield in the tested situations, and that soybean assist N_2O emission reduction in low N conditions. The study also shows that soybean in combination with excessive N fertiliser use can increase N_2O emissions. Achieving N sufficiency of sugarcane while minimising N losses from soil may be possible with prudent N fertiliser use that avoids excess N in soil. How soybean (and potentially other legumes) can best assist in this quest requires further investigation. With N_2O emissions in the spotlight, next-steps research should explore the threshold of N fertiliser input over multiple years to guide the optimisation of sugarcane-legume companion systems across climate and soil gradients. Simultaneous quantification of the overall environmental and agronomic benefits will advance sugarcane production with low pollution footprint.

4.4.1 Sugarcane production with legumes

Legumes are commonly grown as a rotation crop in the sugarcane fallow, with documented benefits including reducing soil pathogens and N input via BNF (White et al., 2011, Park et al., 2010, Wang et al., 2015). Soybean is a compatible intercrop for sugarcane with documented benefits to farm income in subsistence farming (Kaur et al., 2016, Islam et al., 2009, Roodagi et al., 2001a), and can reduce N fertiliser needs (Luo et al., 2016). We observed a pronounced benefit of soybean when N was limiting sugarcane growth when no N fertiliser used, confirming studies of soybean companion crops that benefitted sugarcane growth with no or low rates of N fertiliser (Hauggaard et al. 2016, Jensent et al. 2012). Without N fertiliser, the complementary effects of cereal-legume systems were explained by cereals using soil N and legumes meeting their N requirements with BNF (Ghaley et al., 2005). Sugarcane has a long growth period of nine to 12 months, and we expect a proportion of the N-rich legume residue to become available during the crop season. Our laboratory experiments showed that 20-30% of legume-N mineralises within 60 days, and 30-50% within 150 days (Buckley et al., 2016, Brackin et al., 2013), well within the N uptake phase of sugarcane. The inorganic soil N pool in the zero N+soybean treatment was elevated compared to zero N sugarcane, confirming that soybean increased N availability. Similarly, legume intercrops with low C/N ratios of 13 to 14

increased the pool of inorganic soil N by 38 to 56%, N mineralisation rates by 22 to 34%, and benefitted sugarcane yield (Suman et al., 2006).

Soybean did not improve sugarcane yield with 28 or 50% reduced N fertiliser application rates. The most likely explanation is that the reduced N fertiliser rate supplied sufficient N to sugarcane. Nitrogen use efficiency averages 50% in Australian sugarcane (Robinson et al., 2011) so that, if minimal losses occur and/or if soil contains residual N, a 50% reduced N fertiliser rate theoretically supplies sufficient N to sugarcane. A further mechanism that can explain a lack of benefits from soybean could be that sugarcane uses N fertiliser and residual soil N and outcompetes soybean (Ghosh et al., 2009, Ghaley et al., 2005). However, in our study, soybean biomass and N content increased by 60% in the reduced N fertiliser treatment (1.4 t dry matter and 36.2 kg N ha^{-1}) compared to zero N fertiliser (0.8 t DM and 22 kg N ha^{-1}) confirming that soybean profited from N fertilisation. A further consideration is that high levels of soluble soil N can inhibit or delay nodule formation and reduce BNF. It is well established that BNF decreases with increasing soil inorganic N levels (Peoples et al., 2009, Ghosh et al., 2006, Salvagiotti et al., 2008, Ghaley et al., 2005, Senbayram et al., 2015). In soybean monoculture <16% of legume-N was derived from BNF with 200-300 kg fertiliser-N ha^{-1} while >50% of legume-N was derived from BNF with rates of <80 kg N ha^{-1} (Salvagiotti et al., 2008). By contrast, it was suggested that soybean as sugarcane companion crops fixed 29 to 57% of their N demand when grown with 300-525 kg fertiliser-N ha^{-1} (Shiming and Gliessman, 2016). However, the ^{15}N abundance method used by the authors to quantify BNF is inherently inaccurate, and such high BNF rates in the presence of such massive N fertiliser rates are questionable as they contradict other studies. We did not investigate how much N was fixed by soybean but with high N fertiliser rates (full, reduced) and soybean increasing soil inorganic N levels only under N limiting (zero N fertiliser) conditions, it appears likely that BNF was lower in the N fertilised treatments than unfertilised control.

4.4.2 N$_2$O emissions from sugarcane–legume companion cropping system
Legumes as companion or intercrops of cereals, grass pastures and trees can mitigate N$_2$O emissions but the complex interactions between crops, environmental and agronomic variables prevent accurate predictions of how legumes affect N$_2$O emissions. Legumes can have no effect, decrease N$_2$O emissions by 48%, or increase them by 13 to 50% (Huang et al., 2014, Hauggaard-Nielsen et al., 2016). The impact of legumes on N$_2$O emissions depends on abiotic and biotic factors, especially soil N, moisture and temperature, agronomy, legume and BNF,

among other factors (Senbayram et al., 2015, Luo et al., 2016, Dyer et al., 2012, Huang et al., 2014, Epie et al., 2015, Jones et al., 2007). To the best of our knowledge, only one study on N_2O emissions from sugarcane legume intercropping has been published – they reported that reducing N fertiliser by 40% in a sugarcane-soybean system maintained sugarcane yield but did not significantly reduce N_2O emissions compared to fully fertilised monoculture sugarcane (Luo et al., 2016).

In our study, total cumulative N_2O emissions at the Irrigated site were similar in the N fertilised treatments irrespective of the presence of soybean. At the Rain-fed site, N_2O emissions were elevated in the presence of soybean so that the reduced N fertiliser rate+soybean statistically matched those from the full N treatment. Similarly, with zero-N+soybean, N_2O emission from sugarcane rows where higher than from sugarcane monoculture. Higher levels of soluble ammonium and nitrate in zero-N+legumes compared to sugarcane monoculture confirm that soybean increased soil N levels, but soil inorganic N levels were not increase with reduced N+soybean. These inconsistencies emphasise the need for further research. Our study was restricted to three replicate plots and, typical for field-based experimentation, variability between replicates was considerable. Similarly, intercropped soybean (Luo et al., 2016), or companion crops of soy of mung bean (Chapter 4) had inconsistent effects on N_2O emissions.

In pasture and crop systems, legume rotations are considered a sustainable alternative to N fertiliser (Stagnari et al., 2017, Crews and Peoples, 2005, Jensen et al., 2012) with N_2O emissions from legumes similar to those from unfertilised background (Jensen et al., 2012). However, legumes are not without a N_2O footprint (Rochette and Jansen 2005, Hauggaard-Nielsen et al., 2016, Pappa et al., 2011), and N release from decaying soybean roots and nodules is a likely cause of N_2O emissions during grain filling and maturation (Ciampitti et al., 2008, Yang and Cai, 2005). Our results concur with reports that legumes can increase N_2O emissions when BNF adds further N to fertilised soils (Jensen et al. 2012; Luo et al., 2016; Hauggaard-Nielsen et al., 2016; Ciampitti et al. 2008). N_2O emissions appear to be mostly driven by the gradual turnover and decomposition of N-rich soybean residues during the sugarcane tillering phase (Yang and Cai 2005), rather than being the immediate result of N input from BNF (Rochette and Janzen, 2005, Pappa et al., 2011). Soybean shoots rapidly decompose and stimulate soil microbial growth, mineralisation and nitrification rates (Brackin et al., 2013), and the presence of labile C in soybean biomass increases the activity of nitrifiers and

denitrifiers and promotes N_2O production (Senbayram et al., 2015, Tortosa et al., 2015, Carter et al., 2014).

Compared to non-legume monocultures without added N fertiliser, legumes increased N_2O emissions by 13 to 79% in intercropping systems (barley-pea, barley-white clover, grass-legumes) (Hauggaard-Nielsen et al., 2016). Thus, with different rates of BNF and other effects of legumes on N cycling, the impact of legumes on N_2O emissions will vary. Soybean shoots with zero-N fertiliser had accumulated 22.4 kg N ha^{-1} 124 days after planting. We estimate that at least 50% of legume biomass decomposed over the following 160 days to the end of the season (day 280) (Buckley et al., 2016, Brackin et al., 2013), but the largest driver of N_2O emissions in our study was N fertiliser, not legume-N. This is unsurprising because N fertiliser was the largest N input, and future studies should ascertain in more detailed investigation how N_2O emissions respond to conditions when the largest N input is from legumes rather than N fertiliser.

Alternative processes that contribute to N_2O emissions include *Rhizobium* bacteria (generally added to legumes in farming systems to ensure effective BNF) that can increase N_2O emissions *via* denitrification (Tortosa et al., 2015, Itakura et al., 2013). However, Breitenbeck and Bremner (1989) suggested that the population of symbiotic fixing bacteria such as *Rhizobium* is too small to impact on denitrification rates, yet *Rhizobium* is ubiquitous in soil and rhizosphere (Yeoh et al., 2017). Inoculation of soybean with *Bradyrhizobium japonicum* and grown with 30 kg N fertiliser ha^{-1} increased N_2O emissions by 40% compared to non-inoculated soybean at the same N rate (Ciampitti et al., 2008). However, such experiment does not distinguish between the effects of microbes on N_2O emissions or the increasing N status of the system due to effective BNF of symbiotic microbes. A minor effect that nevertheless highlights the impact of bacterial traits was documented with *Bradyrhizobium japonicum* strains with increased nitrous oxide reductase activity, an enzyme that catalyses the reduction of N_2O to N_2. These strains reduced N_2O emissions by 54 and 60% (0.052 and 0.045 kg N_2O ha^{-1}, respectively) relative to native *B. japonicum* (0.113 kg N_2O ha^{-1}) during decomposition of soybean nodules (Itakura et al., 2013). With the advent of metagenomics and metatranscriptomics such differences in bacterial properties can be explored to potentially assist the selection of microbes to enhance beneficial N cycling processes.

4.4.3 Cumulative greenhouse gas emissions

The N_2O emission factors (EF) from all fertilised treatments at the Rain-fed site were substantially larger than the default 1% N_2O EF of the IPCC (2006) for managed soils and the 1.25% EF for Australian sugarcane soils (Department of Environment, 2014). The EF from full N fertiliser treatments and reduced N sugarcane+soybean ranged from 0.8 to 8.03%. The highest EF at the Rain-fed site was more than twice the global average N_2O emission factor for sugarcane soils (3.87%, Lisboa et al., 2011). The N_2O EF of the Rain-fed Hydrosol in our study was at the higher end of the range of EF of 1.1 to 10% from Australian Hydrosols under sugarcane (Allen et al., 2010, Kingston et al., 2016, Wang et al., 2016). The highest emission factor (21%) in Australia was recorded by Denmead et al. (2010b) from a Hydrosol where trash was burnt before harvest (now an uncommon practice in Australia) and high organic carbon concentration of 9%; by comparison the Hydrosol in our study had <2% organic C content.

N_2O EFs from the Irrigated Vertosol were <1.2% and below the global average EF for sugarcane soil (Lisboa et al., 2011) and Australian sugarcane soils (Department of Environment, 2014). The 28% reduced N fertiliser+soybean had an EF of 1.2%, below the 1.3 to 4.5% from similar soil types (Dermasol, Kandosol, Chromosol) and similar N fertiliser rates (140-150 kg N ha^{-1}) and agronomies (Wang et al., 2016, 2014, 2012, Denmead et al., 2010b). The EF from the Irrigated Vertisol were similar or lower than those from 38% reduced N+soybean or mung bean from Dermasol at our third study site (1.31-1.78% N_2O EF, Chapter 4). The considerable difference in N_2O emissions between the Rain-fed Hydrosol and Irrigated Vertisol, despite similar soil texture and temperature, and higher N fertiliser application at the site with lower N_2O emissions is discussed below.

4.4.4 Influence of soil physical and chemical parameters on N_2O emissions in the field

At the Rain-fed site, it is likely that most N_2O emissions resulted from denitrification as the soil had mostly high soil moisture content and water filled pore space (WFPS) >60%. Nitrification is largely responsible for N_2O emissions at WFPS <55%, while denitrification prevails under anaerobic conditions with WFPS of >80% (Wang et al., 2015, Barton et al., 2008, 2011, Aguilera et al., 2013, Huang et al., 2014). Nitrogen fertiliser was applied at the start of the wet summer season during high temperatures (28-30 ℃ soil temperature), high rainfall (340 mm), and high WFPS (60 to 90%) over the 50 days following N fertilisation. These conditions combined with high concentrations of soil organic carbon and soluble N would have promoted microbial activity and N_2O production (Wang et al., 2016, Signor and Cerri, 2013, Fracetto et al., 2017). Soil microbial activity as quantified with CO_2 respiration

was high, with 400 to 800 mg CO_2-C m^{-2} h^{-1} in all treatments (data not shown). The sugarcane trash blanket can generate favourable conditions for microbial activity, keeping soil moist and providing C-substrate to fuel nitrification and denitrification (Carmo et al., 2013, Denmead et al., 2010b, de Oliveira et al., 2013). The presence of organic carbon as a source of energy for microbial respiration depletes soil oxygen levels and generates favourable conditions for N_2O production (CHAPUIS-LARDY et al., 2007, Robertson and Groffman, 2007, Oertel et al., 2016). In line with previous research, the combination of high soil moisture, temperature, organic C and soluble soil N, promoted high N_2O emissions in our study.

