Full Length Research Paper

Estimating soil available nitrogen with a hot H_2O_2/KCl extraction

T. B. Tié[1], L. N. Diby[1, 2]*, E. Seyo[2] and A. Assa[3]

[1]Department of Water, Forest and Environment, National Institute of Technology FHB, Yamoussoukro, Côte d'Ivoire.
[2]Swiss Center for Scientific Research (CSRS), Abidjan, Côte d'Ivoire.
[3]Department of Earth Sciences and Mine Resources, University of Cocody, Abidjan, Côte d'Ivoire.

Appropriate and simple analysis methods for an accurate estimation of soil N mineralization potential are required to optimize N fertilizer recommendations. We used soils from non-cultivated and cultivated plots in central Côte d'Ivoire to investigate the quantum of ammonium (NH_4^+) that could potentially be mineralized. The soil was first treated with H_2O_2 at 60°C for 2, 4, 6 or 8 h (and referred to as hot extraction) or at room temperature (25°C and referred to as 0 h). The NH_4^+-N extracted increased with the heating time but decline slightly after 6 h. More NH_4^+-N was extracted in non-cultivated plots with the hot extraction, and the difference was significant at 6 h. The NH_4^+-N measured with the hot extraction correlated significantly with the CEC. We obtained the strongest correlation in non-cultivated plots (r^2 = 0.88, p < 0.0001) at 6 h, and the weakest also at 6 h but in cultivated plots (r^2 = 0.64, p < 0.0001). Similarly, we observed a significant correlation between the NH_4^+-N extracted and the amount of N taken up by rice seedlings grown in pots, and the best correlation was again at 6 h. The study illustrated that N mineralization with H_2O_2 at 60°C for 6 h is an appropriate procedure for extracting potentially mineralizable NH_4^+-N in soils.

Key words: Cation exchange capacity (CEC), extractable ammonium, ferralsol, N mineralization, total N.

INTRODUCTION

The estimation of soil organic nitrogen (N) mineralization is important for optimal N fertilization of crops. Non leguminous plants take up mainly inorganic forms of N (NH_4^+-N and NO_3^--N) from soils, while up to 95% of soil N is bound in organic matter. Existing methods include biological (incubation methods) and chemical extraction methods. The biological methods are thought to be more relevant because the mineralization occurring during incubation is a microbial process which better mimics mineralization observed under field conditions (Sahrawat,

1982). However, biological methods are time-consuming and laborious (Haney et al., 2001). In contrast, chemical extraction methods where soil organic matter is oxidized with chemical agents are less time-consuming (Whitehead, 1981) and are also easier to run (Haney et al., 2001). Many authors have reported different chemical methods using KCl solution for mineral N extraction (example: solution concentration, extraction time and temperature) (Oien and Selmer-Olsen, 1980; Whitehead, 1981; Sahrawat, 1982; Gianello and Bremmer, 1986; Smith and Li, 1993; Kowalenko, 2006).

Since organic N mineralization is an oxidation process, all chemical reagents that can oxidize soil organic matter have a potential to release NH_4^+-N from the soil absorbent complex. Hydrogen peroxide (H_2O_2) is a reagent

---

*Corresponding author. Email: lucien.diby@gmail.com. Tel: +225 23 47 27 90. Fax: +225 23 45 12 11.

Table 1. Selected soil properties in non-cultivated and related cultivated plots collected in 2007 in three agro ecosystems in central Côte d'Ivoire.

Treatments	Plot history	Clay (%)	Silt (%)	pH $_{H20}$	CEC (cmol kg^{-1})	Total C (mg g^{-1})	Total N (mg g^{-1})
					Soil properties		
Forest	Non-cultivated	25.3	8.0	6.1	8.72	18.8	1.60
	Cultivated	25.6	8.4	6.0	8.02	17.4	1.49
Fallow	Non-cultivated	22.5	4.4	6.4	7.04	14.6	1.29
	Cultivated	21.0	5.9	6.1	6.82	14.6	1.20
Savanna	Non-cultivated	20.1	7.1	6.1	5.99	12.5	1.21
	Cultivated	16.7	6.9	5.8	4.98	11.0	1.07

