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Effects of nitrogen sources and their split application on some soil properties and tissue nitrogen and sulfur content of rice in Gambella, Ethiopia

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Abstract

Soil and tissue testing are the most reliable ways to assess soil nutrient status to determine fertilizer needs of crop plants. Thus, this field experiment was conducted to study the effect of N fertilizer sources [NH_4NO_3 (34% N), $(NH_4)_2SO_4$ (21% N + 24% S) and $CO(NH_2)_2$ (46% N)], and their split application (½ at sowing + ½ at tillering, ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation, ½ at sowing + ½ at panicle initiation, and ½ at tillering + ½ at panicle initiation) on soil OC, pH, N, P, K and S and tissue N and S contents of NERICA-3 rice (*Oryza sativa x Oryza glaberrima*) for two years (2008-2009) under the climate conditions of Gambella, Ethiopia. The experiment was laid out in a RCBD replicated thrice. The soil characters studied and rice tissue N was significantly influenced by cropping year. The effects of N sources on soil pH, N, K, S and tissue N were significant while split N application had significant effect on soil pH, P, tissue N and S contents. The interaction effects of cropping year and N sources were significant on soil pH, N, S and tissue N only; while cropping year and split application of N interaction significantly influenced soil OC, N, P and K. The N sources and its split application on soil pH, P, K and tissue N; and year by N sources by application time on soil pH and K among the soil and rice tissue parameters studied. The N sources and their split application showed a negative effect on soil OC and N contents. However, split application of N as ½ at sowing + ½ at tillering recorded significantly the highest soil OC content (3.19%). The effects of year by N sources and year by N application time showed significantly the highest soil total N content (each 0.43%) with $CO(NH_2)_2$ and N sources applied as ½ at tillering + ½ at panicle initiation. NH_4NO_3 applied as ½ at sowing + ½ at tillering showed the highest soil pH (7.59) while $(NH_4)_2SO_4$ soil S. The highest soil P value (858.05 mg P kg^{-1} soil) found with N sources applied as ½ at sowing + ½ at panicle initiation while $(NH_4)_2SO_4$ ½ at sowing + ½ at tillering and NH_4NO_3 ½ at sowing + ½ at panicle initiation were obtained significantly higher soil K content than other treatment combination. The highest tissue N content (1.91%) obtained with applied NH_4NO_3 as ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation.

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Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops produced and no food grain is more important than rice from nutritional as well as food security perspectives globally (Assefa *et al.*, 2009; MoARD, 2010). It is one of the most important staple cereals in human nutrition, consumed by about 75% of the global population (Oko and Onyekwere, 2010). Over 90% of the total rice crop is produced in South and East Asia. Africa produced an average of 21.9 million tons (t) of rough rice in the year 2006 on 9.2 million hectare (ha) of land equivalent to 2.5 and 6.0% of the world total production and rice area, respectively (FARA, 2009). Currently, rice is grown in over 75% of Africa countries (MoARD, 2010). Ethiopia is one of Africa's rice growing countries proved to have reasonable potential to grow different rice types for upland and lowland rain-fed, upland and irrigated ecosystems.

Bekur (1997) reported that rice is a recently introduced crop in Ethiopia. Although its research and extension activities are found at the infancy stage, the government, investors and farmers have recognized the importance of rice as a food security crop, and source of income and employment opportunity as compared to other cereals in the country (MoARD, 2010). The Ethiopian government has been encouraging rice growers to increase the area with a system of rice intensification for decreasing the gap between rice production and consumption. As a result, rice production area has been increasing over the years and rice has become one of the important staple foods of Ethiopians.

Among the regions of Ethiopia, Gambella is one of the hot and humid regions known with its best agro-ecological situations for rice cultivation. For example, wild rice (*O. longistaminata* and *O.barthii*) grows very well in its flood plains (Gezahegn, 2007). Most probably, this has instigated the Koreans to introduce rice to Gambella in the 1960s (Bekur, 1997). Now aday's, although Pawe and Gambella Agricultural Research Institutes have released rice varieties like NERICA-3, NNERICA-4 and Superica-1 (tolerant to shattering and high yielding) there is lack of research based information on their agronomy and nutrient management practices related to the region`s soil conditions. Among these, the effects of nitrogen (N) sources and their time of application on soil organic carbon (OC) and pH, and soil and rice tissue N and sulfur (S) contents are not known.

Daniel and Solomon (2008) from their soil nutrient variability study results of the Barro River basin plain, Gambella, reported that the amount of total N ranged from 0.06 to 0.31%. Of the area surveyed 44.4, 40.7 and 14.2% of the total fall under very low, low and medium total N categories, respectively. They added that the amount of available phosphorus (P) ranged from absolutely deficit to excess levels, hence, the N and P fertility of the soils in the study area could be described as low. As of better N fertilizer sources and an adequate supply at the right time can markedly increase the yield and improve the quality of rice and soil in such low soil plant nutrient test value (Manzoor *et al.*, 2006). Assefa *et al.* (2009) reported that di-ammonium phosphate gave the best return in rice grain yield followed by $(NH_4)_2SO_4$, $CO(NH_2)_2$, calcium ammonium nitrate ($5Ca(NO_3)_2$ $NH_4NO_3 10H_2O$) and NH_4NO_3 on Vertisol conditions of Pawe, Ethiopia. The growth and yield parameters and grain N significantly increased by an application of S containing N fertilizer (Super Net) and was at par with $NH_4)_2SO_4$ while $CO(NH_2)_2$ gave the lowest grain yield and N content at Agricultural Research Station, Bilaspur Chhattisgarh, India (Chaturvedi, 2005). Contrarily, Singh *et al.* (1998) reported that S application had no consistent effect on yield of wheat at the Indian Institute of Soil Science, Bhopal, India.

