Potential Total Nitrogen Contributed by Nostoc muscorum

To obtain an estimate of total nitrogen that could potentially be converted to nitrate by nitrifying bacteria, three parameters were examined: (1) excretion of ammonia, the product of nitrogen fixation, directly into the surrounding medium by living tissue, (2) percent nitrogen present in the organism that would be released into the soil upon degradation, (3) growth rates of Nostoc muscorum to determine maximal rates at which degradation could occur.

Ammonia excretion. Excretion of ammonia was examined by using an ammonia ion electrode (Orion, Model 95-10) connected to a Beckman Expandomatic pH meter (Model SS-2). In all cases replicate samples of four were obtained.

Algal samples were washed thoroughly with deionized water to remove excess combined nitrogen. The samples were blotted dry and placed in covered Pyrex containers (no. 3250, approximately 350-mℓ capacity) so as to cover the bottom completely. Double-distilled, ammonia-free water (40 mℓ), containing 1 mℓ of a trace element mixture (2.86 g H_3BO_3, 1.80 g $MnSO_4 \cdot H_2O$, 0.222 g $ZnSO_4 \cdot 7H_2O$, 0.079 g $CuSO_4 \cdot 5H_2O$, 0.018 g MoO_3 in 1 liter of H_2O adjusted to pH 6.7) was added so that the algae was partially immersed. In additional experiments, distilled water containing known amounts of ammonia was used to determine the effects of initial ammonia concentration on ammonia excretion. The preparations were then allowed to incubate for a 24-hr period under ambient light and temperature conditions or, in some cases, in the dark entirely. The water was then poured off and analyzed for ammonia concentration with the ammonia ion electrode. Controls were obtained by incubating the algal preparation for 10 min in order to determine initial contribution of ammonia by the algae. The change in ammonia concentration due to washing was monitored in separate experiments where it was found that the initial ammonia concentration decreased by an average of only 0.02 μg/mℓ as a result of washing. Ammonia excretion is expressed as μg NH_4/g Nostoc/hr, obtained by the following equation:

$$\frac{(\mu g/m\ell\ NH_4\ \text{sample}) - (\mu g/m\ell\ NH_4\ \text{control})/\ (\text{g alga, dry wt})}{24\ \text{hrs incubation time}} \times 40\ m\ell$$

Percent nitrogen. Percent nitrogen content of the alga (dry wt) was determined by digesting about 0.1 g of algal material over heat with 3 mℓ of conc. H_2SO_4 and a 1 cm length of copper wire (no. 12) until the preparation turned white (about 5 hrs). The products were then quantitatively transferred to 100-mℓ volumetric flasks and diluted to that volume. Aliquots of 5 mℓ were basified with 1 mℓ of 10 N NaOH and subjected to microkjeldahl steam distillation (Bremner 1965) to determine TKN (total Kjeldahl nitrogen). Percent nitrogen was determined by the following equation:

$$\%\ N = \frac{(m\ell\ \text{titrated} - \text{blank})\ \ (.014)\ \ (N\ H_2SO_4)}{\text{weight of sample}}$$

Algal growth. Small pieces (.13 - 1.65 g wet wt) of freshly gathered Nostoc muscorum were placed in 50-mℓ beakers partially covered with Parafilm (American Can Co.) to allow gas exchange without excessive evaporation of the media. Initially, 2 mℓ of distilled water containing 1 mℓ/ℓ of the trace element mixture was introduced to the bottom of the beaker. The beakers were then placed in a Biotronette Mark III environmental chamber (Lab-Line Instruments, Inc.) and incubated at 32°C and 350 ft-c for a 12-hr day. The samples were weighed weekly on an analytical balance. Media was added every few days to replace that which was lost due to evaporation.

Soil Percolation Experiments

A series of experimental soil flats were designed to obtain estimates of the ability of Nostoc muscorum, in association with nitrifying bacteria, to contribute nitrate to the groundwater system. Each fiberglass-coated plywood flat (Fig 4A) measured .65 m x 1.33 m x 10 m and had a small drain situated at one end for collecting the soil percolate. Eighteen flats were used and arranged in the manner as shown in Fig. 4B: two control flats having no soil, and eight each of two soil types, referred to as Barrigada clay and San Agustin clay (Fig. 5). Both soils are classified as Guam clays (Dr. J. L. Demeterio, personal communication). At approximately 1-month intervals, water percolate samples were obtained from the flats and analyzed for total nitrogen by microkjeldahl steam distillation (Bremner 1965).