At the Irrigated site, we hypothesise that nitrification was the main pathway for N_2O emissions. Soil aerobic conditions prevailed with WFPS <58% during winter and spring. At this site, several variables differed from the Rain-fed site: application of N fertiliser occurred at the end of winter during comparatively low soil temperatures (~20ºC) and lower soil moisture (26-58% WFPS). Lower levels of soil soluble N occurred during warm summer months and even WFPS of up to 70% did not boost N_2O emissions. Lower N_2O emissions are observed when the controlling factors, especially soluble inorganic N, high WFPS, labile carbon and soil temperature do not interact at the same time (Smith et al., 1998, Wang et al., 2011, Denmead et al., 2010a, Dyer et al., 2012, Zhang et al., 2016). The Irrigated site did not have a trash blanket, and the soil had relatively low soil organic carbon (1.04%) and total N (0.13%) concentrations. Soil microbial activity was substantially lower in most treatments (110 to 410 mg CO_2-C m^{-2} h^{-1}) compared to the Rain-fed site. The only exception was the reduced N sugarcane-soybean treatment in the Irrigated site with significantly higher emissions ranging from 162 to 836 mg CO_2-C m^{-2} h^{-1}, which suggests that labile C additions from decomposition of the soybean crop stimulated microbial activity, as has been observed in previous studies (Brackin et al., 2013, Buckley et al., 2016).

A further factor for N_2O emissions is soil pH; the lower soil pH of 5.2 at the Rain-fed site may have favoured the denitrification pathway, while the higher soil pH of 6.5 at the Irrigated site may have promoted nitrification in line with previous research (Signor and Cerri, 2013, Oertel et al., 2016, Wrage et al., 2001). Which of the two pathways was mainly active at either site requires further investigation (see below), but the net outcome for N_2O emissions, high from Hydrosol with low-drainage and high WFPS, and low from Vertisol with good drainage and comparatively low WFPS, is in line with previous research.

4.4.5 Relationship between soil inorganic N and N2O emissions

Soil NH_4^+-N and NO_3^--N concentration at both locations was high during the first 70 and 80 days after N fertiliser application. All treatments with N fertiliser irrespective of the intercropping treatments had significantly elevated soil soluble NH_4^+-N and NO_3^--N compared to the control treatments (with or without soybean).

At the Rain-fed site, after N fertiliser application, soil NH_4^+-N dominated the soil solution up to 5-fold greater than NO_3^--N during summer. While this would suggest that nitrification could be the main precursor of N_2O emissions; multiple regression analysis indicated that N_2O emissions from all treatments were highly correlated with soil NO_3^--N, followed by NH_4^+-N and water filled pore space. Therefore, denitrification is more likely to have been the main pathway to N_2O emissions. Redding et al. (2016) found a significant presence of bacterial nitrifier communities in a clay soil under denitrification processes and high denitrifier activities independent of availability of NH_4^+-N or NO_3^--N sources. This suggests that simultaneous transformation of NH_4^+-N to NO_3^--N may have occurred within aerobic soil microsites within the predominantly anaerobic soil (Müller et al., 2014, Robertson and Groffman, 2007).

At the Irrigated site, NO_3^--N was the dominant soluble N form in soil, up to 4-fold than higher than NH_4^+-N during spring. Despite the high NO_3^--N concentration and availability of irrigation to compensate the deficit of water (14% less precipitation than the annual average 850 mm), the N_2O emissions were up to 83% lower than N_2O emission from Sunshine Coast. The relatively low soil compaction (soil bulk density; 1.16 g cm^{-3}), good internal drainage and few irrigation events (five) could lead NO_3^--N losses by leaching at the Irrigated site, suggesting that leaching may be the predominant N loss pathway at this site. Relatively low temperatures during winter and spring after early fertiliser application could decrease the rate of N conversion thus less N_2O emissions to the atmosphere. WFPS was less than 58%, suggesting that nitrification was the principal pathway of N_2O production in all treatments. Signor and Cerri (2013) and Oertel et al. (2016) cited that temperature and soil density are the main factors inducing N_2O emission from nitrification while, N_2O emissions from denitrification are related to WFPS.

4.4.6 Relationship between N2O emissions and agronomic efficiency of fertiliser

Minimising and managing N inputs to optimise sugarcane yield is a priority for the Australian sugarcane industry. Approaches include the selection of sugarcane cultivars with superior N use efficiency (Robinson et al., 2011) and agronomic strategies that improve N fertiliser use

(Thorburn et al., 2017, Bell et al., 2014). A simulation study identified N fertiliser rates more prominently affecting N use efficiency (NUE) (Thorburn et al., 2017) than management practices such as timing of fertiliser application, splitting N application, tillage intensity and in-field traffic management. Agronomic efficiency of fertiliser is an indication of how much additional cane (above the amount produced with zero N fertiliser) is produced per kg of N applied, and these values increased in reduced N treatments. Our results are in the range of agronomic efficiencies reported (0.11-to 0.31 t cane ha^{-1} kg^{-1} N applied; Bell et al., 2014), and the agronomic efficiency was similar in reduced N treatments with or without legumes.

Our study, similar to Van Groenigen et al. (2010), showed a significant negative relationship between yield-scaled N$_2$O emissions and Agron Eff, especially in the Rain-fed site (Figure 4). At the two locations, reduced N treatments plots (with or without legumes) decreased yield-scaled N$_2$O emissions from 205 to 64 g N$_2$O-N per tonne sugarcane in Rain-fed site and from 32 to 21 g N$_2$O-N per tonne sugarcane in the Irrigated site while the agronomic efficiency of N use increased from ~0.2 to 0.3 t cane kg N applied at both sites.

4.5 Conclusion

This study confirms previous research that depending on environmental conditions, low to high N$_2$O emissions occur from sugarcane soils. The combination of available N, high bulk density, low drainage, high WFPS and high soil carbon levels (soil organic carbon, trash blanket) resulted in high N$_2$O emissions, while available N in combination with higher drainage, lower WFPS and soil carbon levels had comparatively low N$_2$O emissions. Soybean companion cropping with reduced N fertiliser was associated with higher N$_2$O emissions but did not exceed full N fertiliser rates. This finding indicates that prudent use of N fertiliser has to accompany legume companion cropping. Next steps research has to deepen the observations here that soybean can support sugarcane growth and reduce N$_2$O emissions. Longer term studies are needed to enable maximise the benefits of legumes for N$_2$O mitigation. In addition to soybean, other legumes should be tested for their ability to acquire excess N in the early season, perform BNF and decompose to optimise N cycling in sugarcane systems while minimising competition and maximising facilitation.

Chapter 5 – Responses of sugarcane and soybean to N supply in a glasshouse experiment

The outcomes of this chapter are based on a glasshouse experiment that allowed us to understand the interaction between sugarcane and soybean at different N fertilizer rates under control conditions. The effect of N fertilizer had an important role in sugarcane and soybean biomass. Soybean outcompeted sugarcane growth at low or zero N fertilizer rates while for sugarcane was contrary over medium or high N fertilizer rates.

5.1 Introduction

Legumes are often used as rotation or intercrops because they are an important source of N, which can improve soil fertility, stimulate crop productivity and enhance use of nutrients and water (Jensen et al., 2012, Park et al., 2010). Legume productivity in monoculture and in intercropping systems is influenced by microclimatic conditions, soil properties, crop genotypes and agronomic management (Salvagiotti et al., 2008, Unkovich et al., 2008). These interactions impact on the extent to which legumes can supplement or replace synthetic N fertiliser. The amounts of N fixed *via* BNF varies widely in monoculture and intercropping systems (reviewed by (van Kessel and Hartley, 2000). Nitrogen input from BNF of grain legume monocultures ranges from 14 to 215 kg N ha^{-1}, and in intercropping systems from 8 to 124 kg N ha^{-1}. The legume N becomes available to the surrounding environment via mineralisation of legume shoot and root tissues (indirect transfer), and *via* root exudates and rhizo-deposits, and transfer through mycorrhizas (direct transfer) (van Kessel and Hartley, 2000, Jensen, 1996, Lemaire, 1995, Thilakarathna et al., 2016).

The amount of N transferred to non-legume crops varies widely (Thorsted et al., 2006, Ghosh et al., 2009). High levels of N fertiliser and soil inorganic N inhibit the rate of BNF; for example BNF of soybean decreased by 50% at applications of up to 160 kg N ha^{-1} (Salvagiotti et al. (2008). Likewise, BNF of pea intercropped with wheat dropped by 37% at applications of 80 kg N ha^{-1} (Ghaley et al., 2005). In a sugarcane-soybean intercropping system, BNF was similar at high N fertiliser rates between 300 and 525 kg N ha^{-1} (Shiming and Gliessman, 2016), perhaps because such high N fertiliser rates equally suppress BNF. Application of N fertiliser often increases soil nitrate levels, which suppresses nodule numbers, nodule mass, BNF activity, and accelerates nodule senescence and disintegration ((Ohyama et al., 2011).

Quantifying the facilitative or competitive effects of legumes on nutrient availability in intercropping systems is challenging as growth and resource requirements of component species combinations differ (Ghosh et al., 2009). A way to quantify these interactions is *via* indices of facilitation and competition that describe the type and intensity of interactions between species and/or cultivars (Zhao et al., 2016). Interspecific competition can be linked to crop growth rates, root system architecture, plant density among others factors that affect the competition for water, nutrients and light (Geetha et al., 2015, Ghosh et al., 2009, Yang et al., 2013, Billore et al., 2000). Conversely, facilitation occurs when one species facilitates growth of other species, such as *via* nutrient mobilisation (Li et al., 2016). Intercropping systems often produce higher biomass than monocultures, if two species access different resources and therefore do not compete directly and increasing efficiency (Zhang and Li, 2003, Li et al., 2009).

While sugarcane-legume intercropping is successful in some circumstances, in others a negative or no effect on sugarcane yield was observed (Geetha et al., 2015, Gana and Busari, 2003, Roodagi et al., 2001b). Sugarcane yields were suppressed in a sugarcane-soybean/lupin (*Glycine max*/*Lupinus albus*) intercrop at N fertiliser N rates of 0, 33, 67, and 100% of the full N rate (Ramouthar et al., 2014). Similarly, cowpea (*Vigna unguiculata*), mung bean (*Vigna radiata*) and urad bean (*Vigna mungo*) reduced sugarcane yield by 8 to 14%; however, economically such system was successful with the highest benefit cost ratio at applications of 125% of the recommended NPK in sugarcane-cowpea systems (Kumar et al., 2006).

Overall, most of the experiments using legumes to intercrop with sugarcane have been performed in the field. Assessment of the effect of N in sugarcane-legume intercropping systems to date has largely used different rates of N fertiliser and focussed on sugarcane growth, production and juice quality in field trials. In Chapter 4, at the rain-fed subtropical site, we found that the presence of a legume intercrop benefitted sugarcane yield at 0 kg N ha^{-1} but not at 80 kg N ha^{-1}. It suggests that presence of N fertiliser reduced the facilitation provided by the legume intercrop; or that factors other than N became limiting as crops grew larger in the presence of fertiliser. Although field experiments are important to understand responses under different climate conditions and farm management, greater understanding of the interaction between sugarcane and legume intercrops at different fertiliser rates profit from

controlled experiments than allow to solely assess the effect of N fertilisation on the performance of sugarcane and soybean.

The overall project here is based on the concept of legumes as green manure companion crop, which decays and supplies N to sugarcane. While legumes are grown with the primary purpose of advantaging sugarcane, BNF capacity of soybean, despite being a potential competitor of sugarcane, is crucial for the performance of the system. It remains unclear to what extent growing, living legumes can directly supply N to sugarcane through direct BNF-derive N leakage or transfer (Thilakarathna et al., 2016, Li et al., 2016), or indirect decomposition and mineralisation after legume death (Ghaley et al., 2005, Jensen and Hauggaard-Nielsen, 2003, Ijoyah, 2012, Senbayram et al., 2015).

Here we focus on the first question: can growing, living legumes directly supply N to sugarcane? The use of nodulating and non-nodulating soybean (Lin et al., 2012) allow separating the competitive effects of soybean (non-nodulating soybean) from the beneficial effects *via* N input (nodulating soybean). Additionally, the difference in N uptake of both soybean types allows to estimate the amount of N derived from BNF (Unkovich et al., 2008). The objectives of the research were to assess the interactions between N fertiliser rate, biomass production and N uptake of sugarcane and soybean when grown together, with nodulating or non-nodulating soybean, and compared to sugarcane grown without soybean.

5.2 Material and methods

5.2.1 Plant material and growing conditions

A commercial sugarcane cultivar (*Saccharum officinarum* x *spontaneum*, cv. Q138) was grown in a naturally lit glasshouse during summer-early autumn (December 2016 to March 2017) at the University of Queensland in Brisbane, Australia. Over the experimental period, daytime temperatures in the glasshouse ranged from 21 to 37°C. Mature sugarcane stalks were obtained from the rain-fed subtropical site, Maroochy River, Sunshine Coast, Queensland. Sugarcane plants were grown from setts (nodal stem cuttings; 2.5 cm average length) without N for four weeks in seedling trays with perlite as medium before transfer to pots.

Nodulating soybean cv. Bragg and non-nodulating '139 mutant soybean' (*Glycine max*) were inoculated by adding and mixing moist peat inoculum (Nodule N^{TM} rhizobia, NewEdge Microbials Pty Ltd.). Seeds were provided by the Centre for integrative Legume Research, The

University of Queensland. Seeds were inoculated one hour before planting to ensure maximum BNF potential. Additionally, 20 grams of Nodule N[TM] inoculum was dissolved in 22.5 litres of tap water, and 300 ml was applied to all pots with soybeans.

