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The interactive effects of soil disturbance and residue quality on soil nitrogen mineralisation in a tropical sandy soil

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Abstract. Soil conservation practices, such as reduced and no tillage, have been found to enhance soil nitrogen (N) sequestration through decreasing the rate of N mineralisation of added organic materials. Nitrogen mineralisation is not only affected by tillage, but also by the quality (chemical composition) of the organic residues. This study evaluated the interaction of residue quality and soil disturbance on N mineralisation in a sandy soil. A 112-day incubation experiment was conducted with two levels of soil disturbance (undisturbed and disturbed conditions) and five plant residue amendments of contrasting quality. The contrasting quality (N, lignin (L), and polyphenols (Pp)) (in g kg^{-1}) amendments follow: (i) unamended; (ii) *Sesbania grandiflora* (N 44, L 173, Pp 9.2); (iii) *Indigofera hirsuta* (N 41, L 177, Pp 30); (iv) *Dipterocarpus tuberculatus* (N 8.2, L 203, Pp 71); and (v) *Eucalyptus camaldulensis* (N 9.7, L 126, Pp 110). Residues (ii) and (iii) were fresh legume leaves, while (iv) and (v) were non-legume leaf litter. Disturbance only significantly increased N mineralisation rates in the legume-residue treated soils (increases of 18.8% for *S. grandiflora* and 27.1% for *I. hirsuta*) during the early stage of decomposition (first 14 days). In the legume treatment, disturbance significantly increased the ammonification, but decreased nitrification in soil relative to undisturbed soils. The difference in patterns of ammonification and nitrification was more pronounced in the early than in the later period of decomposition. This indicated an inhibitory effect of soil disturbance on nitrification, which was particularly pronounced in the legume-treated soils. The Pp content of residues was the major quality parameter regulating the soil ammonium-N and nitrate-N concentrations. Minimum soil disturbance should be adopted under legume soil organic amendment so that both ammonification and nitrification components of N mineralisation process can occur normally, and nitrate-loving crops can take up N in the form of nitrate-N which will enhance their yields. Moreover, undisturbed conditions under legume organic amendments reduced N mineralisation, resulting in enhancing soil N sequestration.

Additional keywords: ammonification, nitrification, polyphenols-to-nitrogen ratio, soil tillage, tropical coarse-textured soil.

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Introduction

Developing suitable agronomic management practices to sequester soil nitrogen (N) has proven to be a challenge with respect to restoring degraded soils, maintaining food security, and enhancing environmental integrity. Soil organic matter management can be an effective method because organic N constitutes more than 90% of the total N in soils (Stevenson 1982). Highly-weathered tropical soils have low soil N contents, a critical factor affecting crop productivity (Sanchez and Logan 1992). Conservation tillage practices (e.g. minimum and no tillage) have been widely adopted due to their beneficial effects in rehabilitating degraded soils, including increasing the soil N pools (e.g. total N, microbial biomass N, and active N) (McCarty *et al*. 1995) and making crop production more sustainable (Reicosky and Saxton 2007) under field conditions. In addition, these practices may enhance the profitability of crop production by cutting input costs (e.g. fuel, labour, and equipment maintenance) (Mitchell *et al*. 2012). Reduced soil disturbance has been shown to decrease both the net and gross soil N mineralisation rates (Gómez-Rey *et al*. 2012).

In addition to soil disturbance, N mineralisation is affected by organic residues that are already present in or have been newly applied to agricultural soils. These residues vary in chemical composition or quality depending upon different factors, such as plant type, plant part, and stage of decomposition in soil. In cropping systems practised by smallholder farmers with little purchasing power and limited access to mineral fertilisers, diverse types of locally available

plant materials are employed as soil amendments to reduce production costs (Palm *et al*. 2001; Chivenge *et al*. 2009). However, having a variety of residue qualities, as assessed by carbon (C) : N ratio and the concentrations of lignin (L) and polyphenols (Pp), is recognised as a key factor in determining their decomposability and nutrient release (Handayanto *et al*. 1997; Palm *et al*. 2001; Puttaso *et al*. 2011). According to Handayanto *et al*. (1997), plant residues containing high N, low L, and low Pp concentrations are considered to be of high quality and have rapid N mineralisation. In contrast, those in the low-quality category (low N, high L, and high Pp concentrations) are slow to decompose and produce little net N mineralisation. To our knowledge, few studies have investigated N mineralisation in response to tillage and the quality of organic residues; moreover they have produced inconsistent results. For example, in some studies non-tilled soil produced a lower gross N mineralisation rate than conventionally tilled soil (e.g. Gómez-Rey *et al*. 2012), whereas other studies showed no effect (Cookson *et al*. 2008; Maqsood *et al*. 2013). The effects of tillage practices on gross N mineralisation have been shown to vary across organic matter managements (Thomsen and Sorensen 2006); unfortunately their biochemical quality parameters were not measured. Higher N mineralisation rates have been observed with high-quality residues, i.e. red clover (C : N = 21–22; L : N = 2–3), compared to lower quality counterparts, i.e. peas (C : N = 42–66; L : N = 9–15), canola (C : N = 46–47; L : N = 13–14), and wheat (C : N = 59–76; L : N = 9–11) under cultivation (Lupwayi *et al*. 2004*a*, 2004*b*, 2006). Yet, reduced and no tillage increased the net N immobilisation in low-quality winter wheat residue (C : N = 76–147) compared to high-quality sugar beet (C : N = 14–15) and Italian ryegrass (C : N = 13–15) (Van Den Bossche *et al*. 2009). They found no interaction between tillage practice (no, reduced, and conventional tillage) and residue quality or C : N. Interactions between tillage practices and residue quality parameters with respect to C : N and L : N have been reported. However, interactions between tillage practice and Pp contents, another major quality parameter, which had exhibited a highly negative influence on N mineralisation (Handayanto *et al*. 1997; Vityakon and Dangthaisong 2005), were not determined. Polyphenols can bind with proteins to form Pp–protein complexes that can restrict N release from plant tissue to the soil (Handayanto *et al*. 1997; Mutabaruka *et al*. 2007). Vityakon and Dangthaisong (2005) found that Pp play the most important role in regulating N mineralisation in aerobic conditions.

This study evaluated N mineralisation in a tropical sandy soil as affected by the interactions between soil disturbance (tillage) and residue quality. The interactions were examined with respect to the major soil processes of the soil N cycle (i.e. N mineralisation-immobilisation). We hypothesised that soil N mineralisation would be affected by soil disturbance and would be more pronounced in high-quality than low-quality plant residues.

