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THE RAIN FOREST PROJECT
ANNUAL REPORT

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Table of Contents

DIVISION OF RADIOECOLOGY
TERRESTRIAL ECOLOGY PROGRAM
THE RAIN FOREST PROJECT

INTRODUCTION

The Rain Forest Project is an ecological study of a tropical rain forest located at an elevation of 1500 feet on the side of El Yunque mountain in eastern Puerto Rico. The study has three objectives: 1) to determine the effects of gamma radiation on the tropical ecosystem; 2) to study the cycling of stable and radioactive isotopes through the ecosystem; 3) to investigate basic biological functions of the ecosystem in order to better understand phenomena related to the first two objectives.

The gamma irradiation study has been completed, and results will be published in a volume edited by H.T. Odum. Studies of secondary succession in the forest opened up by radiation are continuing. Changes during the first three years of succession are reported here.

This report is in three major sections. The first section, by Dr. Carl F. Jordan, concerns the stable and radioactive isotope cycles, and a portion of the secondary successional work. The second section, by Dr. George E. Drewry, deals with diversity of the successional forest, and animal ecology studies. The third section consists of reports by visiting scientists, and a manuscript in press.

SECTION I

by
Carl F. Jordan

This section deals with stable element cycling, tritium movement in the tropical rain forest, and secondary succession following irradiation.

The studies on stable element content of the tropical ecosystem, started by Kline in 1966, and the stable element flux in the forest begun by Jordan in 1967, were completed during the past year. The results are brought together in section one so that they are amenable to a systems analysis. Because of the extreme complexity of the ecosystem, a systems analysis is necessary to predict such things as, given a certain amount of fallout: how long will it take for the radioactivity to reach equilibrium in the system?; What will be the levels of radioactivity in each compartment at that time? How long after input will radioactivity be at a maximum in compartments such as leaves and fruits, which are bases of food chains?

Also in section one are some of the results of a series of tritium tracer studies, carried out in conjunction with Dr. Jerry R. Kline of Argonne National Laboratory, and Dr. John Koranda and Mr. John Martin of Lawrence Radiation Laboratory. These studies are of interest, not only because tritium is a tracer for water, but also because tritium will be a principal product of any thermonuclear reaction used to excavate a new canal through Central America.

The secondary successional study was initiated in the summer of 1966, one year after radiation of the forest ceased. Results through 1968 concerning biomass, gross and net photosynthesis, respiration, and efficiency are presented in this section. Results concerning species diversity and information are presented in section two.

PART A - ISOTOPE CYCLES

The movement of radioactive and stable elements through an ecosystem often is termed "mineral cycling" or "biogeochemical cycling". Both these terms are misleading. "Mineral cycling" is misleading because to earth scientists, minerals are substances composed of two or more elements, usually having a definite atomic arrangement. These minerals do not cycle through plants and animals. The term "biogeochemical cycling" also is misleading, because it implies cycling over millions of years whereby an element is deposited on the ocean bottom, becomes sedimentary rock, there is land uplift, erosion, and then the element is again available for cycling through biological systems. The studies at the tropical rain forest at El Verde do not involve this sedimentation, but are concerned only with the movement of biologically available material.

The studies involve transfer and storage of stable chemical elements, as well as the radioactive isotopes of some of these elements. Since stable chemical elements are isotopes, and radioactive elements are radioisotopes, the studies are most accurately called isotope cycling studies.

During the past several years, tracer studies and chemical analyses have been done on many of the compartments and transfer routes shown in Fig. 1 for the tropical rain forest at El Verde. Within the next year, a mathematical model of Fig. 1 will be programmed for a computer, so that with a given input of fallout of stable or radioactive isotopes, concentration in any compartment at any time after the input can be computed.

Studies,relevant to the model, that were completed during the last year by the Terrestrial Ecology Program follow.

Transfer of Stable Isotopes by Water

Water is a principal means of isotope transfer in the ecosystem, as shown in Fig. 1. Concentration of stable isotopes was measured in the water fluxes given in Table 1, and multiplied times the volume of these water fluxes to give total weight of elements moved.

Rainfall was collected in plastic barrels on the top of a tower 12 feet above the top of the canopy. Throughfall was collected in similar barrels placed on the forest floor. Collars around trees to collect stem flow were made with polyvinyl tubing and sealed to the trees. The tubes led to collection barrels. Water moving out of the litter and through the soil was collected with "Tension-free" lysimeters (C.F. Jordan, Soil Science 105: 81-86). Runoff water from the Sonadora River between storms was taken directly from the river. Runoff water from the river during storms was collected in plastic bottles placed

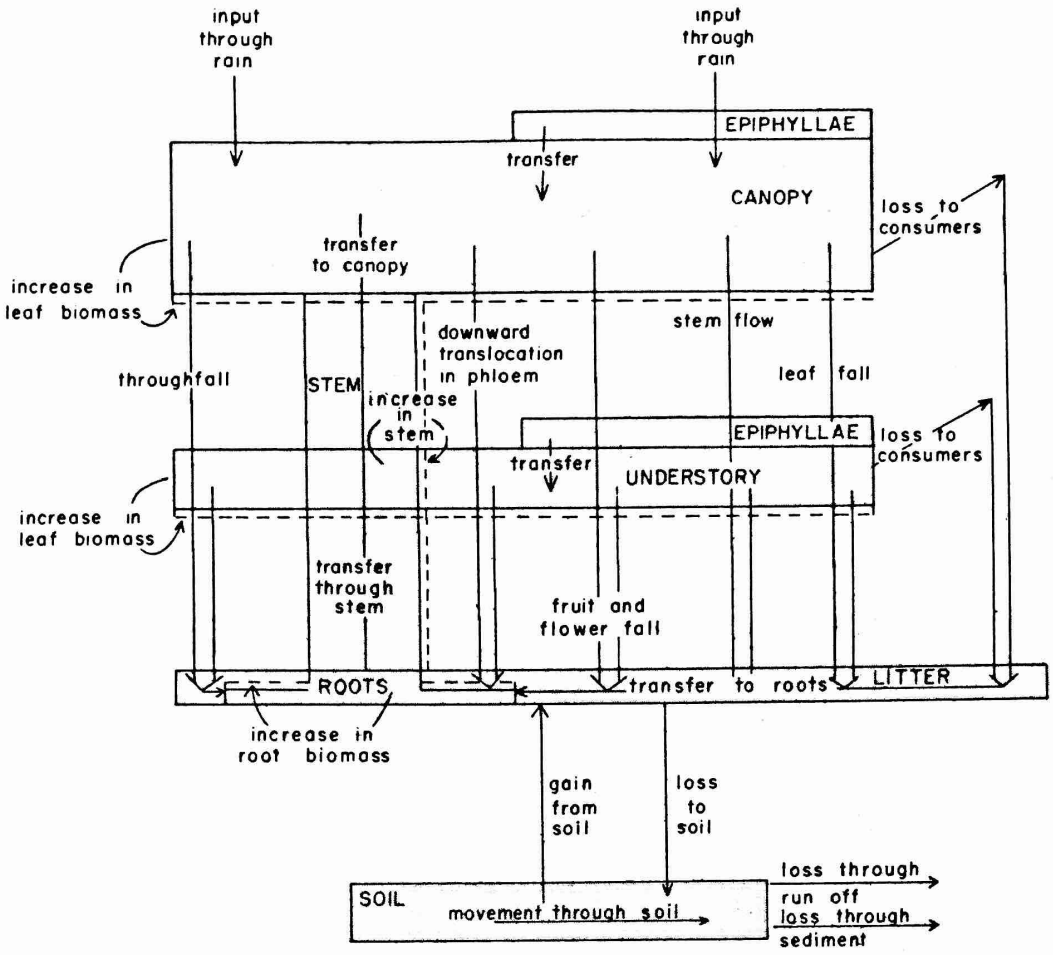

Fig. 1. Block diagram showing major storage compartments and transfer routes in the tropical rain forest.

Table 1. List of water collectors used for studying rate of element movement between compartments.

Flux	Number of collectors
Rainfall	2
Throughfall	10
Stemflow	27
Out of litter	18
Through 5 inch soil depth	13
Through 10 inch soil depth	6
Runoff, during storms	2
Runoff, between storms	4

on the bank at a level of about one foot higher then the normal river level. When the river rose, the bottles filled, and when the river receeded, the water could be collected.

Collections were made once a week. Weekly water samples from each separate collector were pooled proportionately to the amount of water collected. For example, 1/500 of the weekly volume of throughfall collector number three gave a reasonable sized sample for analysis. Therefore, every week, 1/500 of the total amount of water collected in throughfall collector no. three was poured into a plastic bottle labeled "throughfall collector no. three". At the end of the month, the pooled samples were analyzed for conductivity with a conductivity meter; ca, k, mg, mn, fe, and cu by atomic absorption, and na by sodium electrode. Due to various problems, not all the elements could be analyzed every month.

All the concentrations of one group of samples (for example all the throughfall samples) were averaged each month, and the standard deviation was obtained. While it is desired to give the reader an indication of the variation in samples, a listing of averages and standard deviations consumes too much space. Therefore, for each group for each month, the standard deviation was taken as a percentage of the mean concentration. Then the 12 percentages for the year for ca, na, and mg were each averaged, and are shown in Table 2.

Rain shows a fairly high variation in the calcium samples. This was probably because the concentrations were near the lower limit of detection. For example, the same sample could give a concentration of 0.1 ppm the first reading and .2 ppm the second, resulting in an average concentration of .15 with a standard deviation of .071, 47 percent of the mean. Stem flow shows a very high variation between samples. Last year, Jordan (1968, The Rain Forest Project Annual Report) showed that larger trees generally have a higher concentration of isotopes, especially trees of the species Dacryodes excelsa. Variation in runoff is lowest, as might be expected, since samples are taken in virtually the same spot at the same time, while other samples are taken over a wider area.

Concentrations of isotopes in the various water fluxes can indicate certain things about the isotope cycles. Concentrations of ca, na, and mg were compared in water from the A horizon (5 in depth) and B horizon (10 in. depth), in river runoff during high and low water levels, and between the B horizon and river runoff. Average monthly concentrations are shown in Table 3 and 4. Utilizing analysis of variance (Table 5), no differences can be shown between the A and B horizons, the low and high levels, or between the B horizon and the high water level. However, the ranked sign test showed a difference at the 5 percent level between low water and high water for ca, and mg (Table 6). In this case, the signed rank test might be slightly more sensitive than anova, becasue while there are moderate month to month variations, the concentration in the low water is usually just slightly higher than the high water concentrations. Since sodium is a more mobile element than ca and mg, it is not surprising that it is not diluted by rising water, whereas ca and mg are.

Table 2 . Standard deviation as a percentage of mean concentration.

Samples	Percentage		
	Ca	Na	Mg
Rain	57	34	5
Throughfall	45	37	61
Stemflow	87	57	79
Out of litter	34	35	39
Through mineral soil	44	37	49
Run off	14	19	11

Table 3 . Concentration of elements in water collected from the A horizon (5 in. deep) and B horizon (10 in. deep) of soil in the rain forest near El Verde.

	Concentration in parts per million					
	Ca		Na		Mg	
	A hor.	B hor.	A hor.	B hor.	A hor.	B hor.
Oct., 1967	4.2	3.7	7.6	4.0	.74	.56
Nov., 1967	0.7	1.3	3.5	3.5	.37	.50
Dec., 1967	1.8	1.6	6.4	5.2	.51	.60
Jan., 1968	1.0	1.2	6.8	6.0	.56	.55
Feb., 1968	2.3	2.9	4.3	3.2	1.21	1.11
Mar., 1968	1.5	1.2	4.4	3.7	.82	.83
Apr., 1968	1.7	1.6	7.4	6.0	1.11	1.03
May, 1968	1.2	1.7	2.8	3.8	.72	.83
June, 1968	1.0	1.3	4.4	5.5	.24	.30
July, 1968	0.4	0.6	1.8	2.2	.06	ND
Aug., 1968	0.5	0.5	2.3	4.5	.22	ND
Sept., 1968	1.2	1.1	7.0	5.7	.85	.54

Table 4 . Concentration of elements in water collected from the Sonadora River during high water level (during storms) and during low water level (between storms).

	Concentration in parts per million					
	Ca		Na		Mg	
	low water	high water	low water	high water	low water	high water
Oct., 1967	2.0	1.5	7.0	5.0	0.5	0.2
Nov., 1967	1.2	1.0	3.0	3.0	0.5	0.5
Dec., 1967	2.5	1.7	4.7	6.3	0.8	0.5
Jan., 1968	1.5	1.2	4.5	4.0	0.7	0.5
Feb., 1968	2.9	3.1	2.7	2.2	1.6	1.4
Mar., 1968	2.5	2.3	2.8	3.1	1.4	1.0
Apr., 1968	3.2	2.6	4.5	3.4	1.6	1.1
May, 1968	1.4	1.9	2.2	1.5	0.9	0.7
June, 1968	2.1	1.4	2.2	1.8	0.4	0.3
July, 1968	1.0	0.9	1.5	2.1	1.2	1.2
Aug., 1968	1.0	1.0	5.6	6.3	0.6	0.4
Sept., 1968	2.0	1.5	4.5	4.2	0.9	0.7

Table 5

Results of analysis of variance to determine differences in element concentrations between soil water from the A horizon, from the B horizon, river water at low level, and river water at high level.

Test	Element	F ratio (1/22)	Level of significance	Remarks
A & B horizon	Ca	0.06	-	
" "	Na	0.41	-	
" "	Mg	0.22	-	
low & high water	Ca	0.84	-	
" "	Na	0.07	-	
" "	Mg	1.75	75 %	
B hor. - high water	Ca	0.12	-	
" " "	Na	2.14	75 %	
" " "	Mg	0.00	-	
B hor. - low water	Ca	1.29	-	
" " "	Na	1.31	-	
" " "	Mg	2.58	75 %	
A hor. - high water	Ca	0.36	-	
" " "	Na	2.97	90 %	A horizon is higher
" " "	Mg	0.38	-	
A hor. - low water	Ca	1.77	-	
" " "	Na	2.18	75 %	
" " "	Mg	3.73	90 %	A horizon is lower

Table 6

Results of signed rank test to determine differences in element concentrations between soil water from the A horizon, from the B horizon, river water at high level, and river water at low level.

Test	Element	Confidence level of difference
low water - high water	Ca	95 %
" " " "	Na	-
" " " "	Mg	95 %
A hor. - B hor.	Ca	-
" "	Na	-
" "	Mg	-
B hor. - high water	Ca	-
" low "	Ca	-
A hor. - high water	Ca	-
A hor. - low water	Ca	-

Although there is a lower concentration of dissolved ca and mg when the river is high, the river also carries suspended soil material when it is in flood stage. This soil material represents loss of ca and mg, but it is probably a loss as a result of erosion of the river-bed, and does not represent material being carried away from the vicinity of the roots.

The fact that there is no difference in concentration of elements in water moving through the soil at the 5 in. level, the 10 in. level, and river runoff indicates that all the isotopes which are recycled by plant roots are taken up by the roots before the isotopes reach a five inch depth. This evidence is in agreement with the hypothesis of Went and Stark (BioScience 18, 1035-1039) who feel that in the tropics, elements are transferred directly from litter to roots by mycorhiza.

Total amount of isotopes moved by rain, throughfall, stemflow, out of litter, through mineral soil (average of A and B horizons) and runoff (high water only, since that is when the bulk of runoff occurs), were calculated by multiplying isotope concentration in each flux times volume of the flux. Units are:

(Volume of flux) (concentration in ppm) Quantity of isotope moved

10^6 grams of water equivalent to 1 m^3

$$\frac{(m^3/ha/wk)\ \ (g/m^3)}{1000} = Kg/ha/wk$$

Total amount of isotopes moved is given on a weekly basis since collections were always made on the same day of the week (Mon.). Such weekly collections result in some months with four full weeks and some with five full weeks. A month with five Mondays but only 30 days would then have an error of about 14 percent, if there was a monthly base.

Although in reality the rain falls in discrete storms, it is more practical to calculate results on the basis of a steady continuous drizzle throughout the year. Then the total moved for each week is a rate function, and the total amount for each month can be calculated by multiplying rate times the number of weeks plus tenths of a week per month

Rainfall is measured above the canopy with a standard U.S. Weather Bureau recording rain guage. Throughfall is measured in 12 collectors on the forest floor, each measuring 5 ft. by 2 in. by 12 in. Jordan (1968, The Rain Forest Project Annual Report) estimated stem flow to be 18 percent of rainfall, and transpiration to be 105 m^3/ha/wk. Evaporation from soil surface averages 2.5 m^3/ha/wk (Odum and Jordan, A Tropical Rain Forest, in press). Water moving through litter equals throughfall plus stemflow minus evaporation from the surface. The same amount of water moves through

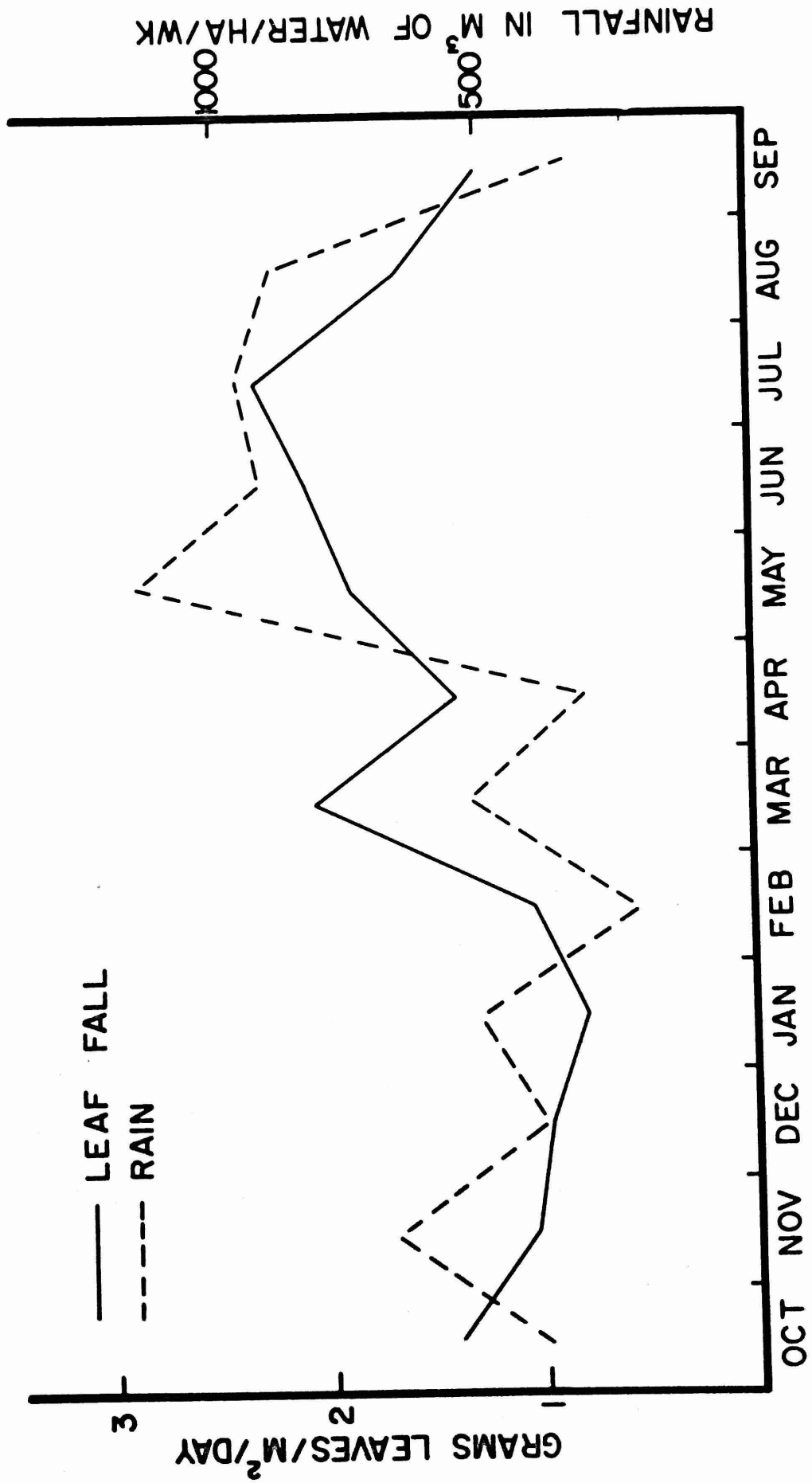

Fig. 2. Average rates of rainfall and leaf fall at the El Verde site.

the mineral soil as out of the litter. Most of the mineral soil lysimeters collect more water than the litter layer lysimeters except on ridge tops, where amounts are roughly equal. This phenomena is caused by the subsurface flow parallel to the sloping soil surfaces described by Jordan (1968, The Rain Forest Project Annual Report). Runoff reaching the river is equal to throughfall plus stem flow minus evaporation minus transpiration.

Centimeters of water flux is quickly converted to m^3 water/ha/wk by the relationship

$$(\text{cm water/wk})(100) = m^3/ha/wk$$

Fig. 2 shows m^3/ha/wk of rain on a monthly basis for the study period.

Kg/ha/wk of isotopes moved by the fluxes on a monthly basis are given in Tables 7-12 and Figures 3-6. Ca, mg, and na moving out of the litter follow the trend of rainfall; the more rain, the more loss from the litter (compare Figs. 3, 4, and 5 with 2). Input of these isotopes into the system via rain does not follow the rainfall pattern. Highest inputs occur around December and January and are probably more closely associated with the frontal passages that occur at that time of year than with total amount of rain.

Gains and losses of isotopes to the ecosystem are calculated by subtracting rate of loss by runoff from rate of input by rain (Table 13 and Figs. 7 and 8). Largest loss from the system occurred during the heavy rains of May, and gains of na and ca around December occurred as a result of the high inputs during that time. Total yearly difference between input and runoff is presumed to be made up by weathering of parent soil material.

Element Concentrations in Ecosystem Compartments

Leaves, wood from trunk, roots, soil, litter, and organic matter in the forest were sampled to determine stable element concentration in each compartment. Concentration, when multiplied times biomass of the organic components gives total amount of elements in each compartment. Biomass of the leaves, trunks, and roots will be calculated from the regressions in Odum (A Tropical Rain Forest, In Press). Biomass of the freshly fallen litter will be taken from the data of the 55 litter collection stations which are sampled monthly. Biomass of the partially decomposed organic material was measured by collecting 300 square meter samples, drying, and weighing them. Average weight was 380 grams per square meter, with one standard deviation of 176. Concentration of elements in the soil extract will be multiplied times weight of the upper layer of soil (Table 14).

Table 7 . Rate of input of elements into ecosystem via rainfall.

	Kg/Ha/Wk							
	Total soluble salts	Ca	Na	K	Mg	P	Mn	Fe	Cu
Oct., 1967	4	0.19	1.34		.077				
Nov., 1967	11	0.40	1.66		.266				
Dec., 1967	8	1.34	1.30		.197				
Jan., 1968	7	0.60	1.79		.204				
Feb., 1968	5	0.36	0.35		.084				
Mar., 1968	13	0.29	0.88		.147				
Apr., 1968	8	0.20	1.00		.120		.000		
May, 1968	19	0.11	1.14		.011		.011		
June, 1968	6	0.65	0.90	0.6	T	1.81	.000		
July, 1968	7	0.31	0.47		T		.036		
Aug., 1968	3	0.30	1.06		T		.026		
Sept., 1968	5	0.30	1.40	0.1	.038		.000	.000	.013
Yearly average	8.00	0.42	1.10	0.35	0.095	1.81	0.012	.000	.013

Table 8 . Rate of movement of elements by throughfall.

	Kg/Ha/Week								
	Total soluble salts	Ca	Na	K	Mg	P	Mn	Fe	Cu
Oct., 1967	8	0.17	1.54		.051				
Nov., 1967	10	0.58	1.46		.195				
Dec., 1967	10	0.70	1.68		.195				
Jan., 1968	10	0.74	2.03		.263				
Feb., 1968	8	0.43	0.52		.150				
Mar., 1968	13	0.70	1.57		.286				
Apr., 1968	13	0.41	1.57		.188		.001		
May, 1968	18	0.14	0.87		.140		.007		
June, 1968	10	0.54	0.53	1.86	.035	1.94	.005		
July, 1968	10	0.47	0.33		T		.000		
Aug., 1968	8	0.40	0.56		.004		.009		
Sept., 1968	9	0.24	0.88	1.23	.110		.000	.013	.015
Yearly average	10.58	0.46	1.128	1.54	.134	1.94	.004	.013	.015

Table 9 . Rate of movement of elements by stem flow.

	Kg/Ha/Week								
	Total soluble salts	Ca	Na	K	Mg	P	Mn	Fe	Cu
Oct., 1967	1	0.12	0.30		.013				
Nov., 1967	3	0.26	0.36		.023				
Dec., 1967	3	0.16	0.30		.028				
Jan., 1968	3	0.18	0.45		.055				
Feb., 1968	2	0.15	0.15		.019				
Mar., 1968	5	0.16	0.50		.058				
Apr., 1968	3	0.10	0.40		.049		.001		
May, 1968	6	0.14	0.30		.067		.001		
June, 1968	4	0.14	0.23	1.50	.016	0.78	.003		
July, 1968	3	0.10	0.08		T		.002		
Aug., 1968	3	0.09	0.16		T		.006		
Sept., 1968	2	0.09	0.25	1.27	.029		.001	.009	.005
Yearly average	3.16	0.14	0.29	1.38	0.029	0.78	.002	.009	.005

Table 10 . Rate of movement of elements out of litter by water.

	Kg/Ha/Week								
	Total soluble salts	Ca	Na	K	Mg	P	Mn	Fe	Cu
Oct., 1967	13	1.78	1.92		.470				
Nov., 1967	21	1.82	2.12		.727				
Dec., 1967	17	1.96	2.43		.564				
Jan., 1968	17	1.74	2.73		.684				
Feb., 1968	14	0.83	1.09		.433				
Mar., 1968	26	1.56	2.94		.849				
Apr., 1968	20	1.24	3.13		.723		.007		
May, 1968	41	2.52	3.03		1.125		.000		
June, 1968	23	2.06	1.75	2.14	.463	6.32	.007		
July, 1968	18	1.88	1.07		.530		.008		
Aug., 1968	16	1.69	1.26		.663		.032		
Sept., 1968	15	1.36	1.97	1.33	.642		.006	.047	.030
Yearly average	20.08	1.70	2.12	1.73	0.656	6.32	.010	.047	.030

Table 11. Rate of movement of elements through soil by bulk flow.

	Total soluble salts	Ca	Na	K	Mg	P	Mn	Fe	Cu
Oct., 1967	9	1.27	2.48		.210				
Nov., 1967	21	1.22	2.12		.484				
Dec., 1967	10	1.78	2.17		.340				
Jan., 1968	15	1.08	3.00		.465				
Feb., 1968	8	0.47	0.81		.213				
Mar., 1968	20	0.65	2.12		.393				
Apr., 1968	15	0.56	2.48		.371		.007		
May, 1968	39	1.23	2.63		.719		.000		
June, 1968	21	0.77	2.92	0.62	.174	5.04* 2.35+	.007		
July, 1968	18	0.74	1.45		.049		.000		
Aug., 1968	14	0.58	1.45		.139		.025		
Sept., 1968	9	0.35	2.07	0.10	.236		.009	.157	.030
Yearly average	16.58	0.89	2.14	0.36	0.316	5.04* 2.35+	.008	.157	.030

* - A horizon
\+ - B horizon

Table 12. Rate of loss of elements from ecosystem via runoff.

Kg/ha/week

	Total soluble salts	Ca	Na	K	Mg	P	Mn	Fe	Cu
Oct., 1967	7	0.44	1.17		.05				
Nov., 1967	17	0.96	1.50		.50				
Dec., 1967	6	0.82	1.42		.24				
Jan., 1968	11	0.84	1.43		.35				
Feb., 1968	4	0.32	0.24		.14				
Mar., 1968	15	0.91	1.24		.40				
Apr., 1968	10	0.67	0.88		.28		.000		
May, 1968	32	1.58	2.47		.58		.000		
June, 1968	22	0.77	1.02	0.70	.14	2.52	.000		
July, 1968	16	1.27	1.55		.25		.000		
Aug., 1968	16	1.08	1.17		.39		.021		
Sept., 1968	5	0.29	0.84	0.10	.23		.000	.002	.012
Yearly average	13.41	0.83	1.24	0.40	0.29	2.52	.003	.002	.012

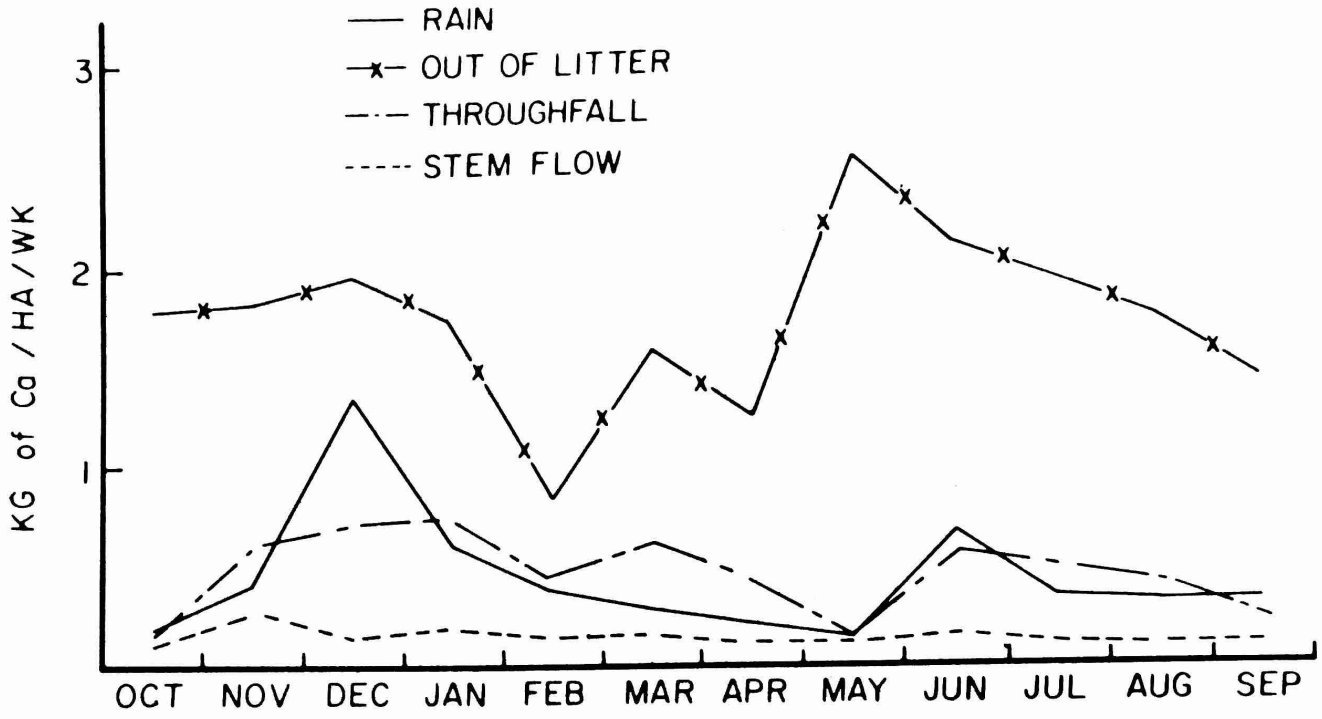

Fig. 3. Average rates of calcium movement through the ecosystem.

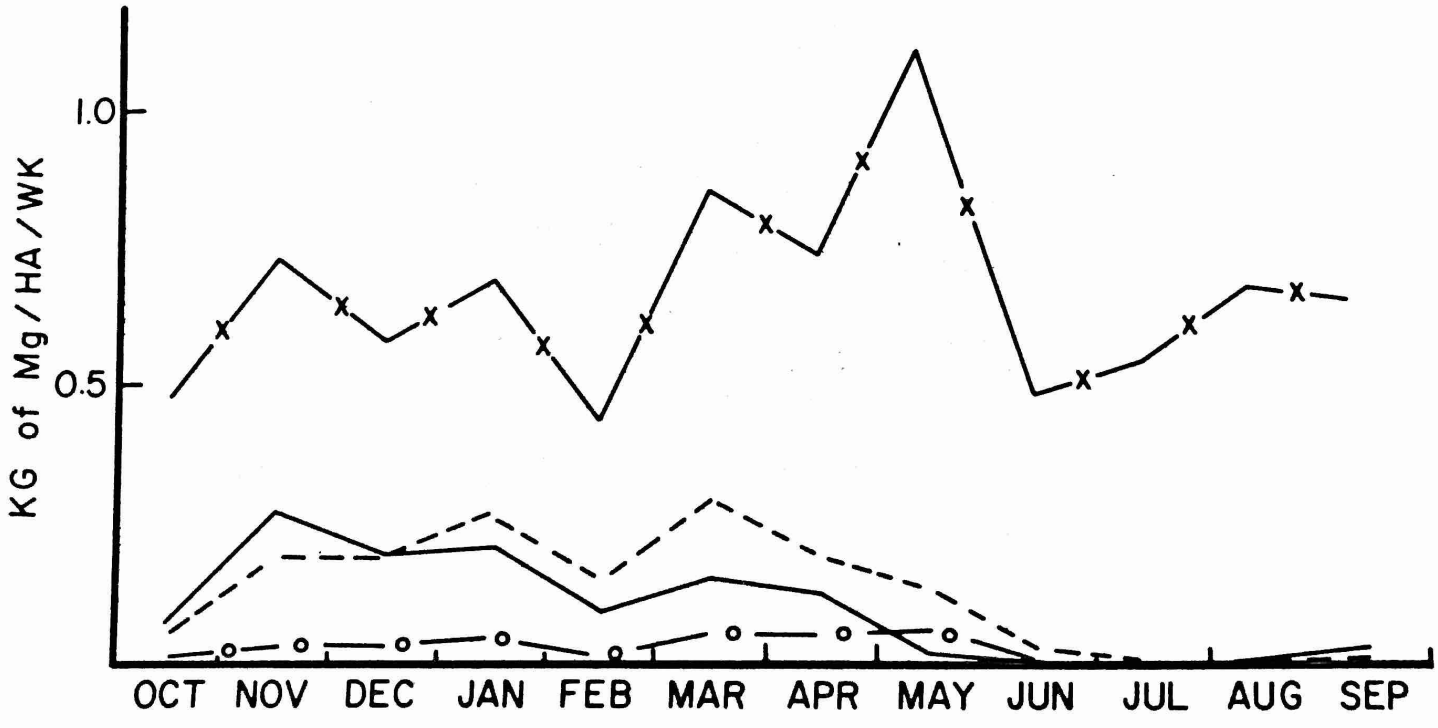

Fig. 4. Average rates of magnesium movement through the ecosystem.