Sugarcane seedlings and soybean seeds were grown together in 8 litre pots (24.8 cm diameter) with a mixture of 25% soil and 75% sand as a potting medium with free drainage. Sugarcane seedlings were planted on the 2nd of December 2016 ('start of the experiment'). Four soybean seeds per pot were sown 14 days later, and re-sown where soybean germination failed. Three soybean plants were removed from each pot and one plant was left to grow. Soybean flowered from 60 to 69 days, and pod formation commenced by day 70. The experiment was harvested on day 88. Nitrogen and basal fertiliser (P, K, S) was applied at day 42 at five cm depth. Basal fertilisation consisted of 1.0 g pot^{-1} of phosphorus (approximately 40 kg P ha^{-1}), 2.4 g pot^{-1} of potassium (100 kg K ha^{-1}) and 0.5 g pot^{-1} of sulphur (20 kg S ha^{-1}), Sugar Research Australia 2013. Urea was source of N, mono-potassium phosphate and sulphate potash were the sources of P, K and S. All pots were supplied with a micro-nutrient solution of pH 6.0 (200 μM FeEDTA, 10 μM MnCl$_2$, 10μM H$_3$BO$_3$, 1μM CuSO$_4$, 2.5 μM ZnSO$_4$, 0.35 μM Na$_2$MoO$_4$) twice a week, and supplied with tap water until the harvest. Nutrient solution or water was added until liquid was dripping from pots. Potting media comprised of 75% sand and 25% soil with a total organic carbon level of 0.16% and total N of 0.05%, available P of 18 mg/kg (Cowell P), CEC of 7.5 cmol (+)/kg, and pH of 6.5 (1:2 soil:water).

5.2.2 Biomass and nitrogen analyses

Plants were harvested 88 days after the start of the experiment. Sugarcane plants were separated into leaves (mature leaf blades and sheaths, immature leaves) and stalks. Soybean plants were separated into leaves (with petiole) and stalks. Roots of sugarcane and soybean were considered whole because separation proved impossible. Plant tissue was dried at 60°C, weighed and ground to a fine powder for N analysis. Tissue N concentration of each component was analysed by combustion (LECO TruSpec analyser, LECO Ltd., St Joseph, MI, USA). Dry matter biomass above and below ground of each intercropping component was expressed in g per pot and multiplied by its respective N concentration to calculate N uptake.

5.2.3 Statistical analyses

Statistical analyses were performed using Minitab (McKenzie, JD 2004) Software. The treatments were arranged as a randomised complete block design (CBD) with two factors and five replicates. The first factor, soybean cultivars, were nodulating (cv. Bragg) and non-nodulating soybean intercropped with sugarcane, and sugarcane monocultures. The second

factor was N fertiliser rate which was applied as one of five rates 0, 1.1, 2.2, 3.3, 4.3 g per pot[-1], comparable to 0, 45, 90, 135, 180 kg N ha^{-1}. Differences and interactions between treatments were calculated by GLM-ANOVA and LSD all-pairwise comparison test at *P<0.05*.

Interspecific competition indices

Relative yield quantifies the productivity of intercropping relative to a sole crop (Ghosh et al., 2009, 2006). For the glasshouse experiment, relative dry matter yield and N uptake (yield) (RDY, RNY) of aboveground intercropped sugarcane aboveground biomass was used to estimate the effect of intercropping with soybean (Ghosh et al., 2009, 2006, Jensen, 1996) and calculated as follows:

$$RDY = \frac{DM_{ISC}}{DM_{MSC}}$$

$$RNY = \frac{N\ uptake_{ISC}}{N\ uptake_{MSC}}$$

Where DM_{ISC} and $N\ uptake_{ISC}$ are intercropped sugarcane dry matter and N uptake, and $_{MSC}$ and the equivalent values of monoculture sugarcane. Theoretically when RDY ≥ RNY, N is limiting for intercropped sugarcane, and if RDY ≤ RNY, more N is accessible in the intercropping system (Ghosh et al., 2009).

5.2.4 Capability for BNF and quantification of BNF

The capability of soybean to fix N_2 and the amount of fixed N were assessed by subtracting the N content of the sugarcane+non-nodulating soybean from the sugarcane+nodulating soybean treatment at each N rate (Unkovich et al., 2008). BNF can be determined by the difference in uptake of N of the N_2 fixing legume and reference plants, in our case the sugarcane with non-nodulating soybean.

$$N_2\ fixed = Total\ N\ Yield_{(ISC+NSB)} - Total\ N\ Yield_{(ISC+Non-NMSB)}$$

Total N Yield $_{(ISC+ NSB)}$ refers to the combined N uptake of sugarcane and nodulating soybean (above and belowground) in the intercropping system. Total N Yield $_{(ISC+ Non-NMSB)}$ refers to the combined N uptake of sugarcane and non-nodulating soybean (above and belowground) in the intercropping system.

Additional assessment of nodulation was used to support to calculated N input. Nodulation was scored visually (Unkovich et al., 2008) with scores from 0 for no nodulation, poor to medium nodulation, to a maximum score of 5 (>20 nodules counted).

5.3 Results

5.3.1 Biomass production

At harvest, 32% of all soybean plants, irrespective of N rates and nodulation ability, were forming pods, the others were flowering. Sugarcane was in the tillering phase. Sugarcane shoot dry matter increased significantly with increased additions of N and plateaued at additions >2.2 g N per pot in intercropped treatments, irrespective of the type of soybean (Figure 5.1a). Sugarcane as sole crop increased dry matter shoot sharply up 1.1 g N per pot^{-1} to 74 g per pot^{-1}, with similar biomass produced at all rates >1.1 g N pot^{-1}. Presence or absence of soybean did not significantly affect sugarcane biomass at 0, 2.2, 3.3, or 4.3 g N, but soybean significantly decreased sugarcane biomass at 1.1 g N pot^{-1} (Figure 5.1a).

Biomass of nodulating soybean was greater with 0, 1.1 and 2.2 g N, decreased gradually at higher rates of N application, and was significantly lower in the 4.3 g N treatment with shoot legume dry matter reduced by 56% ($P<0.05$) compared to non-fertilised soybeans (Figure 5.1b). Aboveground biomass of nodulating soybean was 229 and 37 % greater than biomass of non-nodulating soybean at 0 and 2.2 g N, and similar at all other N rates (Figure 5.1b). Non-nodulating soybean had the highest biomass at 1.1g N per pot^{-1}, a non-significant trend towards reduced biomass with each increasing N level, and significantly lower biomass in the 0 N treatment ($P<0.05$) (Figure 5.1b).

The addition of N fertiliser increased the combined shoot and root biomass with significant ($P<0.05$) differences between treatments (Figure 5.1c). Application of 1.1 g N increased total aboveground biomass 1.8, 3.8 and 14.4-fold and root dry matter biomass by 1.6, 2.6 and 3.4-fold with nodulating soybean, non-nodulating soybean and sole sugarcane treatments respectively, compared to the zero N application (Figure 5.1c). There was no statistically significant effect of N fertiliser or soybean type on total above and belowground biomass at applications > 2.2 g N for intercropped treatments; however, intercropped systems with non-nodulating and nodulating soybean had 1.3 and 1.5 times higher shoot dry matter than sugarcane only ($P<0.05$) (Figure 5.1c). Adding soybean resulted in significant differences ($P<0.05$) in the total shoot dry matter (Table S5.2), especially in the unfertilised treatment

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where the sugarcane monoculture and sugarcane+non-nodulating soybean produced 90 and 56% less aboveground biomass than sugarcane+nodulating soybean ($P<0.05$).

5.3.2 Nitrogen uptake and internal nitrogen use efficiency

Shoot N content of sugarcane and soybean showed similar trends to biomass production (Figure 5.2a). Sugarcane shoot N uptake increased significantly with higher N application. Sugarcane aboveground N uptake was not affected by the presence of either type of soybean. Nodulating soybean was very sensitive to N applications with aboveground N uptake highest in the 0 and 1.1 g N treatments and decreasing significantly by 36 and 60% at 2.2 g N and 3.3 g N applications, respectively. Nitrogen applications ≥3.3 g N resulted in similar soybean aboveground N (Figure 5.2b). Non-nodulating soybean had similar N content at all fertiliser application levels, although there was a trend towards highest N uptake at moderate fertiliser levels (Figure 5.2b). Non-nodulating soybean had significantly lower aboveground N content than nodulating soybean at 0, 1.1 and 2.2 g N application, and N content of both soybean types was similar at higher N applications (>3.3 g N).

Total aboveground N uptake was significantly higher in the non-nodulating sugarcane+soybean than in monoculture sugarcane at all N fertiliser levels (Figure 5.2c). Total combined aboveground N uptake in the unfertilised treatment with nodulating soybean was 6.5 and 37.5-fold higher than that of non-nodulating soybean and sugarcane monoculture, respectively (Figure 5.2c). Additionally, N uptake into root biomass for the unfertilised nodulating soybean treatment was 3.0 to 5.5-fold higher than non-nodulating soybean and sugarcane monoculture, respectively (Figure 5.2c). As a general trend belowground N uptake from the unfertilised treatment was almost similar compared to the all fertiliser treatments. Total N uptake remained stable at applications more than 2.2 g N per, ranging from 0.36 to 0.53 g N pot^{-1} (Figure 5.2c).
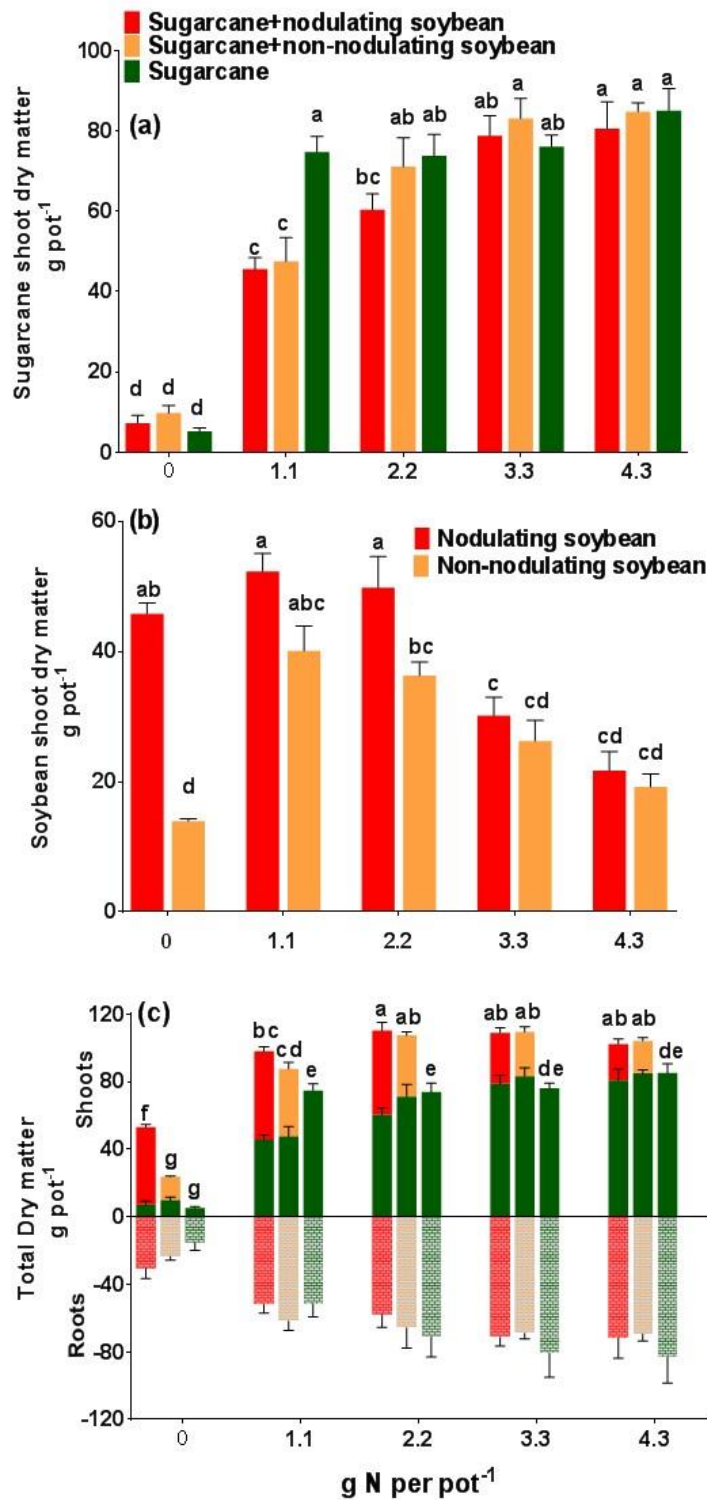
Figure 5. 1 Pot experiment of sugarcane plant grown with a soybean plant or sugarcane grown as sole plant. Shoot dry matter of sugarcane grown in the presence of a soybean plant or as sole sugarcane (a), shoot dry matter of soybean grown with sugarcane (b), shoot and root dry matter of sugarcane and soybean (root dry matter is sugarcane and soybean combined) (c) under different N supply (SE, n=5). Different letters above bars indicate differences at $P<0.05$ between treatments (LSD, Fisher test).