Materials and methods

Soil and plant residues

The soil was a coarse-textured Khorat soil (isohyperthermic Typic Oxyaquic Kandiustults) (Soil Survey Staff 2006) collected at a depth of 0–15 cm in the Fruit Tree Research Section of Khon Kaen University Thailand (16°27′50″N; 102°48′14″E). The soil was air-dried and passed through a 2-mm sieve. The initial physical and chemical properties of the Khorat soil are presented in Table 1.

Four locally available plant residues from North-east Thailand were studied: (i) fresh leaves of sesbania (*Sesbania grandiflora*), (ii) fresh leaves of hairy indigo (*Indigofera hirsuta*), (iii) freshly-fallen leaf litter of dipterocarp (*Dipterocarpus tuberculatus*), and (iv) freshly-fallen leaf litter of eucalyptus (*Eucalyptus camaldulensis*). These residues were selected as representatives of similar quality organic inputs that farmers could apply to their fields. They represented a range of quality parameters, i.e. fresh legume leaves were of high quality and tree leaf litters were low quality. The chemical composition parameters of the residues are shown in Table 2 with detailed methods of laboratory analyses for the residues given in the section below. In order to achieve constant sizes and dimensions for all plant residues, they were cut into squares of ~1 cm^2.

Table 1. Initial physical and chemical properties at 0–15 cm depth of the Khorat soil

Soil properties	Values
Soil particle size distribution	
Sand (%)	75.9
Silt (%)	15.4
Clay (%)	8.7
Soil texture	Sandy loam
Bulk density (g cm^{-3})	1.49
pH (soil : H_2O = 1 : 2.5)	5.04
Total N (g kg^{-1})	0.2
P (mg kg^{-1})	55.3
K (mg kg^{-1})	1453
CEC ($cmol_c$ kg^{-1})	3.22

Table 2. Chemical quality characteristics of plant residues used in this study
Abbreviations: C, carbon; N, nitrogen; L, lignin; Pp, total extractable polyphenol; Cell, cellulose

Residues	C	N	L (g kg^{-1})	Pp	Cell	C : N	L : N	Pp : N	(L+Pp) : N
Sesbania grandiflora	429	43.7	173	9.2	138.3	9.82	3.96	0.21	4.17
Indigofera hirsuta	441	41.1	177	29.8	101.2	10.73	4.31	0.73	5.03
Dipterocarpus tuberculatus	460	8.2	203	71.2	267.4	56.10	24.76	8.68	33.44
Eucalyptus camaldulensis	470	9.7	126	109.6	151.3	48.45	12.99	11.30	24.29

Incubation experiment

A microcosm incubation experiment was conducted under greenhouse conditions from November 2008 to March 2009 with average air temperature of ~30°C. A two-way factorial arrangement, i.e. two soil disturbance levels (undisturbed and disturbed) were used in combination with five plant residue amendments (unamended (control) and four aforementioned residues) in a randomised complete block design with three replications. Five hundred g of air-dried soil was placed in a glass jar (d = 10 cm, h = 15 cm, and v = 1000 cm^3). The soil layer in the glass jar was ~4.3 cm from the bottom of the jar. The soil was thoroughly mixed with 1.67 g (dry weight) of plant residues (equivalent to 10 t ha^{-1}) calculated based on the field soil bulk density (Table 1). Each of these jars constituted an experimental unit watered every other day with distilled water to a pre-determined moisture content of 9.23% w/w, equivalent to 70% of the water holding capacity and equivalent to the field capacity of this particular coarse-textured soil.

The soil disturbance intervals imitated those of the farmers' field practices under major field crops of the region, such as cassava and sugarcane, in which the soil is more frequently disturbed in the earlier stages of the crop cultivation cycle than in the later stages. In the earlier stages of crop establishment employing cuttings, field practices including mechanical cultivation, weeding either manually (hoe) or mechanically (hand-held rotary tiller), and fertiliser application (surface banding followed by manual or mechanical incorporation) cause soil disturbance. Once the crop is established in the later stages, soil disturbance through these various practices is less frequent. In Phase I of the experiment (0–7 days after residue incorporation, DAI), the soil disturbance was carried out daily, Phase II (7–14 DAI) every other day, Phase III (14–28 DAI) every three days, Phase IV (28–56 DAI) alternate weeks, and finally every three weeks in Phase V (56–112 DAI). The disturbance was performed using an electric drill (MAKTEC® MT 651, Rutherford, Gauteng, South Africa) with a modified tip. The drill tip was modified to possess a seven-lobe impeller with a diameter of 3.8 cm. During a soil disturbance operation, the drill tip was positioned into a middle depth of the soil layer at the centre. As the disturbance operation proceeded, the drill tip was moved to the four corners of the jar. Each disturbance operation was at an approximate orbital speed of 450–600 r.p.m. and timed to last 15 s. Soil sampling was undertaken at 0, 3, 7, 14, 56, and 112 DAI.

Laboratory analyses of plant residues and soil

The analyses of the chemical composition of plant residues included the following: total C was measured using the Walkley and Black wet digestion method (Nelson and Sommers 1982), total N using micro-Kjeldahl (Bremner and Mulvaney 1982), L and cellulose using the acid-detergent fibre method (Van Soest and Wine 1968), and total extractable Pp by the colourimetric method according to the Tropical Soil Biology and Fertility Handbook (Anderson and Ingram 1993), i.e. the Follin–Denis reagent with tannic acid was used as a standard, and hot (77°C) extractant of 50% aqueous methanol at a plant material (mg) to extractant (mL) ratio of 37.5 : 1 (w/v) was used.

Soil particle size distribution was determined using the pipette method (Dewis and Fretias 1970), and bulk density was determined using the core method (Dewis and Fretias 1970). Ammonium (NH_4^+) and nitrate (NO_3^-) were extracted with 1 M KCl using a fresh soil to KCl ratio of 1 : 5 w/v and were colourimetrically determined using a flow-injection analyser (FIAstar® 5012, FOSS Tecator, Sweden). Phosphorus was extracted using Bray II solution and determined colourimetrically using Murphy–Riley reagent followed by absorbance (820 nm) determination on a Spectro 2000RS Visible Spectrophotometer (LaboMed, Los Angeles, CA, USA) (Jones 2001). Extractable potassium was extracted using 1 M NH_4OAc at pH 7 (Pansu and Gautheyrou 2006) and was determined with a flame photometer M410 (Sherwood Scientific, UK). Soil cation exchange capacity (CEC) was determined by 1 M NH_4OAc extraction at pH 7 (Pansu and Gautheyrou 2006) and, in order to calculate the CEC, the NH_4^+ concentration was determined using the micro-Kjeldahl method. Microbial activity was determined through capturing evolved CO_2 in a small glass bottle (d = 3.8 cm, h = 6.5 cm) containing 20 mL of 1 M NaOH. The glass bottle with the alkali solution was placed in a tightly-capped jar and left for 24 h. Carbon dioxide was determined by back titration with 0.5 M HCl after carbonate precipitation with excess 0.5 M $BaCl_2$ (Anderson 1982). Microbial biomass N (MBN) was measured in fresh soil samples using the chloroform fumigation–extraction technique of Amato and Ladd (1988). Of the chloroform-fumigated and unfumigated samples, 20 g was extracted with 100 mL of 1 M KCl. The MBN was determined by the ninhydrin-reactive N method and calculated as the difference in values between the fumigated and unfumigated samples multiplied by the k_{EN} factor of 3.1 to convert ninhydrin-reactive N to MBN.

