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NITROGEN MINERALISATION OF LEGUME RESIDUES

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Abstract

Legume break cropping is an important part of the improved sugarcane cropping system, with the potential to supply fixed nitrogen (N) to the succeeding cane crop. However, the effectiveness of a legume crop in achieving this outcome is directly related to the rate of mineralisation of the legume residues. Therefore practices that can potentially manipulate the rate of mineralisation of legume residues are required. A 200 day incubation study was undertaken to: 1) compare the carbon (C) and N mineralisation dynamics of three different legume residues, namely a soybean at maturity, a soybean sprayed out early (green manure) and a peanut at maturity; 2) compare the C and N mineralisation dynamics of three legume management practices, namely surface applied legume residue, incorporated legume residues and legume residues incorporated with a nitrification inhibitor and; 3) examine the effects of legume type and management on nitrous oxide (N_2O) fluxes. The incubation was undertaken on three soils from Bundaberg, Ayr and Ingham, at 50% water holding capacity and 25°C. Mature soybean exhibited net N mineralisation throughout the 200 days, while mature peanut and green manure soybean had initial net N immobilisation followed by net mineralisation. Mature soybean mineralised the largest (80.9 mg N/kg soil) amount of N, followed by green manure soybean (47.6 mg N/kg soil) and mature peanut (30.1 mg N/kg soil). This was due to the lower C:N ratio of the mature soybean, compared with other legumes. On average, incorporated residues mineralised 192% more N than unincorporated residues, while incorporated residues released 324% more N_2O than unincorporated residues. The application of a nitrification inhibitor to the legume residues, was effective at reducing nitrification for 10 days in Ayr and Ingham soils, and up to 30 days in the Bundaberg soil, due to its higher sand content. However, the nitrification inhibitor was only effective at significantly reducing cumulative N_2O flux from mature soybean. This study has shown that the management of residues can manipulate their N mineralisation dynamics, and if used in the appropriate situation could help to maximise legume N supplied to the succeeding plant cane crop.

Introduction

Legume break cropping is an important part of the improved sugarcane cropping system, providing improved soil health and the potential to supply fixed nitrogen to the succeeding cane crop. However, the effectiveness of a legume crop in achieving this outcome is directly related to the rate of mineralisation of the legume residues. If the rate of residue mineralisation is too rapid, the resulting mineral nitrogen may be lost via leaching and/or denitrification prior to the plant cane establishment. Conversely if the rate of mineralisation is too slow the cane crop may not receive the quantity of N required for successful establishment. Therefore practices that can potentially manipulate the rate of mineralisation of legume residues are required.

The mineralisation rate is affected by environmental factors such as rainfall and temperature, and other manipulable factors such as legume type and management. By altering the legume species and/or the management of the resulting legume residues, the release of N may be better synchronised to crop demand.

The aims of this laboratory study were to 1) compare the carbon and nitrogen mineralisation dynamics of three different legume residues, namely a soybean at maturity, a soybean sprayed out early and a peanut at maturity; 2) compare the carbon and nitrogen mineralisation dynamics of three legume management practices, namely surface applied legume residue, incorporated legume residues and legume residues incorporated with a nitrification inhibitor and; 3) examine the effects of legume type and management on nitrous oxide (N_2O) fluxes.

Method

Legume Residue and soil preparation

Above ground components of three legumes were collected from two locations in Queensland, Australia. Mature soybean (*Glycine max*) samples were collected immediately after grain harvest and consisted of the leaves, stems and empty pods. Green manured soybean samples were harvested at early pod development and consisted of the leaves, stems and pods. Peanut (*Arachis hypogaea*) samples were harvested at maturity and consisted of leaves and stems. The collected legumes were dried at 60^0C until constant weight, then cut into 10-20 mm pieces. A sub sample was ground for total carbon (TC) and total nitrogen (TN). Properties of the legumes are given in Table 1.

Table 1-Chemical properties of legume residues

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

Bulk soil samples were collected to a depth of 0-10 cm from multiple points of three sugarcane fields at Bundaberg (S 25°01’31.83” E 152°22’47.67”), Ingham (S 18°41'55.5" E 146°00'58.8") and Ayr (S 19°48'03.67", E 147°9’59.38”) in October 2016. Field bulk density was 1.25, 1.13 and 1.11 g/cm^3, and water holding capacity (WHC) was 0.31, 0.49 and 0.61 cm^3/cm^3 for the soils from Bundaberg, Ingham and Ayr, respectively. The field moist samples were passed through a 4 mm sieve, thoroughly mixed and stored at 4^0C until the start of the pre-incubation. Properties of the soils are given in Table 2.

