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Effect of *Brachiaria Humidicola* Root Exudates, Rhizosphere Soils, Moisture and Temperature Regimes on Nitrification Inhibition in Two Volcanic Ash Soils of Japan

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Abstracts: Experiments were conducted to investigate the effects of soil types, moisture and temperature levels on nitrification inhibitory capability of *Brachiaria humidicola* root exudates on inorganic N applied as $(NH_4)_2SO_4$ in fresh volcanic ash soils of Japan collected from Tsukuba (Andisols) and Ishigaki (Terrace yellow soil), as well as in rhizosphere soils on which *Brachiaria humidicola* and *Panicum maximum* has been cultivated for 3 months. Root exudates collected by root washing method from *B. humidicola* supplied with NH^+_4 or NO^-_3 N in hydroponic media were separated into hydrophobic and hydrophilic phases. Soils were collected from the experimental sites of JIRCAS at Tsukuba and Ishigaki at 0-30cm depth. The fresh soils were weighed into 204 centrifuge tubes each at 5g dry soil weight equivalent per tube. The tubes were separated into 3 batches of 60, 72 and 72 tubes each per soil type. The hydrophobic and hydrophilic phases of *B. humidicola* root exudates were each mixed with mixing solution [$(NH_4)_2SO_4$ + $NaClO_3$] at 1:1 ratio and 0.6ml of the mixtures was added to each tube of the first bath at 15 AT units g^{-1} soil, sealed with Para film and incubated at 30°C. There were 5 treatments (control + 4 root exudates) in 2 replicates per soil type for 6 periods. The second bath of tubes were added 0.6 ml of 1:1 mixture of mixing solutions + root exudates (hydrophobic + hydrophilic mixtures) or without exudates, soil moisture adjusted by addition of 0.6ml or 1.2ml, sealed with Para film and incubated at 30°C. The third bath of tubes were added 0.6ml of 1:1 mixture of mixing solutions + root exudates (hydrophobic + hydrophilic mixtures) or without root exudates and incubated at 20 or 30°C. Rhizosphere soils on which either *B. humidicola* or *P. maximum* have grown for 3 months and the control (no plant) were weighed at 5 g dry soil weight equivalent and added 0.6ml of mixing solution [$(NH_4)_2SO_4$ + $NaClO_3$] at equivalent of 12g N kg^{-1} soil and incubated at 30°C. The NO^-_2, NO^-_3 and NH^+_4-N content of the soils were determined by auto-analyzer 2 after 1, 3, 7, 14, 28 and 56 days of incubation, data analyzed by ANOVA and treatment differences separated by LSD at 5%. Results showed that the hydrophobic, more than the hydrophilic phase of *Brachiaria humidicola* roots exudates, significantly reduced nitrification in soils, while their combined application was more potent than separate usage of the phases. Higher soil moisture and temperature enhanced nitrification, while nitrification was significantly reduced in *B. humidiccla* rhizosphere soils than in *P. maximum*. Nitrification was generally higher in Andisol than Terrace yellow soil due to their inherent properties which significantly influences the functioning of *B. humidicola* root exudates in the level of nitrification inhibition in the two soils. The possibility of *B. humidicola* root exudates to inhibit nitrification showed that for optimal realization of the agronomy efficiency from applied nitrogen fertilizers on the field, root exudates of *B. humidicola* could be concentrated and mixed with fertilizers to checkmate nitrification level, increase N use efficiency (NUE) by crops and guide against N losses to the environment which could result to soil, water and air pollution.

Key words: Brachiaria root exudates · nitrification inhibition · rhizosphere soil · soil moisture · temperature

INTRODUCTION

Nitrogen is a primary nutrient element needed by all plants for optimal growth and yield performance. Insufficient or lack of it results to stunted growth and manifestation of myriads of nutritional deficiency symptoms. Problems of low N use efficiency (NUE) due to loss of N through nitrification, erosion, leaching and gaseous (volatilization) are constantly being reported [1, 2], resulting to low crop productivity and farm returns from applied N fertilizers.

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Presently, about 100Tg (millions Mg) of N is applied globally through chemical fertilizer addition to farms [3]. Approximately about 70% of this amount is lost through nitrification and associated processes [1, 4], which translates to about 17 billion US dollars, plus the unknown costs from environmental consequences such as nitrate pollution of ground water, eutrophication of surface water and atmosphere pollution [2, 4], acid rain and global warming.