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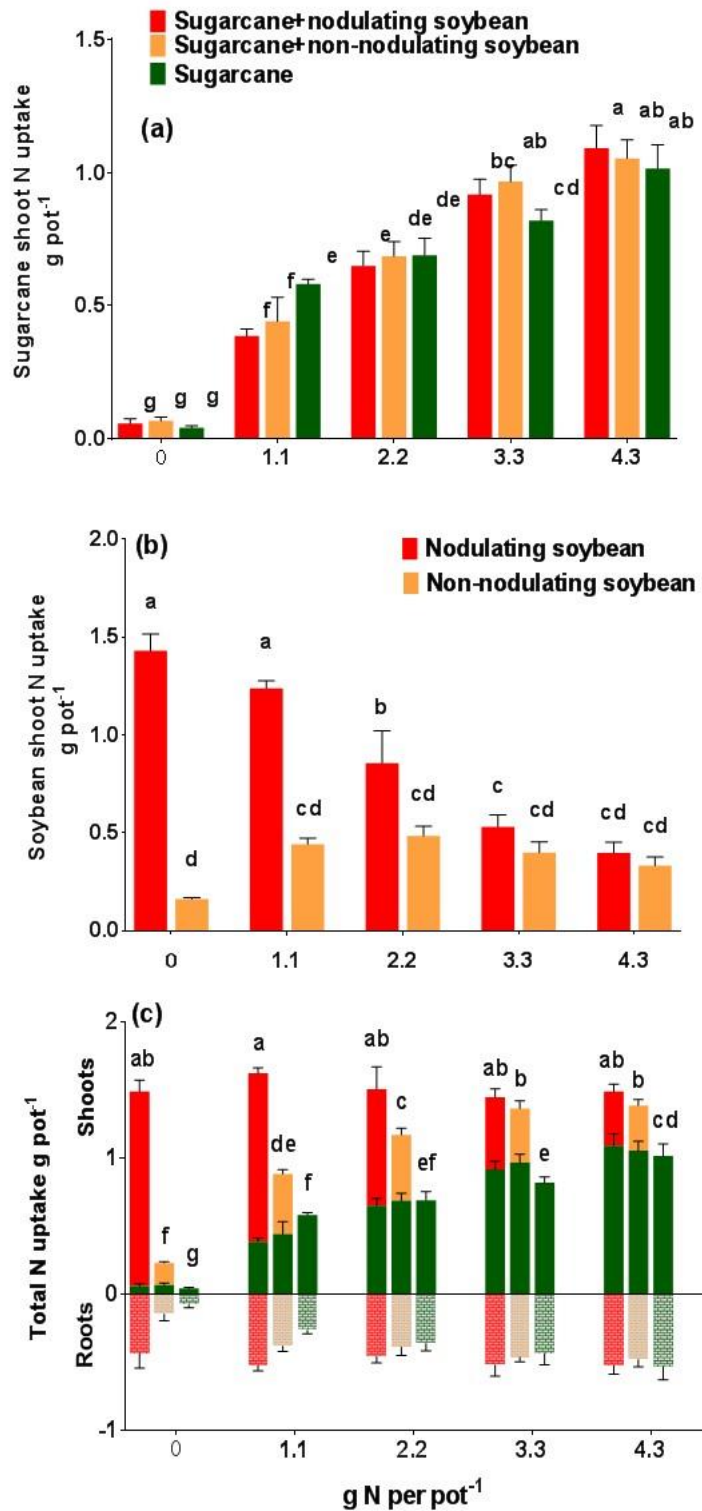
Figure 5. 2 Pot experiment of sugarcane plant grown with a soybean plant or sugarcane grown as sole plant. Shoot N uptake of sugarcane grown in the presence of soybean (a), shoot N uptake of soybean grown with sugarcane (b), and total N uptake of shoots and roots of sugarcane+ soybean, and sugarcane monoculture (root dry matter is sugarcane and soybean combined (c) under different N application rates. (SE, n=5). Different letters above bars show differences at the *P*<0.05 level between N fertiliser and legume treatments (LSD, Fisher test).
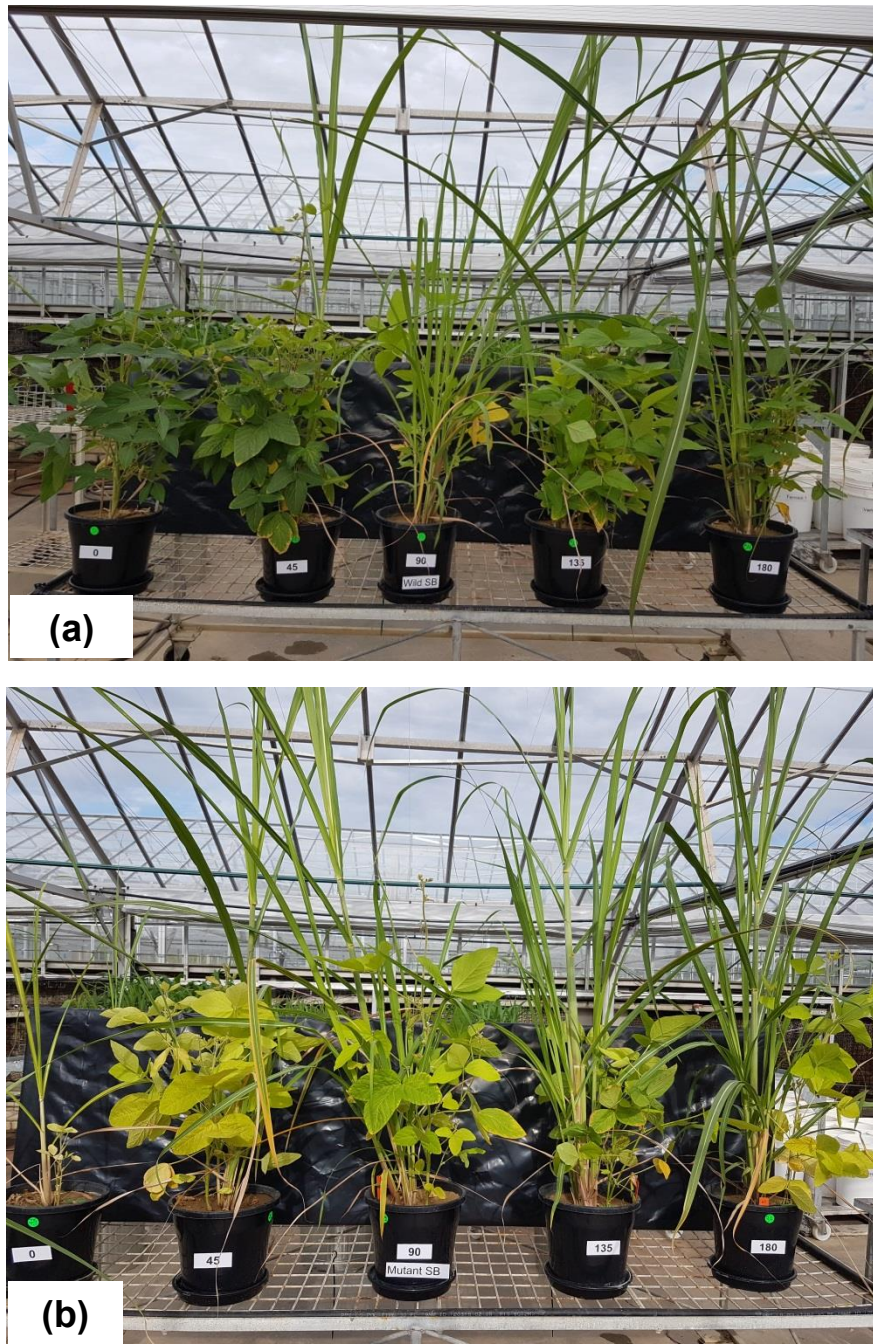
Figure 5. 3 Sugarcane with nodulating soybean plants (a) and sugarcane with non-nodulating soybean plants (b) grown with N fertiliser rates (kg N ha^{-1} are shown, translating to 1.1, 2.2, 3.3 and 4.3 g N per pot^{-1}). Photos were taken day 84 of the experiment.

5.3.3 Tillering

Intercropped soybean cultivars did not affect sugarcane tiller formation. Tillering increased significantly at increasing rates of N fertiliser (P<0.05). Applications from 1.1 to 4.3 g N increased the number of sugarcane tillers from 0 to 5.6, 6.2 and 7.4 tillers in the presence of nodulating soybean, non-nodulating soybean and sugarcane only, respectively (Table 5.1). Sugarcane shoots were thinner in the zero N aplication, and stooling was reduced. With N fertiliser sugarcane and nodulating soybean were markedly greener compared to zero N and compared to non-nodulating (Figure 5.3a and b)

Table 5. 1 Number of sugarcane tillers of sugarcane grown with soybean, and nodulation of soybean under different fertiliser N rates.

g N per pot	Nodule scores*	Number of sugarcane tillers		
		Sugarcane +nodulating soybean	Sugarcane +non-nodulating soybean	Sugarcane only
0	4.8[a]	0.0 [d] ± 0.0	0.0[d] ± 0.6	0.0 [d] ± 0.5
1.1	4.4[a]	4.2 [c] ± 1.1	3.8 [c] ± 1.9	4.8 [c] ± 1.2
2.2	2.6[b]	5.4 [bc] ± 0.5	5.4 [bc] ± 1.0	5.0 [bc] ± 2.2
3.3	2.4[b]	5.6 [bc] ± 2.7	5.5 [bc] ± 1.6	5.4[bc] ± 0.6
4.3	0.4[c]	5.6 [bc] ± 1.1	6.2 [ab]± 0.8	7.4 [a] ± 3.9

Different letters in the column show differences at *P*<0.05 level of significance between N fertiliser rates (LSD, Fisher test). *Unkovich et al., 2008.

5.3.4 Internal nitrogen efficiency of sugarcane

Internal N use efficiency (iNUE) indicates how much biomass is produced per amount of N acquired into shoots. As a general trend, sugarcane iNUE decreased with soybean and in monoculture with increasing N applications. Intercropped sugarcane reached the highest iNUE (up to 149 g biomass g^{-1} shoot N) at N applications <2.2 g N pot compared to sugarcane monoculture (up to 129 g biomass g^{-1} shoot N). Nitrogen applications >3.3 g N reduced iNUE to 74-94, but this was not statistically significant (Table 5.2).

Table 5. 2 Internal nitrogen use efficiency (iNUE) of aboveground biomass from intercropped sugarcane with nodulating and non-nodulating soybeans and sugarcane monoculture under fertiliser N application rates (± SD, n=5).

g N applied	Sugarcane+nodulating soybean	Sugarcane+non-nodulating soybean	Sugarcane
	iNUE (g sugarcane biomass g^{-1} N in sugarcane shoots)		
0	131^b ± 17.5	149^a ± 31.2	125^{bc} ± 10.7
1.1	119^{bcd} ± 10.8	114^{cde} ± 18.9	129^b ± 13.1
2.2	93^{gh} ± 8.1	103^{efg} ± 11.9	108^{def} ± 8.4
3.3	86^{hi} ± 13.1	87^{hi} ± 13.9	94^{fgh} ± 12.9
4.3	74^i ± 4.7	82^{hi} ± 11.1	85^{hi} ± 7.2

Different letters in each column indicate differences at $P<0.05$ between N fertiliser rates (LSD, Fisher test). iNUE: internal nitrogen use efficiency

5.3.5 Quantification of fixed N_2 and nodulation

Nodulating soybean fixed N_2 as indicated by nodules were located in the crown–root zone of sugarcane which intermingled with soybean roots. Visual assessment indicated that nodules were active as indicated by the pink colour of leghaemoglobin (Figure 5.5a and b). A negative relationship was found between the presence of nodules and N applications rates, decreasing from a nodule score of 4.8 without N fertiliser to 0.4 at the highest N rate of N (Figure 5.4).

Table 5. 3 Nitrogen fixed by nodulating soybean grown with sugarcane as estimated by comparison with non-nodulating soybean. (± SD, n=5).

N applied per pot (g)	N uptake into shoots and roots		
	Sugarcane-soybean (nodulating)	Sugarcane-soybean (non-nodulating)	Estimated BNF (g N per pot)
0	1.92^{ab} ± 0.3	0.38^e ± 0.1	1.55^a ± 0.2
1.1	2.15^a ± 0.1	1.26^d ± 0.2	0.89^b ± 0.2
2.2	1.97^{ab} ± 0.3	1.56^c ± 0.2	0.41^c ± 0.5
3.3	1.97^{ab} ± 0.3	1.83^{bc} ± 0.2	0.14^c ± 0.4
4.3	2.01^{ab} ± 0.2	1.87^b ± 0.3	0.15^c ± 0.2

Different letters above bars indicate differences at $P<0.05$ between treatments (LSD, Fisher test).

Total N uptake was 6-fold greater with nodulating soybean compared to non-nodulating soybean in the unfertilised treatments. The nodulating soybean fixed an estimated 1.53 g N per pot at zero N fertiliser addition; this amount decreased to an estimated 0.22 g N per pot with increasing fertiliser addition (Table 5.3).