Soil composition data for the experimental soil types were obtained as follows. Organic content was determined by the Walkley and Black method (Jackson 1958). Soil pH was determined by mixing 10 g of soil with 10 mℓ distilled water and reading the pH of the preparation after a standing period of 15 min. Physical soil composition data was obtained from Ms. Marylou Baccay (personal communication). Percent sand, silt and clay was determined by

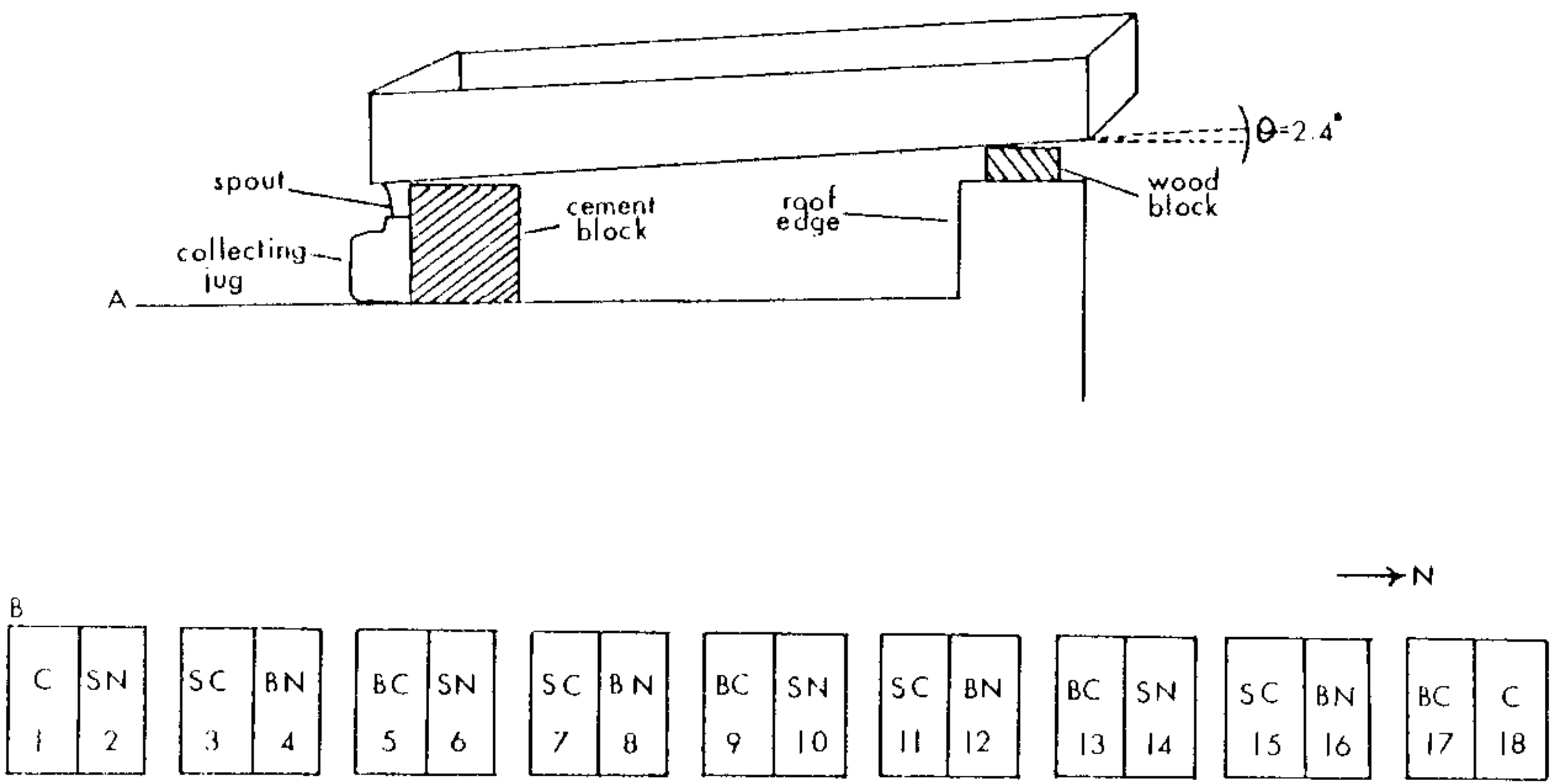

Figure 4. Experimental design for the soil percolate studies. A. Set-up of individual soil flats. B. Arrangement of soil flats. Explanation of symbols: C, control, no soil; SN, San Agustin clay with *Nostoc*; SC, San Agustin clay, bare soil; BN, Barrigada clay with *Nostoc*; BC, Barrigada clay, bare soil.