Table 2-Physio-chemical properties of soils

Site	pH	NH_4^+ mg/kg	NO_3^- mg/kg	Total C %	Total N %	C:N ratio	Particle size analysis Sand %	Silt %	Clay %
Bundaberg	5.7	1.5	28.6	0.87	0.06	14.5	78	12	10
Ingham	5.4	2.0	31.9	1.06	0.09	11.8	44	26	30
Ayr	7.3	0.1	8.2	1.03	0.08	12.9	33	21	46

Incubation setup

Field moist soils equivalent to 81, 82 and 91 g of dry matter, for Ayr, Ingham and Bundaberg soils respectively, were weighed into 120 ml polythene jars (height: 107 mm; diameter 44 mm). The weighed soils were pre-incubated for 3 days at 25°C with a punctured lid on to minimise moisture loss and maintain aeration. Soil moisture was determined gravimetrically at 105°C, to enable calculation of water required to reach 50% WHC.

After the pre-incubation period, ten treatments were applied to each soil (Table 3). Treatments with residue addition received 0.83 g of crop residue per jar, equivalent to 4 060-4 558, 4 037-4 532, 3 983-4 470 mg biomass C/kg soil and 232-261, 184-206, 151-169 mg biomass N/kg soil for the mature soybean, green manure soybean and mature peanut residues, respectively. This application rate of crop residues was equivalent to dry matter of 4 t/ha applied on cropping beds that usually account for about 70% of a cane field. For the unincorporated treatments, the pre-incubated soil was mixed and packed in jars to field bulk density, water was added using a pipette to reach 50% WHC, then legume residues were applied to the surface of the soil. Soil samples for the incorporated treatments were transferred from the jars into bowls, where the residue was added and mixed with the soil, before being added back into the jar, to ensure the residues were evenly distributed. Deionised (DI) water was then added to reach 50% WHC. The legume residues used in the Incorporated+ nitrification inhibitor (3 4-dimethylpyrazole glycolate or DMPG) treatment were sprayed with the nitrification inhibitor equivalent to 3.2 L/ha, and then allowed to dry before being mixed into the soil and watered to 50% WHC. The control treatment had only DI water applied to reach 50% WHC. All soil jars were placed in the dark in an incubator at 25°C. Soil moisture was adjusted by weight difference, at least twice per week, to maintain the soil moisture at 50% WHC.

Table 3-Incubation Treatments

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

where, MR=mature biomass, GM=green biomass

Calculation of carbon and nitrogen mineralisation

Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were determined on day 0, 10, 30, 60, 100, 150 and 200. Each jar was destructively sampled and moist soil was extracted in a 1:5 ratio with 2 *M* KCl. NH_4^+ and NO_3^- concentrations were determined using colorimetric techniques (Rayment and Lyons, 2011). Net N mineralisation from soil and crop residues was calculated as the change in inorganic N ($NH_4^+ + NO_3^-$) content from time zero to each sampling day. Net N mineralisation from legume residues was calculated as the difference in inorganic N between the legume residue-applied treatments and the control treatment on a particular sampling day.

Carbon mineralisation, as CO_2-C respired, and N_2O flux were measured on a subset of jars at day 1, 2, 4, 6, 8, 11, 15, 23, 32, 45, 58, 73 and then every 28 days thereafter. Glass jars (1.5 L) with a rubber seal and septum were sequentially flushed with compressed air for 30 seconds, then a soil jar was placed into each jar and the lid closed. Background air was sampled before closure and used as concentration at time zero. After five to seven hours closure, a 26 ml sample was extracted from the jar headspace using a syringe, and injected into 12 ml evacuated vials (Exetainer; Labco, UK). Samples were analysed for N_2O and CO_2 concentrations on a gas chromatograph, equipped with an ECD and FID with methaniser (GC 2010, Shimadzu Co., Kyoto, Japan). Standards of 500, 1 000, 2 000 and 4 000 µL/L for CO_2 and 0.5, 5.0, 12.0 and 20.0 µL/L for N_2O (BOC Ltd, Sydney, Australia) were used to calibrate the gas chromatograph. The values for days between sampling days were estimated by linear interpolation. Cumulative C mineralisation and N_2O flux were calculated by summing the daily measurements. Carbon mineralisation attributed to residue addition was calculated as the difference between CO_2-C mineralised in the residue treatment minus the CO_2-C in the control treatment at a given sampling time. Similarly, N_2O flux attributed to residue addition was calculated as the difference between N_2O-N flux in the residue treatment minus the N_2O-N flux in the control treatment at a given sampling time.