Figure 5. 4 Intermingled roots of sugarcane and nodulating soybean at different N rates (kg N ha^{-1} are shown, translating to 1.1, 2.2, 3.3 and 4.3 g N per pot). The zero N fertiliser treatment has the largest amount of nodules.
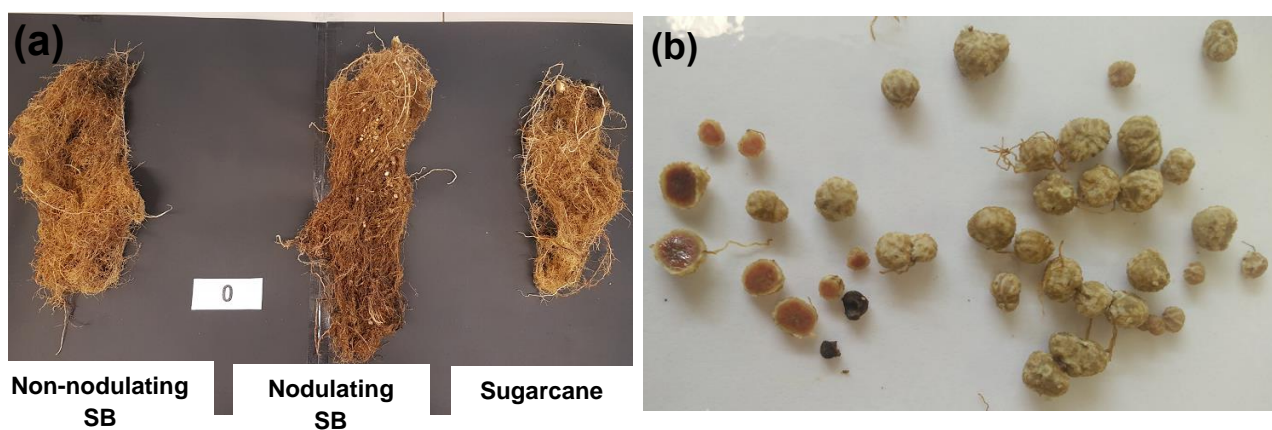
Figure 5. 5 Intermingled roots of sugarcane and soybean (SB: soybean, nodulating and non-nodulating) and root system of sugarcane monoculture without N fertiliser (a) and appearance of active nodules from the nodulating soybean (b).

5.3.6 Competition indices of sugarcane–soybean system

The relative dry matter aboveground biomass (RDY) and relative nitrogen uptake (RNY) from the intercropped sugarcane at harvest varied with the rates of N fertiliser. The unfertilised intercropped sugarcane had significantly higher (*P<0.05*) values of RDY and RNY (regardless the soybean type) than any other fertiliser rate.

Table 5. 4 Shoot relative dry matter yield (RDY) and shoot relative nitrogen uptake (RNY) of intercropped sugarcane with nodulating or non-nodulating soybean, compared to sugarcane monoculture at different rates of N application.

soybean type	N g⁻¹ pot	Relative dry matter or N uptake of intercropped sugarcane	
		RDY	RNY
nodulating soybean	0	1.43[a]	1.33[ab]
	1.1	0.61[c]	0.56[c]
	2.2	0.84 [bc]	0.98 [bc]
	3.3	1.04 [bc]	1.12 [bc]
	4.3	0.99 [bc]	1.09 [bc]
non-nodulating soybean	0	2.19[a]	1.80 [a]
	1.1	0.64 [c]	0.62[c]
	2.2	0.96 [bc]	1.00 [bc]
	3.3	1.09[bc]	1.18 [bc]
	4.3	1.02 [bc]	1.06 [bc]

Different letters in each column indicate differences at *P<0.05* level of significance.

Nitrogen application influences the competitive ability of sugarcane. The RDY of intercropped sugarcane increased gradually from 0.61 to 1.04 and from 0.64 to 1.09 with nodulating and non-nodulating soybean respectively at the application >1.1 g N and <4.3 g N, with a slight reduction at the highest levels of N rate. Similar trends were observed with RNY which increased from 0.56 to 1.12 without significant differences between fertilised treatments (*P<0.05*) (Table 5.4).

Values closer or greater than 1.0 (without considering the zero treatments) at the higher rates of N (3.3 and 4.3 g N) showed an improvement in the use of N fertiliser and production of aboveground biomass for intercropped sugarcaneo but this was not significant compared to respective sole sugarcane. At applications <1.1 g N, the relative dry matter (RDY) was higher than relative N yield (RNY) of intercropped sugarcane with both soybean types, suggesting N

limitation for sugarcane growth. Conversely, with fertiliser N >2.2 g N, RDY was lower than RNY, suggesting resources other than N are limiting (Table 5.4).

5.4 Discussion

5.4.1 Effects of soybean on sugarcane biomass and nitrogen accumulation

The results indicate no significant effects of legumes on sugarcane biomass at 0, moderate or high N application; but significant decreases in sugarcane biomass at low N fertiliser rates. A similar pattern is observed with N accumulation. Presence or absence of BNF did not affect sugarcane biomass or N content. These findings indicate that no significant direct N transfer from soybean to intercropped sugarcane occured in this experiment, despite substantial N fixation in the zero and low-N treatments. Indirect N transfer from soybean to sugarcane (belowground decomposition of root tissue and nodules) was not examined in the experiment but the focus was on direct N transfer that may occur in the early season of legume and sugacane growth. The 'indirect' pathway of N transfer via mineralisation of residues and uptake by the non-legume crop is comparatively understood, direct transfer pathways are less well understood (Peoples et al., 2015). Our results agree with studies that reported that cowpeas (*Vigna unguiculata*) in field and glasshouse experiments and ricebeans (*Vigna umbellata*) in a field trial did not appear to directly transfer N to maize (Rerkasem and Rerkasem, 1988, Ofori and Stern, 1986). Similarly, reviews on the topic have concluded that contribution of direct N transfer are minor or negligible (Peoples and Herridge, 1990). However, pathway may contribute a significant proportion of the N of non-legume crops (Peoples and Craswell, 1992, Thilakarathna et al., 2016, Stern, 1993). Previous studies have shown the importance of root interaction to enhance the direct N transfer via root exudates in a maize-faba bean intercrop (Li et al., 2016) or *via* mycorrhizal hyphae network that increased direct N transfer from soybean to maize from 13.2 to 30.2% (Zhang et al., 2017). Mycorrhizas were not quantified in the current study, although the presence of soil may have provided mycorrhizal spores. The mixture of 75% steam sterilised sand with 25% soil may not have been conducive to mycorrhizal growth. The high root contact between the two crops in pots should have provided conditions highly conducive to direct transfer of N *via* root exudates and another root-derived N. However no significant transfer was apparent, suggesting that this form of transfer may be less important than sometimes claimed. However, it our finding is representative of sugarcane-legume interactions in the field is unknown but should be investigated.

We speculate that the N advantage of legumes for sugarcane (Chapters 3 and 4) could be predominantly occuring *via* indirect N transfer of decomposition and mineralisation of legume litter. If this is the case, the overall success of legume intercropping for sugarcane will be determined primarily by how effectively soybean litter is decomposed and mineralised, and whether the timing of this N release is well matched with sugarcane N demand.

Sugarcane-legume combinations showed a favorable response for iNUE. Sugarcane grown with legume produced more biomass per unit of tissue N acquired, especially at the lowest rates of N fertiliser (<2.2 g N pot). In line with our results, other studies showed that intercrops use soil nutrients more efficiently than monoculture crops due to high recovery (Zhang and Li, 2003, Gao et al., 2014). However, iNUE from intercropped sugarcane was similar as sugarcane monoculture at the highest rates of N (>3.3 g N pot) suggesting that there was no competitive suppression of sugarcane growth by soybean at higher N rates.

5.4.2 Interspecific competition indices for sugarcane-soybean intercropping

Crop yield of intercropping systems is directly influenced by the interspecific competition of each crop (Zhao et al., 2016, Xia et al., 2013a, Yang et al., 2013, Li et al., 2001). In several studies, sugarcane grown with different legumes or other crops showed positive, neutral or negative sugarcane yield responses compared to sugarcane monoculture (Billore et al., 2000, Islam et al., 2009, Ramouthar et al., 2014, Kaur et al., 2016). Intercropping grain legumes with cereals showed high competitive ability of legumes in conditions of low N availability, while cereals outcompeted legumes with high N availability (van Kessel and Hartley, 2000, Lithourgidis et al., 2011, Jensen, 1996, Ghaley et al., 2005). In our study, sugarcane had different N competitive intensities based on dry matter biomass (RDY) and N uptake (RNY) that changed with rates of N fertiliser. Though relative values in zero N applied were high, the absolute values of intercropped sugarcane shoot dry matter and N uptake were significantly lower than those fertilised N treatment (Figure 5.1a and 5.2a). A review by Bedoussac et al. (2015) reported high partial relative dry matter yield occurred only in pot experiments or on a per plant basis where legumes penalised cereal yield because of greater legume growth like it happened in our glasshouse trial.

The no N treatment with soybean resulted in non-significant facilitation of sugarcane growth and N uptake, while low applications of N fertiliser (1.1g N per pot) resulted in a competitive disadvantage to sugarcane crop in the presence of either soybean type. Applications >2.2 g N pot to intercropping treatments with soybean showed no effect of intercropping on sugarcane,

but resulted in yield penalties for soybean, probably due to competition from sugarcane. It concurs with research indicating that moderate to high N fertiliser application tip the balance of interspecific competition towards the non-legume intercrop in intercropping systems (Ghaley et al., 2005, Jensen, 1996).

In a study similar to ours, addition of organic amendments (farmyard manure, and phospho-compost) in combination with a reduction of NPK fertiliser by 25% minimised the competition for N and P between intercopped sorghum and legumes compared to the reduced NPK (without amendments) treatment (Ghosh et al., 2009). By contrast, a study in China on sugarcane-soybean intercropping showed high interspecific competition exerted by soybean on sugarcane crop yield and N acquisition despite high levels of N fertiliser (325 and 500 kg N ha^{-1}). These responses were atributed to a lag of the growth stages between crops, and high capacity of soybean to compete for water, light and nutrients (Yang et al., 2013). In our study, soybean planting and fertilisation schedule may have reduced soybean supression of sugarcane yield. Soybean were planted about 28 days after the sugarcane seedling planting, and all plants received N fertiliser 14 days later. This planting schedule allowed the establishment of sugarcane before the introduction of the soybean intercrop (minicking field conditions), and it is likely that sugarcane was able to respond faster to N supply, as it would have had a larger root system than soybean at the time of fertiliser application.

5.4.3 Roots and nodulation

Yield improvements in intercropping systems have been associated with root traits (Meng et al., 2015, Li et al., 2016, Gao et al., 2010). Root distribution and length, morphology and interaction between intercropped species play important roles in nutrient uptake and water use (Gao et al., 2010, Li et al., 2006). In our study, roots of sugarcane-soybean could only be investigated as combined biomass as both roots systems were entangled. At zero N, combined root biomass and N contents from both intercropped treatments were greater than monoculture sugarcane roots, indicating extra resources were available *via* BNF. Ghaley et al. (2005) noted that interspecific competition decreased under N limitation when intercropped peas fixed N during early growth stages, and that pea and wheat can facilitate for N in soils with low N content. In all levels of fertiliser application here, total root biomass of intercropped and monoculture treatments was similar, potentially indicating limitations on root growth due to lack of available space for root expansion in the relatively small pots.

We calculated that up to 1.53 g N per pot was fixed in the zero N treatment while <0.22 g N per pot was fixed with higher rates of N fertiliser. In line with these findings, the nodulation score decreased significantly from 4.80 in the unfertilised treatment to 0.40 at the highest level of N. Our findings resemble other studies that showed a negative correlation between the % Ndfa (N derived from air) and higher levels of inorganic N (Parsons and Khubone, 1999). For example, Ghaley et al. (2005) found that pea intercropped with wheat decreased %Ndfa by 79 and 90% with additions of 40 and 90 kg N ha^{-1}. This indicates that trade-offs exist with N fertilising legume intercropping systems. While low N fertiliser rates maximise BNF, they may disadvantage sugarcane growth due to a lack of N and/or competition from soybean. High N fertiliser rates on the other hand minimise both N fixation and competition, but reduce the adavantage of lower N fertiliser rates.

5.5 Conclusions

Presence or absence of soybean did not affect sugarcane biomass at zero, moderate or high N application, however, at low fertiliser application rates (1.1g N per pot) significant decreases in sugarcane biomass and N accumulation occurred. Our findings suggest that direct transfer of N from legumes to sugarcane was negligble in the tested conditions at all fertiliser rates and that advantages seen in field are likely to occur predominantly *via* indirect decomposition and mineralisation of legume biomass. This glasshouse study further confirms that finding the ideal N fertiliser rate and legume companion crop will need further research. We also conclude that the overall advantage of legume companion cropping will depend on how effectively soybean litter is decomposed and mineralised and whether this matches the timing of N needs of sugarcane.

Chapter 6 – General Discussion

The research evaluates, for the first time, the relationship between N supply and N_2O emissions in sugarcane-legume companion cropping systems in Australia. This thesis focusses on the questions if legumes can supplement synthetic N fertiliser and lower the emissions of N_2O from the soil, and how sugarcane performs when intercropped with legumes. Several discoveries allow the conclusion that sugarcane-legume cropping has the potential for N_2O mitigation, and that more research is needed to understand better and optimise such systems for particular climates, soil and agronomies.

