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**Application of nitrogen forms affect pH and rice performance in
different soil types**

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Abstract

The shift in rice production systems from aerobic to anaerobic and fertilizer management may create an environment which can affect physicochemical properties of soil. The change in soil pH (acidification or alkalization) consequently alter the availability of macro- and micro-nutrients and their uptake. In this study, we hypothesized that the pH and the performance of rice will be affected differentially by the applications of different nitrogen forms on contrasting soil types. This study was conducted under greenhouse condition of the Institute of Crop Science and Research Conservation in the University of Bonn, Germany. A pot experiment with three soil types with contrasting inherent pH (acidic, neutral, and alkaline), three nitrogen forms (NH_4^+ , NO_3^- , NH_4NO_3) and Nipponbare. Bromocresol purple staining showed that rhizosphere of ammonium-fed rice was acidified down to pH 4 whereas alkalization was observed on the nitrate-fed rice that reached the pH value of 6 – 7. Moreover, NH_4NO_3 and NO_3^- were able to increase the rhizosphere pH of acidic and neutral soil by 0.2-0.4 units and NH_4^+ -N affects the decrease of pH only on alkaline soil. We observed the trade-off between soil types and nitrogen forms since shoot and root dry biomass were not significantly altered by their interaction. However, the dry biomass was tenfold higher in the acidic soil with NH_4^+ as the preferred form of nitrogen in comparison to the lowest mean value of shoot (0.085g) and root (0.048g) found in alkaline soil applied with NO_3^- . Meanwhile, highest proportion of roots were observed on alkaline soil which receives NH_4^+ as a form of N which is 13-45% higher than the other treatments. Our results showed that the inherent soil pH was further influenced by different nitrogen forms thus affecting rice performance.

Keywords: Ammonium, nitrate, nutrient management, *Oryza sativa*, system shift

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Introduction

Rice is the staple food of half of the world's population with 90% of global rice production is in Asia (Bandumula, 2018). Rice is grown in two known systems, rainfed that depends solely on rain as irrigation and irrigated that receives water throughout all stages of the plant. In the Philippines, almost 70% of the total rice area is irrigated and the remaining 30% is rainfed and upland (GRiSP, 2013). Among these systems, yield of upland rice is much lower than lowland, but its production cost is also lower. With these reason, aerobic production system will continue to be an important component of cropping systems (Fageria et al., 2011). Shifting of production systems from lowland to upland will subsequently result to the change in physical and chemical properties of soil. One of the important changes in flooded condition is soil pH. Fageria et al. (2011) suggested that acidic soil is likely to increase its pH due to the reduction of Fe and Mn oxides which consumes H^+ . The decrease in the pH of alkaline soils is attributed by microbial activity that decomposes organic matter and produces CO_2 . The resulted gas then reacts with water resulting in the formation of carbonic acid which dissociates into H^+ and bicarbonate (HCO_3^-) ions. On the other hand, in rainfed rice production, the bicarbonate buffer is off-set and the soil pH varies by soil type (parent material and weathering intensity) and can further be modulated by the type of fertilizer applied, and especially by the prevailing form of mineral nitrogen (nitrate vs. ammonium). Hao et al. (2020) reported perfect correlation between nitrogen input and H^+ production which can induce acidity or alkalinity on specific soil conditions, and nitrogen forms further determine the extent of pH change. Studies on this aspect however remain limited thus, this study aims to investigate the effect of nitrogen forms on the pH of rhizosphere and the response of rice on the prevalent soil types in the Philippines.

