Effectiveness of Nitrification Inhibition on Various Species of *Brachiaria* Grass Rhizosphere

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Abstract. Nitrification has the potential to decrease the efficiency of nitrogen utilization by plants. The use of nitrifying inhibitory chemicals proved to be effective in controlling nitrification, but also affects beneficial soil microbes. Another attempt to inhibit the more environmentally-friendly nitrification is to use plants that have allelochemical nitrification inhibiting compounds such as the grasses of *Brachiaria*. The aim of this research is to know the effectivity of *B.mutica, B.decumbens,* and *B.humidicola* as inhibitors of nitrification rate in soil. The experiment was carried out by pot experimental method based on nondestructive sampling and Complete Randomized Design, consisting of Brachiaria plant types and various doses of N fertilizer, 100 kg/ha, 150 kg/ha, 200 kg/ha. The results of this study show that 1) *B.mutica, B.decumbens, and B.humidicola*, highly significant to the soil potential nitrification, but the treatment of various doses of N fertilizer is not significant to the soil potential nitrification. 2) the highest soil potential nitrification in *B.mutica* rhizosphere was 5.160 mg NO_2^-/g of soil/5h, while the lowest soil potential nitrification in the rhizosphere of *B.humidicola* plant was 0.414 mg NO_2^-/g/5h. 3) From the four treatment of *Brachiaria* plants can be concluded *B.humidicola* plant more effective in inhibition of nitrification.

1 Introduction

One of the main problems in agricultural cultivation is low nitrogen (N) used efficiency due to loss of N through nitrification, erosion, leaching and volatilization [1]. Approximately 67% of N fertilizers in the world's cereal crops (equivalent to US $ 15.9 billion year-1) are leached in NO_3^- form, volatilized as NH_3, N_2O and N_2 gases and lead to complex environmental problems [2]. Chemical compounds of nitrification inhibitors that have been developed include nitrapyrin, dicyandiamide (DCD) and 3,4-dimethyl-pryrazole-phosphate (DMPP) [1]. Although these synthetic compounds effectively reduce soil N losses, they have negative impacts on non-targeting microbes such as N_2 fixing (diazotroph) bacteria and mycorrhiza fungi [3]. Several types of grasses Brachiaria (*B. decumben*, *B. humidicola* and *B. brizantha*) have been shown to be effective in inhibiting nitrification in the rhizosphere plant [1], [4], [5].

Researchers wanted to get models to improve nitrogen use efficiency and nitrate leaching by utilizing *B. mutica*, *B. decumbens* and *B. humidicola* as nitrification inhibitor plants. The purpose of this study was to determine the effectiveness of the rhizosphere of *B. mutica*, *B. decumbens* and *B. humidicola* in inhibiting nitrification in various doses of nitrogen fertilizer, whether the root activity of *B. mutica*, *B. decumbens* and *B. humidicola* plants were able to inhibit the nitrification of Alfisols soil. Of the three plants tested, researchers wanted to know which species of Brachiaria was most effective in inhibiting nitrification in Alfisols soil.

This research method is experimental pots in green house. The results of this study showed that *B. mutica*, *B. decumbens*, and *B. humidicola*, capable of inhibiting nitrification are shown with the result of all three having a very significant effect on the potential of nitrification; *B. humidicola* with a fertilization dose of N 200 kg/ha is most effective in inhibition of nitrification, indicated by the lowest nitrification potential value of 0.414 mg NO_2^- /g soil/5 hours.

2 Methods

The detail of the experiment is as follows:

2.1 Experimental Design

2.1.1 Material

Materials used for this research were *B. mutica*, *B. decumbens* and *B. humidicola*, and chemical for laboratory analysis. The soil used in this study is Alfisols.

2.1.2 Research Design

This research is a pot experiment with functional relationship with variable approach based on nondestructive sampling and using factorial Completely Randomized Design (CRD). The treatment factor of this research is the combination of planting of three species of Brachiaria plant, namely *B. mutica*, *B. decumben* and *B. humidicola*, with the use of three different N fertilizer

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doses of 100 kg/ha, 150 kg/ha and 200 kg/Ha, so there are 9 (nine) different treatments with the control without plants and without fertilizer. Each treatment was repeated three times to obtain 30 treatments.