Data calculation and statistical analysis

A two-way ANOVA, based on a randomised complete block design in a factorial arrangement, was used to determine the effects over all sampling time intervals of the level of soil disturbance, the quality of the plant residues, and their interactions on soil properties including MBN, NH_4^+-N, NO_3^--N, net N mineralisation, and CO_2 evolution. Orthogonal contrast was used to compare effects of residue types (legumes vs non-legumes) under both disturbance regimes at each sampling time interval on the above soil properties. Repeated-measurement analysis was used to evaluate the effects of treatment × time interaction on CO_2 evolution rate, microbial biomass N, NH_4^+-N and NO_3^--N concentrations, and net N mineralisation. Treatment means, including the control were analysed separately between legume and non-legume residues at each sampling time. They were compared using Fisher's least significant difference (l.s.d.). Relationships of quality parameters of plant residues with NH_4^+-N and NO_3^--N concentrations were determined using the Pearson correlation coefficients employing the entire raw data of all factors, i.e. residue quality, disturbance regimes, and sampling time. Stepwise multiple regression analysis was performed to identify the most influential quality parameter on N mineralisation by disturbance regimes and decomposition stages. The effects of

Pp content of plant residues on NH_4^+-N and NO_3^--N concentrations and on microbial N use efficiency were further determined using best-fit regression analysis. The statistical significance of all analyses was evaluated at a probability level of 5%. All statistical analyses were conducted using IBM SPSS statistics Version 20 (SPSS Inc., USA).

Results

Effects of soil disturbance and contrasting quality plant residues on soil chemical and microbiological properties

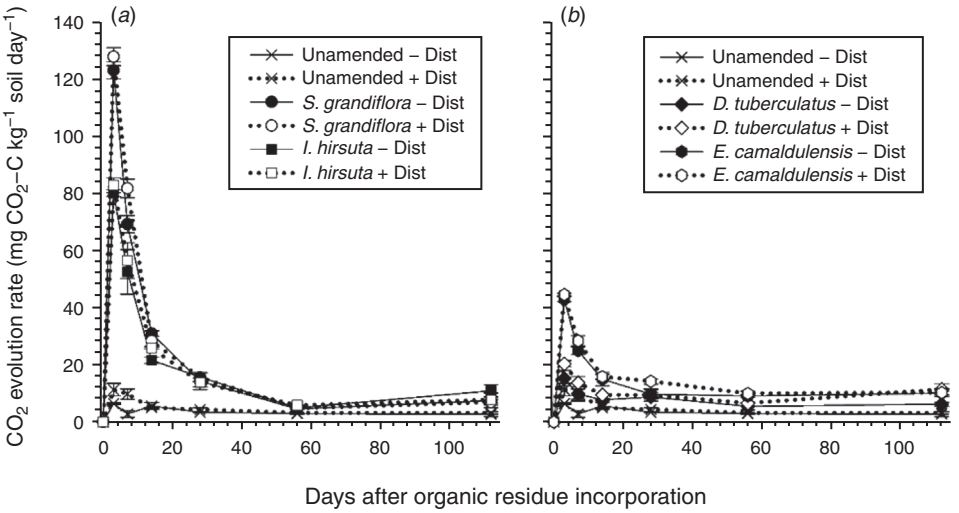
The four contrasting quality plant residues could be categorised as either legumes or non-legumes. Furthermore, these corresponded to their chemical compositions: (i) legumes with high contents of N, but with low L and low Pp (sesbania and hairy indigo) and (ii) non-legumes with low contents of N, but with high L and Pp (dipterocarp and eucalyptus) (Table 2). The treatment × time interactions after residue incorporation from the repeated-measure analysis were highly significant for all measured parameters including CO_2 evolution, MBN, NH_4^+-N, NO_3^--N, and net N mineralisation (Table 3). Significant differences between the residues of legumes (sesbania and hairy indigo) and non-legumes (dipterocarp and eucalyptus) (orthogonal contrast analysis) (Table 3) were shown in higher values of the following parameters in legumes compared to non-legumes: CO_2 evolution (Fig. 1), MBN (Fig. 2), concentrations of NH_4^+-N (Fig. 3) and NO_3^--N (Fig. 4), and net N mineralisation (Fig. 5). The CO_2 evolution rates and MBN in both legume (Figs 1*a* and 2*a*) and non-legume residues (Figs 1*b* and 2*b*) showed sharp increases, reaching their peaks during 3–7 DAI, after which they declined. From 14 DAI onwards, CO_2 evolution and MBN were relatively low. Significantly higher CO_2 evolution rates (Table 3) were found in legume compared to non-legume residues (Fig. 1*a*, *b*). A similar result to CO_2 was also observed for MBN (Fig. 2*a*, *b*). Both legumes (sesbania and hairy indigo) under each disturbance regime had significantly higher CO_2 evolution rates than their respective controls during the first 56 DAI. At the end of the observations (112 DAI), only hairy indigo under undisturbed conditions continued to have significantly higher CO_2 evolution rates than the control. Sesbania had higher CO_2 evolution rates than those of hairy indigo during the first 14 DAI (Fig. 1*a*) but, after 28 DAI, the opposite occurred. In the non-legumes under each disturbance regime, both dipterocarp and eucalyptus had significantly higher CO_2 evolution rates than their respective controls throughout the study period (Fig. 1*b*). The exceptions were observed in dipterocarp under disturbed conditions at 7 DAI and under undisturbed conditions at 14 DAI.