The amount of S absorbed by crops is generally about 9-15% of the amount of N uptake. However, on elemental bases, crops contain as much S as phosphorus (P) and it is about equal in importance with N and P in the formation of protein (Banerjee, 1999; Martin, 2008). Sulfur also increase N use efficiency, improving profit potential and reducing the chance of nitrate leaching into groundwater (Martin, 2008). Depletion of soils S causes decline not only in yield, but also in the response to N, P, potassium (K) and consequently, the economic return from their use (Banerjee, 1999).

Soil analysis provides the basis for assessing soil nutrient status whereas plant analysis is useful in optimizing the timing and rate of nutrient addition (Beegle, 2006). However, published study findings that address the suitable N fertilizer sources and time of application on soil OC and pH and soil and rice tissue N and S contents is limited in the study area. Thus, this investigated the effects of N fertilizer sources and their time of application on soil pH and OC, and N and S contents of soil and rice (NERICA-3) tissue grown in Gambella Zuria District, Gambella, Ethiopia.

Materials and methods

Description` of the experimental site

Field experiment was conducted at *Imla* (8 ° 14' 46.36" N latitude; 34 ° 35' 17.75" E longitude), Gambella Agricultural Research Institute, Gambella, Ethiopia during the 2008 and 2009 main cropping seasons. The site (Fig. 1) is known with hot humid tropical lowland climate (Wikipedia, 2011) at an altitude of 450 meter above sea level.

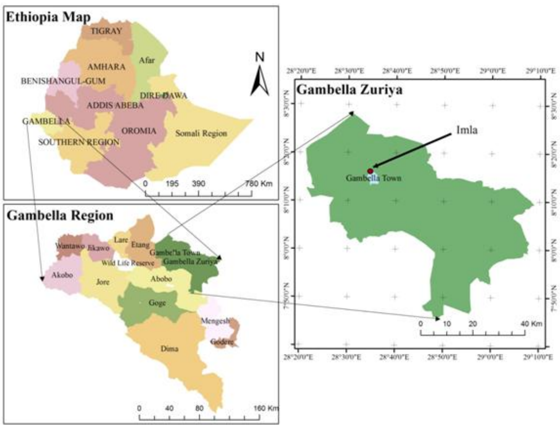

Fig. 1. Location map of the study area, Imla, nearby Gambella town in Gambella Zuria District.

It has mean annual of 19.9 °C minimum and 35.5 °C maximum temperatures, and a mean annual rainfall of 1227.6 mm (NMA, 2009). The location map of the study area and weather data during the two experimental seasons are presented in Fig.1 and 2, respectively. The soil texture was clay, consisting of 4.08% organic carbon (OC), 0.51% total N, and 650.00 mg kg^{-1} available P with a pH of 6.43 (Table 1).

Physico-chemical properties of the experimental soil before sowing and after harvest of rice were listed in Table 1. The experimental site was not cultivated for over 6 years and mostly covered with Sudanese grass and wild sorghum.

Treatments, experimental design and procedures

The experiment was conducted on a permanent field layout in RCBD with three replications. The used treatments were N fertilizer sources [ammonium nitrate/NH_4NO_3 (34% N), ammonium sulphate/$(NH_4)_2SO_4$ (21% N + 24% S) and urea/$CO(NH_2)_2$ (46 % N)], and their split application time (½ at sowing + ½ at tillering; ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation; ½ at sowing + ½ at panicle initiation; and ½ at tillering + ½ at panicle initiation).

Table 1. Physico-chemical properties of the experimental soil before sowing and after harvest of rice during 2008 and 2009 cropping seasons.

Soil analysis	BS (2008)	AH (2008)	AH (2009)
Sand (%)	17.68	-	-
Silt (%)	32.72	-	-
Clay (%)	49.60	-	-
Soil texture	Clay	-	-
pH	6.43	6.80	6.17
Organic carbon (%)	4.08	1.40	2.70
Available N (%)	0.51	0.34	0.29
Available P (mg kg^{-1})	650.00	751.17	448.02
Available K (cmol$_c$ kg $^{-1}$)	0.60	0.66	0.32
S (mg kg^{-1})	6.30	8.79	19.13

BS = Before sowing; AH = After harvesting; pH = The negative logarithm of the hydrogen ion activity of a soil [-log (H$^+$).

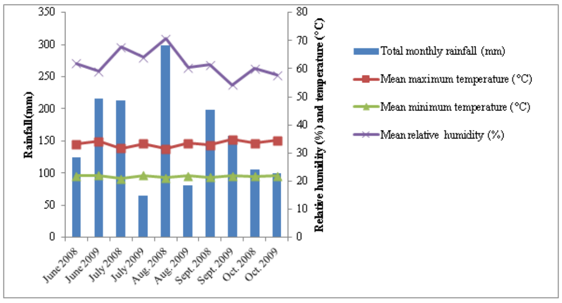

Fig. 2. Monthly weather data for the 2008 and 2009 cropping seasons (Source: Gambella Meteorological Service Branch Office).

Site selection was made considering rice agro-ecological requirement. In the first year, the land was plowed using a tractor in April 2008, while it was prepared manually to control mixing of treatments in 2009. NERICA-3 rice seed germination was determined before sowing each year. Germination (%) was calculated as ratio of number of seeds germinated to the number of seeds on tray multiplied by 100 and recorded as 96.1% and 94.8% in 2008 and 2009, respectively.

