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Influence of Urea and Ammonium Sulfate on Potential Mineralization and Nitrification Rate in Tropical Peat Soil from Oil Palm Cultivation under Lab Condition

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ABSTRACT: *Mineralization and nitrification rate can vary between types of nitrogen (N) fertilizers. In oil palm cultivation, urea and ammonium sulphate (AS) are the two most widely used source of N. However, the mineralization and nitrification rates and patterns under peat soil are still not understood. A 60 days lab incubation, with urea and AS was conducted to understand the mineralization and nitrification rates at 80% water holding capacity (WHC) and 25°C. The N rates tested were 0, 0.11 g, 0.22 g and 0.44 g N per 250 g soil. Soil were sub-samples at day 0, 3,7, 14, 28, 45 and 60. Nitrification happened at very low pH with no N fertilizer added. Addition of urea accelerated nitrification and increases the soil pH at early stage of incubation. AS tend to lower the nitrification status and inhibit nitrification at higher rates (0.22 g N). At the end of the incubation, AS accumulated more inorganic N for N1, N2 and N3 compared to urea added treatment. Urea added treatment have more inorganic N at the start of the incubation, however after it peak at 26 days most of the inorganic are loss presumably via gasses emission. Urea double the potential nitrification rate (PNR) at the end of incubation, however PNR of AS added samples remain the same throughout the incubation period. Urea tends to stimulate nitrification in peat soil. These finding indicated that differential response of N mineralization and nitrification to different input and levels of ammonium fertilizer in tropical peat soil which is important for maximizing N use efficiency. It should be pointed out that the reason for urea being widely used as an N fertilizer worldwide is its lower price per unit N due to its high N content (46% N), compared to those of ammonium nitrate (35% N) and AS (21% N), which reduces the transportation cost of urea–N fertilizer. However, other factors such as its efficiency should also be taken into consideration when choosing N fertilizer as supply.*

Keyword: *urea, ammonium sulphate, mineralization, nitrification, tropical peat soil, oil palm*

INTRODUCTION

The oil palm in Malaysia usually grown on problematic soils and requires large quantities of N fertilizers to achieve good yields. Tropical peat soil has been extensively planted with oil palm. The soil is characterized as highly acidic (pH <4.5) and contain high amount of organic-N (Comte, Whalen, and Gru 2012). However, external N is still needed to ensure crop growth well and produce sustained yields (Mutert, Fairhurst, and Uexküll 1999).

Study have shown that only 50% of the applied N is taken up by plants after fertilizer application and the remainder are lost either via gasses (N_2 and N_2O) to the atmosphere or leached out of the system primarily as nitrate (Cameron, Di, and Moir 2013). This losses contributed by nitrification process in N cycle which is microbial mediated where ammonia/ or ammonium from mineralization of organic matter or fertilizers are oxidized to nitrate. This single process is the most important reaction controlling different type of N loss mechanisms in soil-plant system (Grewal, Virk, and Khind 1999; Subbarao et al. 2006).

Nitrogen mineralization and nitrification are important soil processes in determining the availability of inorganic N to crops (Fageria and Baligar 2005). The factors affecting the rates of these processes and the relationships among biological, physical and chemical factors are reasonably well understood (Ollivier et al. 2011). However, most of the data and understanding of these processes are

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from sites from limited geographical coverage, soil type and crops species.

Nitrification in soil can varies with the type N fertilizers. The input of urea into soil can stimulate nitrification and accelerate nitrification (Tong and Xu 2012; Zhao and Xing 2009) whereas, AS application may stimulate or inhibit nitrification (Chien 2009; Zhao and Xing, 2009). The dynamic effects of different NH_4^+ - based fertilizers and their rates on mineralization and nitrification in tropical peat soil under oil palm cultivation are not well understood.

Therefore, urea and AS which are are two main source used in oil palm cultivation were evaluated in this study to understand the dynamic of NH_4^+, NO_3^-, inorganic N(Ni) and potential nitrification rate (PNR). Awareness of the need to improve our understanding on N cycling after N fertilizer application is important in order to develop fertilizer management for oil palm cultivation in peat soil. Therefore, information on nitrification rate is essential to understand N cycle in oil palm in order to provide knowledge for fertilizer management.