There have been propositions on N loss reduction, to improve on NUE in order to reduce its environmental impacts. Chemical means towards reducing nitrification have been investigated [5] leading to the development of chemicals such as Nitrapyrin, Dicyandiamide (DCD) and 3, 4-dimethyl pyrazole phosphate (DMPP) amongst others on a commercial scale [3]. Application of chemical inhibitors was described as a proven strategy for improving N recovery, agronomic N-use efficiency (NUE) and at the same time limiting environmental pollution [6, 7]. Unfortunately the chemical inhibitors are expensive and there usage serves as additional cost to the low income and poor resource farmers. In addition, they are effective in blocking only the amino monooxygenase (AMO) enzymatic path ways of *nitrosomonas* during nitrification but not including the hydroxylamino oxidoreductase (HAO) path way [8].

Suppression of soil nitrification has been observed to occur naturally and this is termed biological nitrification inhibition (BNI). This biological means was found to be cheaper and more effective in nitrification inhibition than the chemical method due to its ability to block both the AMO and HAO enzymatic path ways of *nitrosomonas* during nitrification process [9]. Hence, they were found to be ecologically more effective than the synthetic inhibitors. *Brachiaria humidicola* has been reported to be very active in producing root exudates with high BNI ability, the activity of which was reported to be affected by some soil factors such as moisture, temperature, texture, pH, organic matter and microbial populations. The specific roles of these factors and their interactions are yet to be established, hence the reason for this investigation.

MATERIALS AND METHODS

Experiment 1: Effects of soil types, moisture and temperature regimes and *Brachiaria humidicola* root exudates on nitrification inhibition.

Experiment 1a: Effects of hydrophobic and hydrophilic phases of *Brachiaria humidicola* root exudates on BNI activities in soils

Root exudates collection: Root exudates of *Brachiaria humidicola* was collected from the roots of *Brachiaria* plants growing in hydroponic nutrient media in 50-liter tanks on floating Styrofoam block having 6 holes with four plants each retained in place with the support of sponges. The nutrient solutions (contains KH_2PO_4 38.31, K_2SO_4 31.02, $CaCl_2.2H_2O$ 10.5, $MgSO_4.7H_2O$ 36.93, Fe-EDTA 15.1, H_2BO_3 0.57, $CuSO_4.5H_2O$ 0.078, $MnSO_4.6H_2O$ 2.35, $Na_2MoO_4.2H_2O$ 0.126 and $ZnSO_4.7H_2O$ 0.22 as mg L^{-1}; with pH adjusted to 5.0, using 1N NaOH or 1N HCl). The tanks were constantly aerated using Nisso CX600 air pump. Two N nutrient sources as NH^+_4-N [$(NH_4)_2SO_4$] or NO^-_3-N (KNO_3) were used.

Root exudates collection commenced at 60 days after transplanting and it continued weekly for 8 weeks. This was conducted by removing intact plants (a sample size of four plants) from the hydroponic media, rinsed the roots twice with de-ionized water, followed by distilled water. The roots of the intact plants were immersed in 800 ml aerated ultra-pure distilled water in 1L sized plastic bottles for 24 hours. The intact plants were then removed from the plastic bottles and replaced in position in the hydroponic media from where they were collected. The collected water from the plastic bottles (that served as medium for collecting root exudates) was filtered using Advantec No 6 filter paper to remove debris and root particles. Exudates content in the water was separated into hydrophobic and hydrophilic phases using the Sep-Pak-Concentrator (SPC-10-P). The solid phase extraction cartridge was used to trap the hydrophobic compounds of the root washed water. Methanol was used to dissolve the hydrophobic compounds trapped by the cartridge. The hydrophilic contents of the root washing water were collected by drying the water using rotary evaporator. The root exudates were determined for biological nitrification inhibition (BNI) activity using a modified bioassay [10] and the activity expressed as the equivalent of the effect of standard inhibitor allythiourea (AT) unit.

Soil collection and processing: Volcanic ash soils collected from the Headquarters experimental site of Japan International Research Center for Agricultural sciences (JIRCAS) at Tsukuba (Andisol soil) and JIRCAS subtropical station, Ishigaki Island, Okinawa, Japan (Terrace yellow soil) were collected between 0-30 cm depth, sieved with 2.0 mm sieve, each mixed thoroughly and bagged in cellophane in the green house. The moisture content of the soils was determined and 5g equivalent dry weight of each soil type was weighed into labeled centrifuge tubes. The soil pH, total N and C were analyzed.