Rice seed (NERICA-3) was drilled by hand at the rate of 100 kg ha^{-1} on 31 July 2008 and 25 July 2009 in a plot size of 4 x 4 m having 20 rows with 20 cm inter-row distance. The outer most row and 0.5 m row length at both ends of plots was considered as border. The second, third and fourth row at both sides of plots were designated for destructive sampling, non destructive sampling and guard row, respectively. Thus, the net plot size was 3.0 m x 2.4 m. Nitrogen was applied as per the treatments at the rate of 92 kg ha^{-1}. The entire dose of P (46 kg ha^{-1}) and K (20 kg ha^{-1}) was drilled at sowing in the form of triple super phosphate and potassium chloride, respectively. Uniform agronomic practices were followed to raise the crop. The crop was harvested in the second week of October each year.

Soil and plant sampling and analysis

To determine N and S contents of soil and rice tissue, soils and plants were randomly sampled from each plot. Determination of soil pH and OC was also followed similar sampling manner. Composite soil samples were collected before the start of the experiment for analysis of selected soil properties. Subsequent soil sampling and analysis were also carried out after harvesting of crop. Soil pH was determined in a 1:2.5 soil-water suspension using a combination of glass electrode. Extracting soil samples by sodium bicarbonate solution as per the procedure outlined by Olsen *et al.* (1954), available P was determined measuring absorbance using spectrophotometer at 882 µm. The extract of K was analyzed using flame photometer (Black, 1965). Organic carbon was estimated by a Walkely and Black (1954) wet digestion method and the organic matter was calculated by multiplying the per cent organic carbon by a factor of 1.724. For tissue analysis, four upper most leaf blades of 35 plants in the destructive rows were randomly sampled at panicle initiation from each plot. The samples were oven-dried in a draft-oven at 60 °C until a constant dry weight and ground using wooden mortar and pestle to pass through a 1 mm sieve (Yoshida *et al.*, 1972). Nitrogen concentration in soil and rice tissue was determined by digestion and distillation of the materials using Micro Kjeldahl method (AOAC, 1994). Sulfur extracted with Ca (H_2PO_4) in 2NHOAc and measured turbid metrically (Hoeft *et al.*, 1973).

Statistical analysis

Data were analyzed using two-way ANOVA following the General Linear Model procedure of Statistical Analysis System processing package, version 9.10 (SAS, 2003). The treatment means were separated using Duncan's Multiple Range Test at 5% probability level.

Results and discussion

Effects of n source and application time on soil OC, pH, N and S contents

Soil organic carbon content: The effects of cropping year ($P \leq 0.01$) and interaction of cropping year with N split application ($P \leq 0.05$) on soil OC were significant while N sources, split application , interactions of N sources with cropping year or their split application and cropping year , N sources and split application were not significant ($P > 0.05$) (Table 2).

The soil OC (2.72%) was significantly higher in 2009 than 2008 (1.39%). Although OC was not significantly affected by N sources, the application of $(NH_4)_2SO_4$ and NH_4NO_3 resulted in higher content of soil OC (2.18 and 2.11%, respectively) than $CO(NH_2)_2$ (1.88%). Similarly, Assefa *et al.* (2009) used $CO(NH_2)_2$, $(NH_4)_2SO_4$, NH_4NO_3, di-ammonium phosphate [$(NH_4)_2HPO_4$)] and ($5Ca(NO_3)_2$ $NH_4NO_3 10H_2O$) each at 120 kg ha^{-1} and reported reduced soil organic matter (5.24%) with $CO(NH_2)_2$ applied ½ at sowing and ½ at panicle initiation, whereas it was increased by 10.47 and 8.38% with the $(NH_4)_2SO_4$ and NH_4NO_3, respectively. The free oxygen created through reaction processes might have oxidized the organic matter of the soil that caused a low-level combustion (burning) of the organic matter which depleted the organic matter (Hermary, 2007). The soil organic matter might have also been depleted by the application of N fertilizer as it promoted microbial carbon utilization and N mineralization (Khan *et al.*,2007; Mulvaney *et al.*, 2009).

Table 2. Combined analysis of variance showing the effects of N sources and application time on soil OC, pH, soil and rice tissue N and S in 2008 and 2009.

Parameter	Mean square for source of variation							
	Year (1)	SN (2)	AT (3)	Y x SN (2)	Y x AT (3)	SN x AT (6)	Y x SN x AT (6)	Error (44)
				Soil				
Organic carbon (%)	31.548**	0.577	0.202	0.272	1.605*	0.640	0.668	0.397
pH 1:1 (H_2O)	7.284**	0.872**	0.375**	0.131*	0.080	0.201**	0.102*	0.033
N (%)	0.054*	0.042*	0.020	0.031*	0.036*	0.005	0.011	0.009
P (mg kg^{-1})	1654089.473**	3431.634	74027.040*	20411.423	86215.448*	77313.569*	55021.605	24155.467
K ($cmol_c$ kg^{-1})	2.118**	0.029**	0.004	0.007	0.012*	0.022**	0.026**	0.003
S (mg kg^{-1})	1923.861**	744.368**	13.913	172.612*	22.998	14.490	16.086	29.069
				Tissue				
N (%)	17.170**	0.438*	0.285*	3.702**	0.195	0.546**	0.154	0.099
S (g kg^{-1})	0.009	0.101	0.466**	0.239	0.082	0.116	0.225	0.102

Figures in parenthesis = Degrees of freedom; ** = Significant at P = 0.01; * = Significant at P = 0.05, SN = Sources of N; AT = Application time; Y = Year

The interaction effect of cropping year with split N application (Table 4) showed no significant difference in OC during 2008, while it was significant ($P \leq 0.05$) in 2009. The N application in two equal splits at sowing and at tillering recorded significantly higher OC content (3.19%) in 2009 but did not vary significantly with three equal splits each at sowing, tillering and panicle initiation (2.86%) in cropping year and time of N application interaction. The lowest level of OC of 1.18% (lower than the treatment with highest soil OC by 63%) was obtained from N application ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation during the 2008 cropping year and had no significant variation with OC obtained from the other N application time in the same year (Table 4). The interaction of cropping year with N split application showed a significant increase in soil OC content in 2009 over 2008 under all the split application treatments (Table 4).