Methodology

The experiment was conducted in one of the tropical greenhouses of the Institute of Crop Science and Research Conservation in the University of Bonn, Germany. Three soil types with different inherent pH from the archive were used to represent Philippine rice soils (acidic, neutral, and alkaline). Growing media was prepared by mixing 50% quartz sand and 50% soil type that filled a total of forty-five (45) pots. Two-week-old Nipponbarre rice seedlings were transplanted individually. Soil moisture was maintained at 70% by weighing the pots daily and deficit irrigation with distilled water. Furthermore, ammonium sulphate ($(NH_4)_2SO_4$), ammonium nitrate (NH_4NO_3) and calcium nitrate ($Ca(NO_3)_2$) were the sources of NH_4^+ -N, NH_4NO_3 -N, and NO_3^- -N forms, respectively. All other nutrients were supplied following the recommended concentration of 5ml per 4L distilled water from readily prepared solution. Six-week-old rice were harvested, and roots were washed for Bromocresol purple staining to semi-quantitatively measure the pH dynamic in the rhizosphere. In addition, soil solution was collected with Rhizon samplers and pH was measured eventually. Roots and shoots were then oven-dried for 48 hours for biomass and were further digested for nutrient content analysis. Results were processed statistically in R and R Studio in which two-way ANOVA was performed to determine significance in main and interactive effects whereas least significant difference was used to evaluate any two individual means.

Results and discussion

pH dynamic

The pH reactions on the Bromocresol purple staining with different N forms are shown in Figure 1. Strong acidification was observed in NH_4^+ -fed rice that can be categorized under the pH range of 4-5 whereas the purple reaction in NO_3^- -fed rice suggests alkalization in the rhizosphere. Moreover, NH_4^+ appeared to be the most preferred form of N of Nipponbare exposed with NH_4NO_3 fertilizer. The pH in the rhizosphere changed in all soil types with the application of different N forms (Figure 2). Additionally, NH_4NO_3 and NO_3^- were able to increase the pH of acidic and neutral soil by 0.2-0.4 units. NH_4^+ -N had a slight effect on the neutral soil and further increased the pH in acidic soil by 0.2 units, however, it led to the decrease in pH value in alkaline soil. These changes are attributed to the consumption and release of identical ions by plants to maintain pH balance in the cells. Ammonium fertilizer input has more potential in acidifying the soil since the transformation from NH_4^+ to NO_3^- by nitrification produces 2 H^+ compare to the transformation of other nitrogen forms (Hao et al., 2020). This was also clearly observed in the staining procedure. In addition to pH, other factors such as temperature and soil moisture content, can also influence plant's sensitivity to N-nutrition (Kotsiras et al., 2005).

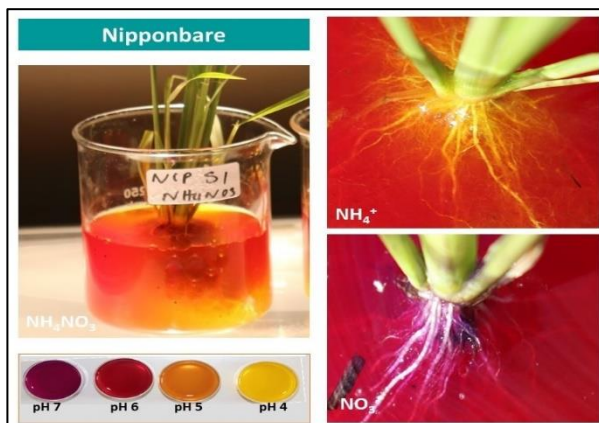


Figure 1. Bromocresol purple staining for semi-quantitative measurement of rhizosphere pH on rice.

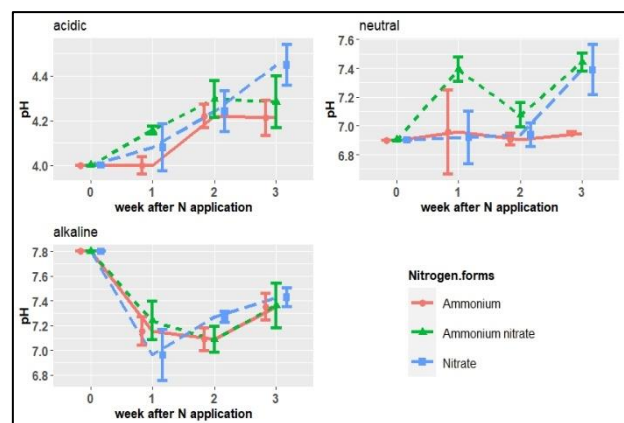


Figure 1. A temporal trend on pH dynamic of soil solution on contrasting soil types applied with different nitrogen forms. Each point is the mean of five individual plants. Week 0 is the initial pH of soil solutions before N application (acidic= pH 4, neutral= pH 6.9, alkaline= pH 7.8). Graphs show mean and standard error ($n = 5$).