Statistics

Results were compared by analysis of variance (ANOVA), using Genstat Version 16. Data were tested for normality using Shapiro Wilks. Where normality could not be satisfied, the data were transformed prior to ANOVA. Where there were significant differences between treatments, means were compared by Duncan's multiple range test.

Results

Mineral nitrogen dynamics

The concentration of NO_3^- in soils without residue addition steadily increased throughout the 200 days (Figure 1). In comparison, soils with residue addition had peak NH_4^+ concentrations at day 10 or 30, while NO_3^- concentrations increased from either day 0 or 10 depending on the treatment. The concentration of NO_3^- in the soil decreased over the first 10 days, predominately in treatments containing soybean green manure residues and peanut residues, while soils containing mature soybean residues generally increased in NO_3^- concentrations from the start of the incubation.

At day 10, treatments with a nitrification inhibitor applied to incorporated residues had a higher proportion of mineral nitrogen as NH_4^+ (22-74%) compared with incorporated residues without an inhibitor (6-31%). By Day 30 the effect of the nitrification inhibitor was only present in the Bundaberg soil, where 21-44% of the mineral nitrogen was in the NH_4^+ form when an inhibitor was applied, compared with 1-3% without an inhibitor. Within each residue type, the unincorporated treatments had the lowest mineral nitrogen concentrations.

