

# Improving the Nitrogen Mineralization of Dried *Azolla pinnata* as a Bio-fertilizer for Increased Rice Production in the Vertisol

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## Abstract

An incubation experiment was conducted to improve the rate of nitrogen mineralization of dried *Azolla pinnata* samples in the Akuse series of the Vertisol. Incubation was done for 60 days at soil moisture content of 40% and at room temperature of 28 °C. The available N (ammonium and nitrate N) was determined for each of the treatment samples on every tenth day after incubation by extracting soil sample with 1M KCl. The treatments were fresh azolla (FA), dry azolla (DA), dry azolla + urea (DA+U), pelleted dry azolla + urea (PDA+U) and the control (C), where neither azolla nor nitrogen fertilizer was applied. From 10 to 30 days after incubation, immobilization of nitrate N was observed for the treatments DA and PDA+U. Mineralization was highest for PDA+U treatment and the least for the DA treatment between 40-60 days after incubation. The addition to pelleted dried azolla of 3 µg/g urea fertilizer improved the nitrogen mineralization.

## Introduction

The availability of nitrogen is vital in the production of rice to ensure high grain yields. With the continual rise in the cost of chemical fertilizer and the introduction of high yielding rice varieties that require higher nitrogen levels, the resource poor farmers have difficulty in accessing and utilizing the recommended application rates of fertilizer for rice production. The introduction and the use of cheaper and easily available forms of biofertilizer such as azolla will be of great benefit to such rice farmers.

Azolla is a genus of Leptosporangiate, an aquatic fern that harbours a heterocyst-forming, nitrogen-fixing blue-green alga, *Anabaena azollae* as a symbiont in the dorsal lobe cavity (Peters & Mayne, 1974). Atmospheric nitrogen fixed by the symbiont can fulfill the nitrogen requirement of the association (Peters *et al.*, 1980), and supplement the nitrogen needs of rice when azolla is grown as a green manure. Azolla can fix about 1.1 kg N /ha/day when used as a green manure, and in 30 days, under favourable environmental condition, about 30 kg N/ha would have been fixed. Apart from azolla being used as a green manure for rice and other such crops, it significantly improves the soil organic carbon content, thus, sequestering carbon in soils (Ramesh & Chandrasekaran, 2004).

While the positive effects of fresh azolla on rice yield and soil physical properties are well demonstrated, little is known of the use of dried azolla in Ghana, thus, indicating that the full potential of azolla as a biofertilizer in rice production in this country has not yet been realized. Such dried azolla can be available all year round. The growth of azolla in the tropics is best during the rainy season when the productivity is high. Low productivity of azolla occurs in the dry season. High environmental temperature during the dry season induces increased insect infestation and fungal infection on azolla, as well as excessive blooming of free living algae in ponds and lakes (Lumpkin, 1987). These factors and others make it difficult to grow fresh azolla in the dry season, thus, compelling the need to explore the use of dry azolla. Studies conducted by Li *et al.* (1982) showed that the lignin content of dry azolla was 21%, and this was higher than fresh azolla lignin of 18%, making the mineralization of dried azolla more difficult. The objective of this study was to improve on the N mineralization status of dried and other similarly treated azolla in the Vertisol.

## Materials and methods

### Soil sampling

The soil was sampled to a depth of 0–15 cm from an uncultivated field at the University of Ghana Agricultural Research Centre at Kpong. All samples were then cooled on ice and transported to the Soil Science Laboratory of the University of Ghana, where sieving was done with a 2-mm sieve.

### *Chemical analysis of soil*

Part of the sieved soil was air-dried and some chemical properties were determined. The pH of soil was determined using pH meter with glass-calomel combination electrode in distilled water and 0.01 M CaCl<sub>2</sub> solution at a ratio of 1:2 soil:solution. The organic carbon content was determined using the Walkley and Black method (1934). The total nitrogen was determined by the Kjeldahl method whilst the cation exchange capacity (CEC) was determined as described by the method of Thomas (1982). The available phosphorus was determined by the Bray 1 method where 0.03 M NH<sub>4</sub>F in 0.025 M HCl was used as the extractant, and the colour development was done using the Watanabe and Olsen's method (1965).

Some of the chemical properties of soil and azolla used are shown in Table 1. The soil is classified as the Vertisol (USDA and the FAO classification systems). The organic carbon content of soil was 8.7 g/kg soil and the dominant exchangeable cations were Ca<sup>2+</sup> and Mg<sup>2+</sup> and has low available P (Table 1). The cation exchange capacity is 43.26 cmol (+)/kg soil and the base saturation of 82.76%. For the azolla strain used, the C:N ratio was 12.9:1 (Table 1), the relative growth rate was 0.20 g/g/day and the doubling time was 3.06 days.