commonly used in soil analysis for organic matter oxidation before mineralogical determinations (Mikutta et al., 2005). Sahrawat (1980) showed that NH_4^+-N can be extracted with a solution of KCl after treating samples with a solution of 30% H_2O_2 for 15 min at room temperature (25°C). In other work, Sahrawat (1982) treated samples with the same H_2O_2 solution at the same room temperature for 1 h, and observed an increase of the amount of $NH4^+$-N extracted, and a better correlation between this $NH4^+$-N and that measured by incubating the samples at 30°C for 2 weeks or at 40°C for 1 week. This indicated that temperature and time during which the soil is left in contact with the H_2O_2 affects NH_4^+-N extraction. Besides, since the ammonification in soil proceeds optimally between 40 - 60°C (Lee et al., 2009), we hypothesized that heating the soil/H_2O_2 suspension at moderate temperatures may improve the subsequent NH_4^+-N extraction. The objectives of this study were to estimate soil NH_4^+-N extracted with a solution of KCl after a prior heating of the soil/H_2O_2 suspension for mineralization, and to determine the contribution of the NH_4^+-N extracted through this method to rice seedlings.

MATERIALS AND METHODS

Study sites

The study sites were located in the forest-savanna transition zone of central Côte d'Ivoire known as the "V-Baoulé" (6° 50' – 7° 50' N and 4° - 6° W). The average annual rainfall in the area is 1000 - 1200 mm with temperature ranging between 26 and 29.5°C (CSRS, Station expérimentale, Bringakro, Côte d'Ivoire). The cropping system in the study area is based on yams which are cultivated mainly at the forest. After one cropping year, the land is left under natural fallow (dominated by *Chromoleana odorata*) for 2 - 5 years before being cultivated again. The savanna was traditionally not cropped; however due to the high population growth rate, it is more and more cultivated by farmers whose have limited access to forest land. Soils in the area are ferralsol (WRB, 2006) derived from granitic and schistose rocks. They are characterized by a high proportion of coarse elements at low depth.

Soil samplings

The soil samples were collected at the onset of the rainy season in 2007. The number of samples collected from the forest, the fallow and the savanna zones were 21, 19, and 10 pairs (total of 100 samples) respectively. A pair included a sample collected from a plot where yams had been cropped during the last growing season (cultivated plot) and left under natural fallow thereafter, and the other collected from an adjacent site under natural vegetation (non-cultivated plot). Fewer samples were collected from the savanna site because these soils are rarely cropped by farmers and it was difficult to find cultivated plots. Each sample was collected with a hoe within an area of 1 m^2 which was ploughed to a depth of 20 cm. The samples were air-dried, sorted from coarse organic residues and ground to pass through a 2 mm sieve and individual sub-samples were used for determining soil properties following the protocols described in Pansu and Gautheyrou (2003). Soil particles size distribution was classified using the pipette Robinson method. The soil pH was measured in a soil to water ratio of 1:2.5. The organic C and total N were determined using the Walkley and Black and Kjeldahl methods respectively. The cation exchange capacity (CEC) was analyzed with a neutral (pH = 7) acetate ammonium solution. Soil properties are reported in Table 1.

Hot and cool NH_4^+-N extraction procedure

Five gram (5g) of a sub-sample from the sample described above (air-dried, sorted from coarse organic residues and sieve at < 2 mm) was placed in a 300 ml Erlenmeyer flask. Fifty ml of 25% H_2O_2 was added and homogenized manually. The suspension in the flask (soil + H_2O_2) was put in a ventilated oven with a temperature of 60°C for 2, 4, 6 and 8 h, respectively. After incubation for the predetermined times, the suspension was cooled and 150 ml of 1 M KCl added. The new suspension was homogenized on a mechanized rotary agitator for 30 min and filtered. The N-NH_4^+ was measured in the filtrate by steam distillation method (Pauwels et al., 1992). These samples will be denoted as 2, 4, 6 and 8 h respectively, based on incubation times.