The NH_4^+-N concentrations in the soil amended with legume residues rapidly increased in the first 7 DAI, were relatively stable during 7–28 DAI, then dropped sharply until 56 DAI, and remained stable to 112 DAI (Fig. 3*a*). In contrast, the NO_3^--N concentrations gradually increased up to 56 DAI after which they remained stable until 112 DAI (Fig. 4*a*). Disturbing the soils led to significant increases in soil NH_4^+-N amended with sesbania at 14 and 56 DAI, and those with hairy indigo at 14 DAI, compared to their undisturbed counterparts (Fig. 3*a*). In contrast to NH_4^+-N, soil disturbance in the legume treatments decreased NO_3^--N concentrations to levels below those of their undisturbed counterparts. Significant differences in NO_3^--N concentrations were found between disturbed and undisturbed conditions for sesbania and hairy indigo at 7 and 14 DAI. The effects were more pronounced during the early than later stages of decomposition, which is reflected in the significant residue type × decomposition time effects on NH_4^+-N and NO_3^--N concentrations (Table 3). Non-legume residues did not show any sharp changes in NH_4^+-N and NO_3^--N concentrations during the decomposition period. Soil disturbance decreased NO_3^--N concentrations in the non-legume amended soil below that of the undisturbed

Table 3. Analysis of variance, repeated-measure analysis, and orthogonal contrast pertaining to effects of soil disturbance, residue type, time after residue incorporation, and their interactions on CO_2 evolution rate, microbial biomass N, mineral N (NH_4^+-N and NO_3^--N), and net N mineralisation

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; and ns = not significantly different (*F*-test).

Source of variance	Period (DAI)	df	*P*-values				
			CO_2 evolution rate (mg CO_2-C kg^{-1} dry soil day^{-1})	Microbial biomass N (mg N kg^{-1} soil)	NH_4^+-N (mg N kg^{-1} soil)	NO_3^--N (mg N kg^{-1} soil)	Net N mineralisation (mg N kg^{-1} soil)
Two-way ANOVA							
Soil disturbance (D)		1	***	ns	***	ns	ns
Residue type (R)		4	***	***	***	***	***
D × R		4	ns	ns	***	ns	ns
Repeated-measure analysis							
Treatment (Trt)		9	***	***	***	***	***
Time (T)		5	***	***	***	***	***
Trt × T		45	***	**	***	***	***
Orthogonal contrast							
Legumes vs	3	20	***	***	***	***	***
non-legumes	7	20	***	***	***	**	***
	14	20	***	***	***	***	***
	28	20	***	***	***	***	***
	56	20	***	***	***	***	***
	112	20	ns	***	***	***	***



Soil amendment	DAI					
	3	7	14	28	56	112
Unamended – Dist	c	d	d	b	c	b
Unamended + Dist	c	d	d	b	c	b
S. grandiflora – Dist	a	ab	a	a	b	ab
S. grandiflora + Dist	a	a	ab	a	b	ab
I. hirsuta – Dist	b	c	c	a	ab	a
I. hirsuta + Dist	b	bc	b	a	a	ab

Soil amendment	DAI					
	3	7	14	28	56	112
Unamended – Dist	d	c	c	d	c	c
Unamended + Dist	cd	b	c	cd	c	bc
D. tuberculatus – Dist	c	b	bc	bc	b	b
D. tuberculatus + Dist	b	b	b	b	b	a
E. camaldulensis – Dist	a	a	a	b	a	a
E. camaldulensis + Dist	a	a	a	a	a	a

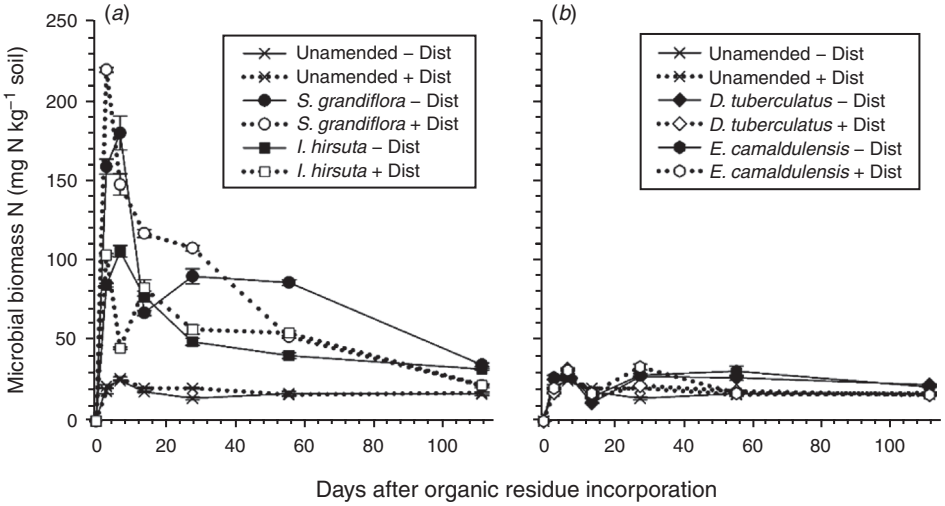
Fig. 1. The CO_2 evolution rates in the sandy Khorat soils without (unamended) and with different quality plant residues: (*a*) legumes (i.e. *Sesbania grandiflora* and *Indigofera hirsuta*) and (*b*) non-legumes (i.e. *Dipterocarpus tuberculatus* and *Eucalyptus camaldulensis*), under different levels of soil disturbance (i.e. undisturbed (- Dist) and disturbed (+ Dist) soils). The inset table accompanying each figure shows comparisons of plant residue treatments (legumes or non-legumes) at each time interval or each period of days after residue incorporation (DAI). Similar letters within a DAI are not significantly different ($P \leq 0.05$; Fisher's least significant difference). Vertical bars represent s.e.m. Note: there are significant differences in CO_2 rates between (*a*) legumes and (*b*) non-legumes ($P \leq 0.05$; orthogonal contrast) (Table 3).

treatment for dipterocarp at 14 DAI (Fig. 4*b*). During the later stage of the incubation period, NH_4^+-N and NO_3^--N concentrations in the disturbed and undisturbed treatments did not change. In summary, the effects of soil disturbance on changes in NH_4^+-N and NO_3^--N concentrations were more pronounced in the legume residues, especially in the early stage of decomposition, than in the non-legume residues. Net N mineralisation occurred immediately after the incorporation of legume residues and continued throughout the decomposition period (Fig. 5*a*). In addition, the disturbed condition showed trends of enhancing net N mineralisation compared to the undisturbed counterparts in both legume residues in 56 DAI. In contrast to the legumes, only net N immobilisation occurred under non-legume residues (Fig. 5*b*).