There was no significant difference among the OC values obtained with N application ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation, ½ at sowing + ½ at panicle initiation and ½ at tillering + ½ at panicle initiation in 2009. However, in both the 2008 and 2009 cropping years these values were lower than the initial soil OC (4.08%). Generally, the influence of N fertilizer as $(NH_4)_2SO_4$ or NH_4NO_3 or $CO(NH_2)_2$ on soil OC efficiency may be mainly due to their effect on soil reaction and nutrient availability as reported by Sharief *et al.*(2004) and Ahmed and Khan(2010).

Soil pH content: Analysis of variance (Table 2) showed that soil pH content was significantly affected by cropping year, N sources, N application time, interactions of N sources by application time ($P \leq 0.01$), cropping year by N source and cropping year by N sources by application time ($P \leq 0.05$), whereas interactions of year by application time was not significant ($P > 0.05$). In the 2008 higher value (6.80) of soil pH was obtained which was 5.75% higher than the initial (6.43) and declined to 6.17 after the harvest in 2009 (Table 3).

Table 3. Main effects of year, N sources and application time on soil OC, pH, P, K, soil and rice tissue N and S contents.

	Soil						Rice tissue	
N sources	OC (%)	pH	N (%)	P (mg kg^{-1})	K (cmol$_c$ kg^{-1})	S (mg kg^{-1})	N (%)	S (g kg^{-1})
Year								
2008	1.39b	6.80a	0.34a	751.16a	0.66a	8.79b	0.95b	1.14
2009	2.72a	6.17b	0.29b	448.02b	0.32b	19.13a	1.93a	1.17
NH_4NO_3	2.11	6.70a	0.29b	586.46	0.52a	15.21b	1.57a	1.21
$(NH_4)_2SO_4$	2.18	6.43b	0.30b	609.86	0.49b	18.79a	1.47ab	1.17
$CO(NH_2)_2$	1.88	6.33b	0.36a	602.43	0.45c	7.87c	1.30b	1.09
Application time (AT)								
AT_1	2.21	6.70a	0.27	506.83b	0.49	13.42	1.56a	1.06b
AT_2	2.02	6.44b	0.32	654.49a	0.47	15.05	1.53a	1.13b
AT_3	2.01	6.38b	0.32	617.32a	0.50	14.13	1.37ab	1.04b
AT_4	1.97	6.42b	0.35	619.71a	0.50	12.93	1.30b	1.39a
CV (%)	30.65	2.82	29.68	25.92	10.92	38.63	21.79	27.66

Means of the same factor in a column followed by the same letter are not significantly different at $P > 0.05$ by Duncan's Multiple Range Test. pH = The negative logarithm of the hydrogen ion activity of a soil [$-\log (H^+)$]; AT_1 = ½ at sowing + ½ at tillering; AT_2 = ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation; AT_3 = ½ at sowing + ½ at panicle initiation; AT_4 = ½ at tillering + ½ at panicle initiation; CV = Coefficient of variation.

Table 4. Interaction effects of cropping year and N application time on soil organic carbon N and P contents of rice field at Gambella.

Applicationtime (AT)	Organic carbon (%)		N content (%)		P content (mg kg^{-1} soil)	
	2008	2009	2008	2009	2008	2009
AT_1	1.23c	3.19a	0.25b	0.29b	654.05bcd	359.61e
AT_2	1.18c	2.86ab	0.34ab	0.31b	801.10ab	507.88de
AT_3	1.53c	2.50b	0.35ab	0.28b	858.05a	376.59e
AT_4	1.63c	2.32b	0.43a	0.27b	691.43bc	547.99cd

Means of the same factor in a column followed by the same letter are not significantly different at $P > 0.05$ by Duncan's Multiple Range Test. AT_1 = ½ at sowing + ½ at tillering; AT_2 = ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation; AT_3 = ½ at sowing + ½ at panicle initiation; AT_4 = ½ at tillering + ½ at panicle initiation.

Table 5. Interaction effects of cropping year, N sources and application time on soil pH content of rice field at Gambella.

Sources of N	2008				2009			
	Application time (AT)				Application time (AT)			
	AT_1	AT_2	AT_3	AT_4	AT_1	AT_2	AT_3	AT_4
	Soil pH							
NH_4NO_3	7.59a	6.81bc	7.02b	6.91bc	6.71bc	6.23d	6.09de	6.22d
$(NH_4)_2SO_4$	6.79bc	6.76bc	6.06de	6.66c	6.25d	6.11de	6.14de	5.86e
$CO(NH_2)_2$	6.81bc	6.68bc	6.75bc	6.81bc	6.05de	6.05de	6.24d	6.06de

Means of the same factor in a row or a column followed by the same letter are not significantly different at P > 0.05 by Duncan's Multiple Range Test. AT_1 = ½ at sowing + ½ at tillering; AT_2 = ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation; AT_3 = ½ at sowing + ½ at panicle initiation; AT_4 = ½ at tillering + ½ at panicle initiation.

As indicated earlier the interaction of cropping year by N sources by split application (Tables 2 and 5) significantly ($P \leq 0.05$) affected soil pH content. Accordingly the highest value of soil pH (7.59) was found with NH_4NO_3 applied ½ at sowing + ½ at tillering in 2008 which was significantly ($P \leq 0.05$) higher than the other interactions (Table 5). Fry *et al.* (2011) also reported fertilizers that contain N in the nitrate form slightly increased soil pH. The magnitude of increase in soil pH over the lowest (pH 5.86) and initial (pH 6.43) values due to application of NH_4NO_3 applied ½ at sowing + ½ at tillering was 22.8 and 18%, respectively.