The cold extraction (cool extraction) was carried out without heating the soil/H_2O_2 suspension following the procedure proposed by (Sahrawat, 1980; Sahrawat, 1982). However, the soil/H_2O_2 suspension was left at laboratory temperature (25°C) overnight before extracting the N-NH_4^+ which was a modification of the method used by Sahrawat (1980, 1982). These samples will be identified as 0 h.
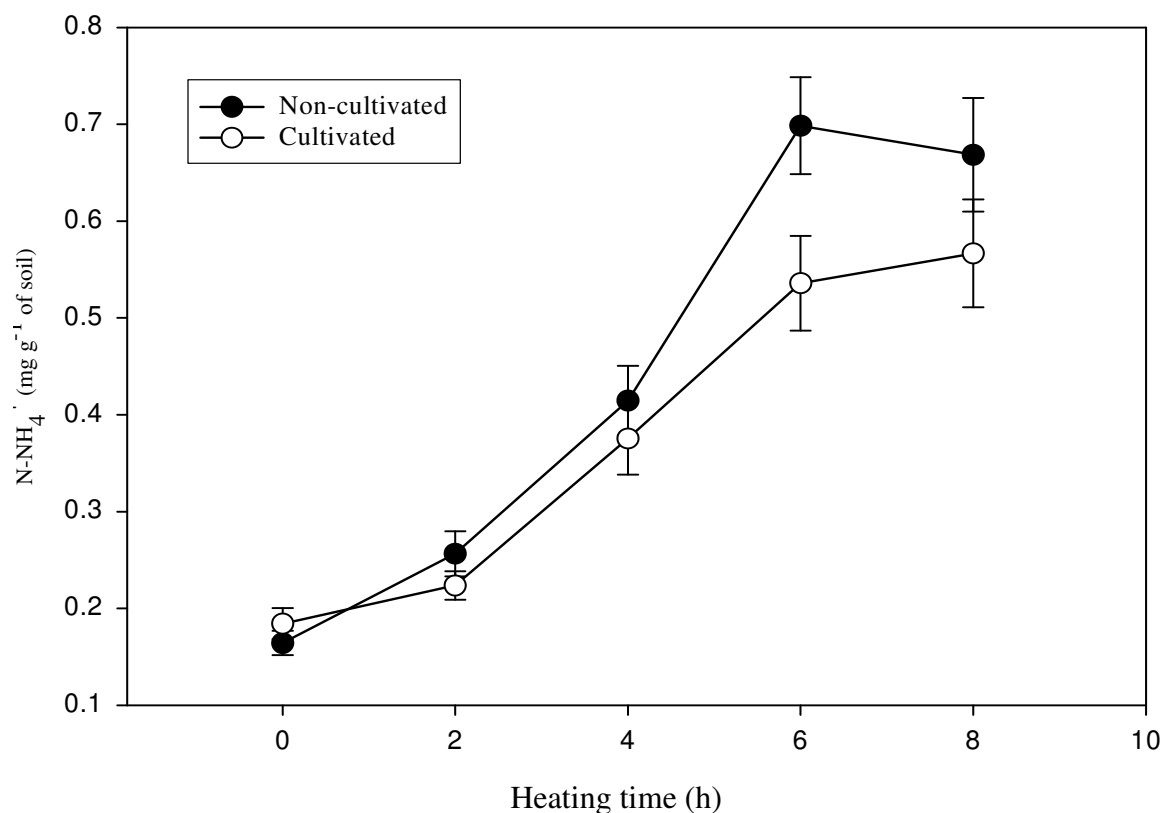
Figure 1. Amounts of NH_4^+-N extracted with a *1M* KCl after allowing the soil samples proceed with a solution of 25% H_2O_2 for 24 hours (0 hour) or after heating the soil/H_2O_2 suspension at 60°C for 2, 4, 6 and 8 hours respectively. Bars represent the standard error of the mean.

Pot experiments

In order to assess the availability for plants of the NH_4^+-N extracted with the hot procedure, pots experiments were conducted with soils sampled at the onset of the rainy season in 2008. Four samples from the forest and four samples from the savanna were used for the experiments. In each agro ecosystem, the samples were collected at different locations with different soil properties (data not shown). The extractable NH_4^+-N of these samples was measured using the hot extraction method described above. The pots used were perforated plastic pots of 200 ml capacity each, having a plastic cap stretched across the bottom. Soil sieved at < 2 mm was added at a rate of 150 g per pot. Each sample was split in eight pots giving a total of 64 pots. Rice (NERICA 2) seeds were pre-germinated, and five seeds were planted in each pot. In order to avoid K and P stresses, each pot received 25 ml of KH_2PO_4 solution with a concentration of 1 gl^{-1}. Each pot was irrigated once or twice a day with 20 - 30 ml of de-ionized water to maintain humidity close to soil water holding capacity. The excess of water was collected in the plastic cap and re-use in order to limit nutrients losses with water flow. After 3 weeks, the plants were carefully removed, and separated into shoots and roots before drying in an oven at 70°C to constant weight and determining their dry weights. The concentration of nitrogen (N) was measured on a sub-sample of shoots and roots using the Kjeldahl method (Pansu and Gautheyrou, 2003).

The PROC REG procedure of SAS v. 8.2 was used to estimate to regression coefficients of the different correlations.

