Additional nitrogen mineralisation and crop uptake following tropical forage legumes is lower if shoot biomass is removed

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Abstract
Tropical pasture legumes used for hay or cut and carry fodder increase dietary protein for ruminants and can also provide nitrogen (N) for subsequent crops. An experiment was conducted at Gatton, Queensland, to quantify N mineralisation and crop uptake following the tropical herbaceous legumes Butterfly pea (*Clitoria ternatea* cv. Milgarra) and Centro (*Centrosema pascuorum* cv. Cavalcade) when shoot biomass was removed or retained during the growing period (8 months). Soil mineralisation (0-0.45 m) was compared to plots following a maize control using in-situ tubes and an oven incubation study. For the incubation study, after 17 weeks soil nitrate following legumes with biomass retained was 115 kg NO_3-/ha higher than following maize. In the in-situ cores, retention of legume biomass increased mineralisation during the oat crop (3 months) from 41-56kg N/ha to 78-125kg N/ha. Consequently, legumes with biomass removed provided an additional 2-23kg N/ha to the oats, compared to maize, but, less than the additional 53-90kg N/ha when biomass was retained. Retention of legume biomass doubled N uptake by the oat crop from 37 kg N/ha to 82 kg N/ha and increased oat biomass by 50%. Despite this, soil N post-oats and mineralisation rates indicate that mineralisation of root material for legumes with biomass removed may still become evident in a subsequent maize crop. These results indicate that removing shoot biomass of Butterfly pea and Centro for cut and carry or hay conservation considerably reduces the N benefit to the subsequent crop.

Key words
Pasture legume, ley legume, defoliation, farming systems, sub-tropical, N-fertiliser substitution value

Introduction

In tropical and sub-tropical mixed farming systems tropical pasture legumes can be used to increase soil nitrogen (N) for subsequent cereal crops as well provide high quality fodder to increase livestock growth rates. In northern Australia, legume-leys can increase the productivity of subsequent cereal crops, however where shoot biomass is removed – such as occurs in haymaking or cut and carry systems – the N benefit to the subsequent cereal crop is reduced. Research comparing tropical pasture legumes with cereal rotations shows that, even with defoliation, a legume rotation can increase grain yield and N uptake of the subsequent cereal crop, however the N benefit is highly variable with changes in the yield of the subsequent cereal crop ranging from -30% to +20% (Smyth et al. 1991; Oikeh *et al*. 1998). While tropical legumes Centro (*Centrosema pascuorum*) and Butterfly pea (*Clitoria ternatea*) also suit haymaking and cut and carry systems, there is limited research into their N contribution to subsequent cereal crops when shoot biomass is removed. This paper describes an experiment to quantify N mineralisation and crop uptake following Butterfly pea and Centro where shoot biomass was removed or retained.

Methods

Field and in-situ micro-plot experiment

An experiment was conducted on a black vertosol at Gatton, Queensland. Four replicate plots (6 m by 12 m) of Butterfly pea (*Clitoria ternatea* cv. Milgarra), Centro (*Centrosema pascuorum* cv. Cavalcade) and maize were planted in a randomised split-plot design, with two legume sub-plots (6 m by 6 m) where shoot biomass was either retained or removed 50 mm above ground level. Inoculated legume and maize seed was planted following recommended district practice on 12 September 2013, after which the experiment was irrigated every 2 weeks. Shoot biomass was removed twice for Centro – 144 days after sowing and at termination – and three times for Butterfly pea – 89 and 144 days after sowing and at termination. Maize grain and biomass above 15 cm was harvested at maturity on 3 February 2014 and then fallowed until legumes were terminated with herbicide 64 days later on 8 April 2014. After spraying, sub-plots with shoot biomass retained were mulched. Following the legume rotation, an oat cover crop was sown over the whole experiment on 28 May

2014. To measure in-crop mineralisation, one PVC tube (100 mm diameter by 0.6 m long) was installed in each plot to exclude oat roots. The tubes – or 'micro-plots' – were driven 0.5 m into the ground and then trimmed to leave 20 mm above ground. The oat cover crop was terminated with herbicide on 14 August 2014.

Soil nitrate (NO_3-) concentrations and soil water content were measured prior to starting the experiment and then in each replicate treatment plot following both the legume and oat rotations. For each sub-plot, soil samples were collected to a depth of 0.45 m for nitrate and 1.5 m for water content and, at oat sampling, soil was collected to 0.45 m in each micro-plot. Samples were separated into 0-0.15, 0.15-0.30, 0.30-0.45, 0.45-0.60, 0.60-0.90, 0.90-1.20 and 1.20-1.50 m layers, with subsamples removed for soil nitrate and gravimetric water content analysis. Soil nitrate was analysed at a commercial laboratory using a 1:5 soil:water extraction and volumetric soil water content was calculated using gravimetric water content and estimated bulk density.