Further, there was no significant difference among soil pH values obtained with the interactions of cropping year by N sources by application times except those recorded from NH_4NO_3 applied ½ at sowing + ½ at tillering and $(NH_4)_2SO_4$ ½ at sowing + ½ at panicle initiation in 2008 and NH_4NO_3 ½ at sowing + ½ at tillering in 2009 (Table 5). Similarly, there was no significant difference among soil pH found with interaction effects of cropping year by N sources by application time except NH_4NO_3 applied ½ at sowing + ½ at tillering and ½ at tillering + ½ at panicle initiation in 2009. Pasha (2005) also reported that there was no significant response of soil pH to S sources [$(NH_4)_2SO_4$ and ammonium thiosulphate], rate (34 and 68 kg S ha^{-1}), and methods of application (pre-plant, drip, and split). However, there was significant difference between soil pH obtained with interactions of NH_4NO_3 ½ at sowing + ½ at panicle initiation and $(NH_4)_2SO_4$ ½ at sowing + ½ at panicle initiation in 2009 (Table 5).

The lowest soil pH (5.86) that recorded from $(NH_4)_2SO_4$ applied ½ at tillering + ½ at panicle initiation had no significant difference with soil pH values recorded from NH_4NO_3 ½ at sowing + ½ at panicle initiation in 2009, $(NH_4)_2SO_4$ ½ at sowing + ½ at panicle initiation in both years, ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation in 2009 and $CO(NH_2)_2$ applied at all application times except ½ at sowing + ½ at panicle initiation in 2009 (Table 5). Wilson *et al.* (1994) and Ahmed and Khan (2010) also reported that $(NH_4)_2SO_4$ contained S fertilizer that reduce soil pH. Whereas Whiting *et al.* (2010) reported that $(NH_4)_2SO_4$ or $CO(NH_2)_2$ as N fertilizer source had a small effect on lowering soil pH in soils without free lime. On the other hand, Khan *et al.* (2007) reported that the application of sulphidic materials at the rate of 75 kg S ha^{-1} for S deficient soils had no negative effect on soil pH and nutrient status of soils.

$(NH_4)_2SO_4$ was no more effective, however, in reducing soil pH than pure S (Fry *et al.* 2011). In this relation, S application at 68 kg ha^{-1} reduced soil pH about 0.3 units at the end of the season in two studies (Susila and Locascio, 2005). Moreover, the soil acidification caused by ammonium sulfate may explain its slow rate of nitrification – it may be associated with ammonium sulfate's higher resistance to leaching and denitrification losses compared to ammonium nitrate and urea (Chien *et al*., 2008).

Fry *et al*. (2011) reported that neither S level nor application time had any effect on soil pH. Separating the soil profile vertically to determine pH revealed no effect of S on reducing pH regardless of rate, timing or soil depth. Finally, using N fertilizer in the form of NH_4NO_3 with all studied time of split application significantly surpassed $(NH_4)_2SO_4$ and $CO(NH_2)_2$ in pH content by 4.03 and 5.52%, respectively (Table 3) since nitrate is a basic form on nitrogen, meaning that it tends to increase the pH of the soil. Generally, the influence of N fertilizer as $(NH_4)_2SO_4$ or NH_4NO_3 or $CO(NH_2)_2$ on soil pH efficiency was mainly due to their effect on soil reaction and nutrient availability (Sharief *et al.*, 2004; Ahmed and Khan, 2010).

Soil nitrogen content: Analysis of variance (Table 2) indicated that the effects of cropping year, N sources, interactions of cropping year by N sources and cropping year by N application time were significant ($P \leq 0.05$) on soil total N but N application time, interactions of N source and application time and cropping year with N sources and application time were not significant ($P > 0.05$).

Table 6. Interaction effects of cropping year and sources of N application on soil N, S and rice tissue N contents in the rice field

Sources of N	Soil N (%)		Soil S (mg kg^{-1} soil)		Tissue N (%)	
	2008	2009	2008	2009	2008	2009
NH_4NO_3	0.28b	0.30b	9.66cd	20.75b	0.80d	2.33a
$(NH_4)_2SO_4$	0.33b	0.27b	11.15c	26.44a	1.43c	1.51c
$CO(NH_2)_2$	0.43a	0.30b	5.55d	10.19c	0.64d	1.95b

Means of the same factor in a row or a column followed by the same letter are not significantly different at P > 0.05 by Duncan's Multiple Range Test

Table 7. Interaction effects of N sources and application time on soil P content (mg kg^{-1} soil) in rice field at Gambella

Sources of N	Application time (AT)			
	AT_1	AT_2	AT_3	AT_4
NH_4NO_3	623.3acd	489.5bcd	684.3ab	548.8a-d
$(NH_4)_2SO_4$	473.2cd	731.4a	602.2a-d	632.7abc
$CO(NH_2)_2$	424.0d	742.6a	565.4a-d	677.7abc

Means of the same factor in a row or a column followed by the same letter are not significantly different at P > 0.05 by Duncan's Multiple Range Test. AT_1 = ½ at sowing + ½ at tillering; AT_2 = ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation; AT_3 = ½ at sowing + ½ at panicle initiation; AT_4 = ½ at tillering + ½ at panicle initiation

Table 8. Interaction effects of cropping year, N sources and application time on soil K content of rice field at Gambella