Incubation process and NO^-_2, NO^-_3 and NH_4-N determination: The hydrophobic or hydrophilic phases of *B. humidicola* root exudates were mixed with mixing solution [$(NH_4)_2SO_4 + NaClO_3$] at 1:1 ratio and 0.6ml of the mixtures at 15 AT units g^{-1} soil was added to soil in the tubes, sealed with Para film and incubated at 30°C.. There were 5 treatments (control + 4 root exudates) in 2 replicates for 6 periods per soil type for a total of 120 centrifuge tubes in a completely randomized design (CRD).Two tubes were retrieved per treatment at 1, 3, 7, 14, 28 and 56 days after incubation for inorganic N extraction using $CaSO_4.2H_2O$. The NO^-_2, NO^-_3 and NH^+_4-N content of the extracts were determined by auto-analyzer 2 and data analyzed using ANOVA and treatment differences separated by LSD at 5%.

Experiment 1b: Effects of soils and moisture regimes on biological nitrification inhibition (BNI) activities of *Brachiaria humidicola* root exudates

Soil samples as used in experiment 1a were weighed at 5g per tube and added 0.6 ml of 1:1 mixture of mixing solutions + root exudates (hydrophobic + hydrophilic mixtures) at 15 AT units g^{-1} soil or without root exudates. Soil moisture was adjusted by addition of 0.6ml or 1.2ml as water treatment levels. The tubes were sealed with Para film and incubated for 1, 3, 7, 14, 28 and 56 days at 30°C. The experiment was a 2 x 2 x 2 factorial in a completely randomized design in 2 replications. The factors considered were soils (Andisols and Terrace yellow), moisture (0.6 and 1.2 ml) and root exudates (with or without). The NO^-_2, NO^-_3 and NH^+_4-N contents were determined as described for experiment 1a.

Experiment 1c: Effects of soils and temperature regimes on Biological Nitrification Inhibition (BNI) activities of *Brachiaria humidicola* root exudates

Representative soil samples weighed at equivalent weight of 5g dry soil per tube were mixed with 0.6ml of 1:1 mixture of mixing solutions + root exudates (hydrophobic + hydrophilic mixtures) at 15 AT units g^{-1} soil or without root exudates and incubated at 20 and 30°C. The experiment was a 2x2x2 factorial in a completely randomized design in 2 replications. The factors considered were soils (Andisols and Terrace yellow soil), temperature (20 and 30°C) and root exudates (with or without). The NO^-_2, NO^-_3 and NH^+_4 N contents were determined at 1, 3, 7, 14, 28 and 56 days of incubation as described for experiment 1a.

Experiment 2: Effects of N sources and *B. humidicola* and *P. maximum* rhizosphere soils on BNI activities in different soil types.

The soils collected and processed as described for experiment 1 were each weighed at 200 g into base perforated and filter paper placed cellophane pots. *Brachiaria humidicola* (CIAT-16028) and *P. maximum* (CIAT-679) seedlings raised in vermiculite growth medium for 2 weeks were transplanted to the labeled potted soils. The experiment included three N sources (NO^-_3, NH^+_4 and-N), two plant types and a control (*B. humidicola, P. maximum* and no plant) and two soil types (Andisols and Terrace yellow) for a 3 x 3 x 2 factorial in a completely randomized design in 4 replications in the green house. The 72 cellophane pots were arranged in 6 plastic trays that contains 12 cellophane pots each (3 trays for Andisols, one each for-N, NO^-_3 and NH^+_4; 3 trays for Terrace yellow soil, one each for-N, NO^-_3 and NH^+_4) and laid with one inch thick foam, watered and allowed to grow for 2 weeks before the commencement of nutrient solution application. All the plants were applied macronutrient solution containing P as 100ppm P_2O_5, K as 150 ppm K_2O, Ca as 200ppm CaO and Mg as 300ppm MgO, at 10 ml/potted plant weekly; micronutrient solution containing Fe as 2ppm, Cu as 0.02ppm, Mo and Zn as 0.05ppm each, Mn as 0.5ppm and B as 0.1ppm at 10 ml/pot/plant weekly. The NO^-_3 was applied as 100ppm KNO_3 and NH^+_4 as 100ppm $(NH4)_2SO_4$, both at 10 ml/pot/ plant weekly for 10 weeks from 2 weeks after transplanting to the 12th week of experimentation. The plants were monitored for 3 months and harvested by cutting the plants at the soil level base.

