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PARACOPRID DUNG BEETLES AND GASEOUS LOSS OF NITROGEN FROM COW DUNG

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Summary—The estimated gaseous loss of nitrogen from cow dung during 4 weeks was almost unaffected by the activity of the dung beetle, *Onthophagus lenzii* H. The loss of gaseous N was the result primarily of NH_3 volatilization during the first week. However, the beetles had a negative effect on NH_3 volatilization by lowering the pH and the NH_4^+-N concentration in colonized cow dung and dung balls. Once NH_3 volatilization had ceased, denitrification became prominent and was the cause of 23.6% or more of the loss of N from the dung balls. N_2-fixation (acetylene reduction assay) had only a negligible role in the estimated N balance. Denitrifying activity was limited by a deficiency in available endogenous NO_3^--N. The action of dung beetles altered environmental conditions and increased ammonification, nitrification and denitrification, as well as N_2-fixation.

INTRODUCTION

Gaseous loss of N is a problem for the efficient recycling of nutrients in cow dung (Fincher, 1981). Gillard (1967) reported that cow dung left on the soil surface loses *ca.* 80% of its N through NH_3 volatilization and that burying activity by dung beetles lowers the loss by 5–15%. However, the mechanisms by which dung beetles affect the process of gaseous loss of N in cow dung are still not clear, and the effects of dung beetles on denitrification have not been studied.

Both NH_4^+-N and NO_3^--N concentrations, which affect the rates of NH_3 volatilization and denitrification, are governed by differences in the rates of mineralization, nitrification, immobilization, leaching and volatilization. Although the dung beetles *Onthophagus lenzii* H. accelerates mineralization and nitrification, they lower the concentration of inorganic N by mixing soil with residual cow dung and with dung balls (Yokoyama *et al.*, 1991). Thus, dung beetle activity should affect gaseous loss of N from cow dung, as the result of chemical, physical and microbiological changes in cow dung and dung balls.

MATERIALS AND METHODS

Experiment 1

Rearing

A rearing system of "beetle" and "control" treatments was used (Yokoyama *et al.*, 1991). In the "beetle" treatment, two pairs of *O. lenzii* H. were introduced into containers with 100 g fresh cow dung on a 10 cm layer of soil. Containers for the "control" treatment contained cow dung and soil without beetles. Both treatments were conducted in triplicate for 3 weeks and in duplicate for 4 weeks. These containers were kept at 25°C, with 60% r.h. and 14 h day light. Residual cow dung colonized by the beetles, dung balls formed by them in the "beetle" treatment and uncolonized cow dung in the "control" treatment were cut up and sampled every week for 4 weeks. Each sample was mixed well with a glass rod. The results were expressed as the means of triplicate or duplicate samples.

Factors affecting gaseous loss of N

Dry weights of collected samples were obtained after exposure to 105°C for 2 days. pH (H_2O) and pH (KCl) were determined for 2–5 g of fresh subsamples suspended in 2.5 times (v/w) distilled water and 1 M KCl by a glass electrode after 1 h of equilibration, respectively. After shaking for 1 h, NH_4^+-N and NO_3^--N were extracted from the fresh subsamples (2–5 g) with 10 fold (v/w) 1 M KCl by centrifugation (8000 *g*, 30 min). The concentration of inorganic N was determined for 5 ml of extract according to the microdiffusion method of Bremner (1965) as modified by Kai and Harada (1972). Inorganic N content was expressed as mg N 100 g^{-1} dry matter. Total C was determined by Tyurin's method on an air-dried and ground sub-sample (Kononova, 1961).

Estimation of NH_3 volatilization, N_2-fixation and denitrification

Fresh sub-samples (0.5–1.5 g) of residual cow dung, dung balls and uncolonized dung were placed in the outer chamber of a Conway unit. 1 ml of 2% boric acid solution was added to the inner chamber of the unit. The units were closed and kept for 24 h at 25°C in the dark. Then the boric acid solution was titrated with 0.005 *N* standardized H_2SO_4 to determine the amount of NH_3-N absorbed. The acetylene-reduction and acetylene-inhibition methods were used to determine N_2-fixing and denitrifying activities

