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Nitrogen Uptake by Sago Palm (*Metroxylon sagu* Rottb.) in Leyte of the Philippines

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Abstract: Nitrogen uptake by sago palm with the application of nitrogen fertilizer (urea) with ^{15}N-labeled ammonium sulfate was studied in Leyte of the Philippines to estimate the effect of nitrogen fertilizers on sago palm growth in the early to middle stages. The first application of nitrogen fertilizers for determining sampling portions of leaflets was carried out at the early growth stage of sago in July of 2005, and the second nitrogen application trial for determining nitrogen uptake was done in the middle growth stage in July of 2007, after combining two 4 m x 2 m plots into one 4 m x 4 m plot. Since the nitrogen contents in the upper, middle, and basal positions of leaflets were different from the sampling in February of 2006, the middle leaflet of the third sago leaf was used for analysis. As the sago palm's fertilizer use efficiency in the middle stage ranged from 10 to 20%, the use of nitrogen fertilizer for sago production should be limited.

Keywords: fertilizer use percent, ^{15}N-labeled ammonium sulfate, nitrogen uptake, sago palm

Introduction

A long research history of nitrogen uptake by sago palms had not shown effects of nitrogen fertilization. Sago palms can grow in Entisols, Inceptisols, and Histosols of Southeast Asia (Okazaki and Sasaki, 2017). No more fertile soils, however, are available in the coastal area in which people are able to cultivate sago palm. In the coastal areas of Malaysia and Indonesia, Histosols prevail, because of the smaller amount of siltation through rivers shorter than those within continents. Histosols are abundant in their natural conditions because of the difficulty of access and crop production, so that researchers in Malaysia and Indonesia have looked at Histosols in these areas. Kueh (1979, 1980) started a sago field fertilizer response experiment with a 9 m x 9 m square at the Stapok Peat Research Station of Sarawak (Histosols, Deep peat soil, Anderson III) in 1976. In 1979, he observed that, in undrained deep peat, leaf production in the third year appeared to indicate a depressing effect of N ($P<0.05$), and that the leaf production of leading palms showed no response to applications of N, P, and K in the fourth year and beyond. However, Kueh (1995) reported that the development speed of leaf and nutrient components in leaves of sago palms were affected by fertilization. Similarly, Paquay et al. (1986) demonstrated the acceleration of leaf development and leaf area increments due to the application of fertilizer to sago seedlings. Furthermore, Kakuda et al. (2005) applied N, P, K, Ca, Mg, Cu, Zn, Fe, and B to 5-year-old sago palms in the thick peat soil of Riau, Indonesia, and obtained data that the application of fertilizer increased the dry matter weight of the petiole. On the other hand, Purwanto et al. (2002) reportedly found no differences in palm height, number of leaves, and leaf N content between the fertilized and unfertilized sago palms in the fifth and twelfth months. The fertilization of slow-release nitrogen fertilizers, Meister 40 and Meister 70, had limited effects on the growth factors of sago palms in the Philippines (Kimura et al., 2007). These studies indicated the limitation of nitrogen fertilization experiments, because of high temperatures, precipitation of more than 2000 mm with dry and wet seasons, and possible water-logged conditions due to irrigation.

The studies on nitrogen fertilization using a ^{15}N tracer were performed by Lina et al. (2009), who reported that sago palms responded to nitrogen fertilizer in the early growth stage under greenhouse conditions, and nitrogen uptake from normal urea fertilizer was 10-20% of the total nitrogen uptake by sago palms with immature root systems. There were few reports on sago palms' utilization rate of nitrogen fertilizer in field conditions with Inceptisols. Especially, the application of nitrogen to sago palms at the middle growth stage was limited.

The objective of this study was to clarify the utilization of nitrogen derived from urea fertilizer in Inceptisols by sago palms using ^{15}N tracers in Leyte of the Philippines.