MATERIALS AND METHODS

Soil sampling and analysis

The peat soil used is taken from topsoil (0-10cm) in weeded circle area of matured oil palm (5 years old) cultivation located at Ladang Sg. Samak, Malaysian Palm Oil Board (MPOB) Research Station in Teluk Intan, Perak, Malaysia. Conventional fertilization has been carried out in weeded circle (WC) zone which received one kg urea palm^{-1} year^{-1}. The soil is classified as Penor Series, Terric Sulfisaprist (USDA Classification 2010). The peat water level was kept 60 cm from the surface. The soil samples are placed in a container with ice and immediately transported to the lab. Visible organic matter such as roots and leaf residues those are thicker than 2 mm were removed manually. The soil samples are then sieved (4.75 mm) and mixed thoroughly before being stored at 4ºC until usage within one week.

Incubation experiments

The soil samples used in the incubation experiment were air-dried and then adjusted to 80% water holding capacity before they were pre-incubate for one week. Next, soil samples (250 g) are placed in plastic container, and urea or ammonium sulphate were added and mixed thoroughly (0, 1.1, 2.2 and 4.4 kg/palm). Deionized water was added to adjust soil moisture to 80% water holding capacity. The entire container were covered with punctured cap to maintain aerobic condition and incubated at 25±2°C for 58 days. Any water loss from evaporation was replaced using deionized water every 3 days. Soils were sub-sampled at 0, 3, 7, 14, 28, 45 and 60 days for NH_4^+ and NO_3^-. There were three replicates for each treatment with control unfertilized. Initial sampling (day 0) were done three hours after the soil were mixed with urea or AS. The soils are then also analyzed for pH (day 0 and day 60). After 60 days of incubation the soil samples were removed from the cup and then determined for potential nitrification rate (PNR).

Soil analysis before and after incubation

The soil samples before incubation were analyzed for pH (1:10 w/w) (Metson, 1971); moisture content (24 hr oven dried at 60°C); total organic carbon (TOC) and total organic N (TON) (Leco 2003) The NH_4^+, and NO_3^- were extracted with 2M KCl using a 1:10 soil:extractant ratio and a 1 hour end-over-end shake followed by filtration (Keeney and Nelson, 1982). Concentrations of NH_4^+ and NO_3^- in solution are measured using auto analyzer with Cadmium-Copper reduction column (Lachat Part No.50277). The summation of NH_4^+ and NO_3^- was referred to as inorganic N (Ni). Potential nitrification rate (PNR) (Hart *et al.*, 1994) was determined by shaking 15 g of field-moist soil sample with 100 mL working solution containing 1.5 mM NH_4^+ and 1mM PO_4^{3-} (pH 7.20). The slurry was collected at 2, 6, 20 and 24 h; filtered and analyzed for NH_4^+ and NO_3^- concentration using auto analyzer with Cadmium-Copper reduction column (Lachat Part No.50277). PNR was determined before and after incubation, whereas soil pH was determine at initial incubation (day 0) and final day of incubation (day 60). Selected soil physicochemical properties of the soil used are listed in table 1 below.

STATISTICAL ANALYSES

SAS was used for statistical analysis of data and the data was subjected to analysis of variance (ANOVA).

Table 1
Selected soil physicochemical properties before incubation

pH	*Soil moisture (%)*	*TC (%)*	*TN (%)*	*C/N ratio*	*NH_4^+ (mg/kg)*	*NO_3^- (mg/kg)*	*Ni (mg/kg)*	*PNR*
	48.81	49.22	1.22	40.45	1.71	0.36	2.07	0.48

RESULTS

Addition of urea resulted in increase of pH at the start of the incubation compared with control. At the start of incubation, pH of urea added treatment increase by 2.84 unit. Whereas, the treatment with AS added had lower pH values compared to urea treatment at the early stage of incubation. However, after 60 days of incubation pH of urea and AS added treatments decreased sharply below the initial level. As for control treatment, both decreased slightly after 60 days of incubation. Increase in rate in both urea and AS did not significantly increase the soil pH. Nitrite was not present in any samples.