RESULTS AND DISCUSSION

Impact of heating time duration on NH_4^+-N extraction

NH_4^+-N extracted increased rapidly by heating up to 6 h. This is due to the increase in soil organic matter oxidation. The rate of increase was lower between 6 h and 8 h in cultivated plots, and declined in non-cultivated plots (Figure 1) suggesting the end of the oxidation process and/or losses of mineral N through volatilization. This oxidation of the organic matter with the heating time duration and the and the subsequent volatilization may explain why Smith and Li (1993) observed similar increase pattern to that observed in our work although they did not use H_2O_2. These authors extracted soil mineral N by boiling soil samples with 1 M KCl for 0.5, 1 and 2 h respectively, and found a 40% increase of the mineral N extracted between 0.5 and 1 h, while only up to

Table 2. Regression coefficients of the linear correlation between the cation exchange capacity (CEC) and the NH_4^+-N extracted at different heating time.

Heating time (h)	Plot history	$CEC = a^* NH_4^+-N + b$					
		Slope (a)	s.e.$_a$	Intercept (b)	s.e.$_b$	r^2	P-values
0	Non-cultivated	18.61	5.77	4.48	1.08	0.18	0.0023
	Cultivated	10.36	3.76	5.05	0.81	0.14	0.0082
2	Non-cultivated	20.81	1.65	2.20	0.50	0.77	<0.0001
	Cultivated	24.32	2.61	1.52	0.64	0.64	<0.0001
4	Non-cultivated	13.98	0.98	1.74	0.48	0.81	<0.0001
	Cultivated	10.03	0.96	3.19	0.44	0.69	<0.0001
6	Non-cultivated	10.39	0.56	0.28	0.44	0.88	<0.0001
	Cultivated	7.36	0.79	3.01	0.50	0.64	<0.0001
8	Non-cultivated	7.04	0.91	2.83	0.72	0.55	<0.0001
	Cultivated	6.63	0.66	3.20	0.45	0.68	<0.0001

s.e.: Standard error of the coefficient; NH_4^+-N: Ammonium nitrate expressed in mg g^{-1}; CEC: cation exchange capacity expressed in cmol kg^{-1}.

10% increase was noticed from 1 to 2 h. NH_4^+-N extracted with the cool procedure was marginally lower in the non-cultivated plots than in cultivated ones (Figure 1), but the difference was not statistically significant. With the hot procedure however, NH_4^+-N contents were greater in the non-cultivated plots compared with the cultivated ones, difference was significant at 6 h. This decrease of NH_4^+-N in cultivated plots suggests that some of the NH_4^+-N have been taken up during the cropping period as shown by different authors (Cheryl et al., 2001; Solomon et al., 2002; Ilnicki and Zeitz, 2003).

The amount of NH_4^+-N extracted in the non-cultivated plots was 12, 30 and 43% higher than in the cultivated plots in the forest, the fallow and the savanna sites respectively (data not shown), when the soils were heated for 6 h. The narrow gap of NH_4^+-N between cultivated and non-cultivated plots at the forest site is in agreement with Chabalier (1986) who did not observe any N fertilizer effect on maize yield after five consecutive years of cropping in the same forest area, while a significant effect was obtained after the fifth year. This was related to an active N mineralization rate which released sufficient N for plants both in fertilized and unfertilized treatments in the first years.

Relationship between the CEC and the NH_4^+-N extracted

For a given sample, the CEC was measured on a sub-sample different from that used to extract the NH_4^+-N. A positive correlation was observed between the CEC and the NH_4^+-N extracted. The CEC represents actually the potential of the soil to exchange cations (including NH_4^+-N) between its solid and its liquid phases. The simple linear regression fitted between the CEC and the NH_4^+-N was highly significant for the different heating time duration (0, 2, 4, 6 and 8 h) (Table 2). The lowest correlation coefficients were obtained at 0 h (cool extraction) both in cultivated ($r^2 = 0.18$, p < 0.0023) and non-cultivated plots ($r^2 = 0.14$, p < 0.0082). With the hot extraction procedure however, higher correlations were observed irrespective of the heating duration. The amount of NH_4^+-N extracted by this procedure explained 55 to 88% of the variation in the CEC. The correlations were stronger in non cultivated plots than in cultivated ones except at 8 h, and the highest correlation was observed at 6 h ($r^2 = 0.88$, p < 0.0001). Similar relationships between the CEC and the NH_4^+-N were observed by Narteh and Sahrawat (1997), who correlated the total C, total N and clay contents, pH and the CEC with $NH4^+$-N extracted with the incubation method and concluded that the CEC was the best index for predicting the mineralized NH_4^+-N based on the coefficient of determination ($r^2 = 0.68$; n = 15). In our study, high coefficients of determination ($r^2 = 0.55 - 0.88$; n = 50) were observed between the NH_4^+-N extracted with the hot extraction procedure and the CEC. This correlation between the CEC and the NH_4^+-N (which derived from the oxidation of soil organic matter) is in agreement with et al. (2003) who reported that 75 - 85% of the CEC in tropical soils depends on soil organic matter because of

the low cation fixation capacity of the clay example, kaolinite in these soils.