Effects of residue chemical quality parameters on mineral N concentration

Most residue quality parameters were correlated with soil concentrations of NH_4^+-N and NO_3^--N, with the exception of L (Table 4). C, Pp, and cellulose, as well as the ratios of C : N, L : N, Pp : N, and (L+Pp) : N were all negatively correlated with both soil NH_4^+-N and NO_3^--N. In contrast, the residue N contents were positively correlated with mineral N. It is notable that although L by itself was not significantly correlated with soil concentrations of NH_4^+-N and NO_3^--N, but L : N was. The C had the highest negative correlations with both NH_4^+-N and NO_3^--N. However, Pp was the recalcitrant carbonaceous compound that exhibited higher correlations with both NH_4^+-N and NO_3^--N than the other recalcitrant C compound, L (Table 4). Additionally, the interaction between Pp and N, represented by Pp : N, showed similar correlations with both NH_4^+-N and NO_3^--N to those of Pp alone; in addition, Pp : N had higher correlation coefficients than L : N and (L+Pp) : N.

Multiple regression analysis showed that the residue quality parameters that exerted significant influence on N mineralisation were consistently C, N, and Pp in all decomposition periods and disturbance regimes (Table 5). In the early period, the ammonification process as indicated by soil concentrations of NH_4^+-N, showed a multiple regression equation with higher R^2 in disturbed ($R^2 = 0.918$, $P \leq 0.001$; Eqn 5.3) than undisturbed ($R^2 = 0.880$, $P \leq 0.001$; Eqn 5.1) soils. In contrast, the nitrification process as indicated by soil concentration of NO_3^--N, was more influenced by these quality parameters under undisturbed ($R^2 = 0.607$, $P \leq 0.001$; Eqn 5.2) than disturbed conditions ($R^2 = 0.411$, $P \leq 0.001$; Eqn 5.4). During the later phase of decomposition (14–112 DAI), the



Soil amendment	DAI					
	3	7	14	28	56	112
Unamended – Dist	e	e	d	e	d	c
Unamended + Dist	c	e	d	e	c	c
S. grandiflora – Dist	b	a	a	a	b	a
S. grandiflora + Dist	a	b	a	a	b	b
I. hirsuta – Dist	d	c	b	d	c	a
I. hirsuta + Dist	c	d	b	c	b	b

Soil amendment	DAI					
	3	7	14	28	56	112
Unamended – Dist	b	d	ab	d	b	b
Unamended + Dist	bc	cd	a	c	b	b
D. tuberculatus – Dist	a	ab	e	b	a	a
D. tuberculatus + Dist	c	a	d	c	b	b
E. camaldulensis – Dist	a	bc	cd	b	a	a
E. camaldulensis + Dist	bc	a	bc	a	b	b

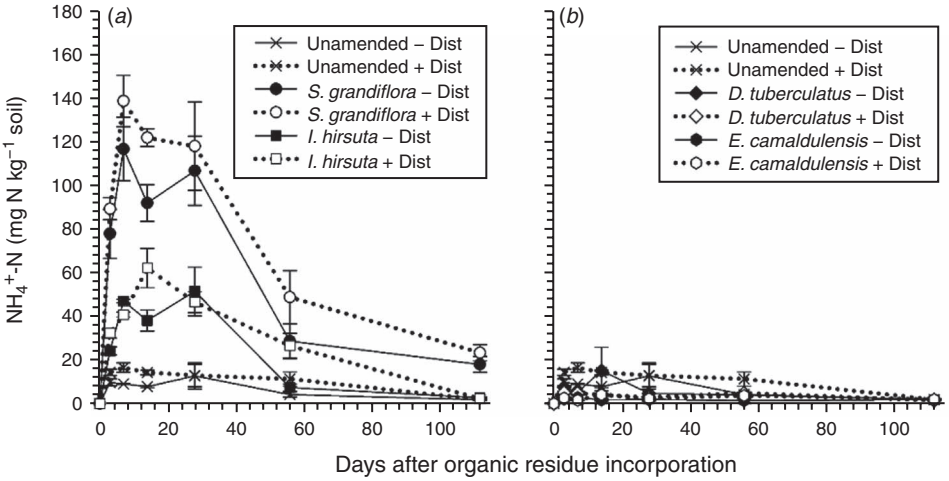
Fig. 2. Microbial biomass N in the sandy Khorat soils without (unamended) and with different quality plant residues: (*a*) legumes (i.e. *Sesbania grandiflora* and *Indigofera hirsuta*) and (*b*) non-legumes (i.e. *Dipterocarpus tuberculatus* and *Eucalyptus camaldulensis*), under different levels of soil disturbance (i.e. undisturbed (- Dist) and disturbed (+ Dist) soils). The inset table accompanying each figure shows comparisons of plant residue treatments (legumes or non-legumes) at each time interval or each period of days after residue incorporation (DAI). Similar letters within a DAI are not significantly different ($P \leq 0.05$; Fisher's least significant difference). Vertical bars represent s.e.m. Note: there are significant differences in microbial biomass N contents between (*a*) legumes and (*b*) non-legumes ($P \leq 0.05$; orthogonal contrast) (Table 3).

influence of these residue quality parameters, i.e. C, N, and Pp, on ammonification was less than during the early period in both disturbed (Eqn 5.5) and undisturbed (Eqn 5.7) conditions (Table 5). Residue quality had a greater influence on nitrification during the later compared to the early stage in both undisturbed (Eqn 5.6 (R^2 = 0.700, $P \leq 0.001$ vs 5.2 (R^2 = 0.607, $P \leq 0.001$)) and disturbed (Eqn 5.8 (R^2 = 0.688, $P \leq 0.001$) vs 5.4 (R^2 = 0.411, $P \leq 0.001$)) conditions (Table 5). Although C had the most significant regression coefficients (Eqns 5.1, 5.3), signifying its dominant influence on ammonification especially in the early stage of decomposition, it is an aggregated variable of many types of carbonaceous compounds. In contrast, Pp is the sole C compound to have highly significant regression coefficients, signifying its influence on ammonification in the early stage (Eqns 5.1, 5.3). It also consistently showed more significant regression coefficients than N (Eqns 5.1, 5.3), another variable identified as influencing N mineralisation. In order to identify the particular influence of residue Pp contents on N mineralisation, non-linear regression analysis was employed (Table 6). In both undisturbed and disturbed conditions, Pp contents had a greater influence on soil concentrations of NH_4^+-N (ammonification) in the early stage (R^2 = 0.867 and 0.897 for undisturbed and disturbed conditions respectively, both $P \leq 0.001$; Eqns 6.1 and 6.3) than later stage (R^2 = 0.391 and 0.498 for undisturbed and disturbed conditions respectively, both $P \leq 0.001$; Eqns 6.5 and 6.7) of decomposition (Table 6). The opposite was the case for soil concentration of NO_3^--N (nitrification) (Eqn 6.2 vs 6.6 and 6.4 vs 6.8) where greater influence was found in later compared to early stages. The regression analysis showed that Pp had a negative influence on soil concentrations of NH_4^+-N and NO_3^--N (Table 6).