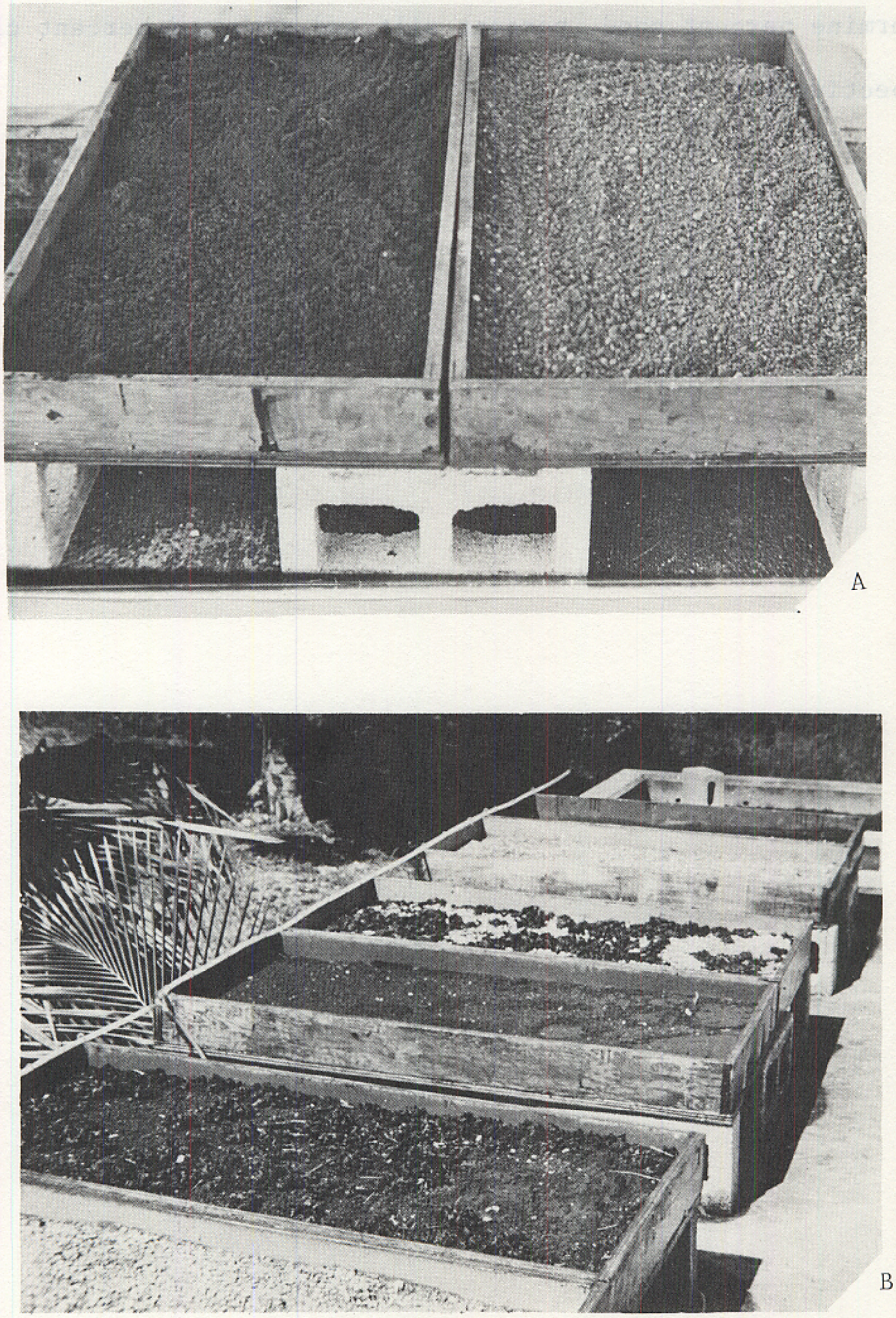

Figure 5. Experimental design for soil percolate studies before (A) and after (B) the addition of <u>Nostoc</u> <u>muscorum</u>.

hydrostatic means, where 50 g of the soil was thoroughly mixed with a known volume of water to which Calgon was added as a dispersing agent. Hydrometer readings were taken at 0 sec, 10 sec and 2 hr to determine percent sand, percent silt and clay and percent clay, respectively.