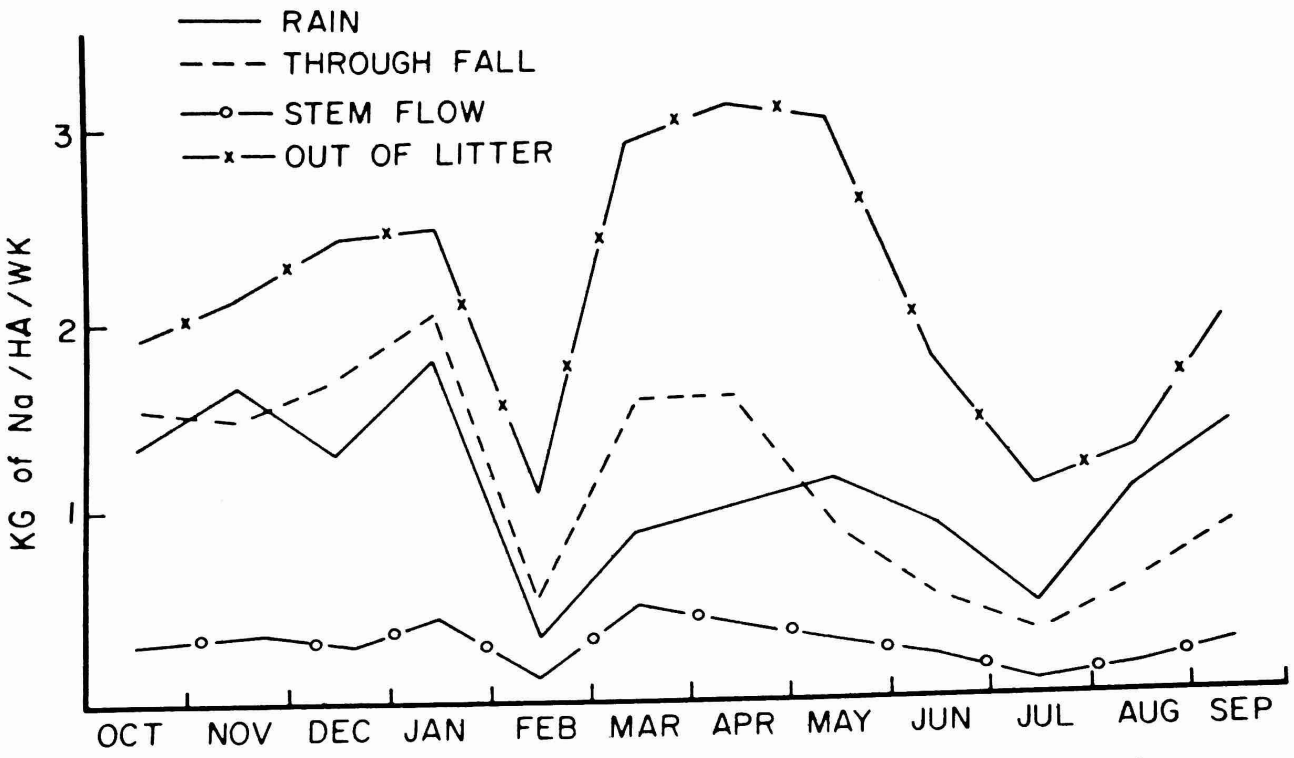

Fig. 5. Average rates of sodium movement through the ecosystem.

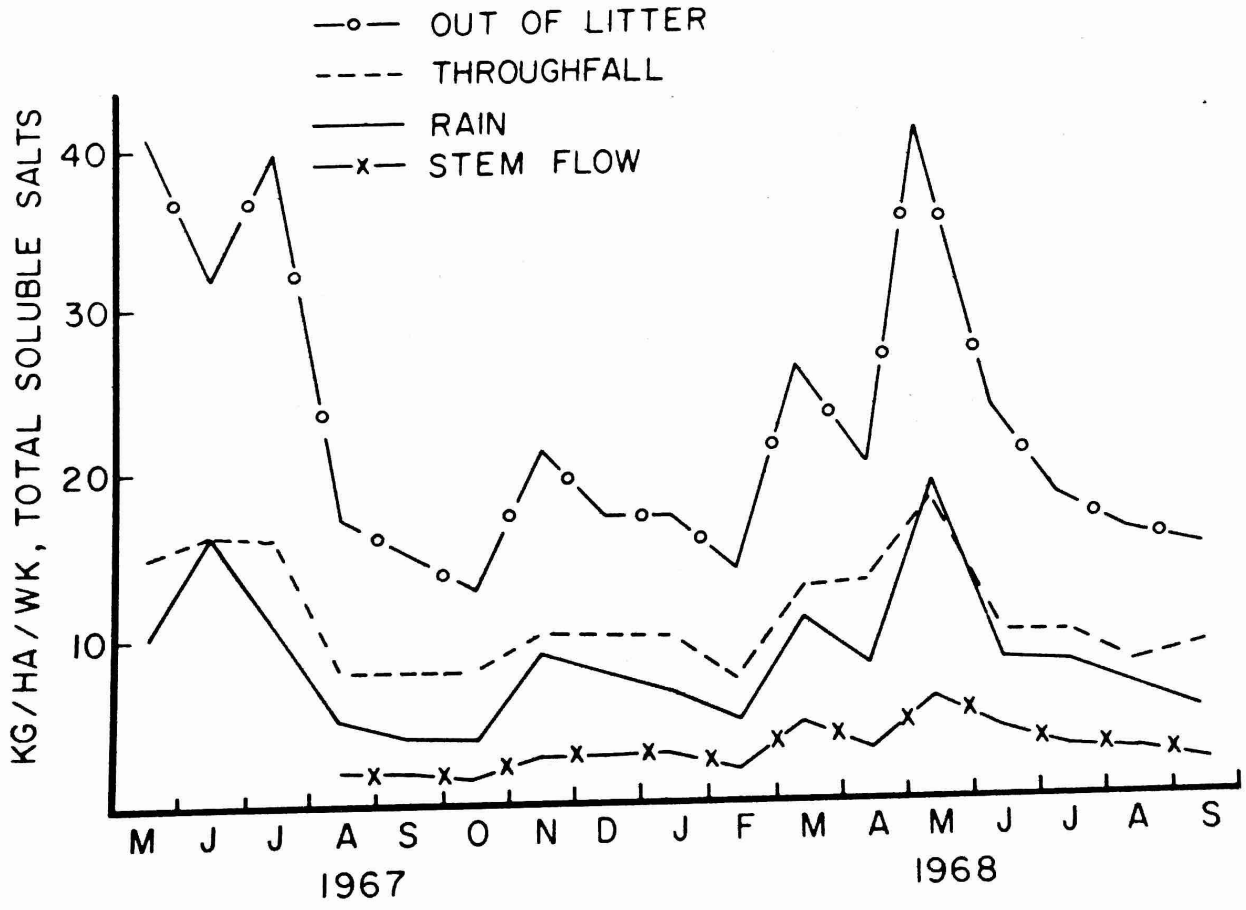

Fig. 6. Average rates of movement of total soluble salts through the ecosystem.

Table 13. Net monthly rates of element gain and loss from ecosystem, determined by subtracting rate of loss by runoff from rate of input by rain.

	kg/ha/week		
	Ca	Na	Mg
Oct., 1967	-.25	+.17	+.02
Nov., 1967	-.56	+.16	-.24
Dec., 1967	+.52	+.12	-.04
Jan., 1968	-.24	+.36	-.15
Feb., 1968	+.04	+.11	-.06
Mar., 1968	-.62	-.36	-.25
Apr., 1968	-.47	+.12	-.16
May, 1968	-1.47	-1.33	-.57
June, 1968	-.12	-.12	-.14
July, 1968	-.96	-1.08	-.25
Aug., 1968	-.78	-.11	-.39
Sept., 1968	+.01	+.56	-.26

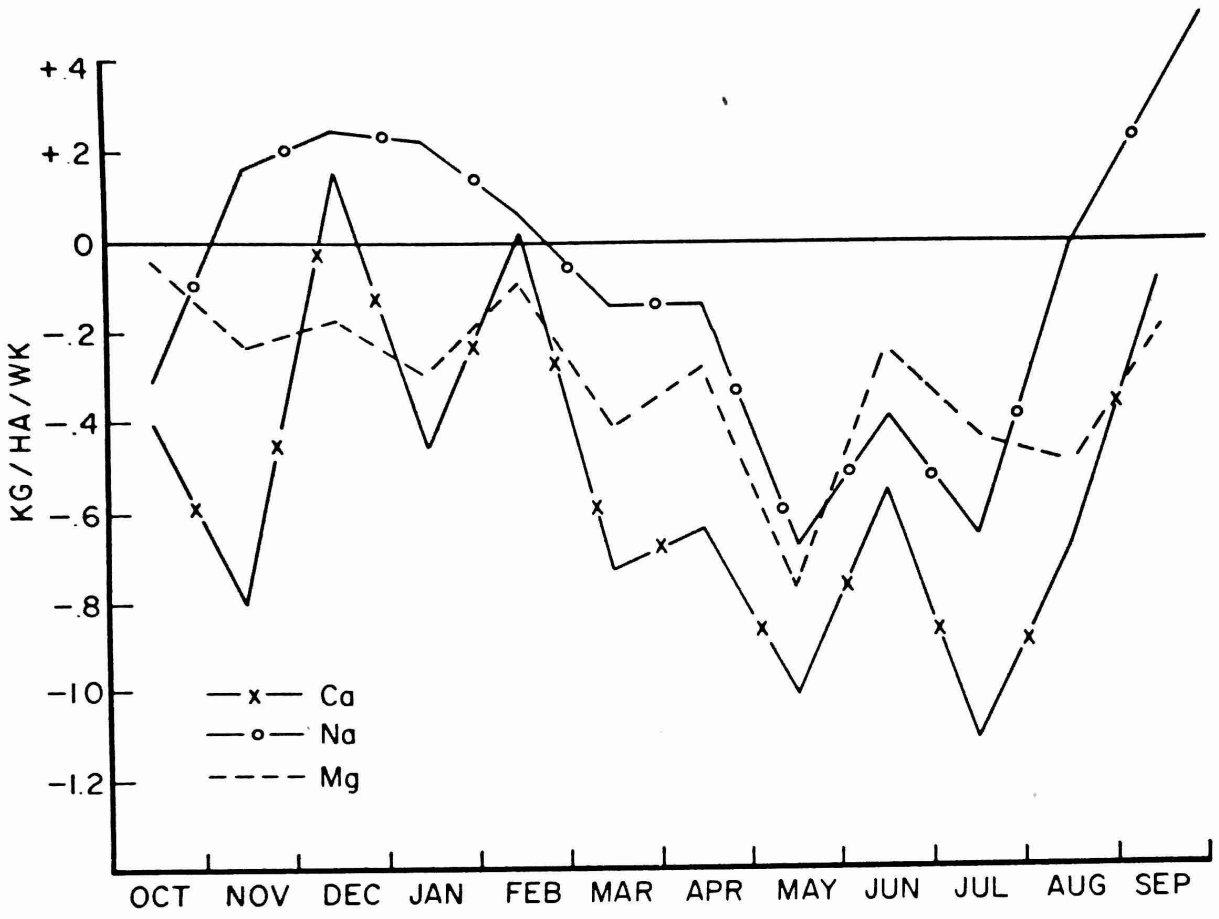

Fig. 7. Rates of gain and loss of isotopes to and from the ecosystem.

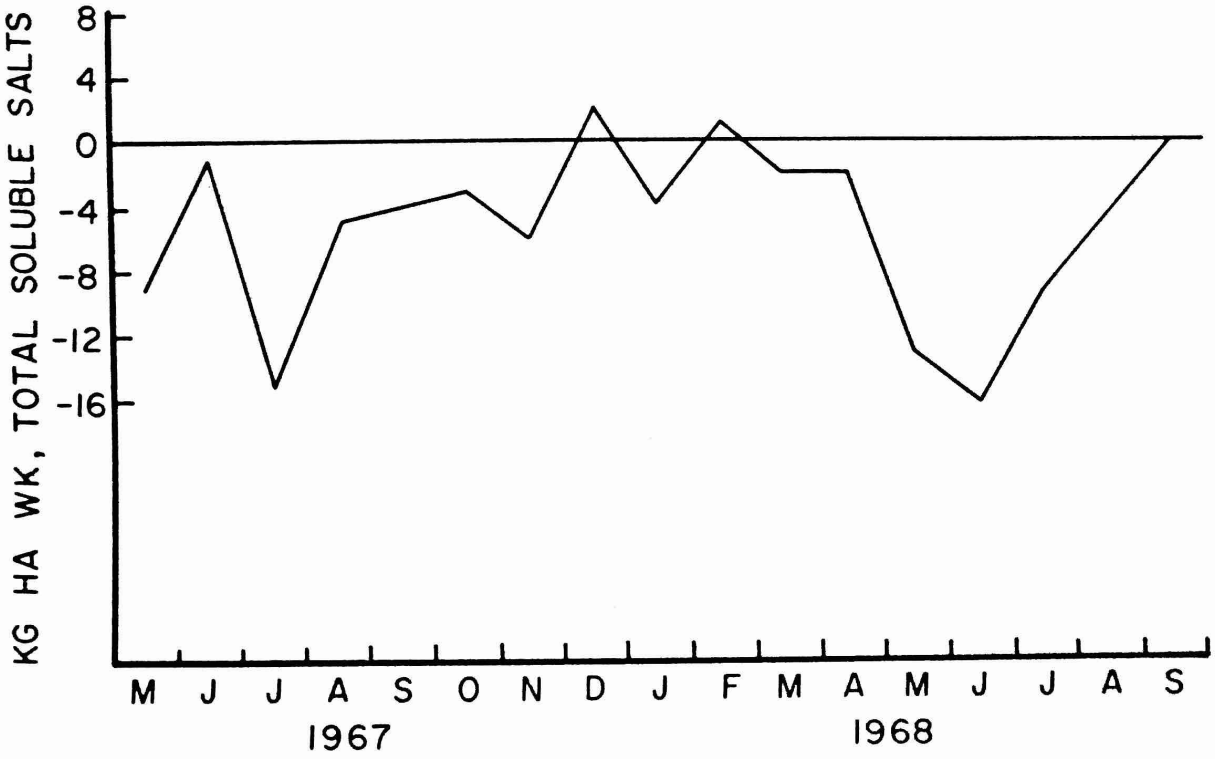

Fig. 8. Rates of gain and loss of total soluble salts to and from the ecosystem.

Table 14

Depth and bulk densities of soil, and weight of upper layer of soil per square meter.

Site	Upper layer of soil				Lower layer of soil	
	average depth, cm.	No. of observations	average bulk density	No. of observations	average bulk density	No. of observations
ridges (oxidized soil conditions)	21.8 ± 6.1	20	0.57 ± .09	10	1.02 ± .10	10
valleys and flats (reduced soil conditions)	27.4 ± 1.6	4	0.47 ± .12	10		
Radiation Center	26.7 ± 10.7	4				
All sites	23.4 ± 6.9	28	0.53 ± .12	20		

average depth x 1 m^2 = vol. of upper layer of soil per m^2 of forest floor

23.4 cm. x 10000 cm^2 = 234,000 cm^3

vol. x g/cm^3 = g/m^2 of forest floor

234000 x 0.53 = 124,020 = 124 kg/m^2

All elements were analyzed by atomic absorption spectrophotometry. Leaves, wood, roots, organic matter and fresh litter were prepared for analysis by the following procedure:

1) Put 2 grams of plant material into a 50 ml. beaker.
2) Burn in the furnace at 250°C for 3 to 4 hours.
3) Increase the temperature to 450°C and ash for 12 hours.
4) Let cool, add 5 ml. of concentrated HCL and evaporate to dryness (Don't let boil).
5) Let cool; add 25 ml. of 0.1 HCL and stir.
6) Let sit for 30 minutes.
7) Stir again and filter through Whatman 40 filter paper (Do not wash filter paper).
8) Run for Co, Cu, Fe, K, Mn.
9) For Ca, Mg, and Sr dilute 1:1 with a solution 2% La; 1000 ppm K; final concentration of La should be 1% and K 500 ppm.
10) Divide every % A by scale expansion, if any, and convert to absorbance.
11) Prepare a standard curve for absorbance (Y) vs concentration in the samples and multiply by dilution factor, if any.

Note: Same procedure as above is used for the complete analysis of organic matter, except for Sr, which has to be analyzed using the method of additions.

250

Elements were extracted from the soil for analysis by the following procedure:

1-3

1) Weigh 2.00 grams of ground, oven dried soil into a 50 ml. plastic centrifuge tube.
2) To the soil in the tube, add 15 ml. of 1 N NH_4OAc and shake at full speed for 30 minutes.
3) Centrifuge at full speed for ten minutes.
4) Decant and save the supernatant.
5) Add another 15 ml. NH_4OAc and shake again at full speed for 15 minutes.
6) Repeat step 3
7) Decant, adding supernatant to the supernatant from step 4.
8) Repeat steps 5, 6, and 7.
9) Make to a total volume of 50 ml. with 1 N NH_4OAc.
10) Filter through Whatman 40 filter paper.
11) Run for Cu, Fe, K, Mn.
12) For Ca and Mg, dilute 1:1 with a solution 2% La; 1000 ppm K to obtain a final concentration of 1% La, and 500 ppm K in the sample.
13) Divide every % A by scale expansion, if any, and convert absorbance.
14) Prepare a standard curve of absorbance (Y) vs concentration (X) with the standards. Determine concentration in the samples and multiply by dilution factor, if any.

Since available elements in the decomposing organic matter may be important in the element cycle, an attempt was made to get an indication of what quantity of elements were available for immediate uptake by plants, as well as total elements as determined by the combustion technique. Therefore, an extraction procedure for the organic material was used, similar to the extraction procedure for the soil. It is as follows:

1) Put 4.00 grams of oven dried organic matter into a 50 ml. plastic centrifuge tube.
2) Add 20 ml. of 0.1 N NH_4OAc and shake at full speed for 30 minutes.
3) Centrifuge at full speed for ten minutes.
4) Decant and save the supernatant.
5) Add another 20 ml. NH_4OAc and shake again at full speed for 15 minutes.
6) Repeat step 3
7) Decant, adding supernatant to the one from step 4.
8) Repeat steps 5,6 and 7 until the extracting solution (IN NH_4OAc) stays clear after shaking.
9) Filter through Whatman 40 filter paper.
10) Run for Co, Cu, Fe, K, Mn, Na (dilute, if necessary).
11) For Ca and Mg, dilute 1:1 with a solution 2% La, 1000 ppm K to obtain a final concentration of 1% La, and 500 ppm K in the sample.
12) For Sr, use the method of addition, in which a standard is added to the sample; two equal volumes of sample are diluted in a 1:1 proportion, one with a known concentration standard prepared in 2% La, 1000 ppm K, and the other with just a solution 2% La, 1000 ppm K. Compare the absorbance of the two samples using the following proportion.

$$\frac{\text{Concentration sample}}{\text{Absorbance sample}} = \frac{\text{Conc. sample + Conc. standard}}{\text{Absorbance of (sample + standard)}}$$

solving the proportion for Conc. sample,

$$\text{Conc. sample} = \frac{\text{A sample} \quad \text{C standard}}{\text{A (sample + standard)} - \text{A sample}}$$

13) Divide every % A by the scale expansion, if any, and convert to absorbance.

14) Prepare a standard curve of absorbance (Y) vs concentration (X) with the standards. Determine concentration in the samples and multiply by dilution factor.

The sampling scheme was designed so that the following statistical tests could be made:

1. Soil, for difference in sites.
2. Soil, for difference in depth.
3. Tree trunks for difference in species.

4. Tree trunks for difference in sites.
5. Freshly fallen litter for difference in season.
6. Organic matter for difference in sites.
7. Leaves for difference in presence and absence of epiphyllas.
8. Leaves for difference caused by location in canopy or understory.

The exact sampling scheme is shown in Table 15.

All statistical tests were made with the analysis of variance technique, except for the leaves, where it was necessary to use a non-parametric sign test.

Results

Leaves with epiphylls contained greater amounts of Co, Mn, Fe, Sr, Ca, and Mg (Table 16). Presumably this is because when rain containing these elements enters the canopy, the elements are more efficiently bound to the leaves when epiphylls are present. It is not surprising that the epiphyll covered leaves did not contain more K and Na, since these are very mobile elements and are less likely to be bound by the epiphylls. It is surprising that Cu showed no difference. Perhaps there is no significant input of Cu via rainfall. Palicourea riparia was excluded from the tests, because in many cases it showed tendencies opposite to that of the other species.

There is a tendency for understory leaves to be slightly higher in element concentration than canopy leaves, (Table 16), but the differences are not great enough nor consistent enough to be statistically significant.

Averages and standard deviations of element concentrations in leaves of each category are given in Table 17.

Differences in element concentrations between species are very great in some, but not all, species (Table 18). However, differences are sufficient for each species to require different treatment in the model. Calcium differences between three species were checked, and the differences are highly significant (Table 19).

There were no differences in six element concentrations in Dacryodes between sites, but there were differences in Mn, Mg, and Na. Therefore the sites were checked again for these elements using Manilkara. Magnesium again showed a difference, so it was checked again in Sloanea. There was no difference between sites (Table 20). For purposes of the model previously discussed, we can assume no difference between sites.

There are apparent differences in concentrations of elements in the roots of the various species (Table 21).

Table 15

Sampling scheme for stable element analysis.

Type of site	No. of sites	Type of sample	Condition	Location	Species	No. of samples of each at each site
Well drained soil on ridge top	5	leaves	clean	canopy	Dacryodes excelsa	1
"	5	"	"	"	Manilkara bidentata	1
"	5	"	"	"	Sloanea berteriana	1
"	5	"	epiphyll covered	"	Dacryodes excelsa	1
"	5	"	" "	"	Manilkara bidentata	1
"	5	"	" "	"	Sloanea berteriana	1
"	5	"	clean	understory	Dacryodes excelsa	1
"	5	"	"	"	Manilkara bidentata	1
"	5	"	"	"	Sloanea berteriana	1
"	5	"	"	"	Palicourea riparia	1
"	5	"	epiphyll covered	"	Dacryodes excelsa	1
"	5	"	" "	"	Manilkara bidentata	1
"	5	"	" "	"	Sloanea berteriana	1
"	5	"	" "	"	Palicourea riparia	1
"	5	wood (trunk)	-	4.5 ft.	Dacryodes excelsa	4
"	5	" "	-	"	Manilkara bidentata	4
"	5	" "	-	"	Sloanea berteriana	4
"	5	" "	-	"	Palicourea riparia	4
Well drained soil on ridge top	5	Organic matter	-	forest floor	-	4
"	5	roots	-	in soil	Dacryodes excelsa	1
"	5	"	-	"	Manilkara bidentata	1
"	5	"	-	"	Sloanea berteriana	1
"	5	"	-	"	Palicourea riparia	1
Well drained soil on ridge top	5	Soil	-	0-2 inches	-	4
"	5	"	-	5-7 "	-	4
"	5	"	-	sub-soil	-	1
All sites	55	fresh litter	-	top of organic matter	all	1 per mon
Poorly drained soil in valley bottom	1	leaves	clean	understory	Croton poecilanthus	1
"	1	"	epiphyll covered	"	Euterpe globosa	1
"	1	"	clean	"	Euterpe globosa	1
"	1	wood (trunk)	-	4.5 ft.	Croton poecilanthus	20
"	1	" "	-	4.5 ft.	Euterpe globosa	20

Table 16

Results of sign tests to test differences in element concentration between leaves with and without epiphylls, Palicourea riparia excluded, and between canopy and understory leaves.

Element	Clean leaves vs. leaves + epiphylls			Canopy leaves vs. understory leaves		
	n	r	level of confidence that leaves plus epiphylls are higher	n	r	level of confidence that understory leaves are higher
Co	18	4	95%	11	4	-
Mn	19	2	99%	10	4	-
Fe	19	1	99%	11	5	-
Cu	20	8	-	11	4	-
K	20	1	-*	11	3	75%
Na	20	8	-	11	5	-
Sr	20	2	99%	11	3	75%
Ca	20	2	99%	11	3	75%
Mg	20	4	95%	11	3	75%

* clean leaves are higher in potassium

Table 17

Averages and standard deviations of element concentrations in leaves of each category. All values are parts per million.

Dacryodes excelsa

canopy

clean leaves (n = 3)

Co	Mn	Fe
4.62 ± 1.07	339 ± 271	52 ± 12
Cu	**K**	**Na**
2.46 ± 1.02	1137 ± 679	1482 ± 190
Sr	**Ca**	**Mg**
8.2 ± 1.41	3321 ± 644	1067 ± 105

Dacryodes excelsa

canopy

leaves + epiphylls (n = 3)

Co	Mn	Fe
5.82 ± 2.21	381 ± 181	102 ± 36
Cu	**K**	**Na**
2.66 ± .44	981 ± 283	1700 ± 368
Sr	**Ca**	**Mg**
10.2 ± 1.8	4529 ± 833	1222 ± 225

Dacryodes excelsa

understory

clean leaves (n = 4)

Co	Mn	Fe
2.90 ± 2.22	2.95 ± 79	89 ± 16
Cu	**K**	**Na**
5.15 ± 2.87	2740 ± 615	1707±773
Sr	**Ca**	**Mg**
6.45 ± 2.54	3713 ± 693	1414±372

Dacryodes excelsa

understory

leaves + epiphylls (n = 4)

Co	Mn	Fe
4.39 ± 3.34	437 ± 146	120 ± 26
Cu	**K**	**Na**
2.72 ± .26	1109 ± 189	1529 ± 621
Sr	**Ca**	**Mg**
11.8 ± 2.96	5173 ±1534	1322 ± 232

Continued Table 17

Manilkara bidentata canopy clean leaves (n = 2)		
Co	Mn	Fe
7.04 ± 0.22	33 ± 12	54 ± 9
Cu	K	Na
4.95 ± 0.64	2656 ± 12	3154 ± 364
Sr	Ca	Mg
22.6 ± 16.1	4991 ±582	2019 ± 371

Manilkara bidentata canopy leaves + epiphylls (n = 2)		
Co	Mn	Fe
8.25 ± 0.36	40 ± 22	128 ± 29
Cu	K	Na
7.40 ± 1.97	1821 ±118	3263 ±571
Sr	Ca	Mg
38.5 ± 10.5	6496 ±453	2647 ±270

Manilkara bidentata understory clean leaves (n = 4)		
Co	Mn	Fe
4.78 ± 2.84	32 ± 13	136 ± 84
Cu	K	Na
8.00 ± 2.59	3663 ± 1099	4285 ± 2116
Sr	Ca	Mg
30.7 ± 13.1	6651 ± 2047	3080 ± 506

Manilkara bidentata understory leaves + epiphylls (n = 4)		
Co	Mn	Fe
4.96 ± 3.44	110 ± 56	253 ± 47
Cu	K	Na
7.85 ± 0.41	1359 ± 323	3828 ± 2589
Sr	Ca	Mg
44.2 ± 13.5	8070 ± 1690	3539 ± 502

Continued Table 17

Sloanea berteriana canopy clean leaves (n = 2)		
Co	Mn	Fe
4.39 ± 1.32	455 ± 424	120 ± 74
Cu	K	Na
9.10 ± 6.50	2224 ± 922	510 ± 180
Sr	Ca	Mg
16.4 ± 9.4	5698 ±3069	2008 ± 608

Sloanea berteriana canopy leaves + epiphylls (n = 1)		
Co	Mn	Fe
5.2	1141	123
Cu	K	Na
4.3	1117	842
Sr	Ca	Mg
34.7	10210	2678

Sloanea berteriana understory clean leaves (n = 5)		
Co	Mn	Fe
4.70 ± 3.29	154 ± 85	77 ± 38
Cu	K	Na
10.22 ± 3.41	2842 ± 607	685 ± 197
Sr	Ca	Mg
15.34 ± 4.65	4587 ± 797	1596 ± 151

Sloanea berteriana understory leaves + epiphylls (n = 4)		
Co	Mn	Fe
5.35 ± 5.95	255 ± 121	139 ± 29
Cu	K	Na
5.32 ± 1.50	1979 ± 359	1135 ± 224
Sr	Ca	Mg
24.0 ± 4.95	7484 ± 843	2039 ± 185

Continued Table 17

Palicourea riparia

clean leaves (n = 5)

Co	Mn	Fe
7.32 ± 3.24	174 ± 93	164 ± 47
Cu	**K**	**Na**
13.96 ± 3.78	4596 ± 954	2202 ±1062
Sr	**Ca**	**Mg**
118 ± 31	10927 ± 943	5300 ± 523

Palicourea riparia

leaves + epiphylls (n = 5)

Co	Mn	Fe
7.32 ± 2.95	191 ± 68	277 ± 126
Cu	**K**	**Na**
18.98 ±18.25	3171 ±1228	5344 ±1112
Sr	**Ca**	**Mg**
114 ± 53	10368 ±1434	5239 ± 803

Croton poecilanthus

clean leaves (n = 1)

Co	Mn	Fe
2.87	828	83
Cu	**K**	**Na**
8.8	5250	6130
Sr	**Ca**	**Mg**
57	6817	4024

Croton poecilanthus

leaves + epiphylls (n = 1)

Co	Mn	Fe
3.37	1204	81
Cu	**K**	**Na**
6.0	3202	8025
Sr	**Ca**	**Mg**
68	8586	4884

Continued Table 17

Euterpe globosa clean leaves (n=1)			Euterpe globosa leaves + epiphylls (n=1)		
Co	Mn	Fe	Co	Mn	Fe
1.75	305	213	4.87	406	224
Cu	K	Na	Cu	K	Na
19.2	4387	926	12.5	2665	375
Sr	Ca	Mg	Sr	Ca	Mg
23.7	4720	2958	19.6	3981	4687

Table 18

Averages and standard deviations of concentrations of elements in tree trunks.

Parts per million

Element	De	Mn	Sb	Eg	Cp	Pr
Co	2.89 ± 2.83	2.54 ± 2.49	3.73 ± 3.88	3.50 ± 2.10	2.30 ± 1.70	5.50 ± 2.20
Mn	67 ± 45	3.38 ± 3.36	24.8 ± 8.7	78 ± 39	124.20 ± 62.05	24.7 ± 5.4
Sr	5.62 ± 1.43	14.15 ± 4.75	11.55 ± 4.39	25.7 ± 10.9	21.70 ± 6.08	10.8 ± 2.98
Ca	1054 ± 334	2246 ± 844	3582 ± 1552	2060 ± 797	2039 ± 1144	1392 ± 488
Mg	268 ± 112	375 ± 154	286 ± 118	863 ± 263	677 ± 167	320 ± 47
Fe	27.9 ± 11.1	18.8 ± 9.0	15.7 ± 4.1	50.2 ± 24.1	27.71 ± 16.08	155 ± 55
Cu	1.78 ± .43	1.97 ± .51	1.80 ± 0.50	3.20 ± 1.60	3.16 ± 0.52	8.03 ± 3.15
Na	360 ± 298	794 ± 530	269 ± 212	5885 ± 3321	1010 ± 648	496 ± 421
K	409 ± 124	323 ± 137	372 ± 132	1191 ± 791	530 ± 245	2084 ± 783

Table 19

Results of analysis of variance to test differences in concentrations of calcium in wood, between species.

Tested	Species	Element	Num./denom. of F ratio	F ratio	Level of significant differences
Species	De-Dm	Ca	1/38	34.0	99.5%
"	Mn-Sb	Ca	1/38	12.0	99.5%
"	De-Sb	Ca	1/38	50.6	99.5%

Table 20

Results of analysis of variance to test differences in concentrations of elements in wood between sites.

Tested	Species	Element	Num./denom. of F ratio	F ratio	Level of significant difference
Sites	De	Ca	4/15	1.27	-
"	De	K	4/15	1.58	-
"	De	Co	4/15	1.84	-
"	De	Mn	4/15	7.80	99.5%
"	De	Sr	4/15	0.24	-
"	De	Mg	4/15	16.22	99.5%
"	De	Fe	4/15	1.84	-
"	De	Cu	2/9	0.00	-
"	De	Na	4/15	2.62	90%
"	Mn	Mn	4/15	1.53	-
"	Mn	Mg	4/15	2.42	90%
"	Mn	Na	4/15	0.62	-
"	Sb	Mg	4/15	1.42	-

Differences in element concentration in the soil extract were tested between the five well drained sites, and all six sites including the poorly drained site. Differences between sites occurred only in Mn and K (Table 22) for the soil 0-2 inches deep, but these differences occurred within the well drained sites, and not necessarily between the well and poorly drained soil. However, with soil from the 5-7 inch level, differences between the well and poorly drained soil existed for Mn, Ca, Mg, and Sr (Table 22). Since differences increase with depth, differences are likely to be caused by differences in parent material.

There are differences in element concentration between the 0-2 and 5-7 inch level for Mn, Ca, Mg, Na, and K. Iron, Cu, and Sr appear to be equally distributed in the soil down to the 7 inch depth (Table 22).

Average and standard deviations of concentrations of element in soil extracts are given in Table 23.

Concentrations of exchangeable and total elements in the organic material are given in Table 24. In the extractable elements, there is a difference between sites only in Mn and Na, and for the total elements, in Na and Co. There is a strong difference between total and extractable elements for all but Sr (Table 25).

Seasonal differences in freshly fallen litter exist only for Na and Mn (Table 26). Exceptionally high sodium concentrations occur during the January collection. This coincides with high sodium input to the ecosystem via rain during Jan. Manganese is low in the litter in the May collection. Yearly averages of elements in the litter are given in Table 27.

Table 21

Average and standard deviations of concentrations of elements in roots.

Parts per million

Element	Dacryodes	Manilkara	Sloanea	Croton	Palicourea	Euterpe
Co	2.72 ± 1.35	3.03 ± .64	3.38 ± .61	3.18 ± .60	4.53 ± 1.16	2.59 ± 0.93
Mn	92.7 ± 51.7	24.9 ± 12.0	32.7 ± 7.2	172 ± 71	39 ± 8.1	69.62 ± 49.89
Sr	12.26 ± 5.11	22.2 ± 4.2	13.0 ± 5.3	43.1 ± 7.7	14.94 ± 2.11	6.98 ± 1.32
Ca	4039 ± 1592	2898 ± 802	3288 ± 1284	3624 ± 979	2499 ± 682	848 ± 181
Mg	984 ± 530	752 ± 208	595 ± 104	1523 ± 369	846 ± 203	2081 ± 545
Fe	224 ± 129	227 ± 56	202 ± 127	146 ± 106	612 ± 247	197 ± 158
Cu	5.00 ± 3.18	6.78 ± 1.62	6.02 ± 2.10	7.18 ± 1.08	12.89 ± 6.00	8.35 ± 2.73
K	691 ± 306	1055 ± 395	981 ± 176	606 ± 307	2509 ± 739	1142 ± 740
Na	416 ± 193	1524 ± 785	132 ± 74	2572 ± 493	267 ± 98	527 ± 308

Table 22

Results of analysis of variance to test soil differences between sites and depths.

Tested	Depth, inches	Element	Num./denom. F ratio	F ratio	Level of significant differences
all sites	0-2	Mn	5/18	2.64	90%
5 well drained sites	0-2	Mn	4/15	3.04	95%
all sites	0-2	Ca	5/18	1.01	-
" "	0-2	Mg	5/18	2.09	-
" "	0-2	Fe	5/18	1.84	-
" "	0-2	Cu	5/18	1.00	-
" "	0-2	Sr	5/18	1.55	-
" "	0-2	Na	1/3	0.55	-
" "	0-2	K	5/18	3.28	95%
5 well drained sites	0-2	K	4/15	3.14	95%
all sites	5-7	Mn	5/18	2.44	90%
5 well drained sites	5-7	Mn	4/15	1.60	-
all sites	5-7	Ca	5/18	2.24	90%
5 well drained sites	5-7	Ca	4/15	0.92	-
all sites	5-7	Mg	5/18	3.96	97%
5 well drained sites	5-7	Mg	4/15	1.17	-
all sites	5-7	Fe	5/18	8.67	99%
5 well drained sites	5-7	Fe	4/15	9.16	99%
all sites	5-7	Cu	5/18	0.50	-
all sites	5-7	Sr	5/18	3.05	95%
5 well drained sites	5-7	Sr	4/15	0.50	-
all sites	5-7	Na	1/1	0.43	-
all sites	5-7	K	5/18	0.49	-
0-2 in. vs. 5-7 in. depth		Mn	1/46	8.00	99%
"		Ca	1/46	7.23	97%
"		Mg	1/46	20.75	99%
"		Fe	1/46	2.15	-
"		Cu	1/46	0.25	-
"		Sr	1/46	1.32	-
"		Na	1/6	4.46	90%
"		K	1/46	35.50	99%

Table 23

Averages and standard deviations of concentrations of elements in soil extracts.

Parts per million

Element	All sites 0-2 in	All sites 5-7 in	W.d. sites 5-7	P.d. sites 5-7	Sub-soil
Mn	4.62 ± 4.88	1.63 ± 1.70	1.21 ± 1.05	3.71 ± 2.85	0.30 ± .55
Ca	209 ± 180	75 ± 96	44 ± 38	230 ± 142	14 ± 3
Mg	166 ± 81	83 ± 36	73 ± 29	134 ± 32	92 ± 8
Fe	25.7 ± 13.5	20.5 ± 11.5	21.4 ± 12.3	15.7 ± 4.3	13.5 ± 7.4
Cu	1.55 ± 0.22	1.51 ± 0.20	1.53 ± 0.20	1.41 ± 0.10	1.40 ± 0.14
Sr	7.76 ± 7.13	5.97 ± 2.55	5.20 ± 1.30	9.68 ± 4.41	3.06 ± 1.28
K	33.5 ± 11.3	16.0 ± 7.7	15.2 ± 8.1	20.0 ± 2.4	5.0 ± 0.0
Na	136 ± 14	116 ± 9	114 ± 10	122	-

Table 24

Averages and standard deviations of concentrations of elements in organic matter on top of soil, as determined by two methods.

