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Denitrification was related to moisture level, organic matter content, pH, and denitrifying population. Gaseous-N losses occurred almost exclusively from the surface soil (0-25 cm) under waterlogged conditions. Losses ranged from 8 to 31% of the applied NO_3 -N. Some loss (7%) also occurred at field capacity from the surface horizon of the Oxisol having the highest organic matter content, Catalina clay. Losses were directly related to organic matter content. Raising the pH of the subsoil only enhanced denitrification slightly. Addition of a dentrifying population and mineral nutrients in the form of a soil inoculant to a subsoil with a raised pH and organic matter content increased denitrification equal to that of surface soil. The lack of denitrification at lower depths is believed to be due to an inadequate supply of rapidly available energy material, low pH and mineral nutrients, and consequent lack of a dentrifying population. Since the surface soil rarely gets waterlogged, it was concluded that little possibility of N losses by denitrification in these humid tropical soils exists.

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Denitrification Losses from Humid Tropical Soils of Puerto Rico[1]

H. D. DUBEY AND R. H. FOX[2]

ABSTRACT

Profile samples from 5 depths (0-25, 25-50, 50-75, 75-100, and 100-125 cm) from 3 soil types (two Oxisols—Piña sandy loam and Catalina clay, and an Ultisol—Humatas clay) were incubated at 23±1C under field capacity and waterlogged conditions in an oxygen-helium atmosphere. Evolved N_2 and N_2O were determined after 1 and 2 weeks by gas chromatography.

Denitrification was related to moisture level, organic matter content, pH, and denitrifying population. Gaseous-N losses occurred almost exclusively from the surface soil (0-25 cm) under waterlogged conditions. Losses ranged from 8 to 31% of the applied NO_3^--N. Some loss (7%) also occurred at field capacity from the surface horizon of the Oxisol having the highest organic matter content, Catalina clay. Losses were directly related to organic matter content. Raising the pH of the subsoil only enhanced denitrification slightly. Addition of a denitrifying population and mineral nutrients in the form of a soil inoculant to a subsoil with a raised pH and organic matter content increased denitrification equal to that of surface soil. The lack of denitrification at lower depths is believed to be due to an inadequate supply of rapidly available energy material, low pH and mineral nutrients, and consequent lack of a denitrifying population. Since the surface soil rarely gets waterlogged, it was concluded that little possibility of N losses by denitrification in these humid tropical soils exists.

Additional Index Words: soil organic matter, soil pH, denitrifying population.

GASEOUS N LOSSES from soils by denitrification are believed to depend principally on pO_2 organic matter content, and pH of the soil (2, 4, 5, 6, 7, 8, 11). Bremner and Shaw (2) concluded that soil pH, temperature, and the amount and type of organic matter were the major factors governing the rate of denitrification in waterlogged soils. They observed no N loss from soils with less than 1% carbon content. Addition of glucose to soils low in organic carbon resulted in N losses equivalent to 67 to 97% of the added NO_3^--N. Cady and Bartholomew (4) found that appreciable denitrification occurred only with an available NO_3^- supply, high levels of organic matter, and O_2 less that 7% by volume.

Ekpete and Cornfield (5) reported greater losses of NO_3^--N under waterlogged conditions than under field capacity or lower moisture levels. In the presence of added organic matter, N losses from waterlogged soil were 48, 55, and 56% at pH 4.7, 6.5, and 8, respectively, showing ability of denitrifying organisms to operate over a wide pH range. Stefanson and Greenland (11) observed 50% denitrification losses at field capacity and 4 times more under waterlogged conditons in a soil-plant system. The gases evolved were N_2 and N_2O. Greenland (6) studied denitrifi-

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Table 1—Some important characteristics of the soils used in the experiment