The content of soil NH_4^+ and NO_3^- of the soil samples before incubation was very low with 1.71 and 0.36 mg/kg soil, respectively. The majority of mineral N at the end of incubation was NH_4^+ in both urea and AS treatment. The AS treatment of 2 kg N palm resulted in 97% composition of NH_4^+ in total of Ni. Total NH4+, NO3- and total Ni production over the 60 day incubation differed significantly among N rates and between N sources.

The soils were incubated with urea and AS at different rates for 60 days. In terms of dynamic, increase in N rate from urea leads to more NH_4^+ .Relationship between NH_4^+ and days of incubation for U1 and U2 were failed to fit to any response curve. However, urea with N2 and N3 rates followed a quadratic response curve. Addition of urea leads to increase in NH_4^+ at the beginning of incubation before achieved maximum NH_4^+ concentration in U3 (482.57 µg g^{-1} soil) and U4 (217.03 µg g^{-1} soil) at day 18 and 17,

Table 2
pH before and after incubation, NH_4^+, NO_3^-, Ni and PNR after 60 days of incubation properties. Small letter indicate significant differences within treatment whereas big letters indicate different between treatment using Tukey test at p<0.05

N source	*Rate*	*pH_0*	*pH_{60}*	*NH_4^+ accumulated after 60 days*	*NO_3^- accumulated after 60 days*	*Total inorganic N accumulated after 60 days*	*PNR After 60 days*
Urea	0	4.28bA	4.04aA	1.02dA	0.76dA	1.78	0.46
	0.5	6.95aA	3.79aA	9.06cB	6.82cA	15.88	0.55
	1	7.17aA	3.77aA	37.91bB	16.19bA	54.10	0.80
	2	7.24aA	3.82aA	58.39aB	24.13aA	82.52	0.81
(NH4)2SO4	0	4.16aA	4.00aA	1.22dA	0.74cA	1.96	0.44
	0.5	4.25aB	3.79aA	63.56cA	3.98bB	67.54	0.48
	1	4.01aB	3.92aA	237.74bA	6.87aB	244.61	0.50
	2	4.14aB	3.90aA			198.80aA	4.03bB
202.83	0.49						