Table 1. Combination Treatment Factor of Plant Type and Urea Fertilizer Dose

Types of plants	Dose of Urea Fertilizer as a source of N			
	N0 (0 kg/ha)	N1 (100kg/ha)	N2 (150kg/ha)	N3 (200kg/ ha)
B1 (*B. mutica)*	B1N0	B1N1	B1N2	B1N3
B2 (*B. mecumbens)*	B2N0	B2N1	B2N2	B2N3
B3 (*B. humidicola)*	B3N0	B3N1	B3N2	B3N3

2.1.3 Data Analysis

Table 2. Methods and Units for Measuring Treatment Variables

Parameter	Units	Method
pH H_2O	pH	1 : 2.5 (soil : H_2O)
Organic Matter	%	Wet oxidation
CEC	cmol kg^{-1}	Elektrometric
Base Saturation	%	Titration
Organic-C	g kg^{-1}	Wet oxidation
Total-N	g kg^{-1}	
Nitrification Potential	mg NO_2^-/g soil/ 5 hours	Schinner *et al*., .

The data of the research were analyzed with F 5% test to know the difference between the treatments. Calculation and comparison between treatments done with Duncan Multiple Range Test (DMRT) 5%. Data analysis used Minitab14 and Excel software.

2.2 Experiment Implementation

The experiments were conducted with the following stages:

(1) Initial soil sampling, to find out Organic-C, total-N, available-N (nitrate and ammonium), CEC, C/N ratio, pH H_2O, temperature, and soil moisture.
(2) Preparation of planting media, the soil Alfisols that pass 2 mm sieve.
(3) Nitrogen fertilizer, ie urea fertilizer mixed into the soil before planting *B. mutica*, *B. decumben* and *B. humidicola* into the pot. Determination of nitrogen fertilizer is to meet the nutrient needs of Brachiaria plants as well as nitrification substrate. The doses administered were adjusted for each treatment.
(4) Planting of Brachiaria plants with plant propagation, then transferred into pots intact with the rhizosphere of the roots.
(5) Maintenance and irrigation by cleaning weeds and watering.
(6) Measurement of variables and soil sampling. Soil samples for potential nitrification measurements were taken aseptically at a depth of 0-20 cm. Potential nitrification was measured from the amount of NO_2^- formed from soil samples after added $(NH_4)_2SO_4$ and incubated at 25° C for 5 hours[6].

3 Results and Discussion

3.1 Characteristics of Alfisols Soil

Table 3. Result of Soil Analysis Before Treatment

No	Soil Properties	Result	Valuation
1.	pH H_2O	5.2	Acid *)
2.	OM	4.1 %	Low *)
3.	CEC	22.28 cmol kg^{-1}	Moderate *)
4.	Base Saturation	36 %	Moderate *)
5.	Organic C	3.89 g kg^{-1}	Moderate *)
6.	Total N	0.28 g kg^{-1}	Moderate *)

Source: Lab. Analysis
Note: *) Valuation according to the Soil Research Institute, 2009.

3.2 The Influence of Various Brachiaria Plants to Soil Nitrification Potential

The amount of NO_2^- formed by a union of time, due to the biological NH_4^+ oxidation process is called nitrification potential [6].

Table 4. Results of Nitrification Potential Diversity Analysis

Source of Variations	F test	P
Plants	18,27	0,000**
Fertilizer	0,15	$0,826^{ns}$
Plants*Fertilizer	1,00	$0,416^{ns}$

**: significant; ns: not significant

Table 4. shows that the Brachiaria plant species has a very significant effect on the value of soil nitrification potential (P <0.01). The treatment of various doses of nitrogen fertilizer as nitrification substrate proved to have no significant effect on the value of soil nitrification potential (P> 0,05).