Element	Parts per million NH_4OAc extraction	Total
Cu	2.85 ± 1.60	25.6 ± 16.5
K	199 ± 41	235 ± 56
Mn	81 ± 52	167 ± 105
Fe	21.1 ± 30.2	6223 ± 5698
Ca	1710 ± 941	2800 ± 1602
Mg	874 ± 243	1512 ± 332
Sr	30.9 ± 8.4	30.3 ± 12.1
Na	198 ± 75	271 ± 110
Co	-	11.61 ± 7.29

Table 25

Results of analysis of variance to test differences between sites, and between analytical methods, for organic matter on top of soil.

Tested	Element	Num./denom. F ratio	F ratio	Level of significant difference
All sites, extractable elements	Cu	4/15	0.44	-
" " "	K	4/15	0.17	-
" " "	Mn	4/15	2.42	90%
" " "	Fe	4/15	2.24	-
" " "	Ca	4/15	1.85	-
" " "	Mg	4/15	1.74	-
" " "	Sr	4/15	0.79	-
" " "	Na	4/15	3.78	95%
All sites, total elements	Cu	4/15	1.13	-
" "	K	4/15	1.45	-
" "	Mn	4/15	2.18	-
" "	Fe	4/15	1.99	-
" "	Ca	4/15	1.49	-
" "	Mg	4/15	1.89	-
" "	Sr	4/15	1.45	-
" "	Na	4/15	3.75	95%
" "	Co	4/15	5.08	99%
Extractable vs. total element	Cu	1/38	37.42	99%
" "	K	1/38	5.18	95%
" "	Mn	1/38	10.76	99%
" "	Fe	1/38	23.57	99%
" "	Ca	1/38	6.87	97%
" "	Mg	1/38	47.80	99%
" "	Sr	1/38	0.05	-
" "	Na	1/38	5.60	97%

Table 26

Results of analysis of variance to test differences in monthly concentration of elements in freshly fallen litter.

Tested	Element	Num./denom. F ratio	F ratio	Level of significant differences
Month (Jan., Mar., May, July, Sept., Nov.)	Ca	5/7	0.50	-
" " " " "	Mg	"	1.00	-
" " " " "	Mn	"	4.57	95%
" " " " "	Co	"	2.02	-
" " " " "	Sr	"	1.68	-
Month (Jan., Mar., July, Nov.)	Fe	3/6	2.32	-
" " " " "	K	"	0.28	-
" " " " "	Cu	"	0.42	-
" " " " "	Na	"	4.87	95%

Table 27

Averages and standard deviations of concentrations of elements in freshly fallen litter.

Element	Parts per million
Co	8.52 ± 6.10
Mn	414 ± 97
Fe	199 ± 72
Cu	5.72 ± 0.75
K	430 ± 54
Na	805 ± 146
Sr	60.5 ± 14.9
Ca	9104 ± 1532
Mg	2200 ± 333

TRANSFER AND STORAGE FUNCTIONS FOR STABLE AND RADIOACTIVE ISOTOPES IN THE TROPICAL RAIN FOREST ECOSYSTEM

1. Input of isotope into system by rainfall is a function of volume of rainfall times concentration of isotope in rain.

2. Isotope movement by throughfall is a function of volume of throughfall times concentration of isotope in throughfall.

3. Isotope movement by stem flow is a function of volume of stem flow times concentration of isotope in stem flow.

4. Isotope movement by leaf fall is a function of biomass of leaf fall times concentration of isotopes is undecomposed litter.

5. Isotope movement from litter to soil is a function of volume of water leaving the litter times concentration of isotope in that water.

6. Isotope movement through the soil is a function of volume of soil water times concentration of isotope in soil water.

7. Isotope loss through runoff is a function of volume of runoff times concentration of isotope in runoff.

8. Loss of isotope through sediment movement is a function of the volume of water during flood stage times concentration of sediment times concentration of isotope in sediment.

9. Turnover time of isotope in canopy is the biomass of the canopy times the concentration of the isotope in the canopy divided by the loss rate from the canopy.

10. Turnover time of isotope in the understory is the biomass of the understory times the concentration of the isotope in the understory divided by the loss rate from the understory.

11. Turnover time of isotope in the litter is the biomass of the litter times the concentration of the isotope in the litter, divided by the loss rate from the litter.

12. Increase of isotope in biomass of canopy is a function of the rate of canopy increase times concentration of isotope in the canopy.

13. Increase of isotope in biomass of stem is a function of the rate of stem increase times concentration of isotope in the stem.

14. Increase of isotope in biomass of roots is a function of the rate of root increase times concentration of isotope in the roots.

15. Transfer rate from epiphyllae to leaves determined by tracer experiment.

16. Transfer rate from canopy to root through phloem determined by tracer experiment.

17. Transfer to roots calculated by subtracting loss from ecosystem from total movement into litter.

Influence of Species, Site, Canopy Position, and Epiphylls on Fallout Distribution

To construct a model which will predict pathways, rates, and turnover times of stable and radioactive isotopes in the tropical rain forest ecosystem, it is necessary to understand inputs to the system. Fallout, of course, is an important input. Once fallout is carried into the ecosystem by rain, a variety of factors might influence its subsequent behavior. This study tests the importance of four of these factors: species, site, location of leaves in canopy or understory; presence or absence of epiphylls on leaves.

The sampling plan was to take one sample of leaves heavily covered with epiphyllae, and one sample devoid of epiphyllae from the top-most canopy leaves, and from the bottom-most understory leaves of three species at five sites. In addition, clean and epiphyllae-covered leaves were to be taken from an understory species at each of the five sites. Because it was difficult to find and reach all the desired samples at all the sites, not all desired samples were obtained (Table 28). Two additional species were sampled at a sixth site for additional comparison (Table 28).

Samples were oved-dried, and counted in bulk by gamma scintillation spectrometry. Data were corrected by computer solution of simultaneous equations. Comparisons between sites, species, canopy position, and presence or absence of epiphylls were made using ^{137}Cs, ^{144}Ce and ^{95}Zr.

First, averages of ^{137}Cs, ^{144}Ce, and ^{95}Zr were calculated for the canopy and understory, for clean and epiphyll covered leaves, by species and site (Tables 29-31). Then differences were tested by analysis of variance techniques for species, sites, clean vs. epiphyll cover, and canopy vs. understory (Table 32). Differences at the 5% error level or less were found between species for ^{137}Cs and ^{144}Ce on clean understory leaves, and between species for all isotopes on epiphyll covered understory leaves (Table 5). Since tables 2,3 and 4 show that average levels of fallout on Palicourea riparia are most different from the rest, species in the understory were again tested for differences, this time without Palicourea riparia. No difference between other species was evident. There also was no difference between species in the canopy.

Two differences between sites, out of the 12 tested (Table 32), were significant. This is not enough to state that there are differences between sites.

Table 28. Actual sampling scheme for determining fallout distribution.

		Dacryodes excelsa	Manilkara bidentata	Sloanea berteriana	*Palicourea riparia	Euterpe globosa	Croton poecilanthus
Site 1	canopy clean	x		x			
	canopy + epiphyll	x	x	x			
	understory clean	x	x	x	x		
	understory + epiphyll	x	x		x		
Site 2	canopy clean	x	x	x			
	canopy + epiphyll	x	x	x			
	understory clean				x		
	understory + epiphyll				x		
Site 3	canopy clean	x					
	canopy + epiphyll	x					
	understory clean	x	x		x		
	understory + epiphyll	x	x		x		
Site 4	canopy clean						
	canopy + epiphyll						
	understory clean	x	x	x	x		
	understory + epiphyll	x	x	x	x		
Site 5	canopy clean						
	canopy + epiphyll						
	understory clean	x	x	x	x		
	understory + epiphyll	x	x	x	x		
Site 6	understory clean						x
	understory + epiphyll					x	

*Understory species

Table 29. Influence of species, site, canopy position and epiphylls on ^{137}Cs distribution. Average values are picocuries per gram.

	Understory					
	Clean leaves			Leaves plus epiphylls		
	N	$\bar{X}$	1 st. deviation	N	$\bar{X}$	1 st. deviation
Site 1	3	3.03	1.02	2	5.98	2.18
Site 3	2	4.07	1.59	2	5.91	2.33
Site 4	3	3.89	0.22	3	5.09	1.82
Site 5	3	2.59	1.30	3	4.33	0.61
Dacryodes excelsa	4	3.62	1.49	4	3.90	
Manilkara bidentata	4	2.73	0.90	4	7.15	
Sloanea berteriana	3	3.88	0.71	2	5.33	
Palicourea riparia	5	5.65	1.59	5	7.82	
	Canopy					
Site 1	2	2.62	0.67	3	4.80	0.48
Site 2	3	3.03	0.55	3	4.29	1.23
Site 3	1	3.76	-	1	4.74	-
Dacryodes excelsa	3	3.50	0.35	3	4.99	0.50
Manilkara bidentata	1	2.84	-	2	3.75	0.92
Sloanea berteriana	2	2.37	0.31	2	4.77	0.80

Table 30. Influence of species, site, canopy position and epiphylls on ^{144}Ce distribution. Average values are picocuries per gram.

	Understory					
	Clean leaves			Leaves plus epiphylls		
	N	$\bar{X}$	1 st. deviation	N	$\bar{X}$	1 st. deviation
Site 1	3	1.38	1.26	2	6.46	0.20
Site 3	2	2.54	2.25	2	4.10	0.55
Site 4	3	1.75	0.04	3	4.17	0.34
Site 5	3	0.63	0.86	3	4.19	1.20
Dacryodes excelsa	4	2.14	1.72	4	4.41	1.26
Manilkara bidentata	4	1.09	0.62	4	4.65	1.42
Sloanea berteriana	3	1.15	0.87	2	4.97	0.78
Palicourea riparia	5	6.59	3.26	5	15.88	4.23
	Canopy					
Site 1	2	2.91	1.14	3	5.99	1.45
Site 2	3	2.46	2.70	3	6.19	6.05
Site 3	1	8.06	-	1	8.81	-
Dacryodes excelsa	3	5.79	2.17	3	8.79	4.23
Manilkara bidentata	1	1.49	0.86	2	3.71	3.19
Sloanea berteriana	2	0.92	-	2	5.78	2.36

Table 31. Influence of species, site, canopy position and epiphylls on ^{95}Zr distribution. Average values are picocuries per gram.

	Understory					
	Clean leaves			Leaves plus epiphylls		
	N	$\bar{X}$	1 st. deviation	N	$\bar{X}$	1 st. deviation
Site 1	3	0.79	0.20	2	1.59	0.12
Site 3	2	0.85	0.14	2	0.92	0.49
Site 4	3	0.32	0.03	3	1.05	0.57
Site 5	3	0.44	0.26	3	1.01	0.21
Dacryodes excelsa	4	0.74	0.31	4	1.46	0.26
Manilkara bidentata	4	0.46	0.30	4	0.93	0.43
Sloanea berteriana	3	0.52	0.16	2	0.81	0.03
Palicourea riparia	5	1.54	0.28	5	4.58	2.78
	Canopy					
Site 1	2	0.58	0.82	3	1.45	0.65
Site 2	3	0.57	0.27	3	0.96	0.49
Site 3	1	1.85	-	1	1.99	-
Dacryodes excelsa	3	1.13	0.73	3	1.51	0.42
Manilkara bidentata	1	0.89	-	2	1.66	0.76
Sloanea berteriana	2	0.22	0.31	2	0.68	0.39

Table 32. Results of analysis of variance to determine significance of differences in fallout.

Test for difference in:	Condition	Species tested	F ratio ^{137}Cs	F ratio ^{144}Ce	F ratio ^{95}Zr	Degrees of freedom in F. ratio
Species	Understory,clean	De,Sb,Mn,Cp,Pr	3.33*	5.10*	2.24	4/12
Species	Understory, plus epiphylls	De,Sb,Mn,Eg,Pr	4.82*	14.76+	3.35*	4/11
Species	Understory, clean	De,Sb,Mn,Cp	0.82	1.39	0.70	3/8
Species	Understory, plus epiphylls	De,Sb,Mn,Eg	1.52	0.41	4.14	3/7
Species	Canopy, clean	De,Mn,Sb	6.66	4.47	1.28	1/4
Species	Canopy, plus epiphylls	De,Mn,Sb	1.92	1.25	2.12	1/5
Site	Understory, clean	De,Mn,Sb	0.83	1.11	5.08*	3/7
Site	Understory, plus epiphylls	De,Mn,Sb	0.81	4.85*	1.11	3/6
Site	Canopy, clean	De,Mn,Sb	1.20	2.31	2.46	3/3
Site	Canopy, plus epiphylls	De,Mn,Sb	0.24	0.16	1.29	2/4
Clean-epiphyll	Canopy	De,Mn,Sb	14.62+	2.45	2.23	1/11
Clean-epiphyll	Understory	De,Mn,Sb,Cp,Eg	8.12+	31.17+	14.10+	1/21
Canopy-understory	Clean	De,Mn,Sb,Cp	0.34	3.91	1.04	1/16
Canopy-understory	Plus, epiphylls	De,Mn,Sb,Eg	0.32	2.74	0.23	1/16

* - significant at 5% level

\+ - significant at 1% level

Table 33. Average values of fallout within and between compartments where no significant differences exist.

	Understory					
	Clean leaves			Leaves plus epiphylls		
	N	$\bar{X}$	1 st.deviation	N	$\bar{X}$	1 st.deviation
All sites and species, except Pr, for ^{137}Cs.	12	3.37	1.13	11	5.30	1.61
Palicourea riparia, for ^{137}Cs	5	5.65	1.59	5	7.82	1.18
All sites and species, except Pr for ^{144}Ce	12	1.65	1.27	11	4.50	1.16
Palicourea riparia, for ^{144}Ce	5	6.59	3.26	5	15.88	4.23
All sites and species, except Pr for ^{95}Zr	12	0.58	0.28	11	1.12	0.41
Palicourea riparia, for ^{95}Zr	5	1.54	0.98	5	4.58	2.78
	Canopy					
All sites, all species for ^{137}Cs	6	3.12	0.68	7	4.57	0.81
All sites, all species for ^{144}Ce	6	3.54	2.83	7	6.47	3.73
All sites, all species for ^{95}Zr	6	0.79	0.65	7	1.31	0.61

There are strong differences between clean and epiphyll covered leaves in the understory, for all isotopes, and for ^{137}Cs in the canopy.

Differences between canopy and understory were not significant. Kline (1967, P.R.N.C. Annual Report) reported differences between canopy and understory leaves for ^{137}Cs, with the understory leaves higher. Epiphyll covered leaves in the understory showed a higher burden than those in the canopy (Table 33), but the difference is not significant. Perhaps differences were obscured because ^{137}Cs levels were lower by a factor of about 4 from the time at which Kline took his samples.

Averages values of fallout within and between compartments where no significant differences existed are summarized in Table 33.

TRITIUM MOVEMENT THROUGH A TROPICAL RAIN FOREST ECOSYSTEM

Movement of tritium through ecosystem is of interest for two reasons: 1) Tritium is a tracer for water, and thus aids in water balance studies of the ecosystem, especially in transpiration studies. 2) Tritium is a major by-product of thermo-nuclear reactions, and could contaminate the environment as a result of both peaceful and military uses of thermonuclear power.

A series of experiments were undertaken to determine rates at which tritium moves through a tropical ecosystem, and the proportion of tritium that is immobilized and thus becomes a long-term radiation hazard in the ecosystem. The experiments were done in cooperative with Dr. Jerry Kline, Argonne National Laboratory, and Dr. John Koranda and Mr. John Martin of Lawrence Radiation Laboratory.

The first experiment involved applying tritiated water to a 0.94 sq. meter plot by simulated rainfall, and collecting runoff water beneath the litter and at a depth of five inches. Results were published in Science 160, 550-551, and the 1968 Terrestrial Ecology Annual Report.

The second experiment consisted of injecting two Dacryodes excelsa and one Sloanea berteriana with a pulse of tritium, and determining the length of time it took for the pulse to reach the canopy, the residence half time of tritium in the free water, and the amount of tritium bound in the leaves by photosynthesis.

The third experiment was called a micro-systems experiment because it was an attempt to measure tritium movement through all portions of a micro-ecosystem, a plot of 3.7 square meters in the middle of the tropical rain forest.

The fourth experiment was a combination of a multi-isotope experiment and a pulsed tritium experiment. The objectives were: 1) to determine if certain gamma emitting isotopes which are similar to nutrient

elements move through trees at the same rate as tritium. 2) to determine the variation in tritium movement through tree throughout a variety of meteorological conditions. 3) to determine if tritium moves uniformly throughout the stem, or if it is concentrated in the outer xylem.

The fifth experiment was to determine tritium uptake, residence half time, and tritium bound by photosynthesis in a secondary successional tropical rain forest.

The sixth experiment consisted of injecting a tree with tritium and one gamma emitter by using a procedure whereby the transpiration stream of the plant was not interrupted, as a check on the other tree injection experiments where the transpiration stream was interrupted.

The first tree injection experiment was reported by Dr. Jerry Kline at the 1969 meetings of the American Nuclear Society. Following is an abstract of the paper.

Measurement of Water Behavior in Tropical Trees Using Tritiated Water

Abstract

J.R. Kline[1], John Martin[2], Carl Jordan[3], John Koranda[2]

Water utilization by plants in one of the most widespread processes in biology. Ecologists seek more detailed information on water relationships in terrestrial ecosystems as part of a general quest for deeper understanding of their functional processes. Modern nuclear technology adds urgency to the acquisition of knowledge on the functions of water in the environment since both peaceful and military nuclear operations could contaminate biological systems with tritium as a major by-product of thermonuclear reactions.

Despite this need for information, there is little detailed quantitative data available. The Rain Forest Project of the Puerto Rico Nuclear Center and the Ecology Group of the Biomedical Division of the Lawrence Radiation Laboratory, Livermore, Ca. have cooperatively initiated a series of experiments on this problem using tritiated water as a tracer. The first experiment in the series is reported here.

The objective of the initial experiment was to determine the response of several tropical rain forest trees to the injection of a pulse of tritiated water. Secondary objectives included monitoring of air surrounding the experimental site and of involved personnel to establish appropriate safeguards in the execution of such experiments.

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[2]Lawrence Radiation Laboratory, Livermore, Ca.
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Three tropical trees representing two species were injected with tritiated water through holes bored in the trunks near ground level. The movement of the labeled water was monitored by sampling leaves from a tower which had been previously erected nearby. Leaf samples were collected, at first several times daily; and later, once daily, and sealed in plastic bags and frozen prior to analysis. Samples were analyzed by extracting tissue water in a specially designed high vacuum freeze drying apparatus, and then counting the water by standard liquid scintillation techniques.

The pulse of tritium reached a peak in all leaves approximately five days following injection, after which concentrations of the isotope declined. The time required for the isotope to reach the crown of the trees was not dependent on the height of the tree. The same time was required for a tree seven meters tall as for two about twenty meters tall. Tritium did not pass through the trees in a symetrical pulse. After the peak was reached, tritium concentrations died away exponentially with half residence times ranging from approximately two to eight days. The largest differences in tritium residence times were found between species, suggesting that they have different adaptations for water use even though they occupy essentially the same environment. The dieaway curves showed several erratically spaced peaks and valleys during the course of the experiment. This was suggested to be due to exchange of leaf tissue water with uncontaminated rain water without corresponding exchange in the xylem elements of the tree.

It was concluded from this experiment that: (1) Tritiated water is a safe powerful tool for the detailed assessment of water use by plants in the field. (2) Tritiated water persists in tropical trees with appreciable residence times even though large amounts of rainfall occurs in the rain forest. (3) Rains bearing tritium will probably cause leaf tissue water to become labeled immediately due to the exchange of water on leaf surfaces. (4) The persistence of tritium in an entire forest may be longer than that shown by single tree experiments due to possible recycling of tritium which may be exchanged at leaf surfaces and carried to the rooting zone of plants by rainfall.

MICRO-SYSTEMS EXPERIMENT

Because an ecosystem, when studied as a whole, often shows properties different than, or not apparent in, the sum of all its parts, an attempt was made to study movement of tritium through all portions of an ecosystem at one time.

METHODS

A plot of ground, 230 cm by 160 cm was outlined with string. One side of the plot was cut away, and soil water collectors ("Zero-Tension Lysimeters", Jordan 1968, Soil Science 105, 81-86) were installed. Each

lysimeter collected water from a 24 sq. inch area. Two lysimeters were installed beneath the litter, two at a depth of 5 inches, two at 10 inches, and two at 15 inches. The bulk density of the soil from the surface down to about 10 inches averages 0.57, at which point there is a region where the bulk density changes quite rapidly to a value of approximately 1.02.

Leaves for analysis for free and bound water were picked from three trees growing inside the plot. They were a Dacryodes excelsa, 2.31 inches basal diameter, Microphilous garcinifolia, 2.06 inches basal diameter and Manilkara bidentata, 1.44 inches basal diameter. Transpiration water was collected from two other smaller trees, a Palicourea riparia, 0.56 inches basal diameter, and a Manilkara bidentata, 0.72 inches basal diameter, by the following method: A plastic bag was put over a bunch of leaves still on the tree; a floodlight was shone on the leaves to increase transpiration; air was pumped out of the bag and through a condensing tube submerged in a dry ice-alcohol mixture, and then back into the bag. About 2 ml. of water collected in the condensing tube in a half hour.

Free water was extracted from the picked leaves by freeze and dry methods using high vacuum apparatus.

Cores of wood were taken from the buttresses of two large trees whose roots extended into the plot. The trees were Buchenavia capitata, 17.75 inches d.b.h., and Tetragastris balsamifera, 8.93 inches d.b.h. Free water was extracted from the wood with the freeze dry apparatus.

Water vapor was collected at 8 points immediately surrounding the plot, at 8 points about 3 meters distant from the plot, and at 4, 100, and 175 cm above the plot. At the 3 meter points, the water was collected in the following manner: An aluminum tube 1 1/2 inches in diameter and about 1 1/2 feet long was inserted in an ordinary wide-mouth thermos bottle so that one end of the tube extended about 8 inches out of the bottle. One cup of liquid nitrogen was poured into the bottom of the thermos. Water vapor condensed and froze on the protruding portion of the aluminum tube. When the liquid nitrogen boiled away, the ice melted and the water ran into the bottle where it could be collected. For the other points, water vapor was collected as follows: Rubber tubing was extended from the collection points to a condensation tube in the same manner as for transpiration water. Air and water vapor were pumped into the tube, the water vapor condensed, and was later collected.

The following method was used for applying tritium to the plot. Fifty millicuries of tritium were diluted into 4 liters of water. The water was siphoned through a polyvinyl tube and ordinary shower head, and applied evenly to the plot. Before the actual application, test runs were made to practice uniform application.

Water vapor samples were collected 15, 80, 165 and 240 minutes, 2 days and 6 days after application. Leaf, wood and water samples were collected daily for a week, and weekly thereafter.

Rainfall was measured above the canopy with a standard U.S. Weather Bureau recording rain guage, and below the canopy with two 5 ft. x 2 in. x 12 in. trough type rain guages. Wet and dry bulb temperatures were measured on every collection date.

Water samples were analyzed by standard liquid scintillation techniques. Known standards were included with the samples and results were converted to decompositions per minute.

EVAPORATION OF TRITIUM FROM THE SOIL

The water vapor from the collectors on the ground surrounding the plot showed decreasing specific activity with distance away from the plot. On the afternoon following the tritium application, specific activity decreased with distance most slowly on the uphill side of the plot, most rapidly on the downslope side, and intermediately on the North and South sides (Table 34). This indicates a slight upslope wind was blowing during the afternoon. Other ground collectors at greater distances showed the same trend as that shown in Table 1.

To calculate the quantity of tritium lost through evaporation from the soil, the collections from 4 cm, 100 cm and 175 cm above the plot were used. Procedures for calculations were as follows. No decay corrections were made because of the relatively long half life of tritium (12 years).

Specific activity of the water vapor was plotted as a function of distance above the plot, for each sampling time (Fig. 9). Specific activity of the water vapor at ground level immediately after application was taken to be the same as the specific activity of the solution applied (50 mCi in 4 liters equals 11×10^{10} dpm in 4000 ml. equals 2.75×10^{7} dpm/ml). Attempts were made to fit a curve to the points by using least square fit to a quadratic ($Y = ax^2 + bx + c$) and least squares fit to a parabolic ($Y = ax^b$), but neither resulting equation yielded a line that fit the data satisfactorily. Therefore, specific activity of water vapor at ground level at times after application was estimated by extrapolation of the curves of Fig. 1 (Table 35), using a flexicurve.

Specific activity of water vapor at ground level, (Table 35) was then plotted as a function of time after application, using three different time scales (Figs. 10, 11,and 12).

Average specific activity for each given time period was then taken from Figs. 10, 11 and 12 (Table 36).

Odum and Jordan (1969) estimated evaporation from the soil to be 36 $g/m^2/day$. If evaporation occurs only during the daytime, the average is $3g/m^2/hr$. during the daylight hours. Since the tritium plot was 3.68

Table 34. Specific activity of water vapor collected around the tritium plot following application.

Location of collector in relation to plot	Date Time	Specific activity, dpm/ml 5/15 12:45	5/15 13:50	5/15 15:15	5/15 16:30	5/17 16:00	5/21 15:00
50 cm. upslope (West)		2.81×10^5	1.34×10^5	1.02×10^5	N.D.	1.71×10^3	893
30 cm. North		1.87×10^5	1.05×10^5	9.04×10^4	6.51×10^4	1.58×10^3	915
30 cm. downslope (East)		6.35×10^4	6.33×10^4	822(?)	N.D.	1.74×10^3	747
60 cm. South		1.4×10^5	1.34×10^5	4.63×10^4	7.13×10^4	1.17×10^3	342
4 cm. above plot		6.74×10^5	3.68×10^5	2.33×10^5	2.24×10^5	5.53×10^5	2.43×10^3
100 cm. " "		6.7×10^3	4.6×10^3	6.1×10^3	N.D.	137	127
175 cm. " "		1.2×10^3	447	1.3×10^3	762	47	78

Table 35. Specific activity of tritiated water at soil surface as dete by extrapolation of curves.

Date	Time	Specific activity dpm/ml
5/15	12:45	2.75×10^7 *
5/15	13:50	1.0×10^6
5/15	15:15	5.0×10^5
5/15	16:30	4.0×10^5
5/17	16:00	1.2×10^4
5/21	15:00	4.0×10^3

* calculated

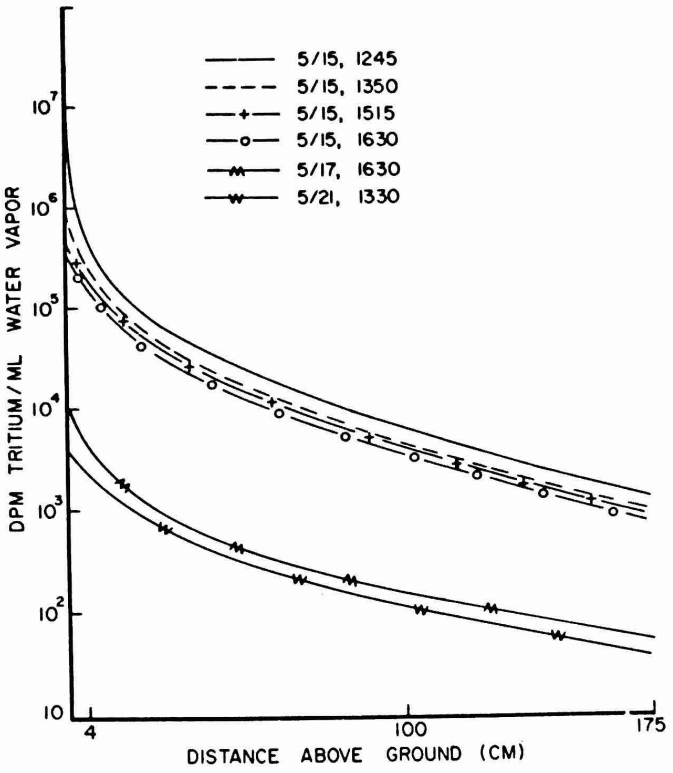

Fig. 9. Specific activity of tritium in the water vapor above the experimental plot as a function of distance above the plot.

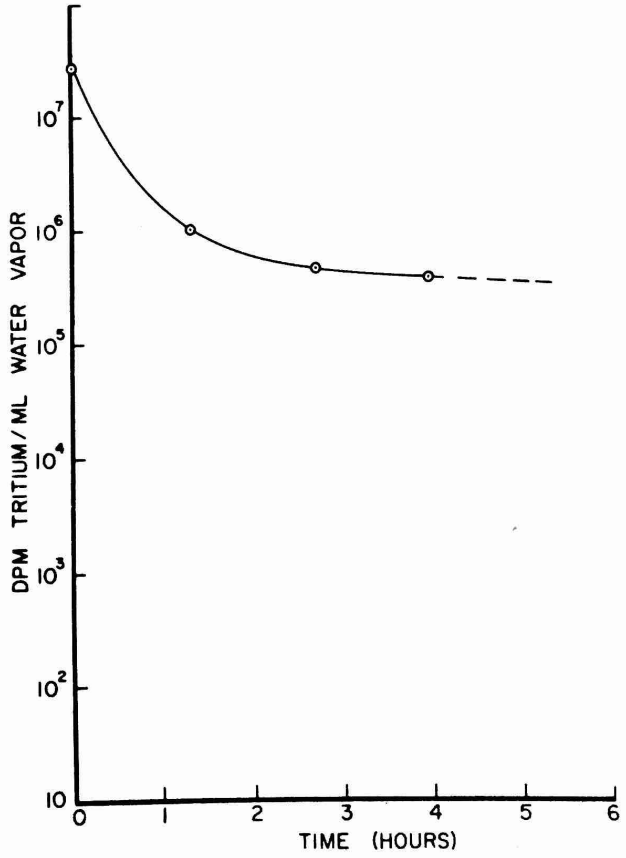

Fig. 10. Specific activity of tritium in the water vapor at ground level as a function of hours since tritium application.

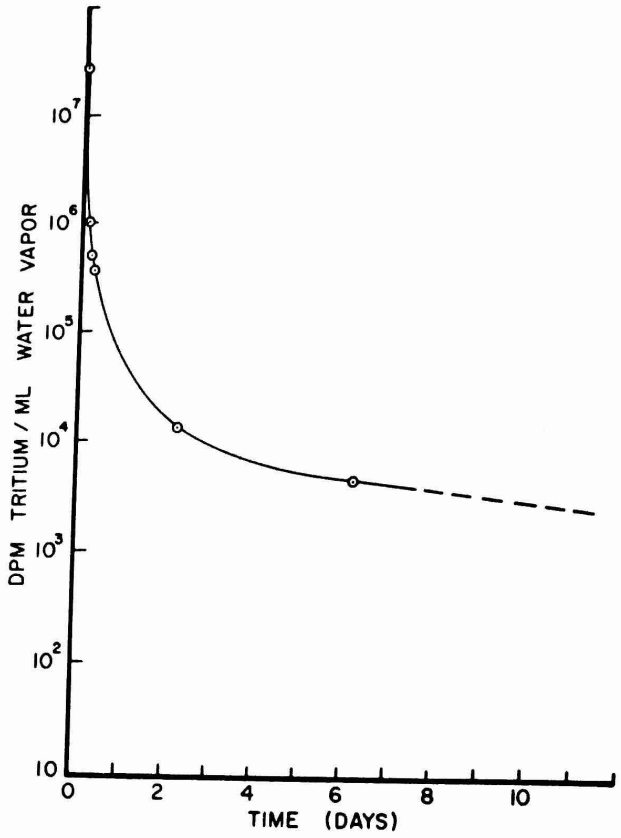

Fig. 11. Specific activity of tritium in the water vapor of at ground level as a function of days since tritium application.

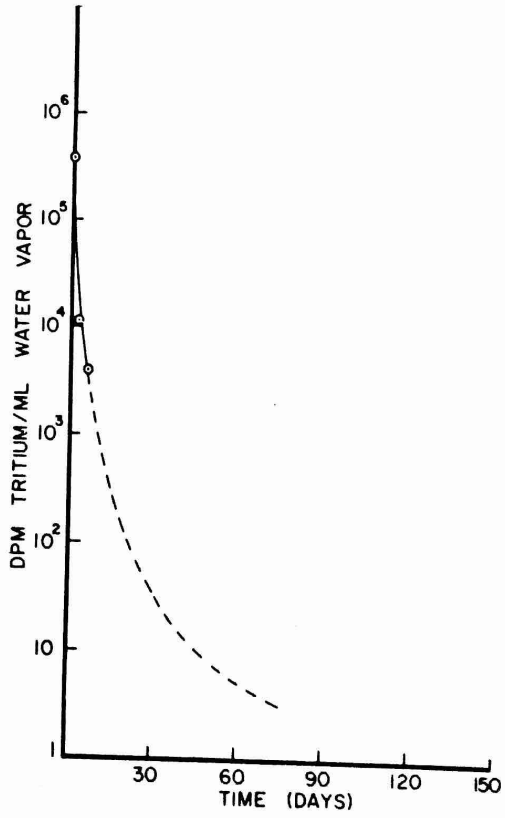

Fig. 12. Specific activity of tritium in the water vapor at ground level as a function of months since tritium application.

Table 36. Specific activity of tritiated water at soil surface as determined from curves, and evaporation of tritiated water from the plot.

Date	Time	Specific activity dpm/ml	DPM evaporated during period	Percent of total dpm applied	Percent of total dpm evaporated	Cumulative total percent evaporated
5/15	12:30-13:00	1.0×10^7	5.5×10^7		51.4	51.4
5/15	13:00-14:00	1.7×10^6	18.7×10^6		17.4	68.8
5/15	14:00-15:00	6.0×10^5	6.6×10^6		6.1	74.9
5/15	15:00-16:00	4.5×10^5	49.5×10^5		4.6	79.5
5/15	16:00-17:00	4.0×10^5	44.0×10^5		4.1	83.6
5/15	17:00-18:00	3.7×10^5	40.7×10^5		3.8	87.4
5/16		4.5×10^4	59.4×10^5		5.5	92.9
5/17		1.6×10^4	21.1×10^5		1.9	94.8
5/18		8.5×10^3	11.2×10^5		1.0	95.8
5/19		6.2×10^3	81.8×10^4		0.8	96.6
5/20		5.1×10^3	67.3×10^4		0.7	97.3
5/21		4.0×10^3	52.8×10^4		0.6	97.9
5/22		3.8×10^3	50.2×10^4		0.4	98.3
5/23 - 5/30		1.5×10^3	138.6×10^4		1.3	99.6
5/31 - 6/30		2.5×10^2	231×10^3		0.2	99.8
7/1 - 7/31		50	204×10^3		0.2	100
		Total	10.7×10^7	.09%		

m^2, 11.04 ml/hr evaporated from the entire plot. Specific activity for a given time period (Table 36) was multiplied times length of time period times 11 ml/hr to give total dpm evaporated during the time period. Total tritium evaporated was 10.7×10^7 dpm, or .09 percent of the total tritium applied.

Fifty one percent of the total evaporation took place during the first one half hour, and 87 percent by the end of the first day (Table 36).

Movement of Tritium Through the Soil and Trees

Specific activity of tritium in the soil water at each depth was plotted as a function of time since application. It is immediately apparent that there are at least two residence half-times of tritium at each depth. Individual points of specific activity vs. time are shown in Fig. 13 to illustrate how clear the break is between the two release rates of tritium. When a least squares straight line regression is calculated for each release rate, the first residence half time in the litter is 1.7 days, and the second is 30 days. The first release rate is approximately equal to that predicted by Odum and Bloom (1969, in press) based on total free water in each ecosystem compartment, and rate of movement of water between compartments. Therefore, it may be safe to assume that this release rate represents total free water turnover in the litter.

The second release rate, however, was not predicted by Odum and Bloom. A hypothesis to explain the second release rate is based on the presence of a thin film of water which surrounds individual soil particles, soil algae, and decomposing organic matter. This water is called hygroscopic water. It is bound to the individual particles, and water molecules in this film are not freely exchangeable with the pool of free water. Some exchange does occur however. As the pulse of high specific activity moves through the litter and soil, some of the tritiated water in the free water pool undoubtedly exchanges with the hygroscopic water. After the peak of specific activity passes downward and the specific activity in the free water becomes lower than that of the hygroscopic water, tritium diffuses outward, the rate of diffusion being governed in part by the amount of bound tritium and the difference in specific activity in the hygroscopic water and the free water.