Materials and Methods

1. Experimental field, soil, and urea application with ^{15}N-labeled ammonium sulfate

In an Af climate, with more than 2400 mm rainfall per year with a maximum temperature of 33 to 35 throughout the year and precipitation of 100 mm in April and 350 mm in December (Fig. 1), this study

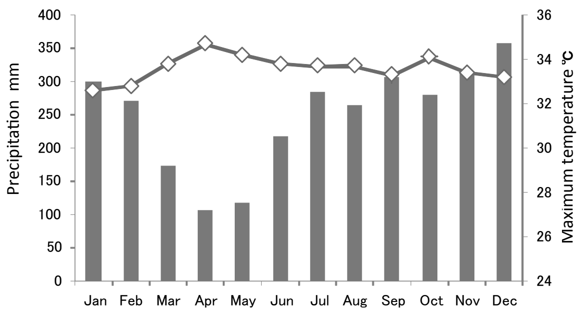


Fig 1. Climate conditions in Leyte, Philippines
Bar shows the monthly precipitation, and the polygonal line shows the maximum temperature.

was started on Eutropepts in Pangasugan, Baybay, Leyte, Philippines, in 2005. Pangasugan faces the Camotes Sea within the Philippine archipelago and is situated between the Eastern Visayas and Central Visayas regions. Hamazaki et al. (2010) suggested that Pangasugan was included in U2, with a dry season in which the shortage of available water was less than 30 days.

Soils were sampled in 2007 and 2008, being air-dried and sieved with a 2 mm mesh sieve for chemical analysis and ground thoroughly with a mortar and pestle for total nitrogen and δ^{15}N analyses. The soil was slightly acidic (pH 6.4) and 3.4 mS m^{-1} (Lina et al., 2008). A sago palm field 50 m x 50 m (the 2005 experimental plot of 4 m x 2 m was combined with another 4 m x 2 m plot into one 4 m x 4 m plot in 2007) was established. Extra control plots (without urea application) in 2005 were prepared for the second trial. The field was intermittently irrigated, and the study of sago palm growth was carried out continuously. Experimental plots in 2007 were settled in three replicates for each treatment: control (no application of urea fertilizer) and urea (50 kgN/ha and 100 kgN/ha) with and without ^{15}N-labeled ammonium sulfate. Hence, three control plots, three urea 50 kgN/ha plots, three urea 100 kgN/ha plots, three ^{15}N-labeled urea 50 kgN/ha plots, and three ^{15}N-labeled urea 100 kgN/ha plots were prepared for the second fertilizer application experiment. Urea fertilizer was applied approximately 10 cm around sago palms. Urea, a common fertilizer in Southeast Asia for nitrogen application, is easily transformed to ammonium nitrogen by urease in soil. It is expected that ammonium nitrogen from ammonium sulfate-tagged ^{15}N and transformed from urea were similarly absorbed by sago palms.

The first application of urea fertilizer with the ^{15}N-labeled ammonium sulfate in 2005 exhibited 3.35 atom %, which implied ^{15}N-labeled ammonium sulfate (8436 ‰) (Lina et al., 2009). The ^{15}N was applied at a rate of 0.094 g per plot for 50 kgN/ha and 0.189 g per plot for 100 kgN/ha. Fertilization was performed within 0.562 m^2 of the position. For the second trial, in 2007, urea fertilizer with 1.52 atom %, which implied ^{15}N-labeled ammonium sulfate (3202 ‰), was applied in the same manner and depth as in the first trial. The ^{15}N was applied to the new plots, which were provided at 50 kgN/ha and 100 kgN/ha levels of common urea for the first trial, at rates of 0.207 g per plot for 50 kgN/ha and 0.416 g per plot for 100 kgN/ha, respectively. The second application of ^{15}N was performed in the former 4 m x 2 m control plot of the first application, so that ^{15}N was added to the virgin soil plots. The 4 m x 4 m plot should have one palm by felling one of the two. The number of leaflets and leaf dry weight of 21 felled palms (early trunk formation stage) in the control (no application of urea fertilizer) plots were determined from October of 2007 to October of 2008 (Fig. 2).