Site location	Soil series	Soil depth	pH	OM	Sand	Silt	Clay
		cm		%			
Corozal	Humatas (Typic Tropohumult; clayey, mixed, isohyperthermic)	0-25	5.20	3.12	59	11	30
		25-50	4.62	1.34	52	9	39
		50-75	4.65	0.86	45	10	34
		75-100	4.65	0.69	52	11	37
		100-125	4.55	0.55	53	12	35
Manatí	Piña (Typic Haplorthox; psammentic, oxidic, isohyperthermic	0-25	4.55	1.04	73	5	22
		25-50	4.57	0.64	65	7	28
		50-75	4.55	0.37	61	4	35
		75-100	4.56	0.21	61	3	36
		100-125	4.48	0.19	60	5	35
Barranquitas	Catalina (Tropeptic Haplorthox clayey, kaolinitic isohyperthermic)	0-25	5.93	4.00	4	26	70
		25-50	5.43	1.36	2	16	82
		50-75	5.18	0.99	2	17	81
		75-100	4.88	0.82	2	19	79
		100-125	4.50	0.52	3	28	69

cation losses from cultivated, grassland, and forest soils in Ghana; he noticed insignificant N losses at field capacity, but larger and rapid losses in the saturated soils.

Soil profile studies in some Canadian soils revealed a higher rate and total quantity of N_2 evolution from the surface layer of the soil than from the lower depths (7). Similarly, in a study involving 17 solodized solonetz profiles in Australia, generally greater denitrification losses occurred from surface soils than from the lower depths (9). This was believed to be due to higher organic matter in the surface soil as compared to the lower horizons.

Cady and Bartholomew (3) using tagged N in denitrification studies observed that N_2O and N_2 were the principal gases evolved during the soil denitrification process.

Although considerable literature is available on denitrification studies in the temperate regions of the world, there have been fewer studies reported on the extent of denitrification losses from soils in the tropics and especially in the Neotropics. A study was, therefore, conducted to determine the extent of N losses by denitrification in humid tropical soils of Puerto Rico.

METHODS AND MATERIALS

Soil samples were collected from three different sites—Corozal (Humatas series; Typic Tropohumult), Manatí (Piña series; Typic Haplorthox) and Barranquitas (Catalina series; Tropeptic Haplorthox) where field experiments on N fertilizer utilization are being conducted. Some characteristics of the soils appear in Table 1. The 5 soil sample replicates at each site were single plot composites of 5 to 7.5-cm cores taken from the 5 plots receiving no N fertilizer at each site. The samples were allowed to air-dry in the greenhouse for 1 day, then crushed and passed through a 2-mm sieve.

Fifty-gram samples of the screened soil were weighed in 250-ml glass beakers. Two ml of distilled water containing 400 μg of NO_3^-—N as KNO_3 were applied to the samples receiving N and allowed to air-dry. The crust that formed was then crushed and uniformly mixed with the rest of the soil and transferred to 125-ml Erlenmeyer flasks. To samples not receiving N, 2 ml of distilled water were added. When pH was adjusted, the required amount of $Ca(OH)_2$ was added to the samples before transferring to Erlenmeyer flasks. Distilled water was added to maintain two moisture levels—field capacity and waterlogged. In the latter case, enough water was added to have a water level 1 cm above the soil surface. The flasks were made gas-tight by closing with one-hole rubber stoppers through which passed a small glass tube closed with a serum cap. The joints between the flask and the rubber stopper were covered with a "heavy grip" contact cement. Flasks were then evacuated by means of a vacuum pump and filled with a 1:4 oxygen/helium mixture. This was done 3 to 4 times to ensure complete replacement of air. Gas samples were obtained from the flasks through the serum cap with a gas-tight syringe. The apparatus used for this purpose was essentially that described by Smith and Clark (10) with minor modifications. The treatments were replicated 6 times and incubated in the laboratory at 23 ± 1C.

One of the replicates was used for determining the pH potentiometrically in a 1:1 soil/water mixture in the case of field capacity samples. No additional water was added to the waterlogged treatments for pH determinations. The gas samples from the flasks were analyzed for molecular N (N_2) and nitrous oxide (N_2O) after 1 and 2 weeks, using a Perkin-Elmer Vapor Fractometer Model 154 coupled with a Varian G 2000 Recorder. Molecular sieve 5A for N_2 and silica gel + ascarite for N_2O were used in 0.64 cm outside diameter (OD) copper tubing. The N_2 and N_2O gases were determined on separate samples after changing the columns for the respective gases. A standard of N_2O was prepared by reacting cold solutions of hydroxylamine hydrochloride and sodium nitrite. Air was used as the standard for N_2. Column length, rate of flow and other particulars were conducted according to the method of Smith and Clark (10).