Further evidence for this hypothesis is shown in Fig. 14, where following a period of heavy rainfall, specific activity drops rapidly, due to the high dilution of the tritium diffusing out from the hygroscopic shell, and then jumps up again during a relatively dry spell, when the out-diffusing tritium is less diluted.

Specific activity as a function of time, for each of the four depths sampled, is shown in Fig. 15. The buildup of specific activity at the 10 and 15 inch depths is clear, as the peak of specific activity moves downward and broadens. After outward diffusion of tritiated water from the

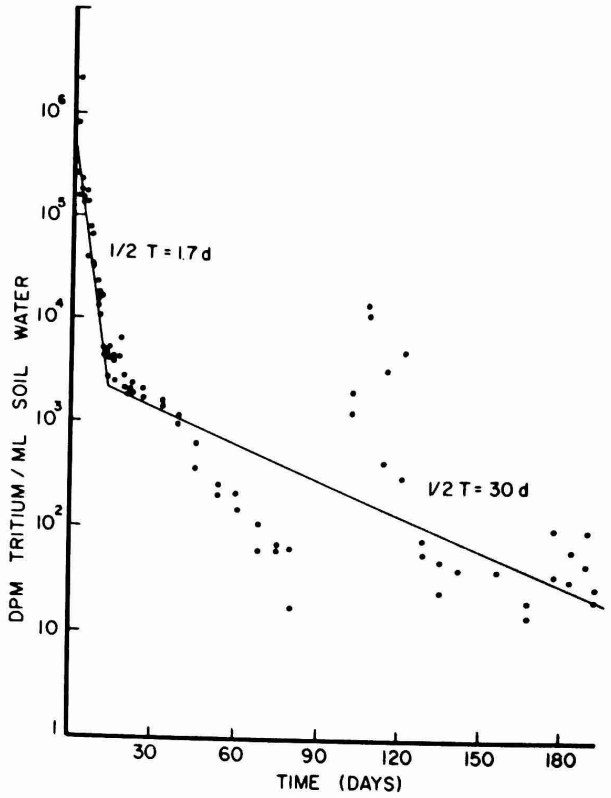

Fig. 13. Specific activity of tritium in the water leaving the litter layer as a function of time since tritium application.

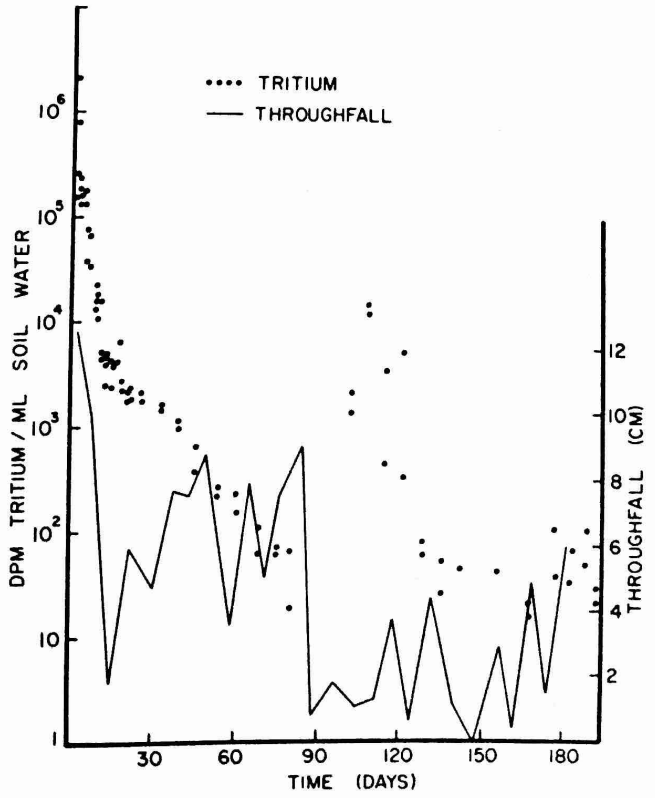

Fig. 14. Specific activity of tritium in the water leaving the litter layer compared to amount of rain reaching the forest floor.

hygroscopic shell begins, one half residence time at the 5 inch depth is 15 days, and at the 10 and 15 depth it is 32 days. The differences in half times between the different soil depths could be explained by differences in soil structure. At the 5 inch depth, the clay particles are well aggregated (bulk density is 0.57) and therefore the reservoir of bound tritium is not as large as at the lower depths, where there are more clay particles per unit volume (bulk density is 1.02).

The release rate of tritium after 165 days (Nov. 1) should change again for the 5 inch depth. Theoretically, the specific activity at any depth cannot be lower than that in the soil above, because if it starts to get lower, inward diffusion of tritiated water into the hygroscopic shell begins, as the water from above moves down, thus increasing the specific activity again.

When specific activity is plotted as a function of depth on a given day, much less scatter appears in the data points (Fig. 16). When a series of these functions is plotted on a single graph, a picture emerges of the movement through the soil of the peak of maximum specific activity (Fig. 17). The pattern is wave-like, moving downward through the soil, gradually decreasing in wave height.

Specific activity as a function of time for soil water at the 5 inch depth is compared for two experiments in Fig. 18. In the experiment initiated on Feb. 14, 1967, (Kline and Jordan, 1968), the tritium has a residence time similar to that of the micro-systems experiment. A big difference, however, occurs in the initial few days of the experiment. In the earlier experiment, specific activity increased during the first few days, whereas in the micro-systems experiment, maximum specific activity occurred the first day. The difference can be explained by the rainfall pattern following tritium application. In the earlier experiment, only 0.24 inches of rain fell during the first 2 days following tritium application. Thus there was relatively little free water in the soil, and little opportunity for diffusion of the tritiated water downward into the free water at the 5 inch depth. In the micro-systems experiment however, 2.3 inches of rain fell the night following tritium application, and apparently there was sufficient water in the soil to permit diffusion of the peak of maximum activity down to the 5 inch level the first night. In fact, there was sufficient water to permit diffusion down to a depth of 15 inches the first night (Fig. 15).

The pattern of tritium movement through the trees is influenced by the pattern of movement through the soil. Since tritium has a long residence time in the litter and soil, the roots of trees are exposed to tritiated water for a relatively long time. Specific activity of tritium in the transpiration water is affected by several factors: 1) Distribution of roots with depth in the soil 2) Specific activity of tritium at each depth 3) Water vapor deficit of the air, which affects rate at which water is pulled through the plant 4) Light, which indirectly controls transpiration through regulation of stomatal openings. 5) Proportion of roots which are in the contaminated plot (not applicable, of course, in a wide-

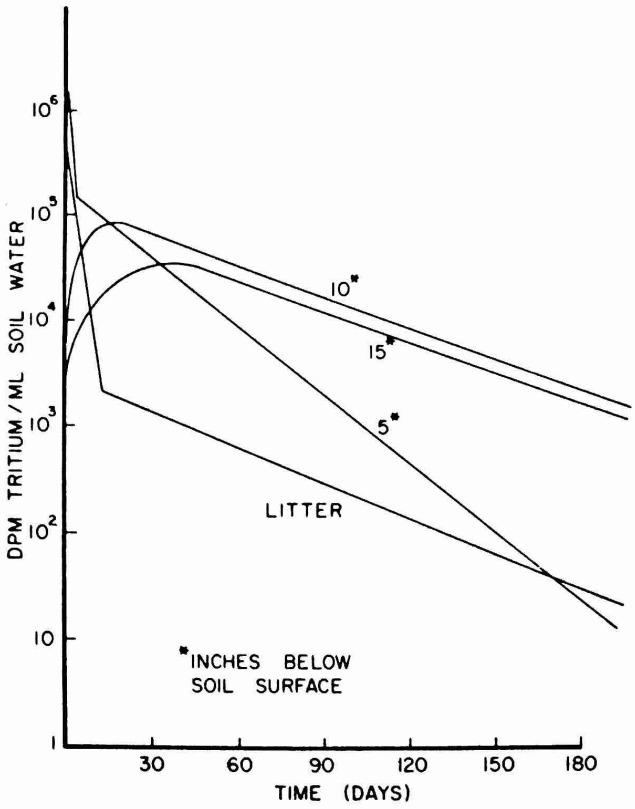

Fig. 15. Specific activity of tritium in the soil water at four depths as a function of time.

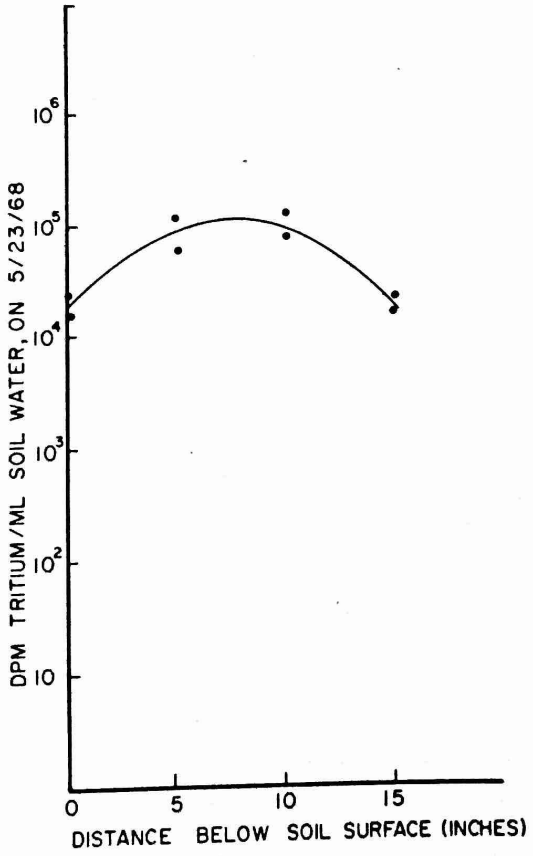

Fig. 16. Specific activity of tritium in the soil water as a function of depth, eight days after tritium application.

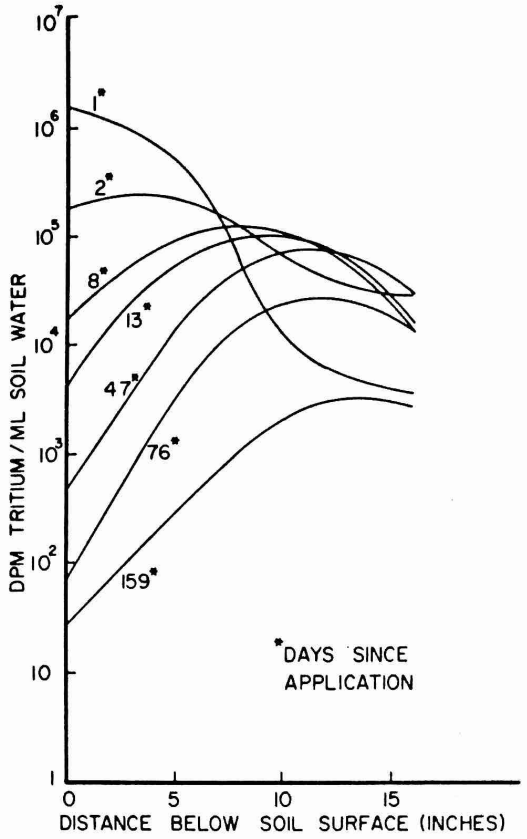

Fig. 17. Specific activity of tritium in the soil water as a function of depth, at intervals following tritium application.

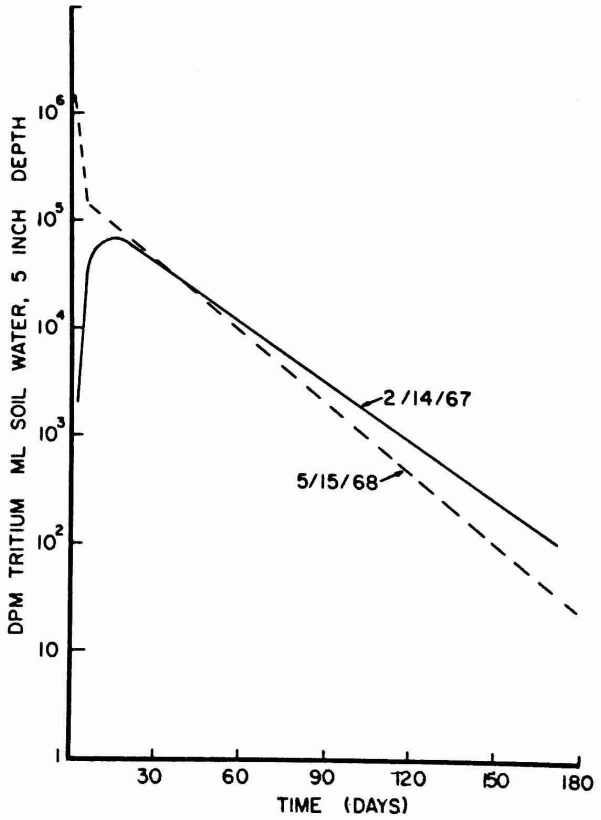

Fig. 18. Specific activity of tritium in the soil water at the five inch depth as a function of time since tritium application, for two experiments.

spread fallout situation). As a result of all these factors, data points of specific activity of tritium in leaf and wood water as a function of time show much scatter, after the initial buildup. If a least squares regression of DPM on time is performed, residence half times range from 25 to 50 days. However, if data points are averaged together (weekly averages for the first month, then monthly averages), a die-away curve appears that follows the trend of specific activity in the litter and soil (compare Figs. 19, 20 and 13).

In the roots of the larger trees, yet another phenomena seems to be involved (Fig. 21). The first little peak may represent water taken out of the litter by rootlets in that layer, while the second, more diffuse peak, may represent water taken up by rootlets deeper in the soil.

A comparison of the prediction of specific activity of tritium in ecosystem compartments based on total water content only, and experimental results of the micro-systems experiment are shown in Fig. 22. Because of the hypothesized diffusion of tritium into and out of the hygroscopic shells, residence half time in the tropical rain forest ecosystem is increased by a factor of five to ten.

Movement of ^{137}Cs, ^{86}Rb, ^{85}Sr, and ^{54}Mn through Canopy Trees

Movement of gamma emitters through large trees was measured in two ways. (1) A portable rate-meter with a G-M tube was connected by a coaxial cable to a portable scaler that was carried to the area of injected trees. The G-M tube was fastened to a pole in such a way that the tube could be held flush against the tree without the field assistant getting closer than 8 feet from the radioactive tree. As the field assistant placed the tube against the tree from the adjacent tower, the operator determined gross counts per minute with the scaler. (2) Various parts of the tree were collected periodically, oven dried, and counted for 100 minutes in a 400 channel gamma analyzer. When more than one isotope was present in a sample, it was necessary to solve simultaneous equations to quantify each isotope in the sample.

A tree of the species Matayba domingensis, 31 cm. d.b.h. and 52 ft. high was injected with .46 millicuries of ^{137}Cesium on Sept. 18, 1968. Table 37 shows the portable scaler readings. At the base of the tree there was an increase in activity for seven days, followed by a gradual decrease. This downward movement is confirmed by Table 38 which shows the wood at the base of the tree to be somewhat radioactive, and the bark to be very radioactive 20 days after the injection. The high level of activity 1 ft. above the injection hole 20 days after injection (Table 38) and the low level between holes indicates very little translocation laterally across the xylem cells as compared with longitudinal movement. Portable scaler counts between the injection holes, and at an injection hole (Table 37) show a gradual decline in activity, indicating a movement of the ^{137}Cs away from the injection holes. The activity rose to a maximum at six feet,

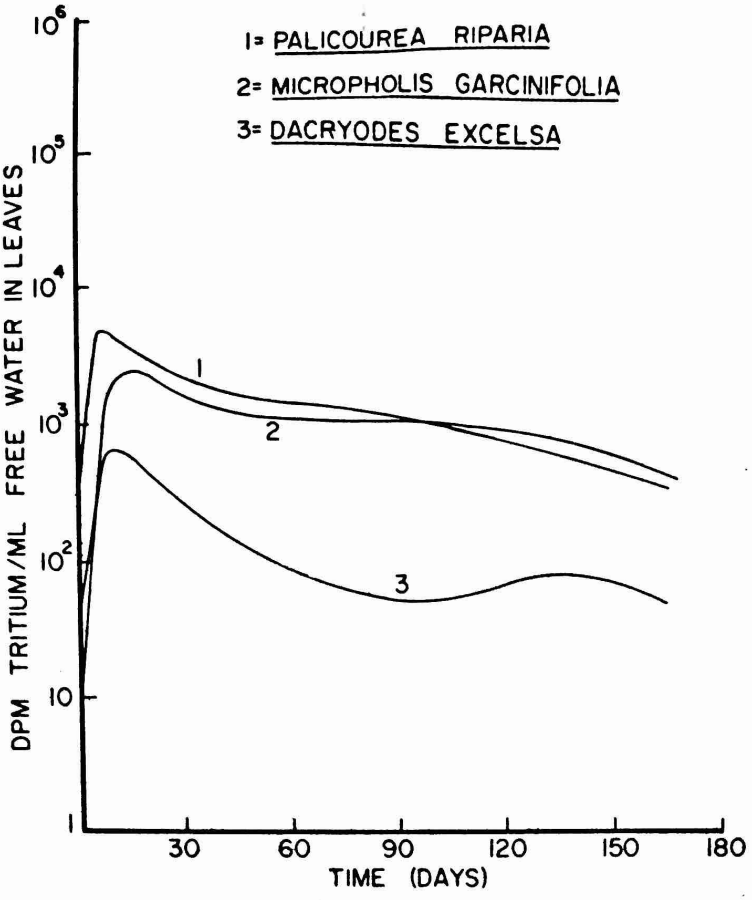

Fig. 19. Specific activity of tritium in the free water of leaves of three species, as a function of time since initiation of the experiment.

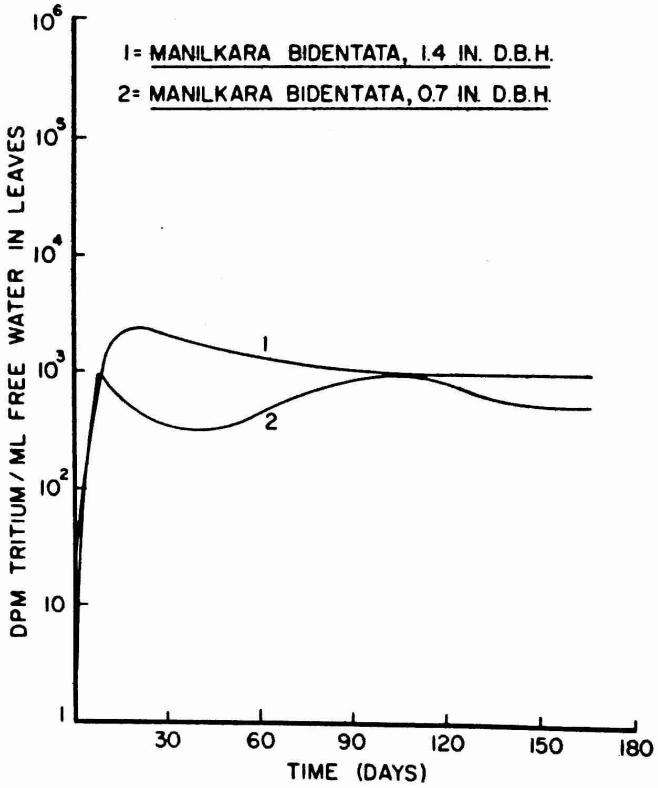

Fig. 20. Specific activity of tritium in the free water of leaves of two trees of the same species, as a function of time since initiation of the experiment.

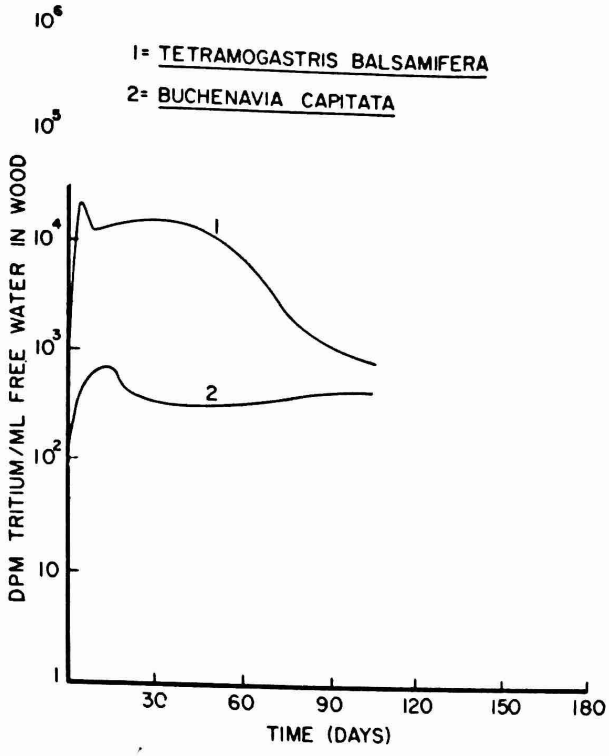

Fig. 21. Specific activity of tritium in the free water of root buttresses of two trees as a function of time since initiation of the experiment.

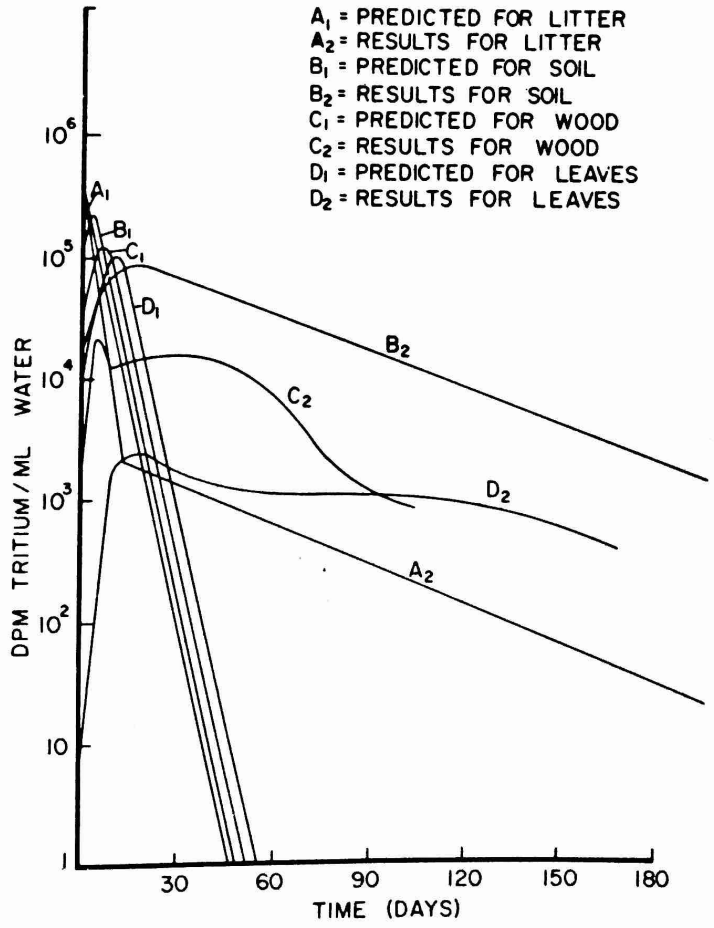

Fig. 22. Experimental results of tritium residence time in the forest compared to predicted residence times.

Table 37

Portable scaler readings on Matayba domingensis
Background is 30 cpm.

		Location					
		Counts per minute					
Date	Days since injection	Base of tree	Between injection holes	At injection holes	6 ft.	21 ft.	35 ft.	42 ft.
9/18	0	198	10,049		251			
9/19	1	255	10,997	20,555	257	28		
9/20	2				315	49		
9/21	3	781	9,216	15,311	328	50		
9/22	4	951	9,071	15,821	307	50		
9/23	5	986	8,190	15,778	265	65	30	
9/25	7	1010	8,282	13,987	203	98	41	22
10/1	13	943	7,256	10,213	188	56	64	44
10/8	20	973	6,677	10,302	174	60	70	74
10/15	27	946	5,711	9,016	142	67	58	50
10/29	41	921	4,570	7,514	130	45	59	46
12/2	75	500	614	405	103	48	40	
1/28	132	473	1,783	2,502	130	61	48	47

Table 38. Movement of ^{137}Cs through Matayba domingensis

	Days since injection			
Sample	20	37	75	132
	Activity in picocuries per gram dry weight			
Leaves	65	398	384	407
Twigs			348	508
Wood, 1 ft. above injection holes	5,569		144	464
Wood, at level of injection holes	46			704
Wood, base of tree	5,929		125	373
Bark, base of tree	170,382		17,213	6205

three days following the injection; a maximum at 21 ft., seven days after; and a maximum at 35 and 42 ft. 20 days after. Table 38 shows that the leading edge of the pulse of activity reached the leaves sometime between the 20th and the 37th day. The relatively stable level of activity after the 37th day could indicate that a steady rate of input to the leaves had occurred, and that cesium was being leached from the leaves at the same rate it was being supplied to the leaves. By 132 days, there was a fairly uniform distribution of the cesium throughout the tree, with the exception of the bark near the base.

A tree of the species Dacryodes excelsa, 51 cm. d.b.h. and 60 ft. high was injected with 17.69 millicuries of ^{86}Rb, 0.19 millicuries of ^{85}Sr, and 0.34 millicuries of ^{54}Mn on Sept. 18, 1968. Interpretation of the portable scaler data (Table 39) is more difficult than for the $^{137}Cesium$ injected tree because of the presence of three isotopes, and their relatively short half lives. Nevertheless, the same trends as in the Matayba can be detected (Table 37). The peak of the downward moving pulse occurs at the base of the tree about 7 days after injection, and activity at the level of the injection holes gradually declines. The peak passes the 6 ft. level on the 5th day, and the 21 ft. level and above at about 3 weeks.

Tables 40, 41, and 42, show downward movement of all three isotopes, presumably in the phloem which was included in the bark samples, with ^{86}Rb showing the fastest movement. At 132 days after injection, ^{86}Rb was still increasing in the leaves (Table 40). Data for day 132 indicates that the peak of upward moving ^{86}Rb is somewhere between 20 ft. above the injection hole and the twigs in the canopy.

During Jan., 1969, a large increase in fallout in the El Verde area resulted in an obscuration of ^{85}Sr and ^{54}Mn data after the 75th day. However, it is clear that both isotopes had only reached approximately 24 ft. (20 ft. above injection holes) by the 75th day.

All isotopes not only moved downward in the trees, they also moved out of the roots into the litter and soil (Table 43). All isotopes were found in litter and mineral soil samples except ^{86}Rb, which was found only in the litter. As a check to see that the isotopes actually were transferred out of the tree, all organic matter was separated (by agitation and flotation) from mineral soil, and the mineral soil only checked for activity. All isotopes except ^{86}Rb were present.

A curious phenomena occurred on Dec. 2, the 75th day. In the portable scaler readings on Matayba domingensis (Table 37), the values at the injection hole level and above all declined, then increased again on the 132 day. Table 38, which shows the results of the gamma analysis, indicates the same thing. Portable scaler readings on Dacryodes excelsa at the injection level (Table 39) show the same trend, as well as Table 40 for ^{86}Rb. No explanation is apparent for this phenomena.

Table 39

Portable scaler readings on Dacryodes excelsa
Background is 30 cpm.

		Location						
		Counts per minute						
Date	Days since injection	Base of tree	Between injection hole	At injection hole	6 ft.	21 ft.	35 ft.	42 ft.
9/18	0	232	8,712	34,902	341			
9/19	1	202	9,023	30,867	340	33		
9/20	2				229	34		
9/21	3	234	9,807	33,111	446	36		
9/22	4	228	8,366	32,342	415	53		
9/24	5	214	7,604	31,513	493	65	49	39
9/25	7	246	7,622	29,609	338	51	40	36
10/1	13	239	6,476	24,732	381	69	54	43
10/8	20	242	5,684	17,914	279	80	54	46
10/15	27	204	4,558	15,792	310	69	46	46
10/29	41	179	3,210	12,140	151	64	38	43
12/2	75	97	2,246	3,327	107	53	47	
1/28	132	68	1,493	5,632	74	41	47	27

Table 40. Movement of ^{86}Rb through Dacryodes excelsa

	Days since injection			
Sample	20	37	75	132
	Activity in picocuries per gram dry weight			
Leaves	2,271	3,386	10,120	15,193
Twigs			20,405	65,116
Wood, 20 ft. above injection hole	19,991		2,267	14,496
Wood, 1 ft. above injection hole	26,142		9,784	13,721
Wood, base of tree	87,990		26,439	42,403
Bark, base of tree	626,450		not detectable	

Table 41. Movement of ^{85}Sr through Dacryodes excelsa

Sample	Days since injection: 0	20	75
	Activity in picocuries per gram, dry weight		
Leaves	—	6.0	12.0
Twigs			23.6
Wood, 20 ft. above injection hole			63.4
Wood, 1 ft. above hole			0.0
Wood, base of tree		0.0	2.2
Bark, base of tree			3,385

Table 42. Movement of ^{54}Mn through Dacryodes excelsa

Sample	Days since injection: 20	75
	Activity in picocuries per gram, dry weight	
Leaves	7.9	16.9
Twigs		50.7
Wood, 20 ft. above injection hole	12.7	68.1
Wood, 1 ft. above injection hole		15.4
Wood, base of tree	0.0	27.9
Bark, base of tree	0.0	8,020.0

Table 43. Activity in litter and soil 96 days after tree injections. Matayba and Dacryodes are 15 feet apart.

Isotope	Location				
	Litter, 5 ft. from Matayba	Soil, 5 ft. from Matayba	Litter, 5 ft. from Dacryodes	Soil, 5 ft. from Dacryodes	Mineral soil only, 5 ft. from Dacryodes
	Activity in picocuries per gram, dry weight				
^{137}Cs	2,532	3,506	622	68	149
^{85}Sr	31	70	77	8	11
^{54}Mn	0	0	127	2	4
^{86}Rb	0	0	4	0	0

PART B - SECONDARY SUCCESSION IN THE IRRADIATED AREA

METHODS

Description of Site

The study area is located near El Verde, in the Luquillo Experimental Forest of eastern Puerto Rico. The site is at an elevation of 1500 feet, in a forest described as Tabonuco type (Wadsworth 1951). Annual rainfall is approximately 240 cm. per year, with more than 10 cm. every month. The terrain consists of a series of sharply sloping ridges and ravines. Average height of the forest top is 65 feet.

The studies of early succession were made in the area affected by gamma radiation. In 1966, the area surrounding the source location, out to about 15 meters, was virtually barren of canopy leaves (Figs. 1, 2). By August 1966, canopy dieback had ceased (Table 1).

There are two distinct soil types in the irradiated area, one reddish yellow (7.5 YR/6/8) (Munsell 1954) and associated with the ridges, and another dark brown (10 YR/4/3) and associated with the ravines. Richards (1957) states that the reddish-yellow color of the soil formed under conditions of unimpeded drainage in the tropics is due to the abundance of iron oxides, while non-peaty swamp soils often have a grey or brown color, and occur under conditions of superabundance of water and poor aeration. For convenience, the reddish-yellow soil will be called "oxidized" soil, and the brown soil "reduced".

Soil color was used to delimit boundaries of two communities within the irradiated area.

Studies in a later stage of succession were made in the forest surrounding the irradiated area. To simplify discussion, the surrounding forest will be called the "mature" forest, even though it contains some successional species, and the irradiated area will be called the "successional" area.

Grid

To facilitate measurement of vegetation in the irradiated area, a grid-work of nylon line was laid out in one meter squares, 26 meters on each side, with the center of the gridwork coinciding with the source location. On the four cardinal axis, a strip of squares two meters wide was run out to 30 meters from the source.

Fig. 1. Photograph of the irradiated area in August, 1966. Pipes in the center of the picture supported the source during irradiation.

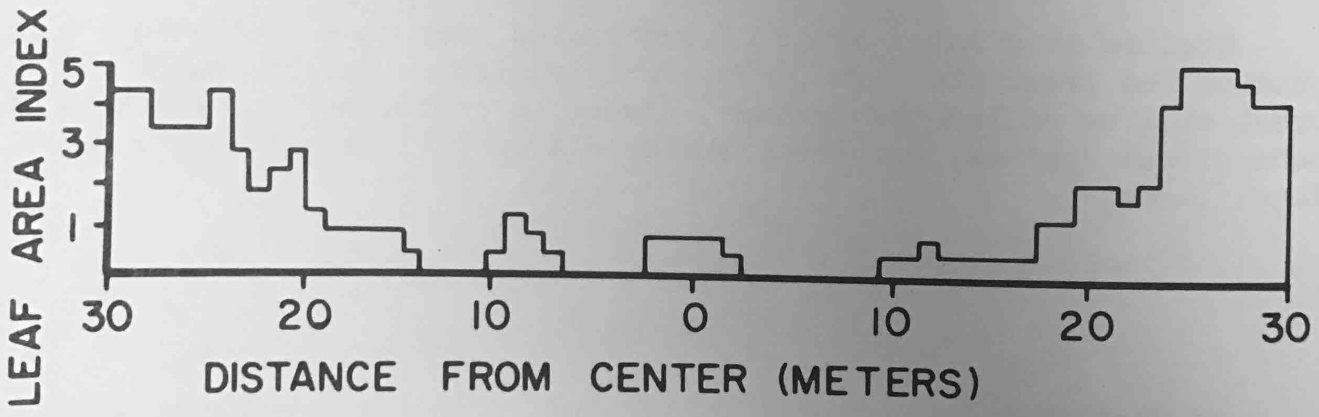

Fig. 2. Leaf area index of the irradiated area in August, 1966. Leaves directly at the center are of the large Cyrilla racemiflora which were partially shielded from radiation by the plug above the source.

Sampling

Measurements of all the plants within the 676 square meter grid were made in the fall of 1966, 1967, and 1968. In 1966 and 1967, measurements were made of basal diameter, diameter at 30 cm., and of height, of all plants with single stems, including individual sprouts. Since it was later determined that basal diameter alone was an adequate predictor of biomass (see next section), basal diameter only was measured in 1968. For plants with stilt roots such as Cecropia peltata, basal diameter measurements were made above the roots. Basal diameters were measured to the nearest 1/128 of an inch with micrometer calipers.

For ground cover species such as grasses, sedges, and Desmodium, percent cover of each square meter was estimated, and then percent of total possible density within areas covered was estimated.

Leaf Fall

Square meter leaf fall collection baskets had been placed throughout the site during the radiation experiment (Odum In Press). Leaf fall during the period following radiation was high in the area surrounding the source due to die-back of the canopy. After August, 1966, there was no further measurable dieback of the canopy (Table 1) and therefore, presumably, no leaf fall in the irradiated area due to canopy die-back. From June, 1966, 14 months after cessation of radiation, through March, 1968, the 10 collection baskets within the area where canopy die-back had occurred yielded a relatively constant amount of leaves, except during the period of May through July, when the amount increased, as does leaf fall throughout the forest (Kline and Jordan, 1967 and 1968 Annual Reports). Average leaf fall during the post die-back period was 0.63 $g/m^2/day$.

Leaf fall in the mature forest was taken from Odum and Jordan (In Press).

Biomass of Successional Vegetation

Ten individuals of each of 15 common successional species, ranging in diameter from 1/8 inch to two inches, were taken from other successional sites in the vicinity of the study area.

The above ground portions of the plants were clipped off, and the roots were carefully extracted from the soil. The entire plant was then dried and weighed.

Correlation coefficients were made between heights, diameters, and weights (Table 2). Since basal diameter and height were closely correlated there was little to be gained by using height in addition to basal diameter as a predictor of biomass. Because basal diameter and diameter at

Table 1. Average leaf area indexes of canopy leaves measured from 0 to 30 meters from source location in irradiated area.

Date	Leaf Area Index
Aug. 1966	2.20
Feb. 1967	2.10
Aug. 1967	2.21
Feb. 1968	2.25
Aug. 1968	2.19

Table 2 . Correlation coefficients of measurements of successional plants.