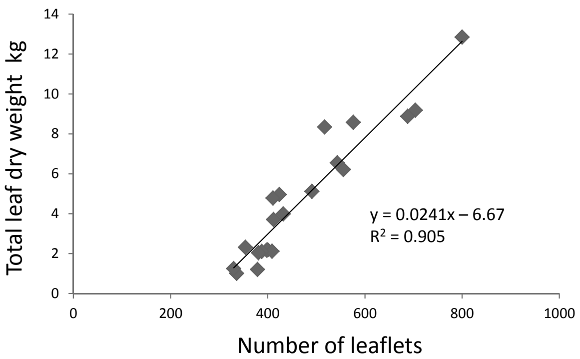


Fig 2. Relationship between the number of leaflets and the total leaf dry weight of sago palms measured in 2006 to 2008

The monthly fertilizer use percent (MFUP) of nitrogen of initially applied fertilizer from October of 2007 to October of 2008 was calculated using the following equation:

$$MFUP\ (\%) = (\delta^{15}N_{lab} - \delta^{15}N_{cont})\ (TN_{leaflet}) / (\delta^{15}N_{fert}\ TN_{fert}) \times (\text{total leaf dry weight per palm}) \times 100, \quad (1)$$

where $\delta^{15}N_{lab}$ is the $^{15}N/^{14}N$ ratio of the labeled palm, $\delta^{15}N_{cont}$ is the $^{15}N/^{14}N$ ratio of the control palm, $\delta^{15}N_{fert}$ is the $^{15}N/^{14}N$ ratio of the fertilizer, $TN_{leaflet}$ is the total nitrogen content in leaflets, and TN_{fert} is the total amount of fertilizer nitrogen initially applied. The total leaf dry weight was obtained by the equation of y = 0.0241 x - 6.77 (R^2 = 0.905), where y is the total leaf dry weight, and x is the number of leaflets per palm (Fig. 2).

The total fertilizer nitrogen uptake of initially applied fertilizer nitrogen per palm (total fertilizer use percent: TFUP) from October of 2007 to October of 2008 was calculated as the product of the total weight of leaves and the difference between the N content in the leaflets of the plot with and without fertilizer.

$$TFUP\ (\%) = (TN_{leafletx} - TN_{leaflet0}) / TN_{fert} \times (\text{total leaf dry weight per palm}) \times 100, \quad (2)$$

where $TN_{leafletx}$ is the total nitrogen content in leaflets at the urea application plot of 50 kgN/ha or 100 kgN/ha, $TN_{leaflet0}$ is the total nitrogen content in leaflets at the plot without fertilizer application, and TN_{fert} is the initially applied total amount of fertilizer nitrogen. The total leaf dry weight is obtained by the equation of y = 0.0241 x - 6.77 (R^2 = 0.905), where y is the total leaf dry weight, and x is the number of leaflets per palm.

2. Nitrogen in soil and sago leaflet samples

Soil samples in the 4 m x 4 m plot were collected from the experimental field in August of 2007 and January of 2008, air-dried, sieved with a 2 mm mesh sieve, and stored in a dark room. Soil analysis was described in the previous report (Lina et al., 2008).

Since July of 2005, sago leaflet samples have been taken from different positions of the sago leaf and upper, middle, and basal leaflets on the third leaf from the top, as Sim and Ahmed (1991) recommended the sampling of two leaflets from the middle portion of the third leaf from the top on both sides of the rachis. Seven months later, leaflet samples were taken from the third leaf from the top of the standing sago palms every month, because sago palms grow 10 leaves per year, on average. The leaflets were cut and dried at 70 °C for 4 hours in the Philippines.

