N_2O EMISSIONS AND SOIL MINERAL N EFFECT FROM COTTON RESIDUES DURING FALLOW PHASE

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Statement of original authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

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N_2O EMISSIONS AND SOIL MINERAL N EFFECT FROM COTTON RESIDUES DURING FALLOW PHASE

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Abstract

The incorporation of crop residues post-harvest can provide an important source of nitrogen (N) for the subsequent crop but can simultaneously lead to elevated nitrous oxide (N_2O) emissions. The magnitude of N supply and corresponding N_2O emissions strongly depends on the quality of the added crop residues, and soil and climatic conditions. However, little data is available for subtropical cotton systems. The primary aims of this study were to (i) quantify carbon dioxide (CO_2) and N_2O emissions, and corresponding emission factors (EFs) following cotton residue incorporation, and (ii) determine the contribution cotton residues have to soil N content for the subsequent crop. Using a semi-automated sampling system, CO_2 and N_2O emissions were monitored during a cotton fallow period following cotton residue incorporation under varying rainfall conditions compared to residue removal. The high C/N of the incorporated residues, low temperatures and the limited C substrate resulted in low N_2O emissions. EFs were found to be 0.016 and 0.064% for average and high rainfall conditions, respectively which is significantly lower than the IPCC EF of 1%. The majority of decomposed residues were recovered in the soil of up to 68% with the remaining undecomposed and a very minimal amount was lost. There was a net mineralisation or loss/immobilisation observed across sites indicating soil conditions and management practices significantly influence N dynamics. The study suggests that the current IPCC methodology should be re-evaluated and that EFs need to be lowered to reflect the low N_2O emissions from high C/N cotton residue N inputs. It also shows that effect of crop residues on soil N dynamics needs to be considered in N fertiliser management strategies in order to maximize the benefits from crop residue incorporation.

Keywords

Nitrous oxide, mineralisation, cotton, crop residues, ^{15}N, emission factor

1.0 Introduction

Improving the efficiency of applied nitrogen (N) fertiliser is a key challenge in many agricultural systems, particularly cotton. Cotton is a highly valued commodity providing the textile industry with fibres used in many everyday products. Australia produces the most efficient cotton in the world (3-fold the world average) and is the third largest exporter behind the United States and India (CRDC, 2015). Cotton is a resource intensive crop requiring large quantities of N fertiliser to maintain high yield and quality. However, the application of N fertiliser poses significant environmental threats through the release of vast amounts of nitrous oxide (N_2O). N_2O is a potent greenhouse gas (GHG) implicated in stratospheric ozone depletion and global warming (Ravishankara et al., 2009). With an atmospheric residence time of 114 years, N_2O is 296 times more potent than carbon dioxide (CO_2) over a 100-year horizon (IPCC, 2006). N fertiliser application is the main source of anthropogenic N_2O emissions and with N fertiliser rates expected to increase over the coming years (FAO, 2015), global N_2O emissions will most likely follow. It is evident that improvement in fertiliser N use efficiency (NUE) is required to reduce the reliance on N fertiliser and associated N_2O emissions.

The incorporation of cotton residues post-harvest is a potential strategy to reduce N fertiliser rates by increasing soil N content. The mineralisation of the organic N bound within the residues can reduce the fertiliser N demand of the subsequent crop, and thus reduce fertiliser-induced N_2O emissions during the growing season. The incorporation of crop residues is mainly seen as a method to improve overall soil biological, physical and chemical characteristics (Lehtinen et al., 2014). However, crop residues contain large amounts of N and their contribution to soil N content is rarely considered in N fertiliser management strategies. As a consequence, soil N content can exceed crop needs and be prone to being lost as N_2O. Integrating this information into N fertiliser management strategies could lead to improved NUE in cotton systems (Roberts et al., 2015).

The incorporation of crop residues post-harvest also leads to elevated N_2O emissions which are vital to quantify for accurately estimating N_2O budgets and subsequently, development of mitigation strategies. Many studies have indicated that post-harvest N_2O emissions contribute a substantial amount of total N_2O emissions over the entire cropping season. For example, in vegetable cropping systems, Scheer et al. (2014) observed 70 to 90% of total N_2O emissions occurring across the season following broccoli residue incorporation and Baggs et al. (2003) observed 65% of total N_2O emissions occurring following lettuce residue incorporation. The decomposition of easily mineralisable N in low C/N residues can lead to increased soil mineral N content and thus, the potential for N to be lost as N_2O via nitrification and denitrification processes. However, N_2O emissions from residue incorporation are highly dependent on the soil and climatic conditions, and residue quality (size, C/N, moisture, composition) (Chen et al., 2013). Crop residues with increasing C/N have a negative effect on N_2O emissions. For instance, Velthof et al. (2002) observed no increase in N_2O emissions from the incorporation of barley straw residues (C/N– 69:1) in comparison to the control treatment of no residue incorporation. The IPCC currently estimate 1% of residue N will be lost as N_2O (IPCC, 2006). However, there is a general consensus that the IPCC default emission factor (EF) needs to be re-evaluated and specific to each crop residue (Velthof et al., 2002).

Currently, there is limited data available on N_2O emissions from residue incorporation during the cotton fallow in subtropical regions. Several studies have reported N_2O emissions during the cotton fallow, however, these were carried out either in semi-arid environments, laboratory experiments or residues were removed (Mahmood et al., 2008; Muhammad et al., 2011; Scheer et al., 2016). Additionally, to our knowledge, no study has reported the N contribution of cotton residues for the subsequent crop.

Therefore, the primary aims of this research were to (i) quantify CO_2 and N_2O fluxes, and corresponding EFs from a cotton fallow in sub-tropical Australia following residue incorporation, and (ii) determine the contribution cotton residue incorporation has to soil N content and thus, the potential for N fertiliser rates to be reduced for the subsequent crop.

Soil N_2O emissions and inorganic N concentrations were measured in a cotton fallow following residue incorporation and compared to residue removal. High intensity rainfall events were simulated each month to assess the influence climatic variation has on soil N_2O emissions and inorganic N dynamics over the fallow.

This is the first study to quantify N_2O emissions in a subtropical cotton fallow following residue incorporation and the contribution of residues to soil N for the subsequent crop using a ^{15}N tracer technique. The outcomes of this study will assist with defining local EFs for the cotton fallow under residue retention practices and N fertiliser management strategies throughout the growing season.