RESULTS

Biomass Estimates of *Nostoc muscorum*

Nostoc muscorum, on Guam, is found primarily along lightly traveled roads and in disturbed areas where ground cover is generally sparse, such as abandoned roads (Fig. 6A), old foundations, unused runways and on hard-packed clay surfaces (Fig. 6B) that are exposed to surface run-off.

Table 1 lists the dry weights of *Nostoc muscorum* for samples typical of sparse, medium and heavy coverage along various transects. Average dry weights per 100 m of roadway obtained using the values from Table 1, are listed in Table 2. The total biomass estimates are obtained by multiplying the average for each road type by 2 (to account for the other side of the road), divided by the transect length 0.1 km, and multiplied by the number of kilometers of each road type as determined from aerial photographs. Table 3 shows these calculations. An estimate of 10^{-2} kg/m^2 is obtained by adding the values obtained from the disturbed areas (see Table 1). If the area over the lens is 2.6×10^2 km^2, then an upper estimate of 2.6×10^6 kg for the biomass of *N. muscorum* is derived.

Guam's Department of Public Works (1969) requires that ponding basins be in existence where the natural seepage of groundwater is retarded due to development. These ponding basins provide a means for recharging the lens. Therefore, any *Nostoc muscorum* growing in these basins would directly affect the soil percolate originating as runoff from urban areas. The biomass estimates for these ponding basins are listed in Table 4.