	2008				2009			
	Application time (AT)				Application time			
Sources	AT_1	AT_2	AT_3	AT_4	AT_1	AT_2	AT_3	AT_4
of N	Soil K ($cmol_c$ kg^{-1} soil)							
NH_4NO_3	0.63cd	0.63cd	0.75ab	0.70bc	0.32gh	0.42ef	0.29gh	0.45e
$(NH_4)_2SO_4$	0.80a	0.60cd	0.65cd	0.65cd	0.24hi	0.19i	0.34fgh	0.42ef
$CO(NH_2)_2$	0.65cd	0.70bc	0.58d	0.58d	0.29gh	0.27ghi	0.37efg	0.19i

Means of the same factor in a row or a column followed by the same letter are not significantly different at P > 0.05 by Duncan's Multiple Range Test. AT_1 = ½ at sowing + ½ at tillering; AT_2 = ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation; AT_3 = ½ at sowing + ½ at panicle initiation; AT_4 = ½ at tillering + ½ at panicle initiation

Table 9. Interaction effects of N sources and application time on tissue N content (%) of rice at Gambella

Sources of N	Application time (AT)			
	AT_1	AT_2	AT_3	AT_4
NH_4NO_3	1.75abc	1.91a	1.35cde	1.25de
$(NH_4)_2SO_4$	1.83de	1.43b-e	1.14e	1.47b-e
$CO(NH_2)_2$	1.11e	1.27de	1.63a-d	1.19e

Means of the same factor in a row or a column followed by the same letter are not significantly different at P > 0.05 by Duncan's Multiple Range Test. AT_1 = ½ at sowing + ½ at tillering; AT_2 = ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation; AT_3 = ½ at sowing + ½ at panicle initiation; AT_4 = ½ at tillering + ½ at panicle initiation

The N content of the soil declined after the crop harvest due to N sources and application time (Tables 6 and 4) compared to that was observed before sowing of rice (0.51%). However, interaction effects of both cropping year with N source and cropping year with N application time showed significant differences in total N content during 2008, while there was no significant ($P > 0.05$) difference during 2009 (Tables 6 and 4), respectively. The interaction effect of cropping year 2008 with $CO(NH_2)_2$ as a source of N resulted in significantly higher soil total N content (0.43%) than the other interaction effects. This was followed by $(NH_4)_2SO_4$ as a source of N in the same year which did not show a significant difference in soil N content recorded with the application of NH_4NO_3 in 2008 and all the three sources of N in 2009. Although the N sources resulted in a decrease in soil N compared to the initial (0.51%) value over the cropping years, the $CO(NH_2)_2$ significantly increased soil N by 34.9 and 23.3% compared to NH_4NO_3 and $(NH_4)_2SO_4$ in 2008. However, the maximum total N content depletion in soil was observed in 2009 with $(NH_4)_2SO_4$. This depletion of soil N content represented a decrease of 37.2 and 47.1% over the highest (0.43%) and the initial (0.51%) soil total N content, respectively.

On the other hand, the interaction effects of cropping year with N application time (Table 4) showed significantly ($P \leq 0.05$) higher soil total N content (0.43%) with N application in two equal splits (at tillering and panicle initiation stages) which was at par with soil N contents obtained with interactions of application of N ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation as well as ½ at sowing + ½ at panicle initiation in 2008 cropping year. However, this accounted for a decrease of 15.7% over the initial soil total N value (0.51). The soil N content remained statistically at par with the application of N ½ at sowing + ½ at tillering, ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation and ½ at sowing + ½ at panicle initiation in 2009 when compared to 2008 (Table 4). The highest depletion of soil total N content (0.25%), however, was recorded with N application ½ at sowing + ½ at tillering in 2008. This depletion of soil N content represented a decrease of 41.9 and 51.0% over the highest (0.43%) and the initial (0.51%) soil total N values, respectively. NH_4NO_3 increased soil total N by 3.28% while $(NH_4)_2SO_4$ decreased it by 7.10% and $CO(NH_2)_2$ by 3.83% (Assefa *et al.*, 2009).

Soil phosphorus content: The significant differences on soil P was recorded due to cropping year ($P \leq 0.01$), N application time, interaction of year with N application time and N source with application time ($P \leq 0.05$) while no significant difference ($P > 0.05$) was observed due to N sources, interactions between year and N sources, as well as between year, N source and application time (Table 2). After the first year, there was an increase of 15.6% in soil P content over the initial available P content (650.0 mg kg^{-1} soil), but after the second cropping year it declined by 40.4 and 31.1% compared to soil P contents recorded after the first cropping year and before the start of the experiment, respectively (Table 3).

The interaction of cropping year with N application time (Table 4) revealed the highest P (858.1 mg kg^{-1} soil) with the application of N ½ at sowing + ½ at panicle initiation which was statistically at par only with N applied in three equal splits at sowing, tillering and panicle initiation stages in 2008. The available P content of the soil decreased significantly in 2009 compared to 2008 due to absorption by rice plants and the strong retention of phosphate ions by reactive soil components (Sample *et al.* 1980).

Further, the interaction of N source with its application time indicated the highest soil P content (742.64 kg^{-1} soil) with $CO(NH_2)_2$ applied ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation that was statistically in parity with soil P content obtained with the other interactions except NH_4NO_3 applied ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation, $NH_4)_2SO_4$ and $O(NH_2)_2$ applied ½ at sowing + ½ at tillering (Table 7). The magnitude of increase in soil P value over the lowest (424.03 mg kg^{-1} soil) and initial (650.0 mg kg^{-1} soil) due to application of $CO(NH_2)_2$ ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation was 42.9 and 14.3%, respectively in interaction of N sources and application time.