3. Total nitrogen and $\delta^{15}N$ determination in soil and sago leaflet samples

Leaflet samples were washed with deionized water, oven-dried for 2 to 3 days at 70 ˚C using a forced-draft oven, and ground with a ball mill (Model MM 301, Retch, Haan, Germany) in Japan. These samples were stored in a dark, cool room until analysis. Total nitrogen was analyzed with a CHNS/O analyzer (PerkinElmer 2400, Waltham, USA). The ^{15}N was determined by an elemental analyzer (Euro-EA-Elemental Analyzer; EuroVector, Milano, Italy) connected to a continuous flow-isotope ratio mass spectrometer (IsoPrime, GV Instruments, Manchester, UK). USGS-40-2 (glutamic acid) was used as a second reference material (Lina et al., 2010). The values of $\delta^{15}N$ of sago palm leaves ranged from 1.34 ± 1.64 ‰ in Talau, Sarawak, Malaysia to 5.05 ± 0.61 ‰ in Kendari, Sulawesi, Indonesia (unpublished).

Table 2. Total nitrogen content and $\delta^{15}N$ of soil (n = 3) in the study field

				N0	N50	N100
Total nitrogen	2007	August	Mean g/kg	1.97	1.90	1.89
			SD	0.41	0.96	0.47
			CV %	20.6	50.4	25.0
	2008	January	Mean g/kg	2.12	1.20	2.23
			SD	0.52	0.25	0.40
			CV %	24.5	21.1	18.0
^{15}N‰	2007	August	Mean	1.96	2.86	2.45
			SD	0.59	0.55	1.15
			CV %	30.1	19.3	46.7
	2008	January	Mean	2.53	3.56	4.73
			SD	0.60	1.24	2.73
			CV %	23.8	34.9	57.8

N50:^{15}N labeled urea, 50 kg/ha application
N100:^{15}N labeled urea, 100 kg/ha application
SD:Standard deviation, CV:Coefficient of variation

Soil samples were taken from the surface of the experimental field.

Results

1. Total nitrogen content in soil and sago palm samples

The mean height of sago palms in July of 2007 and July of 2008, shown in Table 1, are 269±45 cm and 377±83 cm (n = 15), and the mean number of living leaves are 14 and 16, respectively.

Table 1. Mean height and number of living leaves of sago palms

	July of 2007	July of 2008
Mean palm height cm	269±45	377±83
Mean number of living leaves	14±2	16±1

The total nitrogen content and $\delta^{15}N$ values of soil samples taken in August of 2007 (one month after starting the second trial) and January of 2008 (6 months after starting the second trial) are shown in Table 2. The total nitrogen content in Eutropepts of sago fields (N0: control), Pangasugan, Leyte, was low (1.97 gN/kg in August of 2007 and 2.12 gN/kg in January of 2008). The urea application of 50 kgN/ha and 100 kgN/ha did not necessarily increase the total nitrogen content. Meanwhile, the $\delta^{15}N$ values (N0) of soil samples were 1.96 in August of 2007 and 2.53 in January of 2008, which implies annual variation. The application of urea remarkably increased the $\delta^{15}N$ values in this study field. The increment of $\delta^{15}N$ values did not always depend on the amount of urea applied.

The nitrogen contents of the different portions of leaflets in Table 3 indicate the decreasing order of upper, middle, and basal portions. However, the $\delta^{15}N$ values with large coefficients of variation do not represent a trend within this study.

Table 3. Total nitrogen content and $\delta^{15}N$ in different portions of sago leaflets

		Portion of sago leaflet Upper	Middle	Basal
Total nitrogen	g/kg	21.3	18.3	16.4
SD		2.6	2.2	3.6
CV %		12.4	12.2	22.9
$\delta^{15}N$‰		2.81	1.88	2.03
SD		0.83	0.46	0.73
CV %		29.7	24.4	36.2

The sampling of sago leaflet (n = 3) was carried out in February of 2006.
Leaflet sample of the third leaf from the apical from was collected.
SD: Standard deviation
CV: Coefficient of variation

2. Nitrogen uptake by sago palms

The relationship between the number of leaflets and the total leaf dry weight was obtained from 2006 to 2008 (Fig. 2). Based on the equation from Fig. 2, the total leaf dry weight was estimated from the number of sago palm leaflets, and the monthly and total fertilizer use percent of sago palms were calculated.