X	Y	Correlation coefficient
basal diameter	weight	.97
basal diameter	height	.93
height	weight	.94
height	weight (adjusted for dia.-wt. correlation)	.24
basal diameter	diameter, 30 cm.	.99

30 cm. were almost perfectly correlated, nothing could have been gained by using both as predictors of weight. Therefore, basal diameter alone was used to predict biomass.

Regression of biomass on basal diameter for all 15 species were tested for differences by covariance analysis. There was no detectable difference in slopes and y intercepts in the regressions. Therefore, all 150 individuals were used to calculate a single regression. The regression line was curved on linear paper, so the most general equation for a curved line ($y = ax^2 + bx + c$) was derived from the data. The equation is:

$$Y = .0289x^2 - .2525x - 13.4557$$

where Y equals biomass in grams of dry weight, and X equals basal diameter in 1/128 of an inch.

Due to lack of perfect correlation, diameter values less than 3/16 of an inch give negative values for biomass. All plants less than this diameter were arbitrarily given a weight of one gram in the calculations of total ecosystem biomass.

Equations for Phytolacca icosandra, a common successional species with an unusual shape, and for all sprouts (above ground portions only) were derived in a similar manner. For grasses and sedges, and Desmodium procumbens, biomass was determined by digging up 10 individual square meters of each type, and regressing biomass on the quantity (% coverage) x (% density). Biomass was directly proportional to this quantity.

Regressions were programmed into a desk-top computer, and total biomass of every plant (or every m^2 in the case of grass etc.) was computed. Total biomass of various categories (as shown in the results section) was then obtained by adding together all plants in the appropriate category.

Biomass of Mature Forest

To calculate the biomass of mature forest trees, the equations of Ogawa et al. (1965) were used. These equations were based on measurements made in southern Thailand, in stands which, from their description, closely resembled the forest of this study. Calculations were made for trees in every 2-inch diameter size class, from 4 to 26 inches, diameter breast height. Biomass of trees in each size class was then multiplied times number of trees in each size class per hectare. Tree density data is from Dr. Frank Wadsworth, Director of the Institute of Tropical Forestry, who has transect information from over 20 years of observation in the area. Finally, biomass/sizeclass/hectare for each size class was added together to give total biomass/hectare.

Net Photosynthesis (Assimilation)

Net photosynthesis in the successional area was determined by subtracting total biomass of standing crop of one year from that of the next. Biomass of successional vegetation in 1965 was assumed to be zero.

Net photosynthesis for the mature forest was determined as follows. Total biomass/hectare was determined as described in the section "Biomass of mature forest", using 4 in., 6 in., 8 in., etc. as the diameters for calculating biomass in each size class. Change in diameter per size class was measured on 194 trees from July 1, 1966 through Dec.1, 1967. Each tree was fitted with an aluminum tape that expanded as the tree grew. The tapes were marked with a vernier scale. Change in diameter/size class/ year was computed. Total biomass of the forest was again calculated, but this time the diameters used for each size class determination were the original diameters plus average change in diameters of each class tree per year. For example, in the 4 inch class trees, the new diameter was 4 inches plus average yearly diameter increase of 4 inch trees.

Diameter growth was measured only on dicotyledonous trees, while density data included palm trees. Therefore, if rate of biomass increase in palms is different from rate of biomass increase in other species, an error was introduced. It is not known if the rates differ.

Respiration

Leaf respiration of successional vegetation was determined during night time hours only, using the following technique. A plastic bag was inverted over the leaves to be studied; the bottom of the bag was left open. Air was slowly pumped from 92 feet above ground (to ensure a source of air with a stable CO_2 content) through a plastic tube into the top of the bag. A relay switched attached to a timer set for 15 minute intervals directed air into an infra-red CO_2 analyzer, alternately from the stable air source, and from the bottom of the bag. Differences in CO_2 concentration between source and bag were converted into grams of carbon respired/m^2 leaf area/hour. (Lugo, in press, describes calculations).

Total leaf respiration (T.L.R.) for the successional ecosystem was calculated by the equation:

$$T.L.R. = ax + by$$

where
- $a = 1$, when leaf area index = or $>$ 1
- a = leaf area index, when leaf area index $<$ 1
- b = (leaf area index) - 1
- x = respiration rate of top leaves
- y = respiration rate of bottom leaves

Gross Photosynthesis

Gross photosynthesis was calculated for the successional area by the following formula:

G.P. = biomass + leaf fall + leaf respiration + root respiration.

Biomass and leaf fall were converted into carbon by multiplying times 0.44 (carbon = 44% $C_6H_{12}O_5$).

Gross photosynthesis of the mature forest was calculated by adding change in biomass to total forest respiration (Odum and Jordan, In Press).

Solar Radiation

Solar radiation above the canopy was measured with an Epply pyranometer from April 1967 through Jan. 1968. Data was recorded on Rustrak tape, and daily records were integrated with a compensating polar planimeter.

Leaf Area Index

Leaf area index is an index of the quantity of vegetation. An index of three, for example, indicates that there are three square meters of leaf surface for every square meter of soil surface.

Leaf area index of vegetation less than 6 ft. high was determined by dropping a plumb bob on a string directly over each corner of the grid, and counting the number of leaves touching the string. Leaf area index of vegetation greater than 6 ft. was determined as follows: A mirror with a hairline cross in the center was mounted at 45 degrees on one end of a level; on the other end was mounted a peep sight. When the device was level, a vertical line of sight was obtained, and the number of sprays of leaves through which the line of sight passed was counted. It was assumed that a spray of leaves averaged one leaf in thickness.

Leaf area index of the mature forest was derived from the infra-red/red light ratio on the forest floor (Jordan, In this volume). Leaf area index is proportional to the light ratio.

Chlorophyll Content

Chlorophyll A content of leaves was taken from results of 773 determinations which constituted part of another study (Cintrón, In Press).

Total chlorophyll (C_t) in the successional ecosystem was calculated by the equation

$$C_t = ax + by$$

and for the mature forest b the equation

$$C_t = ar + bs$$

where

a = 1, when leaf area index = or $>$ 1
a = leaf area index, when leaf area index $<$ 1
b = (leaf area index) - 1
x = chlorophyll concentration of sun leaves, successional plants
y = chlorophyll concentration of shade leaves, successional plants
r = chlorophyll concentration of sun leaves, trees in mature forest
s = chlorophyll concentration of shade leaves, trees in mature forest

"Equivalent" Age of the Mature Forest

To plot long term changes in ecosystem functions with succession, it was necessary to establish an age for the forest surrounding the irradiated area. The forest, however, had been affected in the past by hurricanes, and some selective logging, with the result that it is an uneven aged stand. Therefore, an "equivalent" age was determined by dividing the biomass of the mature forest (22,853 g/m^2 from Table 3) by the average of the four values of assimilation/year (Table 5). The equivalent age of the forest in 1966 was 59 years.

RESULTS

Standing Crops

Total standing crop increased every year from early succession up through the 60-year-old forest (Table 3, Fig. 3). Standing crop was greater on the oxidized soil of the irradiated site than on the reduced soil (Table 4, Fig. 3). Sprouts played a decreasingly important part during succession (Table 4).

The six most common species in the mature forest, in decreasing order of importance, are Euterpe globosa, Croton poecilanthus, Dacryodes excelsa, Cecropia peltata, Sloanea berteriana, and Manilkara nitida (Wadsworth, 1967). Cecropia is a secondary successional species, while the rest produce seedlings capable of germinating beneath a closed canopy, and thus can be called "climax" species. The standing crop of the five most important climax

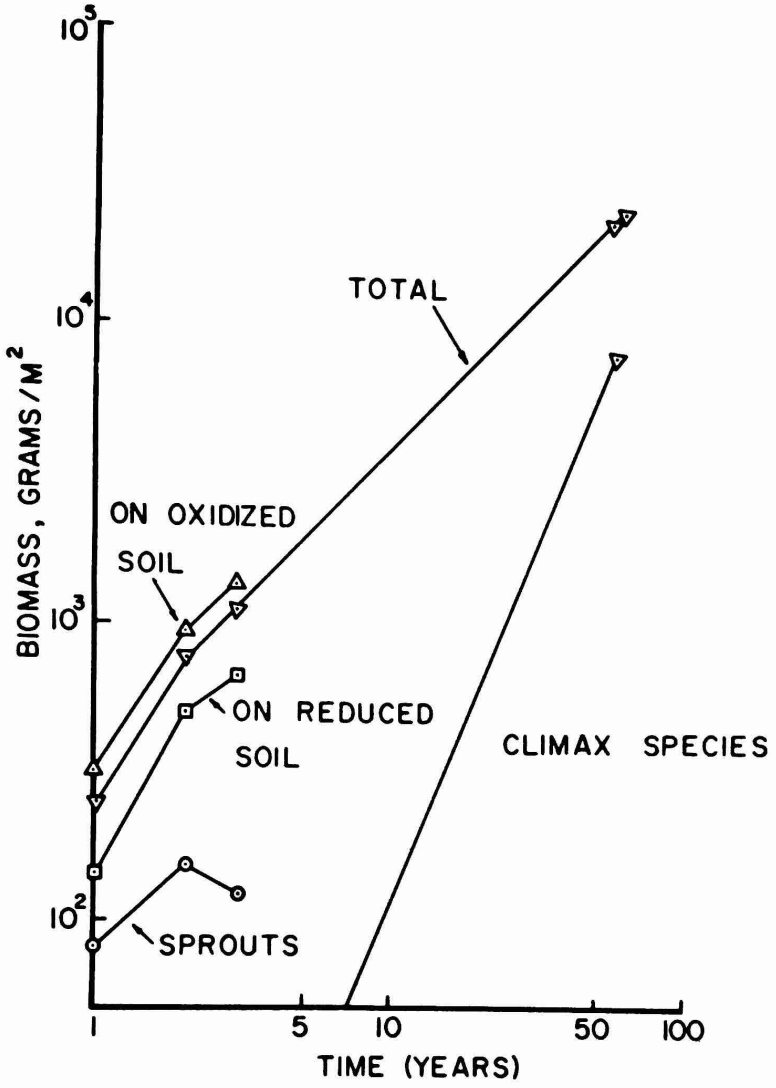

Fig. 3. Change in biomass of plant material during succession.

Table 3 . Total standing crops (biomass) of vegetation in the irradiated area and the surrounding forest.

Years from start of secondary succession	grams/square meter				
	Total standing crops	Sprouts	Oxidized soil (total veg.)	Reduced soil (total veg.)	Five most common climax species
1	242	46	316	142	0.297
2	768	83	950	512	2.973
3	1,060	120	1,352	671	6.441
59	22,853				8,429.000
60	23,339				

Table 4. Percentage of standing crop contributed by vegetation in various categories.

Years from start of secondary succession	Percent of average total standing crop/square meter			
	Sprouts	Plants on oxidized soil	Plants on reduced soil	Five most common climax species
1	19.00	130.57	58.67	0.12
2	10.80	123.69	66.66	0.39
3	11.32	127.54	63.30	0.61
59				36.88

Table 5. Net photosynthesis (assimilation) in the irradiated area and mature forest.

	Net Photosynthesis					
	$g/m^2/yr$ organic matter		$g/m^2/day$ organic matter		$g/m^2/day$ carbon	
Years from start of secondary succession	Total vegetation	Five common climax species	Total vegetation	Five common climax species	Total vegetation	Five common climax species
1	242	0.297	0.663	0.0008	0.292	0.0004
2	526	2.676	1.441	0.0073	0.634	0.0032
3	292	3.486	0.800	0.0095	0.352	0.0042
59	486	179.23	1.331	0.464	0.585	0.2150

species was much lower than that of other species during early years of succession, but these climax species had a rate of increase much greater than the average of all vegetation (Table 5, Fig. 3).

During succession, percentage of total biomass of sprouts decreased, percentage of climax species increased, and percentage on each of the two soil types remained constant (Table 4, Fig. 4).

Net Photosynthesis

Total net photosynthesis for the ecosystem went up to a maximum value of 0.634 $gC/m^2/day$ only two years after succession began (Table 5, Fig. 5). From the second through the 59th year, total net photosynthesis showed neither a distinct increasing nor decreasing trend.

Net photosynthesis of the five most common climax species increased by a factor of 537 times from the first through the 59th year (Table 5).

Respiration

There is very little difference in respiration rate between leaves in the same position in different species (Table 6). Only Cecropia peltata has a decidely higher respiration rate. Much greater differences occurred between leaves toward the top of the plant and leaves toward the bottom of the plant, with the top leaves having a greater respiration rate.

No clear differences occurred in rate of soil respiration between the mature forest and the secondary successional area (Table 7). The low rate of respiration in the mature forest on Feb. 14, 1968 may have been caused by an unusually dry condition on the floor of the mature forest. Between Feb. 5 and Feb. 14, no moisture was collected in 12 below-canopy rain fall collectors (Jordan 1968), while about 1/10 of an inch fell in the open.

Soil respiration consists of the respiration of microorganisms decomposing fallen leaves and plant parts, and root respiration. Total soil respiration of the secondary successional ecosystem could not have been equal to that of the mature forest, since the mature forest contained about 22 times as much biomass as the successional site. High soil respiration in the successional area is probably due to decomposition of dead and fallen trees which were killed by radiation, plus roots of these trees which were at least partly living, as evidenced by the presence of sprouts. Therefore, to calculate total respiration of the successional ecosystem, root respiration of the successional plants was taken to be 37% of the total respiration of the ecosystem, because in the mature forest, root respiration was 37% of total ecosystem respiration (Odum and Jordan In Press).

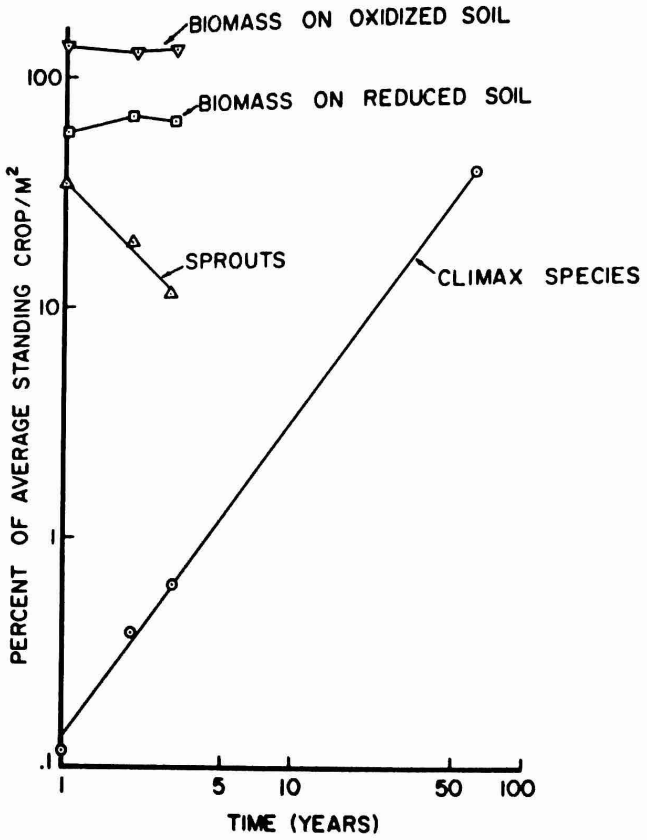

Fig. 4. Contribution toward total biomass contributed by various categories of plants during succession.

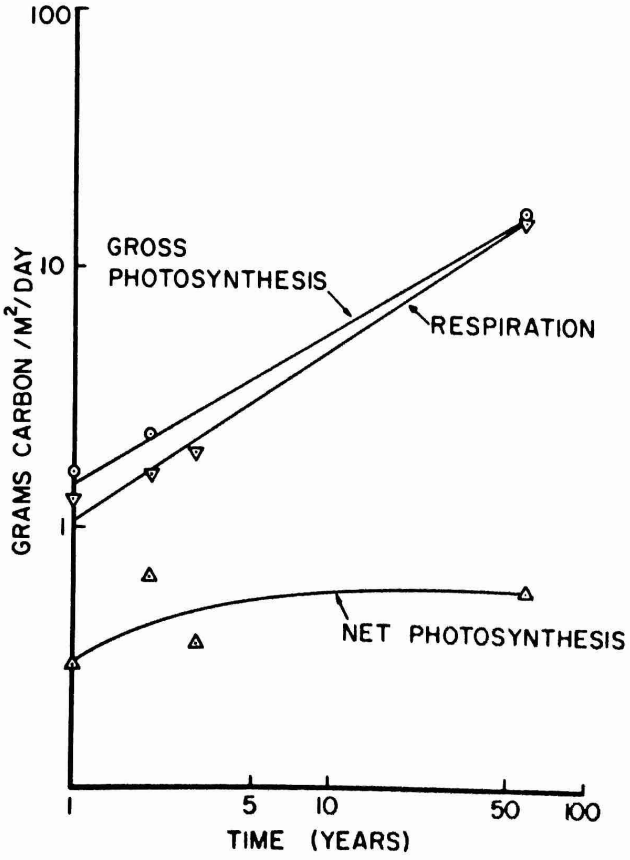

Fig. 5. Change in net photosynthesis, gross photosynthesis, and respiration during succession.

Table 6 . Respiration of leaves in the irradiated area

Species	Location of leaves on plant	Number of nights measured	$gC/m^2/hr$ respired	$gC/m^2/day$ respired
Psychotria berteriana	top	5	0.0096	
Palicourea riparia	top	3	0.0196	
Tabebuia pallida	top	3	0.0136	
Didymopanax morototoni	top	3	0.0218	
Cecropia peltata	top	3	0.0343	
Desmodium procumbens	top	3	0.0156	
Psychotria berteriana	bottom	2	0.0029	
Palicourea riparia	bottom	1	0.0093	
Tabebuia pallida	bottom	3	0.0061	
Didymopanax morototoni	bottom	3	0.0067	
Average	top		0.0190	0.4560
Average	bottom		0.0062	0.1488

Table 7 . Soil respiration in the irradiated area and the mature forest.

Area	Grams carbon respired/m^2/hr. 11/22/67	1/5/68	2/14/68	6/27/68	7/31/68	8/27/68
Mature forest, oxidized soil		.06	.008	.05	.07	.13
Mature forest reduced soil	.11	.07	.03	.06	.09	.08
Irradiated area, oxidized soil 100% grass covered		.12	.10	.07	.08	.09
Irradiated area, oxidized soil, no grass cover		.10	.09	.07	.06	.10
Irradiated area, reduced soil, 50% grass cover		.09	.07	.11	.05	.15

Limb respiration was not measured in the successional vegetation. However, when leaves were covered with a plastic bag for respiration measurements, the bag covered the twig on which the leaves were growing except for Cecropia peltata, and thus respiration due to small limbs were included in the leaf respiration data. In the case of Cecropia peltata, there were no limbs on the young trees. All leaves originated from the main stem.

Trunk respiration and animal respiration were not measured in the successional area. In the mature forest, trunks contributed 4.7% and animals 0.7% total ecosystem respiration (Odum and Jordan, In Press). Respiration due to trunks and animals in the successional area was calculated by taking the same percentage of total ecosystem respiration as was found for the mature forest.

Ecosystem respiration increases during succession (Table 8). The least squares line of regression of respiration on years since start of succession is shown in Fig. 4.

Gross Photosynthesis

Gross photosynthesis was calculated by adding change in biomass to total respiration (Table 8). The least squares line of regression of gross photosynthesis on years since start of succession and the regression line of respiration on years converge with passage of time during succession (Fig. 4).

Ratio of gross photosynthesis to respiration decreases with time during succession (Table 8).

Leaf Area Index and Chlorophyll

Leaf area index increased rapidly during the first years of succession (Table 9, Fig. 6). After only three years, leaf area index in the successional area was greater than half of the leaf area index of the mature forest.

Chlorophyll content is slightly higher in shade leaves than in sun leaves, and higher in the leaves of the mature forest than in those of the successional vegetation (Table 10).

Chlorophyll content of the ecosystem increased more rapidly than leaf area index, because of the proportion of shade leaves and mature leaves increases with succession (Table 9, Fig. 6).

Table 8 . Respiration and gross photosynthesis in the successional and mature forest.

Years from start of secondary succession	Leaf fall	Leaf respiration	Root respiration	Limb respiration	Trunk respiration	Animal respiration	Total respiration	Change in biomass	Gross photosynthesis	Gross photosynthesis / respiration
	grams carbon/m^2/day									
1	.28	.44	.46	*	.06	.01	1.25	.29	1.54	1.23
2	.28	.63	.59	*	.07	.01	1.58	.63	2.21	1.39
3	.28	.82	.71	*	.09	.01	1.91	.35	2.26	1.18
59	.66	4.77	6.07	3.98	.78	.12	16.38	.58	16.96	1.03

*Included with leaf respiration

Table 9 . Leaf area index and chlorophyll content of forest during succession.

Years from start of secondary succession	Leaf area index	Total chlorophyll A in ecosystem g/m^2
1.0	.96	.226
1.5	1.64	.454
2.0	2.90	.883
2.5	3.26	1.006
3.0	3.53	1.098
59.0	6.60	2.745

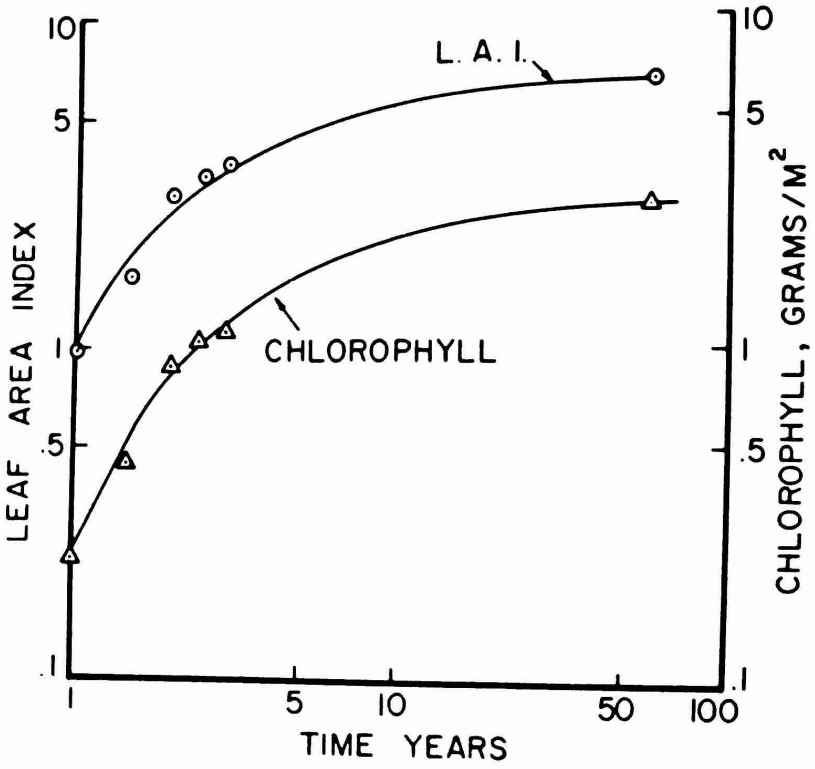

Fig. 6. Changes in leaf area index and amount of chlorophyll during succession.

Table 10. Chlorophyll A content of leaves (from Cintrón In Press).

Species	grams/m^2 of leaf area			
	sun leaves		shade leaves	
	$\bar{x}$	n	$\bar{x}$	n
Cecropia peltata Psychotria berteriana Phytolacca icosandra Commelina sp.	.236	35		
Cecropia peltata			.341	20
Sloanea berteriana Euterpe globosa Manilkara bidentata Dacryodes excelsa Cecropia peltata Croton poecilanthus	.404	384	.416	334

Solar Radiation

The average total solar radiation during 197 days during the period April 1967 through January 1968 was 206.6 cal/cm^2/day, with one standard deviation of 69.9. This is 2.066 x 10^6 cal/m^2/day. If one gram of glucose yields 3730 calories (Wilson and Loomis, 1962), then 2.066 x 10^6 calories would yield 553 grams glucose/m^2/day at 100% efficiency.

Efficiencies and Taxes

Trophic level efficiency, which is defined as the ratio of gross photosynthesis to total light (Lindemann in Odum, 1957) increases during succession (Table 11, Fig. 7). Total light is the total sunlight as measured by an Epply pyranometer, and converted to grams of glucose/m^2/day by taking one gram to be equivalent to 3730 calories. Gross photosynthesis also was converted to grams of glucose/m^2/day.

Tissue growth efficiency, which can be defined as the ratio of assimilation to gross photosynthesis, decreases during succession (Table 11, Fig. 7).

Property tax (Olson, 1964), taken here as the ratio of respiration in grams of organic matter/m^2/year to standing crop in grams/m^2/decreases during succession (Table 11, Fig. 7).

Comparison of Functions

A comparison of several of the functions, as they change with succession, is shown in Fig. 8. Especially striking is that several functions (i.e. net photosynthesis, leaf area index) approach a maximum just a few years after start of succession. Diversity will be considered in the next section.

Correlation

Species whose seeds are carried by wind or animals might be expected to have a random distribution shortly after germination in a cleared area. With a perfectly random distribution, the correlation coefficient between any two species is necessarily zero, because random distribution implies there are no positive or negative correlations between species. As succession proceeds and competition increases, some species which are better adapted to one micro-habitat (group A, for example) will crowd out other species which are less well adapted (group B). In another habitat, the situation could be reversed. All pairs of species within group A will be positively correlated, while pairs, one from each group, will be negatively correlated.

Table 11. Efficiencies and taxes during succession

Years after start of secondary succession	Gross photosynthesis, grams glucose/m^2/day	Trophic level efficiency	Tissue growth efficiency	Property tax
1	3.85	0.007	1.88	428%
2	5.52	.010	.285	171%
3	5.65	.010	.155	149%
59	42.40	.076	.034	59%

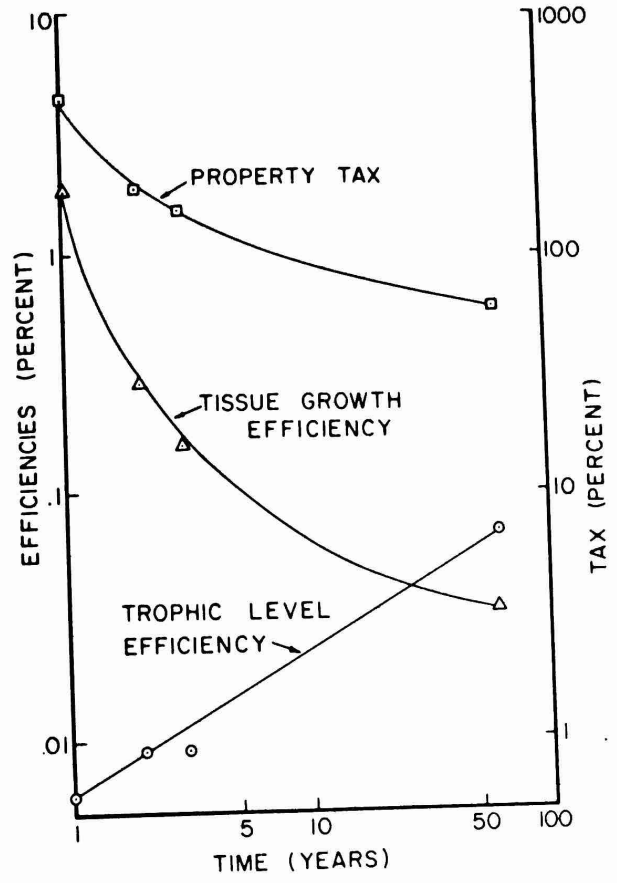

Fig. 7. Changes in various types of efficiencies during succession.

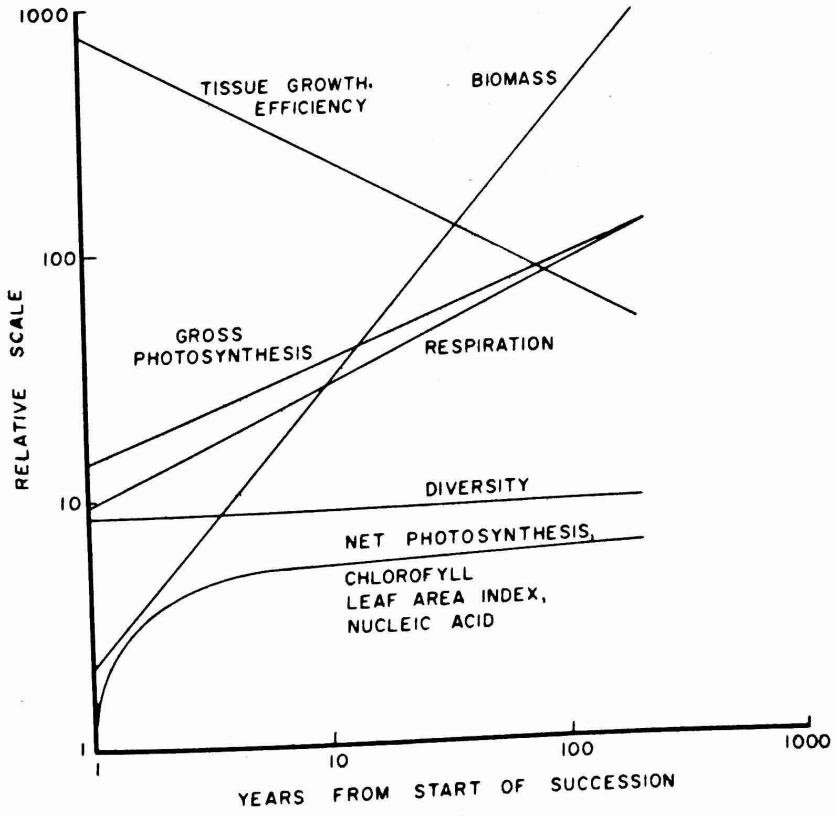

Fig. 8. Comparison of trends of various functions during succession.

Part of this study was to determine how correlation between species of plants changes with succession in the tropical rain forest.

As described previously, all the plants within a 676 m^2 grid were tallied according to the quadrat into which they fall. The same was done in a nearby area of the mature forest. Correlation coefficients between pairs of species were determined by counting the number of each species in every quadrat, and then determining the correlation coefficient. For example, the input data into the correlation coefficient calculation for positively correlated species might be as follows: quadrat 1, species X, 5 individuals, species Y, 4 individuals; quadrat 2, species X, 2 individuals, species Y, 1 individual, etc. For negatively correlated species, the data might be: quadrat 1, species X, 8 individuals, species Y, 0 individuals; quadrat 2, species X, 1 individual, species Y, 12 individuals, etc. In the successional area where the grass Icnanthus pallens was very common, it was given a valve of one if present, regardless of its coverage of the quadrat.

Correlation coefficients depend on the size of the quadrats (Grieg Smith, 1962). Therefore, several size quadrats were used. One square meter quadrats were too small, even for the successional area. Too many quadrats occurred with zero of both members of each pair. For the successional area, four contiguous square meter quadrats were used to make one size quadrat, and 16 contiguous quadrats were used to make a larger size, four meters on a side. In the successional area, quadrats larger than 16 square meters could have included two or more single-species clusters of plants, and thus could have shown positive correlation whereas negative correlation actually existed. Therefore, 16 m^2 was the maximum quadrat size in the successional area. For the mature forest, quadrat sizes of 16 m^2 and 64 m^2 were used. Quadrats smaller than 16 m^2 resulted in too many zeros, and quadrats larger than 64 m^2 presented the same problem as quadrats larger than 16 m^2 in the successional area.

Correlation coefficients were determined for all possible combinations of the nine most important species (according to biomass) in each area. This amounted to 36 correlation coefficients for each quadrat size for each area. Correlations for the successional area were taken from the 1966 data.

Coefficients were calculated with the aid of a desk top computer.

Apparently there is no increase or decrease in correlation in the area studied (Tables 12 and 13).

Table 12. Correlation coefficients between species in the successional area, 1966.

Pair no.	Species X	Species Y	Correlations coefficient $4m^2$ quadrats	$16m^2$ quadrats
1	Psychotria berteriana	Linociera domingensis	0.02	0.05
2	" "	Tabebuia pallida	0.19	-0.29
3	" "	Icnanthus pallens	0.11	0.07
4	" "	Cecropia peltata	0.42	0.60
5	" "	Alchornia latifolia	0.33	0.44
6	" "	Palicourea riparia	-0.04	-0.16
7	" "	Didymopanax morototoni	0.07	0.28
8	" "	Casearia bicolor	0.52	0.67
9	Linociera domingensis	Tabebuia pallida	0.05	0.06
10	" "	Icnanthus pallens	0.06	0.31
11	" "	Cecropia peltata	-0.03	-0.38
12	" "	Alchornia latifolia	0.15	0.18
13	" "	Palicourea riparia	-0.03	-0.01
14	" "	Didymopanax morototoni	-0.02	-0.01
15	" "	Casearia bicolor	-0.06	-0.05
16	Tabebuia pallida	Icnanthus pallens	0.12	0.09
17	" "	Cecropia peltata	0.00	-0.07
18	" "	Alchornia latifolia	0.01	-0.15
19	" "	Palicourea riparia	-0.03	-0.03
20	" "	Didymopanax morototoni	0.01	0.06
21	" "	Casearia bicolor	-0.13	-0.02
22	Icnanthus pallens	Cecropia peltata	0.04	0.02
23	" "	Alchornia latifolia	0.01	0.02
24	" "	Palicourea riparia	-0.01	-0.07
25	" "	Didymopanax morototoni	-0.05	-0.10
26	" "	Casearia bicolor	-0.03	-0.03
27	Cecropia peltata	Alchornia latifolia	0.11	0.18
28	" "	Palicourea riparia	-0.01	-0.15
29	" "	Didymopanax morototoni	0.17	0.37
30	" "	Casearia bicolor	0.55	0.65
31	Alchornea latifolia	Palicourea riparia	0.17	0.38
32	" "	Alchornic latifolia	0.15	0.64
33	" "	Casearia bicolor	0.36	0.52
34	Palicourea riparia	Didymopanax morototoni	0.07	0.48
35	" "	Casearia bicolor	0.07	0.08
36	Didymopanax morototoni	Casearia bicolor	0.06	0.43
	Average of 36 pairs, all signs changed to plus		0.12±0.14	0.22±0.21

Table 13. Correlation coefficients between species in the mature forest.

Pair no.	Species X	Species Y	Correlation coefficient 16m² quadrats	64m² quadrats
1	Dacryodes excelsa	Euterpe globosa	-0.01	0.45
2	" "	Manilkara bidentata	-0.11	-0.55
3	" "	Eugenia stahlii	-0.13	-0.40
4	" "	Palicourea riparia	-0.16	-0.17
5	" "	Drypetes glauca	-0.04	-0.39
6	" "	Linociera domingensis	0.01	-0.21
7	" "	Sloanea berteriana	-0.07	-0.31
8	" "	Croton poecilanthus	-0.06	0.15
9	Euterpe globosa	Manilkara bidentata	-0.16	-0.60
10	" "	Eugenia stahlii	-0.35	-0.48
11	" "	Palicourea riparia	0.05	0.44
12	" "	Drypetes glauca	-0.24	-0.66
13	" "	Linociera domingensis	-0.24	-0.64
14	" "	Sloanea berteriana	0.11	0.42
15	" "	Croton poecilanthus	0.07	0.33
16	Manilkara bidentata	Eugenia stahlii	-0.02	-0.09
17	" "	Palicourea riparia	-0.31	-0.46
18	" "	Drypetes glauca	0.39	0.39
19	" "	Linociera domingensis	0.07	0.06
20	" "	Sloanea berteriana	-0.16	-0.44
21	" "	Croton poecilanthus	-0.26	-0.38
22	Eugenia stahlii	Palicourea riparia	0.09	0.18
23	" "	Drypetes glauca	0.18	0.35
24	" "	Linociera domingensis	0.22	0.57
25	" "	Sloanea berteriana	0.22	-0.03
26	" "	Croton poecilanthus	0.20	0.27
27	Palicourea riparia	Drypetes glauca	-0.22	-0.36
28	" "	Sloanea berteriana	0.14	0.51
29	" "	Croton poecilanthus	0.06	0.48
30	" "	Linociera domingensis	-0.21	-0.24
31	Drypetes glauca	Linociera domingensis	0.40	0.79
32	" "	Sloanea berteriana	-0.10	-0.09
33	" "	Croton poecilanthus	-0.30	-0.40
34	Linociera domingensis	Sloanea berteriana	-0.12	-0.01
35	" "	Croton poecilanthus	-0.27	-0.49
36	Sloanea berteriana	Croton poecilanthus	0.05	-0.12
	Average of 36 pairs, all signs changed to plus		0.16 ± 0.11	0.36 ± 0.19

SECTION II

by
George E. Drewry

In section two, current animal ecology studies including tracer work, and territoriality and other work with amphibians are reported.

