

Figure 3. Growth rates for Nostoc muscorum.

Table 7. Characteristics of the two experimental Guam clays used for soil percolate studies. The data were provided by Ms. Baccay, chemist, Soil Testing Laboratory, College of Agriculture and Life Sciences, University of Guam.

Parameter	Barrigada Type Guam Clay	San Agustin Type Guam Clay
Drainage	well-drained	moderately well-drained
pH	6.4	7.3
Organic Matter (% total)	1.39	5.07
Physical Composition (% total)		
Sand	36.7	22.0
Silt	14.6	30.0
Clay	48.7	48.0

Table 8. Nitrate-nitrogen ($\mu\text{g}/\text{ml}$) in rainwater percolates from soil flats containing Nostoc muscorum.

Sampling Date	12/29	1/1	3/16	6/30	7/20	9/1	10/6	11/18	12/28
No. 6	5.85	5.61	9.17	8.78	11.48	10.99	15.42	8.92	15.41
8	11.86	6.51	9.69	1.40	-0.29	-5.74	-0.15	-0.15	1.63
12	0.19	-1.18	11.38	4.48	4.89	9.31	8.55	6.74	16.52
16	-7.50	-0.54	0.10	5.93	0.23	1.40	8.16	4.13	10.04
\bar{Y} (s_y)	10.87(4.12)	7.63(2.01)	12.28(2.54)	9.04(1.54)	8.62(2.71)	17.50(3.86)	13.06(3.18)	16.03(1.58)	19.25(3.39)
\bar{Y} control ($s_{\bar{Y}}$)	8.27(3.32)	5.03(1.11)	4.70(1.93)	3.92(0.97)	4.57(1.06)	13.51(5.27)	5.06(2.40)	10.08(3.34)	8.18(1.93)
\bar{Y} \bar{Y} control	2.60	2.60	7.58	0.13	4.05	3.99	8.00	5.95	10.87
No. 2	6.31	-51.19	47.06	-0.55	5.67	15.36	7.96	5.15	20.38
6	-19.36	41.46	44.08	-2.11	5.15	17.69	-1.94	9.65	23.25
10	94.92	48.13	15.30	-3.02	4.24	-0.46	4.28	2.10	5.88
14	-6.38	19.91	2.20	-0.62	9.82	13.15	6.62	11.82	15.16
\bar{Y} (s_y)	54.76(25.88)	102.98(22.78)	82.19(11.83)	17.70(0.63)	14.29(0.30)	25.51(4.07)	10.05(4.22)	9.97(2.03)	33.00(1.53)
\bar{Y} control ($s_{\bar{Y}}$)	35.88(29.47)	88.45(15.28)	56.13(13.98)	19.22(2.33)	9.24(1.22)	14.07(2.98)	5.83(1.65)	5.15(1.62)	17.33(4.68)
\bar{Y} \bar{Y} control	18.88	14.53	26.06	-1.52	5.05	11.44	4.22	4.82	15.67