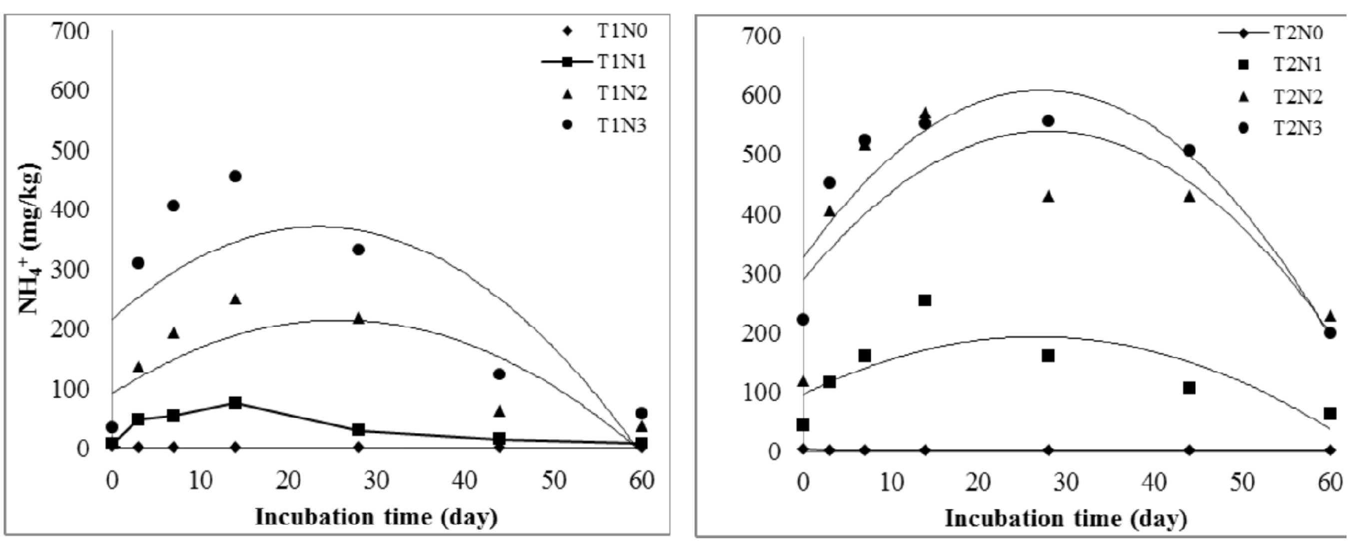

Figure 1: Dynamic of soil NH_4^+ during the incubation with urea and ammonium sulphate added. T1 and T2: urea and ammonium sulphate; N0, N1, N2 and N3: 0, 0.11, 0.22 and 0.44 g N, respectively

respectively. Without N addition (control) for both treatment, the NH_4^+ and NO_3^- remained constant at very low concentration throughout the incubation period. Following the addition of urea, the NH_4^+ concentration increased steadily with increase in rate of N. For U2 and U3 treatment, the NH_4^+ concentration of urea-N peaked at day 26 respectively. The highest NH_4^+ recorded at U3 with 454.12 on day 14. After day 26 or 27 the NH_4^+ start to steadily decline to day 60 (58.39 mg/kg).

At the start of incubation (day 0) urea produced more NH4+ in N1, N2 and N3. However, after 3 days of AS addition, N1, N2 and N3 produced more NH_4^+ concentration. For AS, increase in N rate from 0 to 1 kg N/per palm leads to increase in NH_4^+ (from 0.48 to 554.21 mg/kg). However, 2 kg N/ per palm didn't show distinct NH_4^+ difference from 2 kg N/per palm rate. As for control unit without any N sources addition, NH_4^+ remained constant with small fluctuation throughout the incubation time. Addition of AS tend to produce more NH_4^+ in longer time frame. However, AS also have the similar pattern with urea with quadratic shape but NH_4^+ peak at longer incubation days before steadily decrease. At the end, AS still have more NH_4^+ compared to urea.