2.0 Methods

2.1 Study sites

The experimental field trials were conducted during the 2016 cotton fallow period, running from May to August/September. Two field sites used as part of this trial were situated within the Darling Downs region of Queensland, Australia, approximately 160-170km west of the state’s capital, Brisbane. The primary site was located just outside of Oakey (27˚28’ S, 151˚ 37’ E, 399m above mean sea level) on the Nass farm. The secondary site was situated near Pittsworth (27˚49’ S, 151˚30’ E, 393m above mean sea level) on the Clapham farm. The Darling Downs region is of subtropical conditions, exhibiting mild cool winter and hot humid summer months. Both field sites generally have dry winters (June-August) with monthly rainfall averaging 33mm in Oakey and 31mm in Pittsworth. Over the same period, mean daily minimum temperatures average 3.5˚ C and 7.1˚ C, and mean daily maximum temperatures are 19.3˚ C and 17.3˚ C in Oakey and Pittsworth, respectively (Bureau of Meteorology, 2016). The soil at both sites is classified as a black, self-mulching, heavy-clay vertosol according to the Australian Soil Classification (Isbell, 2002). Both farms were under conventional practices and the Pittsworth farm received an application of feedlot manure equivalent to 20t/ha at the beginning of the fallow. Soil physical and chemical characteristics at both sites are outlined in table 1.

Table 1: Main soil physical and chemical characteristics (0-10cm) of the experimental field sites at Oakey and Pittsworth, Queensland, Australia.

Soil Property (0-10 cm)	Oakey	Pittsworth
pH (H_2O)	7.8	7.4
Bulk Density (g/cm^3)	0.77	0.88
Total Nitrogen (%)	0.13	0.11
Total Carbon (%)	1.77	1.34
Texture (USDA)	Clay	Clay
Clay (%)	75	50
Silt (%)	13	20
Sand (%)	12	30

2.2 Experimental design

The field trial was set up in a randomised split-plot design with four replications of each treatment. The split-plot design contained two varying cotton residue and irrigation management strategies. Each plot was 50cm x 50cm, with a 50cm buffer between adjacent plots and an approximate 5m buffer between blocks. The four treatments included:

- Control (C) – i.e. no added residues
- Control + irrigation (C+I) – irrigation was applied monthly at a rate of 50mm
- Cotton residues (R)
- Cotton residues + irrigation (R+I) – irrigation was applied monthly at a rate of 50mm.

Treatments receiving irrigation (12.5L/plot) were designed to simulate a high intensity rainfall event. Irrigated treatments were watered individually with a watering can to achieve uniformity of application and to avoid irrigation of adjacent plots. Irrigation was done with bore water.

2.3 ^{15}N labelled cotton residues

Cotton (Gossypium *hirsutum* L. cv. Bollgard® II) residues used in this trial were harvested from a previous trial on the sites using urea fertiliser labelled with 5 atom % ^{15}N. The two sites had varying fertiliser N rates of 180 and 125 kg N/ha at the Oakey and Pittsworth site, respectively. The plant density at each site was approximately 10 plants per m^2 as recommended for Bollgard® II. Harvested cotton plants were cut close to the soil surface from ^{15}N enriched plots and subsequently, dried at 60 ° C for several days in an oven. Following drying, lint was separated from all cotton samples and discarded. The remaining plant samples were weighed for total biomass and a separate subsample was analysed for total C, N and ^{15}N atom % via isotope-ratio mass spectrometry (IRMS).

During the incorporation process, cotton residues were mixed into the soil with a hoe. Mixing was done carefully to achieve a uniform spread of the cotton residues within the plots. The cotton residues incorporated were cut prior similar to that experienced in the mulching process. To avoid differences in the amount of N added via the cotton residues, the same amount of cotton residues were added to each plot. The total amount of N added via the cotton residues equated to 68 kg N/ha at the Oakey site and 55.4 kg N/ha at the Pittsworth site. Additional chemical characteristics of the cotton residues are displayed in table 2.

Table 2: Chemical and physical properties of cotton residue biomass added (t/ha), total C and N (%), C/N, average atom% ^{15}N abundance, and total C and N (kg/ha) incorporated at the Oakey and Pittsworth sites, Queensland, Australia.

Plant Property	Oakey	Pittsworth
Biomass (t/ha)	5.55	4.82
Total C (%)	36.6	37.5
Total N (%)	1.2	1.1
C/N	29.8	32.6
Average ^{15}N abundance (%)	1.51 ± 0.13	1.37 ± 0.08
Total N added (kg/ha)	68	55.4
Total C added (kg/ha)	2030	1804

2.4 CO_2, N_2O and ^{15}N-N_2O measurements

Following cotton residue incorporation, a semi-automated GHG sampling system was set up at the Oakey site. The sampling system utilised a static closed chamber technique (non-steady state, non-through flow) to capture GHG samples from soil. The system involved extracting air within the headspace of a closed chamber into pre-evacuated 12mL Exetainer® tubes

(Labco Ltd, Buckinghamshire, UK) for further analysis. Twelve chambers were fixed on stainless steel frames inserted to a 10cm depth in the soil to minimise lateral gas diffusion and to provide an airtight enclosure with the soil. Each chamber had a length and width of 0.5m and a height of 0.15m, equating to a volume of 0.0375m^3.

A typical measurement cycle involved the extraction of gas samples three times over a 60 min closure time (0, 30 and 60 min). An electronic actuator installed allowed the lids to open and close automatically. Gas sampling always occurred between 9am-12pm to avoid diurnal variations and this time has shown to be the best approximation of mean daily N_2O emissions (Reeves & Wang, 2015). Gas samples were taken every second day throughout the trial period. All chambers were situated within 50m of the sampler unit and gas dilution was considered negligible. Following gas extraction, samples underwent gas chromatographic measurement for N_2O and CO_2 concentrations using a Shimadzu GC-2014 equipped with an electron capture detector (ECD) for N_2O and a thermal conductivity detector (TCD) for CO_2.

2.5 Soil and environmental parameters

The environmental parameters of soil temperature and moisture were measured at both sites. Soil moisture was measured gravimetrically and subsequently converted into volumetric water content and then water-filled pore space (WFPS). Soil temperature was recorded via external soil sensors connected to the GHG chamber system at the Oakey site and via a HOBO® soil temperature logger at the Pittsworth site. At each site, daily rainfall and minimum and maximum air temperature data was collected from the nearest weather station.