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(Yoshinari *et al.*, 1977; Kamata *et al.*, 1987). The sub-samples (0.5–2.0 g) were placed in 105 ml assay flasks. The flasks were closed with double-layer serum stoppers, and the atmosphere in the flasks was replaced by assay gas (C_2H_2, 9.8%; CO_2, 370 $\mu l\,l^{-1}$; Ar balance). The flasks were then kept for 24 h in the dark at 25°C. Ethylene emission from decomposing organic matter under anaerobic conditions was determined by replacing the atmosphere by He instead of assay gas. After incubation, 1 ml of the gas phase in each flask was analyzed for C_2H_4 by g.c. (Kamata *et al.*, 1987). A theoretical mole ratio of 3:1 (C_2H_4:N_2) was used to calculate the amount of N_2 fixed. Another portion of the gas phase (1 ml) in each flask was used to determine the concentration of N_2O from endogenous NO_3-N. The g.c. conditions for N_2O determination were as follows: g.c., Shimazu GC-8AIT (TCD); column, Porapack Q (80–100 mesh) 1.5 m plus Porapack N (60–80 mesh) 0.75 m by 3 mm i.d.; column temperature, 60°C; detector temperature, 100°C; filament current, 120 mA; carrier gas, He 43 ml min^{-1}. The rate of the three processes examined were expressed as the weight of N volatilized from or fixed on 1 g of dry matter during 24 h.

Daily rates of these processes and daily dry weights of collected samples were extrapolated from differences between weekly readings. Changes in values were assumed to be linear on these days. The N balance of cow dung in each treatment was calculated as the sum of daily N balance estimated by multiplying daily rates by daily oven dry weights. The processes in soil were not measured.

Potential denitrifying activity was quantified using a sufficient amount of exogenous NO_3-N. After determination of the rate of denitrification and N_2-fixation, 5 ml of KNO_3 solution (1 mg ml^{-1}) were added to each flask. The gaseous atmosphere in the flask was again replaced by assay gas. After 12 h of incubation in the dark, N_2O in the gas phase was determined as above. The results of both actual and potential denitrification, as well as N_2-fixation, were transformed on the basis of 1 g of total C for examination of microbial activity.

Experiment 2

Fresh cow dung (100 g) was placed on a 5 cm deep layer of soil (300 g) in a 1 l glass bottle. Two pairs of *O. lenzii* were introduced into the "beetle" treatment. The "control" treatment was consisted of cow dung (100 g) on a 5 cm layer of soil, and the "untreated" was soil alone. The treatment "blank" was an empty bottle for background correction. Each bottle was connected to a rubber stopper containing aerating tubes. Air temperature was kept at 25°C under 14 h of light. All treatments were conducted in triplicate. Ammonia volatilized was introduced into a 2% boric acid solution by NH_3-free and water-saturated air (8 ml s^{-1}). This solution was newly replaced twice a day for the first 3 days, once a day from days 3 to 14, and once every few days thereafter. The cumulative amount of NH_3-N volatilized from each bottle was calculated. The beetles did not make dung balls, as it was not their reproductive season.

Statistical analyses

Mean values of duplicate or triplicate samples were used for statistical analyses. A set of homoscedastic data was analyzed according to a 2-way ANOVA (Edwards, 1967), where the main effects were dung beetle activity and days. This was followed, if necessary, by Duncan's multiple range test (Rickmers and Todd, 1967) for values on beetle effect. When a set of variance was not homoscedastic, a Kruskal–Wallis rank order test was conducted (Edwards, 1967). The mean value of the cumulative amount of NH_3-N volatilized in Experiment 2 was analyzed by the Duncan's multiple range test (Rickmers and Todd, 1967).

Table 1. Estimated values of N balance[1]

Duration (weeks)	Gain	Loss		Weekly balance[3] (mg N)	Cumulative balance[3] (mg N)
	N_2-fixation[2] (μg N)	Denitrification[2] (mg N)	NH_3 volatilization[2] (mg N)		
Uncolonized cow dung					
0–1	7.06	0.00	8.86	−8.85	−8.85
1–2	7.69	0.05	2.85	−2.89	−11.74
2–3	2.65	0.75	0.96	−1.71	−13.45
3–4	2.50	0.95	0.00	−0.95	−14.40
Sum	19.90	1.75	12.67	−14.40	
Residual dung					
0–1	2.17	0.00	10.49	−10.49	−10.49
1–2	1.26	0.00	1.63	−1.63	−12.12
2–3	2.85	0.16	0.07	−0.23	−12.35
3–4	3.64	0.51	0.00	−0.51	−12.86
Sum	9.92	0.67	12.19	−12.86	
Dung balls					
0–1	6.15	0.00	2.40	−2.40	−2.40
1–2	1.72	0.00	0.00	0.00	−2.40
2–3	3.79	0.22	0.00	−0.22	−2.62
3–4	2.72	0.52	0.00	−0.52	−3.14
Sum	14.38	0.74	2.40	−3.14	

[1]The values are based on one rearing container where 100 g fresh cow dung (total N: 293 mg) was applied.
[2]Values are calculated by the following equation: the value to day $s = (W_0V_0 + W_1V_1 + \cdots + W_tV_t + \cdots + W_{s-1}V_{s-1})$ where, W_t:a estimated dry weight on day "t", V_t:a estimated rate (oven-dry basis) on day "t".
[3]Minus expresses loss as a whole.