TABLE 1  
*Some chemical properties of Akuse series and Azolla pinnata*

<i>Property</i>	<i>Soil (Vertisol)</i>	<i>Azolla pinnata</i>
pH in water (1:2)	7.58	
pH in 0.01M CaCl <sub>2</sub> solution	6.88	
Organic carbon g/kg	8.7	492.4
Total nitrogen g/kg	6.0	38
C: N		12.9:1
Available phosphorus mg/kg	0.52	
Exchangeable cations (cmol (+)/kg/soil		
Ca <sup>2+</sup>	23.6	
Mg <sup>2+</sup>	10.6	
K <sup>+</sup>	0.08	
Na <sup>+</sup>	1.52	
Cation exchange capacity (cmol (+) /kg/soil	43.26	

### *Azolla collection, preparation and cultivation*

*Azolla pinnata* was collected from the Crop Science Department of the University of Ghana, Legon. It was gently washed with tap water and the azolla was dried with tissue paper. The azolla was then cultured using the International Rice Research Institute Medium (IRRI Medium, 1979; Appendix 1). Fresh *Azolla pinnata* was weighed and cultured to obtain enough azolla biomass for the experiment. The relative growth rate (RGR) of the fresh azolla was determined by using the following formula

$$\text{RGR} = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1} \text{ g/g/day}$$

where W<sub>1</sub> and W<sub>2</sub> were the dry weight of azolla at times t<sub>1</sub> and t<sub>2</sub>, respectively. Growth of azolla was maintained at the exponential growth phase.

### *Nitrogen mineralization studies*

There were five azolla treatments for the nitrogen mineralization study. These treatments were fresh azolla (FA), dry azolla (DA), dry azolla + urea (DA+U), pelleted dry azolla + urea (PDA+U) and for the control (C) no azolla nor mineral N fertilizer was added to soil sample. Each of these treatments contained total N of 32.5 mg N but the PDA+U treatment contained 32.49 mg N +0.01 mg urea N. The pelleted azolla was prepared by taking a known amount of dry azolla and mixing with a few drops of starch solution to form pellets. A small hole was made in the pellet and allowed to air-dry for 3 days at room temperature. After that, urea was inserted into it using a spatula and sealed with the rest of the dry azolla.

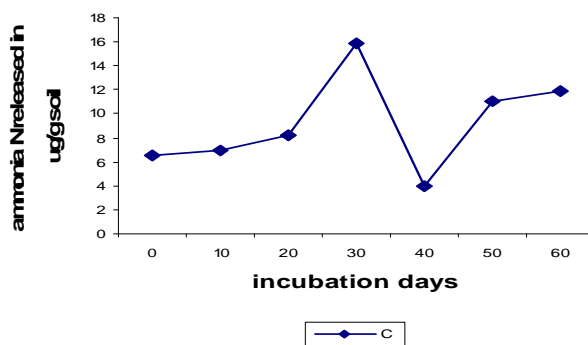
Thirty grams of sieved soil was put into a black polyethylene bag. The moisture content of the soil was adjusted to 40% moisture holding capacity, and each of the azolla treatment was incorporated into the soil. The samples were incubated at room temperature in the laboratory at 28 °C setting them up in a completely randomized design with 21 replicates. Sampling of soil was carried out on days 0, 10, 20, 30, 40, 50 and 60 of incubation (for each sampling time, replicate treatment was taken for analysis). Soil sample of 10 g was taken from each treatment after mixing the soil thoroughly in the polyethylene bag and 50 ml of 1M KCl added to soil in a plastic tube. The tube was capped and shaken for 1 h on a mechanical shaker and the mixture filtered, using Whatman No. 44 filter paper. The method of Ogoshi *et al.* (1999) was used to determine both ammonium and nitrate N, using 10 ml of the above filtrate.

#### Statistical analysis

The data was subjected to analysis of variance using Genstat software (Rothamsted Experimental Station, Harpenden, Hertfordshire, UK) and significance of treatment effects, and was tested at the 5% level of probability. The least significant difference (LSD) was used to separate the means.

### Results and discussion

The ammonium-N mineralization patterns for the different azolla treatments are shown in Fig. 1. The same pattern of ammonia released was observed for almost all the treatments. Treatment PDA + U had a high release of ammonia on day 20 after incubation. Generally, the amount of ammonium N released was lower than total nitrate N released (Fig. 1 and 2). The release pattern of nitrate was low from day 0 to day 30 of incubation. From days 40 to 60, release of nitrate N was high, resulting in high total N released (Fig. 2). On the day 10 to day 30, immobilization of nitrate N was observed for the dry azolla (DA) and the other treatments.