Soil sampling to a depth of 10cm occurred weekly within the first month and fortnightly in the proceeding months to determine soil inorganic N (NH_4^+ and NO_3^-) concentrations. Following collection, soil samples underwent mineral N extractions using a modified Carter and Gregorich (2008) method which briefly involved the agitation of 20g of fresh soil with 100mL of 2M potassium chloride (KCl) for 60 mins. The agitated samples were then allowed to settle and subsequently filtered with Whatman no. 42 filter papers. The extracted solution was then frozen until analysis by a Gallery™ Automated Photometric Analyzer (Thermo Fisher Scientific, California, USA). Furthermore, a total soil ^{15}N recovery was conducted at the conclusion of the experiment down to 50cm at four different depths (0-10, 10-20, 20-30 and 30-50cm). This was done by drying a soil subsample from each replicate and depth at 60 °

C. Subsequently, dried samples were ground to a fine powder for ^{15}N analysis via IRMS. Additionally, any undecomposed cotton residues at the end of the fallow period were collected, sieved to 2mm, ground to a fine power and analysed via IRMS for ^{15}N enrichment.

2.6 Statistical analysis and calculations

CO_2 and N_2O fluxes were calculated across the linear decrease or increase from 3 concentrations (T_0, T_1, T_2) collected during the chamber closure time. Fluxes were corrected for chamber temperature and atmospheric pressure as described in Scheer et al. (2014). Chamber temperature was collected from internal sensors in the chambers. All N_2O and CO_2 fluxes were quality checked using the coefficient of determination (R^2). Subsequently, T_2 samples for CO_2 fluxes were discarded to avoid the effect of CO_2 saturation in the GHG chambers. Cumulative emissions were calculated by summing the mean of all days across the cotton fallow period. Linear interpolation was used between sampling periods to fill any gaps in the dataset. EFs for residue-induced N_2O emissions were calculated using the following equation:

$$\mathrm{EF}(\%) = \frac{\text{Total } \mathrm{N_2O} \text{ flux (residue)} - \text{Total } \mathrm{N_2O} \text{ flux (control)}}{\text{Total N applied}} \times 100$$

^{15}N recovery was calculated as that described in Mitchell et al. (2016).

Statistical analyses were undertaken in R 3.1.1 (R Foundation for Statistical Computing, Vienna, AUT). A one-way analysis of variance (ANOVA) was used to determine whether cotton residue incorporation or removal had a significant influence on N_2O and CO_2 emissions, inorganic N concentrations, and ^{15}N recoveries. All analyses were deemed significantly different when $p \leq 0.05$. The *post-hoc* Tukey test was used to compare significant differences between treatments.

3.0 Results

3.1 Environmental and soil conditions

Over the cotton fallow period, a total of 106mm and 186mm of rainfall was recorded at the Oakey and Pittsworth sites, respectively. This rainfall was similar to the monthly average in both areas with the exception of June and August at Pittsworth which received double the monthly average. An additional 150mm was applied in three split applications to the irrigation treatments over the fallow period equating to 256mm at Oakey and 336mm at Pittsworth. The mean daily air temperature over the study period at Oakey was 12.7℃ with maximum daily temperature reaching 29.9℃ while minimum daily temperatures fell to -2.4℃. Similarly, Pittsworth's mean daily air temperature ranged from 0.4 to 27.9℃ with a mean of 12.7℃. Temperature in the surface soil (0-10cm) responded to air temperature and was approximately the mean of the maximum and minimum air temperature. Average soil temperature over the study period was 12℃ and 13.8℃ at Oakey and Pittsworth, respectively.

Topsoil WFPS (0-10cm) responded to rainfall and irrigation events. At Oakey, WFPS ranged between 23 and 54% (average 41%) in the C and R treatments while the C+I and R+I treatments ranged slightly higher between 23 and 58% (average 45%). WFPS was similar at Pittsworth ranging between 22 and 51% (average 34%) in the C and R treatments. The irrigation treatments were slightly higher peaking at 55% WFPS (average 36%). WFPS was highest at both sites following the first irrigation and rainfall event. The irrigated treatments at both sites were 1-9% higher in WFPS compared to non-irrigated treatments. The lowest WFPS was recorded at the beginning of the fallow period for both sites after the three prior months received well below average rainfall (figure 1).