The surface of the potted soils were scrapped to an inch level, plant roots removed, air dried for 30 minutes, sieved and moisture contents determined. The soil pH was determined at soil/KCl ratio and soil/water ratio of 1:2.5. Five gram equivalent dry weight of each of the rhizosphere soils were weighed into 6 centrifuge tubes for a total of 432 tubes. To each tube content 0.6 ml mixing solution containing equivalent of 12 mg N/kg soil was added, sealed with Para film and incubated at 30°C. NO^-_2, NO^-_3 and NH^+_4-N contents were determined using auto-analyzer 11 at 1, 3, 7, 14, 28 and 56 days after incubation as described for experiment 1.

RESULTS

Experiment 1a: Effects of root exudates' phases: The amount of $NO^-_2 + NO^-_3$ N accumulated after 3 days of incubation showed that the hydrophobic phase of *B. humidicola* root exudates resulted to between 2.03-4.48% of $NO^-_2 + NO^-_3$ accumulation compared with 4.88-10.66% for hydrophilic phase and 4.70% for the control in Andisol. The values were 0.63-2.62, 2.04-4.63 and 2.27% for hydrophobic, hydrophilic and control respectively in

Table 1: Effect of *Brachiaria humidicola* root exudates phases on accumulation% of inorganic NO^-_2+NO^-_3 N over incubation period in Andisol and Terrace yellow soils

		Incubation time					
Treatments	Exudates phases	1 day	3 day	7 day	14 day	28 day	56 day
Andisol soil							
Control		0	4.70	13.38	29.77	39.70	76.58
Bh-NO^-_3	Hydrphobic	0	4.48	10.92	27.26	27.37	49.65
Bh-NO^-_3	Hydrophilic	0	10.66	19.10	36.19	46.45	66.87
Bh-NH^+_4	Hydrphobic	0	2.03	9.00	23.53	27.21	47.70
Bh-NH^+_4	Hydrophilic	0	4.88	8.62	32.43	37.79	57.58
LSD 5%		-	NS	NS	1.09	NS	7.30
CV (%)		-	10.54	20.81	1.15	15.82	7.13
Terrace yellow soil							
Control		0	2.27	10.58	13.56	26.60	39.65
Bh-NO^-_3	Hydrphobic	0	0.63	2.40	6.00	8.14	9.20
Bh-NO^-_3	Hydrophilic	0	4.63	5.67	7.85	13.76	16.88
Bh-NH^+_4	Hydrphobic	0	2.62	5.65	5.57	6.08	8.98
Bh-NH^+_4	Hydrophilic	0	2.04	6.45	6.89	8.08	17.23
LSD 5%		-	ns	7.89	ns	10.99	21.02
CV (%)		-	5.14	3.58	6.50	3.56	5.26

Bh-NO^-_3 = Root exudates from *B. humidicola* grown in NO^-_3-N medium; Bh-NH^+_4 = Root exudates from *B. humidicola* grown in NH^+_4-N medium; LSD = Least significant difference; CV = Coefficient of variation; NS = Not significant

Table 2: Effect of *Brachiaria humidicola* root exudates and moisture levels on% of NO^-_2+NO^-_3 released over incubation period in Andisol and Terrace yellow soils

		Incubation time					
Treatments	Moisture levels	1 day	3 day	7 day	14 day	28 day	56 day
Andisol soil							
Control	0.6ml	0	11.87	14.57	21.21	29.24	64.74
Control	1.2ml	0	8.84	12.97	15.35	31.44	53.93
Bh-NO^-_3	0.6ml	0	1.23	0.71	3.01	4.88	35.06
Bh-NO^-_3	1.2ml	0	10.87	14.06	15.32	20.12	61.44
Bh-NH^+_4	0.6ml	0	2.75	8.81	13.50	13.39	46.66
Bh-NH^+_4	1.2ml	0	5.23	11.34	11.20	15.35	57.68
LSD (5%)		-	7.16	3.19	5.08	13.33	NS
CV (%)		-	3.01	1.25	1.92	4.59	5.69
Terrace yellow soil							
Control	0.6ml	0	2.52	4.49	6.32	7.68	12.69
Control	1.2ml	0	4.35	5.46	8.63	11.11	23.62
Bh-NO^-_3	0.6ml	0	0.76	2.89	3.37	0.73	7.80
Bh-NO^-_3	1.2ml	0	2.22	4.06	5.04	3.48	14.41
Bh-NH^+_4	0.6ml	0	2.95	3.30	3.99	1.19	8.44
Bh-NH^+_4	1.2ml	0	3.40	3.46	2.60	1.06	10.62
LSD (5%)		-	3.26	NS	2.79	2.85	10.37
CV (%)		-	2.80	4.13	2.21	2.30	6.60