In contrast to the CEC, the correlation between the NH_4^+-N extracted and the C/N defined by Jansen (1996) and Powlson et al. (2001) as a mineralization index was very weak in our study. Similar weak correlation was also observed between the NH_4^+-N extracted and the clay content which protect the OM against microbial activity (Feller and Beare, 1997).

Total N extracted in the form of NH_4^+-N

The NH_4^+-N extracted was correlated with the total N content. This correlation was however significant only when NH_4^+-N was measured with the hot extraction procedure (Figure 2). Similar correlations have been reported by Stanford and Smith (1972) and Narteh and Sahrawat (1997) who measured the NH_4^+-N with incubition methods. This suggests that the hot extraction method used in our study can be used to predict the potentially mineralizable NH_4^+-N. The amounts of NH_4^+-N extracted at 6 h was about 40 and 50% of the total N in non-cultivated and cultivated soils respectively. Pansu and Gautheyrou (2003) used biological extraction method and observed similar values, but they obtained much lower values (12 - 20%) when they used chemical extraction method. The proportions of 40 and 50% are also much higher compared to the proportion of free organic matter in tropical soils (15 - 20%) reported by Feller (1994). The proportions of total N extracted in the form of NH_4^+-N in this study may include both the fraction of NH_4^+-N that is readily available for plants, and the fraction that is mineralizable on a long term basis. This suggests that the hot extraction method presented in this work does not allow distinguishing between both fractions as already reported by N'Dayegamiye et al. (2007). Further investigations are therefore needed to quantify the different proportions of these two fractions.

These two fractions may represent the part of the total N in the active pool of the soil organic matter while the rest could be considered as linked to the recalcitrant pool which is hardly mineralized (Bidisha et al., 2007). The proportion of total N extracted in the form of NH_4^+-N was significantly lower at the savanna site than at the forest, but no difference was observed between the forest and the fallow (data not shown). This low proportion at the savanna site was presumably due to the fact the organic matter (OM) at this site is derived essentially from roots biomass which are more lignified. Indeed, the savanna site is burnt annually during dry seasons for hunting and also for feeding cattle with the re-growth of grasses (*Imperata* sp*., Panicum* sp*., Pennisetum* sp.) (Adou-Yao C., personal communication) at the beginning of the rainy

season. The aboveground biomass and the resulting litter are therefore poorly developed at this site. In the forest and the fallow sites however, the OM is dominated by residues of aboveground organs which are less lignified and thus easily degradable (Floret et al., 1993).

The total N extracted in the form of NH_4^+-N was significantly increased in non-cultivated plots only in the savanna site (data not shown). This can be related to a susceptibility of the cultivated soils in the savanna to erosion and volatilization of its N content due the sandy nature of the soil at this site.

Relationship between the amounts of N in plant total biomass and the NH_4^+-N extracted

There were significant linear correlations between the amounts of N taken up during the pot experiments and the NH_4^+-N extracted for all heating durations (Figure 3). Different authors used different NH_4^+-N extraction methods and observed the same relationships. For example, Smith and Li (1993) extracted the potentially mineralizable N with a solution of hot 1 M KCl and observed correlation between the mineral N measured and the amounts of N in dry grass ($r^2 = 0.90$), barley ($r^2 = 0.81$) and oats ($r^2 = 0.81$) grown in pots. Similarly, Sahrawat (1980) found significant correlation between the amount of NH_4^+-N released with an anaerobic incubation method and the N taken up by rice crops grown in a greenhouse pot experiment. The highest correlation ($r^2 = 0.87$, p < 0.0007) observed at 6 h confirmed that this period is an appropriate heating duration of the soil/H_2O_2 suspension to estimate the mineralizable NH_4^+-N that contribute to soil N supplying capacity.

