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Dried *Azolla pinnata* as a supplementary nitrogen source for lowland rice production in a Calcic Natraquert

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A pot experiment was conducted in the experimental field of the University of Ghana, Legon to examine the effectiveness of dry *Azolla* as N source in flooded rice field. The treatments included incorporating fresh *Azolla* (FA at 90 kg N/ha), dry *Azolla* (DA at 90 kg N/ha), dry *Azolla* + Ammonium sulphate (DA at 45 kg N/ha + AS at 45 kg N/ha), fresh *Azolla* + dry *Azolla* (FA at 45 kg N/ha + DA at 45 kg N/ha), ammonium sulphate (AS at 90 kg N/ha) and a control (C at 0 kgN/ha). The treatments were applied 8 days after transplanting rice. Results showed that the DA + AS treatment, that is, the treatment where dry *Azolla* + ammonium sulphate were used to fertilize the rice had the highest dry weight and total N yield followed by the treatment AS. Total N for the DA + AS treatment was 36.67% over the control whilst that for the AS was 25% over the control. Dry *Azolla* has the potential for supplementing for the nitrogen requirement for irrigated rice.

Key words: *Azolla*, tiller number, total N.

INTRODUCTION

Rice is gaining much research attention owing to an astronomic increase in its consumption relative to lower domestic production in some countries in Africa. The increase in consumption of rice in Africa is related to rapid population growth, incessant urbanization, increased income, and favourable government pricing policies, easy and long storability of rice and ease of cooking. About 90% of the world rice output is produced in Asia (66% in India, Indonesia and China).

Most rice fields in the tropics require the application of nitrogen fertilizer for cost-effective yields (Tilman et al., 2002). Ammonium and amide forms of nitrogen are preferable to nitrate forms in the lowland rice fields because nitrate forms are prone to leaching and losses through denitrification in flooded rice fields. The use of *Azolla* is beneficial in increasing rice production because *Azolla* can supplement more than half of the nitrogen requirements of lowland rice crop. As an aquatic fern, *Azolla* fixes atmospheric nitrogen and makes available some of the fixed nitrogen to the planted rice. *Azolla* can excrete 3 to 4% of the total N fixed to the exterior solution and of the total N released; 15 to 30% was available to the first rice crop (Liu, 1985). Rice field inoculated with *Azolla* before and after transplanting rice resulted in grain

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yields being increased by 54% over the control fields without *Azolla*. The complementary use of *Azolla* and N fertilizer for rice cultivation is receiving attention. Thus, incorporating 5 tons fresh *Azolla*/ha just before planting increased yields of rice by 44% over the control whilst a combination of 30 kg N/ha and 5 tons of *Azolla*/ha increased yields 86.4% (Singh and Singh, 1986). The application of 30 kg N/ha urea in combination with *Azolla* inoculated basally or with the *Azolla* intercropped gave an equal grain yield compared to 60 kg N/ha urea. Plant height, number of effective tillers, dry mass and nitrogen content of rice plants were increased with the use of *Azolla* and N-fertilizers and combinations (Bhuvaneshwari and Singh, 2015).

Azolla also prevents rise in flood water pH, reduce water temperature, curb NH_3 volatilization and suppresses weeds and mosquito proliferation in wetland rice fields (Pabby et al., 2004). When intercropped or incorporated into paddy rice fields, *Azolla* mitigated the rapid loss of nitrogen from chemical fertilizer since the release of the nutrient is progressively slow making it a viable alternative source of nitrogen in lowland rice production (Lumpkin and Plucknett, 1985). The applied *Azolla* increased both total and available nitrogen of the paddy soil. Methane emission was also reduced because *Azolla* improved the content of NO_2^- and NO_3^- in the floodwater and also of the soil. The NO_2^- and NO_3^- oxidized CH_4 to CO_2 (Mujiyo et al., 2016). Organic matter, potassium and the organic nitrogen contents of the soil significantly increased with the use of *Azolla* in rice production (Yadav et al., 2014). Both biochemical and biological properties of the field were improved by *Azolla* incorporation. Thus, the soil urease and phosphatase activities improved and there were increased cellulolytic and urea hydrolyzing activities in addition to significant increase in the population of heterotrophic bacteria due to *Azolla* application (Thanikachalam et al., 1984; Kannaiyan and Subramani, 1992). *Azolla* enhanced the soil biological health and optimized the use of organic, inorganic and biological inputs in an integrated manner (Yadav et al., 2014).