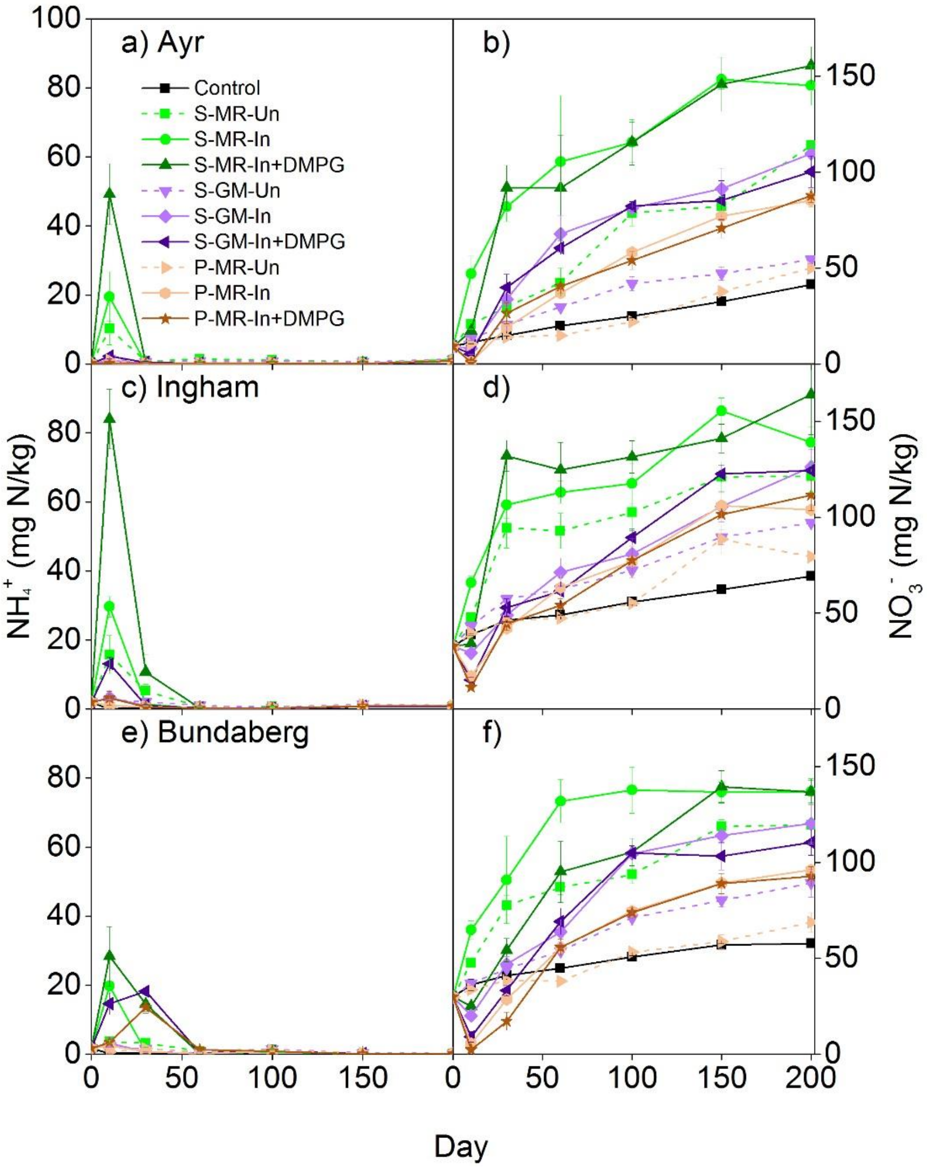

Fig. 1- Mineral nitrogen dynamics (a,c,e ammonium; b,d,f nitrate) in three soils applied with residues under different management practices, over 200 days incubation. Soybean, S; peanut, P; mature residue, MR; green manure, GM; unincorporated, Un; incorporated, In; incorporated with nitrification inhibitor applied; In+DMPG. Mean ± standard error.

Nitrous oxide flux

Although the Bundaberg soil produced the lowest cumulative N_2O flux and the Ayr soil produced the highest, the difference was not significant due to the large variability in fluxes (Figure 2). Residue type significantly ($p<0.001$) influenced cumulative N_2O flux at Day 200, with mature soybean having the highest flux (1 056 µg N_2O-N/kg), while peanut (172 µg N_2O-N/kg) and green manure soybean (212 µg N_2O-N/kg) had similar fluxes. The unincorporated residue management practice had significantly lower emissions than both incorporated management practices. The addition of a nitrification inhibitor significantly reduced N_2O flux from the incorporated mature soybean treatment in the Ayr soil, but there was no significant effect in other cases. An interactive effect of residue type and management was evident, with mature soybean releasing significantly higher emissions when incorporated without DMPG (1 821 µg N_2O-N/kg) compared with green manure soybean and mature peanut, which emitted similar amounts of cumulative N_2O whether incorporated or unincorporated (152-227 µg N_2O-N/kg).

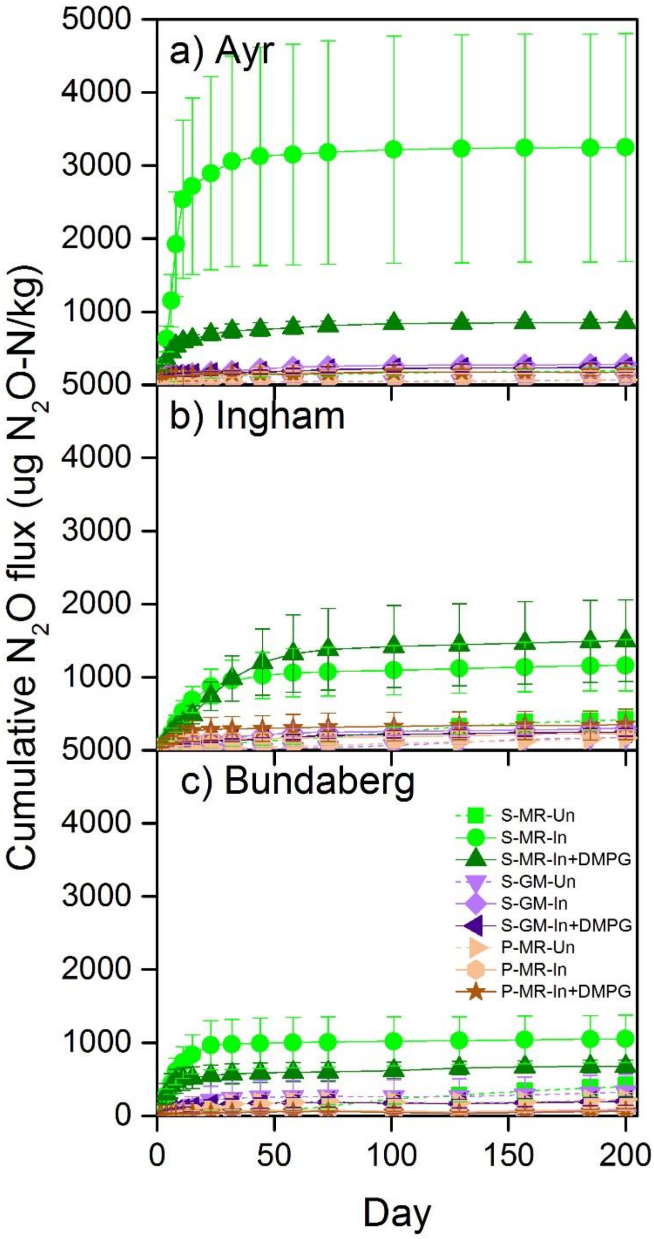

Fig. 2-Cumulative N_2O-N flux attributed to residue addition in three soils. Soybean, S; peanut, P; mature residue, MR; green manure, GM; unincorporated, Un; incorporated, In; incorporated with nitrification inhibitor applied; In+DMPG. Mean ± standard error.