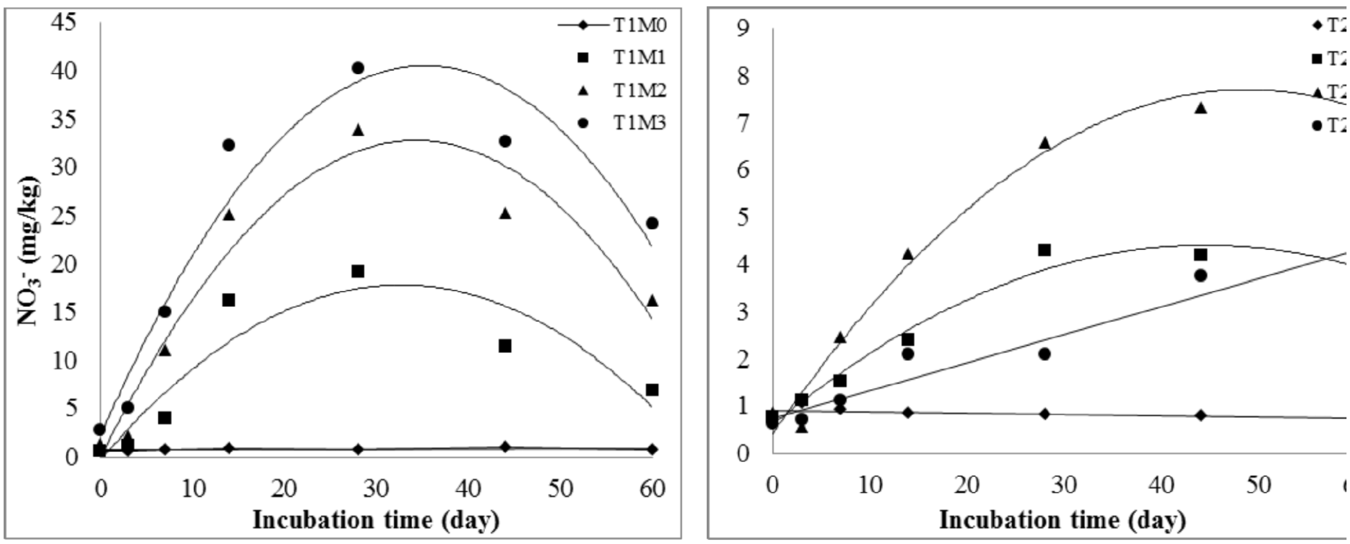

Figure 2 Dynamic of soil NO_3- during the incubation with urea and ammonium sulphate added. T1 and T2: urea and ammonium sulphate; N0, N1, N2 and N3: 0, 0.11, 0.22 and 0.44 g N, respectively

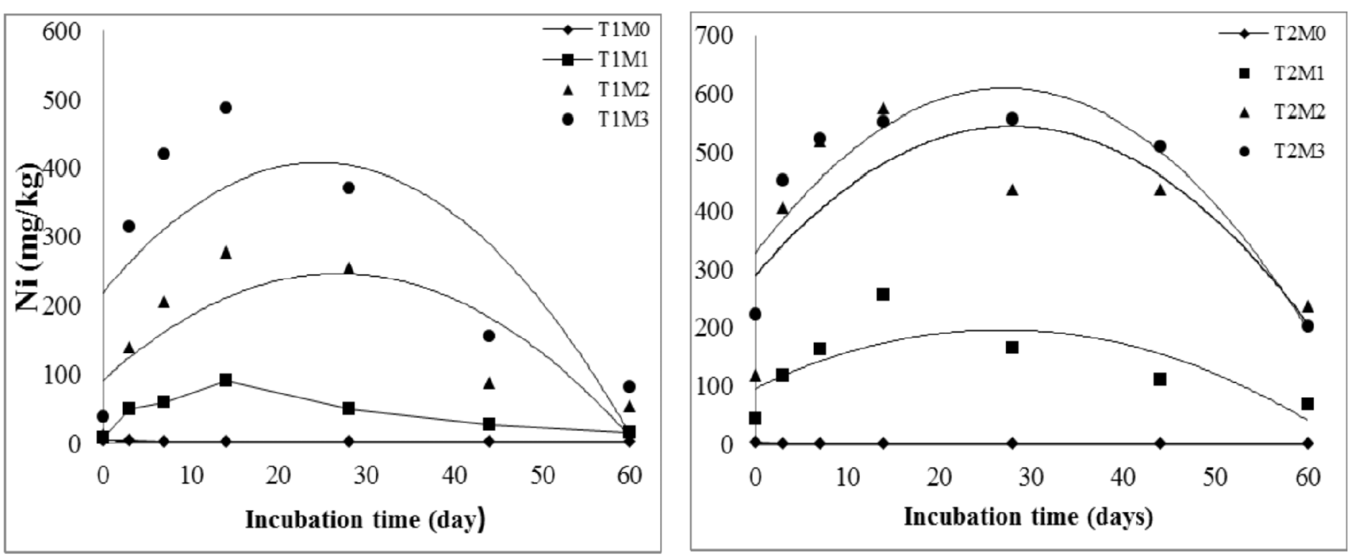

Figure 2: Dynamic of soil Ni during the incubation with urea and AS added. T1 and T2: urea and AS; N0, N1, N2 and N3: 0, 0.11, 0.22 and 0.44 g N, respectively.