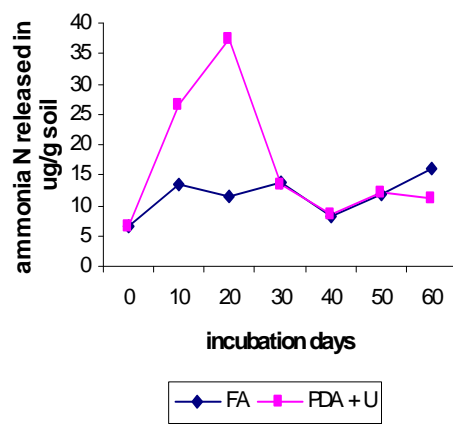
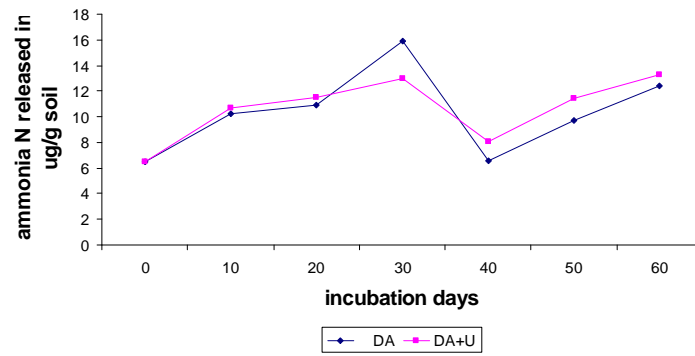
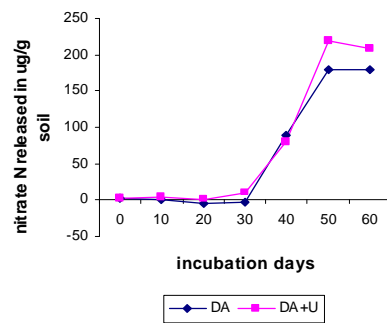
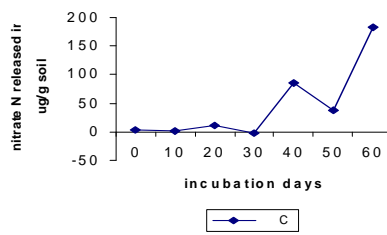


Fig. 1. Ammonia N released from differently treated azolla samples in the Akuse series



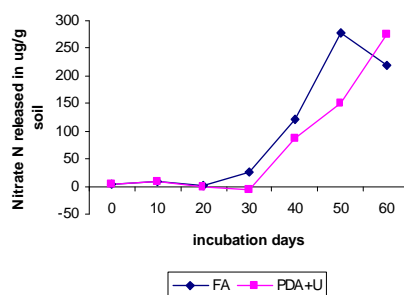


Fig. 2. Nitrate N released from differently treated azolla samples in the Akuse series

Total N mineralized from the control was highest on day 60 of incubation (Table 2). Treatment DA had a lower N mineralized at 20, 30 and 60 days as compared to C even though the difference was not significant. On day 50, N mineralized in DA was significantly higher than C (Table 2). Mineralization of DA was improved when urea was added, thus, from day 50, significantly higher mineralization rate was obtained for DA+U than for DA alone (Table 2).

TABLE 2  
Total N in  $\mu\text{g/g}$  soil (ammonium N + nitrate N) mineralized in different soil treatments

Incubation days	Control	DA	DA+U	FA	PDA+U	LSD(0.05)
0	9.20	9.20	9.20	9.20	9.20	18.82
10	7.60	11.00	15.40	21.90	36.50	24.41
20	9.20	5.60	11.70	13.20	36.60	21.21
30	14.00	12.20	22.20	39.80	7.80	19.59
40	90.00	95.10	88.50	129.40	94.50	21.26
50	49.00	189.00	231.00	290.00	163.00	13.87
60	194.70	192.70	222.30	235.00	286.90	61.99
LSD(0.05)	60.70	19.31	30.87	48.68	96.80	

Molding a ball with dried azolla and placing urea in the centre of it, improved significantly the mineralization rate, possibly because of the better contact of urea with the dried azolla. The starch might have contributed to this observation and this could be validated in a later study. Total N mineralized for PDA+U was highest on 60 days of incubation. However, on 30 days of incubation, total N was very low for the PDA+U treatment. This might be due to the immobilization of nitrate N at this period. Total N mineralized for FA was high from day 40 and the highest total N mineralized was on day 50, where N mineralized was significantly different from the control. But on day 60, the total N mineralized began declining with the FA treatment. A similar observation was made by Watanabe *et al.* (1977) when within 40–50 days after incorporating fresh azolla in paddy fields, about 60% of its organic nitrogen had been mineralized. Beyond that period, nitrogen mineralization was low.