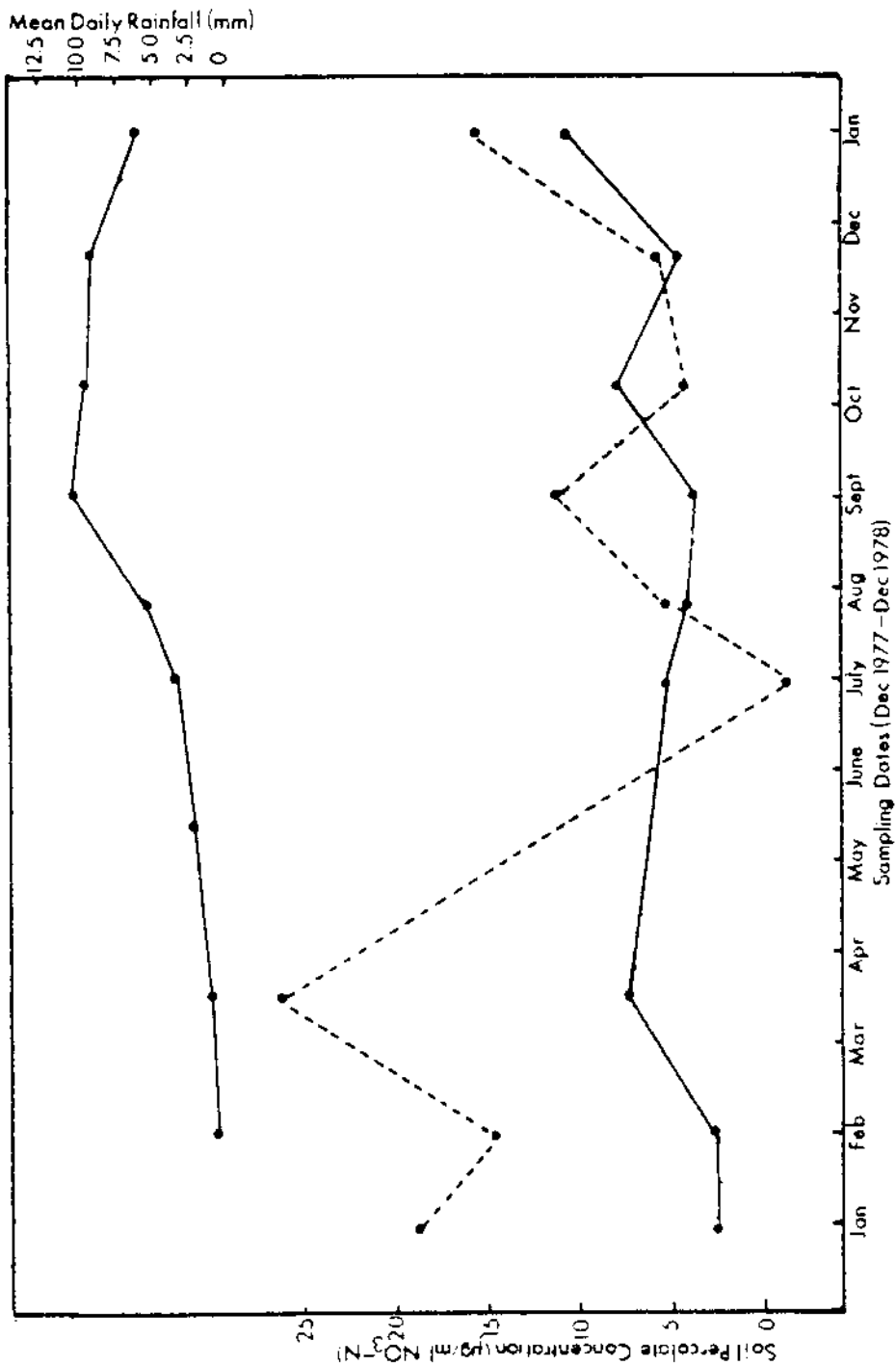


Figure 9. Mean nitrate-nitrogen percolate concentrations in excess of controls for Barrigada (-----) and San Agustin (-----) Guam clays and the corresponding mean daily rainfall.

Table 9. Compiled rainfall data for each sampling period.*

Dates	Total Rainfall (mm)	Mean Daily Rainfall (mm)	Days With No Rainfall (%)	Days Exceeding 13 mm Rainfall (%)**
12/29/77				
2/1/78	37.6	1.02	51	0
2/2/78				
3/14/78	54.6	1.27	60	2
3/15/78				
6/30/78	378	3.56	34	8
7/1/78				
7/20/78	106	5.33	40	10
7/21/78				
9/1/78	441	10.4	17	26
9/2/78				
10/6/78	323	9.40	6	26
10/7/78				
11/18/78	380	9.14	12	26
11/19/78				
12/28/78	238	6.10	18	13

*Data from the first three sampling periods were obtained from Fleet Weather Central (NAS rain gauge). Subsequent data were the result of daily monitoring of a rain gauge situated at the study site.

**Amount of rainfall required to saturate flats.

in the percolate from the San Agustin clay was initially higher than for the Barrigada clay because of the relatively high organic content of the San Agustin clay; but these values dropped to levels comparable to the Barrigada clay after an increase in mean daily rainfall, presumably due to leaching of nutrients by excess rainwater.

In Fig. 10, the absolute values for $\text{NO}_3\text{-N}$ levels in the percolate are obtained, indicative of the high organic content of the San Agustin clay. If mean control values are subtracted, obtaining net $\text{NO}_3\text{-N}$ production values, then the mean of the two soils becomes $5.50 \mu\text{g}/\text{m}\ell$ $\text{NO}_3\text{-N}$ and $6.61 \mu\text{g}/\text{m}\ell$ $\text{NO}_3\text{-N}$ for Barrigada and San Agustin clays, respectively. Any discrepancy in these values is probably due to a greater degradation of Nostoc muscorum on the San Agustin clay discussed later.

During the first six months (three sampling periods of the experiment), little rain fell. Samples were obtained by artificial flooding. Therefore, values from these flats most likely reflect residual $\text{NO}_3\text{-N}$ in the soils as the alga was almost continuously in a desiccated state (Table 9). Also, it can be noted in Table 8 that the standard error is large for these points indicating that several outside factors were operating. For this reason, the analysis of experimental results will be confined to the months of July through December when sufficient rain fell for the Nostoc muscorum to remain in a hydrated state for a large percentage of the time (Table 9). Also, standard errors drop sharply at the time of this sampling (Table 8).