About two thirds of the total nitrogen that was in fresh *Azolla* was mineralized within 6 weeks and the nitrogen was made available to rice plants after the *Azolla* had been incorporated into the soil (Ito and Watanabe, 1984). However, nitrogen mineralization from dried *Azolla* is slower than fresh *Azolla* (Watanabe et al., 1977) when used to grow lowland rice. Dry *Azolla* has been used in feeding livestock and poultry (Giridhar and Rajendran, 2013).

The full potential of *Azolla* as a biofertilizer is yet to be realized since much emphasis has been made on fresh *Azolla* than on dried *Azolla*. Thus, the potential use of fresh *Azolla* in combination with dried *Azolla*, the use of dry *Azolla* and inorganic fertilizer, the combined use of fresh *Azolla*, dry *Azolla* and inorganic N fertilizer have not been fully explored for lowland rice production. This study was carried out to elucidate the use of dried *Azolla* in increasing lowland rice production.

The objective of the study was to examine the effectiveness of dry *Azolla* in influencing lowland rice growth in the Bumbi series (Calcic natraquert).

MATERIALS AND METHODS

Bumbi series is a vertisols of the Accra plains. It lies in a valley with a slope of 0 to 2% and is made up of montmorillonite clay derived from amphibolite and/or hornblende schists with little or no stones in the surface or subsurface horizons. The Bumbi series is deep and very poorly drained thus swelling when wet and very hard and cracks when dry. The Bumbi series has been classified by Amatekpor and Dowuona (1995) as Calcic Natraquert under the USDA Soil Taxonomy System. The soil texture is silty clay loam. The physical and chemical characteristic of the soil is shown in Table 1.

Soil sampling

Soil samples were taken from the plough layer (0 to 20 cm) of the Bumbi series from an uncultivated field at the Irrigation Development Authority (IDA), Ashaiman located on latitude 05° 41.400, and longitude 00°03.018. The area has an annual rainfall between 700 and 1000 mm. All samples were transported to the Department of Soil Science Laboratory of the University of Ghana. The soil was air dried, crashed and passed through a 2-mm sieve to obtain the fine earth fraction that was used for pot experiments later. Part of the fine earth fraction was used for laboratory analysis.

Bulk density was determined using the core sampler method of Blake and Hartge (1965). A core sampler was driven into the soil with a mallet far enough for all of its volume to be filled with the soil while a flat wood plank covered the uppermost opening of the cylinder. The core sampler was then removed by digging around it and then inserted a cutlass just beneath its bottom opening. The core sample was leveled by trimming the excess soil. The sampler was then covered and placed in a polythene bag to prevent water loss. At the laboratory, the soil was transferred into an initially weighed moisture can, reweighed and oven dried at 105°C for 24 h. The bulk density was then calculated.

The pH of the soil was determined using a pH meter with glass-calomel combination in distilled water and 0.01 M $CaCl_2$ solution at a ratio of 1:2 soil: solution. Determination was done in duplicate.

Some of the soil sample was sieved through 0.5 mm sieve and treated with HCl to destroy any calcium carbonate concretions present in the soil. Organic carbon content in that soil sample was determined using the Walkley and Black method (1934) which is based on the reduction of $Cr_2O_7^{2-}$ ion by organic matter, and the unreduced $Cr_2O_7^{2-}$ measured by titration with ammonium ferrous sulphate. The quantity of organic carbon oxidized was obtained from the amount of $Cr_2O_7^{2-}$ reduced. The organic carbon content obtained was adjusted using the factor of 1.32 since the 60 to 86% of soil organic carbon is only oxidized in the Walkley and Black method (1934).