Dynamic of soil NO_3^- in relation to N sources and rates are shown on figure 2. The availability of nitrate response to days of incubation, for both urea and AS treatment was quadratic except in U0 and S0 (0 kg N $palm^{-1}$) and S1. However, incubation with urea produced more NO_3^- (up to five folds higher) in N1, N2 and N3. Incubation with AS tend to inhibit nitrification process and very small amount of NO_3^- accumulated at sampling time. The maximum NO_3^- obtained from each treatment (U1, U2 and U3) of urea are 17.75, 32.72 and 40.22 mg/kg soil. Each of the rate peaks at day 33, 34 and 35, respectively. As for AS treatment, S1and S1peaked at day 43 and 48 with 4.24 and 6.88 mg/kg soil, respectively. Whereas, S3 followed a linear trend and peak at day 103 with 4.80 mg/kg soil of NO_3^-. Over applying AS tend inhibit nitrification greatly.

In terms of Ni, AS addition tend to have more Ni at each rate and incubation time. Even at the end of incubation, AS accumulated more Ni compared to urea. However, most of Ni in AS are in NH_4^+ (ranged above 90% for all rates). However, urea tends to have more balanced proportion of Ni. At the end of incubation, NO3- percentage for urea treatment ranged from 29% to 43%.

Net mineralization is defined as the accumulation of NH_4^+ at the end of experiment divided by the number of incubation days. Urea incubated soil showed a linear relationship with increase in rate of N. whereas, AS incubated soil followed a quadratic response relationship with the N rates. Net mineralization peaked at 1 kg N/palm for AS and then steadily decrease.

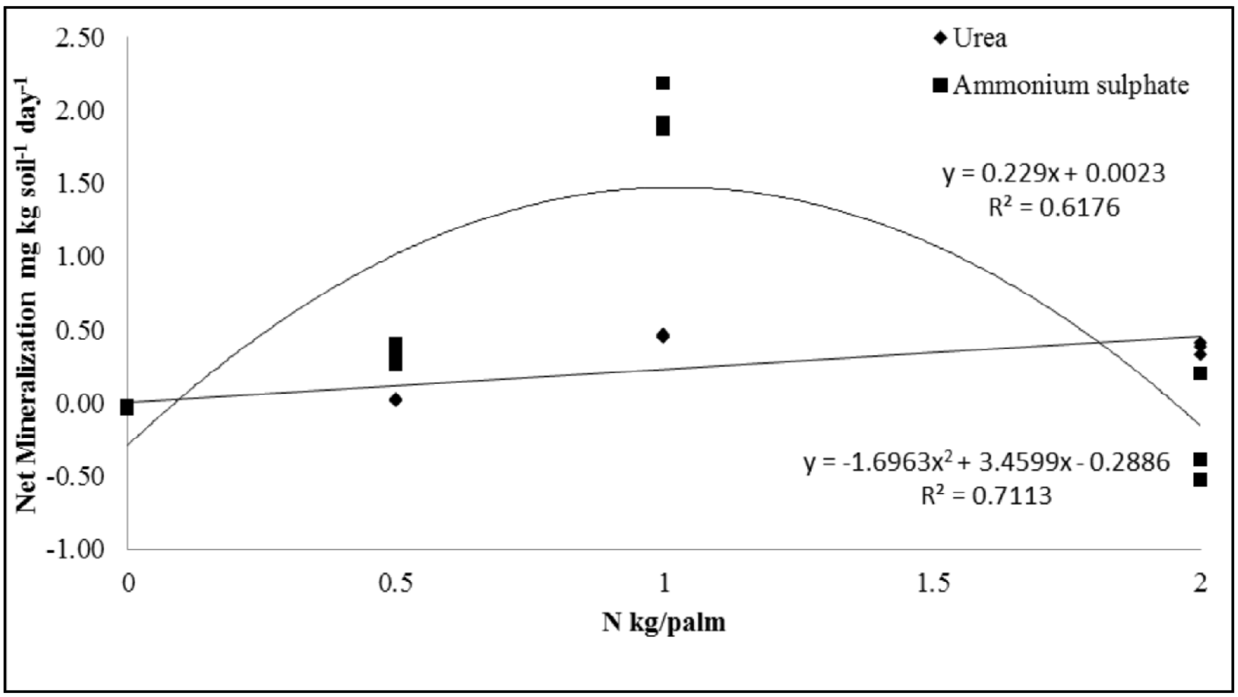

Figure 3: The relationship between rates of nitrogenous fertilizers with net N mineralization