RESULTS AND DISCUSSION

Nitrogen balance

Estimated amounts of NH_3 volatilization, denitrification and N_2-fixation in Experiment 1 are shown in Table 1. Nitrogen lost over the 4 weeks came mainly from NH_3 volatilization during the first week. Denitrification became prominent following completion of HN_3 volatilization. The contribution of N_2-fixation to the N balance was negligible. The cumulative amount lost during the experiment was less than 5% of the total N in the cow dung. This agrees well with the results of MacDiarmid and Watkin (1972). However, dung beetles affected the pathway of gaseous loss of N. In fact, NH_3 volatilization from dung balls almost ceased during the first week (Fig. 1). In contrast, denitrification was found to cause 23.6% of the loss of N from dung balls. This was extremely higher than the contribution of denitrification in uncolonized dung (12.2%). Moreover, the contribution of denitrification to gaseous loss of N in dung balls was probably greater, as dung balls were formed at the bottom of the containers, and NH_3 volatilizing from dung balls should become absorbed by surrounding soil (Freney *et al.*, 1983; Simpson and Steele, 1983).

Conditions in which NH_3 volatilization was observed are summarized as follows: moisture content, >40%; pH, >6.5 to 7.5 and NH_4^+-N, >30 to 35 mg N $100\ g^{-1}$ (Fig. 1). The beetles caused a decrease in pH ($P < 0.01$) and in the actual NH_4^+-N concentration ($P < 0.05$) by mixing soil with cow dung and dung balls. They also enhanced nitrification (Yokoyama *et al.*, 1991) by producing aerobic conditions (Stevenson and Dindal, 1987), which should result in part of the NH_4^+-N pool being consumed under such conditions.

The results obtained from Experiment 2 clearly indicate that the beetles inhibited NH_3 volatilization ($P < 0.01$, Fig. 2). The slow volatilization during the first few days and the small amount of N volatilized as NH_3 in Experiment 2 may have resulted from differences between the two experiments. The possible reasons are as follows: (1) the cow dung in Experiment 2 contained only 29.1 mg NH_4^+-N $100\ g^{-1}$ dry matter, which was considerably less than that in Experiment 1 (118.6 mg N $100\ g^{-1}$ dry matter), and was less than the lower limit of NH_4^+-N concentration required for NH_3 volatilization as determined in Experiment 1 (Fig. 1); (2) the method used for preparing the sub-samples in Experiment 1 may have possibly increased the surface area of the sub-samples and, thus, enhanced volatilization.

Enhancement of microbial activity in denitrification and N_2-fixation

Table 2 shows actual and potential denitrification activity. At the end of the experiment, actual denitri-