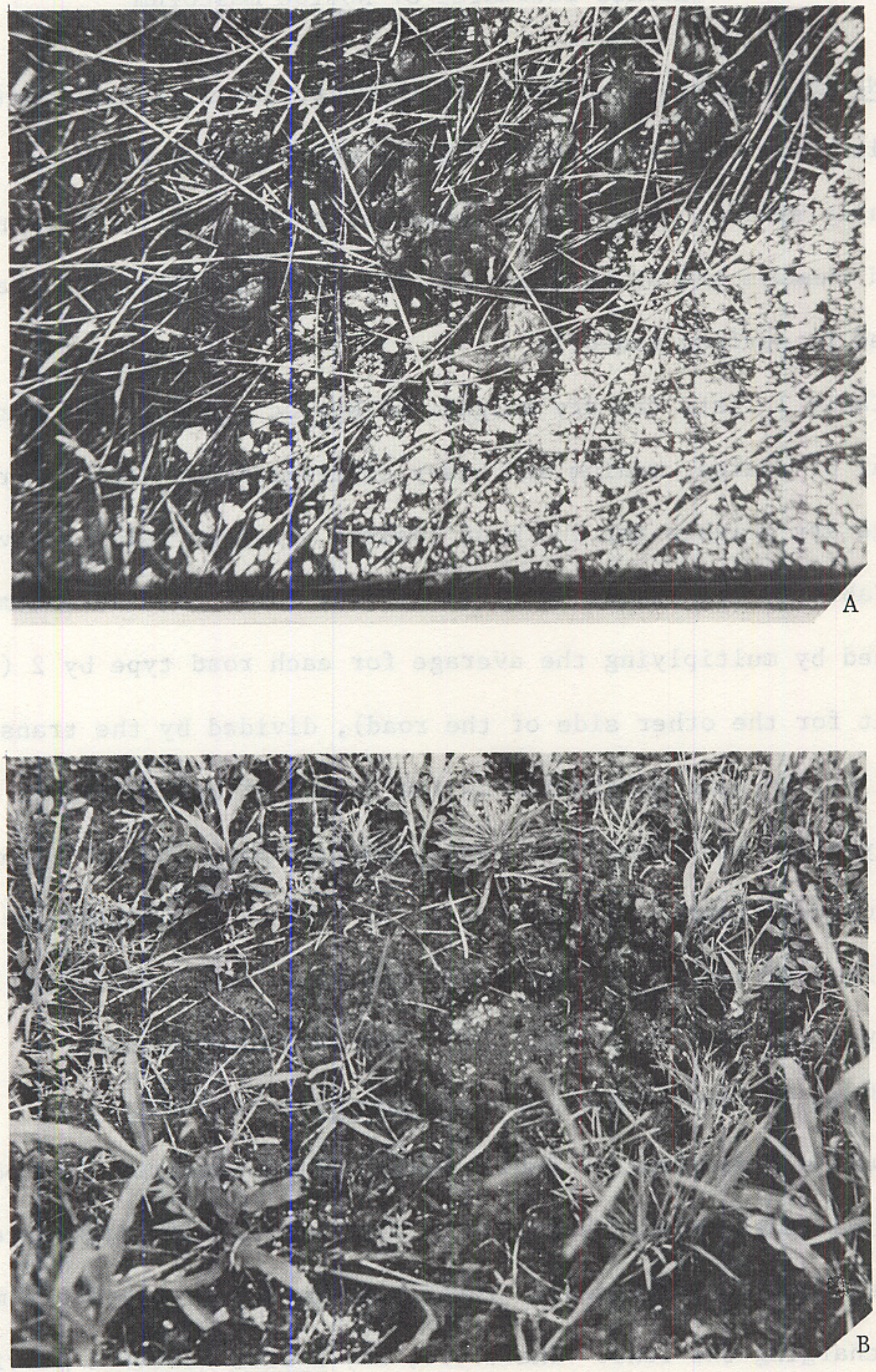

Figure 6. Habitat of Nostoc muscorum. A. abandoned asphalt road, B. hard-packed Guam clay.

Table 1. Dry weights of Nostoc muscorum for samples obtained from transects during October, November, and December 1978. See Fig. 1 for location of transects.

Transect	Date	Dry Weight (g)	Sample Size (m^2)	g/m^2
		SPARSE COVERAGE		
1	10-10	15.1	2.50	6.0
5	10-10	15.2	2.50	6.1
6	10-11	9.0	2.50	3.6
12	10-17	14.9	2.50	6.0
8	11- 8	18.0	2.50	7.2
3	12- 4	16.7	2.50	6.7
7	12- 4	12.6	2.50	5.0
2	12- 4	9.6	2.50	3.8
18	12-18	6.1	2.50	2.4
19	12-18	13.3	2.50	5.3
			$\overline{Y}$ = 5.2 g/m^2	
			s = 1.52	
		MEDIUM COVERAGE		
2	10-10	52.7	2.50	21.1
6	10-11	46.6	2.50	18.6
1	10-10	35.4	2.50	14.2
15	10-17	158	2.50	63.2
17	10-17	182	2.50	72.8
9	10-17	35.4	2.50	14.2
20	10-17	114	2.50	45.6
19	11- 8	148	2.50	59.2
8	11- 8	128	2.50	51.2
7	12- 4	48.8	2.50	19.5
			$\overline{Y}$ = 38.0 g/m^2	
			s = 22.7	
		HEAVY COVERAGE		
2	10-10	226	2.50	90.4
21	11- 8	122	1.25	97.6
8	11- 8	186	1.25	149
2	11- 8	172	1.25	138
20	10-19	188	1.25	150
7	12- 4	150	1.25	120
6	12- 4	126	1.25	101
1	12- 4	215	1.25	172
13	12-18	165	1.25	132
15	12-18	237	1.25	190
			$\overline{Y}$ = 134 g/m^2	
			s = 32.7	
		DISTURBED AREAS		
1	10-19	375	2.50	150
2	12-19	238	1.25	190
3	10-19	636	1.0	636
			$\overline{Y}$ = 325	

Table 2. Dry weight (g) of *Nostoc muscorum* per 100 m on different types of roads.

Transect No.	Sparse $\overline{Y}$ = (5.2 g/m^2)	Medium $\overline{Y}$ = (38 g/m^2)	Heavy $\overline{Y}$ = (134 g/m^2)	Total
		ROAD TYPE 1		
2	16	342	3216	3.57×10^3
3	130	-	-	1.30×10^2
4	-	-	-	-
				1.23×10^3
		ROAD TYPE 2		
7	109	4522	804	5.44×10^3
9	109	38	-	1.47×10^2
10	-	-	-	-
11	10	-	-	1.00×10^1
12	5	1140	268	1.41×10^3
13	36	1216	-	1.25×10^3
14	-	-	-	-
15	21	304	402	7.27×10^2
16	36	1216	134	1.39×10^3
17	78	1254	268	1.60×10^3
18	88	266	-	3.54×10^2
19	52	570	-	6.22×10^2
21	5	874	4288	5.17×10^3
				1.61×10^3
		ROAD TYPE 3		
1	42	76	8308	8.43×10^3
5	78	38	-	1.16×10^2
6	57	304	6164	6.52×10^3
8	26	3534	5226	8.79×10^3
20	135	228	9648	1.00×10^4
				6.77×10^3

Table 3. Total biomass of *Nostoc muscorum* for a selected 1 km^2 area.