Conclusions

Heating the soil/H_2O_2 suspension before extracting the potentially mineralizable NH_4^+-N was more effective than when the suspension was not heated. The dynamics of the NH_4^+-N extracted between cultivated and non-cultivated plots and/or its correlation with the amount of N taken up by plants may suggest the contribution of the extracted NH_4^+-N to plant N nutrition. We conclude that a moderate heat of the soil/H_2O_2 suspension at 60°C for 6 h is a convenient procedure for a quick estimation of potentially mineralizable NH_4^+-N. The subsequent NH_4^+-N measured and its relationship with the CEC may be useful for predicting soil-derived nitrogen availability for crops. It is recommended that the fraction of the NH_4^+-N extracted which is directly available for plants be studied further.
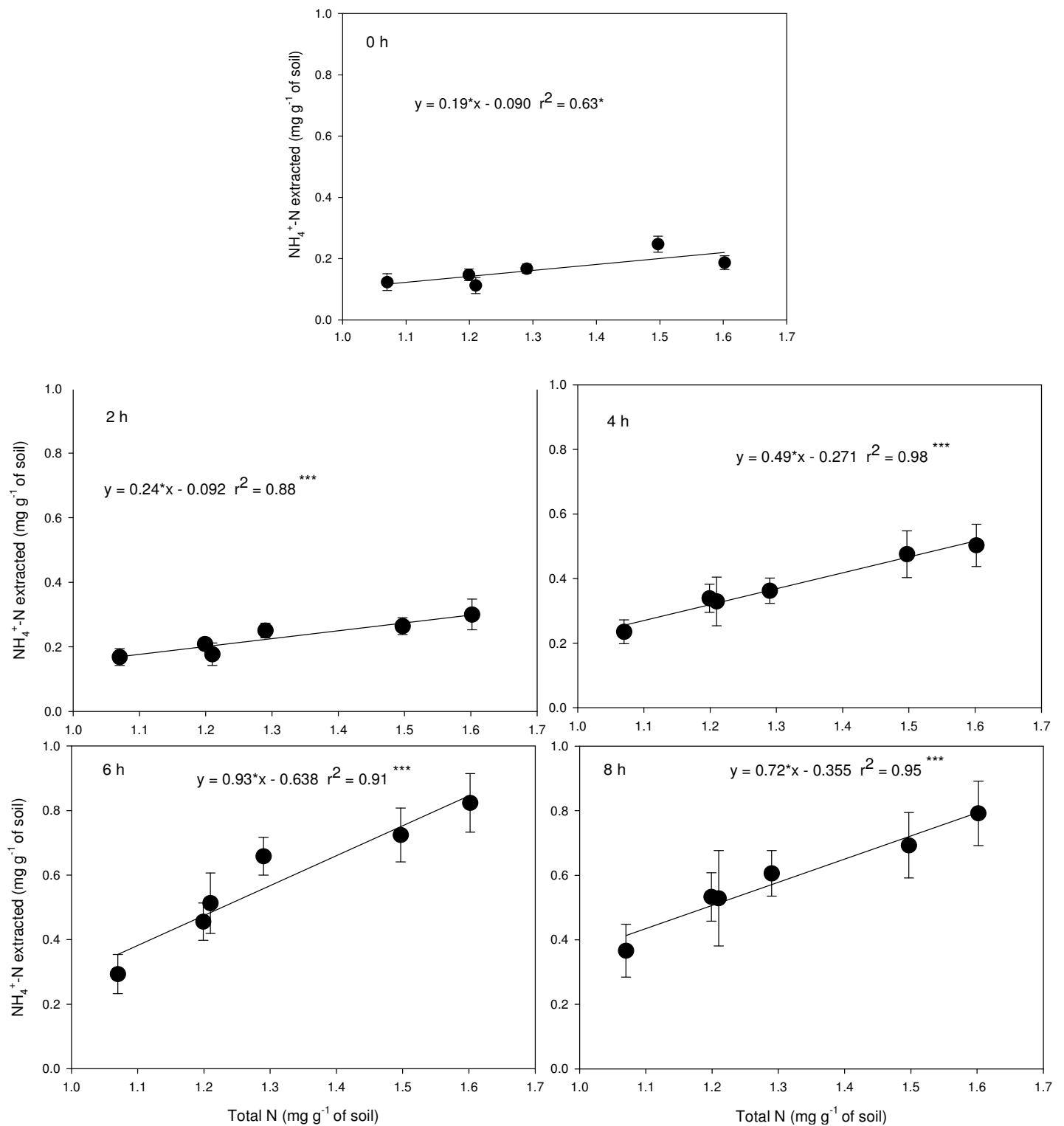
Figure 2. Relationship between the NH_4^+-N extracted with a *1M* KCl after allowing the soil samples proceed with a solution of 25% H_2O_2 for 24 hours (0 h) or after heating the soil/H_2O_2 suspension at 60°C for 2, 4, 6 and 8 h, respectively and the total N. Bars represent the standard error of the mean.
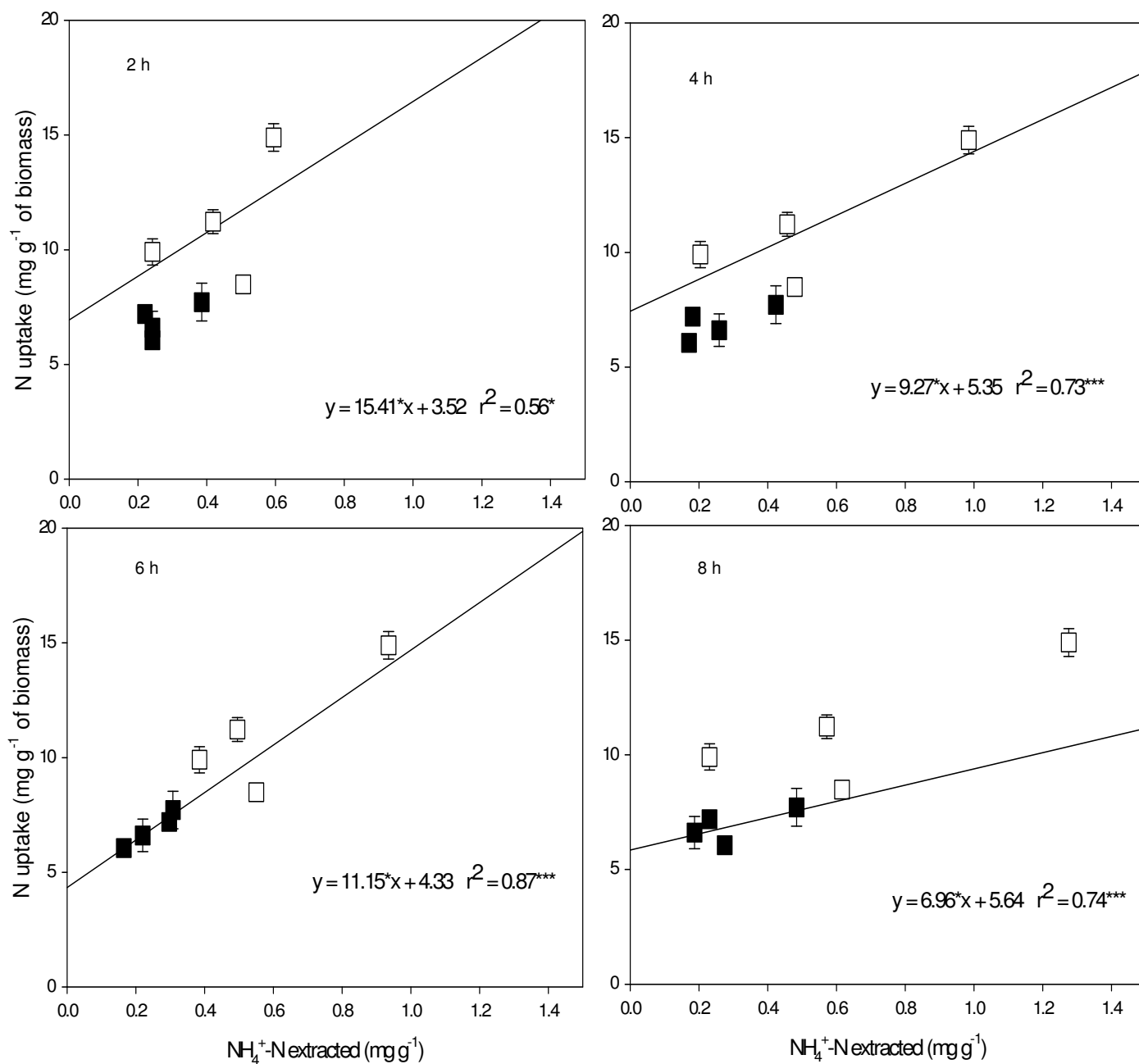
Figure 3. Relationship between the amount of N exported by the total biomass (root and shoot) of rice seedlings grown on non-cultivated forest (□) and savanna (■) soils (n = 8) and the amounts of NH_4^+-N extracted after heating the soil/H_2O_2 suspension at 60°C for 2, 4, 6 and 8 h, respectively. Bars represent the standard error of the mean.