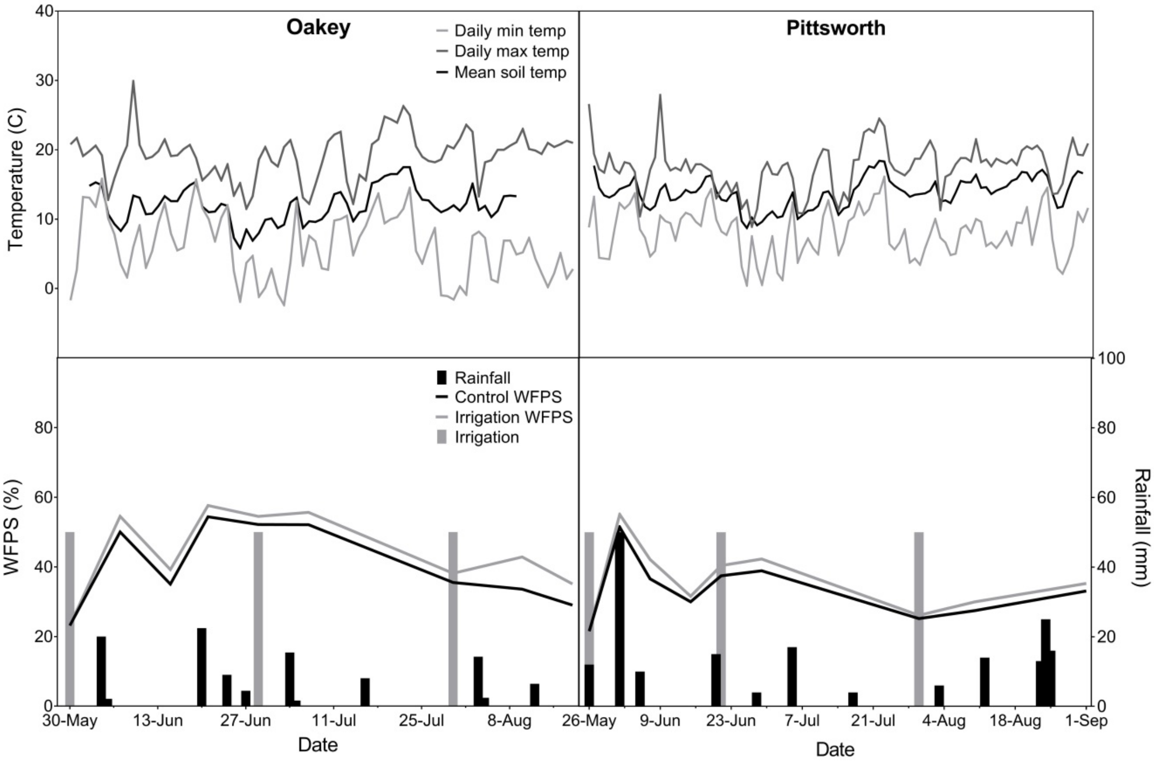

Figure 1: Mean daily maximum and minimum air temperature, mean soil temperature (0-10cm), rainfall, and topsoil WFPS (0-10cm) for irrigated and non-irrigated treatments over the cotton fallow period at the Oakey (left) and Pittsworth (right) experimental sites, Queensland, Australia.

3.2 CO_2 and N_2O emissions

Temporal N_2O fluxes measured over the cotton fallow period are depicted in figure 2a. The soil acted as a N_2O source and sink over the 74-day study period. Emissions ranged from -0.71 to 3.42 g N_2O-N ha^{-1} day^{-1} while averaging 0.33, 0.28, 0.52 and 0.87 g N_2O-N ha^{-1} day^{-1} for the C, C+I, R and R+I treatments, respectively. The highest N_2O flux was observed following maximum air temperatures of 30℃ on 5 June in the R+I treatment of 3.2 g N_2O-N ha^{-1} day^{-1} which was significantly higher than all other treatments ($p < 0.05$). Another N_2O peak was observed in the R+I treatment on 2 August of 1.2 g N_2O-N ha^{-1} day^{-1} following the third irrigation event, however, was not significantly higher than other treatments ($p > 0.05$). There were no other pronounced effects observed on N_2O from the incorporation of cotton residues and the application of irrigation with emissions predominately at background levels.

Cumulative N_2O emissions over the 2 month cotton fallow period were 24.5, 20.5, 35.1 and 63.7 g N_2O-N ha^{-1} for C, C+I, R and R+I, respectively. Corresponding EFs, corrected for background emissions, were 0.016% for R and 0.064% for R+I (table 3). The incorporation of cotton residues and the application of irrigation did not have a significant effect on cumulative N_2O emissions (table 3).

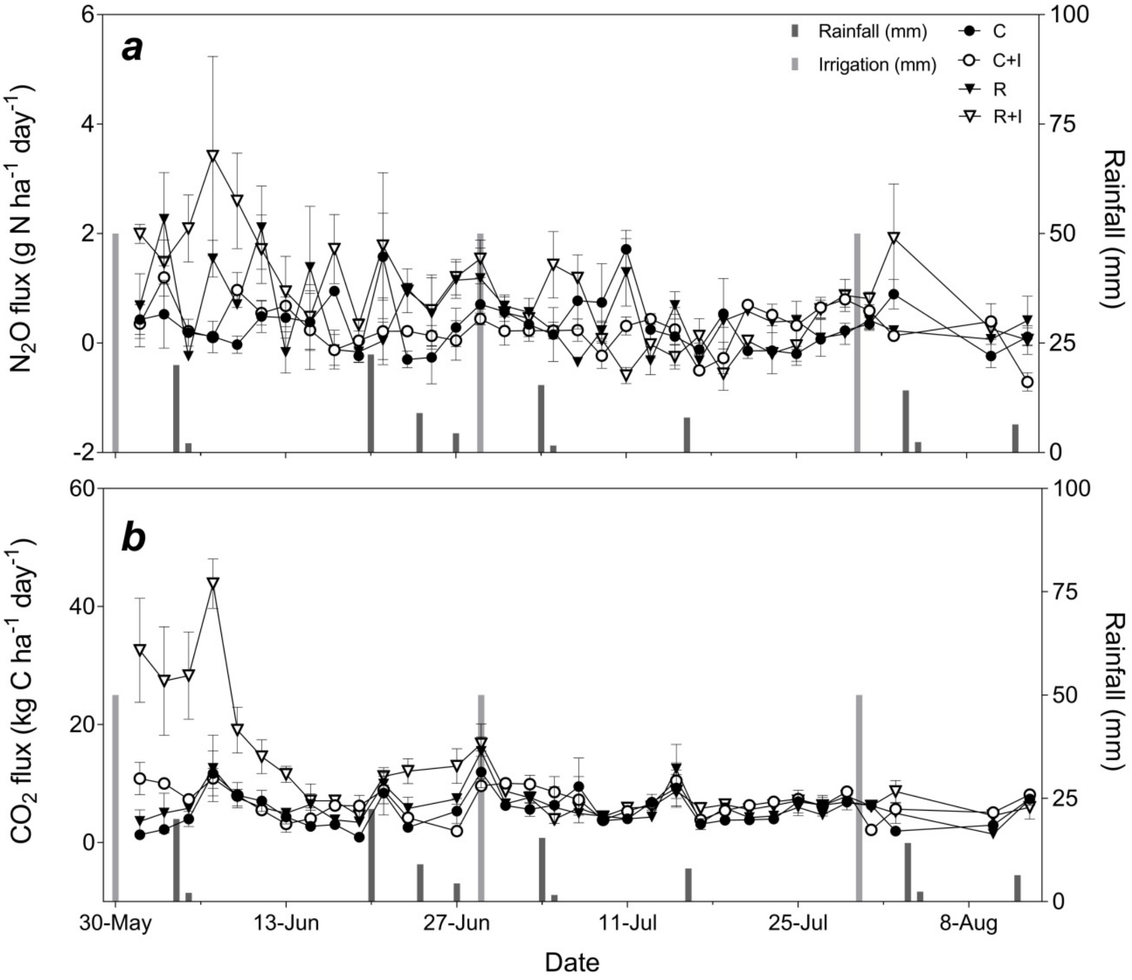

Figure 2: N_2O (*a*) and CO_2 (*b*) fluxes, and irrigation/rainfall for the four treatments over the cotton fallow period (30 May to 13 August, 2016) at Oakey, Queensland, Australia. Error bars indicate the standard error of the mean fluxes (n = 3).