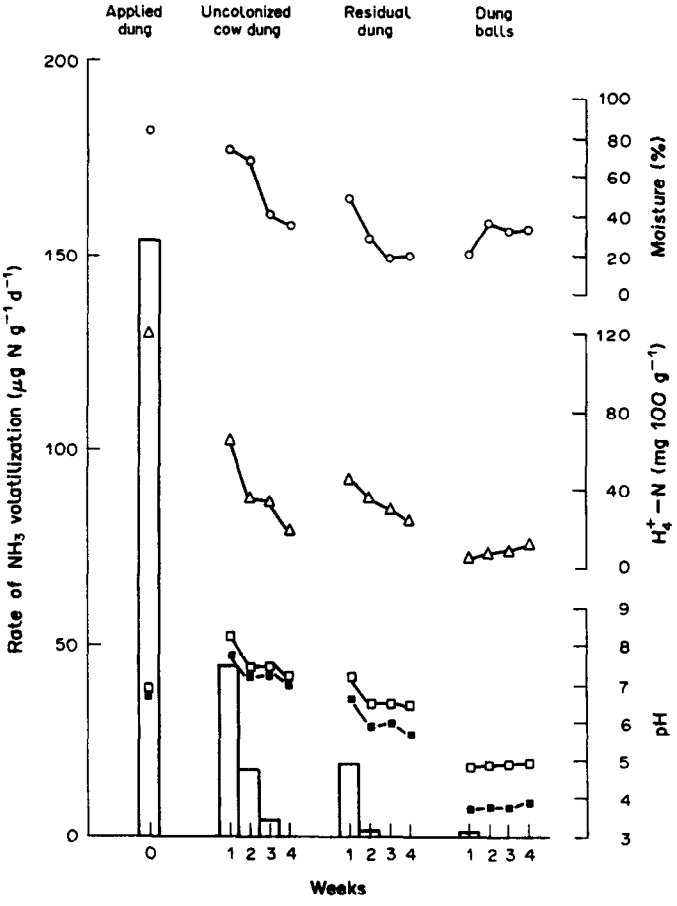

Fig. 1. Changes in the rate of ammonia volatilization and in the factors affecting ammonia volatilization in Experiment 1. ○: moisture; △: NH_4^+-N; □: pH(H_2O); ■: pH(KCl).

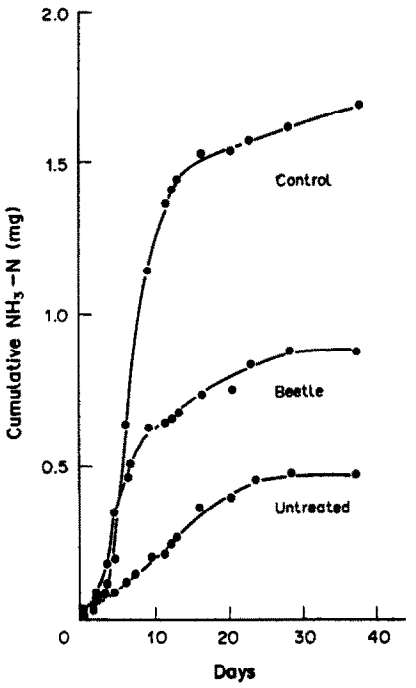

Fig. 2. The cumulative amount of NH_3-N volatilized in Experiment 2.

fying activity among the three samples was proportional to increases in the intensity of dung beetle activity, although the variation was insignificant. Activity in the three samples was enhanced by the addition of exogenous NO_3^--N, especially in uncolonized cow dung ($P < 0.01$), suggesting that NO_3^--N was the most important limiting factor for denitrification. Thus, dung beetles enhance actual denitrifying activity by increasing the endogenous NO_3^--N pool as a result of enhanced ammonifying and nitrifying activity (Yokoyama *et al.*, 1991). Our results indicate that dung beetles affect the denitrification in a way somewhat similar to that noted for earthworm casts, in which the supplies of available energy and NO_3^--N and the consumption of O_2 by active microorganisms enhance the potential for denitrification (Svensson *et al.*, 1986).

Table 2. Changes in actual and potential denitrifying activity

	Weeks	Actual denitrification ($\mu g\,N\,g^{-1}\,C\,d^{-1}$)	Potential denitrification ($mg\,N\,g^{-1}\,C\,12\,h^{-1}$)
Cow dung applied	0	ND*	0.15
Uncolonized cow dung	1	ND	1.28
	2	3.1	1.71
	3	43.8	1.83
	4	6.7	1.19
Residual dung	1	ND	1.13
	2	ND	0.92
	3	11.0	0.56
	4	20.5	1.00
Dung balls	1	ND	0.21
	2	ND	0.15
	3	65.3	0.44
	4	116.3	0.67

*Not determined.

Table 3. Changes in N_2-fixing activity ($ng\,N\,g^{-1}\,C\,d^{-1}$)

	Weeks				
	0*	1	2	3	4
Uncolonized cow dung	55.0	350.0	94.7	51.8	73.1
Residual dung	55.0	51.9	22.2	179.0	82.0
Dung balls	55.0	460.9	194.7	493.3	376.1

*Cow dung applied.

Although the actual contribution of N_2-fixation to the N balance was very small (Table 1), N_2-fixing activity was enhanced in dung balls ($P < 0.05$, Table 3). Dung beetles could support N_2-fixing activity in dung balls by lowering the actual concentration of inorganic N ($P < 0.05$, Fig. 1) and supplying easily-decomposable organic matter (Yokoyama *et al.*, 1991). The reason for a decrease in N_2-fixing activity in both colonized dung and dung balls in the second week (Table 3) cannot be deduced from Fig. 1. It might be explained by floral changes in N_2-fixers as the result of beetles activity and time.

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