According to the University of California research as well as field experience, the economic value of soil analysis before planting and leaf tissue analysis during the growing season indicated that the minimum soil levels of P necessary for satisfactory rice yields is 6-9 ppm orthophosphate (PO_4-P) using $NaHCO_3$ soil test methods. Therefore, the soil test result revealed that the studied site soil had excess soil P content (Tables 3, 4 and 7). The upper leaves of rice also developed yellowish color/chlorosis that might be indicated the deficiency of micronutrients due to excess availability of soil P.

Soil potassium content: The effects of cropping year, N source, interactions of N source with application time, year with N source and application time ($P \leq 0.01$) and year with application time ($P \leq 0.05$) on soil K were significant whereas application time and interactions of year with N source were not significant ($P > 0.05$) (Table 2). In the 2008 higher value of soil K (0.66) was obtained than that of the 2009 (0.32) which increased (9.1%) after the first year of cropping and then showed a decrease of 46.7%, over the initial soil K content (Table 3).

The interaction of cropping year, N source and application time (Table 8) significantly ($P \leq 0.01$) affected soil K content. Accordingly the highest value of soil K (0.80 $cmol_c$ kg^{-1} soil) resulted with the application of $(NH_4)_2SO_4$ ½ at sowing + ½ at tillering in 2008 which was significantly ($P \leq 0.01$) higher than the other interactions except NH_4NO_3 ½ at sowing + ½ at panicle initiation in the same year. The magnitude of increase in soil K value over the lowest (0.19 $cmol_c$ K kg^{-1} soil) and initial value (0.60 $cmol_c$ K kg^{-1} soil) due to application of $(NH_4)_2SO_4$ ½ at sowing + ½ at tillering was 76.3 and 33.3%, respectively.

Based on the University of California research as well as field experience the economic value of soil analysis before planting indicated that the minimum soil levels of K necessary for satisfactory rice yields is 0.15 $cmol_c$ kg^{-1} soil using NH4Ac soil test methods. Therefore, the soil test result revealed that the studied site soil had excess soil K content (Tables 3, and 8). Further, there was no significant difference among soil K content recorded with interactions of year, N source and N application time except with $NH_4)_2SO_4$ applied ½ at sowing + ½ at tillering and NH_4NO_3 applied ½ at sowing + ½ at panicle initiation in 2008. In 2009, there was no significant difference among soil K contents due to application of NH_4NO_3 ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation and ½ at tillering + ½ at panicle initiation and $(NH_4)_2SO_4$ ½ at tillering + ½ at panicle initiation. Moreover, soil K contents obtained with NH_4NO_3 applied ½ at sowing + ½ at tillering and ½ at sowing + ½ at panicle initiation, $(NH_4)_2SO_4$ ½ at sowing + ½ at panicle initiation and $CO(NH_2)_2$ ½ at sowing + ½ at tillering, ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation and ½ at sowing + ½ at panicle initiation had no significant variation among them during 2009. Similarly, there was no significant difference among soil K content obtained with $(NH_4)_2SO_4$ ½ at sowing + ½ at tillering, ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation and $CO(NH_2)_2$ ½ at tillering + ½ at panicle initiation in 2009 (Table 8). However, Some mechanisms of soil fertility and the interaction effects among factors could only be recognized rightly after 10 years (Xu *et al.* 2006; Wu *et al.* 2008)

Soil sulfur content: Analysis of variance (Table 2) indicated that the effects of cropping year, N sources ($P \le 0.01$), interaction of year with N source ($P \le 0.05$) were significantly influenced the soil S content, while N application time, interactions of year with application time, N source with application time and year, N source and application time had no significant ($P > 0.05$) effect. The soil S content increased by 39.5 and 203.7% than that of the initial value after the 2008 and 2009 crop harvests, respectively due to applied N source (Table 3).

The interaction effects of year with N source (Table 6) showed significant ($P \le 0.05$) variation in soil S content. Accordingly, the interaction effects of year with N source treatment showed significantly ($P \le 0.05$) higher soil S content (26.44 mg kg^{-1} soil) with applied $(NH_4)_2SO_4$ ½ than the other interactions of year by N source and accounted for an increase of 319.7% over the initial soil S content (6.30 mg kg^{-1} soil). This highest soil S content also showed an increase of 79.0% than the lowest soil S content that obtained with applied $CO(NH_2)_2$ in 2008.

Application of S bearing materials was found to increase the content of available S in the soils and the increments were significantly ($P \le 0.05$) stronger with the passage of time (Ahmed and Khan, 2010). The $(NH_4)_2SO_4$ exerted better response to the increment of S because of the contents of other essential nutrients, especially N, which enhanced S uptake by the rice compared with the $CO(NH_2)_2$ and NH_4NO_3 treated plots. In Assefa *et al.* (2009) study, the lowest soil S content (8.37 mg S kg^{-1} soil) also recorded with $CO(NH_2)_2$ treated plots. It could be stated that using N fertilizer in the form of $(NH_4)_2SO_4$ surpassed soil S content by 19.1% and 58.1% as compared to NH_4NO_3 and $CO(NH_2)_2$, respectively (Table 3).

Effect of N sources and their application time on rice tissue N and S contents

Rice tissue nitrogen content: The effects of cropping year, interaction of year with N sources, N sources with application time ($P \le 0.01$), and N source and application time ($P \le 0.05$) on rice tissue N content were found to be significant while interactions of year with application time and year, N source with application time were not (Table 2) significant. The rice tissue N content was significantly higher in 2009 than in 2008 (Table 3). This difference may be due to changes in soil properties as well as the meteorological conditions like temperature and rainfall that enhanced the availability of nitrogen for plant uptake.