Net nitrogen mineralisation

Soil net N mineralisation (i.e. without residue addition) after 200 days was 42, 70 and 58 mg N/kg for the Ayr, Ingham and Bundaberg soils, respectively. Applying the same crop residue to different soils produced similar net N mineralisation, except for the Ayr soil with mature soybean applied, which was significantly higher than mature soybean applied to the Ingham and Bundaberg soil.

Mature soybean residues displayed net N mineralisation throughout the 200 day incubation, whereas the soybean green manure residue and peanut residue displayed initial net N immobilisation followed by net mineralisation (Figure 3). The highest net N mineralisation occurred in the incorporated mature soybean treatments (S-MR-In: 84.4 mg N/kg; S-MR-In+DMPG: 96.0 mg N/kg) and the lowest occurred in the unincorporated peanut residue treatments (10.1 mg N/kg). In soils with incorporated mature soybean residues, >60% of the net N mineralisation during the 200 days occurred in the first 60 days of the incubation. In contrast <45% of the net N mineralised during the incubation was accumulated by day 60 of the incubation for incorporated peanut residues.

The types of residue applied significantly affected the net N mineralised (p<0.001), with all three residues being significantly different to each other (Table 4). On average across different soils, soybean mature residue had the highest amount of net N mineralised (80.9 mg N/kg), followed by soybean green manure (47.6 mg N/kg) and peanut mature residue (30.1 mg N/kg). Net N mineralised on an applied residue N basis followed the same trends to the net N mineralised when considering residue type (Table 4).

Unincorporated residues had significantly lower (p<0.001; 32.3 mg N/kg) net N mineralisation compared with incorporated treatments (In: 62.0 mg N/kg; In+DMPG: 64.3 mg N/kg), with unincorporated treatments mineralising approximately half of the N mineralised in incorporated treatments. Spraying residues with a nitrification inhibitor did not affect the net N mineralised by the end of the incubation, although lower values were observed earlier in the Bundaberg soil.

Table 4-Net crop residue N Mineralisation, residue N mineralised, C mineralisation and cumulative N_2O flux, from residue amended soil after 200 days of incubation

	Net legume residue N mineralisation mg N/kg soil	Residue N mineralised mg/g residue N	C mineralisation mg CO_2-C/kg soil	Cumulative N_2O flux µg N_2O-N/kg soil
Means of individual effects*				
Soil				
Ayr	59.1^{b}	240	1964^{a}	589^{ab}
Ingham	49.3^{a}	206	2369^{b}	505^{b}
Bundaberg	50.2^{a}	231	2663^{c}	345^{a}
Residue				
Soybean mature	80.9^{c}	297^{c}	2437	1056^{b}
Soybean green manure	47.6^{b}	210^{b}	2279	212^{a}
Peanut mature	30.1^{a}	170^{a}	2280	172^{a}
Management				
Unincorporated	32.3^{a}	132^{a}	2273	226^{a}
Incorporated	62.0^{b}	269^{b}	2343	734^{b}
Incorporated+DMPG	64.3^{b}	276^{b}	2380	479^{b}
Source of Variation (p-value)				
Soil	0.015	0.066	<.001	0.012
Residue	<.001	<.001	0.077	<.001
Management	<.001	<.001	0.394	<.001
Soil x residue	0.021	0.103	0.203	0.784
Soil x management	0.086	0.13	<.001	0.002
Residue x management	0.262	0.077	0.032	0.023
Soil x residue x management	0.978	0.992	0.724	0.445