Also in this section a new approach to species diversity is developed, and applied to plant diversity in the radiation recovery area, and to insects in the surrounding forest.

Insect keys constructed in the past year are also presented as an appendix .

PART A - ANIMAL ECOLOGY

Work begun earlier on two animal ecology projects was continued by visiting investigators. Both studies involve termites, which are among the most important insects of the rain forest animal community. One study includes a census of individuals and relative metabolic rates of each caste in the termite nest by measurement in a microrespirometer. The other study, reported by Dr. E. McMahan in section three, is a follow-up of radiation effects on nest survival and includes some interesting experiments on worker behavior and direct responses to gamma radiation.

Staff efforts continue to include studies of isotope tracers, insect diversity, and amphibian ecology. Isotope studies were enlarged to include uptake and bioelimination of tritium in the form of HTO. Tritiated water sprayed at ground level was absorbed by direct contact and respiration in insects, snails, frogs and lizards. No uptake was exhibited by insects, frogs or lizards subsequent to 36 hours after treatment. Snails continued to show uptake as long as 72 hours after treatment when collected after crossing contaminated litter surface. A method for live testing snails consisted of teasing them back into their shells at which time they released 1 to 4 ml. of urine. Urine samples exhibited approximately the same count rate as tissue fluids obtained by dissection. Biological half life of tritium in snails was very short, just under 24 hours.

Tracer and bioelimination studies of Zinc 65 in a natural population of the snail Caracolus caracolla moved into the second year, with resolution of some of the mysteries of the first year. Area of home range was found to be a function of age, increasing until the second year after puberty and decreasing after that. Adult size, previously demonstrated to be independent of home range area, is likewise independent of age, shell growth ceasing at maturity. Present estimate of life span in this species is up to 18 years, with sexual maturity not developing until 8 years of age. On the basis of last year's growth these estimates appear to be within 2 years of the true values.

Insect diversity studies involved research on methods of obtaining and expressing diversity measurements as well as the slow, continuing labor of separating and identifying the species of some of the poorly known groups. In some of these groups the recorded fauna for the whole island has been as much as quadrupled in this study alone. Comparisons are being made between diversities obtained with various trapping methods such as sticky traps, pitfall traps, light traps and Malaise (flight) traps. Attractant traps such as light traps avoid the distorting effects of irregular natural concentrations or foci, but impose their own artificial focus on the distribution. The effect of natural foci is illuminated in this comparison as giving a curvature to the normally linear relationship between number of species and log number of individuals.

Progress in the study of amphibian ecology has moved into an area of collaboration with two graduate students at the University of Texas. With James P. Bogart, who finished a doctoral thesis on the evolution of anurans in the family Bufonidae and in the process accumulated considerable data for the family Leptodactylidae, a cooperative study on Puerto Rican Leptodactylids is well advanced. Karyotypic analysis of 11 of the 12 species of this family in the Luquillo National Forest is completed and forms the basis for a set of hypotheses about insular trends in the evolution of the family and the role of ecological specialization in their evolution. A joint publication in which analytical data is presented by Bogart, and ecological data by Drewry, is to be the result of this study. Preliminary information indicates that several of the speciation events giving rise to separate genetic lines may have been due to ecological separation within the geographical limits of the island, and not to separate migrations from elsewhere. A list of chromosome counts from species of this family is given as Table I. Of particular taxonomic interest is the rediscovery after several years of Eleutherodactylus unicolor Stegner and the discovery of its call, its habitat and methods for collecting it, and the fact, revealed by its karyotype, that it may not belong in the genus at all, but to the genus Syrrophus.

The second collaboration is with William Martin, who is finishing a doctoral thesis on the biophysics and mechanics of vocalization in anurans. Some of the hypotheses tested and supported by his research were originally proposed by Drewry, and others grew out of a long period of correspondence, so that the basic model is considered a joint achievement and is in early manuscript stages. Data on rain forest species is to be included in this publication.

Possible ecological role of the call of male eleutherodactylid frogs as a population spacing device is presently being studied. Mate attraction as one primary role is well documented, but recent observations of increased calling activity after introduction of tape recorder playbacks or natural imigrations of calling males suggest additional functions. Agonistic behavior toward calling intruders immediately after their calls has also been observed. Tape recording equipment and additional electronic circuitry to create a "responsive" artificial competitor are now on order. If quantitative behavioral responses are obtained, options designed into the equipment can control the timing and acoustic characteristics of the competing call, providing data of ecological, evolutionary, and behavioral relevance.

Table 1

Chromosome counts of Puerto Rican leptodactylid frogs.

Species	Diploid Chromosome Count
Leptodactylus albilabris	22
Eleutherodactylus unicolor	30
E. portoricensis	26
E. antilliensis	26
E. brittoni	26
E. wightmannae	26
E. richmondi	30
E. eneidae	26
E. hedriki	26
E. locustus	26
E. gryllus	24

PART B - SPECIES DIVERSITY

Introduction and Methods

Most methods devised to numerically describe population structure in ecological communities have been extensions of one or the other of two basic approaches. The earliest, and still most common, approach rests on an assumption that there is an underlying mathematical relationship that governs the complex ratio of numbers of individuals of various species to one another. Very little has been published of causative factors that might generate such a relationship, although it has been repeatedly observed that species similar enough to be included in a sample collected by a single method are almost never equally abundant, and that real samples never seem large enough to include all of the species known to exist in even a relatively homogeneous area. Williams (1964) has brought together a large amount of the evidence for the existence of such a relationship, and has proposed some sophisticated methods for utilizing this assumption. Although the methods are somewhat difficult to apply and require use of a set of computer generated nomographs, he has carried them to some remarkable lengths, and even proposes a model for the rate of species formation over the earth as a whole based on these methods.

The mathematical relationship most commonly assumed to exist between organisms of a single habitat is an exponential one, that the number of species in a given sample is some function of the logarithm of the number of individuals in it. The simplest function would be a fixed ratio between these parameters (Odum 1953) and the index of diversity would be species per decade of sample size. A statement of species per thousand individuals (or per any other fixed number) taken in conjunction with this assumption would provide sufficient information to extrapolate in either direction to expected species number for any sample size. Although widely used, this method of description has only occasionally been validated by the total distribution of numbers in a field sample, and the validating graph has normally been constructed by counting species in a few subsamples of various sizes or by noting accumulated individuals each time a new species is encountered in a random sorting of the sample. Both tally methods require a randomizing procedures for sorting and recording, and neither uses as information the relative numbers of each species present. Williams has pointed out that assumption of a linear relationship between species number and log number of individuals violates mathematical reality, because zero species must involve zero individuals, while zero does not occur on a logarithmic scale, which is infinite in both directions. He substitutes the mathematically valid log series curve, which is defined by a parameter calle α, and whose graph in semi-log plot is linear for large numbers and curves to the intersection of one and one. He claims validity for this relationship in many stands of vegetation and for light trap collections of lepidopterous insects, but has used the above mentioned methods of validation, with their limitations.

Williams also suggests that when collections are expanded to include organisms from more than one habitat type the relationship shifts from a log series to a log normal series, in which the logarithm of species number is linearly related to the logarithm of number of individuals. MacArthur and Wilson (1967) have utilized this assumption in a recent book on the theory of island biogeography. Again their validating data is a widely scattered series of points, although it seems clear that the relationship holds in a general way.

Margalef (1957) has introduced a new (to biologists) measurement of species diversity that does not rest on prior assumptions of relationship between species and individual numbers, but has the disadvantage of not describing community structure beyond diversity. This is actually a family of measurements derived from formal information theory as used in communications engineering, and has been subsequently utilized by Pielou (1966a and b), Lloyd and Ghelard; (1964), Lloyd et al (1968), Dickman (1969), and others. Information content of an individual in the sense of species diversity is not all of the information possessed, but rather that required to distinguish it from the other species of the sample, i.e. possession of feathers is sufficient information to separate a sample of two chickens and a cat, but must be supplemented if the sample also contains turkeys.

Two equations are available for calculating diversity in terms of mean information content per individual, known as Brillouin's measure and Shannon's measure. Both are given in several forms by Lloyd et al (1968). Shannon's measure estimates the diversity of an unlimited population composed of a known number of species or classes from the proportions of each in a sample and is largely inapplicable to biological diversity where the total number of species obtainable is almost never known or present in a single sample. This measure will not be discussed further here. Brillouin's measure is:

$$H = \frac{c}{N} \quad (\log_{10} N ! \; - E \log_{10} ni ! \;)$$

where H is mean diversity or information content per individual, c is a scale factor to convert to any number base desired (binary bits are often used in information theory where c = 3.321928), N is total individuals in the sample and ni are numbers of individuals of each species. This measure gives only the sample diversity and does not extrapolate to a larger population unless the population has the same structure as the sample. At this point the discussion has come full circle and focuses on the problem of structure.

Many biologists have attempted to describe the structure of communities with graphs of relative abundance or relative frequency or with various arbitrary abundance classes, but apparently have not attempted to relate these to species diversity. In this research an effort has been made to relate all of the measures of structure and diversity in the simplest possible way. If species of a sample are ranked in order of decreasing representation a curve can be drawn connecting the number

of individuals of each. Because of the large range of category size usually encountered in a sample such a graph has little resolution at the level of the rarer species. Resolution can be improved by plotting number of individuals on a log scale. Figure 1 has such a plot, using individuals of tree species originating after radiation in a radiation recovery area, labelled cumulative species versus log n. The curve would normally be viewed with the left edge at bottom. Note that the logarithm of one is zero, so species represented by one individual are in the line corresponding to zero. This graph contains all species information available in the sample and has not required assumptions to be made about structure. The curve can be inverted by dividing N, the total number of individuals in the sample, by the number of individuals in each species. The curve so formed is labelled cumulative species versus log N/n and can also be called a reciprocal frequency curve or a composite ratio curve. It retains the slopes of the relative abundance curve because division is algebraically subtractive on a log scale, but differs from the relative abundance curve in that, given that the composite ratio of species remains constant as sample size is increased, each point on the relative abundance curve moves to the right as sample size increases while the composite ratio curve only adds points at the upper end. Points in this curve representing species may be read as a ratio, 10:12:15 or as one part in 10, 12, 15 etc. The composite ratio curve in this form is similar, but not identical, to a diversity index curve developed by classical methods or to William's log series curve. The upper right hand point, which represents a single species in the composite ratio, also represents total species and log total individuals and will be used as a data point in any diversity index method. It is the only data point normally used in William's method. It is important to note, however, that the break near log 1.5 in the composite ratio curve is not a concession to mathematical reality but represents a real property of the vegetation, separating a group of abundant species having one ratio slope from a group of rarer species having quite a different slope. Such a break has characterized most, but not all, of the vegetation communities studied and has interesting and predictable properties of its own.

The mathematical relationship between the composite ratio curve and the diversity index curve generated by any method is a rigorous exercise in probability theory and has been substituted here by an empirical correlation method covering the range of curves and slopes encountered in this study (some are not linear at any point). An example of the theoretical complexity is provided by the fact that the probability that a single species whose frequency is one part in one hundred will be missing from a random sample of one hundred is its probability of absence in a sample of one (.99) raised to the 100th power (approximately 36%). The probability that it will be absent, but replaced by a still rarer species, involves all of their probabilities in a manner almost too complex for computation. Common sense indicates that for even a single sample there is not one but an indefinite family of diversity index curves depending on the order of sorting and recording individuals, and that there is a maximum likelihood curve having the highest probability of occurrence that will best represent this family.

Such a curve should run approximately parallel to the composite index curve, always to the left of it (randomly varying individual curves might cross), and be convergent with it because it is subject to the same constraints as the log series curve at low number. It must be emphasized that no theoretical reasons exist for linearity of either diversity index or composite ratio curves in any type of graph. Semi-log plots are merely a representational convenience.

The empirical method of comparing these two types of curves consisted of creating easily sampled populations of known composite ratio for the mean and extreme types of populations encountered in the field. These do not include all theoretical curves, some of which obviously deviate from the conclusions reached. For example, in the ultimately diverse population, each of whose members is a different species, the diversity index is a log series whose α is infinity, while the composite ratio is a vertical line at whatever sample size is chosen. The other extreme is an indefinite population of one species; diversity index is a straight line at one, α is zero and composite ratio is a single point at whatever sample size is chosen. The composite ratios chosen for this experiment ranged from 10 to 500 species per thousand individuals and were either straight, broken, or continuously curving upward in semi-log plot. "Collections" were made from a random numbers table whose digits were "identified" in groups of three by assignment of number groups from 000 to 999 to relative frequency categories dictated by the composite ratio. Diversity index curves were only evaluated to 100 individuals because of the constraint of a finite number of species per 1000 rather than the natural situation of an indefinite number of species of progressively lower frequencies. Distribution of data points in large numbers of series of 100 individuals confirmed the common sense expectation; the curves followed a log series at low numbers shifting to a curve similar to the composite ratio curve past 10 individuals. Distributions appeared to be normal on the species per fixed number of individuals axis, for which the mean is an adequate measure of central tendency, while on the log individuals per fixed number of species axis distributions resembled the Poisson distribution and the median was taken as the best measure of central tendency. Convergence of the curves predicted from the necessity that the diversity index curve pass through the upper point of a realistic composite ratio curve was supported in curves having a straight segment from at most 50 to 1000; the curve straighted and passed through log 60 individuals at the same number of species as the composite index curve had at log 100 individuals; when extended it passed log 640 individuals at the species level of log 1000 individuals in the other curve and also intersected the upper point of the composite ratio curve, which is always above the line of the curve itself. A maximum likelihood diversity index curve consistent with these observations is given in figure 1. If such a maximum likelihood curve continues to be supported by theoretical and/or empirical evidence it provides a method of stating the slope of a linear segment of the diversity index curve above 100 individuals in terms of the slope of the composite ratio curve (it will be 1.0280 times the slope of the composite ratio curve or log 640-log 60) and for extrapolating the number of species in any fixed sample size such as 1000 believed

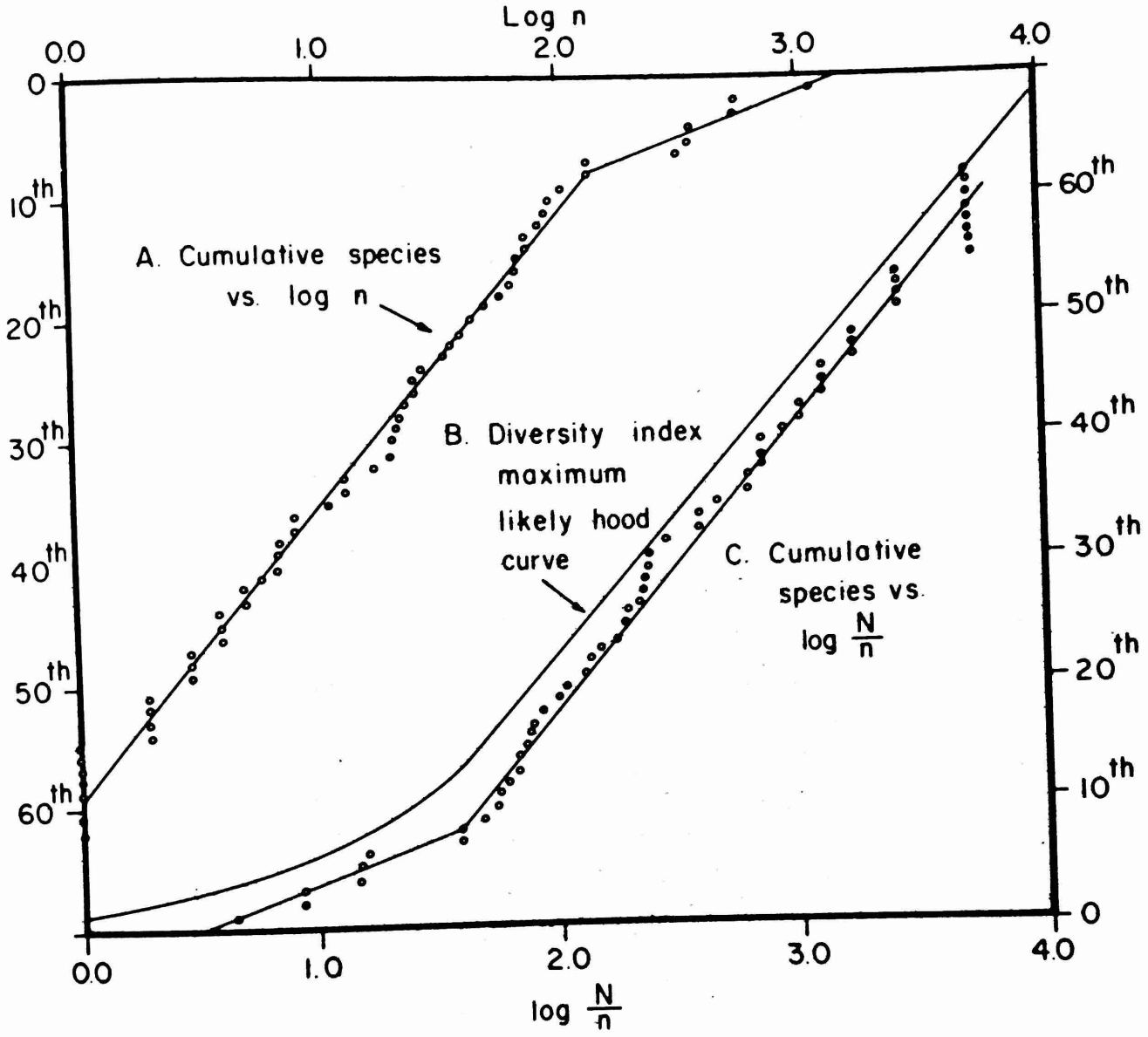

Fig. 1. Graph showing inter-relationship between relative abundance (curve A) composite ratio (curve C) and the postulated maximum likelihood curve of traditional diversity index methods (curve B). Semilog plot is for representational convenience and does not reflect assumptions about species inter-relationships.

to lie on the linear portion of the composite ratio curve. Species per thousand is approximately the intersection of the composite ratio curve with log 1000 plus .2 times the C.R. curve slope (log 1000-log 640 times log 640-log 60 or .1928x1.0280 = .2). Basing the standard measurement of species per thousand on the slope of the composite ratio curve, rather than on the number of species collected in a sample of 1000 individuals, has already proved to be a valuable strengthening of the foundation for this useful measurement. When notified that the number of rare species in a certain collection was theoretically inadequate, one collector was reminded that a small group of very rare species had been put aside for detailed identification and forgotten. The relative taxonomic abilities of several collectors have also been evaluated by this method and the evidence obtained has been consisted with other evidence available. Extrapolation of such measurements as species per thousand upward beyond the limits of the sample has for far been confined to communities having linear composite ratio curves, but downward extrapolation along non linear diversity curves agreeing in shape to the composite ratio and passing the log 640 and 60 points has given realistic estimates.

Information theory measurements have proved difficult to relate in a simple way to the above measurements of diversity. Independence of sample size has been found in a very few cases where the composite ratio curve was linear and the usual group of common species exhibiting a different slope was absent. Normally Brillouin's measurement is very sensitive to sample size, making our necessarily differing samples difficult to compare. In addition, the output measurement is subtractive rather than multiplicative and scaling to a comparable sample size must be done on the input data before computation. This scaling is most readily accomplished by computer manipulation of the data. Log factorials are most easily handled in tabular form (Lloyd et. al. 1968) but fortunately an alternative exists in the form of Sterling's approximation:

$$\text{Log } n\,! \;=\; (n + 0.5) \log n - 0.434294482\, n + 0.39909$$

which can be written into a computer program. For rigorous accuracy, logarithms should be taken to six places but it was determined that the uncertainty in Brillouin's H of four random subsamples of 100 square meter plots from a sample of 676 square meter plots outweighed by at least an order of magnitude the error in H occasioned by using four place logarithms, so these were used. Five programs have been written for computing this measurement on the Olivetti Underwood Programma 101 desk top computer. The first two are alternate programs yielding total information content in bits, H in mean bits per individual and N, the total number of individuals. One is for unskilled operators, requiring only the entry of species number (n) for each species but is much slower owing to the calculation of log n, which it prints for each entry. The other accepts n and log n in pairs and runs at approximately 50% entry time and 50% computing time for a skilled operator. The remaining programs differ only in stored constants and scale data downward to 1000, 500 or 100 individuals distributed according to the composite ratio curve. They require entry of N, log N and n and log n for each species in pairs. By utilizing Sterling's approxi-

mation they calculate log n ! on a continuum rather than as discrete whole numbers and thus avoid rounding errors. Species are entered from commonest to rarest and readout is automatic when N/n for an entry is less than 1.5 (log n ! for single individuals is zero and does not contribute to the index). Programs and constants for this computer are stored on magnetic memory cards and entered by passing the card through a reader. Copies of these programs are available to anyone on request. Equations have been rewritten and constants consolidated to minimize memory space. The entire memory capacity is utilized in each program.

Processing of data has been consistent and diversity measurements are now available for several communities of plants and animals. These include a semi-log plot of composite ratio, called the CR plot, the slope of linear portions of the CR curve in species per decade, designated A slope for abundant species and B slope for rare species when two linear slopes are present, an estimate of species per hundred, per five hundred and per thousand based on the slope of the CR curve (sometimes involving extrapolation upward, if the CR curve is linear), Brillouin's H and information content of the sample in bits per individual and binary bits respectively, and scaled H and information content for samples scaled downward to 100, 500 and 1000 individuals where appropriate designated H_{100}, H_{500}, H_{1000} and Inf./100, Inf./500 and Inf./1000.

Of these measurements the CR plot has proven most informative to the experienced evaluator. It opens the way to further research by pointing out real discontinuities in ratio between abundant and rare species that are smoothed over or concealed by the random fluctuations of traditional diversity index plots. Species per fixed number of individuals, particularly per thousand, which form an easily remembered diversity statement can be rapidly and reliably estimated from CR plots and arduous randomizing procedures are unnecessary. The only danger seems to lie in its apparent ability to conceal the combination of certain unrelated types of communities (it readily reveals others) and the consequent possibility of publishing diversity figures that are meaningful only for the unnatural combination. A non linear CR plot immediately reveals the fallacy of applying linear diversity index methods and can be used to expose such inappropriate applications in past research. It is hoped that both the advantages and limitations of information theory measurements will be realized by the bulk of workers in this field and that uncritical and inappropriate application and resulting false conclusions can be avoided.

CR plots in the remaining portions of this manuscript will be presented with log N/n on the ordinate and cumulative species on the abcissa. This is done deliberately to avoid confusion with traditional species diversity curves.

The Development of Plant Community Structure

Plant succession following a dose of gamma irradiation that either killed old vegetation outright or greatly reduced its ability to compete illustrates well some of the trends in the development of communities. A grid measuring 676 square meters has been mapped in detail each year beginning one year after the 1965 period of irradiation.

Taken as a whole, the vegetation had reached its maximum level of mean information content within a year after irradiation, H decreasing from 4.922 bits per individual in 1966 to 4.889 in 1967. At the same time the species per thousand increased from 64 to 76, an increase of more than 5 percent in this measurement of diversity. The apparent discrepancy is explained by the CR plots in figure 2 (data points are omitted in this and the following plots. All are very similar to fig. 1 in fit). The diversity changes reflected in H occurred in the A slope or abundant species group, while species per thousand responded primarily to large increases in the number of rare or B slope species. Breaks in the composite ratio curve delineate 17 abundant species in 1966, having a total of 4,002 individuals, and only 10 species in 1967, the number of individuals increasing to 5,433. If H is calculated for the abundant species only the drop in diversity is from 3.824 to 3.097. At the same time the number of rare species was extended from 79 to 109, and while rare individuals increased 1,244 to 3,090, diversity measured by H increased from 5.147 to 5.439 on these species alone. The B slope increased from 33.28 species per decade to 41.14 while the A slope decreased from 19.90 to 11.46. Thus only the CR plot tells the whole story. The dimensionless indexes appear to contradict one another until their bias is revealed. Three trends are noteworthy: a rapid and early increase in both numbers and diversity of abundant species, which seem to be well adapted to the situation; a subsequent reduction in number of abundant species with further increase in number of their individuals; and a slower increase of species and individuals of rare species bringing the total species count to a maximum. 1968 data on total vegetation is still being processed but the numbers of both species and individuals declined as individuals grew and space became a limiting factor. Overall species diversity has probably increased as intraspecific competion eliminates individuals of common species more rapidly than those of rare species, but the exact effects on H and species per thousand can not be predicted.

Data processing is complete for tree species, and CR plots for trees originating from seed after the radiation treatment are given in figure 3. In this figure the 3 graphs on the left are for seedlings in 1966, 1967, and 1968 respectively, while the two right hand graphs are for saplings more than 4.5 feet tall for 1967 and 1968. Only Cecropia peltata saplings exceeded this height in 1966, so no ratio was obtained. The composite ratio of seedlings in 1966 is different from any of the other curves shown and reflects the effects of open, well lighted ground for germination. The A portion of the curve includes 20 species and the fast growing species had not yet gained the numerical advantage they enjoyed in the next two years. The slope and H of the A curve were the highest measured for any A trees, being 11.38 species per decade and 3.639 bits per individual, respectively.

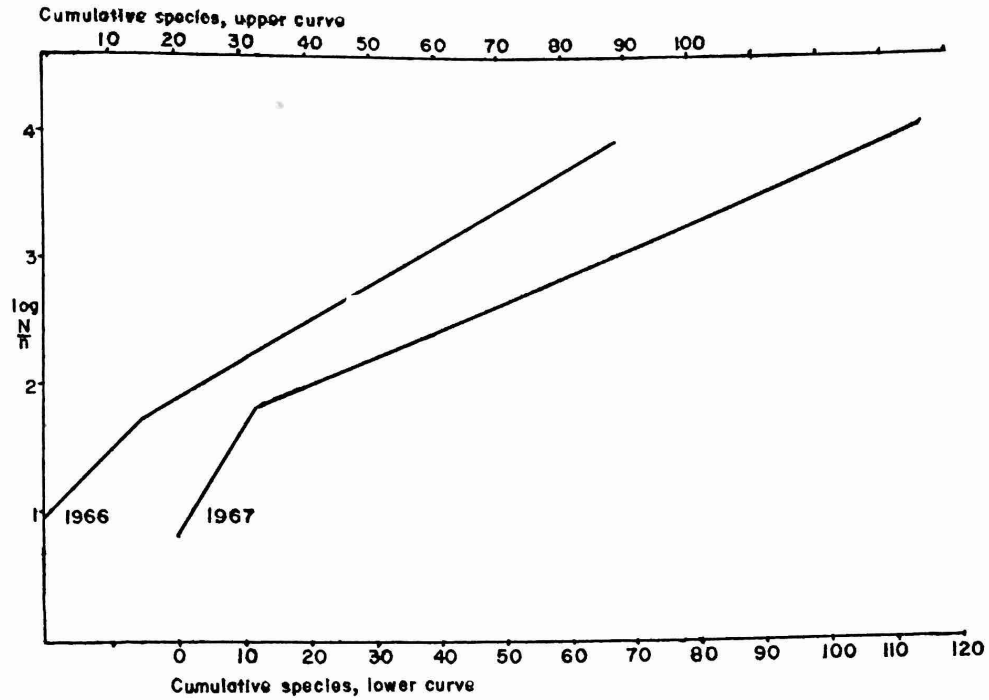

Fig. 2. Composite ratio curves for total vegetation in a radiation recovery area one and two years after irradiation. Ordinate and abscissa have been reversed from figure one and species data points ommitted.

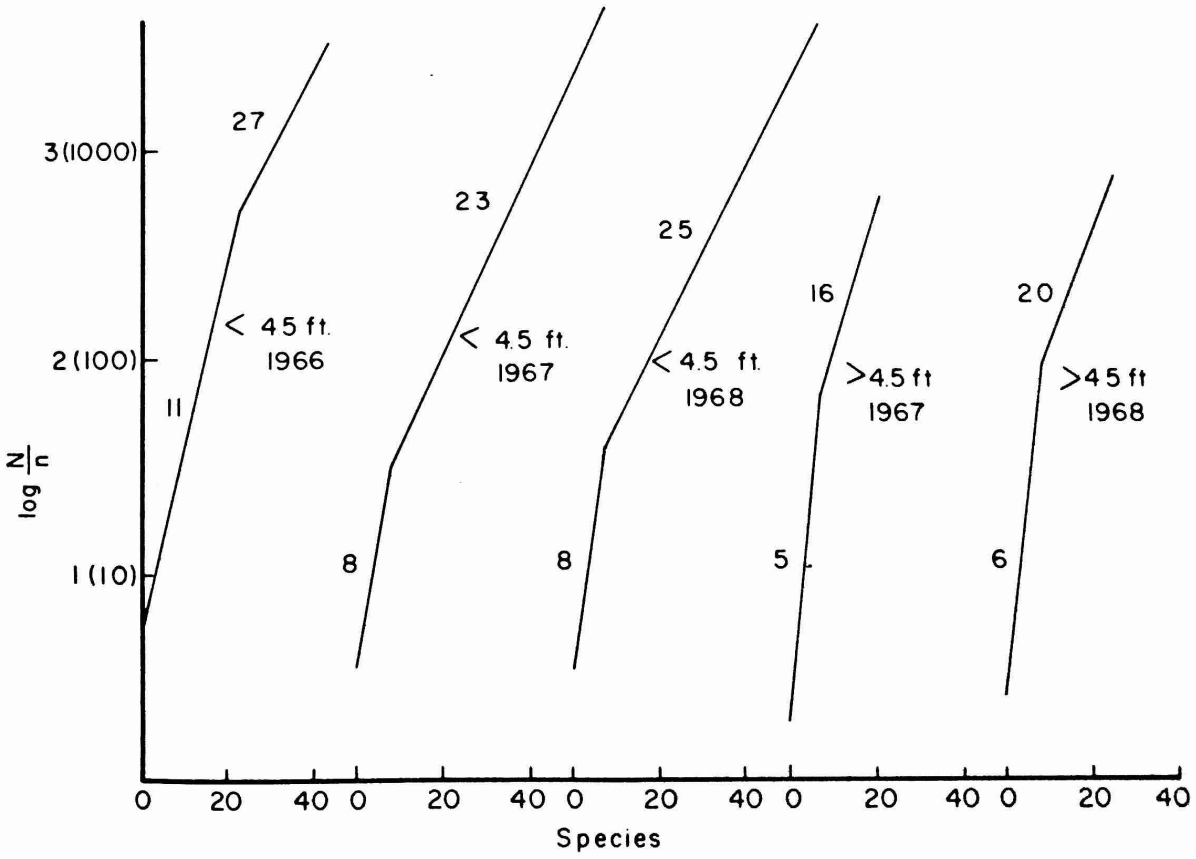

Fig. 3. Composite ratio curves of seedlings ($<$ 4.5 ft. tall) and saplings ($>$ 4.5 ft. tall) of tree species one, two and three years after irradiation. Slopes given to nearest whole number. Scale of species axis shorter than figure 2.

The B curve is not well differentiated and contains only 20 species. Species per thousand at 35 is the lowest registered for seedlings. Combination of this curve with the curves for non-tree plants, sprouts, old trees and saplings obliterated all traces of the break in the CR curve, (see Fig. 2) one of the few cases in which this happened.

The seedling trend in 1967 was similar to that in the total vegetation but more pronounded. Six species produced more seedlings than 20 had possessed the year before. The H measurement for the A segment dropped more than a whole unit to 2.413 and the slope likewise decreased to 8.04 species per decade. The B segment increased even more than the A segment decreased, with an increase from 20 to 55 species, 37 to 1,584 individuals, and 3.377 to 4.748 in average information content of the segment. Overall diversity thus increased species per thousand from 35 to 47, H from 3.732 to 4.071 and the scaled H_{1000} from 3.674 to 3.977. Seedling changes in 1968 represented the same diversity trends to a much smaller degree, except for the A segment, which changed very little. The number of abundant species remained the same, about 14 percent of the individuals moved into the sapling category or died (mostly the former), raising H from 2.413 to 2.444. The B segment lost two species and 10 percent of its individuals, but this was a net increase in diversity of 8 percent in slope and 2 percent in H. Overall diversity rose by 6 percent in species per thousand to 50.5 and by 2 percent in H and H_{1000}. Perhaps it is purely coincidental that these values all correspond very closely to those found in climax trees in this general type of terrain and soil, but it is very intersting that seedling trees, consisting of a large percentage of successional species that will be replaced in the mature canopy by other species, should in three years time establish such a mature community structure. Of course, if species versus area were under consideration, the seedling diversity would appear to be enormous, but it would seem that species versus individuals is the more appropriate measure of diversity when communities of very different individual size are being compared. It will be interesting to see whether the large reductions necessary in species and individuals for this 676 square meters to be occupied again by mature forest can be accomplished without disturbing the diversity structure, or if oscillations are inevitable. Sapling changes in the first three years have involved slower but steady increases in all of the diversity parameters. Species increased from one the second year to 16 the third year to 21 the third. Individuals increased from 17 to 557 to 707. Extrapolated species per thousand were 1, 29, and 34. H has gone from zero to 2.923 to 3.196. Slope A has been 0, 5 and 6, while slope B was 0, 15.02 and 19.54. The only probable overshoot so far is in number of A segment species which went to 10 in 1968. This category would appear to be at a diversity stage somewhat similar to that of the seedlings before the first measurements were made in 1966, so diversity overshoot and subsequent correction are probably to be anticipated among the common species. There appears to be little competition among saplings at this time.

The area of this study has been divided by Dr. Carl Jordan into two soil types, well and poorly drained. Vegetation from these two types was processed separately before being combined into the categories already dis-

cussed, and although a great deal of labor was involved, trends in the two were so similar as to warrant little discussion here. Development did proceed more rapidly in well drained soil and it seemed always at a more advanced stage. Although several individual species showed strong preferences for one or the other type of soil, any slight differences in diversity parameters were averaged rather than additive when the two were combined. In distinction to different habitats to be discussed below, these seemed to be complementary parts of the same habitat from a diversity viewpoint. One difference that was amazing in its consistent repetition was the number and corresponding slope of A segment species. These were always more numerous and diverse in well drained soil. In what is taken as mature ratio, ie. climax vegetation, old radiation center vegetation and 1966, 1967 seedlings the A slope species numbered 9 to 11 with a slope near the same value in well drained soil and about 6 in poorly drained soil, averaging 8 in combination. This phenomenon appeared identical in manifestation with the sun-adapted abundant species of recovery vegetation and the entirely different dominants of mature forest. The explanation seems to be that fewer species are well adapted to the anerobic soils of poorly drained areas, so that the competitive advantage these few have is greater. Figure 4 illustrates this phenomenon. Table 1 shows some of the stronger individual soil preferences. These were computed by multiplying by a scale factor to correct for inequality of soil areas, substracting less preferred from preferred and dividing by the sum. No preference would be zero percent, while 50 percent indicates that three fourths of the individuals are found in the preferred soil.

To discuss trends in the development of rain forest community structure after irradiation, the often stated rule that successional communities develop higher diversity in early stages than they will exhibit at maturity (Odum 1959) seems to require qualification. The statement seems to be very true for the more abundant species, which seem to be the ones best adapted for rapid germination and growth and which will always be collected and identified in quantitative studies. These species will also bear the brunt of competition during the inevitable overcrowding as individuals grow, however, and competition may be most fierce at the intraspecific level, with the result that formerly common species may recede toward rarity without disappearing more rapidly than less well adapted species are completely eliminated, all of which would dictate gradual increases of diversity in species per individual. Something of the sort seems to have happened here, because at no time has there been a reduction in total diversity among plants in comparable size categories. This generalization does not hold at all if species per unit area taken as a measure of diversity, for the growth process itself dictates that a large percentage in early colonists can not survive to reach tree size and selective forces favoring rarer species would have to be many times stronger than they apparently are to overcome this elimination process. Thus the generally held belief that diversity in terms of individuals is the same as diversity in terms of area must be strongly restricted to situations in which size and/or density are equivalent.