TOTAL ALONG ROADS

Type 1 Roads -

$$\frac{(1.23 \text{ kg})\ (2)}{(0.1 \text{ km})} \times (34 \text{ km}) = 836 \text{ kg}$$

Type 2 Roads -

$$\frac{(1.61 \text{ kg})\ (2)}{(0.1 \text{ km})} \times (70 \text{ km}) = 2254 \text{ kg}$$

Type 3 Roads -

$$\frac{(6.77 \text{ kg})\ (2)}{(0.1 \text{ km})} \times (35 \text{ km}) = 4739 \text{ kg}$$

total $7829 \text{ kg} / 10^6 \text{ m}^2$

TOTAL IN DISTURBED AREAS

Total calculated area of disturbed areas: 4709 m^2

Average dry weight for disturbed areas: 0.325 kg/m^2

Total biomass in disturbed areas: 1530 $kg/10^6\ m^2$

TOTAL FOR 1 KM^2 STUDY AREA

Road + Disturbed areas = $9359 \text{ kg}/10^6 \text{ m}^2 = 9.36 \times 10^{-3} \text{ kg/m}^2 = 10^{-2} \text{ kg/m}^2 = 10^4 \text{ kg/km}^2$

Table 4. Dry weight (kg) estimates for _Nostoc_ _muscorum_ in four ponding basins situated over the groundwater lens.

Site	Soil Type	Area	Dry Weights of Algae
Dededo	Barrigada limestone; overlying Guam clay*	31,600 m^2	423 kg
Barrigada No. 3	Mariana limestone; overlying Guam clay	2,500 m^2	22 kg
Latte Estates No. 2	Barrigada limestone; overlying Guam clay	2,000 m^2	7.3 kg
Mariana Terrace	Mariana and Barrigada limestone; overlying Chacha-Saipan and Saipan-Yona-Chacha clay*	25,000 m^2	0.0 kg

*The U.S. Soil Taxonomy method describes Guam clay as belonging to the Inceptisol order, subgroup Lithic Ustropepts; and Chacha clay as belonging to the Inceptisol order, subgroup Oxic Ustropepts (Park 1979).

In most cases, the flora in the ponding basins is of sufficient density to exclude any large amounts of N. muscorum growth. One ponding basin (Dededo) did support a biomass of 423 kg of N. muscorum presumably attributable to a scarcity of dense plant growth. This particular ponding basin is a possible influx point for NO_3-N into the lens which will be discussed later in the discussion section. Since it has been shown that NO_3-N levels of water entering the ponding basins is low at an average of 0.033 μg/mℓ NO_3-N (Zolan et al. 1978) and the biomass of N. muscorum in these basins is not generally high, the ponding basins in themselves are probably not a significant source of NO_3 contribution with respect to N. muscorum.

Potential Total Nitrogen Contributed by Nostoc muscorum

Ammonia excretion. The results of the ammonia excretion experiments are presented in Fig. 7. The change in the ammonia concentration in the media is plotted against initial ammonia concentration. Data points from samples incubated in the dark for the entire 24-hr period were grouped together with those samples exposed to daylight after an analysis of covariance (Sokal and Rohlf 1969) showed them to be on the same regression line.

This lack of light dependency is not in accordance with nitrogen fixation studies (Steward 1973) that indicate a definite positive correlation between light and rates of nitrogen fixation. However, since excretion, rather than fixation, is being measured, the same correlation would not be expected to exist, as excretion rates are more strongly dependent on the ammonia concentration of the surrounding media (Fogg 1971).