Bh-NO^-_3 = Root exudates from *B. humidicola* grown in NO^-_3-N medium; Bh-NH^+_4 = Root exudates from *B. humidicola* grown in NH^+_4-N medium; LSD = Least significant difference; CV = Coefficient of variation; NS = Not significant

Terrace yellow soil (Table 1). The change in $NO^-_2 + NO^-_3$ accumulation increased with the increase of incubation time in a similar trend as observed for the first 3 days. After 56 days, about 47.7-49.65, 57.58-66.87 and 76.58% of $NO^-_2 + NO^-_3$ N has been accumulated under hydrophobic, hydrophilic and control treatments in Andisol, while about 8.98-9.20, 16.88-17.23 and 39.65% of $NO^-_2 + NO^-_3$ has been accumulated under similar treatments in Terrace yellow soil. The $NO^-_2 + NO^-_3$ N accumulation was significantly (P=0.05) higher in Andisol than in Terrace yellow soil across the treatments.

Experiment 1b: Brchiaria humidicola root exudates and moisture levels: Effects of *B. humidicola* root exudates and moisture levels on $NO^-_2 + NO^-_3$ N accumulation showed that nitrification was significantly (P=0.05) reduced due to the application of the root exudates compared to control in both Andisol and Terrace yellow soils. Higher soil moisture regime (1.2 ml) however, resulted to significantly (P=0.05) higher $NO^-_2 + NO^-_3$ N accumulation than lower moisture regime (0.6 ml) (Table 2). About 1.23-11.87% at 0.6 ml moisture and 5.23-8.84% at 1.2 ml moisture, of $NO^-_2 + NO^-_3$ was

Table 3: Effect of *B. humidicola* root exudates and temperature levels on% of NO^-_2+NO^-_3 released over incubation period in Andisol and Terrace yellow soil

		Incubation time					
Treatments	Temperature	1 day	3 day	7 day	14 day	28 day	56 day
Andisol soil							
Control	20°C	0	3.38	9.94	18.98	19.32	50.22
Control	30°C	0	6.90	14.13	23.88	33.01	62.76
Bh-NO^-_3	20°C	0	4.04	10.11	17.86	15.29	52.97
Bh-NO^-_3	30°C	0	6.32	12.41	17.23	20.01	51.09
Bh-NH^+_4	20°C	0	0.65	7.59	10.59	18.64	40.16
Bh-NH^+_4	30°C	0	1.49	7.74	10.58	15.32	40.01
LSD (5%)		-	5.58	6.32	12.33	12.90	NS
CV (%)		-	2.90	2.68	5.31	4.70	7.42
Terrace yellow soil							
Control	20°C	0	1.90	3.86	4.75	1.41	9.10
Control	30°C	0	2.66	3.065	6.56	9.09	15.39
Bh-NO^-_3	20°C	0	3.05	3.42	2.68	3.18	4.42
Bh-NO^-_3	30°C	0	1.42	1.26	3.26	1.88	9.63
Bh-NH^+_4	20°C	0	1.59	1.18	2.55	1.81	2.91
Bh-NH^+_4	30°C	0	2.36	3.04	3.28	2.56	5.75
LSD (5%)		-	NS	NS	1.65	4.01	5.97
CV (%)		-	4.55	2.67	1.38	3.47	4.43

NO^-_3-N = Root exudates from *B. humidicola* grown in NO^-_3-N medium; NH^+_4-N = Root exudates from *B. humidicola* grown in NH^+_4-N medium; LSD = Least significant difference; CV = Coefficient of variation; NS = Not significant

Table 4: Effect of *B. humidicola* and *P. maximum* rhizosphere soils and N sources on inorganic NO^-_2+NO^-_3 N accumulation % over incubation period