The Kjeldahl method was used in the total nitrogen determination whereby soil (0.1 g) was weighed into Kjeldahl flask and selenium catalyst was added to accelerate the digestion process. This was followed by addition of 5 ml of concentrated H_2SO_4. The mixture was digested until the digest became clear. It was cooled and transferred into a volumetric flask and made to volume. An aliquot of 5 mL was taken into Markham distillation apparatus and 10 mL of 40% NaOH was added. The solution was distilled and the distillate was collected in 2% boric acid (H_3BO_3) solution which was then titrated with 0.01 M HCl from green to purplish endpoint. The

Table 1. Some physical and chemical properties of Bumbi series*.

Properties	Value
Bulk density (Mg/m^3)	1.45
pH	
H_2O (1:2)	7.6
0.01 M $CaCl_2$ (1:1)	7.1
Organic carbon (g/kg)	35.1
Available nitrogen (mg/kg)	410.0
Total nitrogen (g/kg)	1.46
Available phosphorus (mg/kg)	29.4
CEC (cmol/kg)	50.2
Exchangeable bases (cmol/kg)	
Ca^{2+}	12.3
Mg^{2+}	14.5
K^{+}	0.32
Na^{+}	0.78

percentage N was then calculated.

The available P was extracted from the soil using the Olsen method. Soil (5 g) was weighed into an extraction bottle and 50 ml of 0.5 M $NaHCO_3$ adjusted to pH 8.5 was added and shook on a mechanical shaker for 30 min. The suspension was filtered through a No.42 Whatman filter paper into a volumetric flask and 5 ml aliquot of the filtrate was transferred into another volumetric flask and the pH was adjusted using P-nitrophenol indicator and neutralized with a few drops of 4 M NH_4OH until the solution turned yellow. The concentration of the available P was determined using the method of Watanabe and Olsen (1965).

Soil (10 g) was weighed in duplicate into an extraction bottle and 100 ml of 1M ammonium acetate solution buffered at pH 7.0 was added. The bottle and its contents was placed on a mechanical shaker and shaken for 1 h. The supernatant was filtered through No.42 Whatman filter paper. The extract was used for the determination of Ca, Mg, Na, and K.

An aliquot (10 ml) of the extract was used for the readings of Ca^{2+} and Mg^{2+} on the Atomic Adsorption Spectrometer (AAS).

The flame photometer was standardized and used to determine the concentration of potassium and sodium in the extract.

Azolla collection, preparation and analysis for total nutrient content

Azolla pinnata was collected from Irrigation Development Authority (Ashiaman, Tema, Ghana). It was gently washed with tap water and dried with tissue paper. The fresh weight, dry weight and total N content of the *Azolla* were determined.

Greenhouse to determine *Azolla* influence on rice growth

Rice seeds (IR841) of 90% germination were nursed in pots and seedlings transplanted later.

Basal phosphorus and potassium were applied to all treatments at the rate of 45 kg P_2O_5/ha and 40 kg K_2O/ha. The experimental design was completely randomized (CRD). The experimental treatments were as follows:

(1) Fresh *Azolla* (FA), 90 kg N ha was applied (that is, 45 kg N/ha FA was basally applied and 45 kg N/ha FA topdressed at booting stage);
(2) Dry *Azolla* (DA) 90 kgN/ha was applied (that is, 45 kgN/ha DA was basally applied and 45 kg N/ha DA topdressed at booting stage);
(3) Ammonium sulphate (AS) 90 kgN/ha (that is, 45 kgN/ha AS was basally applied and 45 kg N/ha AS topdressed at booting stage);
(4) Dry *Azolla* + sulphate of ammonia (DA + AS), 45 kg N/ha DA basal application + 45 kg N/ha AS topdressing;
(5) Dry *Azolla* + Fresh *Azolla* (DA + FA that is, 45 kg N/ha FA basally applied + 45 kg N/ha DA topdressed
(6) Control (C), no *Azolla* nor ammonium sulphate was added to soil sample

Each treatment was replicated three times.