Total N for the soil was 0.06% (Table 1). It was expected that total N mineralized in that soil would be around 600  $\mu\text{g N/g}$  soil but such an observation was not made. Except for the PDA +U and FA treatments, which had total N mineralized around 220–300  $\mu\text{g N/g}$  soil, the rest of the treatments had a lower total N of < 200  $\mu\text{g N/g}$  soil. Probably some of the ammonia N released during mineralization was trapped in the lattice layer of the expanding clay of the Akuse series, and the rest lost through volatilization considering the neutrality of the soil pH.

From the study, dry azolla mineralization can be improved when urea fertilizer (3 µg/g dry azolla) is added and is even more improved in the pelleted form (PDA+U form). However, the nitrogen in PDA+U becomes more available after 50 days of incorporating in the soil. PDA+U treatment can be used with fresh azolla so that within the first 6 weeks, N is released from fresh azolla for rice plant use and later, N released from PDA+U treatment.

### Conclusion

The mineralization of dry azolla can be improved by molding the azolla in the form of a pellet and introducing small amount of urea fertilizer to the pelleted azolla. High amount of N is mineralized from days 50 to 60, and such a bio-fertilizer can be useful to long duration crops, such as long duration lowland rice varieties.

### References

- Li Z., Zu S., Mao M. and Lumpkin T. A.** (1982). Study on the utilization of eight Azolla species in agriculture.1. An investigation of utilization properties (in Chinese). *Sci. Agric. Sin.* **1**: 19–28.
- Lumpkin T. A.** (1987). Environmental requirements for successful Azolla growth. In *Azolla Utilization*. International Rice Research Institute Publication. 296 pp.
- Ogoshi R. M., Cagauan B. G., Jr and Tsuji G. Y.** (1999). Field and laboratory methods for the collection of the minimum dataset for calibrating DSSAT crop growth models. In *DSSAT v3*, vol. 4. (G. Hoogenboom, P. W. Wilkens and G. Y. Tsuji ed.), pp. 217–283. University of Hawaii, Honolulu, Hawaii .
- Peter G. A. and Mayne B. C.** (1974). The Azolla-Anabaena azollae relationship. II. Localization of nitrogenase activity as assayed by acetylene reduction. *Pl. Physiol.* **58**: 820–835.
- Peter G. A., Toia jr R. E., Evans W. R., Crist D. K., Mayne B. C. and Poole R. E** (1980). Characterization and comparison of five N<sub>2</sub>- fixing N<sub>2</sub> Azolla-Anabaena associations. 1. Optimization of growth conditions for biomass increase and N content in a controlled environment. *Pl. Cell Envir.* **3**: 261–269.
- Ramesh K. and Chandrasekaran B.** (2004). Soil organic carbon bulk-up and dynamics in rice-rice cropping systems. *J. Agron. Crop Sci.* **190**: 21–27.
- Thomas G. W.** (1982). Exchangeable Cations. In *Methods of Soil Analysis*. (A. L. Page, R. H. Miller and D. R. Keeney, ed.), pp. 159–165. Part 2-Chemical and Microbiological Properties.
- Walkley A. and Black I. A.** (1934). An examination of the different methods for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* **37**: 29–38.
- Watanabe F. S. and Olsen E. R.** (1965). Test of an ascorbic method for determining phosphorus in water and NaHCO<sub>3</sub> extract from soil. *Soil Sci. Am. Proc.* **29**: 677–678.
- Watanabe I., Espinas C. R., Berja N. S. and Alimagno B. V.** (1977). Utilization of Azolla - Anabaena complex as a nitrogen source. *International Rice Research Newsletter*. 30 pp.

### Appendix 1

#### *Composition of culture solution for growth of symbiotic Azolla.*

<i>Element</i>	<i>Conc (p.p.m.)</i>	<i>Reagent</i>
P	20	NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O
K	40	K <sub>2</sub> SO <sub>4</sub>
Ca	40	CaCl <sub>2</sub> ·2H <sub>2</sub> O
Mg	40	MgSO <sub>4</sub> ·7H <sub>2</sub> O
Fe	0.5	Fe EDTA
Mn	0.5	NaMoO <sub>4</sub> ·2H <sub>2</sub> O
B	0.20	H <sub>3</sub> BO <sub>3</sub>
Zn	0.01	ZnSO <sub>4</sub> ·7H <sub>2</sub> O
Cu	0.01	CuSO <sub>4</sub> ·5H <sub>2</sub> O
Co	0.01	CoC <sub>12</sub> ·6H <sub>2</sub> O

(2/5 of Hoagland's N-free medium)

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