Plant biomass cuts were taken from each replicate sub-plot at legume biomass removal, legume termination and oat termination. Biomass was measured by collecting shoot material above ~10 mm in two quadrats (0.5 m^2 each). Shoot samples were divided into leaf and stem, dried at 80°C for 3 days and a ground subsample was analysed for total N (mg N g^{-1}) using a calibrated NIRS. Total and retained biomass N was calculated as:

Total shoot biomass N = [(total shoot DM) x (shoot %N/100)] [1]
Retained shoot biomass N = [(retained shoot DM) x (stem %N/100)] [2]

As root nitrogen is not accounted for in biomass N calculations, total plant N was calculated by multiplying biomass N by a root factor estimating the proportion of below-ground plant N.

Total plant N = [(total shoot biomass N) x (root factor)] [3]

A root factor of 1.49 was used for Centro (33% below ground N), 1.8 for Butterfly pea (45% below ground N), 1.85 for oats (46% below ground N) and 1.58 for maize based on mean below ground N (37%) of a range of temperate cereals (Wichern *et al*. 2008; Unkovich et al. 2010; Peoples *et al*. 2012).

Oven incubation experiment
At legume termination seven soil cores (32 mm) were collected in each replicate sub-plot to 0.6 m deep. Each core was placed in a PVC tube (33 mm diameter x 0.6 m long) and sealed with a PVC cap on the base and 4 layers of Gladwrap™ at the top. Soils were placed in an oven at 33°C and were re-wet to original weight every two weeks. Tubes were destructively sampled at 6 times over the incubation period, 0, 14, 47, 76, 119 and 191 days and gravimetric water content and nitrate were measured for one tube from each sub-plot. Samples were processed as described above, except soil nitrate was analysed using a 2M KCl extraction. Analysis of variance in Genstat 16.1 (VSV International Ltd. Hemel Hempstead, UK) was used to determine statistical differences in soil N for field and incubation studies as well as biomass production and nitrogen uptake.

Results
All legume treatments produced >12t DM/ha, however estimated total N inputs were 266-322 kg N/ha higher ($p<0.05$) when shoot biomass was retained (Table 1). Maize grain yield averaged 4.36 t/ha and 12.2 t/ha of stover was produced.

Table 1. Total accumulated N retained on plots following legumes with shoot biomass removed or retained

Legume	Shoot biomass	Total retained shoot N (kg N/ha)	Total retained plant N (kg N/ha)*
Butterfly pea	Retained	339	610
	Removed	17	288
Centro	Retained	293	437
	Removed	27	171
Maize	Removed	4	49

*Adjusted to include below-ground contributions of nitrogen using root-factors

Incubation nitrogen mineralisation study

In the incubation study, N mineralisation rates following legumes with shoot biomass retained averaged 1 kg NO_3-/ha/d, compared with 0.43 kg NO_3-/ha/d where shoot biomass was removed and 0.25 kg NO_3-/ha/d following maize (Figure 1). Consequently, after 17 weeks incubation, soil nitrate following legumes with shoot biomass retained was 115 kg NO_3-/ha higher ($p<0.05$) than following maize. However, where shoot biomass was removed there was no significant difference between legumes and maize.

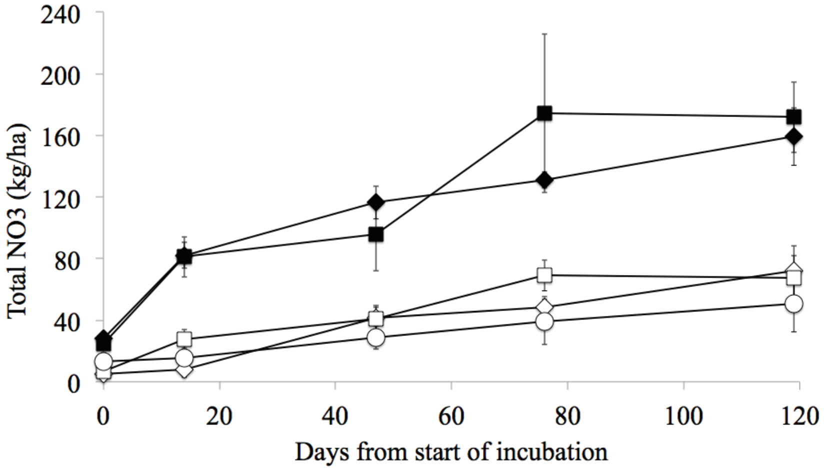

Figure 1. Soil nitrate (0-0.45 m) during incubation of soil cores at 33°C following a legume – Butterfly pea with biomass retained (black square), Centro with biomass retained (black diamond), Butterfly pea with biomass removed (white square), Centro with biomass removed (white diamond) – or maize (white circle) rotation