Each pot was filled with 3 kg of 2 mm sieved soil which was then flooded with water and left for a period of time for an equilibrium to be established between the soil and water. Three weeks after transplanting the rice (that is, 21 days), the plant height and tiller numbers were measured and recorded weekly. The plants were harvested 60 days after transplanting by cutting them just above the soil surface. The plants were washed with distilled water and oven-dried at 68°C for analysis. The plant samples were then grinded.

Plant sample analysis

Ground plant material of 0.1 g was weighed into conical flask and 5 ml of concentrated H_2SO_4 was added and left to stand for about 1 h. The mixture was then heated. Hydrogen peroxide was added dropwise until the digest became clear. The digest was cooled and then filtered.

Total N uptake in plant material was determined as follows: A 5 mL aliquot of the digest (described earlier) was taken into a Markhan distillation apparatus. Five millilitres of 40% NaOH solution

Table 2. Height of rice plants in cm of different nitrogen treatments with different sampling times.

Treatment	21 DAT*	28 DAT	35 DAT	42 DAT
AS	20.23	42.6	48.53	54.20
AS + DA	36.13	45.9	49.90	54.27
DA	35.73	45.9	49.43	54.07
DA + FA	31.17	44.6	49.99	55.17
FA	36.13	49.3	53.27	56.83
C	39.77	48.9	50.40	51.93
LSD ≤ (0.05)	7.45	7.71	5.83	5.58

*DAT: Days after transplanting.

was added to the aliquot and the mixture distilled. The distillate was collected in 5 mL of 2% boric acid that contained three drops of a mixed indicator containing methyl red and methylene blue. The distillate was then titrated against 0.01M HCl acid solution (Bremner, 1965). The percentage of nitrogen was then calculated.
The shoot total N (mg/plant) was derived from the shoot biomass (shoot dry matter yield: **SDW**) and the plant N content (% N) as:

$$\text{Shoot Total N}\ (mg/plant) = SDW\ (g\ /plant) \times \frac{\text{shoot \% N}}{100} \times 1000$$

Statistical analysis

The data on plant height, tiller number, dry weight and Total N were subjected to analysis of variance using Genstat software, 9th edition. The significance of treatment means was tested at the 5% level of probability and the least significant difference (LSD) was used to separate the means.

RESULTS AND DISCUSSION

The Bumbi series has a bulk density of 1.45 Mg/m^3, clay content of 36%. Considering the bulk density, root penetration by most crops in the soil may be inhibited. The soil is slightly alkaline with pH of 7.6 in water and 7.1 in 0.01M $CaCl_2$ (Table 1). The organic carbon content of 35.1 g C/kg could be the consequence of the long fallow period the field was subjected to and the accumulation of organic matter from the sedges growing on the field. Available P content of the soil was 29.4 mg/kg. The exchangeable Ca^{2+} and Mg^{2+} values were 12.3 cmolc/kg and 14 cmolc/kg whilst the Na^+ (0.78 cmolc/kg) and K^+ (0.32) are quite low. The high Ca^{2+} content of the Bumbi series is due to the presence of calcium carbonate concretions.

The height of rice plant increased for all treatments with time (Table 2). In the first week of sampling, the least plant height was recorded by the treatment AS and the highest height by the control, whilst the different treatments (FA, DA, FA + DA, AS + DA) attained almost the same heights with no significance difference among the various heights of the various treatments. For the subsequent weeks, that is, week 2 through to week 4, there was no significant difference among treatments for a particular week. The rice variety IR 841 has been bred to medium height to avoid lodging.