Table 5. Results of Diversity Analysis Influence of Planting of Some Species of Brachiaria Plants to Soil Nitrification Potential

Source of Variations	F tetst	P
B. mutica	20,14	0.000**
B. decunbens	98,98	0.005**
B. humidocola	21,75	0.009**

**: significant; ns: not significant.

Table 6. Results of Potential Diversity Analysis of Nitrification Influence of Planting of Some Species of Brachiaria Plant on Each Incubation

Source of Variations	Nitrification Potential (mg NO_2/g soil/5 hours) per incubation (Weeks)					
	1	2	3	4	5	6
B. mutica	0.029*	0.010*	0.006**	0.012*	0.012*	0.017*
B. decunben	0.013*	0.007**	0.000**	0.006**	0.031*	0.061*
B. humidicola	0.018*	0.032*	0.073*	0.110 ns	0.100 ns	0.137 ns

Note:*) significant;**)very significant; ns:not significant.

Based on result of analysis of variance Table 5 it is known that the planting of various Brachiaria have a very significant effect (P value <0.01) to the potential of nitrification. The results of the analysis of variance of table 6 of the treatment of various Brachiaria species showed different effects on the potential value of nitrification at each incubation time. *B. mutica* plants at each incubation period showed a significant effect on the potential value of nitrification, and very significant during the third incubation period. Plant *B. decumben* at the second incubation until the fourth incubation showed a very significant effect (P value <0.01) on the nitrification potential, whereas for *B. humidicola* plant the fourth incubation until the end of incubation did not significantly affect the nitrification potential.

Differences in the effect of planting of various Brachiaria plants during the incubation period can be indicated that each of the Brachiaria plant rhizosphere has different inhibitory activities.

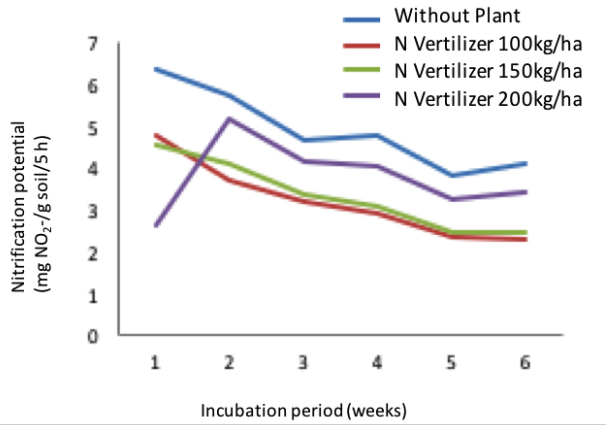

Fig. 1. Potential Nitrification (NO_2^-) of soil in *Brachiaria mutica* plant per incubation time

The potential value of nitrification of *B. mutica* plants has a similar pattern at each N fertilization dose, except in the N fertilizer treatment of 200 kg/ha at incubation time of the first week showed the lowest nitrification potential value of 2.582 mg NO_2^-/g/5 hours later Increased to the highest value of 5.160 mg NO_2^-/g of soil/5 hours at the second incubation period, and subsequently began to decrease. The decrease in the potential value of nitrification after the third incubation period indicates the onset of inhibition of nitrification by the *B. mutica* root activity. Treatment of *B. mutica* cultivation with N 100 kg/ha fertilization has the lowest nitrification potential value (2,245 mg NO_2^-/g of soil/5 hours in fifth incubation) compared with N 150 kg/ha fertilizer and N 200 kg/ha fertilizer.