Discussion

N mineralisation was more affected by residue quality and soil disturbance in the early than the later stage of decomposition

Plant residue decomposition can be divided into two stages: a rapid decomposition phase during the first 14 days and a slower decomposition phase from 14 DAI onwards. The division was based on the rates of change of microbial activity as indicated by the CO_2 evolution rate (Fig. 1) and MBN (Fig. 2). During the early stage of decomposition, there was an abundance of labile substrates in the forms of soluble sugars, starch, polysaccharides, and proteins available to microbial decomposers which promoted their activities and growth as



Soil amendment	DAI					
	3	7	14	28	56	112
Unamended – Dist	d	c	e	c	d	b
Unamended + Dist	cd	c	e	c	b-d	b
S. grandiflora – Dist	a	a	b	a	b	a
S. grandiflora + Dist	a	a	a	a	a	a
I. hirsuta – Dist	bc	b	d	b	cd	b
I. hirsuta + Dist	b	b	c	bc	bc	b

Soil amendment	DAI					
	3	7	14	28	56	112
Unamended – Dist	b	b	ns	ns	b	ab
Unamended + Dist	a	a	ns	ns	a	ab
D. tuberculatus – Dist	bc	c	ns	ns	b	ab
D. tuberculatus + Dist	cd	cd	ns	ns	b	b
E. camaldulensis – Dist	e	cd	ns	ns	b	ab
E. camaldulensis + Dist	de	a	ns	ns	b	a

Fig. 3. The NH_4^+-N concentrations in the sandy Khorat soils without (unamended) and with different quality plant residues: (*a*) legumes (i.e. *Sesbania grandiflora* and *Indigofera hirsuta*) and (*b*) non-legumes (i.e. *Dipterocarpus tuberculatus* and *Eucalyptus camaldulensis*) under different levels of soil disturbance (i.e. undisturbed (- Dist) and disturbed (+ Dist) soils). The inset table accompanying each figure shows comparisons of plant residue treatments (legumes or non-legumes) at each time interval or each period of days after residue incorporation (DAI). Similar letters within a DAI are not significantly different ($P \leq 0.05$; Fisher's least significant difference). Vertical bars represent s.e.m. Note: there are significant differences in NH_4^+-N concentrations between (*a*) legumes and (*b*) non-legumes ($P \leq 0.05$; orthogonal contrast) (Table 3).

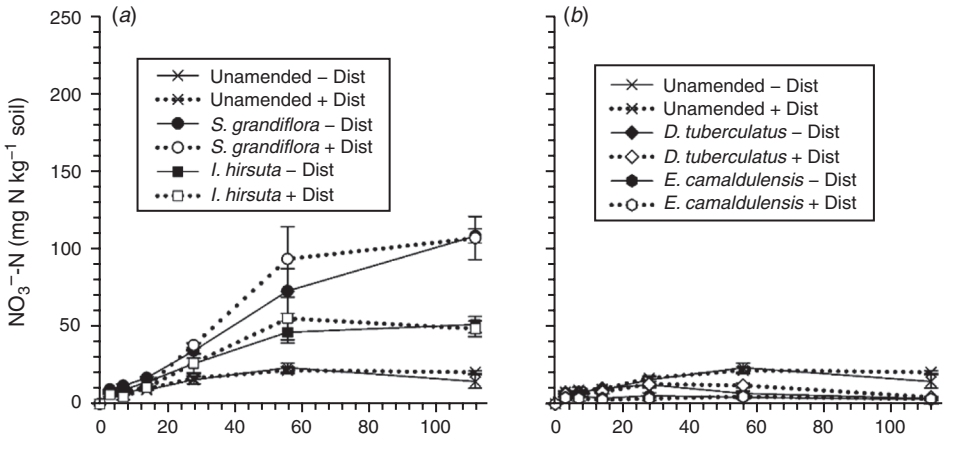
shown by the CO_2 evolution rate and MBN respectively (Benbi and Richter 2002). During the later stage of decomposition (14 DAI onwards), not only were labile substrates exhausted but also the presence of Pp resulted in reduction in microbial activity and growth compared to the early stage (Sall *et al*. 2003). Similar patterns of change with time in microbial activities, MBN, and ammonification reflected utilisation of C compounds by heterotrophic microorganisms and the transformation of some organic C and N into CO_2 and NH_4^+ and immobilisation into microbial biomass (Benbi and Richter 2002). The pattern of nitrification (Fig. 4) countered that of ammonification (Fig. 3). The two decomposition periods were more pronounced with the addition of legume residues compared to the non-legume treatments. Similar decomposition stages for different types of leaf litter, applied to a sandy-loam tropical soil, were also presented by Sall *et al*. (2003). Swift *et al*. (1979) and Coûteaux *et al*. (1998) found that decomposition processes in the early stages were regulated by soluble phenolic compounds. Whereas Giller and Cadisch (1997) stated that N mineralisation and immobilisation in the early stage was related to the N content and C : N of plant residues.

In the current study, ammonification was heavily regulated by residue quality in the early stage of decomposition (0–14 DAI). Residue quality as well as the soil disturbance regime regulated N mineralisation in the early stage of decomposition through inhibition of nitrification in legume residues.

Nitrification inhibition can result from increases in soil pH. Most ammonium-producing microorganisms and ammonium-oxidising bacteria, such as *Nitrosomonas* and *Nitrobacter* prefer a near-neutral pH (Roberson and Groffman 2015); however, in acid soils, archaea have been recognised as the key nitrifiers (He *et al*. 2012; Roberson and Groffman 2015). We found significantly higher soil pH in the early stage of decomposition in legume treatments under disturbed than undisturbed treatments (data not shown). This inhibition effect on archaeal activity with respect to increases in soil pH corroborated results from He *et al*. (2012) and Nicol *et al*. (2008). In addition to the pH effects, NH_4^+ toxicity may have inhibited the nitrification process (He *et al*. 2012) under soil disturbance.