ACKNOWLEDGEMENTS

REFERENCES

Bidisha M, Biswapati M, Bandyopadhyay PK, Chaudhury J (2007). Soil organic carbon pools and productivity relationships for a 34 year old rice–wheat–jute agroecosystem under different fertilizer treatments. Plant Soil 297(1-2): 53-67.

Chabalier PF (1986). Evolution de la fertilité d'un sol ferrallitique sous

culture continue de maïs en zone forestière tropicale. Agron. Trop. 41(3-4): 179-185.

Cheryl AP, Giller KE, Mafongoya PL, Swift MJ (2001). Management of organic matter in the tropics: translating theory into practice. Nutr. Cycl. Agroecosys. 61: 63-75.

Feller C (1994). La matière organique dans les sols tropicaux à argile 1:1: recherche de compartiments organiques fonctionnels: une approche granulométrique. Thèse Doctorat. Université de Strasbourg (ULP) 247 pp. + annexes.

Feller C, Beare MH (1997). Physical control of soil organic matter dynamics in the tropics. Geoderma 79: 69-116.

Floret C, Pontanier R, Serpentié G (1993). La jachère en Afrique tropicale. UNESCO. Paris.

Gianello C, Bremmer JM (1986). A simple chemical method of assessing potentially available organic nitrogen in soil. Commun. Soil Sci. Plan. 17: 195-214.

Haney RL, Hons FM, Sanderson MA, Franzluebbers AJ (2001). A rapid procedure for estimating nitrogen mineralization in manured soil. Biol. Fert. Soils 33: 100-104.

Ilnicki P, Zeitz J (2003). Organic soils and peat materials for sustainable agriculture. CRC Press, Boca Raton, Florida pp. 15-32.

Jansen BH (1996). Nitrogen mineralization in relation to C:N ratio and decomposability of organic materials. Plant Soil 181: 39-45.

Kowalenko CG (2006). Evaluation of hot KCl extraction as an index of mineralizable N: Ammonium and ultraviolet absorption measurements. Can. J. Soil Sci. 86(5): 911-920.

Lee C, Fletcher TD, Sun G (2009). Nitrogen removal in constructed wetland systems. Eng. Life Sci. 9(1): 11-22.

Mikutta R, Kleber M, Kaiser K, Jahn R (2005). Review: organic matter removal from soils using hydrogen peroxide, sodium hypochlorite, and disssodium peroxodisulfate. Soil Sci. Soc. Am. J. 69: 120-135.

Narteh LT, Sahrawat KL (1997). Potentially mineralizable nitrogen in West African lowlands rice soils. Geoderma 76: 145-154.

N'Dayegamiye A, Giroux M, Gasser M (2007). La contribution en azote du sol relié à la minéralisation de la matière organique; facteur climatique et régies agricloes influençant les taux de minéralisation d'azote. Colloque sur l'azote, Université de Laval, CRAAQ-OAQ, p. 17.

Oien A, Selmer-Olsen AR (1980). A laboratory method for evaluation of available nitrogen in soil. Acta Agric. Scand. 30: 149-156.

Oorts K, Vanlauwe B, Merckx R (2003). Cation exchange capacities of soil organic matter fractions in a Ferric Lixisol with different organic matter inputs. Agric. Ecosyst. Environ. 100: 161-171.

Pansu M, Gautheyrou J (2003). L'analyse du sol - minéralogique, organique et minérale. Springer-Verlag, Paris.

Pauwels JM, Van Ranst E, Verloo M, Mvondo Ze, A (1992). Manuel de laboratoire de pédologie: Méthodes d'analyses de sols et de plantes, équipement, gestion de stocks de verrerie et de produits chimiques. Publications agricoles 28. Centre Universitaire de Dschang - AGCD.

Powlson DS, Hirsch PR, Brookes PC (2001). The role of soil microorganisms in soil organic matter conservation in the tropics. Nutr. Cycl. Agroecosyst. 61: 41-51.

Sahrawat KL (1980). Nitrogen supplying ability of some Philippine rice soils. Plant and Soil 55(2): 181-187.

Sahrawat KL (1982). Evaluation of some chemical indexes for predicting mineralizable nitrogen in tropical rice soils. Commun. Soil Sci. Plan. 13(5): 363-377.

Smith KA, Li SX (1993). Estimation of potentially mineralizable nitrogen in soil by KCl extraction. 1. Comparison with pot experiments. Plant Soil 157(2): 167-174.

Solomon D, Fristzche F, Lehmann J, Tekalign M, Zech W (2002). Soil organic matter dynamics in the subhumid agroecosystems of the Ethiopian highlands: Evidence from Natural 13C abundance and particle-size fractionation. Soil Sci. Soc. Am. J. 66: 969-978.

Stanford G, Smith SJ (1972). Nitrogen mineralization potentials of soils. Proc. Soil Sci. Soc. Am. 36: 465-472.

Whitehead DC (1981). An improved chemical extraction method for predicting the supply of available soil nitrogen. J. Sci. Food Agric. 32(4): 359-365.

WRB (2006). World reference base for soil resources 2006. FAO, Rome.