Growth responses

Results of shoot and root dry biomass and root/shoot ratio are shown in Table 1. The interaction between Soil and N-forms did not influence significantly to the abovementioned parameters (two-way ANOVA, p -value < 0.05 , $df = 4$, $n = 5$). Interestingly, main effects of soil and N-forms had a significant influence for example on the root to shoot ratio with alkaline soil has significantly higher root proportion to acidic (11-12%) and neutral (10-14%). Additionally, there was a significant response to N treatments with 8% difference of NO_3^- -N from NH_4^+ and 4% to NH_4NO_3 -N. Rice preferred specific N forms in particular soil type in attaining highest production of shoot and root dry biomass. For instance, rice in acidic and alkaline soil preferred NH_4^+ -N and NH_4NO_3 -N, respectively whereas NO_3^- -N in neutral soil for shoot biomass. Similarly, NH_4^+ -N had the highest proportion of roots irrespective to the soil types while their interaction remains insignificant. The application of NH_4^+ -N in all soil types might have translocated more photosynthates to the roots thus the highest root ratio among other N forms (13-35%). High root to shoot ratio in alkaline soil can be attributed to the increased capture of both nitrate and ammonium from the soil (Weligama et al., 2010). Moreover,

these growth responses can be further explained and understood based on the nutrient status of individual plant given the difference in soil environment.

Table 1: ANOVA and mean shoot and root dry biomass and root/shoot ratio of Nipponbare on contrasting soil types and different nitrogen forms

Treatment		Dry biomass (g plant ⁻¹)		Root/shoot ratio (%)
Soil	Nitrogen	Shoot	Root	
Acidic	NH ₄ ⁺	0.814 ± 0.053 a	0.470 ± 0.058 a	58.6 ± 8.44 ab
	NH ₄ NO ₃	0.809 ± 0.066 a	0.391 ± 0.033 ab	48.6 ± 3.26 b
	NO ₃ ⁻	0.656 ± 0.098 ab	0.310 ± 0.054 b	47.4 ± 3.70 b
Neutral	NH ₄ ⁺	0.579 ± 0.039 ab	0.310 ± 0.021 b	54.5 ± 6.60 ab
	NH ₄ NO ₃	0.510 ± 0.024 b	0.306 ± 0.012 ab	53.8 ± 4.24 ab
	NO ₃ ⁻	0.604 ± 0.020 ab	0.281 ± 0.012 b	48.0 ± 3.46 b
Alkaline	NH ₄ ⁺	0.115 ± 0.056 c	0.078 ± 0.034 c	73.0 ± 0.87 a
	NH ₄ NO ₃	0.135 ± 0.074 c	0.080 ± 0.003 c	60.4 ± 1.38 ab
	NO ₃ ⁻	0.085 ± 0.083 c	0.048 ± 0.024 c	54.0 ± 3.48 ab
ANOVA	Soil	***	***	*
	N-forms	ns	*	*
	Soil x N-forms	ns	ns	ns

* same letters following \pm se on the same column are not significantly different at 0.05 level (Test= tukey, n=5). ANOVA: ns= not significant, *** significant at $p < 0.001$, * significant at $p < 0.05$, \pm = se

Conclusions and outlook

In conclusion, the change in status of pH in the soil is greatly affected by the forms of nitrogen applied which consequently affect rice plant growth and development. This dependency effect can be used for proper management of fertilizers on rice crop production systems under changing rhizosphere environments.

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