		Incubation time					
Treatments	Previous crop	1 day	3 day	7 day	14 day	28 day	56 day
Andisol soil							
No N	NP	0	42.23	55.31	58.51	92.32	98.17
	Pm	0	31.65	41.49	52.39	85.41	93.20
	Bh	0	17.24	35.82	46.54	70.98	75.43
NO^-_3-N	NP	0	32.67	63.05	69.45	92.82	95.08
	Pm	0	26.92	46.39	65.73	88.51	87.39
	Bh	0	16.79	35.94	46.35	81.04	85.12
NH^+_4-N	NP	0	25.41	48.56	54.03	83.40	99.64
	Pm	0	21.22	45.86	51.35	77.28	96.59
	Bh	0	19.33	38.48	44.65	64.26	87.05
	LSD (5%)	-	2.19	2.75	3.89	3.26	3.51
	CV (%)	-	14.02	11.41	15.69	10.77	7.92
Terrace yellow soil							
No N	NP	0	4.39	20.40	47.61	85.63	85.83
	Pm	0	3.89	17.89	41.14	76.40	75.73
	Bh	0	2.21	11.75	36.77	58.45	60.75
NO^-_3-N	NP	0	5.85	26.86	61.84	80.47	87.77
	Pm	0	4.67	21.16	52.98	76.43	81.76
	Bh	0	3.51	16.58	40.88	67.03	71.15
NH^+_4-N	NP	0	8.19	27.61	57.23	83.53	96.28
	Pm	0	5.20	22.23	51.61	72.09	98.23
	Bh	0	4.68	17.71	40.08	59.12	71.11
	LSD (5%)	-	1.06	1.45	2.68	2.57	2.59
	CV (%)	-	15.83	12.19	13.87	9.92	7.31

NP = No plant; Pm = *Panicum maximum*; Bh = *Brachiaria humidicola*; LSD = Least significant difference; CV = Coefficient of variation, No N = no nitrogen source

accumulated after 3 days of incubation, which increased to about 35.06-64.74% at 0.6 ml moisture and 53.93-61.44% at 1.2 ml moisture in Andisol after 56 days of incubation. The values were 0.76-2.95% at 0.6ml moisture and 2.22-4.35% at 1.2 ml moisture after 3 days of incubation, which increased to about 7.80-12 69% at 0.6 ml moisture and 10.62-23.62 at 1.2 ml moisture in Terrace yellow soil after 56 days of incubation (Table 2). The change in NO^-_2 + NO^-_3 N accumulation over the incubation periods was observed to be higher in Andisol than in Terrace yellow soil, as reported for *B. humidicola* root exudates' phases (Table 1). The application of *B. humidicola* root exudates, moisture regimes and their interactions resulted to significant (P=0.05) differences in mean $NO^-_2 + NO^-_3$ N accumulated over the incubation periods.

Experiment 1c: Brchiaria humidicola root exudates and temperature levels: Higher temperature regime of 30°C resulted to higher nitrification of NH^+_4-N thereby leading to higher accumulation of $NO^-_2 + NO^-_3$ N than obtained at 20°C, over the incubation periods in both Andisol and Terrace yellow soils. The values were 0.65-4.04% at 20°C and 1.49-6.90% at 30°C in Andisol after 3 days of incubation, while it was 1.59-3.05% at 20°C and 1.41-2.66% at 30°C in Terrace yellow soil for the same period (Table 3). These values increased over the incubation periods to 40.16-52.97% at 20°C and 40.00-62.76% at 30°C in the Andisols and 2.91-9.10% at 20°C and 5.75-15.39% at 30°C in Terrace yellow soil after 56 days of incubation. The values for Terrace yellow soils were generally lower than those observed for Andisols across the treatments, as reported for experiment 1a above (Table 1).

Experiment 2: Effects of rhizosphere soils and N nutrient sources on nitrification: For both Andisols and Terrace yellow soils, values of $NO^-_2 + NO^-_3$ N accumulated over the incubation periods for rhizosphere soils that has been used to grow *P. maximum*, *B. humidicola* for 3 months and the control (no plant-NP) indicated a sharp reduction in the nitrification of applied NH^+_4-N for all the *B. humidicola* rhizosphere soils compared to the *P. maximum* and NP rhizosphere soils (Table 4). Nitrification was generally higher in NP rhizosphere soils thereby resulting in more $NO^-_2 + NO^-_3$ N accumulation than in *P. maximum* and *B. humidicola* rhizosphere soils for both soil types. The amount of $NO^-_2 + NO^-_3$ N released and accumulated over the incubation periods showed a range of 25.41-42.22% for NP, 21.22-31.65% for Pm and 16.78-19.33% for *B. humidicola* rhizospheres in Andisols; while it was 4.39-8.19% for NP, 3.89-5.20% for *P. maximum* and 2.21-4.68% for *B. humidicola* in Terrace yellow soils after 3 days of incubation. The values increased with length of incubation to 95.08-99.63 for NP, 87.39-96.57% for *P. maximum* and 75.43-87.39% for *B. humidicola* in Andosols; while it was 85.82-96.28% for NP, 75.73-98.23% for *P. maximum* and 60.75-71.15% for *B. humidicola* in Terrace yellow soil (Table 4). Nitrification was higher in Andisol relative to Terrace yellow soil, in a similar trend as observed in experiments 1a-c on the use of *B. humidicola* root exudates (Table 1-3).