DISCUSSION

It is interesting to note that nitrification still can occur at strongly acidic conditions and additions of substrates accelerate the nitrification process in peat soil. This result coincides with previous findings that strong nitrification occurred in acid soil with a pH range from 3.71 to 4.22 (Xue et al. 2006), and the lowest limit for nitrification could bearound pH 2.9 (Hayatsu 1993). Substantial evidence supports the role of chemolitho-autotrophic bacteria as the main nitrifying agents in most acid soils (De Boer and Kowalchuk 2001) and other microorganisms such as archaea and heterotrophs also make great contribution to nitrification in acid soils (Hayatsu et al. 2008). As for microbial from peat soil, none can be found from tropical peat soil. Our results suggest that acid tolerant nitrifiers do exist in these soils and have potential for high activity, and N-substrate (urea) most often increased nitrification. In terms of mineralization, It is speculated that the peat that been incubated were dried from 100% WHC to 80% WHC which enhanced the soil aeration and increase the mineralization.

Urea addition accelerated nitrification

This study shows that urea addition stimulates more nitrification than $(NH_4)_2SO_4$ addition and also resulted in greater pH changes during the early incubation. This result is consistent with recent study on acidic soil (Zhao and Xing 2009). At the same time, the high linear correlation between urea mineralization and net nitrification hinted that high stimulation effect. When urea was added, the pH of the microsite below the urea granule can increase above a value of 8 (Sommer *et al*., 2004). The increase in soil pH for urea-added systems was ascribed to the consumption of H^+ during urea hydrolysis process in the soil (De Vries and Breeuwsma, 1987). The hydrolysis of urea increased the soil pH at early stage which may have stimulated the nitrifiers activity and the availability of soluble substrate enhanced the nitrification status. Therefore, there was high nitrification potential in urea-added soils. This process occurs over the first few days following application; however, once nitrification processes start to become significant then the pH of the soil is decreased. At the end of incubation the two highest N rates in urea resulted in twice the amount of PNR which means that urea has doubled the nitrification activity. In addition, fertilized soils had higher nitrification activity due to the increase release of NH_4^+ from fertilizers and accelerated growth of nitrifying population (Mendum *et al.* 1999; Chu *et al.* 2008; Xue *et al.* 2009). Other studies also found that addition of urea stimulated the increase in AOB population and thus accelerated nitrification and soil acidification; while addition of AS inhibited the increase in AOB population and thus inhibited nitrification (Tong and Xu 2012). In other study, application of urea on subsoil had enhanced the growth of nitrifying bacteria was proved by viable numbers (MPN) where bacteria numbers increase after incubation with urea (Swensen and Bakken 1998). A laboratory based study on low pH soil in China suggested high-pH in combination with NH_4^+ addition stimulated nitrification was associated with increase in number of ammonia-oxidizing bacteria. Altering the pH alone using lime materials did not change the nitrification activity (Che *et al.* 2014). However, the results vary depending on the soil type, geographical location and land uses. In other study, high pH rather than N amendment stimulated the growth of AOB and their nitrifying activity in acidic (pH around 3.0) forest soil (Nugroho 2006).

Most recent study with molecular quantitave PCR approach concluded that AOB abundance was significantly higher in high N-loading treatments than control without N addition in acid soil (Shen, Zhang, and He 2014). Other results in peat soil, nitrification is enhanced in boreal peat forest with additional of N sources (Potassium Nitrate, ammonium chloride and urea) which leads to increase in N_2O and NO emission (Regina *et al.* 1998). Similar results in flooded rice systems where nitrification potential was distinctly higher in urea amended plots compared to control and green manure amended plots (Adhya *et al.* 1996). Finally, a study showed that drained N-rich peat soils may be significant sources of N_2O and NO and that their production of nitrogenous trace gases is enhanced by additional N (Regina *et al.* 1998).