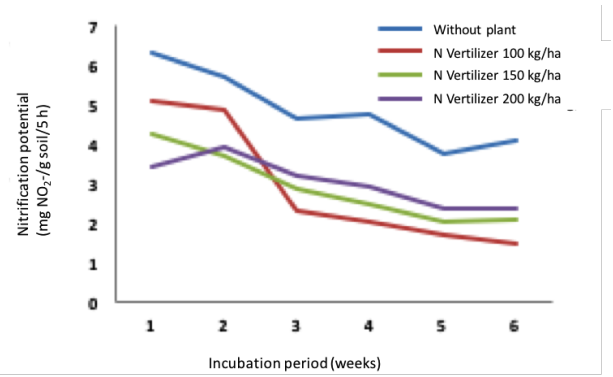

Fig. 2. Potential Nitrification (NO_2^-) soil in *Brachiaria decumben* plant per incubation time

Figure 2 shows that the potential value of nitrification at planting *B. decumben* has a large value difference compared to the potential value of nitrification on soils without planting *B. decumben*. The highest value of 5.114 mg of NO_2^-/g of soil/5 hours was found in the first N 100 kg/ha incubation fertilization treatment, but then it will substantially decrease from the second incubation to the sixth week. In the third incubation period until the last incubation period the measured potential value of nitrification was 1.493 mg NO_2^-/g of soil/5 hours. In general, treatment of addition of N 100 kg/ha fertilizer has the lowest nitrification potential value from the addition of N 150 kg/ha and N 200 kg/ha fertilizer.

Based on figure 2 above it can also be seen that in each treatment, the decrease of potential nitrification value occurs in the third incubation which then shows a stable nitrification potential value until the end of incubation.

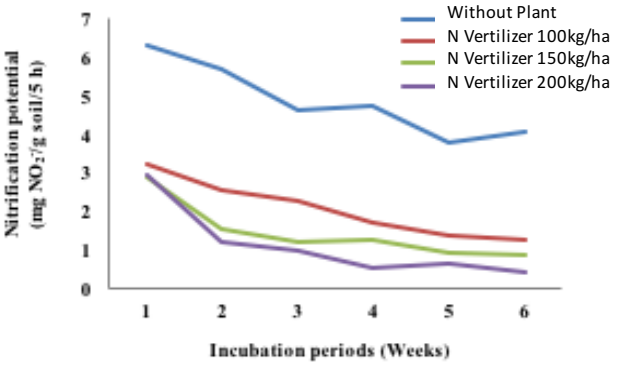

Fig. 3. Potential Nitrification (NO_2^-) soil in *Brachiaria humidicola* plant per incubation time

From the observation of potential value of soil nitrification on *B. humidicola* plant has a measured value that is much lower than the potential value of soil nitrification without planting Brachiaria species. The potential value of nitrification on the N 200 kg/ha fertilizer treatment was the lowest compared to the treatment of N 100 kg/ha and 150 kg/ha, ie 0.414 mg NO_2^-/g/5 hours. In the treatment of *B. humidicola* plant it

can be seen that the decrease of nitrification potential indicates a value that is not too different from each incubation.

Treatment with planting of three types of Brachiaria plants showed a lower nitrification potential (NO_2^-) value than a plantless and non-fertilized treatment. This indicates that all three species of Brachiaria plants are capable of inhibiting nitrification. Soil without Brachiaria plants assumed to be a common condition of Alfisols soil proved to show a high potential value of nitrification. The activity of root exudate of *B. humidicola* plant is able to decrease NO_2^- quantities in soil during incubation period, compared with control[16]. This suggests that the hydrolysis of N-fertilizers in soils at *B. humidicola* planting treatment is still largely in NH_4^+ and not oxidized to NO_3^-

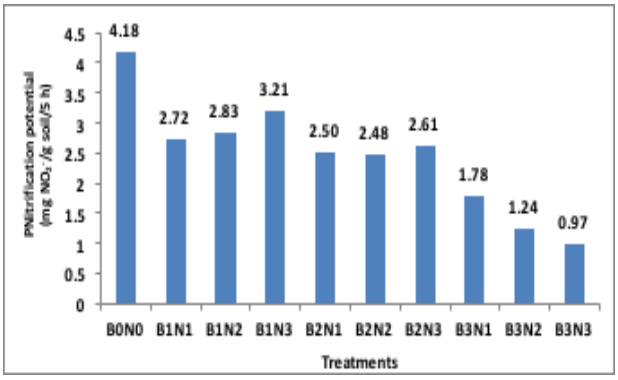

Fig. 4. Potential Nitrification at Various Treatments

From the average range of treatments compared with control (without crops and without fertilizers) showed that treatment with the Brachiaria plant had a lower nitrification potential value than that of non-plant treatment. This is because most of NH_4^+ of urea fertilizer will be utilized as nitrification substrate so the nitrification potential is higher than treatment with Brachiaria plant. In the three treatments of the Brachiaria plant, (Figure 4), the treatment of *B. mutica* plants has a higher nitrification potential value, followed by the treatment of *B. decumben* and *B. humidicola* plants.