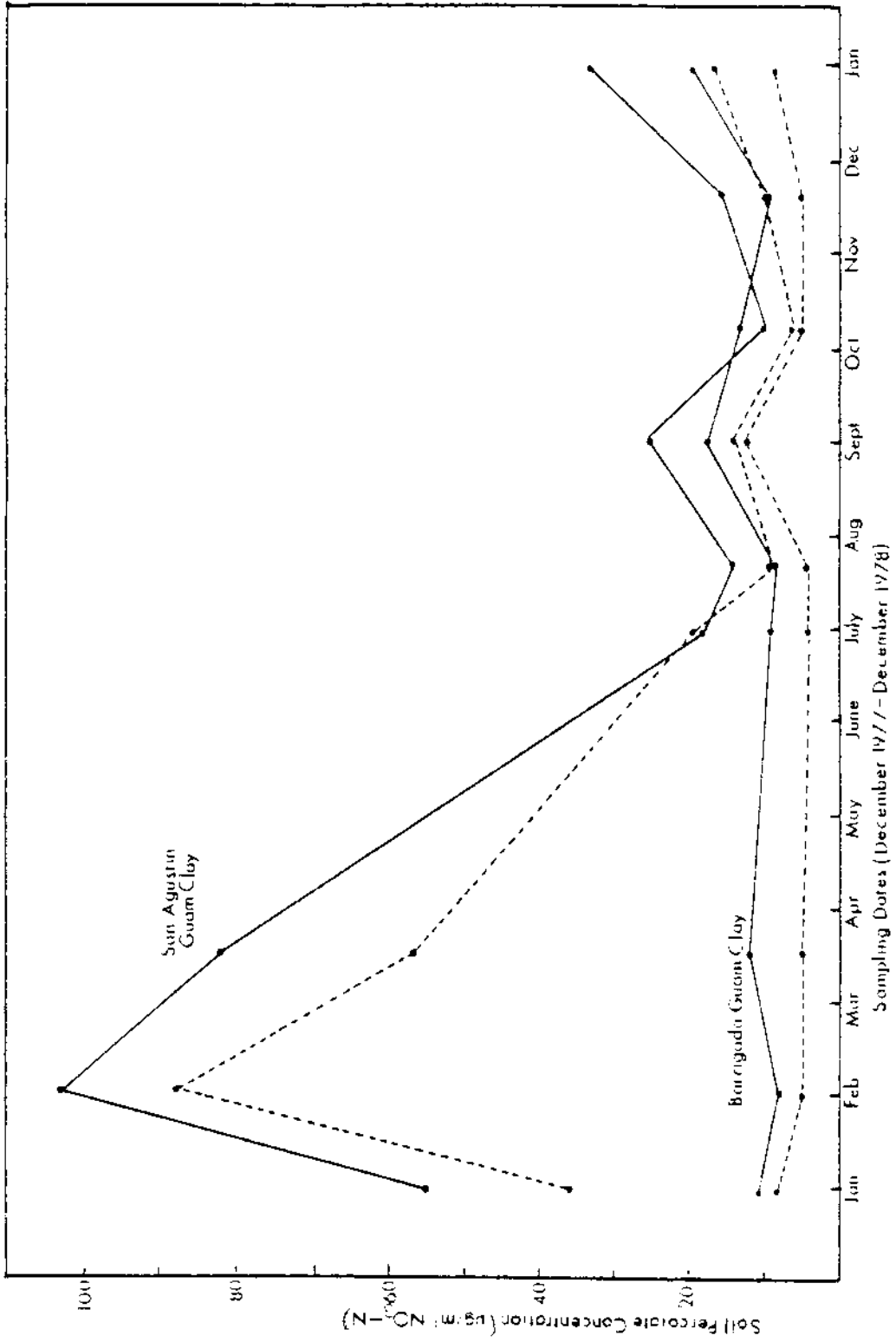


Figure 10. Mean nitrate-nitrogen percolate concentrations ($\mu\text{g/ml}$) for Barrigada and San Agustín Guam clays (----) and their respective controls (---).

It can be seen from Fig. 10, in all cases but one (San Agustin clay in July), that the control flat percolates are lower than the percolates from those flats containing Nostoc; the means of the ratios (Nostoc: control) for July through December are 2.06:1 for San Agustin soil and 2.05:1 for the Barrigada soil. Also evident is the fact that values from the San Agustin clay percolates are more erratic than those from the Barrigada clay. Since Nostoc is not normally seen growing on San Agustin clay it is possible that a stable microbe-Nostoc ecology is prevented, as evidenced by the fact that significant algal decay occurred throughout the experiment on that soil, while the algae on the Barrigada clay appeared healthy throughout the experiment (Fig. 11).