Ammonium and NO_3^-—N were determined by alkaline distillation, and NO_2^-—N by the sulfanilic acid and 1-napthtylamine method as described by Bremner (1).

RESULTS

The extent of gaseous N losses by denitrification from different depths in the Humatas soil are shown in Table 2. There was practically no loss of N at field capacity, but under waterlogged conditon, 12 and 22% of the applied NO_3^--N was lost from the surface soil (0–25 cm) in 1 and 2 weeks, respectively. Denitrification also resulted in the production of NO_2^--N. Slight denitrification loss (5%) as N_2 also occurred in soil from 25 to 50 cm.

In the Piña soil, as in the Humatas soil, there were no denitrification losses at field capacity (Table 3). Under wa-

Table 2—Nitrogen losses by denitrification from Humatas soil*

Moisture level	Soil depth	1 week								2 weeks							
		pH	NH_4^+	NO_2^-	NO_3^-	N_2O	N_2	Total	Gaseous N loss	pH	NH_4^+	NO_2^-	NO_3^-	N_2O	N_2	Total	Gaseous N loss
	cm		ppm						%		ppm						%
Field capacity	0-25	5.8	17	-	383	-	5	405	1.4	6.1	22	-	382	-	-	404	-
	25-50	4.1	-	-	397	-	-	397	-	4.0	-	-	391	-	-	391	-
	50-75	4.2	-	-	396	-	-	396	-	4.1	-	-	395	-	-	395	-
	75-100	4.1	-	-	396	-	-	396	-	4.0	-	-	395	-	-	395	-
	100-125	4.0	-	-	395	-	-	395	-	3.8	-	-	396	-	-	396	-
Waterlogged	0-25	6.4	-	45	2	26	20	371	11.5	6.2	5	41	250	54	33	383	21.6
	25-50	4.2	-	10	374	-	7	391	1.8	4.1	3	10	365	-	19	397	4.6
	50-75	4.1	-	1	396	-	-	397	-	4.1	1	1	395	-	-	397	-
	75-100	4.1	-	-	401	-	-	401	-	4.0	1	-	393	-	-	394	-
	100-125	4.0	-	-	398	-	-	398	-	3.9	-		394	-	-	394	-

* 400 ppm of NO_3^--N was added to the soil at the start of the experiment.

Table 3—Nitrogen losses by denitrification from Pina soil*

Moisture level	Soil depth	1 Week								2 Weeks							
		pH	NH_4^+	NO_2^-	NO_3^-	N_2O	N_2	Total	Gaseous N loss	pH	NH_4^+	NO_2^-	NO_3^-	N_2O	N_2	Total	Gaseous N loss
	cm		ppm						%		ppm						%
Field capacity	0-25	4.5	4	-	378	-	-	382	-	4.4	4	-	379	-	-	383	-
	25-50	4.1	-	-	376	-	-	376	-	4.1	-	-	373	-	-	373	-
	50-75	4.1	-	-	375	-	-	375	-	4.1	-	-	374	-	-	374	-
	75-100	4.1	-	-	386	-	-	386	-	4.1	-	-	371	-	-	371	-
	100-125	4.4	-	-	386	-	-	386	-	4.4	-	-	380	-	-	380	-
Water-logged	0-25	5.0	5	4	361	6	24	400	7.5	4.6	2	4	344	9	24	383	8.3
	25-50	4.2	-	1	363	2	-	372	-	4.1	1	1	369	2	-	373	0.5
	50-75	4.1	-	-	371	-	-	372	-	4.1	-	-	373	-	-	373	-
	75-100	4.2	-	-	379	-	-	371	-	4.1	-	-	372	-	-	372	-
	100-125	4.4	-	-	374	-	-	374	-	4.3	-	-	382	-	-	382	-

* 400 ppm of NO_3^--N was added to the soil at the start of the experiment.

terlogged conditions 8% of the added NO_3^- was lost as gaseous N (N_2O and N_2) from the surface soil in 2 weeks. No gaseous N loss occurred from soils from the lower depths.