6.1 Effect of companion cropping on sugarcane yield (Chapters 2, 3, 4 and 5)

In Chapter 2, review of the literature showed that in subsistence agriculture, sugarcane intercropped with oil legumes, pulses and other food crops benefit farm income, land use efficiency, resource use and soil health. Why then, if sugarcane intercropping is successful in subsistence agriculture, is it so rarely practiced in modern agriculture? Brooker et al. (2015a) postulated that in mechanised agriculture, intercropping hinders the management of monoculture crops by increasing labour and equipment needs. A key difference between subsistence and commercial systems is whether the intercrop can be harvested for profit – while this is feasible in small plots with manual labour, it may be more difficult to implement in mechanised systems. In the research here, legumes were planted solely as green manure crop with the aim to benefit sugarcane through BNF. Following the development of other successful intercropping systems in mechanised agriculture, we see an opportunity to advance sugarcane-legume systems with customised agronomies. Multi-species cropping, whether as intercropping (all crops are harvested) or companion cropping systems (one crop is harvested), has considerable potential to improve crop yields with similar input, or to maintain or improve crops yields with reduced input; in all instances agronomic efficiency increases (Brooker et al. 2015).

The review of sugarcane intercropping (Chapter 2) and the empirical field experimentation (Chapters 3 and 4) provide evidence that legume intercropping can benefit sugarcane yield but also that it can have neutral or negative effects. It is clear that a wide range of parameters influences the outcomes of such system including spatial (row spacing, planting distance) and temporal (time of legume planting, size and growth of sugarcane) factors, water availability, legume type, soil and fertiliser rate.

Site characteristics and management of the three experimental locations

Location	N Application	Planting legumes	Sugarcane harvest
2014-2015	days after the harvest of previous cane ratoon		
Wet tropic	57	126	388
Rainfed	25	25	328
2015-2016			
Wet tropic	42	122	377
Irrigated	15	15	410
Rainfed	46	46	359

	Number of cane ratoon where the N_2O emissions begun to sample		
Location	Wet tropic	Irrigated	Rainfed
Ratoon	2nd to 3rd	2nd	6th to 7th

Location	Rainfall (mm)	Irrigation	WFPS (%)
Wet tropic	802*/1211*	no	15-51*/30-60**
Irrigated	850	yes	24-59
Rainfed	1359*/1139*	no	70-82

* 2014-2015 season. **2015-2016 season

Soil physical characteristics					
Location	Type	Texture	T°C	pH	bulk density
Wet tropic	Dermosol	sandy clay loam	30	5.6	1.20
Irrigated	Vertosol	clayed soil	21-28	6.5	1.16
Rainfed	Hydrosol	silty light clay	27-29	5.2	1.35

Location	Total Carbon (%)	Total N (%)	CEC cmol(+)/kg	Trash Blanket
Wet tropic	1.74	0.15	5.2	yes
Irrigated	1.99	0.22	9.8	no
Rainfed	1.04	0.13	26	yes

C/N ratio soybean (15)

Figure 6. 1 Summary of the three experiments and land-use management of sugarcane-legume companion cropping systems.

In the three field experiments, soybean subtly enhanced sugarcane yield in several settings, and a pronounced positive effect occurred in N limiting conditions at the Rain-fed subtropical site when soybeans were planted early in the season into small sugarcane (~50 cm). Contrary, at the zero-N rain-fed treatment in the wet tropics, soybean did not improve sugarcane yield, possibly because legumes were planted late in the season (December) and into large sugarcane (>1 m). These results indicate that planting time is a major determinant if legumes benefit sugarcane yield (Figure 6.1).

Sugarcane yield was not penalised when grown with reduced N and legumes (an exception was reduced N+mung bean at the Wet tropics site, see below). We hypothesise that facilitative and competitive interactions were in balance. Nitrogen benefits from legumes would have been less obvious because the reduced N fertiliser rate mostly sufficiently supplies sugarcane. Next-step research should pay attention to studying the water relations of both crops as under Rain-fed and low-rainfall conditions companion crops may compete with sugarcane for water. Interestingly in the year with 50% below the long-term average rainfall at the Wet Tropics site, sugarcane yield is not impacted by legumes. At the Irrigated site the same about of water was

applied all treatments, and the reduced N+legumes treatment had a (statistically non-significant) trend towards higher sugarcane yield than the full N treatment (Figure 6.3), which requires further investigation. Whether this benefit is derived from BNF or other legume effects requires further investigation. Clearly, a better understanding of competition and facilitation is needed to dissect the interactions of sugarcane and legumes. Our study at three contrasting sites gives an early indications of potential problems (in our study herbivory by wildlife affected each site in one year), the need to test legume systems in N limiting conditions (which means reducing N fertiliser applications rates substantially over several years), and, as outlined above, the temporal effect: if legumes are planted too late, their benefits are likely to be low because they will be shaded out by sugarcane resulting in low input of N through BNF.

The glasshouse experiment (Chapter 5) showed that the N fertiliser rate strongly affects sugarcane and legume biomass production. Soybean benefitted sugarcane biomass in the absence of N fertiliser, possibly because breakdown and decomposition of soybean nodules and roots occurred during the 88 days of the experiment, supplying some N to sugarcane. This notion is in line with the field experiments, where a significant increase in the soluble inorganic soil N pool occurred in several instances the presence of soybean. We conclude that higher resolution of soil N dynamics will improve understanding of how legumes affect soil N pools and budget. Such resolution can be achieved by quantifying not just the static N pools, but also N fluxes. Microdialysis is a sophisticated tool for studying soil N fluxes and discerning small changes in soil N availability with strong temporal resultion (Buckley et al., 2016). Further, in addition to inorganic N, the low molecular weight organic N pool should quantify as a source of N for sugarcane (Brackin et al., 2015) and account for up to 60% of the soluble low molecular weight N pool in sugarcane soils (Holst et al., 2012).

The glasshouse experiment confirmed previous research that legume BNF is highest under low soil N availability and decreases with increasing N fertiliser. This change in N availability may well have been a factor in the field trials, where legumes benefitted sugarcane most with no N fertiliser application at the Rain-fed Subtropical site (but not the Dry Tropics site), and smaller or no benefits to sugarcane were apparent at higher fertiliser applications. In the glasshouse experiment, the presence of soybean decreased sugarcane biomass at low (but not at high or zero) fertiliser rates indicating that facilitation, competition and neutral effects occur depending on resource availability. A small but significant decrease in sugarcane yield was observed at the Wet Tropics site with reduced N+mung bean. Reasons for this are unknown, potentially

because BNF of mung bean was lower than soybean or because the competition was more pronounced. With sufficient N in glasshouse and field (reduced N treatments), we did not observe a competition between sugarcane and soybean. These results suggest that N application rates have to be tailored carefully to achieve a balance between maximising BNF and avoiding sugarcane yield penalties. In Australia, new government directives on N fertiliser use are being discussed because N pollution of the Great Barrier Reef lagoon is not being curbed as quickly as desirable. Some demand 'disruptive innovation' rather than the incremental improvements of the sugarcane farming system that have occurred over the past two decades. If farmers are limited in their N fertiliser use in the future, legume companion cropping may become of considerable importance to ensure N sufficiency with a lower N pollution footprint.

6.2 Effect of fertiliser rate and legume intercropping on N_2O emissions (Chapters 3 and 4)

N_2O emissions from companion cropped systems were overall below those of the full N rate and higher than those from sugarcane only with the same level of N (Figure 6.3). This effect of increased N_2O emissions with legume companion cropping was strongest where the sugarcane crop had the greatest yield benefit (Figure 6.2). At the Irrigated Dry Tropics site, in particular, the reduced N+soybean treatment and at the Rain-fed Subtropical site zero N+soybean treatment had greater N_2O emissions, suggesting that successful BNF by soybean is accompanied by N losses.

Peak N_2O emissions occurred at each location soon after N fertiliser application confirming that urea fertiliser is readily converted by soil bacteria to inorganic N and N_2O. The lowest N_2O emissions occurred where soil moisture was comparatively low at sites with low rainfall and well-drained soils. At the Rain-fed Subtropical site, considerable rainfall and high soil moisture content over the crop season generated the highest cumulative N_2O emissions. The main driver for N_2O emission was soil nitrate, contributing 64 and 81% of the variation in N_2O emissions in the Rain-fed Subtropical and Wet Tropical sites, respectively, followed by WFPS with 18%. At the Irrigated site only, the interaction of nitrate*WFPS contributed 63% of the variation in N_2O emissions. The high soluble N pool and aerobic soil conditions that dominated at the Rain-fed and Irrigated tropical sites with WFPS <60% mostly provided conditions where N_2O was generated *via* nitrification. In contrast, the Rain-fed Subtropical site had in addition to a high soluble N pool, high soil moisture contents of 60 to 90% WFPS and highest N_2O

emissions, most likely from denitrification. Avoiding the accumulation of inorganic N in soil using legumes has potential to reduce N_2O emissions.
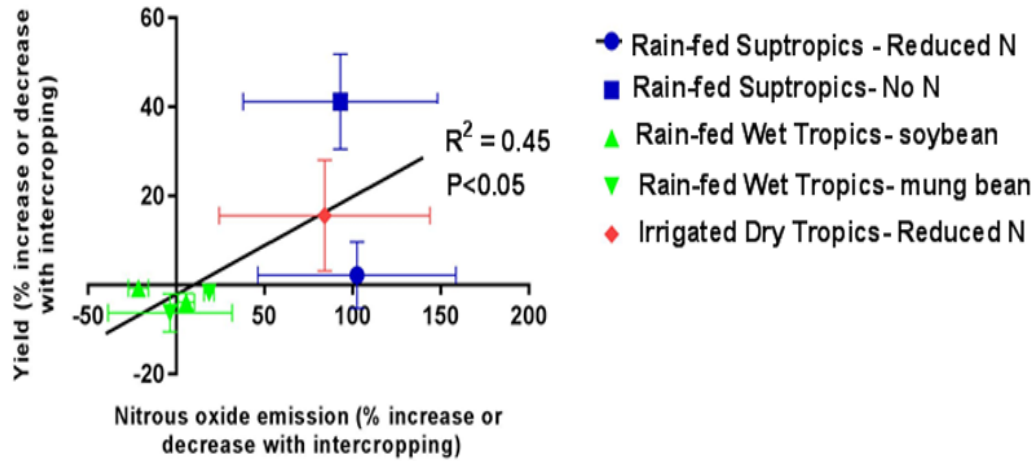
Figure 6. 2 Effects of legume companion crop on sugarcane yield (y-axis) and nitrous oxide emissions (x-axis). Both axes show percentage increase or decrease relative to the control treatment with the same fertiliser application without companion crop. Error bars showed the standard error of the mean from three field replicates.

Brackin et al. (2013) reported that sugarcane soils have high nitrification rates, converting N from fertiliser or soil organic matter, increasing nitrate concentration in the soil and therefore allowing N_2O emissions *via* denitrification. The findings in Chapters 3 and 4 provide some evidence that legumes BNF and the gradual addition of legume residues stimulated N_2O emissions. Some evidence exists that labile C (such as that in legume biomass) supplied alongside with N promotes N_2O production by stimulating soil microbial activity. It is possible that this was the reason here for generally higher N_2O emissions in treatments with legumes present versus the same N fertiliser rate without legumes, similar to previous studies (Senbayram et al., 2015, Dyer et al., 2012, Huang et al., 2014, Epie et al., 2015, Carter et al., 2014, Jensen et al., 2012, Pappa et al., 2012).

The zero-N treatment with soybeans at the Rain-fed Subtropical site had sugarcane yield increases of 41%, achieving a similar yield as full N fertiliser sugarcane with 83.3% lower N_2O emissions than the full N rate. This indicates that soybeans can facilitate sugarcane yield while reducing N_2O emissions. However, these findings also suggest that N derived from legume N-

fixation like N derived from chemical fertiliser has a risk of losses to the environment, similar to findings from previous studies examining N losses from legume-fertilised compared to synthetic-fertilised cropping systems (Robertson et al., 2000, Crews and Peoples, 2004). Other potential environmental benefits have not been quantified – we did not measure N losses via leaching and runoff, which are a larger N loss pathway than N_2O emissions (Zhou et al., 2012). A further consideration is that BNF has no pollution footprint compared to Haber-Bosch N fixation that has a high energy demand (approximately 2% of global energy use) in addition to emissions linked to transportation from manufacturing plant to the field (Crews and Peoples, 2004). Future studies should include life-cycle analyses to examine the whole pollution footprint of both cropping systems.

6.3 Future challenges and research directions

Sugarcane farming in Australia has caused changes in soil fertility, decreased soil organic carbon, altered soil microbial community structure and nutrient cycling (Brackin et al., 2017, 2013). Sugarcane-legume companion cropping has potential to be a sustainable alternative to current practices. Sugarcane would rely partialy on legume BNF and the soil's capability to recycle nutrients. This system has drawbacks as highlighted by this study, including the unsuccessful or partial establishment of legumes due to wildlife or adverse weather conditions preventing timely legume planting and growth.