The average number of potential nitrification (3.21 mg NO_2/g soil/5 hour) on a variety of plant species Brachiaria of the entire incubation period is the planting of *B. mutica* treatment with dosages of 200 kg/ha, while the lowest value (0.97 mg NO_2^-/g of soil/5 hours) was found in the treatment of *B. humidicola* with fertilizer N 200 kg/ha.

The treatment plant *B. mutica* and *B. decumben* with fertilizer dose of 200 kg N/ha shows nitrification potential value that is greater than the treatment plant *B. mutica* and *B. decumben* with fertilizer dose of 100 kg N/ha and a dose of fertilizer N 150 kg/Ha. This is thought to be due to a larger N source so that inhibition of nitrification is less effective than the treatment of smaller N fertilizer doses. Based on comparative test (DMRT 5%) between interaction treatment of various species of Brachiaria and N fertilization dose to nitrification potential showed no significant difference.

Figure 4 shows that *B. humidicola* plant yields the lowest potential nitrification potential of the two other Brachiaria species. The rhizosphere activity of the three *B. mutica*, *B. decumben* and *B. humidicola* plants was shown to have a lower nitrification potential value than the non-plant and without fertilizer treatment. *B. humidicola* plant looks most effective in inhibiting nitrification with nitrification potential value of the lowest (0.414 mg NO_2^-/g soil/5 hour) at a dose of fertilizer N 200 kg/ha than *B. mutica* and *B. decumben*.

Some of these nitrifying inhibitors have been isolated and identified from plant root exudates using bioassay purification tests. Biological nitrification inhibitor compound at several plants Brachiaria, have been identified in the form of free fatty acids are unsaturated[7],[8],[9],[10]. In a further study, it was found to be more specific that *B. humidicola* plants contain linoleic and alpha-linoleic acid compounds in appropriate amounts as a nitrification inhibitor. Nitrification inhibitors compound inhibit the chain allows the enzyme ammonia monooxygenase (AMO) and the enzyme hydroxylamin oxidoreductase (HAO) on nitrification [7], [11].

By proving the nitrification inhibition of various plant species Brachiaria in this study is expected to be developed model of the nitrification inhibition by utilizing Brachiaria plant as a crop mix (multiple cropping) together with other types of forage crops or other crops so as to increase the effectiveness of the use of fertilizer N.

4 Conclusion

Nitrification is an adverse process in relation to the nitrogen use efficiency of plant and raises complex environmental problems. This study aims to find a way of controlling biological nitrification that is environmentally friendly by utilizing plants that produce alelochemical nitrification inhibitors compounds. The study was conducted by pots experiments in a greenhouse.

The research result of a variety Brachiaria planting with various doses of fertilizer N sources indicate that: (1) *B. mutica*, *B. decumben*, and *B. humidicola* plant, capable of inhibiting nitrification is indicated by the results of three very significant effect on nitrification potential; (2) *B. humidicola* with a dose of fertilizer N 200kg/ha of the most effective in the inhibition of nitrification, nitrification potential value indicated by the low at 0.414 mg NO_2^-/g soil /5 hours. *B. humidicola* planting as mixed crops along with other types of grasses or other cultivation plants can be used to increase the efficiency of N nutrient utilization in the soil. To ensure the effectiveness of nitrification inhibition in the rhizosphere of Brachiaria plants need to do further research on the influence of the rhizosphere of various Brachiaria species on the ratio of NH_4^+ and NO_3 content in soil (actual Nitrification in soil).

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