In the Catalina soil, as in the previous two soils, significant denitrification losses occurred only from the surface soil under waterlogged condition—20 and 31% during the 1- and 2-week incubations, respectively (Table 4). Greater denitrification losses and from greater depths (up to 50–75 cm) occurred from this soil than from the other two soils. No nitric oxide (NO) evolution was observed in any of the three soils studied.

Gaseous N losses in the form of N_2 and N_2O ranged from 8% in Piña soil to 31% in the Catalina soil and occurred almost exclusively in the surface soil and only when waterlogged. These results are in accord with the findings of other workers studying denitrification from different soil depths (7, 9).

Since a denitrifying population is favored by organic matter and high pH (2,5) the higher organic matter content and relatively higher pH of the surface soil (0–25 cm) compared to those of the lower depths may have been responsible for denitrification occurring almost exclusively in the surface soil. In order to confirm this, another experiment was conducted with one of the three soils.

Rapidly available energy material in the form of sucrose was added to soil samples from the 50 to 75 cm depths of the Humatas soil to raise its initial organic matter content (0.87%) to the level present in the 0 to 25 cm soil (3.07%). The initial pH (4.4) of this soil was raised to 4.8 by the addition of $Ca(OH)_2$. After application of these treatments, 400 ppm NO_3^--N was applied and the samples were incubated under a flooded condition. Samples were analyzed after 1 and 2 weeks as described earlier.

Gaseous N loss from the surface soil was 22% in 2 weeks while from the untreated deeper soil (50–75 cm) there was no such loss (Table 5). When sucrose was added to the 50- to 75-cm zone, about 3% gaseous loss was observed. When the pH of this zone was raised close to that of the surface soil without the addition of sucrose, there was no gaseous loss, indicating that pH adjustment alone was not sufficient for denitrification to occur. When sucrose was added with concomitant pH adjustment, there was a gaseous loss of 6% of the added N.

In spite of the addition of energy material and raising the pH, denitrifying activity in the 50 to 75 cm depth remained far below that of the surface soil. It was hypothesized that the lack of sufficient energy material and mineral nutrients, and prevalence of an unfavorably low pH at the lower depths caused the denitrifying population to remain very low. Because of the low denitrifying population the addition of sucrose and raising the pH did not bring about as active denitrification as was observed in the surface soil. In order to confirm this hypothesis another experiment was conducted in which, besides the addition of organic material and the adjustment of pH to approximate that of the surface soil, a denitrifying population in the form of 1 g soil from the 0 to 25 cm depth was added to the 50 to 75 cm depth soil, and denitrification was determined after the usual incubation period under waterlogged conditions. Results are given in Table 6. Fresh samples were used for these experiments so that the soils for Tables 4 and 5 were different samples from those used for earlier experiments.

Gaseous N losses by denitrification from the surface soil (0–25 cm layer) amounted to 24%, while from the 50- to 75-cm layer there was no such loss without pH adjustment and the addition of organic material, and a soil inoculant. Upon addition of the energy material to the 50- to 75-cm

Table 4—Nitrogen losses by denitrification from Catalina soil*

Moisture level	Soil depth	1 Week								2 Weeks							
		pH	NH_4^+	NO_2^-	NO_3^-	N_2O	N_2	Total	Gaseous N loss	pH	NH_4^+	NO_2^-	NO_3^-	N_2O	N_2	Total	Gaseous N loss
	cm		ppm						%		ppm						%
Field capacity	0-25	5.6	3	-	377	-	-	390	-	5.5	6	2	351	-	29	388	7.3
	25-50	5.0	-	-	369	-	6	375	1.5	5.1	-	-	367	-	5	372	1.2
	50-75	4.4	-	-	373	-	-	373	-	4.5	-	-	368	-	-	368	-
	75-100	4.3	-	-	368	-	-	368	-	4.4	-	-	365	-	-	365	-
	100-125	4.2	-	-	377	-	-	377	-	4.3	-	-	371	-	-	371	-
Water-logged	0-25	6.0	20	1	276	23	58	378	20.3	5.9	27	1	239	16	108	392	31.0
	25-50	5.0	-	1	372	-	12	385	3.0	5.2	3	2	354	-	15	374	3.8
	50-75	4.5	-	1	387	-	-	388	-	4.5	2	2	355	-	12	371	3.0
	75-100	4.3	-	-	376	-	-	376	-	4.4	3	1	371	-	-	375	-
	100-125	4.2	-	-	387	-	-	387	-	4.3	-	-	377	-	-	377	-