As outlined above, legume inter/companion cropping can improved sugarcane yield but also have not measurable effect or even reduce yield. The exact reasons for this remain unclear, and we can only speculate about likely processes (Figure 6.3). Legumes were more successful at the Irrigated and Rain-fed Subtropical sites (Chapter 4), both of which had relatively high-water availability. Less or no competition for water may have been the reason for the benefits observed at the wetter sites, compared to the neutral or negative effects at the drier Wet Tropics site (Chapter 3).
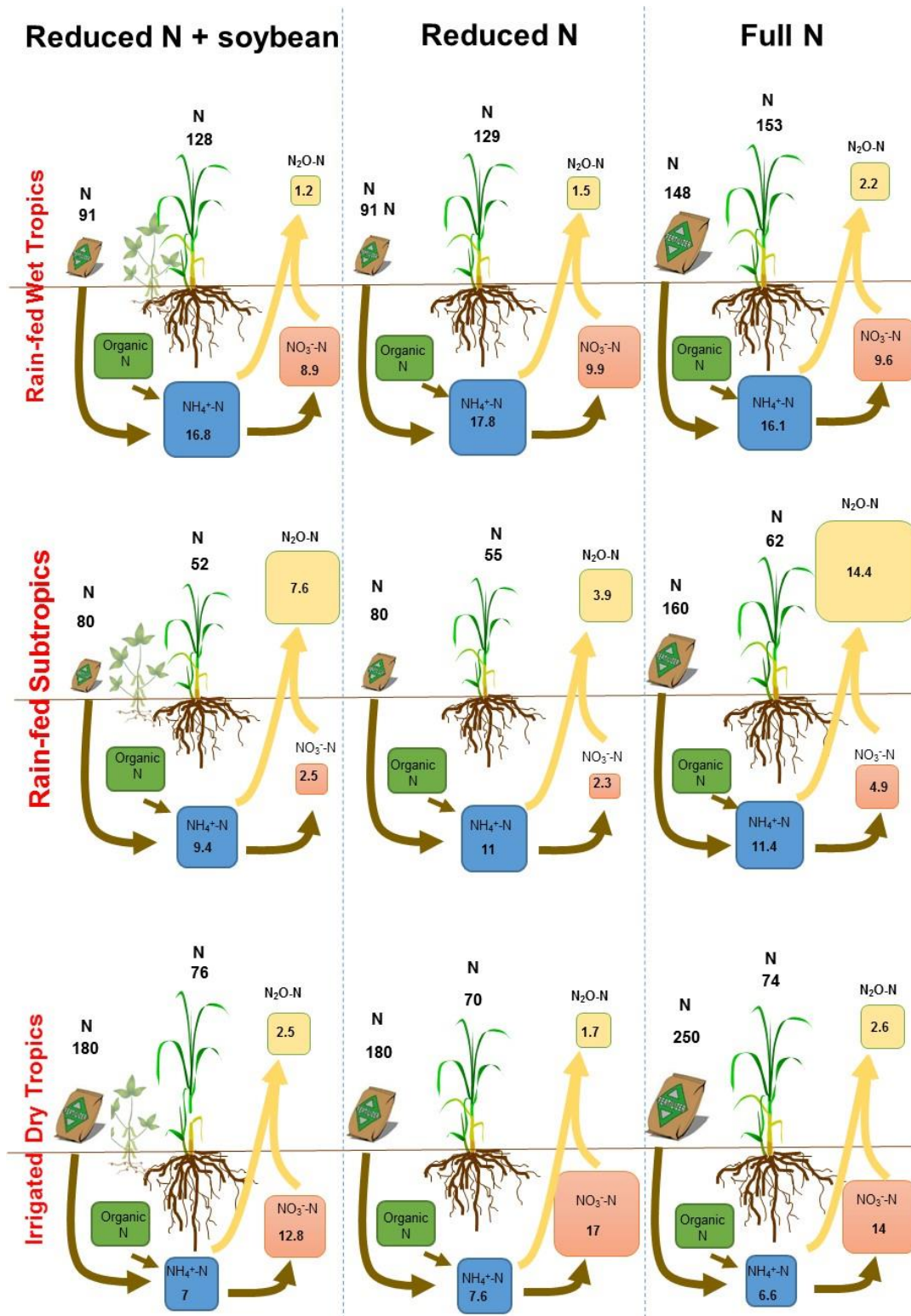
Figure 6. 3 Summary of field experimentation: reduced N+soybean (left); reduced N sugarcane (middle), and full N sugarcane (right). Soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) content in the soil is in mg kg^{-1} of soil. Pools soluble N are annual averages. N_2O are cumulative emissions (kg N_2O-N ha^{-1}) over the crop cycle. The amount of N acquisition for the crop (sugarcane shoots at harvest is shown) and the amount of N applied to the crops are in kg N per hectare.

Legume planting dates relative to the sugarcane varied at each site depending on local conditions – ranging from an early planting into small sugarcane at the irrigated site, to very late planting into tall sugarcane at the wet tropics site. At the rain-fed subtropical site, legumes were planted at an intermediate stage. Planting date potentially had a strong outcome on the system, and future trial research has to investigate legume planting dates and water regimes. Conditions of high-water availability and early legume planting date may maximise benefits from legumes as BNF can increase and legume decomposition occur earlier in the season, to better align with sugarcane N needs (Figure 6.1)

The legume species and cultivar is likely to be important but was not a focus of the investigations here. We chose soybean for all location because suitable soybean varieties adapted to local conditions and *Bradyrhizobium* inoculum were available. At the Wet Tropics site (Chapter 4), mung bean was planted in addition to soybean. Both legumes had a similar effect on N_2O emissions and sugarcane yield in the first year, but in the second-year sugarcane yield with soybean outperformed that of mung bean. Other intercropping studies have highlighted the importance of selecting suitable legume species and cultivars to maximise the potential for facilitation and minimise competition (Jones et al., 2007, Huang et al., 2014). Considerations should include the legume's chemical composition (e.g. polyphenol content, protein binding capacity of polyphenols, other) as these impact on N_2O emissions (Millar and Baggs, 2004). In a given soil and climate, the combination of total N, C/N ratio and chemical makeup of legume tissues will dictate the speed of breakdown and N release. It would be ideal if the timing of legume decomposition coincides with the N needs of sugarcane and if low molecular weight organic N or ammonium are the end products, rather than nitrate.

With a couple of exceptions where the presence of soybean temporarily increased the levels of inorganic soil N, legumes did not affect the inorganic N pools. Reasons include that N uptake and release processes were in equilibrium,decomposition from litter was gradual, mineralisation was slow, or that sugarcane uptake acquired legume N. A further possibility is that the resolution of soil N analysis was insufficient (see discussion above) to discern what may be subtle changes in the total soluble N pool and/or fluxes. Future studies should examine the patterns of litter deposition and N release.

The complex interactions between species may also influence the rhizosphere including microbial communities, which drive nutrient cycling as well as nitrification and denitrification

processes. Soil microorganisms from the domain archaea (encoding ammonia-oxidising genes, AOA) may produce lower N_2O emissions than ammonia-oxidising bacteria (AOB). AOA were dominant in sugarcane, pasture and vegetable soils (Bowatte et al., 2009, Liu et al., 2016, Paungfoo-Lonhienne et al., 2017, Hink et al., 2016). Thus, sugarcane-legume intercropping may have potential to mitigate N_2O emissions if the combination of both species fosters the presence of AOA, especially where nitrification is the main pathway for N_2O emissions.

Further research on a wide range of land uses and management practices could add information about how legumes affect soil physical, chemical and biological parameters. Like other organic amendments, the gradual contribution of dying legumes biomass will increase the flow of nutrients through decomposition. Future research should also examine the effect of sugarcane-legume intercropping on soil health. Soil health is a major issue within the Australian sugarcane industry, and legume rotation crops are considered one of the best management practices to mitigate the accumulation of soil pathogens (Brackin et al., 2017). Legume intercropping may have potential for improving soil biological health, and research has to explore this possibility.

The research completed here may be useful for sugarcane industries elsewhere. For example, the sugar industry in Ecuador (the PhD candidate's country) faces similar challenges as in Australia. The Ecuadorian sugarcane industry heavily relies on synthetic N fertiliser and the use has increased by up to 63% in the past decade (FAOSTAT, 2016) to 100 to 180 kg N ha^{-1}. Current practices include planting of velvet bean (*Mucuna pruriens*, the only available legume species) or rice (*Oriza sativa*) as rotation crops before planting the new sugarcane crop. Velvet bean was able to supply about 105 kg N ha^{-1} through aboveground biomass, reducing weeds and increasing beneficial native microorganisms in the soil (*Rhizobium* and *Gluconacetobacter* spp.) (CINCAE, 2017). Abiotic factors differ greatly between Australia and Ecuador, the main constraint to sugarcane production in Ecuador is limited sunlight due to high cloud cover. For example, the watershed of the Guayas River where the Ecuadorian sugarcane industry is located has a third of the sunlight per day (CINCAE, 2017) compared to the Australian industry (Bureau of Meteorology, 2016) . In our study, competition for light between the two crops was probably a minor factor (except after closure of the sugarcane canopy when desirable shading out of legumes occurred). Light limitation may be a bigger problem in Ecuador and research is needed to determine if, for example, early vigour of legumes can hinder sugarcane establishment. A current constraint in Ecuador is the availability of legume seeds, which would have to be addressed to expand from velvet bean to test legumes.

Overall, the insight generated from the current study provides foundations for sugarcane-legume companion cropping. If successfully implemented, sugarcane-legume systems may extend the lifespan of the sugarcane ratoon cycle, reduce N fertiliser needs, improve soil health, and, importantly, reduce N losses.

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Appendices

Appendix A – Supplementary Information (Chapter 3)

Table S3.1. Concentrations of soluble NO_3^- and NH_4^+ N averaged across seasons in the top 10 cm of soil ($\pm$ S.D, n=3), from sugarcane rows and inter-rows with different N fertiliser rates in sugarcane monoculture and sugarcane–legume companion cropping system at Abergowrie, North Queensland.

N (kg ha^{-1})	Spring	Summer	Spring	Summer
2014-2015				
sugarcane row	**NO_3^--N, mg kg^{-1} soil**		**NH_4^+-N, mg kg^{-1} soil**	
148	9.4[a] ± 6.4	11.0[a] ± 1.9	36.9[b] ±5.6	14.6[a] ± 3.6
91	7.7[a] ± 7.5	11.1[a] ± 2.6	42.4[ab] ±9.7	13.0[a] ± 5.4
91+soybean	6.6[a] ± 7.8	12.4[a] ±1.2	40.1[ab] ± 7.6	12.4[a] ± 1.7
91+mung bean	6.6[a] ± 2.6	9.8[a] ± 5.0	50.7[a] ±12.7	12.3[a] ± 1.2
0	0.4[b] ± 0.8	1.3[b] ± 0.3	3.3[c] ± 1.3	7.5[b] ± 2.5
sugarcane inter-row	1.2 ± 0.2	2.2 ± 0.7	2.4 ± 0.5	5.7 ± 1.4
2015-2016				
sugarcane row				
148	14.3[a] ± 11.3	18.8[a] ± 1.8	36.4[a] ± 4.7	17.8[a] ± 3.0
91	19.3[a] ± 11.4	18.8[a] ± 1.4	42.3[a] ± 5.1	18.3[a] ± 1.2
91+soy	8.8[a] ± 4.1	19.7[a] ± 2.6	36.9[a] ± 3.2	19.4[a] ±2.5
91+mung	12.5[a] ± 6.5	19.5[a] ± 3.1	38.8[a] ± 4.4	18.4[a] ± 5.1
0	0.3[b] ± 0.5	1.6[b] ± 0.5	15.6[b] ± 3.9	3.9[b] ± 0.4
sugarcane inter-row	2.1 ± 1.3	3.8 ± 0.8	5.8 ± 3.5	3.2 ± 0.8

Different letters in the column show differences at *P<0.05* level of significance between N fertiliser and legume treatments in sugarcane rows. Concentrations for sugarcane inter-rows are averaged across treatments as no significant differences were observed.

Appendix B – Supplementary Information (Chapter 4)

Table S4.1 Seasonal N_2O-N fluxes ($\pm$ S.D, n=3), from the sugarcane row and inter-row at different nitrogen rates in sugarcane as monocrop and sugarcane–soybean as intercropping system at Rain-fed site. 2014-2015 season.

Rain-fed site	Summer	Autumn	Winter-Spring
N fertiliser (kg N ha^{-1})	N_2O-N, mg m^{-2} h^{-1}		
sugarcane row			
160	$2.37^a \pm 1.14$	$0.21^a \pm 0.16$	0.01 ± 0.005
80	$0.68^b \pm 0.23$	$0.01^b \pm 0.00$	0.02 ± 0.02
80+soybean	$1.25^{bc} \pm 0.61$	$0.04^b \pm 0.03$	0.01 ± 0.00
0+soybean	$0.36^c \pm 0.41$	$0.02^b \pm 0.01$	0.01 ± 0.00
0	$0.08^d \pm 0.03$	$0.01^b \pm 0.00$	0.01 ± 0.00
sugarcane inter-row	0.036 ± 0.020	0.007 ± 0.005	0.008 ± 0.004
Irrigated site	Winter	Spring	Sumer-Autumn
N fertiliser (kg N ha^{-1})	N_2O-N, mg m^{-2} h^{-1}		
sugarcane row			
250	$0.56^a \pm 0.04$	$0.28^a \pm 0.04$	0.006 ± 0.003
180	$0.50^a \pm 0.03$	$0.25^a \pm 0.11$	0.008 ± 0.003
180+soybean	$0.29^b \pm 0.15$	$0.16^a \pm 0.13$	0.011 ± 0.003
0	$0.02^c \pm 0.01$	$0.01^b \pm 0.01$	0.001 ± 0.002
sugarcane inter-row	0.01 ± 0.008	0.01 ± 0.015	0.002 ± 0.003

Table S4.2 Seasonal NO_3-N and NH_4-N concentration in the soil ($\pm$ S.D, n=3), in the top 10 cm of soil profile, from the sugarcane row and inter-row at different nitrogen rates in sugarcane as monocrop and sugarcane– soybean as intercropping system at Rain-fed site. 2015-2016 season

N (kg N ha^{-1})	Sumer	Autumn	Winter-Spring	Sumer	Autumn	Winter-Spring
sugarcane row	NO_3^--N, mg kg^{-1} soil			NH_4^+-N, mg kg^{-1} soil		
160	$7.2a \pm 2.9$	$4.1^a \pm 4.0$	$0.1^a \pm 0.2$	5.8 ± 3.2	5.5 ± 6.3	1.4 ± 0.4
80	$6.5^a \pm 1.5$	$1.5^b \pm 4.2$	$0.4^a \pm 0.2$	4.8 ± 4.5	5.7 ± 5.3	1.2 ± 0.8
0	$1.7^b \pm 1.5$	$0.3^b \pm 0.1$	$0.4^b \pm 0.4$	6.4 ± 4.6	6.4 ± 7.2	1.5 ± 1.2
sugarcane inter-row	0.4 ± 0.5	0.3 ± 0.4	0.4 ± 0.2	3.0 ± 1.3	3.6 ± 2.3	1.8 ± 1.9

Different letters in the column show differences *at P<0.05* level of significance between N fertiliser and legume treatments in sugarcane rows. Concentrations for sugarcane inter-rows are averaged across treatments as no significant differences were observed.