Residue chemical quality interacts with soil disturbance in regulating N mineralisation

The biochemical properties of plant residues (e.g. N, L, Pp, cellulose, C : N, L : N, Pp : N, and (L+Pp) : N) have been found to regulate soil N mineralisation sub-processes including ammonification and nitrification, and can affect N availability (Handayanto *et al*. 1997; Palm *et al*. 2001; Puttaso *et al*. 2011). The interaction of these quality parameters with soil disturbance has not been found in the past (Lupwayi *et al*. 2004*b*; Thomsen and Sorensen 2006; Van



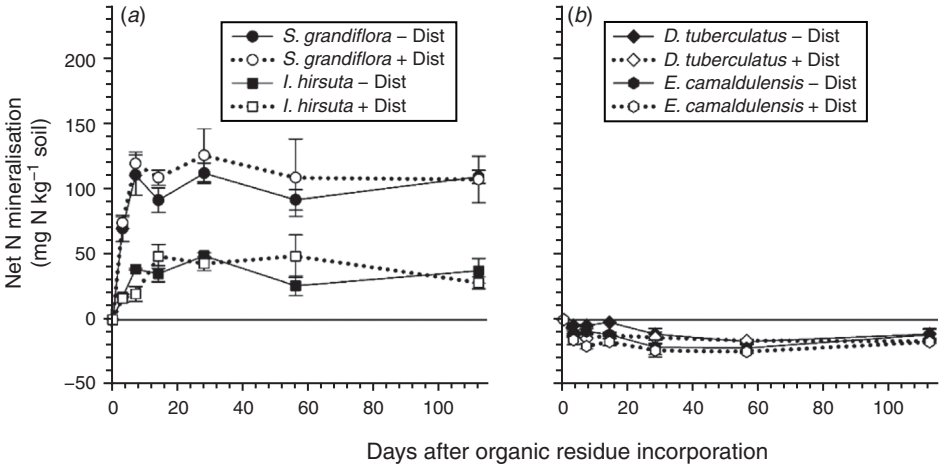
Soil amendment	DAI					
	3	7	14	28	56	112
Unamended – Dist	ab	a-c	b	b	cd	c
Unamended + Dist	ab	ab	b	b	d	c
S. grandiflora – Dist	a	a	a	a	ab	a
S. grandiflora + Dist	ab	bc	b	a	a	a
I. hirsuta – Dist	ab	ab	a	ab	b-d	b
I. hirsuta + Dist	b	c	b	ab	bc	b

Soil amendment	DAI					
	3	7	14	28	56	112
Unamended – Dist	a	ab	ab	a	a	b
Unamended + Dist	a	a	ab	a	a	a
D. tuberculatus – Dist	a	ab	a	a	bc	c
D. tuberculatus + Dist	ab	a-c	b	a	b	c
E. camaldulensis – Dist	b	bc	c	b	c	c
E. camaldulensis + Dist	b	c	c	b	c	c

Fig. 4. The NO_3^--N concentrations in the sandy Khorat soils without (unamended) and with different quality plant residues: (*a*) legumes (i.e. *Sesbania grandiflora* and *Indigofera hirsuta*) and (*b*) non-legumes (i.e. *Dipterocarpus tuberculatus* and *Eucalyptus camaldulensis*) under different levels of soil disturbance (i.e. undisturbed (- Dist) and disturbed (+ Dist) soils). The inset table accompanying each figure shows comparisons of plant residue treatments (legumes or non-legumes) at each time interval or each period of days after residue incorporation (DAI). Similar letters within a DAI are not significantly different ($P \leq 0.05$; Fisher's least significant difference). Vertical bars represent s.e.m. Note: there are significant differences in NO_3^--N concentrations between (*a*) legumes and (*b*) non-legumes ($P \leq 0.05$; orthogonal contrast) (Table 3).

Den Bossche *et al*. 2009). In the present study, this interaction was limited to the enhancement of NH_4^+-N (Table 3) in legume residues only (Fig. 3). Legumes are highly degradable due to their low leaf toughness related to their low L concentration. Leaf toughness is correlated with L contents and has been a characteristic used to determine leaf decomposition by various invertebrates and microbial decomposers (Graça and Zimmer 2005). Legume residues had lower L : N than non-legume residues. We found negative correlation between L : N of the residues and N mineralisation (NH_4^+-N and NO_3^--N concentrations) (Table 4) but residue L content alone did not correlate with N mineralisation. The L : N has often shown negative correlations with organic residue decomposition (Handayanto *et al*. 1997; Palm *et al*. 2001; Puttaso *et al*. 2011). Lignin retards decomposition rates due to its physical protection of not only C compounds (Melillo *et al*. 1982) but also, to a lesser extent, N compounds (Talbot and Treseder 2012). Lignin also biochemically protects polysaccharides (cellulose and hemicelluloses) by interfering with enzymes degrading polysaccharides (Melillo *et al*. 1982) through competitive adsorption of L to cellulase enzymes (Guo *et al*. 2014; Li and Zheng 2017). Lignin can also be adsorbed to substrates (e.g. cellulose) (Vermaas *et al*. 2015). In contrast to residue C, residue N also enhances decomposition through alleviating N limitation to microbial decomposers (Talbot and Treseder 2012).

Correlation analysis did not produce a clear picture of the relationship of residue quality parameters to N mineralisation (i.e. C, Pp, C : N, Pp : N, and (L+Pp) : N) as they all had comparable negative correlations. However, stepwise multiple regression identified three quality parameters (C, N, and Pp) as joint factors controlling N mineralisation, both ammonification and nitrification (Table 5). The C content of residues is an aggregated quality parameter which does not distinguish various types of carbonaceous compounds. The multiple regression analysis again highlighted the dominant roles of recalcitrant carbonaceous compounds, Pp, and N compounds. The Pp, particularly condensed tannins, have a high protein-binding capacity to form Pp–protein complexes that slow the decomposition rate and delay N release to the soil (Handayanto *et al*. 1997; Mutabaruka *et al*. 2007). Condensed tannins are highly active and have a higher protein affinity relative to soluble tannins (Handayanto *et al*. 1997; Mutabaruka *et al*. 2007). Protein-binding capacity can be measured by the reaction of Pp with bovine serum albumin (BSA). Vityakon and Dangthaisong (2005) found sesbania foliage to have protein-binding capacity 1.6-fold lower than the litter of dipterocarp (a forest tree) with 97 and 154 µg BSA mg^{-1} plant dry weight respectively. The two legume residues would likely have less condensed tannin. For example, Seresinhe *et al*. (2012) reported that the condensed tannin content of foliage of non-legume trees was 4.9% for *Ceiba perntandra* and 6.2% for *Carallia*



Soil amendment	DAI					
	3	7	14	28	56	112
S. grandiflora – Dist	a	a	a	a	ab	a
S. grandiflora + Dist	a	a	a	a	a	a
I. hirsuta – Dist	b	b	b	b	c	b
I. hirsuta + Dist	b	b	b	b	bc	b

Soil amendment	DAI					
	3	7	14	28	56	112
D. tuberculatus – Dist	a	a	a	ns	a	ns
D. tuberculatus + Dist	ab	bc	bc	ns	a	ns
E. camaldulensis – Dist	ab	a	b	na	ab	ns
E. camaldulensis + Dist	b	c	c	na	b	ns

Fig. 5. Net N mineralisation in the sandy Khorat soils amended with different quality plant residues: (*a*) legumes (i.e. *Sesbania grandiflora* and *Indigofera hirsuta*) and (*b*) non-legumes (i.e. *Dipterocarpus tuberculatus* and *Eucalyptus camaldulensis*) under different levels of soil disturbance (i.e. undisturbed (- Dist) and disturbed (+ Dist) soils). The inset table accompanying each figure shows comparisons of plant residue treatments (legumes or non-legumes) at each time interval or each period of days after residue incorporation (DAI). Similar letters within a DAI are not significantly different ($P \leq 0.05$; Fisher's least significant difference). Vertical bars represent s.e.m. Note: there are significant differences of net N mineralisation between (*a*) legumes and (*b*) non-legumes ($P \leq 0.05$; orthogonal contrast) (Table 3).