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

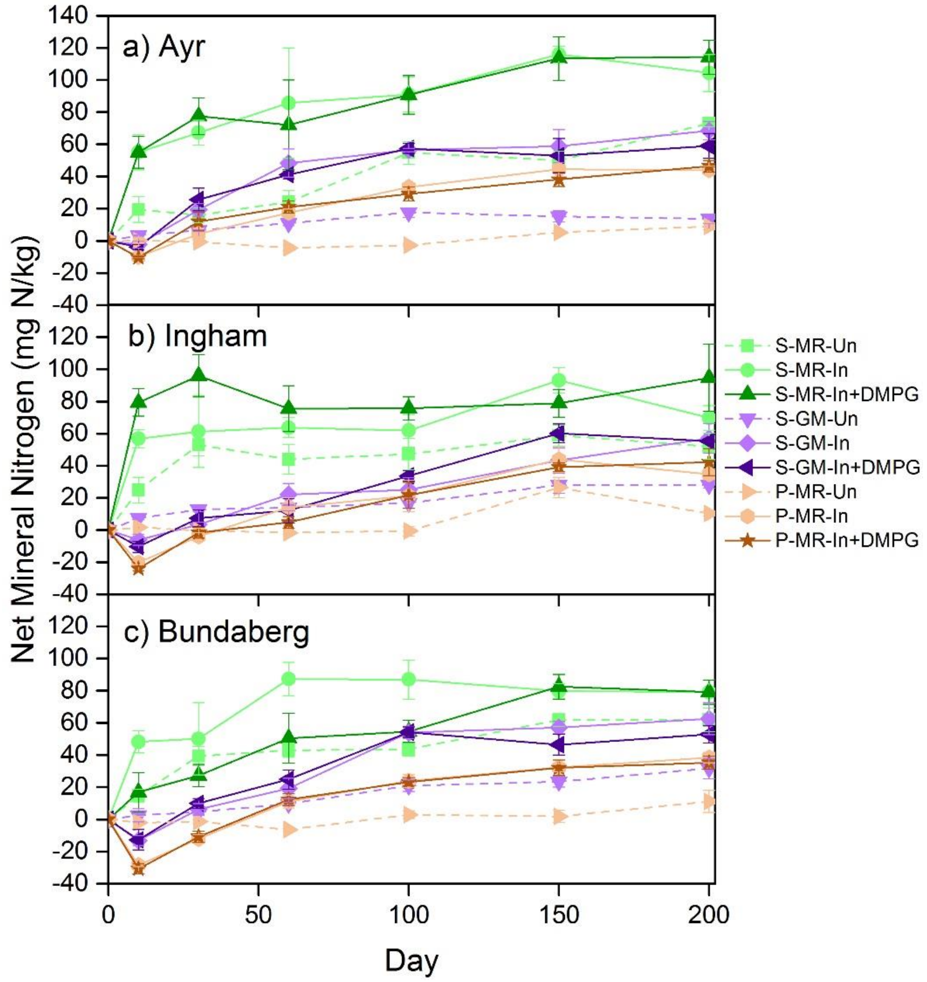

Fig. 3-Dynamics of net nitrogen mineralisation from legume crop residues applied to different soils under different treatments. Soybean, S; peanut, P; mature residue, MR; green manure, GM; unincorporated, Un; incorporated, In; incorporated with nitrification inhibitor applied; In+DMPG. Mean ± standard error.

Carbon mineralisation

After 200 days of incubation, the cumulative soil C mineralisation (i.e. without residue addition) was 782, 1 118 and 836 mg CO_2-C/kg for the Ayr, Ingham and Bundaberg soils, respectively. Carbon mineralisation in the incorporated treatments proceeded at a higher rate directly after incorporation and slowed down over time. In comparison, the unincorporated treatments initially proceeded at a slower rate than the incorporated treatments, but exceeded the C mineralisation rate of the incorporated treatments in latter stages of the incubation (Figure 4). There was a significant effect of soil type on carbon mineralisation of the crop residues ($p<0.001$), however effects of residue type and management were not significant at the end of the incubation (Table 4). On average, the total cumulative carbon mineralisation from crop residue addition was highest for the Bundaberg soil, followed by the Ingham and Ayr soils, with 2 663, 2 369 and 1 964 mg CO_2-C/kg, respectively, accounting for 66%, 53% and 43% of the added crop residue C. Soil type significantly interacted with management practice, whereby management practice did not affect cumulative C mineralisation of the crop residues in the Bundaberg soil, but produced significant differences with other soils. Overall, there was also an interactive effect of residue type and management practice, whereby unincorporated green manure soybean had significantly less C mineralisation than incorporated treatments. In contrast, unincorporated and incorporated residues had similar C mineralisation for peanut and mature soybean. The total C mineralisation was similar for the same crop residue type in most cases, but the unincorporated treatments mineralised less C than the incorporated treatments with the Ayr soil and, in contrast, the unincorporated peanut residue released more C than the incorporated treatment with the Ingham soil.