Tiller number increased with time and by the fourth week of sampling, that is, 42 DAT, the highest tiller number was produced by the AS > AS + DA > DA + FA > DA > FA > C (Table 2). The application of ammonium sulphate produced more tillers than any other treatment and the least tiller number was produced by the control. Tiller number ranged from 11 to 30 by the fourth week of sampling. Ammonia sulphate being an inorganic fertilizer released the nitrogen early enough for the plant to use for tiller production as compared to the N released from *Azolla* which is organic N that had to be mineralized to release inorganic forms of N for rice plant to use.

The highest dry weight was observed for the AS + DA treatment followed by the AS treatment and there was no significant difference between them (Table 3). The treatment AS + DA was significantly different from all the other treatments. Even though the dry weight of DA + FA was higher than the individual dry weights of DA and FA, there was no significant difference between dry weight of DA + FA, DA and FA. This shows that the combined use of DA and FA has the potential to increase rice dry matter yield more than the use of dry *Azolla* alone and the use of fresh *Azolla* alone in growing lowland rice. The control had the lowest dry weight. Correlation of 0.879 and 0.853 existed between the dry weight of the rice plant and the tiller number at 35 DAT and 42 DAT implying that tiller number contributed immensely to the dry matter yield of the rice plant in this study.

The AS + DA treatment had the highest total N and this was significantly different from all other treatments (Table 3). The AS treatment had the next highest total N and it was significantly different from DA + FA treatment. The individual treatments of DA and FA had lower total N compared to the combined treatment of DA + FA. Similarly, the treatment AS + DA had higher total N yield than the individual treatments of AS and DA. Combining DA with AS, ensures prolonged N availablility for rice plant use. This is because AS releases N at the initial stage of rice plant growth and at a later stage, DA mineralizes and releases N making that combination a

Table 3. Tiller number, dry matter yield and total N of rice plant of different nitrogen treatments at different sampling times.

Treatment	Tiller number for 21 DAT	Tiller number for 28 DAT	Tiller number for 35 DAT	Tiller number for 42 DAT	Dry matter yield (g)	Total N (mg)
AS	3.67	19.33[c]	27.00[c]	32.00[c]	11.59[bc]	139.1[c]
AS +DA	3.67	14.67[b]	23.00b[c]	26.33[b]	12.83[c]	163.3[d]
DA	6.00	17.00[bc]	21.00[b]	22.67[a]	9.46[ab]	114.8[b]
DA + FA	3.33	14.00[b]	19.00[b]	23.33[ab]	9.53[ab]	124.1[b]
FA	4.00	8.00[a]	14.00[a]	17.33[d]	7.28[a]	98.9[a]
C	5.00	10.33[a]	11.00[a]	12.00[e]	7.52[a]	103.6[a]
LSD ≤ (0.05)	3.24 NS	3.55	4.29	2.9	2.9	13.99

Means with the same letters are significantly not different.

superior treatment to use than DA alone. Similarly, the DA + FA treatment performed better than the DA and FA treatments because the rice plant had available N for the growth of rice over a longer span of time. There was no significant difference in the treatments for total N of DA and DA + FA. The lower total N recorded for the treatment FA as compared to the treatment DA is difficult to explain because one would have expected higher total N yield from the fresh *Azolla* than the dried *Azolla* since dried *Azolla* mineralizes and releases N more slowly (Ito and Watanabe, 1984) than fresh *Azolla* and that can retard the vegetative growth of rice plant.

Conclusion

The application of dried *Azolla* and ammonium sulphate performed better in all the parameters examined than the other treatments. Thus, dry *Azolla* can be used to supplement the nitrogen fertilizer supplement of lowland rice. Also, the full potential of *Azolla* can be realized when both fresh and dried forms of *Azolla* are considered for rice and other crop production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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