The monthly total uptake of nitrogen per palm to fertilizer nitrogen (MFUP) initially applied with the urea application of 50 kgN/ha and 100kgN/ ha in 2007 to 2008 is shown in Fig. 3. The nitrogen in sago palms on the control plot (without fertilizer) was derived from the soil only. There were few differences in sago leaflet nitrogen content between the control (without fertilizer) plot and nitrogen application (50 kgN/ha and 100 kgN/ha) plots, which suggests that the nitrogen uptake of sago palms was from the soil, not from fertilizer. Although from August to December of 2007 the urea application did not stimulate the nitrogen uptake of sago palms, the MFUP increased until March of 2008 and gradually decreased with 50 kgN/ha and 100 kgN/ha applications. The MFUPs of sago palms, with more- or less-developed root systems, at the middle stage were, at most, 6.5% (50 kgN/ha application) and 3.3% (100kgN/ha), respectively, of the initial amount of nitrogen fertilizer, based on the nitrogen content in the middle leaflet of the third leaf. Since July of 2008 (10 months after urea application), the nitrogen uptake from fertilizer by sago palms was obscure. The nitrogen supply as a fertilizer did not affect sago palm effectively.

3. Nitrogen uptake by sago palms from fertilizer

Figure 4 represents the total fertilizer use percent (TFUP) of sago palms at the middle stage with urea fertilizer application of 50 kgN/ha and 100 kgN/ha.

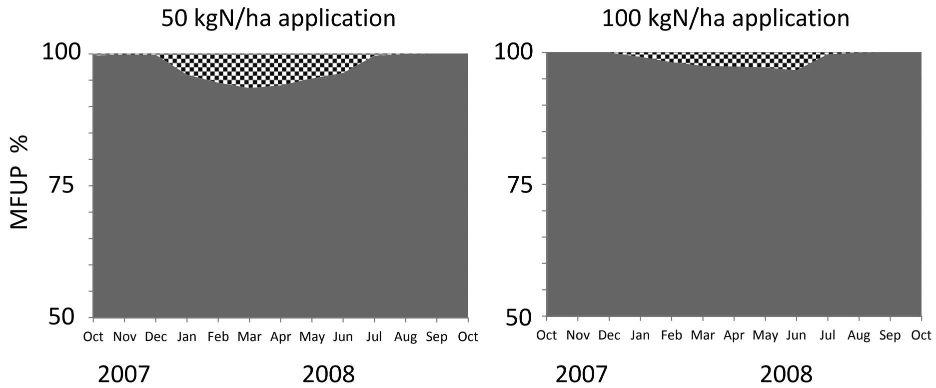
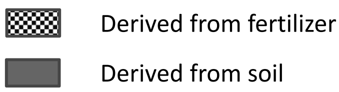


Fig 3. Monthly fertilizer use percent in sago leaflets of the third leaf from the top with the initial application of nitrogen



MFUP: Monthly fertilizer use percent (%)

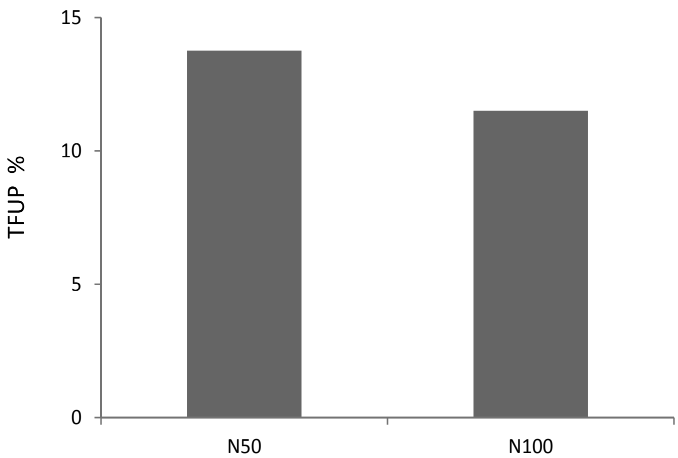


Fig 4. Total fertilizer use percent of sago palm with urea application (Oct. 2007 to Oct. 2008)

N50: Urea application of 50 kgN/ha
N100: Urea application of 100 kgN/ha
TFUP: Total fertilizer use percent (%)

The TFUPs were 13.8% for 50 kgN/ha application and 11.5% for 100 kgN/ha application. These values are relatively lower than those of rice (Kaneta et al., 1994) and wheat (Ishimaru et al., 2016).