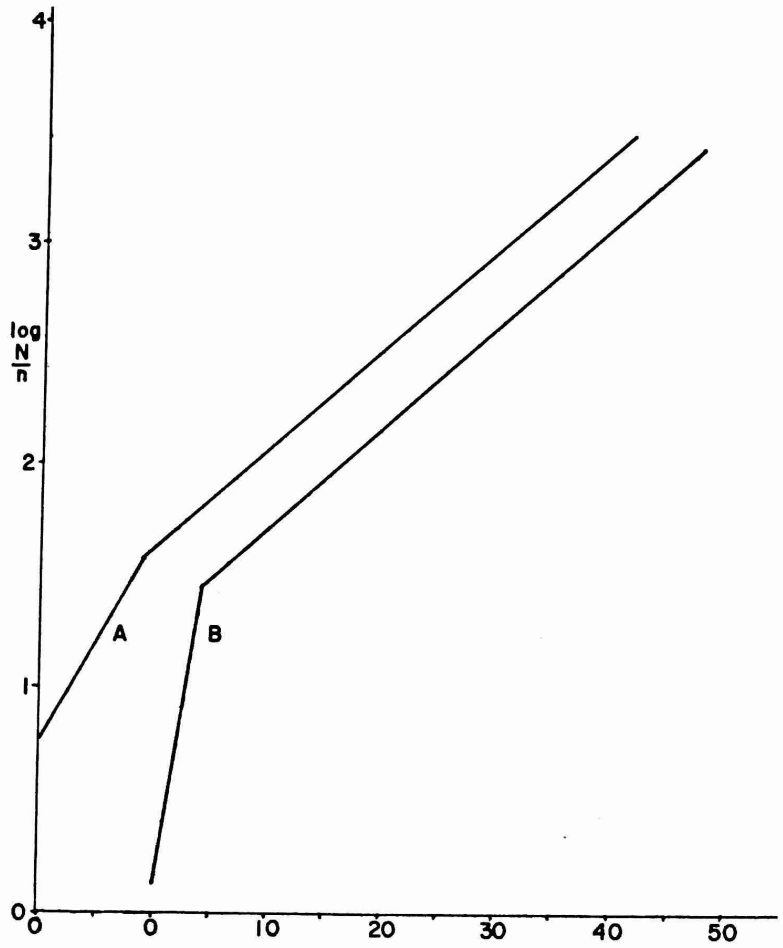

Fig. 4. Comparision of composite ratios for 1967 tree seedlings in well and poorly drained soils. (A) well drained (B) poorly drained.

Table 1

Strong soil type preferences exhibited by individual species in the irradiated study area. Method of computation explained in text. Only preferences greater than 40% listed, except in support of another entry.

Species	Sample Size	Preference %	Soil prefered	Size of plants
Myrcia leptoclada	14	100*	Well drained	≥ 4.5 ft.
Melisona herberti	12	100*	" "	≥ 4.5 ft.
Guarea ramiflora	11	92a	Poorly "	" "
Tabebuia pallida	6	85	" "	" "
Tabebuia pallida	297	65*	" "	Seedlings
Sloanea berteriana	123	75*	Well "	≥ 4.5 ft.
Cordia boringuensis	11	70	Poorly "	" "
Euterpe globosa	25	69*	" "	" "
Miconia tetandra	11	63	" "	" "
Miconia tetandra	63	25	" "	Seedlings
Casearia arborea	9	58	Well drained	≥ 4.5 ft.
Palicourea riparia	57	52*	Poorly "	" "
Alchornea latifolia	7	46	" "	" "
Ixora ferrea	9	45	" "	" "
Dacryodes excelsa	36	41a	Well "	" "

* Significant by chisquare test at .01 level.
a " " " " " .05 " .
Sample inadequate to establish significance of others.

In summary, the seedling population of this recovery area was able in three years to produce a diversity structure comparable to that of mature rain forest in every way except species per unit area. In that regard it achieved a species density that can only be reduced with the passage of time. In addition two general classes of abundance that characterized the composite ratio of every vegetational unit studied were manifested very early in the succession. These classes exhibit, within themselves, a remarkable exponential relationship between species and individuals having a characteristic slope, and the differences in slope and information content between classes increased with time to a plateau level. Overall tree species diversity in this successional vegetation reached a level near 50 species per thousand individuals by the third year.

Several studies have aimed at discovering community structure and diversity of lower montane rain forest in Puerto Rico. Smith (in press) studied preirradiation diversity in the El Verde site by conventional diversity index methods and arrived at a figure of approximately 48 species per thousand individuals for the mature forest. In a later study involving transects into different habitats, he obtained 60 species per thousand. The present techniques were applied to a sample of 116 trees in 676 square meters of the control center sampled especially for the purpose and yielded a composite ratio having an A slope of 8 species per decade, extrapolating to 50 species per thousand individuals (figure 5). In an attempt to avoid extrapolation, a sample of 2000 trees was made in a 10 meter wide transect encircling the irradiated area at a distance of 160 meters. One thousand trees were taken on well drained soil and the transect was lengthened by spiralling to include 1000 trees on poorly drained soil. The composite ratio for poorly drained soil had the expected shape and reduced A segment, but had 53 species and a B slope indicating 58 species per thousand, while the well drained soil sample had an unexpected shape with three segments, had 55 species and would require 62 for the usual symmetry (figure 5).

As the more or less linear transect had been taken in order, the trees were divided into groups of ten and a search made for frequency correlations of certain species in neighboring groups. The data under this treatment fell into three groups having high internal correlation and low correlation with other groups. One group was dominated by Croton poecilanthus, a tree of ravines and flats that is rare on ridges and slopes and was rare in the other two groups. A second group was dominated by Euterpe globosa, which was also a dominant in the Croton group but had a complex of species almost absent from the other groups, including Myrcia deflexa, Trichilia pallida and Ixora ferrea. The habitat of this group seemed to be gentle slopes having mostly soils of high moisture content, but not easily separated into well and poorly drained categories. The third group was dominated by Sloanea berteriana and seemed to be a ridge top and steep slope flora but included also a river bank flora having distinctive species which was impossible to separate with this technique.

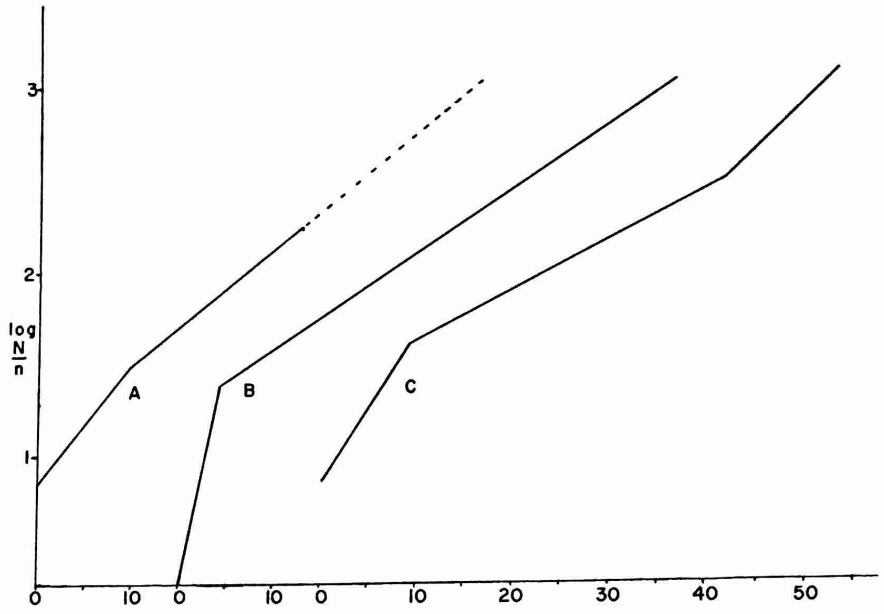

Fig. 5. Composite ratio curves obtained for mature forest. (A) 676 square meters in radiation control center (B) 1000 trees growing in poorly drained (reduced) soils (C) 1000 trees from red or yellow (presumably well drained soils).

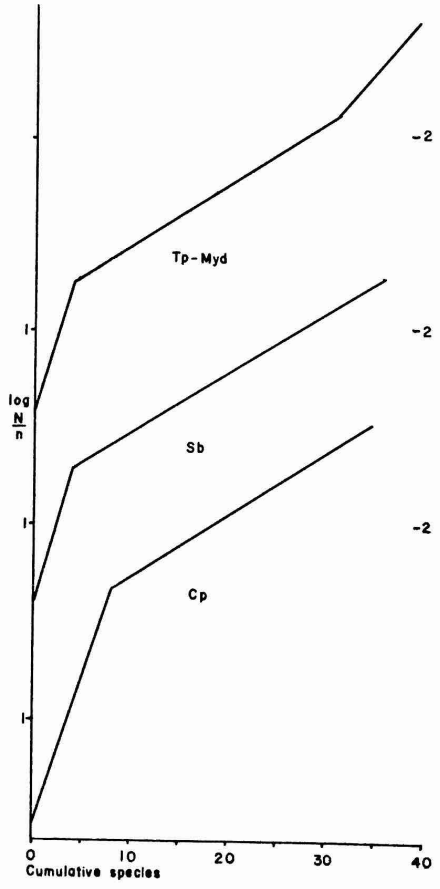

Fig. 6. Further breakdown of figure 5C into three apparent tree associations which correlate with topography. Lower curve characterizes flat areas and is dominated by Croton poecilanthus. Middle curve includes steer slopes and river bank and is dominated by Sloanea berteriana. Upper curve includes gentle slopes with Euterpe globosa and Dacryodes excelsa as dominants but has Trichilia pallida and Myrcia deflexa as exclusive subdominants.

When processed separately all three of the groups had higher diversities than expected, but the unique third segment proved to be a phenomenon of the Myrcia - Trichilia group (upper curve in figure 6). The A segment of this group has only three species; Euterpe globosa at 1 part in 5, Dacryodes excelsa at 1 part in 10, and Myrcia deflexa at 1 part in 18. Almost all of the remaining tree species found in any forest habitat occurred as rare species among the 360 individuals sampled and very rare species were inadequate in number. The only other place such a composite ratio was encountered was in post radiation sprouts to be discussed below. Explanation is very hypothetical at this point but may involve a sublethal environmental stress such as strong seasonal fluctuation in moisture content. Specialist species, such as those in the chronically poorly drained soils, could be discouraged by temporal physical diversity of the environment from exerting strong competitive pressures, leaving the habitat relatively open for sub-optimal, subsistence utilization by any comer. Such a situation could lead to development of a condition of maximum diversity and may have, to the extent that the diversity limits of a small island land mass are being reached. The composite ratio for this habitat would apparently allow for a species per thousand diversity of about 70, which approaches that recorded in continental rain forests, and it is doubtful that so many sufficiently unspecialized species are available. An analogous situation exists in the human economic situation of Puerto Rico, where an infusion of foreign capital has acted to depress competition. Aggressive entrepeneurs are able to amass fortunes and there seems to remain plenty, yet many specialized occupational niches remain mysteriously open or are filled by relatively non-aggressive immigrants; the explanation being that the human technological diversity of the island, developed under conditions of stronger competition, is inadequate to fill the niches as rapidly as they would be filled in a larger area having a broader economic base. If applicable, the hypothesis may further indicate that maximum diversity, although not to be expected under conditions of strong environmental stress, may appear under conditions that are not conducive to the most rapid utilization of resources, as these promote keen competition and stress of biological origin. Chronic or recurrent sublethal stress could therefore be the key to maximum diversity.

Community Destruction

Only two categories of plants seemed to show diversity effects attributable directly to the radiation stress. They were the plants that survived until the postradiation measurements were taken, and a subcategory, those that sprouted and began regrowth after sustaining visible damage. All plants in the sampled area had shown visible damage by 1967. From the standpoint of the composite ratio the plants maintained an orderly retreat (figure 7). Individuals decreased from 824 in 1966 to 386 in 1967, the number of species from 54 to 42, extrapolated species per thousand from 51 to 44, and H from 3.821 to 3.481. The number of species in the A segment decreased from 8 to 5 and the

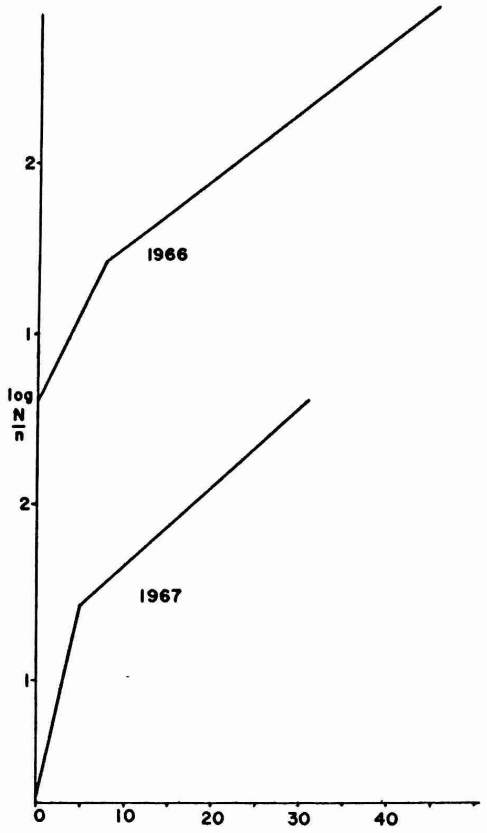

Figure 7. Composite ratios of old vegetation surviving from before irradiation until 1966 and 1967, one and two years respectively.

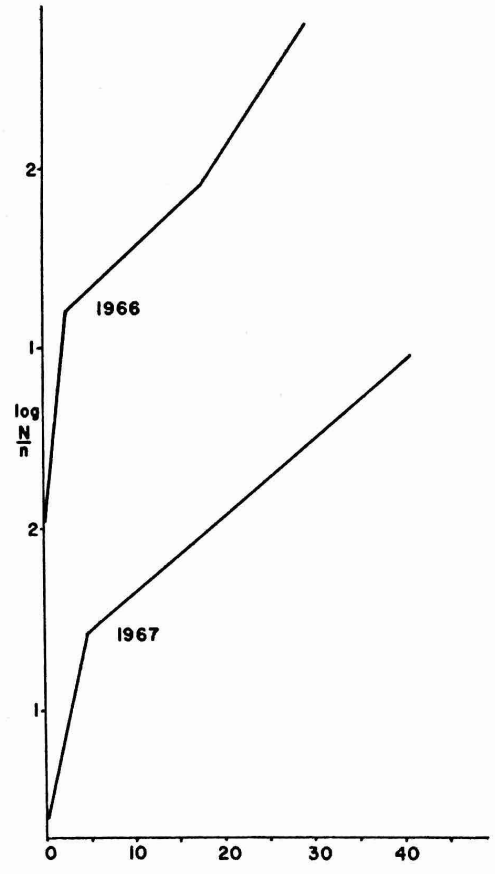

Figure 8. Composite ratio curves of sprouts populations one and two years after irradiation.

A slope from 9.5 to 5.3, while a comparable decrease occurred in the B slope, from 20.25 to 22.55. Thus, although individual species differed in radiosensitivity, the decreases were distributed throughout the composite ratio, in contrast to the development pattern, in which diversity changes in the common species were not synchronous with those among rarer species. Processing is not complete in 1968 old vegetation, but the overall trend continued without major discrepancy.

Sprouts from old vegetation were placed in a separate category from the parent plant, which remained in the group just discussed. Although sprouts have taken a respectable position in the community of recovery vegetation, and have increased in numbers and diversity, they did exhibit an unusual composite ratio during the first year that may represent a reaction to the radiation stress (figure 8). Separation of the usual B segment into two separate linear segments having different slopes was observed earlier in what appeared to be a community of natural occurrence. Here the explanatory hypothesis has a more tangible form. Removal of the canopy created conditions conducive to rapid growth, and undamaged meristematic tissue near or below the ground surface, where rock and slope shielding had reduced radiation dosage, found itself with a strong competitive advantage over seedlings by virtue of possessing extensive and relatively undamaged root systems and food reserves. Spacing of the old plants reduced competition between sprouts to a very minimal level. The controlling factors for diversity therefore became the diversity of old plants, the diversity of meristematic tissue near the ground among them, the rate at which this tissue could be stimulated into growth, the radiation dose received and the relative radiosensitivity of each. Meristem diversity may be the factor most responsible for the fact that sprouts had a lower diversity than old vegetation as the sprout lists are very similar to the old vegetation lists but lack certain of the species completely. The 1966 anomaly in composite ratio, on the other hand, seems to be partly a function of sprouting rate, as several rarer species did not join the sprout community until 1967. Other factors may have also operated to smooth out the anomaly, competition with the fastest growing saplings probably intensified and the inroads of disease and insect predators increased the rarity of some species. The B slope ultimately achieved was the one predicted by the more diverse of the two B segments of 1966.

Animal Diversity

Animal diversity studies have lagged behind the studies of vegetation because fauna are more poorly known than the flora, sampling methods are more biased due to motility and secretiveness of the organisms, and fewer investigators have studied the question of diversity in this area. In particular, the problem of mobility becomes almost insurmountable in some groups. Turner (in press) discussed problems he encountered in attempting to assess vertebrate populations and how greater mobility in one lizard species made data obtained for it incompatible with data

gathered for a more sedentary member of the same genus. The census data for birds gathered by Recher (1964, 1965, in MacArthur and Wilson 1968) is good in many respects, but numbers were obtained on both sexes of some species, only males of others, and others were observed but could not be counted. Insectivorous birds are rare in the resident populations but the pattern is complicated by massive seasonal influxes of migrants, particularly insectivorous warblers of numerous species. Wiegert (in press) made population determinations of soil and litter microarthropods but could not carry separations to the species level in some groups.

Continuing efforts have been made over the past three years to achieve sufficient familiarity with the insect fauna for meaningful diversity estimates to be made. To this end keys have been written separating distinguishable species designated by code letters. When sufficient material is accumulated in a family group, the group is sent first to the U.S. National Museum under an agreement with Dr. William Anderson, and, if the museum specialists recommend, it is forwarded to a recognized specialist for the group. Of some 30 families sent so far, none has failed to contain some undescribed species, and some have contained more unknown than known forms. A sample key written for tne Dolichopodiae is included as an appendix to this report. This is a Dipteran family for which determinations have just been made by Dr. Harold Robinson of the U.S. National Museum. For diversity purpose, all Dolichopodids are assigned a letter and the abbreviation Dol. Other abbreviations are explained at the beginning of the key. Insect collections have been made using sticky traps, malaise flight traps and light traps. Composite ratio plots have now been made for significant numbers of insects collected in single 24 hour periods. Numerous other collections have been made and are in various stages of sorting; some are waiting on taxonomic work for only a very small percentage of rare species in difficult groups.

A sample of 6,377 insects was taken in 31 small mosquito type light traps on the night of Sept. 24, 1965. Total diversity calculations have been made on these insects, 5,789 of which were Diptera with 98 species, 268 Lepidoptera with 60 species, 145 Homoptera with 26 species, 79 Trichoptera with 13 species, 62 Coleoptera with 16 species, 42 Psocoptera with 9 species, 35 Hemiptera with 9 species, 32 Hymenoptera with 7 species and 13 Neuroptera with 9 species. All CR plots with the exception of Lepidoptera were smooth curves with no clearly discernible segments such as were characteristic of vegetation. Lepidoptera exhibited a sharp break beyond the fifth species but tended to curve a little beyond the break (see figure 9). Samples of other families are also presented in figure 9, with Trichoptera the least diverse and Coleoptera more diverse. Such curvature makes H a very inadequate measure because of its high sensitivity to sample size. Scaled H seems to be the only information measurement giving valid comparisons between groups, although no routine scaling procedure for samples less than 100 was used. Crude extrapolations for H 100 were made on Trichoptera and Coleoptera, yielding H100 of 1.6 and 2.8 respectively. H100 for Homop-

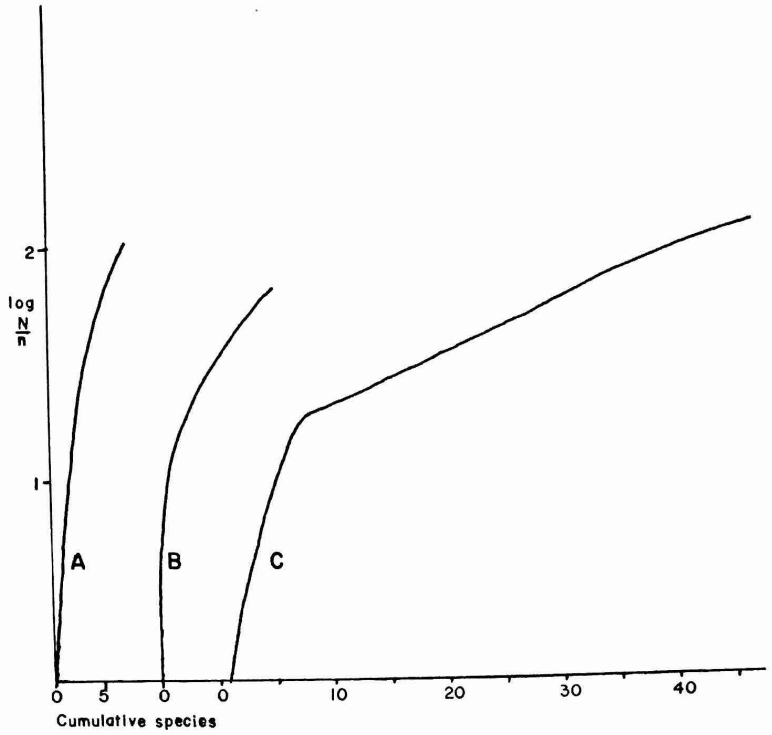

Fig. 9. Composite ratio curves for insect families taken in light traps Sept. 24, 1965. (A) Trichoptera (B) Coleoptera (C) Lepidoptera.

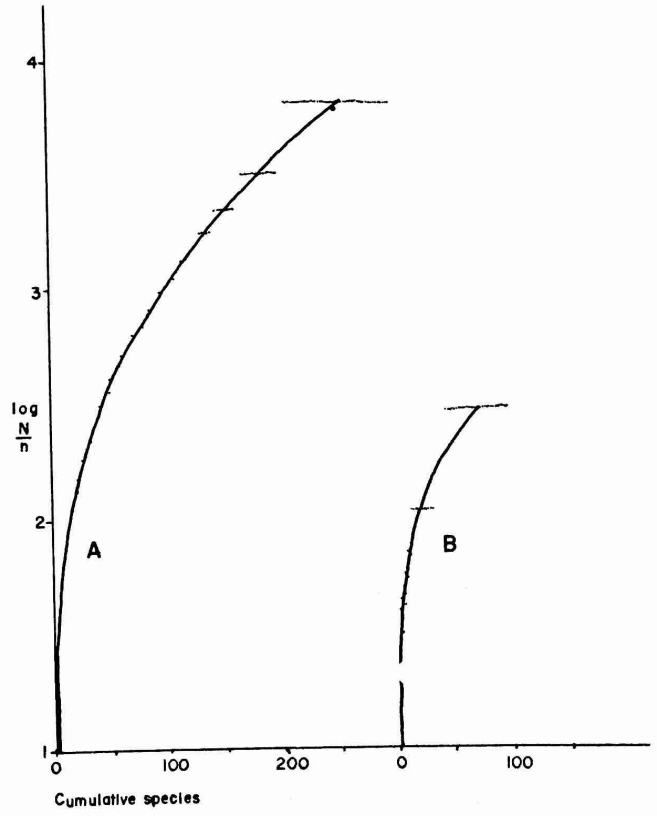

Fig. 10. Composite ratio curves for total insects taken in (A) light traps Sept. 24, 1965. (B) malaise flight trap Mar. 22, 1969. Note scale on species axis is very different from previous curves.

tera was 3.5, for Lepidoptera 3.9, for Diptera 4.2 and for total insects 4.3. As no insect family can be said to form a community in the sense the word was used for plants, perhaps only the total insects (figure 10) should be compared to plants. An estimate of species per thousand by the maximum likelihood method is 125, while H1000 = 5.108, both much higher than in any community of plants measured.

Malaise trap insects exhibited similar patterns but the largest sample in 24 hours was 199 insects of 120 species (figure 10). H100 of this sample was the same as in the light trap at 4.3, but curvature was much stronger as is suggested by the fact that almost as many species were obtained in 200 individuals as the estimated species per thousand from the light trap. A very crude estimate of species per thousand would fall between 200 and 300 and suggests that the light trap is more selective than the flight trap, which uses no bait but depends on the tendency of flying insects when encountering an obstacle to veer upward. When one considers that this trap is almost limited to flying insects and is immune to many of them as evidenced by sticky trap collections we see that total insect diversity must be very high with a truly unbiased and random sample of 1000 insects containing perhaps 500 species.

Conclusions and Discussion

From the standpoint of diversity methods we have concluded that each method mentioned makes a valuable contribution to our understanding of community structure and diversity, and that there are pitfalls in the uncritical applications of any of them. In particular, linear methods such as William's log series or a falsely assumed constancy of Brillouin's H measurement with increasing sample size can be misapplied. Actual plotting of a diversity index curve can give warning of nonlinearities at high number levels and the even more easily computed composite ratio provides a view of total sample composition, giving a solid foundation to whichever diversity index is chosen.

Details of community structure noted are a break in composite ratio of most plant communities which is not a mathematical artifact and which divides the community into a group of common species with lower diversity and rare species of higher diversity, an occasional second break farther out which sets off a group of the rarest species having lower diversity than the intermediate species also thus formed, and the absence of such breaks in trap samples of insects of most groups. It is probably inappropriate to call trap samples of insects or their taxonomic subgroups communities, or to compare them in any rigorous way to communities of macroscopic vegetation. When more knowledge of the ecological role and trophic levels of the particular insects is gained it may be possible to assign species to communities. An example is the fact that some phorid flies, a group dominating sticky trap collections, are scavengers and some are known to be insect parasites. These are members of the same community only in the sense that vines and mushrooms among the plants are,

and diversity of this scope has not yet been measured in the plants. It is even possible that the continuous curvature in the composite ratios of the insects is the result of single breaks at different points in many combined community curves, but this unlikely in view of curvature in such groups as Trichoptera, which are ecologically very narrow. A more probable explanation is that the insects collected are adults, and many, if not most, species from breeding aggegrations of varying density and dimensions. There is thus a potential non linearity in the distribution of each species and random soundings should yield many valleys and few peaks. The combined sample of many independent species should therefore show the same trend. In addition to possible selective attraction of light traps, this phenomenon might provide additional reason to expect less ratio curvature and diversity, as an attractant should tend to shift several distribution peaks into register and sample the tops of all.

It was hypothesized that anamalous double-breaking vegetation CR curves could be due to the effects of reduced competition combined with some sort of limit on the number of species able to take advantage of this. In post-radiation sprouts the lack of competition was clear and the limit was suggested to be the rate at which species could sprout. This anomaly disappeared in the second post radiation year. A similar anomaly was found in mature vegetation in certain soil types apparently intermediate between well and poorly drained and containing a complex of medium to rare trees species almost absent elsewhere. Reduced competition was inferred from the comparative absence of abundant specialized species, and the limit on rare species was suggested to be the island's lack of sufficiently unspecialized species able to grow there.

In the development of plant community structure, abundant species were found to overshoot the ultimate levels of diversity very early and return more slowly to the mature levels. Rare species were found to slowly increase to the mature levels with no overshoot yet observed. Total species diversity as species per thousand was found to be more sensitive to changes in rare species, but Brillouin's H did not show diversity overshoot in tree species only. The latter measurement followed the common species in overshoot and correction when total vegetation including herbs and vines was considered. Seedlings of tree species considered as a class established mature levels of diversity in every parameter by the third year, and served to point up the fact that species per fixed number of individuals is a very different measurement from species per unit area unless individual size is strictly comparable.

Reduction of diversity in vegetation showing radiation damage did occur, but the reduction was orderly in that disruption of the composite ratio did not occur as plants died.

In projected diversity studies it is desireable to follow radiation recovery with annual surveys for several more years. The sapling class of new trees seems to be duplicating in slow motion diversity events that

happened too rapidly in the seedling class for good resolution. More study should be given to the areas of mature trees showing the anomaly in composite ratio and extremely high diversity to test hypotheses about their cause. After four years of groundwork, diversity studies in insects are on a firm foundation and should be expanded into community studies by discovery of ecological roles of species. Methodology in animal diversity study for all groups is in need of a great deal of further research. Isotope tracer studies in the El Verde site are also now yielding conclusions of significance to animal community studies and studies of trophic level exchanges.

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Appendix A

Key to Dolichopodidae of the Rain Forest at El Verde by George Drewry (identifications by Harold Robinson, U.S. National Museum). Abbreviations used: A - anterior, P - posterior, D - dorsal, V - ventral, T1 - protibia, T2 - mesotibia, T3 - metatibia.

Wing veins after Curran (North American Diptera, Second edition, Henry Tripp, Pub.).

1 Fourth vein with a widely divergent fork; all metallic flies more than 3 mm. long 2
1' Fourth vein without a fork 11

2 Scutellum with 4 strong bristles (Condylostylus) 3
2' Scutellum with only 2 strong bristles (Sciapus) 6

3 Wings pictured 4
3' Wings clear 5

4 Wing pattern distinct; PV bristles of T1 elongate. C. pilosus (Loew) Dol V

4' Wing pattern indistinct; AD bristles of T1 elongate. C. diffusus Wied ..... Dol AA

5 AD and AV bristles of T2 greatly elongated; 2 long PV bristles on T1. C. pruinosus (Coq.) ..... Dol W
5' T2 bristles normal; no PV bristles on T1. C. graenicheri (Van Duzee) ..... Dol T

6 Gap between tips of third and fourth veins much wider than diameter of tibiae; all coxae yellow; bristles and wing veins yellowish. S. sp. near bellimanus (Van Duzee) ..... Dol M
6' Third and fourth veins almost meeting at tips, gap no wider than diameter of tibiae; wing veins dark 7

7 Pleura light in color (except a small anterior spot); two stripes on each abdominal tergite. S. dorsalis Loew ..... Dol U
7' Pleura a dark metallic color 8

8 Meso and metacoxae dark in color 9
8' Metacoxae yellow, mesocoxae slightly darker; sclerotized portion of first abdominal tergite two narrow wedges joinded by a thin line. S. sp. near unicinctus (Van Duzee) ..... Dol Q

9 Sclerotized portion of first abdominal tergite divided into two separate thin slivers; second tergite with triangular posterior band having an anterior lobe. S. sp. not identified ..... Dol X
9' An undivided band on first tergite, which may have an anterior notch 10

10 Three medium - sized bristles on each side of first abdominal segment, an anterior notch in band; all antennal segments dark. S. sp. near inaequalis (Van Duzee) ..... Dol D

10' A single very large bristle on each side of first abdominal segment, no notch in band; antennal segments yellowish, especially first. Probably S. inaequalis (Van Duzee) ..... Dol XX

11 Pronotum mostly bare of fine hairs, those present confined to strips in front of bristle rows; color always metallic 24

11' Front of pronotum with numerous scattered fine hairs; some not metallic 12

12 Fourth vein bent strongly forward beyond posterior crossvein 13

12' No strong bend in fourth vein, may be curved 15

13 Posterior crossvein almost perpendicular to fourth vein; legs yellow. Sarconius lineatus (Ald.) ..... Dol O

13' Posterior crossvein oblique to fourth vein; legs dark 14

14 Fourth vein ending near wing tip. Tachytrechus sp. ..... Dol PP

14' Fourth vein ending well before wing tip. Plagioneurus univittatus Loew ..... Dol QQ

15 Front of pronotum yellow 16

15' Front of pronotum metallic 18

16 Sixth (anal) vein present; a large, round green spot in front of scutellum. Neurigonia signifer Ald. ..... Dol K

16' Sixth vein absent: thorax wholly yellow. Xanthina 17

17 T2 with a comb of ventral bristles, having in addition long hairs in the male; posterior half of postscutellum and postnotum yellow X. sp. ..... Dol Z

17' T2 without ventral bristles; postscutellum dark and a thin dark line down postnotum. X. sp. ..... Dol YY

18 Posterior crossvein would intersect third vein if extended forward by its own length; bristles yellowish; very small. Thrypticus 19

18' Posterior crossvein too short to intersect third vein if extended forward by its own length; bristles dark 23

19 Coxae and femora dark 20

19' Coxae and femora yellow 21

20 Wing veins yellowish; a minute AD bristle about one fifth of way down T2, I. sp. ..... Dol NN

20' Wing veins dark; a normal AD bristle about one third of way down T2, T. fraterculus (Wheeler) ..... Dol MM

21 Basal abdominal tergite yellow. T. abdominalis (Say) ..... Dol YY

21' Basal abdominal tergite dark 22

22 Basal abdominal sternites yellow. T. setosus H. Robinson ..... Dol ZZ
22' Basal abdominal sternites dark. T. sp. undescribed ..... Dol KK

23 Aristae longer than tibiae; only one anterior bristle on T2. Medetera sp. ..... Dol E
23' Aristae normal; 2 anterior bristles on T2. Coeloglutus concavus Ald. ..... Dol OO

24 Slender elongate flies with wings about 3 times as long as wide; third antennal segment triangular with aristae on basal half of upper edge; a ventral bristle on T2. Sympycnus 25
24' Not exactly as above 27

25 Basal metatarsal segment about as long as second segment; 3 preapical AD bristles on T2, S. sp. ..... Dol L
25' Basal metatarsal segment shorter than second segment; 2 preapical AD bristles on T2 26

26 Pleura light in color; 2 AD bristles on T3, S. sp. ..... Dol BB
26' Pleura dark in color; only 1 AD bristle on T3 (disregard dorsal bristles). S. sp. ..... Dol N

27 Four strong bristles on end of male abdomen; face of male usually wide; females difficult to separate so all can start here. Diaphorus 28
27' No strong bristles on end of male abdomen; face narrow, eyes of males almost touching below antennae. Chrysotus 35

28 Front coxae yellow 29
28' Front coxae dark 32

29 A strong ventral aristae on T2, T2 also with 2 AD and 2 PD preapical bristles; legs yellow with a distal dark band on metafemora. D. dimidiatus Ald. ..... Dol SS
29' No strong ventral bristle on T2; mostly small species 30

30 Aristae of male apical on a slender neck, pleura dark; wing tip behind long axis of wing; female unknown. D. flavipes Ald. ..... Dol G

31 Not as above. Chrysotus females 35

32 Eyes contiguous above base of antennae. D. contiguus Ald. ..... Dol R
32' Eyes not contiguous above antennae 33

33 A median AD bristle on T2 about half way between upper AD and apex of leg, but median D or PD of T1, if present, is much smaller than basal D of T1; third segment of antennae little higher in lateral view than second segment; basal metatarsal segment of males with a strong ventral bristle; large species over 2.5 mm long 34
33' Median AD bristle of T2 absent, or much closer to upper AD than to apex, or a median D or PD present on T1 that is as long as upper D. Chrysotus females 41

34 Median AD of T2 more than half as long as upper AD. Third vein of males not strongly arched. D. sp. ..... Dol DD

34' Median AD of T2 less than half as long as upper AD. Third vein of males strongly arched. D. simplex ..... Dol GG

35 At least front coxae yellow; small species less than 3 mm long 36

35' All coxae dark; size variable 41

36 Distance between tips of second and third veins little more than distance between tips of third and fourth; femora solid yellow; only one well developed preapical bristle on T2, C. sp. ..... Dol C

36' Distance between tips of second and third veins almost twice that between third and fourth 37

37 Pleura, legs, and antennae yellow; very small flies 1-2 mm. long; third antennal segment of male with slender processes above and below aristae. C. sp. ..... Dol A

37' Pleura and antennae dark; usually with some dark shading on femora 38

38 A small PD bristle opposite or basal to upper AD on T2..... 39

38' Any PD bristle on T2 distal to upper AD; profemora usually not shaded with dark color 40

39 Some dark shading on all femora; all segments of antennae dark; T3 of male shorter than first metatarsal segment, that of female normal. C. brevitibia Van Duzee ..... Dol RR

39' All femora yellow; second antennal segment yellow, third dark; T3 of male normal. C. mexicanus H. Robinson ..... Dol Y

40 A median AD bristle on T2 and T3, although minute in males; dark bands on femora variable but usually strong on mesofemora of males. C. flavohirtus Van Duzee ..... Dol B

40' Upper AD only noticable bristle on T2 and T3; dark distal band on metafemora only. C. sp. ..... Dol H

41 At least basal three fourths of all femora dark 42

41' Metafemora solid black, others yellow with slight dark shading in female, heavy shading in male; third antennal segment of male spearhead - like with a long tapering point; aristae apical. C. excavata Van Duzee ..... Dol EE

42 Third antennalsegment disc - shaped, its height in lateral view more than twice that of second segment; median AD bristle on T2, if present, much closer to basal AD than to T2 apex; medium sized, all longer than 2 mm. 43

42' Height of third antennal segment little more than that of second, median AD of T2, if present, midway between upper AD and T2 apex..... 46

43 At least front tibiae yellow 44

43' All tibiae black; median AD bristle of T2 developed but almost D rather than AD; second metatarsal of male shorter than third. C. sp. near excisus Ald. ..... Dol CC

44 Only basal AD of T2 well differentiated from hairs 45

44' Three AD bristles differentiated on T2, the uppermost a small one basal to the usual basal AD; metatibiae of males brown; aristae of males not recessed but females very similar to C. proximus. C. sp. Dol JJ

45 Aristae of male recessed in a deep notch, those of female slightly so; basal metatarsal segment of male lacking a ventral bristle. C. proximus Ald. ..... Dol J

45' Aristae of male not recessed; a long ventral bristle on basal metatarsal segment of male; female not discovered but possibly indistinguishable from C. proximus. C. spinipes Van Duzee ..... Dol WW

46 Median AD bristle of T2, if developed, less than half as long as basal AD; small species mostly less than 2 mm. long 47

46' Median AD bristle of T2 almost as long as basal AD; very similar to Diaphorus species DD and GG but distinguished by a median D to PD bristle on T1 that is as long as basal D; males lack a ventral bristle on basal metatarsal segment. C. sp. ..... Dol VV

47 Wings dusky, veins dark; tibiae of males dark, females light; femora dark but with little metallic sheen; basal AD of T2 present in both sexes 48

47' Wings clear, veins yellowish; pleura and femora with bright green metallic sheen; male abdomen with violet reflections; T2 of male lacking bristles. C. humilis Parent ..... Dol FF

48 Basal metatarsal segment longer than next segment; T3 dark on basal one fifth in female; third segment of male antennae rounded in front and somewhat bean-shaped in lateral view. C. sp. near niger Ald. ..... Dol TT

48' Basal metatarsal segment no longer than next segment; T3 of female all light; third segment of male antennae triangular and pointed; aristae barely subapical. C. sp. ..... Dol HH

Appendix B

Key to Muscidae (sensus latus) of the Rain Forest at El Verde (Anthomyiidae and Muscidae) by George Drewry (some identifications provided and all checked by Silverio Medina, U.S. Department of Agriculture, University Experimental Station, Rio Piedras).