Soil properties and pH change: The physical and chemical characteristics of Andisol and Terrace yellow soils respectively, were: clay = 548 and 120 g/kg; silt = 263 and 120 g/kg; sand = 189 and 760 g/kg; pH (KCl) = 5.13 and 4.84; (H2O) = 6.0 and 6.41; total C = 29.2 and 5.8 mg/g soil; total N = 2.5 and 0.62 mg/g soil and C/N ratio = 11.7 and 9.4. Thus the two soils contrast greatly in their physical and chemical properties. Pre-cropping soil pH values for Andisols (KCl: 5.13 and H_2O: 6.25) did not show wide margins compared to the post-cropping values (KCl: 5.49-5.69 and H_2O: 6.26-6.42), while the pre-planting pH values for Terrace yellow soil (KCl: 4.84; H_2O: 6.41) were of wider range to the post-cropping soil pH values (Kcl: 5.65-5.72; H_2O: 6.53-7.08) (Table 5). The variation in soil pH levels across the N sources and plant

Table 5: Effect of nitrogen sources and Plant growth (*P. maimum* and *B. humidicola* on rhizosphere soil pH after cropping

Treatments		Andisol soil pH		Terrace yellow soil pH	
Nitrogen source	Per cropping plant	KCl	H_2O	KCl	H_2O
No N	No plant	5.64	6.42	5.72	7.01
	P. maximum	5.56	6.26	5.7	6.75
	B. humidicola	5.49	6.28	5.67	6.79
NO^-_3 N	No plant	5.51	6.30	5.67	7.08
	P. maximum	5.61	6.40	5.65	6.76
	B. humidicola	5.69	6.30	5.79	6.88
NH^+_4 N	No plant	5.59	6.35	5.51	6.66
	P. maximum	5.65	6.31	5.65	6.62
	B. humidicola	5.49	6.33	5.66	6.53
LSD (5%)		0.14	NS	0.11	0.13
CV (%)		1.67	1.15	1.44	1.23

LSD = Least significant difference; CV = Coefficient of variation; NS = Not significant

types were significantly (P=0.05) different both in KCl and H_2O media for the two soils. When N was not applied, the soil pH was highest under NP, while the values were relatively similar for *P. maximum* and *B. humidicola* rhizosphere collected soils for both soil types.

DISCUSSION

The application of *B. humidicola* root exudates had reductive impact on accumulated total NO^-_2+NO^-_3 N over the incubation periods, compared to the control soil samples. This shows that more of the mineral N was retained as NH^+_4-N form in the soils as a result of nitrification inhibition due to the root exudates of *B. humidicola* applied. *Brachiaria humidicola* have been reported to possess inherent ability to release some biochemical substances from their roots that could inhibit nitrification in the soil [3, 11]. Generally, the hydrophobic phase of the *B. humidicola* root exudates resulted to lower NO^-_2+NO^-_3 N accumulation compared to the hydrophilic phase. This tends to show the higher potency of the hydrophobic phase over the hydrophilic phase in nitrification inhibition, which might be as a result of the complex constituent organic compounds in the hydrophobic phase, that are stronger than those contained in the hydrophilic phase. *Brachiaria humidicola* root exudates have been reported to contain arrays of complex organic compounds [9], which are currently undergoing isolation and classification. These compounds are believed to individually and or in combination involve in blocking both the Ammonia Monoxygenase (AMO) and hydroxylamino oxidoreductase (HAO) enzymatic pathways of nitrification processes by *Nitrosomonas europaea* in the soil.