In contrast to N_2O, there was a pronounced effect of rainfall, irrigation and soil temperature on CO_2 emissions (figure 2b). CO_2 emissions ranged from 0.9 to 43.9 kg C ha^{-1} day^{-1}. The daily average CO_2 flux was significantly higher in the R+I treatment (11.2 kg C ha^{-1} day^{-1})

than the C (5.4 kg C ha^{-1} day^{-1}), C+I (6.8 kg C ha^{-1} day^{-1}) and R treatments (6.2 kg C ha^{-1} day^{-1}) ($p < 0.05$). Soil CO_2 flux from R+I was significantly higher from 1 to 13 June compared to the other treatments reaching a peak of 44 kg C ha^{-1} day^{-1} on 7 June from high soil temperatures ($p < 0.05$). Approximately 43% of total C losses in the R+I treatment occurred within this period of significantly higher fluxes. Following 22mm of rainfall on 20 June, CO_2 emissions from R+I remained significantly higher for a week compared to all other treatments but declined to background emissions following the second irrigation ($p < 0.05$). In contrast to the first and second irrigation event, the third irrigation event had no effect on CO_2 emissions in C+I and R+I. There were no other pronounced differences observed between treatments. Overall, cumulative R+I CO_2 emissions were significantly higher than all other treatments at 800.3 kg C ha^{-1} (table 3). C+I had the second highest cumulative flux at 485.7 kg C ha^{-1}, followed by R and then C with 435.4 and 381.6 kg C ha^{-1}, respectively.

Table 3: Daily and cumulative CO_2 and N_2O emissions, corresponding EFs and total rainfall and irrigation for the four treatments over the cotton fallow period at Oakey, Queensland, Australia. Means with different letters indicate significant differences ($p <$ 0.05).

Measurements	**Treatment**			
	C	**C+I**	**R**	**R+I**
Average N_2O flux (g N_2O-N ha^{-1} day^{-1})	0.34 ± 0.06^a	0.28 ± 0.03^a	0.51 ± 0.06^a	0.85 ± 0.2^a
Cumulative N_2O emissions (g N_2O-N ha^{-1})	24.5 ± 5.7^a	20.5 ± 1.4^a	35.1 ± 4.1^a	63.7 ± 18.9^a
Emission factor (%)	-	-	0.016	0.064
Average CO_2 flux (kg C ha^{-1} day^{-1})	5.4 ± 0.8^a	6.82 ± 0.5^a	6.12 ± 0.7^a	11.4 ± 0.7^b
Cumulative CO_2 emissions (kg C ha^{-1})	381.6 ± 52.6^a	485.7 ± 34.8^a	435.4 ± 40^a	800.3 ± 36.8^b
Rain + irrigation (mm)	106	256	106	256

3.3 Soil inorganic N variability

Soil inorganic N concentrations (0-10cm) displayed great temporal variability over the cotton fallow at both the Oakey and Pittsworth sites (figure 3). At Oakey, NH_4^+ concentrations ranged between 4 to 14 kg N ha^{-1} and 4 to 20 kg N ha^{-1} for NO_3^-. An immediate decrease in NH_4^+ concentrations was observed following residue incorporation in the R and R+I treatments, however, was not apparent in the control treatments (figure 3a). NH_4^+

concentrations remained low in R and R+I for the entire fallow period except for small increase on 7 June. There was a sharp increase in NH_4^+ concentrations for C and C+I following 22mm of rainfall on 20 June reaching 11 kg N ha^{-1} in both treatments. Following the second irrigation event on 27 June, C and C+I NH_4^+ content gradually decreased to 6.3 and 6.7 kg N ha^{-1} similar to that observed in R (6.3 kg N ha^{-1}) and R+I (4.1 kg N ha^{-1}) at the end of the fallow. In terms of NO_3^-, concentrations displayed higher temporal variability compared to NH_4^+ (figure 3b). All treatments increased following the rainfall event on 4 June except in the C+I treatment which slightly decreased to 4 kg N ha^{-1}. NO_3^- reached a peak at the end of the fallow season for all treatments with the C having the highest NO_3^- content of 20 kg N ha^{-1}, followed by R+I (16 kg N ha^{-1}), R (15.5 kg N ha^{-1}) and C+I (11.2 kg N ha^{-1}). The C treatment was significantly higher at the end of the fallow than C+I ($p < 0.05$).

At Pittsworth, NH_4^+ concentrations ranged from 0.1 to 18 kg N ha^{-1} and NO_3^- ranged from 4 to 28 kg N ha^{-1} across all treatments. Similar to Oakey, the incorporation of the cotton residues immediately decreased the size of the NH_4^+ pool across the entire fallow period (figure 3c). Minimum NH_4^+ concentrations in the R and R+I treatments were 0.3 and 0.1 kg N ha^{-1}, respectively while C and C+I remained constant at approximately 10 kg N ha^{-1}. As a result, NH_4^+ levels for C (12.1 kg N ha^{-1}) and C+I (13 kg N ha^{-1}) were significantly higher than R (1.6 kg N ha^{-1}) and R+I (1.7 kg N ha^{-1}) at the end of the fallow ($p < 0.05$). In contrast, there was no significant effect of the residues on NO_3^- content over the fallow. NO_3^- content was highest at the start of the fallow but decreased in all treatments after the 50mm rainfall on 1 June. Concentrations increased over the next two consecutive weeks in all treatments but decreased again following 15mm of rain on 20 June. Final NO_3^- concentrations for C, C+I, R and R+I were similar at 11.4, 10.1, 10.3 and 8.3 kg N ha^{-1}, respectively.