Discussion

In this study, urea was used as a nitrogen fertilizer. Urea is easily adsorbed on the soil surface with a hydrogen bond. Urease mediated ammonification from urea, which caused the pH to increase slightly in lowland soils with the production of ammonium carbonate. However, there was no significant difference before and after urea fertilization (Kumazawa, 2000). The maximum activity of ammonification from urea was observed at 30 °C, and all urea was changed to ammonium nitrogen within 2 to 3 days. The nitrification proceeded continuously after ammonification, and the maximum nitrification occurred at 27 to 29 °C in soil with 24 to 27% water content. In the Philippines, Fillery et al. (1986) reported that the loss of ammonia volatilization from paddy fields where urea was applied, broadcast into floodwaters 14 to 21 days after transplanting in Munoz and Los Baflos, accounted for 45 and 60% of the ^{15}N applied, respectively. In contrast, ammonia volatilization in Vietnamese paddy rice fields, where urea was applied at 20 to 40 kgN/ha as a split application for a total of 80 to 100 kgN/ha throughout the cropping period, was 1.7 to 14.6% of the applied N (Watanabe et al., 2009). Ammonia volatilization is the important nitrogen loss mechanism and has equal importance to nitrification/denitrification in tropical wetlands.

There has been a long time to discuss the effect of nitrogen application to sago palms in Sarawak of Malaysia. Since 1976, Kueh (1979, 1980, 1981, 1982, 1983) found no effect of nitrogen (ammonium sulfate) application on leaf production in undrained Fibrists at Stapok of Sarawak. In case of the eleventh year of growth, trunk heights in Histosols with minimal drainage at Stapok Station seemed to increase with N application (Kueh, 1987). However, there was no significant effect of N application on trunk height in thirteen years of growth (Kueh, 1989, 1990, 1995). Similarly, Jaman (1983) found in Sarawak that NPK fertilizer application had no significant positive effect on the rate of leaf production of sago palms in the first year at the Sungai Talau Peat Research Station. Jaman (1984, 1985) reported that NPK fertilizer application, pruning, weeding, seed germination, spacing, and intercropping had no significant positive effect on the annual leaf production rate of sago palms. Kakuda et al. (2005) also reported that applying seven times the usual amount of N, P, K, Ca, Mg, Cu, Zn, Fe, and B to Histosols provided a significant difference in petiole and sucker dry weight and that any difference was not found in other growth factors of sago palms. Our results on nitrogen uptake from urea fertilizer in Inceptisols indicate a limitation of up to 13%, which indicates that nitrogen fertilizer has a small effect on growth and final starch production.

The small effect of nitrogen (N) application on sago palm growth is explained by the findings of the endophytes' activities on the N fixation (Shrestha et al., 2006, 2007). When sago palm becomes easily able to absorb N from the soil, it does not require N support by endophytes. Accordingly, it is concluded that endophytes play an important role in sago palm growth without N application. Another explanation is the emission of nitrous oxide (N_2O) and nitric oxide (NO) production through nitrification and denitrification in both reductive and oxidative conditions of sago fields intermittently after applying urea fertilizer, accompanied with the dissolution and elution of nitrogen by irrigation water. The production of nitrous oxide (N_2O) and nitric oxide (NO) (Firestone and Davidson, 1989) easily occurs in sago fields intermittently irrigated by irrigation water in Histosols (Inubushi et al., 2003). Lan et al. (2014) represented that N_2O and NO emissions were observed in a Typic Epiaquept in US Soil Taxonomy with paddy rice cultivation. Sago palm fields also have the repetition of oxidative and reductive conditions seasonally, in a situation similar to that of the paddy field.

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