Figs. 12 and 13 show the $\text{NO}_3\text{-N}$ content of the percolate from the separate flats; Fig. 11 illustrates the condition of the flats on November 18, 1978, toward the end of the experiment. The fact that significant decay occurred on the San Agustin clay and not on the Barrigada clay could account, in part, for the fact that $\text{NO}_3\text{-N}$ levels in the soil percolates from San Agustin clay flats are generally higher than in their Barrigada counterparts.

Since the ammonia excretion data show that, at most, only a small amount of fixed nitrogen can be contributed by metabolizing Nostoc muscorum, the decaying alga on the San Agustin clay would contribute more fixed nitrogen than would the healthy alga on the Barrigada soil. This is supported by the data in Table 10 which show that the San Agustin clay flats, where decay was greatest, produce high concentrations of nitrate in their respective soil percolates.



Figure 11. Representative soil flats showing the condition of Nostoc muscorum on San Agustin Guam clay (A) and on Barrigada Guam clay (B).

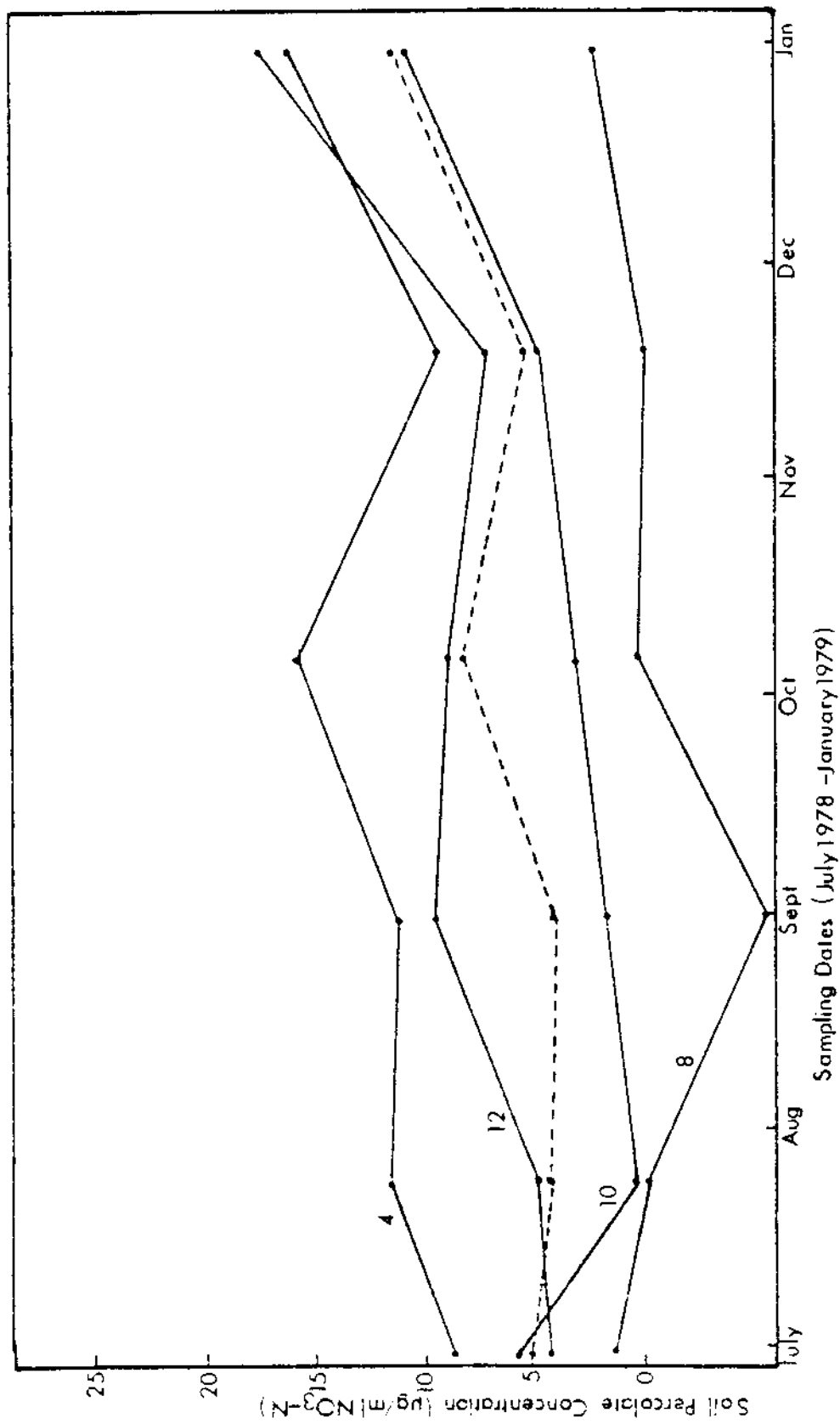


Figure 12. Nitrate-nitrogen percolate concentrations in excess of controls for individual flats containing Barrigada Guam clay. The mean of the four values is indicated by the dashed line.