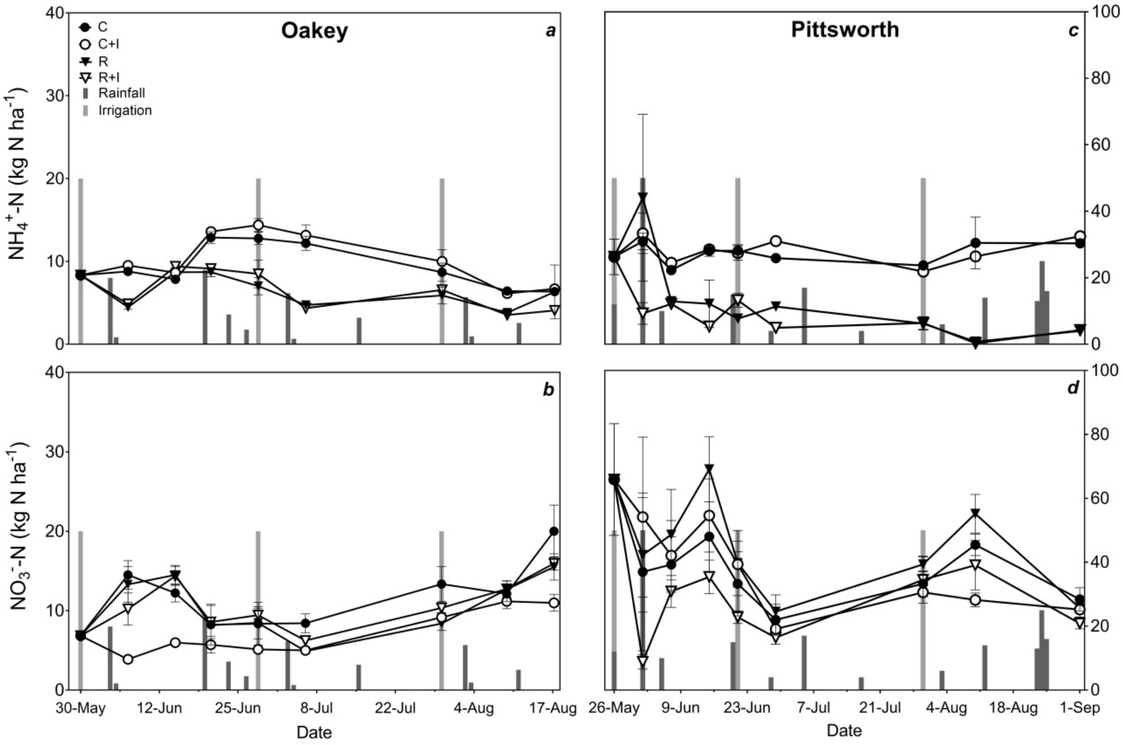

Figure 3: Soil inorganic N variability, NH_4^+ (top) and NO_3^- (bottom), in the topsoil (0-10cm) and irrigation/rainfall for the four treatments over the cotton fallow period at the Oakey and Pittsworth sites, Queensland, Australia. Error bars indicate the standard error of the mean (n = 4).

Total soil inorganic N content in the top 30cm over the fallow period displayed a net mineralisation or immobilisation/loss (figure 4). At Oakey, total NH_4^+ concentrations ranged between 12.6 and 23.1 kg N ha^{-1} (figure 4a). R+I was found to be significantly lower than the starting NH_4^+ concentrations and C+I at the end of the fallow ($p < 0.05$). In terms of total NO_3^-, there was an increase observed in all treatments at the end of the fallow compared to the start. C displayed the highest NO_3^- content at the end of the fallow with 70 kg N ha^{-1}, followed by R with 61 kg N ha^{-1}, R+I (50 kg N ha^{-1}) and C+I (46 kg N ha^{-1}). Total soil inorganic concentrations at the end of the fallow ranged from 63 to 90 kg N ha^{-1} across all treatments and were similar to the starting concentration of 66 kg N ha^{-1}.

At the Pittsworth site, NO_3^- concentrations at the end of the fallow were significantly lower than the NO_3^- content at the start of the fallow in both the C+I and R+I treatments ($p < 0.05$) but were not significantly different between treatments at the end of the fallow ($p > 0.05$)

(figure 4b). In terms of NH_4^+, concentrations were significantly higher in both the control treatments at the end of the fallow by more than double compared to the start of the fallow. However, there was a significant reduction observed in the residue incorporation treatments where NH_4^+ concentrations were 3.3 and 3.2 kg N ha^{-1} for treatments R and R+I, respectively. There was a significant reduction in the NH_4^+ pool in residue treatments down to 50cm in the soil profile compared to the control treatments ($p < 0.05$). Total soil inorganic N concentrations were 84, 71, 52 and 36 kg N ha^{-1} for treatments C, C+I, R and R+I, respectively which are lower than the total soil inorganic N at the start of the fallow of 112 kg N ha^{-1}. The incorporation of residues significantly decreased the total inorganic N content compared to the start of the fallow ($p < 0.05$).

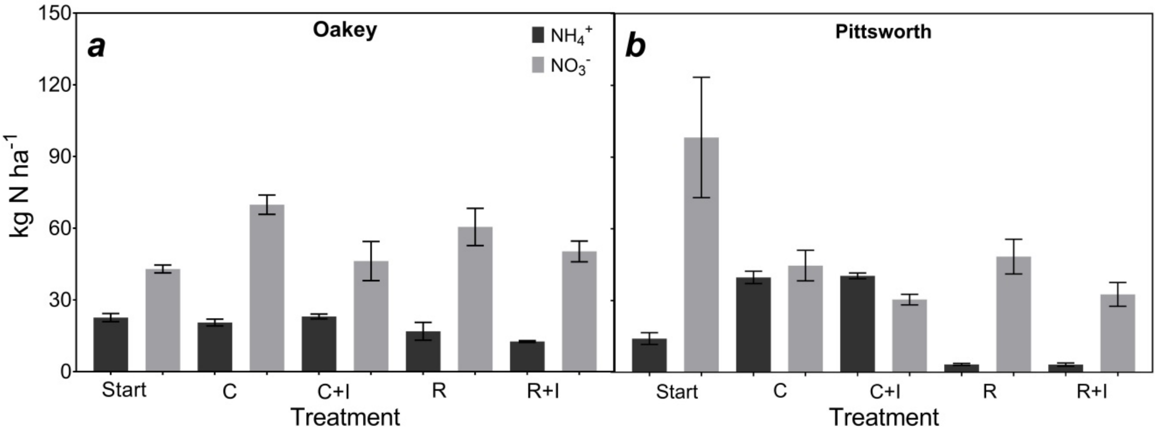

Figure 4: Total soil inorganic N concentrations (0-30cm) in the NH_4^+ and NO_3^- pool in the four treatments at the end of the fallow period compared to the start of the fallow period for the Oakey (*a*) and Pittsworth (*b*) sites, Queensland, Australia. Error bars indicate the standard error of the mean (n = 4).