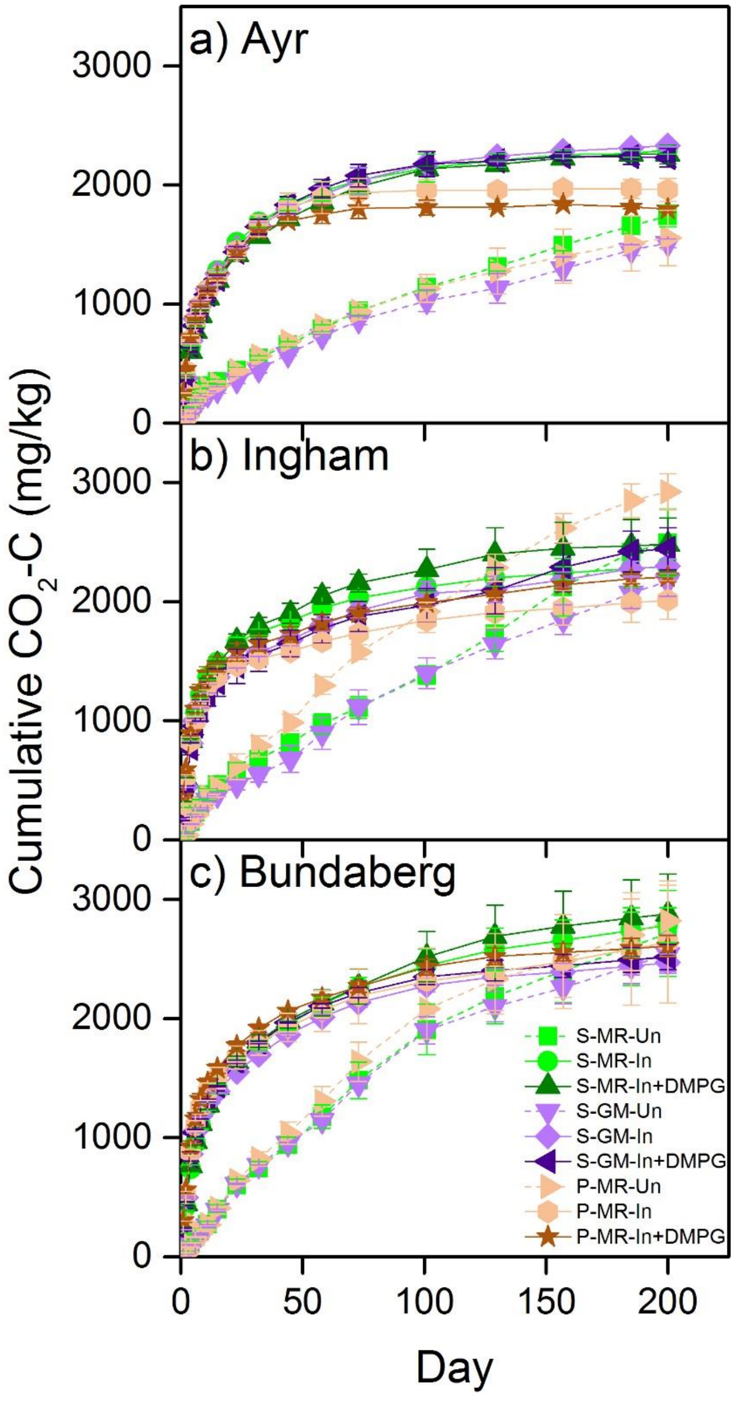

Fig. 4-Cumulative C mineralisation from crop residues in three soils. Soybean, S; peanut, P; mature residue, MR; green manure, GM; unincorporated, Un; incorporated, In; incorporated with nitrification inhibitor applied; In+DMPG. Mean ± standard error.