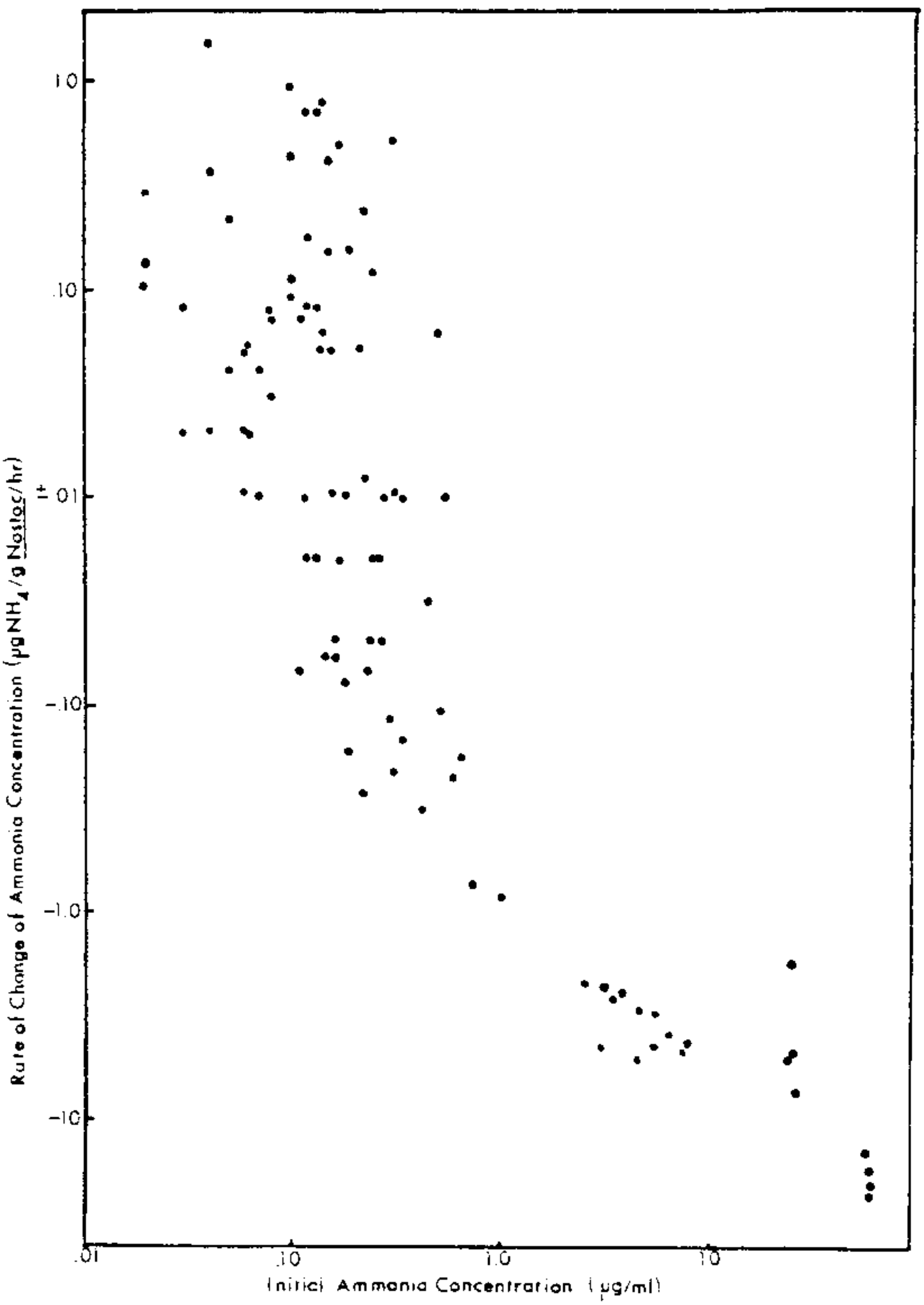

Figure 7. Initial ammonia concentration versus rate of ammonia uptake or excretion by Nostoc muscorum.

Regression analysis of 96 data points (Sokal and Rohlf 1969) shows a significant negative correlation between initial ammonia concentration and rate of ammonia excretion ($t = -13.21$; $t_{.001}$ (60) = 3.460), and further shows that the best relationship approximating the data is logarithmic with the y-intercept at 1.39 μg NH_4/g Nostoc/hr and the x-intercept at 1.05 μg/mℓ. In Fig. 7, it appears that the x-intercept (the initial ammonia concentration where no ammonia excretion exists) may be somewhat to the left of this value, but the y-intercept (the theoretical maximum ammonia excretion at zero initial ammonia concentration) seems to be a good approximation.