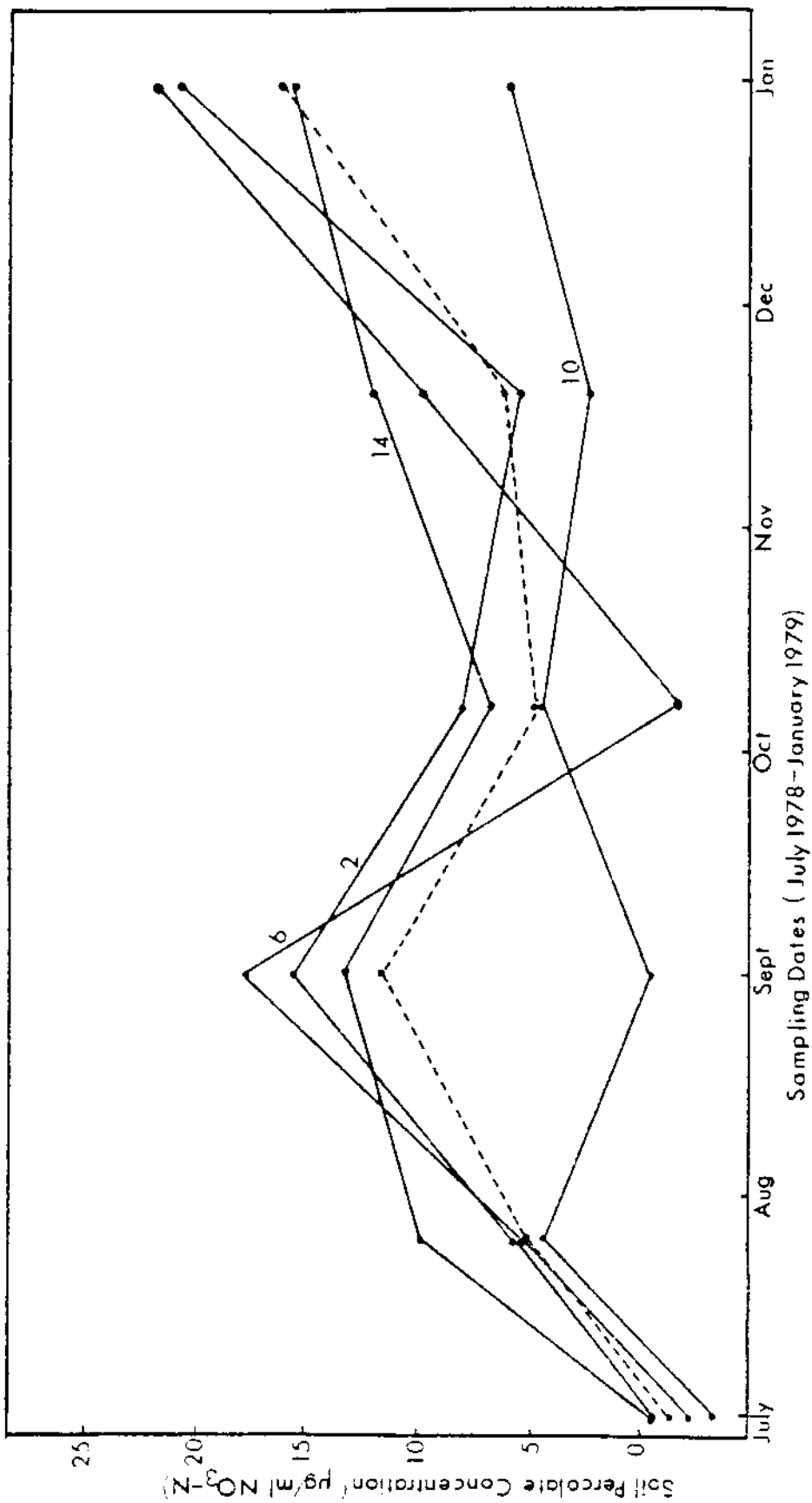


Figure 13. Nitrate-nitrogen percolate concentrations in excess of controls for individual flats containing San Agustin Guam clay. The mean of the four values is indicated by the dashed line.