Discussion

Legume type

The legume species chosen in a fallow rotation was an important determining factor in N mineralisation. The three legume residues studied here demonstrated large variability in residue N mineralisation, with mature soybean mineralising 269% more N than peanut residues over 200 days. The C:N ratio of residues is one of the main determining factors of residue N mineralisation, with a C:N ratio of between 20-40 regarded as the switch over point between net immobilisation and mineralisation (Cabrera *et al.*, 2005). The C:N ratio of mature soybean (C:N ratio: 17) used in the current study falls below this range, which helps to explain why mature soybean exhibited mineralisation continuously throughout the 200 days. In comparison, the other two legume types used had C:N ratios within 20-40, and both exhibited varying periods of initial immobilisation before net mineralisation. The similar trends observed between net N mineralised when on an applied residue N basis and on a dry soil basis indicates that it is not purely the increased N content that is causing increased net N mineralisation, but other residue quality factors. A review by Cabrera *et al*. (2005) highlighted groups of compounds (e.g. polyphenols, proteins and lignin) that further affect residue mineralisation rates in addition to the C:N ratio. Planting legumes with contrasting N mineralisation profiles in a mixed planting may also provide an additional method to alter N provided to the following crop, however this requires further investigation.

Whether a residue is sprayed out and used as a green manure or left until maturity to harvest grain, can greatly affect the resulting residue mineralisation. The green manure soybean had a higher C:N ratio in this study, resulting in a period of immobilisation after incorporation, followed by an overall lower net N mineralisation compared to the mature soybean during the 200 days. Franzluebbers *et al*. (1994) also found mature cowpea mineralised more than double green manured cowpea, due to greater N accumulation and more rapid mineralisation. These outcomes should be taken into account when considering the use of green manures.

Unincorporated vs. incorporated residues

Residues retained on the soil surface are generally reported to mineralise more slowly than incorporated residues due to less contact with soil and microbes (Garside and Berthelsen, 2004). This was supported in the current study with incorporated residues mineralising 192% more N overall than unincorporated residues. In addition, 324% more N_2O was lost from incorporated residues than unincorporated residues. In a paddock situation where cane is not planted shortly after incorporation of the legume crop residues, the accumulated inorganic N in the soil would also be prone to leaching (Garside and Bell, 2001).

Nitrification inhibitor application to residues

The nitrification inhibitor applied on incorporated residues affected both nitrogen mineralisation dynamics and N_2O flux from residues. Nitrification was effectively reduced by the inhibitor in all soils for at least 10 days after incorporation as evidenced by the higher NH_4^+ concentrations. In the Bundaberg soil with the highest sand content, the effect of the inhibitor persisted until day 30, which supports other studies that have found higher sand contents increases efficacy of the nitrification inhibitor (Barth *et al.,* 2001). The increased NH_4^+ concentration potentially can reduce N leaching as NH_4^+ is less mobile than NO_3^-. By maintaining N in the NH_4^+ form, NO_3^- was effectively reduced, therefore there was less substrate for denitrification to convert to N_2O. However, N_2O flux was only significantly reduced when mature soybean was incorporated. This is because green manured soybean and mature peanut both produced net N immobilisation soon after incorporation, reducing the N available for nitrification and subsequent denitrification during this time period. In comparison soil with mature soybean incorporated had net N mineralisation directly following incorporation, providing higher concentrations of inorganic N for nitrification and denitrification. Where residue incorporation may likely cause initial net N immobilisation the benefits of applying an inhibitor may not be significant, compared with residues that cause net N mineralisation.

The supply of N is only one factor required for denitrification to occur in soil, the others are a C supply and anaerobic conditions. In this study excess C is supplied by the residues; however, it is often assumed that anaerobic conditions do not prevail in soil at 50% WHC. With the addition of residues, the microbial community proliferates, consuming oxygen and creating anaerobic microsites within the soil, where denitrification can occur. The rapid proliferation of the microbial population is evidenced by the rapid increase in C mineralisation in incorporated treatments at the beginning of the incubation. Most incubation studies examining residue N mineralisation do not consider this issue, and therefore N mineralisation may be underestimated. Furthermore, whether or not in-situ residue incorporation creates anaerobic conditions, needs to be investigated.

Conclusions

It is evident from this study that the management of residues can manipulate their N mineralisation dynamics, and if used in the appropriate situation could help to maximise N provided to the succeeding crop. In situations where a plant cane crop would be established soon after residue management, residues exhibiting net mineralisation would be beneficial to avoid N deficiencies in the sugarcane plant crop, caused by net immobilisation. In situations where a longer period of time is expected to elapse between residue management and plant cane crop a higher C:N ratio legume could be planted and incorporated or a lower C:N ratio legume could be left on the soil surface. In situations expecting heavy rainfall after residue management, leaving the residues on the surface to slow mineralisation, or the application of a nitrification inhibitor prior to incorporation would be beneficial in avoiding N leaching and gaseous N losses.

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