Table S4.3 Seasonal N_2O-N fluxes (± S.D, n=3) from the sugarcane row and inter-row at different nitrogen rates in sugarcane as monocrop and sugarcane–soybean as intercropping system at Rain-fed site. 2015-2016 season.

	Sumer	**Autumn**	**Winter-Spring**
N (kg N ha^{-1})		N_2O-N, mg m^{-2} h^{-1}	
sugarcane row			
160	$0.91^a \pm 0.78$	$0.13^a \pm 0.14$	0.002 ± 0.002
80	$0.72^b \pm 0.58$	$0.04^b \pm 0.03$	0.004 ± 0.004
0	$0.14^c \pm 0.15$	$0.01^c \pm 0.01$	0.001 ± 0.002
sugarcane inter-row	0.08 ± 0.06	0.01 ± 0.01	0.003 ± 0.006

Table S4.4 Cumulative N_2O-N emissions and emission factors (EF) (±S.D, n=3) from the sugarcane rows and inter-rows at different nitrogen rates in sugarcane monoculture and sugarcane–legume companion cropping system at the Rain-fed site, 2014-2015 season.

2014-2015	Cumulative N_2O-N (kg ha^{-1})			
N kg ha^{-1}	row	inter-row	Total[2] (row+inter-row)	N_2O-N EF[3] (%)
160	$18.1^a \pm 10.4$	2.4 ± 1.8	$6.8^a \pm 3.6$	3.0
80	$11.9^b \pm 4.9$	4.3 ± 2.4	$5.4^b \pm 1.5$	4.3
80+Soy	$2.4^c \pm 2.1$	3.7 ± 2.3	$2.0^c \pm 1.3$	
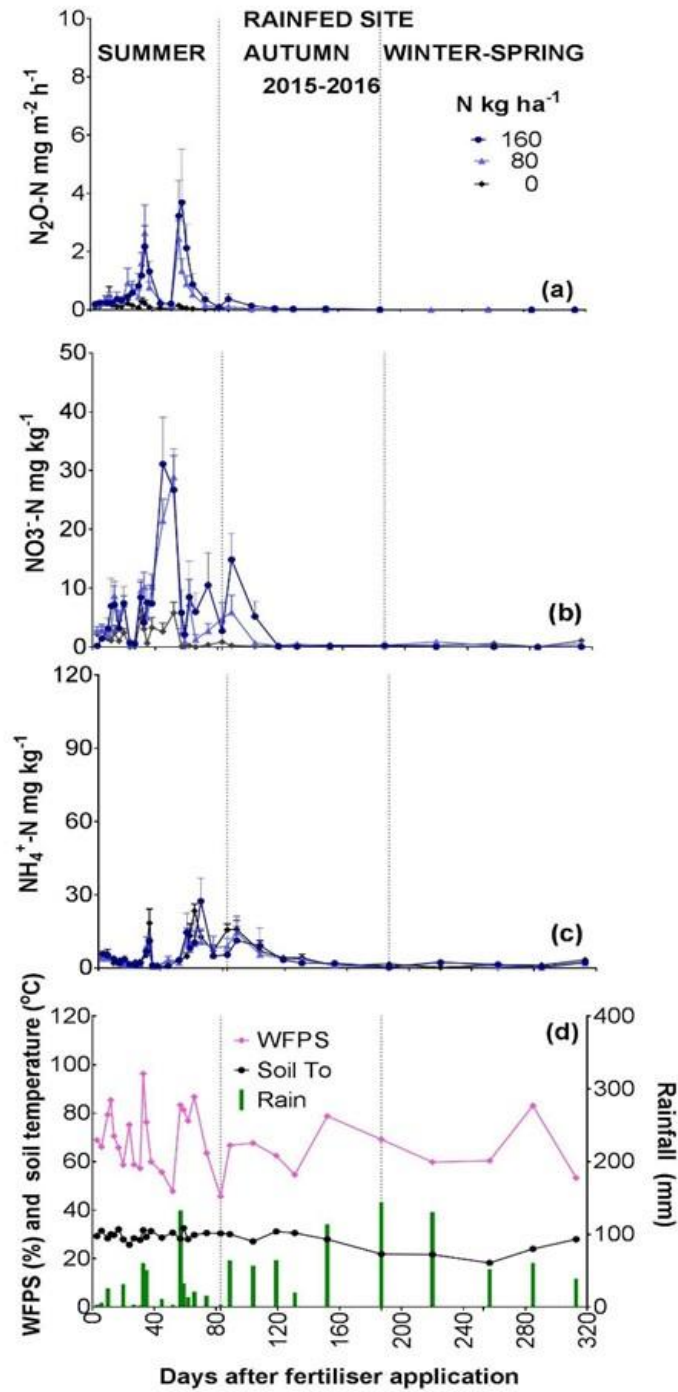
Figure S4.1 Seasonal patterns from sugarcane row of N_2O production (a), soluble soil NO_3-N (b), and NH_4^+-N (c), concentrations and weather patterns (d) (S.E, n=3) at different N fertiliser rates in sugarcane monoculture and sugarcane-legume companion cropping system in the Rain-fed site, 2015-16 season. Trends across the seasons of rainfall (mm), soil temperature (^{o}C) and water filled pore space (WFPS %) measured in the top 10 cm soil layer. Control was a sugarcane monoculture without N fertilisation (No N).

Figure S4.2. Tonnes of sugarcane per hectare (TCH) at different N rates as monoculture and grown with soybean. Data are means S.E, n=3) from sugarcane soil i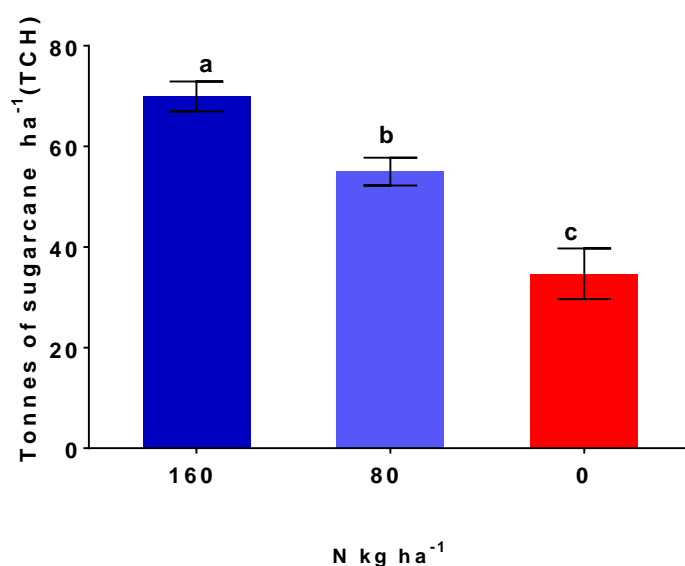n the Rain-fed site, 2015-16 season, Queensland. Different lowercase letters above bars indicate significant differences between treatments at *P< 0.05* (LSD, Fisher test).

Appendix C– Supplementary Information (Chapter 5)

Table S5.1. Averaged dry matter and N uptake of above and below ground of total shoot (sugarcane and soybean), roots, sugarcane and soybean, and sugarcane monoculture under fertiliser N application rates (S.E, n=5). Root biomass consisted of intermingled soybean and sugarcane roots.

N kg ha^{-1}	*Total Shoots	Roots	Intercropped sugarcane	Intercropped soybean	Total Shoots	Roots	Intercropped sugarcane	Intercropped soybean
	Dry matter				**N uptake**			
	g dry matter per pot[-1]				g N uptake per pot[-1]			
0	27.3 c	23.1 a	7.4 d	29.9 b	0.6 d	0.2 c	0.1 e	0.8 ab
1.1	86.8 b	55.0 a	55.9 c	46.2 a	1.0 c	0.4 b	0.5 b	0.8 a
2.2	97.1 a	64.9 ab	68.4 b	43.0 a	1.1 bc	0.4 b	0.7 c	0.7 b
3.3	97.2 a	73.7 b	79.3 a	28.2 b	1.2 ab	0.5 ab	0.9 b	0.5 c
4.3	98.1 a	74.8 c	83.5 a	20.5 c	1.3 a	0.5 a	1.0 a	0.4 c

Different letters in the column show differences at *P*<0.05 level of significance between N fertiliser and legume treatments (LSD, Fisher test). *Total shoots is the sum of sugarcane shoots + soybean shoots

Table S5.2. Averaged dry matter and N uptake above and below ground of total shoot (sugarcane and soybean), roots, intercropped sugarcane and soybean of three cropping systems. Root biomass was formed by intermingled soybean and sugarcane roots.

Cropping system	Dry matter			N uptake		
	*Total Shoots	Intercropped sugarcane	Intercropped soybean	Total shoots	Roots	Intercropped soybean
	g dry matter per pot[-1]			g N uptake per pot[-1]		
Sugarcane-nodulating soybean	94.5 [a]	63.0 [a]	40.0 [a]	1.5 [a]	0.5 [a]	0.9 [a]
Sugarcane-non-nodulating soybean	86.4 [b]	59.3 [ab]	27.1 [b]	1.0 [b]	0.4 [b]	0.4 [b]
Sugarcane	63.0 [c]	54.5 [b]		0.6 [c]	0.3 [b]	

Different letters in the column show differences at *P<0.05* level of the three cropping systems (LSD, Fisher test).
*Total shoots is the sum of sugarcane shoots + soybean shoots

Table S5.3. Averaged dry matter and N uptake above and below ground of total shoot (sugarcane and soybean), roots, intercropped sugarcane and soybean of the interaction cropping systems and N fertiliser rates. Root biomass was formed by intermingled soybean and sugarcane roots.

Crop	N g/pot[-1]	Dry matter				N uptake			
		*Total Shoots	Roots	Intercropped sugarcane	Intercropped soybean	Total Shoots	Roots	Intercropped sugarcane	Intercropped soybean
Sugarcane-nodulating soybean	0	53.0[f]	**30.6**	7.2 [e]	45.8 [ab]	1.5 [ab]	0.4	0.1	1.4 [a]
	1.1	97.9 [bc]	51.9	45.6 [ab]	52.3 [a]	1.6 [a]	0.5	0.4	1.2 [a]
	2.2	110.2[a]	57.8	60.4 [ab]	49.8 [a]	1.5 [ab]	0.5	0.7	0.9 [b]
	3.3	108.9 [ab]	71.3	78.8 [ab]	30.1[de]	1.4 [ab]	0.5	0.9	0.5 [a]
	4.3	102.3[ab]	71.8	80.6 [ab]	21.7 [fg]	1.5 [ab]	0.5	1.1	0.4 [a]
	Average	**94.5**	**56.7**	**54.5**	**40.0**	**1.5**	**0.5**	**0.6**	**0.9**
Sugarcane-non-nodulating soybean	0	23.6[g]	23.5	9.7 [e]	13.9 [g]	0.2 [f]	0.1	0.1	0.2 [a]
	1.1	87.6[cd]	61.8	47.5 [d]	40.1 [b]	0.9 [de]	0.4	0.4	0.4 [a]
	2.2	107.4 [ab]	65.7	71.1 [bc]	36.3 [fg]	1.2 [c]	0.4	0.7	0.5 [a]
	3.3	109.4 [ab]	69.0	83.1 [ab]	26.3 [ef]	1.4 [b]	0.5	1.0	0.4 [a]
	4.3	104.1 [ab]	69.4	84.9 [a]	19.2 [fg]	1.4 [b]	0.5	1.1	0.3 [a]
	Average	**86.4**	**57.9**	**59.3**	**27.2**	**1.0**	**0.4**	**0.6**	**0.4**
Sugarcane	0	5.2[g]	15.3	5.2 [e]		0.04 [g]	0.1	0.0	
	1.1	74.8[e]	51.5	74.8 [ab]		0.6 [f]	0.3	0.6	
	2.2	73.8[e]	71.2	73.8 [ab]		0.7 [ef]	0.4	0.7	
	3.3	76.1[de]	80.7	76.1 [ab]		0.8 [e]	0.4	0.8	
	4.3	85.1[de]	83.1	85.1 [a]		1.0 [cd]	0.5	1.0	
	Average	**63.0**	**60.3**	**63.0**		**0.6**	**0.3**	**0.6**	

Different letters in the column show differences at *P<0.05* level of the three cropping systems (LSD, Fisher test).
*Total shoots is the sum of sugarcane shoots + soybean shoots