Table 10. Mean drainage, biomass and combined nitrogen data for the individual soil flats.

Barrigada Type Clay	Drainage (μ /min)	Final Biomass (g dry wt.)	NO ₃ -N/Sampling (μ g/ml)	NO ₃ -N kg	NO ₂ -N kg	NH ₄ -N kg
#4	0.84	308	11.8	38	2.1	0.81
#8	1.2	214	-0.54	-2.0	1.7	0.23
#12	0.096	430	8.4	20	0.32	0.070
#16	0.34	302	5.0	16	1.5	0.30
San Agustín Type Clay						
#2	0.044	270	9.0	33	0.56	0.71
#6	0.18	47	8.3	180	0.94	0.45
#10	0.28	205	2.2	11	2.7	0.66
#14	0.78	80	9.4	120	0	0

Referring to the $\text{NO}_3\text{-N}$ levels in percolates from San Agustin clay flats (Fig. 13), the trends of the individual flats are erratic in comparison to the $\text{NO}_3\text{-N}$ concentration profiles on the Barrigada soils (Fig. 12). Again, it is possible that the conditions are not optimal for an algae-Nitrobacter system and that the upward trend at the end of the experiment is due to algal decay.

Since Nostoc muscorum was most often observed growing on either concrete, asphalt or hard packed Guam clay, of the Barrigada type, it is the results of Fig. 12 that are probably most significant as far as nitrate contribution to the groundwater lens is concerned. Three of the four flats had $\text{NO}_3\text{-N}$ percolate levels higher than the control levels. Flat 8 was consistently below control levels except for an apparent "attempt" at recovery during the last sampling period. Referring to Table 10, this can perhaps be explained by the drainage rate on this particular flat. Post-flooding drainage from this flat was 1.2 ℓ/min , the fastest for any of the flats. Since water was not retained during any length of time, it could be that the $\text{NH}_4^+ \rightarrow \text{NO}_2 \rightarrow \text{NO}_3$ conversion could not take place; or that simply an artifact of sampling was introduced, i.e., that $\text{NO}_3\text{-N}$ in the soil was immediately washed from the soil and subsequently diluted.

Another factor evident in Table 10 is that besides having the highest drainage rate, flat 8 has the lowest algal biomass for its soil type. An attempt to correlate drainage rate with biomass was undertaken. If both soil types are taken together, there appears to be no correlation ($r_{12} = -0.005$) where r_{12} is the product-moment correlation coefficient of Sokal and Rohlf (1969). If the two soil

types are analyzed separately, the data points fall into definite groups. Nostoc muscorum on the Barrigada clay maintained or increased its biomass in three out of four cases; the correlation between drainage and final dry weight was $r_{12} = -0.90$. This value for the product-moment correlation coefficient must be viewed with caution, since the small sample size dictates that $\alpha = 0.1$ for the t distribution (Rohlf and Sokal 1969).

Still, it does seem that a correlation between drainage rate and final biomass does exist at least on the Barrigada soil. That is, where drainage is slow resulting in standing water, N. muscorum is increased.

Contribution of Nitrate to the Groundwater by Nostoc muscorum

From the data obtained, two estimates for nitrate contribution by all Nostoc muscorum affecting the lens were made. The first estimate was derived from the potential total nitrogen contribution based on the ammonia excretion data (in $\mu\text{g NH}_4/\text{g}/\text{yr}$) which was converted to $\text{g NO}_3\text{-N}/\text{yr}$ assuming total conversion of $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$ and considering the total biomass of Nostoc muscorum to be 2.6×10^6 kg. Nitrogen released by decaying algae was calculated in $\text{g NO}_3\text{-N}/\text{yr}$ considering that 3.38 percent of the algae was nitrogen (again assuming total conversion) and that there was a steady-state growth and decay rate of 40 percent of the biomass per week. These two values were combined and divided by the amount of yearly rainfall (mm) calculated from Mink's (1976) mean yearly rainfall for the northern

plateau (235 cm) and the area over the lens ($2.6 \times 10^8 \text{ m}^2$). The final value is expressed as $\mu\text{g}/\text{m}^2$.

Soil percolate data were handled in the following manner: positive $\text{NO}_3\text{-N}$ values (flat nos. 4, 12, 16) were expressed in terms of $(\mu\text{g}/\text{m}^2)/(\text{kg}/\text{m}^2)$ and the mean divided by the total density of Nostoc muscorum over the lens (kg/m^2), obtaining the value in $\mu\text{g}/\text{m}^2$. Similar manipulations were performed for the Dededo Ponding Basin. The estimated nitrate contribution of Nostoc muscorum to the groundwater is $2.6 \times 10^{-2} \mu\text{g NO}_3\text{-N}/\text{m}^2$ by ammonia excretion and $1.5 \mu\text{g NO}_3\text{-N}/\text{m}^2$ through algal decay for a combined estimate of $1.5 \mu\text{g NO}_3\text{-N}/\text{m}^2$ if the parameters of total potential nitrogen are considered. Since the contribution by ammonia excretion is two orders of magnitude lower than the decay value, it can be considered to be negligible.