3.4 ^{15}N recovery

Cumulative ^{15}N recovery by the soil at Pittsworth showed no significant effect from rainfall conditions (table 4). Total ^{15}N recoveries were 107 and 97% for treatments R and R+I, respectively. However, there was a substantially high variation observed in the recovery of 24 and 20% for the respective treatments. The greatest portion of the recovered ^{15}N was found in the soil pool ranging between 63% (35 kg N ha^{-1}) and 68% (38 kg N ha^{-1}) with approximately half recovered in the top 10cm. More than a third of the total ^{15}N was recovered in the undecomposed residues in both the R (39%) and R+I (33.9%) treatments. Corresponding losses were higher in the R+I treatment compared to the R treatment of 2.8% compared to nil. There were no significant differences found between treatments in any of the recovery pools. At Oakey, the ^{15}N recovery displayed substantial variability and as a consequence, the data is not shown.

Table 4: Fate of incorporated cotton residues as determined by ^{15}N isotope recovery over the cotton fallow period at Pittsworth, Queensland, Australia.

	Pittsworth			
	R		R+I	
	kg N ha^{-1}	%	kg N ha^{-1}	%
Residue added	55.4		55.4	
Undecomposed residue	21.6 ± 0.83	39	18.8 ± 0.5	33.9
Soil (0-10cm)	17.4 ± 7.9	31.4	20 ± 9	36.1
Soil (10-20cm)	7.4 ± 2.9	13.4	4 ± 0.5	7.2
Soil (20-30cm)	4.8 ± 1.1	8.7	3.8 ± 0.2	6.8
Soil (30-50cm)	8.1 ± 0.4	14.6	7.3 ± 0.9	13.2
Total soil ^{15}N recovery	37.7 ± 12.3	68.1 ± 22.2	35.1 ± 10.6	63.4 ± 19.1
Total ^{15}N recovery	59.3 ± 13.1	107 ± 23.6	53.9 ± 11.1	97.2 ± 20
Unaccounted ^{15}N (losses)	-	-	1.5 ± 11.1	2.8 ± 20

4.0 Discussion

4.1 CO_2 emissions

This is the first study to quantify N_2O emissions during a cotton fallow period coupled with CO_2 emissions in subtropical Australia under varying residue retention and rainfall conditions. Cumulative CO_2 emissions over the cotton fallow ranged from 381.6 kg C ha^{-1} to 800.3 kg C ha^{-1} and average daily CO_2 fluxes ranged between 0.9 and 43.9 kg C ha^{-1} day^{-1} (table 3). There is only one reported study by Mahmood et al. (2008) that coupled N_2O emissions with CO_2 emissions over the cotton fallow. In this study, Mahmood et al. (2008) observed average soil respiration rates of 29.1 kg C ha^{-1} day^{-1} while ranging between 16.4 and 35.5 kg C ha^{-1} day^{-1} using a dataset of 38 sampling events over a 6-month fallow. These values closely align with peak CO_2 fluxes observed in the R+I treatment, however, average daily CO_2 emissions were much higher compared to values found in this study of 6.1 kg C ha^{-}

^{1} day^{-1} and 11.4 kg C ha^{-1} day^{-1} in the R and R+I treatments, respectively. CO_2 emission pulses were regulated by variations in soil moisture and temperature. In all treatments, CO_2 fluxes increased following rainfall events within the first month. However, there was little to no increase in CO_2 fluxes later in the cotton fallow in any treatment indicating a limited C substrate. CO_2 fluxes were mainly controlled by soil temperature with strong relationships observed with higher temperatures. For example, peak CO_2 emissions occurred on 7 June when maximum daily temperatures reached 30℃. These relationships support the results of previous studies where C substrate, soil moisture and temperature regulate soil respiration rates (Han et al., 2007; Scheer et al., 2008).

There was a significant effect of cotton residue incorporation with high rainfall conditions on CO_2 emissions as significantly higher soil respiration rates occurred. CO_2 emissions were significantly higher than all other treatments during the first 2 weeks and decreased to similar values in the other treatments. It is known that the incorporation of organic material results in elevated soil respiration rates as a consequence of high microbial activity to decompose the material (Beare et al., 1994; Vargas et al., 2014). In this study, the decomposition of easily mineralisable C from the cotton leaves in the R+I treatment was apparent but was not in the R treatment indicating a lower soil moisture restricted microbial activity.

4.2 N_2O emissions and corresponding EFs

In terms of N_2O, several studies have quantified N_2O emissions under field conditions during the cotton fallow when residues were removed and incorporated (Mahmood et al., 2008; Muhammad et al., 2011; Scheer et al., 2016). However, to our knowledge, no study has yet to quantify cotton residue-induced N_2O emissions under subtropical field conditions in Australia. N_2O emissions in this study ranged from -0.71 to 3.42 g N_2O-N ha^{-1} day^{-1} with cumulative emissions between 20.5 and 63.7 g N_2O-N ha^{-1} over the cotton fallow period. Higher N_2O emissions were observed in the R and R+I treatments compared to C and C+I. Increases in N_2O fluxes following crop residue incorporation has been frequently observed (Baggs et al., 2003; De Antoni Migliorati et al., 2015; De Rosa et al., 2016; Scheer et al., 2014). The emissions observed in this study are at the lower end of other reported fallow periods with residue incorporation, although limited data is available for the cotton fallow. Scheer et al. (2016) reported post-harvest emissions averaging 54.4g N_2O-N ha^{-1} over 60 days with cotton residues removed. In contrast, a study by Muhammad et al. (2011) observed

N_2O emissions of 240g N_2O-N ha^{-1} in an 84-day incubation study. Higher cotton residue-induced emissions were observed by Mahmood et al. (2008) of 648g N_2O-N ha^{-1} over a 6-month fallow under semi-arid subtropical conditions.

The comparatively lower emissions observed in this study could be due to the lower soil temperatures, extended dry period prior to residue incorporation, the limited C substrate and the size of the cotton residues. The surface area of organic material available to microbes is known to have a significant effect on the mineralisation rate and thus, N_2O emissions (Ambus et al., 2001). In the current study, cotton residues were large ranging from 5 to 10cm in length. Consequently, there was a negative effect on the mineralisation rate. Additionally, a limited C substrate reduces the chances of soil anaerobic microsites occurring which stimulates denitrification processes. In this study, soil respiration rates were low indicating low microbial activity. Furthermore, nitrification was dominant (average 40% WFPS) and with the majority of the NH_4^+ pool immobilised, N substrate was limited to nitrifying bacteria resulting in low N_2O losses. These findings are supported in the literature where soil temperature and the NH_4^+ pool regulate N_2O emissions (Skiba et al., 1998). Moreover, the cumulative N_2O emissions in this study may have been significantly lower than other reported studies due to the correction of background emissions.