Percent nitrogen. Table 5 presents results of analysis for the nitrogen content in Nostoc muscorum in various stages of hydration. Since the raw data are expressed as a percentage, they were transformed using the arcsine transformation and then subjected to a one-way analysis of variance (Sokal and Rohlf 1969). The means were found to differ significantly to a level of $\alpha = 0.001$ which represents the area to the right of the critical value of the F distribution. The data were then subjected to a two-way analysis of variance without replication (Sokal and Rohlf 1969) to determine if the means differed significantly between locations or condition of algae. This was found to be not significant in both cases.

A possible explanation for the random variation among means is that certain samples contain differing amounts of extraneous materials. Although every effort was made to remove the materials, the small size of the sample (0.1 g) would allow any small amount of debris to enter into the calculations.

Table 5. Percent nitrogen in *Nostoc muscorum* plant tissue from various sites, sampled while in various stages of hydration. The data represented are means of the actual data points, where the number of samples from which they were derived is given in parentheses.

	Dededo Ponding Basin	Harmon Transect 21	Harmon Transect 8	Nimitz Hill
Moist Bloom	3.15%(n=2)	3.05%(n=3)	2.67%(n=3)	4.14%(n=6)
Dry Top Crust	2.74%(n=3)	3.46%(n=3)	3.10%(n=6)	3.40%(n=3)
Completely Desiccated	3.55%(n=3)	3.96%n=3)	3.40%(n=4)	3.34%(n=4)

The means were determined by multiplying each mean by its respective value of n, then dividing the resulting sum by the sum of n to obtain a value of 3.38 percent nitrogen or 33.8 g per kg dry weight of Nostoc muscorum. In terms of potential nitrate contribution, this is equal to 150 g NO_3 per kg N. muscorum if the ratio of the molecular weights of NO_3:N is taken to be 4.43.

Algal growth. Table 6 presents the results of the Nostoc muscorum growth experiments, which are graphed in Fig. 8. It can be seen readily in Fig. 8 that biomass increase per week is a function of initial biomass. The percent increase per week for each of 18 samples is listed in Table 6. The average of the means is 42 percent with a standard error of the mean of 0.306, setting the 95 percent confidence limits between 27 percent and 57 percent for weekly algal biomass increase (Sokal and Rohlf 1969).

Since frequent observations of field areas indicate that the yearly increase in the biomass of N. muscorum is not noticeable, it is assumed that approximately 40 percent of the algal biomass per week undergoes degradation during the periods of algal bloom.

Nitrate-Nitrogen Percolation Through Soil

The characteristics of the two experimental Guam clays used in this phase of the study are presented in Table 7. Table 8 lists the values obtained for NO_3-N in μg/mℓ for those soil flats containing Nostoc muscorum. The mean values for each soil type minus their respective controls are presented in Fig. 9 with the mean daily rainfall for each sampling period (Table 9). As expected, the NO_3-N

Table 6. Weekly wet weights (g) of Nostoc muscorum growth samples and their respective mean percent weekly increase in parenthesis.

	Sample Number																	
Date	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
11/21/78	.164			.201		.286	.266			.250			.233		.222		.289	
11/28/78	.254 (55)			.435 (116)		.451 (58)	.391 (73)			.309 (24)			.335 (44)		.283 (28)		.542 (88)	
12/5/78	.260 (2)			.462 (6)		.537 (19)							.403 (20)				.589 (9)	
12/12/78	.375 (44)					.581 (8)							.483 (20)				.627 (6)	
12/19/78		.237			.340			.574			.367					.520		.389
12/26/78		.429 (81)						1.43 (149)			.715 (100)							.461 (18)
1/2/79		.561 (31)			.663 (48)			1.78 (24)			.830 (13)					.992 (46)		.925 (101)
1/9/79		.661 (18)			.973 (47)			1.89 (6)			.948 (14)			.115		1.44 (45)		1.02 (10)
1/16/79			1.65		1.09 (10)				1.58			1.12		.134 (16)		1.76 (22)		1.07 (5)
1/23/79			3.27 (98)		1.81 (71)				2.24 (42)			2.70 (141)		.139 (4)		2.36 (34)		
Y % Weekly Increase	34	43	98	61	45	28	73	60	42	24	42	140	28	10	28	38	34	34

% Weekly Increase for 18 Samples $= \frac{Y_{(n)1}}{n1} = 40\%$