If the soil percolate data are extrapolated to account for the entire area of northern Guam, a value of $6.5 \times 10^{-4} \text{ g NO}_3\text{-N}/\text{m}^2$ is obtained. The significance of these results and possible reasons for the large discrepancy between the methods is discussed in the next section. The same calculations in relation to the Dededo ponding basin yield $2.1 \mu\text{g NO}_3\text{-N}/\text{m}^2$ and $8.7 \times 10^{-4} \mu\text{g NO}_3\text{-N}/\text{m}^2$ for total potential nitrogen and soil percolate derived estimates, respectively.

DISCUSSION

The results indicate that the total estimated Nostoc muscorum biomass over Guam's lens could potentially account for 1.5 $\mu\text{g NO}_3\text{-N/m}^2$ if all algal nitrogen were converted to NO_3 and found its way into the lens. Extrapolating the soil percolate data, a more conservative figure of 6.5×10^{-4} $\mu\text{g NO}_3\text{-N/m}^2$ is derived. These figures are well below the $\text{NO}_3\text{-N}$ concentrations of groundwater from the various wells compiled by the Guam Environmental Protection Agency (Table 11).

The large discrepancies between potential NO_3 contribution and extrapolated soil percolate data are not surprising. Potential NO_3 contribution estimates should be considerably higher than those extrapolated from percolate data since in the former, uptake of combined nitrogen by soil bacteria and plants is not considered. The potential NO_3 estimate may also be inherently high because the 40 percent weekly algal growth rate is based on the assumption that the alga is hydrated constantly six months of the year.

Even though N. muscorum appears not to be a major contributor of NO_3 to the lens, it could possibly be a factor in some areas where growth is particularly high. The ecology of this particular alga is such that, in general, it is either found in abundance or not at all. Since this study was concerned only with estimates of algae actually affecting the lens, no quantitative ecological studies were attempted; although, in the course of running transects, numerous observations were made.

Table 11. Mean nitrate-nitrogen (NO₃-N) levels (in µg/ml) of the various wells on Guam compiled from Guam Environmental Protection Agency monitoring.

Date	Tumon-Maui Tunnel	Marbo Well (Air Force)	Mangilao	Marbo (Gov-Guam)	Finegayan	Dededo	Agafa Gumas	Yigo
3/14/78						2.32	1.85	2.77
3/15/78				1.59	1.40			
3/16/78			2.64					
3/30/78	2.07	1.95						
4/27/78	2.46	1.96						
6/27/78				1.83	1.56			
9/12/78			3.84					
9/25/78						3.50	2.77	3.83
9/28/78	3.06	3.48						
11/2/78	2.97	3.15						
12/26/78	2.88	2.94				3.33	2.64	
1/25/79	2.91	2.76						

The type of clay surface on which N. muscorum is normally found corresponds closely to the Barrigada Guam clay soil type on which the alga appeared healthy and did not undergo the noticeable decay as seen on the San Agustin variety. It is evident that the only differences in soil characteristics are in soil pH, organic content, and physical soil composition. The higher organic content of San Agustin clay can account for the higher moisture retention (Brady 1974) which in conjunction with physical composition would affect drainage rates. While drainage on Barrigada type clay was negatively correlated with algal biomass, no such correlation exists for San Agustin clay. This is probably a result of the fact that more moisture is retained in contact with the alga for extended periods of time, contributing to its decay. Organics in the soil also yield ammonia and nitrate upon decomposition (Brady 1974). This may give plants that require nitrogen a competitive edge over N. muscorum that is not present on the organic-deficient Barrigada clay types.

Drainage seems to be a prime ecological factor in relation to the growth of N. muscorum. The alga requires frequent drenching to maintain its hydrated state, yet if allowed to stand for several days in puddles, it undergoes rapid decay. This characteristic is especially evident in the ponding basins where no N. muscorum is seen in the main ponding area or on the steep sloping sides, but rather in areas that receive run-off which does not accumulate for extended periods of time.

The drainage data from the experimental flats indicate that a negative correlation between drainage and biomass exists where slower

draining flats had a higher final algal biomass. In all cases, drainage rates were such that each flat was completely drained within a 24-hr period. This correlation probably breaks down when drainage rates are low enough that significant algal decay occurs.