Corresponding EFs in this study were found to be 0.016% for residue incorporation under average rainfall conditions and 0.064% for residue incorporation under above average rainfall conditions (table 3). The EFs found in this study are much lower than the IPCC default EF of 1% indicating a significant overestimation of N_2O from cotton residues under the current IPCC methodology. However, it aligns more closely with the study by Muhammad et al. (2011) where an EF of 0.17% has been reported. In a meta-analysis by Chen et al. (2013), EFs for crop residues can range anywhere from 0.62 to 2.8%. As a result, there is a general consensus that EFs need to be crop specific due to the variation in N content and the C/N (Velthof et al., 2002). This study clearly illustrates that crop specific EFs are required to accurately estimate N_2O emissions derived from cotton residue incorporation. The data of this study suggests that the current IPCC methodology should be re-evaluated and that EFs need to be lowered to reflect the low N_2O emissions from high C/N cotton residue N inputs.

4.3 Effect of residue retention and rainfall conditions on soil N dynamics

To date, there is no research on the effect cotton residue incorporation has on soil N dynamics during the fallow. This is the first study to investigate the mineralisation of cotton residues under varying rainfall conditions in subtropical regions. At both sites, the incorporation of cotton residues resulted in the immobilisation of NH_4^+ across the season. The consumption of N by soil microbes is common when the C/N content of the applied organic material is high, typically >25:1 (Congreves & Van Eerd, 2015). In the current study, cotton residues at Oakey had a C/N of 30:1 and 33:1 at Pittsworth. Immobilisation following the incorporation of high C/N residues/amendments has been observed in other studies (Huang et al., 2004; Pimentel et al., 2015).

Immobilisation in this study was clearly pronounced in the NH_4^+ pool more so than the NO_3^- pool. It has been noted that NH_4^+ is the preferred N source to be immobilised compared to NO_3^- by heterotrophic bacteria (Azam et al., 1993). Recous et al. (1990) hypothesised that the preference to immobilise NH_4^+ could be due to either few microbes capable of assimilating NO_3^-, NO_3^- assimilation by microbes is inhibited when both NH_4^+ and NO_3^- is present in the soil, or the amount of C required to immobilise NO_3^- is too high. Furthermore, NH_4^+ was found to be immobilised down to 50cm but only at the Pittsworth site. The higher C/N of the cotton residues at the Pittsworth site is thought to be responsible for the variation across sites. However, it is unknown why immobilisation occurred this deep in the soil profile but could be due to the leaching of water soluble organic C from the residues. Pittsworth had a higher sand and lower clay content compared to Oakey and therefore, a greater leaching potential.

Overall, there was a net loss/immobilisation of soil inorganic N over the fallow period at the Pittsworth site (figure 4b). The application of feedlot manure at the beginning of the fallow resulted in high NO_3^- concentrations. There was an immediate decrease in NO_3^- concentrations following the first irrigation and rainfall event of 50mm indicating the majority of NO_3^- was lost via denitrification processes as N_2O and dinitrogen (N_2) or via leaching.

In contrast, there was a total net N mineralisation observed at the Oakey site in all treatments (figure 4a). At both sites, NO_3^- concentrations were consistently lower in the high rainfall treatments indicating net losses over the season via denitrification or leaching processes.

There was a substantial difference of 150mm of rainfall between the high rainfall treatments compared to average rainfall conditions. As a result, ideal conditions for N loss via denitrification or leaching were created.

This study indicates that unless a winter crop is planted to assimilate the available N from the feedlot manure, the majority may be lost to the environment. However, the practice of incorporating residues can prevent N loss by immobilising the N pool.

4.4 Influence of residue retention and rainfall conditions on ^{15}N recovery

This is the first study to investigate the influence of residue retention and rainfall conditions using a ^{15}N tracer technique. Of the incorporated cotton residues at Pittsworth, a large portion of up to 39% was found undecomposed at the end of the fallow. The recovered residues were predominately stems which contain a high concentration of lignin. Lignin is highly resistant to decomposition due to its complex nature. However, the majority of residues were found in the SOM pool down to 50cm. As there was no apparent increase in soil inorganic N content, the residues recovered in the soil pool were mostly likely part of the particulate organic matter (POM) fraction or immobilised by microbes. The POM fraction would have comprised of partially decomposed cotton residues between 0.053 and 2mm in size, most likely derived from the leaves. However, this would probably be confined to the top 20cm and the deeper depths would be from the leaching of dissolved organic N. In terms of unaccounted N losses, they were found to be minimal. However, based on the cumulative N_2O losses at Oakey, very minimal losses would be expected at Pittsworth.

At Oakey, the ^{15}N recovery displayed great variability and the data was not included in this study. A heterogeneous distribution of ^{15}N in the cotton plant may have resulted in the high recovery and variability observed. This heterogeneous distribution needs to be considered in further ^{15}N studies. The cotton residues in this study also had a low ^{15}N enrichment (1.15 to 2.04 ^{15}N atom %), which increases the detection limit of residue derived N and thus, may have emphasised the data variability.

5.0 Conclusion

To our knowledge, this is the first study to quantify N_2O and CO_2 emissions, and cotton residue soil N contribution during the fallow period using ^{15}N tracers. Such data is crucial to accurately estimate N_2O budgets and improve NUE in cotton systems. N_2O emissions were low with cumulative losses between 20.5 and 63.7 g N_2O-N ha^{-1} over the cotton fallow. Cotton residue EFs were found to be 0.016 and 0.064% for average and high rainfall conditions which are much lower than the IPCC's default EF of 1%. The study suggests that the current IPCC methodology should be re-evaluated and that EFs need to be lowered to reflect the low N_2O emissions from high C/N cotton residue N inputs. It also shows that effect of crop residues on soil N dynamics needs to be considered in N fertiliser management strategies in order to maximize the benefits from crop residues incorporation. Further long term studies are needed investigating the mineralisation potential of cotton residues over the subsequent growing season to improve NUE in cotton systems.

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