Micro-plot nitrogen mineralisation study

Soil N was 42 kg N/ha before legume planting and 16 kg NO_3-/ha at legume termination; there was no difference ($p=0.553$) in soil N amongst all treatments immediately after the legume crop. Despite this, in-crop mineralisation during the oat crop was higher ($p<0.001$) were shoot biomass was retained, with up to an additional 88 kg NO_3-/ha mineralising when shoot biomass was retained instead of removed (Table 2). However, when biomass was removed there was no significant difference in mineralisation between the two legumes or maize. After oat termination, residual soil N was higher following Butterfly pea where shoot biomass was retained ($p<0.05$); there was no significant difference between the other four treatments.

Table 2. Soil volumetric water content at oat planting, soil mineral nitrate-N following legume and oat rotations and in-crop mineralisation (water content 0-1.5 m; nitrate 0-0.45 m)

Previous rotation	Shoot biomass	Volumetric water content at oat planting (mm)	Soil N at oat planting (kg NO_3-/ha)	Residual soil N in field at oat termination (kg NO_3-/ha)	Soil N in micro-plots at oat termination (kg NO_3-/ha)	N mineralisation after legume termination (kg NO_3-/ha)*
Butterfly	Retained	190	12	73	137	125
pea	Removed	201	9	22	49	41
Centro	Retained	184	22	33	100	78
	Removed	189	13	37	70	56
Maize	Removed	217	22	20	47	25

*Estimated for 132 day period by subtracting soil N at legume termination from soil N inside the micro-plots at oat crop termination

Oats biomass production and N uptake

Oat DM production was greatest ($p<0.001$) following maize and when legume shoot biomass was retained with a mean yield of 3.2 t DM/ha, or 70% more than when legume biomass was removed (Table 3). Although oat DM production was similar following both Centro and Butterfly pea, oat shoot N % was higher ($p<0.05$) following Centro when shoot biomass was retained. Retaining legume shoot biomass increased ($p<0.001$) oat shoot N % and increased estimated uptake of soil N by the oat crop by up to 152% ($p<0.001$).

Discussion

This study indicated that removing shoot biomass of Butterfly pea and Centro for cut and carry or hay conservation considerably reduces the N uptake of the subsequent crop, resulting in little to no N benefit when compared to a previous cereal crop. There were no differences in N mineralisation in the incubation and micro-plot studies between soils following maize and legumes with biomass removed.

Table 3. Oat biomass and nitrogen accumulation following a legume rotation

Legume	Shoot biomass	DM production (t DM/ha)	Leaf nitrogen (%N)	Accumulated shoot N (kg N/ha)	Accumulated total plant N (kg N/ha)
Butterfly pea	Retained	3.03	1.23	38	70
	Removed	1.97	1.00	20	36
Centro	Retained	3.09	1.66	51	94
	Removed	1.86	1.09	20	37
Maize	Removed	3.60	1.20	44	81

However, as approximately 90% of total legume plant N was below ground following biomass removal, there is potential for additional mineralisation over a longer period given that root decay is commonly slower than the decay of shoots and stems (Abiven *et al*. 2005).

Retaining shoot biomass significantly increased soil N mineralisation, oat crop N uptake and biomass production. These differences were driven by the additional 266-322 kg N/ha of plant N that was retained and mulched and subsequent higher soil N mineralisation rates. Consequently, when legume shoot biomass was retained rather than removed, N uptake by the oat crop increased from 37 to 82kg N/ha and oat DM production increased from less than 2t DM/ha to over 3t DM/ha. These differences between retention and removal of shoot biomass are considerably larger than other research in Northern Australia, which found that following *Stylosanthes hamata*, N uptake by the subsequent maize crop was only 4 kg N/ha higher where shoot biomass was retained rather than removed (Jones et al. 1996).

Comparison of maize and legumes with biomass retained showed that oat DM production and N uptake were similar following both treatments. This is attributed to a longer fallow period after maize, resulting in an additional 2 months for accumulation of soil water and N mineralisation, with an extra 26 mm of soil water available at sowing following maize compared with legumes. In contrast to this, research shows that, given the same fallow period, a legume phase can provide significantly more soil N at sowing than a cereal rotation (Armstrong *et al*. 1997).

Conclusion

Using Centro or Butterfly pea in hay or cut and carry systems considerably reduces the N benefit to the subsequent crop, resulting in N inputs that are too small to substitute N fertiliser or increase crop yield.
In contrast, results showed that green manuring Centro and Butterfly pea can potentially increase the productivity of the subsequent cereal crop. While these results indicate that neither of these options can provide both high quality fodder and increased soil N, careful grazing of Centro and Butterfly pea is another option which could potentially achieve these dual benefits.

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