The fact that N. muscorum is hardly ever seen along well-traveled roads is something of a curiosity. It may be that stress due to the likelihood of automobiles pulling off the roads onto the shoulder is a factor, or possibly, carbon monoxide, a competitive inhibitor of nitrogenase (Fogg 1974), is indirectly retarding growth by preventing nitrogen fixation.

Nostoc muscorum also seems to have trouble competing with other vegetation for space and sunlight. Off roadways and in ponding basins, there is a noticeable decrease in algal density as other types of ground cover become dominant. Basically, N. muscorum is mostly to be found in areas where surface run-off collects but not for more than a couple of days, and where competition from other forms of vegetation is limited. This condition exists in several of the ponding basins, Dededo in particular. If the same operations are performed on the data as for total lens area with respect to algal biomass and surface area of Dededo ponding basin, the estimates show 2.1 $\mu\text{g}/\text{m}^2$ and 8.7×10^{-4} $\mu\text{g}/\text{m}^2$ for potential and extrapolated $\text{NO}_3\text{-N}$ contribution, respectively. These values are higher than those which take the entire area of northern Guam into account, yet still too low to be considered significant. Still, if N. muscorum were of sufficient density in a localized area, its nitrate contribution might be a significant factor for that particular area.

In any case, contribution of combined nitrogen by Nostoc muscorum appears to be almost entirely through algal decay. The regression equation (see Fig. 7) estimates a maximum possible ammonia excretion rate of $1.4 \mu\text{g NH}_4/\text{g Nostoc}/\text{hr}$ if the surrounding media contains no combined nitrogen. Even under such hypothetical conditions, nitrate contribution through ammonia excretion would be two orders of magnitude lower than that which would be contributed through algal decay. Since ammonia release is most likely determined by an equilibrium between the cell and its surrounding media (Fogg 1971), nitrate concentrations resulting from excretion are most likely negligible.

Nostoc muscorum can take up ammonia from the media if provided in adequate concentrations. The findings here support Ohmori and Hattori (1974), who, in studying nitrogen-fixation in Anabaena cylindrica Lemm., found that a concentration of $1 \times 10^{-3}\text{M}$ ammonia ($18 \mu\text{g}/\text{m}^3$) was sufficient to completely inhibit nitrogenase activity.

Since excretion is inhibited at about $1 \mu\text{g}/\text{ml}$, it may be that N. muscorum nitrogenase is more sensitive to ammonia inhibition than A. cylindrica nitrogenase. More likely, nitrogenase is only partially inhibited by $1 \mu\text{g}/\text{ml}$ ammonia in the media since these experiments measure excess ammonia excreted. This partial inhibition is most likely enough to slow down nitrogen fixing activity to where an excess of ammonia is not being produced.

It is interesting that Stewart et al. (1967), using the acetylene reduction technique, found that a species of Nostoc was capable of

reducing $0.51 \text{ M}\mu \text{ moles N}_2/\text{mg protein min.}$ Converting units, this works out to be $7 \times 10^4 \text{ }\mu\text{g NH}_4/\text{g Nostoc/hr.}$ Since the results show that a maximum of $1.4 \text{ }\mu\text{g NH}_4/\text{g Nostoc/hr}$ is excreted, it can be seen that only a very small fraction of nitrogen that is fixed by Nostoc muscorum is excreted. Thus, the major nitrogen source will not be a direct result of nitrogen excretion, but rather, the degradation of complexes (primarily protein) that contain nitrogen. Even so, algal biomass of N. muscorum is not of sufficient density to contribute a significant amount of fixed nitrogen to the groundwater system.

CONCLUSION

Nostoc muscorum is not a major contributor to the high nitrate content of Guam's groundwater. The alga, however, may contribute small amounts of nitrate in areas where it is particularly abundant.

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ACKNOWLEDGMENTS

The author would like to express her appreciation to the members of her Masters thesis committee, Drs. R. T. Tsuda, J. A. Marsh, Jr., J. L. Demeterio and S. J. Winter, for their help on various phases of this project. The help of Dr. Charles E. Birkeland with the statistical analysis of the data and Dr. Stephen G. Nelson with the ammonia probe analysis is also appreciated. Thanks, too, go to the marine technicians John R. Eads and Frank Cushing, Jr. and fellow graduate students Russell N. Clayshulte and William J. Zolan for their aid as "body guards" on various occasions while working in the "wilds" of the Harmon field area. Thanks, also, are in order for the valuable assistance provided by Marylou Baccay in soils and soil percolate analysis, and to U. S. Fleet Weather Central, Guam, for making their rainfall data records available to us. Finally, the author is especially grateful to Mrs. Evelyn Paulino for her patience and skill in the preparation of the final manuscript.

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