AS lower nitrification status, inhibit nitrification at higher rate

Compared with other N fertilizers, AS may have some potential agronomic and environmental benefits. Overall in this study, AS accumulated more inorganic N at the end of the incubation. This is probably because AS tend to lower the nitrification rate proved by PNR at start and the end of the incubation. Therefore, more inorganic N in the form of NH_4^+ accumulated. In addition, AS addition did not increase the soil pH unlike urea and therefore minimize microbial mediated process such as nitrification. In contrast, a recent study showed addition of AS enhanced nitrification compared to urea which effectively stimulate nitrification (Zhao and Xing 2009). Again, soil type, land use and geographical location resulted in different interpretation. Other study, using NH4SO4 as soil pre-treatment before urea application significantly reduced NH_3 volatilization by half compared to urea application alone (Goos & Cruz 1999). Furthermore, replacing urea with AS at the same rate significantly reduced CH_4 emissions by 40% (Linquist *et al.* 2012). High NH_4^+ produced by AS content may inhibit nitrification (Hadi *et al.* 2000). Unlike urea, AS is a nitrogen with minimal or no surface volatilization when applied to soils. Its disadvantage is that it is the most acidifying form of N fertilizer where it requires 2 or 3 times much lime to neutralize the same amount of acidity of formed by other common N carriers.

Urea losses more N after peak

Both added substrates, showed quadratic curve response and reached a peak area before declining in concentration for both NH4+ and NO3-. In this lab incubation system, leaching is not possible since it was a closed system. Therefore, it is expected that most of inorganic N are losses via gasses emission after certain period of mineralization. Urea which is the most widely used N-fertilizers, is not considered to lost by leaching although it is a water soluble compound. This is due to high urease activity found in most top soils which rapidly hydrolyse urea to CO_2 and NH_3 (Boyd and Mortland,1985). Addition of N enhanced N cycling and emission of nitrogenous trace gas. Fertilization has commonly increase N_2O fluxes (Ref). At the same time, peat soil contained high amount of Carbon substrates which is known to be required in denitrification process. Lack of Carbon substrates known to limit denitrification (Koops et al. 1996; Sotomayor and Rice 1996). Application of urea in combination with high moisture content increase the emission of N_2O in laboratory condition (Serrano-Silva et al. 2011). This is because wetting and substrate availability increase the microbial activity of the soil. Under ureolysis, provides a mechanism for nitrification in acid soils but ceased when urea hydrolysis was complete (Burton, Prosser, and Prosser 2001). Gaseous losses of NH_3 is enhanced especially in soils with low buffering capacity and soil with high organic C content (Fenn and Hossner, 1985). However, leaching losses was the lowest with urea application alone compared urea combined with nitrification inhibitors (Gioacchini *et al.* 2002). Ammonia volatilisation losses from urea applied at <50 kgNha"1 are generally in the range of 5–15% of the N applied, whereas ammonia losses from ammonium sulphate and calcium ammoniumnnitrate are typically less than 2–3% of the N applied at this rate (Black *et al.*, 1984, 1985*a*,*b*).

CONCLUSION

Nitrification and inorganic N availability in tropical peat soil were affected by type of N fertilizers and the rates. In this study, nitrification was very low with no N fertilizer added. Addition of urea accelerated the nitrification at higher rates. Whereas, high input of AS inhibited nitrification. However, addition of AS accumulated more inorganic N. These finding indicated that differential response of N mineralization and nitrification to different input and levels of ammonium fertilizer in tropical peat soil which is important for maximizing N use efficiency. It should be pointed out that the reason for urea being widely used as an N fertilizer worldwide is its lower price per unit N due to its high N content (46% N), compared to those of ammonium nitrate (35% N) and AS (21% N), which reduces the transportation cost of urea–N fertilizer. However, other factors such as its efficiency should also be taken into consideration when choosing N fertilizer as supply.

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