Higher moisture and temperature regimes (1.2 ml and 30°C) resulted into significantly (P=0.05) higher NO^-_2+NO^-_3 inorganic N accumulation than the lower regimes (0.6ml and 20°C) in the two soils. The amount of NO^-_2+NO^-_3 of the inorganic N accumulated after 56 days of incubation was 7.80-12.69% at lower moisture and 10.62-23.62% at higher moisture in Terrace yellow soil, while it was 35.06-64.74% and 53.93-61.44% respectively for the moisture levels in Andisol. The values were 40.16-52.97% at 20°C and 40.0-62.76% at 30°C in Andisol; 2.91-9.10% at 20°C and 5.75-15.39% at 30°C in Terrace yellow soil. The significant (P=0.05) effects of moisture and temperature regimes on actions of *B. humidicola* root exudates in the amount of NO^-_2+NO^-_3 N accumulated in the soils indicates that the level of soil water and temperature influenced greatly the quantum of nitrification that was taking place in the soil at any given time. Biochemical reactions in soils have been reported to be influenced by the amount of soil water [12] and level of temperature [13]. Considering the moisture level at 0.6ml and temperature at 30°C under which experiment on the effectiveness of the root exudates' phases were conducted, the combined effects of hydrophobic + hydrophilic phases of *B. humidicola* root exudates were more effective in reducing nitrification than the separate actions of the two phases.

There was significant (P=0.05) reduction in the level of nitrification in all the *B. humidicola* rhizosphere soils compared to the rhizosphere soils of *P. maximum* and control (no plant) soils. After 56 days of incubation, mineral N in NH^+_4 form in Andisol was 13-24.6%, 3.5-13% and <1.0-5% in the rhizosphere soils of *B. humidicola, P. maximum* and control respectively, while it was 30-41%, 1.8-24.2% and 3.73-14.2% for *B. humidicola, P. maximum* and control respectively in Terrace yellow soil. This informs that *B. humidicola* must have released some biochemical compounds from their roots, which were active in checkmating the activities of nitrifying bacteria in the soil medium from converting mineral N from NH^+_4 form (immobile) to NO^-_3 form (mobile). Such biochemical compounds are generally referred to as biological nitrification inhibition (BNI) compounds [10]. The suppressive effect on nitrification would enhance nitrogen use efficiency (NUE) of applied N fertilizers, thereby reducing environmental pollution impacts that may results from leaching, gaseous and erosion losses into underground water, atmosphere and bodies of water [2, 4]. The trend in the level of inorganic N retention in NH^+_4 forms in the *B. humidicola* rhizosphere soils agreed with previous reports that a near total suppression (>90%) on nitrification was observed when rhizosphere soils from a land under *B. humidicola* cultivation for 10 years were investigated for BNI activities [9].

There was a greater change in pH for the Terrace yellow soil between pre-cropping and post-cropping values compared to the narrow margins obtained for Andisols. This reflected the contrasts in the physical and chemical properties between the two soils. The Andisols with higher total organic C (29.2 mg/g) and clay content (548 g/kg soil), must have been endowed with more buffer capacity than the Terrace yellow soil with lower total C (5.8 mg/g) and clay (120 g/kg soil). The buffering capacity play major role in the adsorptive and desorptive behaviors of soils [14] which affects the changes in soil reactions.

On the overall, nitrification was higher in Andisol than in Terrace yellow soil which suggests that the physical and chemical property differences in the soils have contributed in influencing the pace of nitrification in the soils. The high total organic C content of 29.2 mg g^{-1}

soil for Andisol, compared to 5.8 mg g^{-1} soil for Terrace yellow soil indicated a higher microbial numbers, biomass and amount of activities in the Andisol than in Terrace yellow soil. Similar trend of high microbial populations and activities had been reported [15, 16] when rice straw and compost were used on some Andisols of Japan. The higher soil organic matter also indicated the presence of arrays of organic compounds that could interfere in nitrification inhibition processes more in Andisol than would be expected in Terrace yellow soil. To achieve similar level of nitrification inhibition in the two soils, more *B. humidicola* root exudates would be needed in the Andisol than in Terrace yellow soil.

The results have helped to show that the hydrophobic, more than the hydrophilic phase of *Brachiaria humidicola* roots exudates, reduced nitrification in soils, while their combined application was more potent than their separate usage for this purpose. The soil moisture, temperature and inherent properties of the soils serves as major factors that significantly influence the functioning of *B. humidicola* root exudates in the level of nitrification inhibition in the two soils. For optimal realization of the agronomy efficiency from applied nitrogen fertilizers on the field, root exudates of *B. humidicola* could be concentrated and mixed with fertilizers to checkmate nitrification level, increase NUE and guide against N losses to the environment that could result to soil, water and air pollution.

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