Table 4. Pearson correlation coefficients of initial concentrations of chemical constituents (quality) of plant residues as related to soil mineral N (NH_4^+-N and NO_3^--N) concentrations (n = 144)

Abbreviations: C, carbon; N, nitrogen; L, lignin; Pp, polyphenols; Cell, cellulose. * $P \leq 0.05$; ** $P \leq 0.01$; and ns = not significantly different

Soil mineral N	C	N	L	Pp	Cell	C : N	L : N	Pp : N	(L+Pp) : N
NH_4^+-N (mg N kg^{-1} soil)	–0.790**	0.759**	0.141^{ns}	–0.750**	–0.439**	–0.740**	–0.639**	–0.744**	–0.720**
NO_3^--N (mg N kg^{-1} soil)	–0.536**	0.487**	0.125^{ns}	–0.509**	–0.232**	–0.467**	–0.388**	–0.485**	–0.450**

Table 5. Stepwise multiple regression analysis and coefficients showing effects of residue chemical properties, i.e. carbon (C), nitrogen (N), and polyphenol (Pp), on soil mineral nitrogen (NH_4^+-N and NO_3^--N) concentrations in the early (0–14 days after residue incorporation) and later stages (14–112 days after residue incorporation) of decomposition under different soil disturbance regimes (n = 36)

*$P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Dependent variable	Independent variables				R^2	P-value	Equation no.
	y-intercept	C	N	Pp			
				0–14 days after residue incorporation			
Undisturbed soil							
NH_4^+-N	4075***	–9.16***	–1.62**	2.27***	0.880	***	5.1
NO_3^--N	43.4	–0.063	–0.071	–0.080	0.607	***	5.2
Disturbed soil							
NH_4^+-N	5072***	–11.4***	–1.62**	3.00***	0.918	***	5.3
NO_3^--N	11.5	0.005	–0.096	–0.085	0.411	***	5.4
				14–112 days after residue incorporation			
Undisturbed soil							
NH_4^+-N	2142*	–4.83*	–0.710	1.24	0.400	***	5.5
NO_3^--N	1974*	–4.44*	–0.191	1.07	0.700	***	5.6
Disturbed soil							
NH_4^+-N	2730*	–6.16*	–0.845	1.63*	0.509	***	5.7
NO_3^--N	2315*	–5.19*	–0.447	1.217	0.688	***	5.8

Table 6. Non-linear regression analyses showing effects of polyphenol (Pp) content of residues on soil mineral nitrogen (NH_4^+-N and NO_3^--N) concentrations and ratios of NH_4^+-N and NO_3^--N to microbial biomass nitrogen (MBN) in the early (0–14 days after residue incorporation) and later stages (14–112 days after residue incorporation) (n = 36)

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Regression equation		R^2	P-value	Equation no.
0–14 days after residue incorporation				
Undisturbed soil				
NH_4^+-N	$= -1.95 + (912/Pp)$	0.867[A]	***	6.1
NO_3^--N	$= 9.09 - 0.076Pp - 0.0001Pp^2$	0.598[B]	***	6.2
Disturbed soil				
NH_4^+-N	$= -5.00 + (1141/Pp)$	0.897[A]	***	6.3
NO_3^--N	$= 6.94 - 0.042Pp - 0.0004Pp^2$	0.380[B]	***	6.4
14–112 days after residue incorporation				
Undisturbed soil				
NH_4^+-N	$= -1.14 + (490/Pp)$	0.391[A]	***	6.5
NO_3^--N	$= 16.7 - 0.753Pp - 0.010Pp^2$	0.700[B]	***	6.6
Disturbed soil				
NH_4^+-N	$= 6.98 - 0.660Pp + 0.011Pp^2$	0.498[B]	***	6.7
NO_3^--N	$= 18.5 - 0.825Pp - 0.010Pp^2$	0.686[B]	***	6.8

[A]Inverse first order non-linear regression
[B]Quadratic non-linear regression

integerrima, while the tannin content for foliage of legume trees ranged from 0.78% for *Gliricidia sepium* to 1.58% for *Leucaena leucocephala*. The Pp content of the residues consistently showed a more significant influence on ammonification compared to N content (Table 5). Non-linear regression analysis highlighted the high negative influence of Pp in controlling ammonification, especially in the early stage of decomposition. The regression equations with Pp as the sole influencing factor could explain up to 90% of the variation in NH_4^+-N contents produced under disturbed conditions.

Conclusions

Our results support the hypothesis of an interaction of residue quality and soil disturbance affecting N mineralisation. High-quality residues (fresh legume leaves) were more affected by soil disturbance than low-quality residues (non-legume leaf litter) in both the ammonification and nitrification components of N mineralisation. Ammonification was enhanced under disturbed condition in legume amendments during the early stages (first 14 days) of decomposition. In contrast, nitrification was inhibited by disturbance under legume residues. When the soil was disturbed, the low Pp content of the legumes led to higher nitrification inhibition effects compared to undisturbed condition. This highlights the significant effects that the residue Pp contents have in controlling ammonification and nitrification under disturbed conditions. The findings of this study showed that minimal soil disturbance should be adopted with legume amendments. An undisturbed condition is more conducive to reducing N mineralisation and hence increasing soil N sequestration under legume-residue amendments. For the non-legume residue amendments, soil disturbance has little impact on N mineralisation, with immobilisation occurring during decomposition. The application of non-legume residues is more suitable for the purpose of soil surface cover for soil conservation. To enhance their decomposition, mixing of the non-legume (low quality) residues with legume (high quality) residues can be recommended. The mixture alleviates N deficiency, which can enhance microbial decomposition, leading to soil organic C and N stabilisation and conservation which is desirable for improving productivity and sustainability of degraded tropical sandy soils.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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