* 400 ppm of NO_3^--N was added to the soil at the start of the experiment.

Table 5—Effect of energy material and pH on denitrification losses from lower depths of Humatas soil*

Soil depth	Energy material	pH Amendment	1 Week pH	NH_4^+	NO_2^-	NO_3^-	N_2O	N_2	Total	Gaseous N loss	2 Weeks pH	NH_4^+	NO_2^-	NO_3^-	N_2O	N_2	Total	Gaseous N loss
cm				ppm						%		ppm						%
0-25	-	-	5.0	2	37	269	65	-	373	15.2	5.2	7	27	259	70	17	380	21.8
50-75	-	-	4.4	-	2	384	-	-	386	-	4.2	-	5	372	-	-	377	-
50-75	Sucrose	-	4.3	-	4	380	-	-	384	-	4.2	-	3	358	11	-	372	2.6
50-75	-	Amended	4.8	2	7	378	-	-	387	-	4.8	1	8	372	-	-	391	-
50-75	Sucrose	Amended	4.8	-	16	371	-	-	387	-	4.8	-	9	337	23	-	369	5.7

* 400 ppm of NO_3^--N was added to the soil at the start of the experiment. All the treatments were incubated under waterlogged condition (1 cm of standing water above the soil surface).

Table 6—Effect of energy material, pH and inoculant on denitrification from lower depths of Humatas soil*

Soil depth	Soil inoculant	Energy material	pH Amendment	1 Week pH	NH_4^+	NO_2^-	NO_3^-	N_2O	N_2	Total	Gaseous loss	2 Weeks pH	NH_4^+	NO_2^-	NO_3^-	N_2O	N_2	Total	Gaseous loss
cm					ppm						%		ppm						%
0-25	-	-	-	4.8	5	23	283	55	-	366	13.8	4.8	7	16	255	95	-	373	23.8
50-75	1 g. 0-25 cm soil	-	-	5.0	1	1	384	-	-	385	-	4.1	3	4	380	-	-	387	-
50-75	"	Sucrose	-	4.2	-	2	378	-	-	380	-	4.1	-	5	361	9	-	375	2.3
50-75	"	-	Amended	4.9	2	11	373	-	-	386	-	4.8	3	12	356	3	-	374	0.9
50-75	"	Sucrose	Amended	4.8	1	22	337	10	-	370	2.5	4.9	-	5	287	84	-	376	21.0

* 400 ppm of NO_3^--N was added to the soil at the start of the experiment. All the treatments were incubated under waterlogged condition (1 cm standing water above the soil surface).

soil samples, 2% nitrogen loss was observed. With pH adjustment but without addition of energy material only 1% denitrification loss occurred; with soil inoculant alone, no denitrification occurred. When the pH was adjusted and both energy material and soil inoculant were added, denitrification losses were increased to 21%, almost equal to that from the surface soil. Apparently the soil inoculant, besides adding the denitrifying population, supplied sufficient mineral nutrients essential for the microbes. Thus, it appears that denitrification occurs only in surface soil and not at lower depths in these soils because of the lack of sufficient energy material and mineral nutrients, low pH, and consequent lack of sufficient denitrifying population in the lower depths of the profile.

In practice the surface soil seldom gets waterlogged, as all these soils have rapid surface drainage. The possibility of waterlogged conditions exists at lower depths after heavy rains, but, as shown in this study, denitrification does not occur at these depths. In view of these observations it is concluded that there is very little possibility of denitrification losses occurring in these humid tropical soils.

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