The interaction effects of cropping year with N source and N source with its time of application (Tables 9 and 10) showed significant ($P \leq 0.01$) difference in rice tissue N content. According to the interaction effects of year with N source (Table 6) highest tissue N content (2.33%) was obtained from NH_4NO_3 application in 2009 than the other interactions and the lowest value (0.64%) was obtained from plants receiving $CO(NH_2)_2$ as a source of N in 2008. Increase in N concentration was obviously due to the better supply of this nutrient in soil associated with relatively higher uptake by plants (Mahmood *et al.*, 1993). Less uptake of N in case of urea than $(NH_4)_2SO_4$ or NH_4NO_3 could be due to volatilization losses of urea under high temperature of study area.

According to two year average interaction effects of N source with application time on the tissue N content of rice, the highest value (1.91%) was obtained from NH_4NO_3 applied ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation which had no significant variation with tissue N value obtained from NH_4NO_3 applied ½ at sowing + ½ at tillering and $CO(NH_2)_2$ applied ½ at sowing + ½ at panicle initiation (Table 9). Lowest (1.11%) tissue N content, however, obtained with $CO(NH_2)_2$ applied ½ at sowing + ½ at tillering and had no significant variation with most of the interactions except with NH_4NO_3 ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation and ½ at sowing + ½ at tillering and $CO(NH_2)_2$ ½ at sowing + ½ at panicle initiation.

University of California research clearly demonstrates the economic value of leaf tissue analysis during the growing season, to assess the fertilization requirement of rice. For example, the critical and adequate tissue N necessary for satisfactory rice yields is 3.3 and 3.3-3.8 (% total N). High N concentration and accumulation at flowering stage was the basis of large post-flowering dry matter production and N redistribution (Wen-xia *et al.*, 2007). In a paddy field, rather than nitrate (NO_3^-), ammonium (NH_4^+) is considered the main source of N for rice. However, in recent years, researchers have paid more and more attention to the partial NO_3^- nutrition of rice crops, and the results have shown that lowland rice was exceptionally efficient in absorbing NO_3^- formed by nitrification in the rhizosphere (Kirk and Kronzucker, 2005; Duan *et al.*, 2006). To summarize, in line with this reality, average tissue N (1.57%) observed in NH_4NO_3 at all application time showed an increase of 6.4 and 17.2% than $(NH_4)_2SO_4$ and $CO(NH_2)_2$, respectively. The average tissue N value obtained with N sources ½ at sowing + ½ at tillering showed an increase of 1.3, 12.2 and 16.7% over application of ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation, ½ at sowing + ½ at panicle initiation and ½ at tillering + ½ at panicle initiation, respectively (Table 3), however, there was no significant difference among them except with N source applied ½ at tillering + ½ at panicle initiation.

Wen-xia *et al.* (2007) arranged seven N treatments in different fertilizer N management strategies, including control and three fixed N split treatments. The three fixed N split treatments were with total N rates of 60, 100 and 140 kg N ha^{-1} with 20% applied at three leaf stage, 35% at early tillering, 45% at panicle initiation in Jinzao22. Whereas total N rates of 60, 120 and 180 kg N ha^{-1} with 35% applied at sowing, 20% at mid-tillering, 30% at panicle initiation and 15% at heading in Shanyou 63. Finally, they concluded that increasing fixed N split rates enhanced dry matter and N accumulation at different stages in Jinzao 22 and Shanyou 63 rice cultivars, while there was no significant difference between 120 and 180 kg N ha^{-1} (Mae *et al.*, 2006; Peng *et al.*, 2006; Wen-xia *et al.*, 2007).

Tissue sulfur content: For tissue S content, only the effects of N application time was significant ($P \leq 0.01$) while the effects of year, N sources, interactions of year by N source, year by application time, N source by application time and year by N source by application time were found not to be significant ($P > 0.05$) (Table 2).

The two years average highest rice tissue S content (1.39 g kg^{-1} tissue) obtained with application of N ½ at tillering + ½ at panicle initiation over than the other application times. All rice tissue S values that were recorded from N application times, however, did not show significant variation to each other except with application ½ at tillering + ½ at panicle initiation. Fry *et al.*(2011) showed as S is an essential plant nutrient, with tissue levels of approximately 0.2% thought to be reflective of adequate S nutrition. Similarly, Aggarwal and Nayyar (1998) reported that soil having an available S status of 22.2 ppm significantly and positively correlated with S concentration in wheat plants and S uptake by wheat grain and straw.

Conclusion

Due to the economic and agronomic importance of rice, it is becoming popular in Gambella Region of Ethiopia. The source of N and its application time has a paramount importance to harvest a good yield. Hence, determining of N fertilizer sources and their time of application is important to ensure adequate soil OC, pH, P, K soil and tissue N and S contents. The study showed that application of different inorganic N sources and their split application had a negative effect on soil OC and N content whereas, NH_4NO_3 and $(NH_4)_2SO_4$ applied ½ at sowing + ½ at tillering showed positive effect on soil pH, and $(NH_4)_2SO_4$ on soil S. $(NH_4)_2SO_4$ applied ½ at sowing + ½ at tillering resulted in better soil OC. The highest soil P was found with $CO(NH_2)_2$ ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation while $(NH_4)_2SO_4$ ½ at sowing + ½ at tillering and NH_4NO_3 ½ at sowing + ½ at panicle initiation obtained significantly higher soil K content than other treatment combination. The highest tissue N content obtained with applied NH_4NO_3 as ⅓ at sowing + ⅓ at tillering + ⅓ at panicle initiation. Generally, evidence suggested that even balanced application of inorganic fertilizers alone does not sustain the soil productivity under continues cropping. Therefore, to reduce the inorganic fertilizer requirement and to restore organic matter in soil, enhance nutrient use efficiency and maintain soil quality in terms of physical, chemical and biological properties and to ensure sustainable rice production, combined and balanced use of inorganic and organic nutrients following site and season-specific nutrient management is important.

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