Abbreviations same as in Appendix A.

1 Lower calypter rounded posteriorly and dorsally. Anthyomyiidae..... 2
1' Lower calypter broad, somewhat flattened posteriorly and triangular dorsally (Muscidae). Aristae bare. Synthesiomyia nudiseta Van der Wulp ..... Mus A

2 Sixth vein very short, seventh curved outward so that it would intersect sixth only a short distance beyond end of latter. Subfamily. Fanniinae 3
2' Not as above 4

3 A small preapical AD bristle on T1; palpi broad, flat and yellow; tibiae yellow, femora black. Probably Euryomma sp..... Anth U
3' No preapical AD on T1; palpi cylindrical and black; head bristles all shorter than third antennal segment; color shiny black overall. Fannia sp. ..... Anth V

4 Less than two presutural dorsocentral bristles. Subfamily Coenosiinae 5
4' Two presutural dorsocentral pairs 24

5 One pair of presutural dorsocentral bristles 6
5' Dorsocentral bristles not differentiated from thoracic hairs; profemora black, meso and metafemora yellow. Atherigona excisa Thomson ..... Anth W

6 Two pairs of postsutural dorsocentral bristles. Bithorachaeta 7
6' Three pairs of postsutural dorsocentral bristles. Neodexiopsis 8

7 Legs yellow. B. leucoprocta (Wiedemann) ..... Anth F
7' Legs black. B. varicornis (Coquillett) ..... Anth Q

8 Apical scutellar bristles more than three fourths as long as subbasals 9
8' Apical scutellars less than three fourths length of subbasals 17

9 One pair of postsutural IA (intralar) bristles; procoxae yellow 10
9' Two pairs of postsutural IA bristles; procoxae yellow or black 12

10 Palpi and third segment of antennae yellow (males presently unknown) 11
10' Third antennal segment dark below aristae; palpi dark with some light shading; female with distal fifth of all femora darkened, three preapical bristles on T3 (AD, D, AD), distal AD on apical eighth of T3; male with an additional PD bristle on T3 basal to the other 3. N. rex Curran ..... Anth B

11 Two large median anterior bristles on mesofemur; proboscis light yellow; female with posterior side of profemora dark, others banded distally, same T3 bristles as N. rex but situated more basally so distal AD on apical third. N. sp. undescribed near N. rex..... Anth G

11' One large median anterior bristle on mesofemur; proboscis brown; female with all femora yellow; same T3 bristles as N. rex but distal AD on apical fourth. N. sp. undescribed near N. rex. ..... Anth H

12 Procoxae gray or black and same color as adjacent mesopleura..... 13

12' Procoxae yellow and much lighter than adjacent mesopleura 15

13 A preapical AV bristle on T3 14

13' No preapical AV on T3 (the usual AD, D, AD present); no longitudinal stripes on thorax. N. sp.undescribed near ditiportus.. Anth J

14 Four preapical T3 bristles (AD, AV, D, AD); indistinct longitudinal stripes on thorax; tibiae black; median parafrontal bristles reclinate. N. ditiportus Snyder..... Anth C

14' Five preapical T3 bristles, a PD almost even with basal AD; distinct longitudinal stripes on thorax; tibiae brown; median parafrontals cruciate, N. sp. undescribed near ditiportus Anth K

15 No preapical AV bristles on T3 16

15' A small AV near median AD of T3; palpi yellow; third antennal segment dark with a prominent yellow band basally (male unknown); femora of female yellow with posterior of profemora shaded black, others banded distally with black. N. sp. undescribed near N. discolorisexus ..... Anth T

16 Third antennal segment dark; 3 preapical T3 bristles (AD, D, AD); legs of female black, those of male yellow with black tarsi. N. discolorisexus Snyder ..... Anth E

16' Third antennal segment yellow; female with 4 preapical T3 bristles (AD, D, PD, AD); T3 of male with numerous long, bristly hairs. N. calvalata Snyder (probably a synonym of N. medinai Snyder) ..... Anth A

17 Femora, all coxae and palpi dark, tibiae light 18

17' Femora, coxae and palpi yellow 19

18 One pair of postsutural IA (intralar) bristles; median parafrontal bristles cruciate; 3 preapical bristles on T3 (AD, D, AD). Male unknown. N. ebinfemur Snyder ..... Anth O

18' Two pair of postsutural IA bristles; median parafrontals reclinate; 6 preapical T3 bristles (AD, A, PD, D, AD, PD). N. maldonadoi Snyder..... Anth N

19 Stigmatal and propleural bristles duplicated (4 small bristles near base of front coxae); tibiae of males fairly straight 20

19' Only 2 small bristles above base of front coxae; tibiae of known males bowed considerably and enlarged distally; third antennal segment dark 22

20 Third antennal segment clear, light yellow 21
20' Third antennal segment brown with a yellow area adjacent to base of arista; males with long hairlike bristles on tibiae, 1A and 1D on T2, a basal PD, AD pair then PD, D, AD, on T3; sides of male abdomen shiny with few setulae, two postsutural IA on at least some specimens, one is listed for holotype. N. crispiseta Snyder ..... Anth M

21 One pair postsutural intralar bristles; male with 1A and 3D bristles on T2, numerous curly hairs on T3 and dorsum of basal metatarsal segment; posterior hairs of oral margin yellow in female. N. sp. undescribed ..... Anth R
21' Two pair postusutural intralar bristles; both sexes with 1A and 1D on T2, AD, D, and AD on T3; posterior hairs of oral margin black in female. N. neoflavipes Snyder ..... Anth P

22 An AV bristle on T3; otherwise intermediate between next two species. N. sp. undescribed or possibly hybrid ..... Anth W
22' No AV on T3 23

23 Males with one A to AD bristle on T2, numerous posterior T2 hairs and bristles, abdominal setulae sparse and abdomen shiny; female with all hairs on oral margin black, one posterior bristle on T2. N. micans Snyder ..... Anth S
23' Males with numerous, long curly A and P bristles on T2, abdominal setulae normal; female with posterior hairs of oral margin light, two posterior bristles on T2. N. crassicrurus Snyder ..... Anth L

24 Fourth vein curving forward at end toward convergence with third or small species less than 4 mm.long 25
24' Fourth vein parallel or divergent with third at end; larger than 4 mm. long; Aristae long-plumose. Subfamily Phaoniinae 29

25 Aristae long-plumose; middle thoracic stripe light in color; third vein with a tuft of setulae at base. Subfamily Mydaeinae 30
25' Aristae short-plumose to bare; middle thoracic stripe dark in color; no setulae on third vein. Subfamily Limnophorinae 26

26 Aristae almost bare; color a dirty blue-gray with indistinct darker pattern; small species less than 5 mm. long. Gymnodia 27
26' Aristae short-plumose; color pattern fairly distinct when dry, a clear demarkation on mesopleura between a smooth, dark anterior half and a silvery pollinose posterior half; large species mostly longer than 5 mm. Limnophora 28

27 Fifth vein reaching margin of wing; 3.5-5 mm. long. G. sp..... Anth DD

27' Fifth vein stopping short of wing margin; 2.5-3.5 mm. long G. sp. ..... Anth JJ

28 Two posterior preapical bristles on T2; first postsutural dorsocentral bristle much longer than second. L. sp. ..... Anth EE

28' Only one posterior preapical T2 bristle; first two postsutural dorsocentrals similar in size and considerably shorter than last two. L. sp. ..... Anth CC

29 Third vein setulose; three sternopleural bristles. Scenetes cardini Malloch ..... Anth FF

29' Third vein bare; two sternopleural bristles. Phaonia. No preapical bristles on T1; 1 AV, 2 AD and 1D on T3. P. sp..... Anth BB

30 Posterior crossvein almost twice as long as segment of fifth vein distal to it; first postsutural dorsocentral bristle slightly longer than second; humeri;and scutellum black. Myospila obsoleta (Brauer and Bergenstamm) ..... Anth GG

30' Posterior crossvein little longer than segment of fifth vein distal to it, first postsutural dorsocentral much shorter than second; humeri yellow, scutellum red posteriorly. Neomuscina farri (Dutch)..... Anth KK

Notes: Key expanded in part from Snyder, F.M. 1957. Puerto Rican Neodexiopsis (Diptera Muscidae: Coenosinnae) J. Agr. Univ. of Puerto Rico 41: 207-229. His characters involving intralar bristles and distal mesofemoral bristles did not hold for all specimens of N. crispiseta and N. micans examined. Species identified as N. calvalata on basis of wing shape were more like medinai in all less exact characters and cast doubt on specific distinction.

SECTION III

Section three consists of a manuscript submitted for publication by Dr. Carl F. Jordan, and two reports by visiting scientists who were supported by the Terrestrial Ecology Program.

"Nitrogen Fixation by Epiphyllae at El Verde" was prepared by Dr. Joe A. Edmisten, of the University of Georgia, and his graduate student, M.A. Harrelson. Dr. Edmisten spent two weeks during the summer of 1968 at the El Verde site, to initiate the project, and Mr. Harrelson spent two months on site completing the work.

Dr. Elizabeth McMahan of the University of North Carolina has been visiting the El Verde site yearly since the termination of radiation, to measure long term changes in termite populations as a result of radiation. Her report for the 1968 check is included.

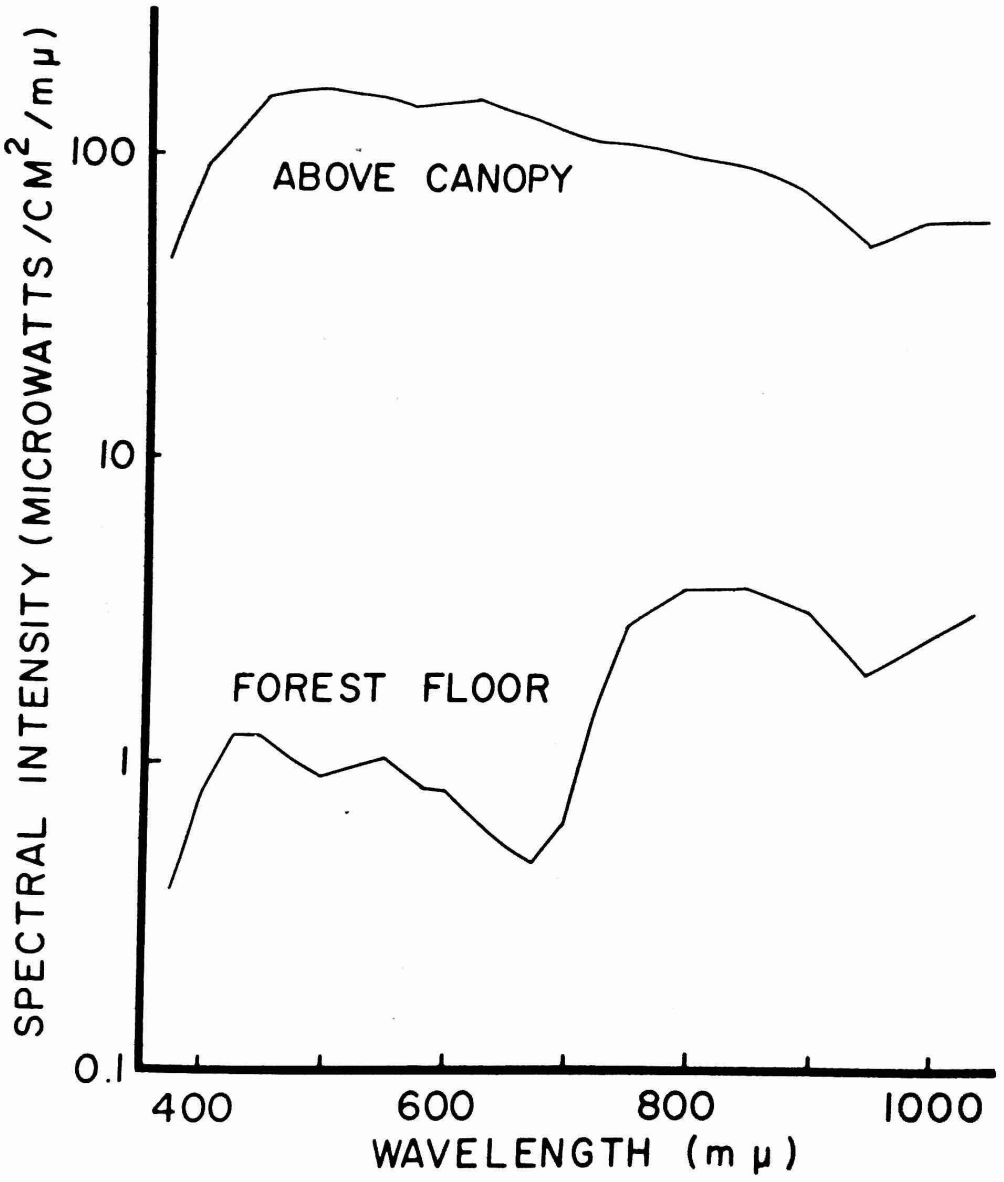

Fig. 1. Intensity of radiation vs. wavelength, measured above the canopy at noon on Nov. 16, 1967, and measured on the forest floor a few minutes later.

DERIVATION OF LEAF AREA INDEX FROM QUALITY OF LIGHT ON FOREST FLOOR

By
Carl F. Jordan

Introduction

Ecosystem studies such as those of productivity and chemical element cycling require measurements of the quantity of leaves in the canopy. This quantity is often expressed as leaf area index, that is, area of leaf per areaof ground. In herbaceous communities, it can be determined directly by clipping (Monsi and Saeki, 1953), but forest measurements are more difficult to make. In order to estimate leaf area index throughout a large area of tropical rain forest, Odum, Copeland, and Brown (1963) measured leaf area index directly in 10 locations, correlated it with optical density measured with silicon solar cells, and then made optical density determinations throughout the forest.

There are two disadvantages in using optical density determined by solar cells as a measure of leaf area index. One is practical, in that it is inconvenient in a field survey to have one cell above the canopy and the other in the investigator's hand, both of which must be read simultaneously, or nearly so. The second is theoretical, in that solar cells respond to light over a broad band of the spectrum including infra-red whereas extinction of light is due primarily to chlorophyll which absorbs light in a relatively narrow band. Much of the light recorded by a solar cell on the forest floor is due to scattered light of wavelengths other than the chlorophyll absorption band. The quantity of this scattered light could be influenced by shape, orientation, and spacing of canopy leaves.

This paper presents an indirect method of measuring leaf area index. The method may be superior to the optical density method.

Theory

Intensity of red light reaching the canopy is slightly greater than that of near infra-red, but on the forest floor, the relative intensity of the infra-red is many times greater (Fig. 1; Federer and Tanner, 1966). This is due to the selective absorption of radiation by leaf pigments. The more leaves that are present, the greater will be the difference in red and infra-red radiation at the forest floor.

The intensities of infra-red and red light can be expressed as a ratio, and this ratio can be calibrated with leaf area index measured

directly at several points in a forest. Leaf area index throughout the entire forest can then be derived from ratios measured at the forest floor.

To maximize the ratio as leaf area index increases, the ratio should be between light at 800 and 675 millimicrons. Absorption of light by the canopy is at a maximum at 675 millimicrons, and transmission has a maximum at 800 millimicrons (Fig. 1).

Since absorption of light is greatest at 675 millimicrons, scattering of light at this wavelength will be less than at most other wavelengths. The less scattering, the less the ratio is influenced by the angles and spacing of leaves, and hence, the more reliable the correlation of ratio and leaf area index. However, even at 675 millimicrons there probably is some light scattering. To minimize this scattering, ratios should be measured only in direct sunlight, and when the sun is high overhead. At 800 millimicrons, it is not clear how much of the transmission through the canopy is due to scattering of light, and how much is due to absorption and re-emission by leaves. Here again, however, scattering is probably at a minimum in direct sunlight, and with the sun overhead.

To use the ratio as a measure of leaf area index within the forest, the ratio must be constant above the canopy. Figure 2 shows that although quantities of light vary during midday hours of sunny days, the ratio 800/675 remains almost constant. The ratio is also independent of time of year (Table 1). The slight variations could be caused by human and instrumental factors. In any case, the variations are minute compared to changes due to the light passing through the canopy.

Since chlorophyll content per unit leaf area varies between species, the correlation between the ratio and leaf area index will be valid only in the forest type where the calibration was accomplished. However, a correlation between ratio and chlorophyll concentration per square meter of forest floor could be valid for many vegetation types. With such a correlation, if mg. of chlorophyll per square meter of leaf area were determined for a given vegetation type, leaf area index could easily be derived by dividing chlorophyll concentration per square meter of forest floor by concentration per square meter of leaf area.

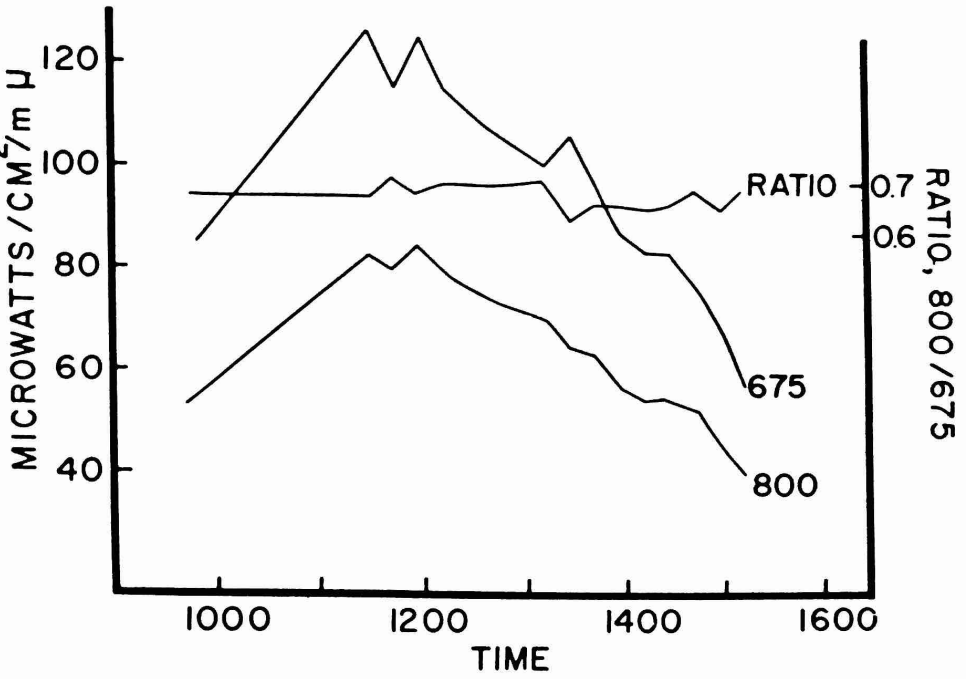

Fig. 2. Absolute intensity of light at wavelengths 800 and 675 millimicrons during the day, and ratio between these intensities.

Table 1 . Ratio of light of wavelengths 800 and 675 millimicrons above canopy at various dates. All readings made with no clouds covering the sun.

Date	Number of Readings	Average Ratio
May 4, 1967	5	0.72 ± 0.03
May 10, 1967	1	0.71
June 5, 1967	1	0.76
June 16, 1967	1	0.75
June 30, 1967	1	0.81
July 5, 1967	1	0.70
Sept. 15, 1967	1	0.81
Sept. 18, 1967	2	0.72 ± 0.01
Oct. 18, 1967	1	0.76
Nov. 16, 1967	15	0.66 ± 0.02
Dec. 5, 1967	2	0.77 ± 0.02
May 2, 1968	6	0.80 ± 0.03
July 1, 1968	4	0.69 ± 0.04
Average of all dates		0.74 ± 0.05

Methods and Results

Leaf area index was measured at three locations in the Luquillo Experimental Forest near El Verde, Puerto Rico, by the following method. Scaffold type towers were erected to a height equal to the top of the canopy, and with a minimum disturbance to the forest. A string with a weight on the end was thrown out from the top of each tower 16 times in such a way as to hook over a twig and then fall straight to the ground, and the number of leaves which each string touched was recorded. Leaf area index at each site was taken to be the average number of leaves touched by the string on each throw. Leaf area index at a fourth site in a ravine was taken to be 2.2, the value Odum, Copeland, and Brown (1963) determined for that site by clipping and measuring leaves.

Light readings at each location were made with a spectroradiometer manufactured by Instrument Specialties Co. The first wavelength was dialed in and a light intensity reading was taken. Immediately, the second wavelength was dialed, and a second reading taken. The process took about 15 seconds. Since the ratio method proposed here assumes that both readings are made simultaneously, the first wavelength was dialed in a second time to assure that the intensity had not changed while the second reading was being made. On clear, sunny days, there was no measureable change.

The spectroradiometer was calibrated with a spectral standard lamp supplied by Instrument Specialties Co. All readings were corrected to absolute values, and the 800/675 ratio was calculated in the office some time after the field measurements were made.

Results of the correlation are given in Table 2 and Figure 3. The equation for the regression line in Fig. 3 is

Eq. (1) $$\log Y = 0.3813 + 0.0989X$$

where Y is the ratio of light at the wavelengths 800 and 675 millimicrons, and X equals leaf area index. Using a value of 340 mg. chlorophyll A per square meter of leaf area for this forest (Odum, Copeland, and Brown, 1963), the relation shown in Fig. 4 was derived. The equation here is

Eq. (2) $$\log Y = 0.3813 + 0.0002908X$$

where Y again is the ratio, and X is mg. chlorophyll A per square meter of forest floor.

Equation 1 is probably not valid for values of leaf area index less than one, since it is known from Table 1 that with a leaf area index of zero, the ratio is 0.78.

If light scattering were not a factor, Fig. 4 could be used to determined chlorophyll A concentration in any forested area, and from

Table 2 . Data for correlation between leaf area index and light ratio.

Also, leaf area index determination for entire forest.

Site	Leaf area index	Average light ratio, 800/675 mu	No. of readings taken	Date of readings
Slope	6.68	10.57	16 16	Aug. 15, 1968 Aug. 19, 1968
Slope	5.60	8.84	4 4 30	May 2, 1968 July 23, 1968 Aug. 15, 1968
Ridge	8.60	17.37	10 16 8	April 24, 1968 May 2, 1968 July 1, 1968
Ravine	2.2*	3.98	32	Aug. 22, 1968
Total for forest	6.6	10.90	114 123 130	April 24, 1968 May 2, 1968 July 26, 1968

* Value taken from Odum, Copeland and Brown, 1963.

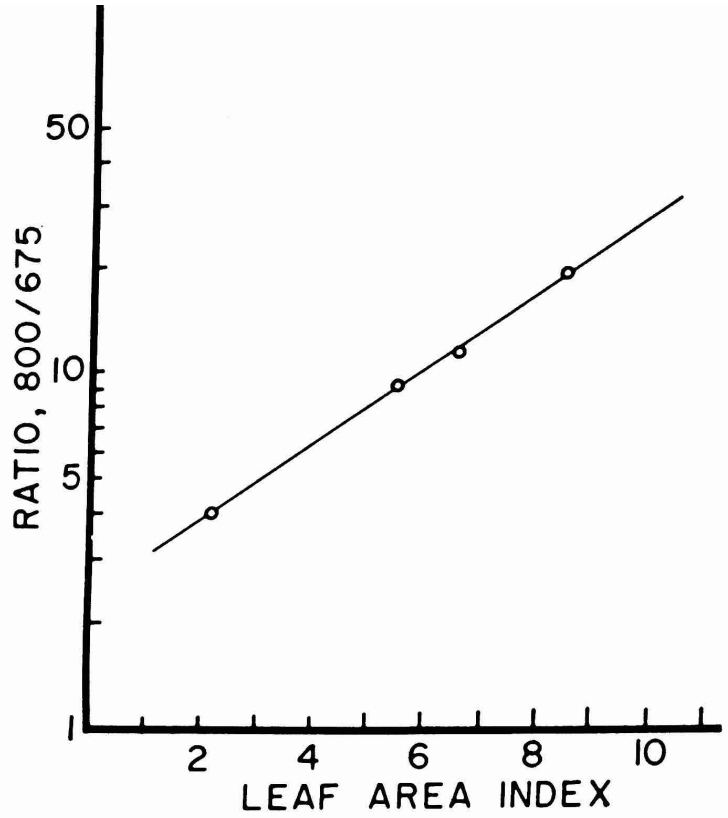

Fig. 3. Ratio of light intensities at 800 and 675 millimicrons measured on the forest floor, as a function of leaf area index.

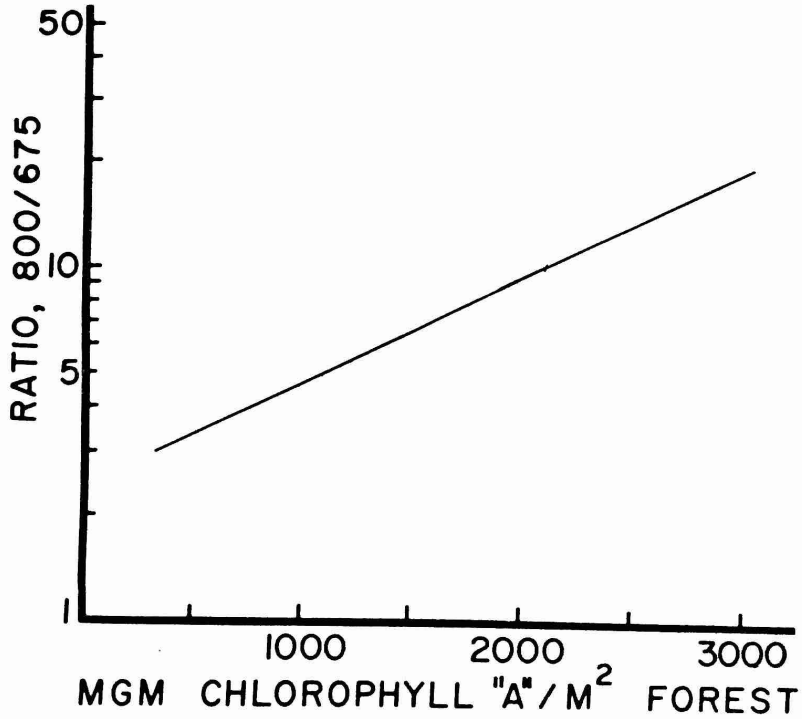

Fig. 4. Relation between ratio of light intensities at 800 and 675 millimicrons measured on the forest floor, and milligrams of chlorophyll A per square meter of the forest.

this, leaf area index could be derived, as previously described. Since the calibration was made in a broad leaved forest with the canopy top at about 65 feet, and very little shrub vegetation, the closer another forest resembles this structure, the more applicable this relation will be.

Light readings were done in a systematic manner, and values were recorded regardless of whether a light speck fell on the meter, or whether a limb was in a direct line between the sun and the meter. As a result, individual ratios taken at a given site varied greatly, but the averages (Table 2) were almost perfectly correlated with leaf area index (Fig. 3).

Average leaf area index for the entire forest as determined by light ratios measured every 5 feet along three 600 foot transects was 6.6 (Table 2). Odum, Copeland, and Brown (1963) determined an average value of 6.4 for the same forest by optical density means.

Light ratios were always higher at the calibration sites during early morning and late afternoon hours, and any time during winter months. The higher ratios were a result of relatively less light at 675 millimicrons, at the forest floor. This could result from the chlorophyll of the forest not being saturated at these times. Only during noon hours, during the summer, was it possible to get repeatable results. This suggests that trees of the forest have evolved so that their chlorophyll content is such that only during periods of maximum insolation is there no excess capability of chlorophyll for absorbing red light.

If this is true, this means that actual determinations of leaf area index of a forest, by the ratio method, must be done under the same solar conditions as those which exist during calibration, and that this is best accomplished during the noon hours during summer months, north of the equator. It also means that if Fig. 4 is used for other forests, it must be assumed that these forests have chlorophyll contents adapted to the maximum light levels which exist at their location, probably a safe assumption for mature forests.

Cloudy skies are not suitable for using the ratio method of determining leaf area index for two reasons. First, the thickness of the cloud cover could change without the observer on the forest floor being aware of a change in incoming light intensity. Secondly, with relatively more diffuse light entering the forest under cloudy conditions, there is more light scattering, and consequently the calibration is less reliable.

A spectroradiometer is not necessary in order to use the ratio method. Any of many types of light meters can be used in combination with narrow band pass filters for wavelengths of 675 and 800 millimicrons. The only requirement is that the meter be calibrated so that field readings can be converted into absolute light energies.

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NITROGEN FIXATION BY EPIPHYLLAE AT EL VERDE

J.A. Edmisten* and M.A. Harrelson**

Abstract

Acetylene reduction techniques with gas chromatography have been used to demonstrate that epiphytic plants on leaves could fix atmospheric nitrogen. These experiments confirm earlier ^{15}N tests with the same organisms. Leaves with intact mixed epiphyllae populations both on the tree and in flasks have been shown to reduce acetylene to ethylene. Mixed epiphyllae populations scraped from leaves produced more ethylene than scraped leaves. Mixed bacteria populations from leaves were shown to reduce acetylene. Three genera of blue-green algae isolated from leaves were found to have the ability to fix nitrogen as evidenced by the acetylene reduction tests.

Introduction

Root nodule experiments by Edmisten show that the generally accepted methods of nitrogen entering the tropical rain forest ecosystem at El Verde were not sufficient for the existing growth rates. Edmisten (1968) suggested that epiphyllae might be contributing factors in the nitrogen cycle. Kline and Edmisten (1968), in ^{15}N experiments, reported on a high rate of N-fixation by mixed epiphyllae on Citrus leaves and showed that some of the fixed ^{15}N was transferred to leaves. The mixed epiphyllae included bacteria, algae, fungi, lichens, and liverworts.

To explore this idea, the acetylene reduction technique (Stewart, 1966) was used on whole leaves, scraped leaves, and bacterial and blue-green algae cultures isolated from leaves. This technique involves the fact that the same enzyme complex which converts nitrogen to reduced usable forms will also reduce acetylene to ethylene. It was generally expected that certain bacteria and blue-green algae were responsible for the nitrogen fixation.

Seven genera of plants, representing shrubs and trees, were tested. These are shown in Table 1.

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TABLE 1

Plants from El Verde Forest Used in Acetylene Tests

Genus	Growth Habit	Niche
Citrus	small understory tree	escaped
Croton	slender canopy tree	climax
Dacryodes	large spreading canopy tree	climax
Euterpe	medium palm	follows streams
Manilkara	large canopy tree	climax
Psychotria	small understory shrub	successional
Sloanea	large canopy tree	climax

Materials and Methods

Two basic methods were used in preparing specimens for testing. For testing of whole leaves with epiphyllae on trees, plastic bags were sealed around the leaves at the twig with plastic tape. A piece of plastic tape about two inches long was used as a reinforcement for hypodermic needle insertion during gas exchange. After completion of gas exchange, a smaller piece of tape was used to seal the needle hole.

After securing the bag in place around the leaf, air was withdrawn by mouth vacuum through a plastic tube and hypodermic needle. The bag was then filled with a mixture of 22% O_2, .04% CO_2, and 77.95% Argon. The bag was again evacuated and refilled with the same mixture to insure the elimination of nitrogen. Acetylene was added to account for one-tenth of the volume of the bag. After varied exposure times, ranging from 1 to 6 days, the leaf in the sealed bag was clipped from the twig and taken to the laboratory for testing with G.C. techniques for the presence of ethylene.

For testing organisms isolated from leaves, and leaves with epiphyllae removed from trees, Erlenmeyer flasks of suitable size were used. Rubber serum stoppers were used to seal the flasks while allowing the replacement of gases through hypodermic needles. Flasks were flushed (an inlet for flushing gases and an outlet for escaping air) by about 10 volumes of the O_2, CO_2 and Argon mixture. Acetylene was added to make up one-tenth of the flask volume.

For isolating the various organisms suspected of fixing nitrogen, sterile disposable gloves and sterile scissors were used to detach and place leaves in sterile flasks. The leaves were taken to the field lab where the isolations were done.

Whole leaves were placed in Erlenmeyer flasks in media specific for either algae or bacteria. Agitation was used to free the organisms from the leaf surface. Transfers were made to suitable media.

Bacteria were grown in Ruinen's Medium (1965) at pH 7 and pH 4.5.

Algae were grown in soil extract media for flush growth, then to N-free media (Ruinen, 1965) for testing.

Fungi were isolated by cutting strips of leaves 3x20 mm and placing them on Martin's Rose Bengal Medium, soil-extract medium, and V-8 juice medium. Transfers were made to N-free medium for testing.

The surface of leaves was scraped to get a mixture of lichens and liverworts. These were tested as fresh materials and not cultured.

After adding acetylene, cultures were tested on a gas chromatograph for conversion of acetylene to ethylene.

Controls were run on the gas chromatograph with pure acetylene, pure ethylene, air and the flushing gas mixture.

The total number of cultures prepared for testing by gas chromatography were as follows:

Whole leaves on trees	20
Whole leaves in flasks	8
Whole leaves in flasks, scraped clean	8
Epiphyllae in Flasks, from scraped leaves	8
Bacterial cultures	70
Algal cultures	70
Fungal cultures	20

Results and Discussion

Positive results were obtained for epiphyllae as follows: 1. bacteria grown in culture, 2. blue-green algae grown in culture, 3. whole leaves with epiphyllae intact, 4. epiphyllae scraped from leaves.

The bacteria tested for figure 1 were isolated from the older leaves of an understory palm Euterpe globosa. The presence of these and other nitrogen fixing bacteria on leaves has been reported by

Ruinen (1965) when Beijerinckia, Azotobacter and Rhizobium were said to have increased total nitrogen in, on, and around leaves of bean and coffee grown in culture.

Figure 2 shows a very efficient conversion of acetylene by epiphyllae on old Citrus leaves in a flask. Citrus leaves with epiphyllae removed (figure 3) show less conversion of acetylene than those in figure 2. The epiphyllae scraped from the leaves in figure 3 show good conversion of acetylene to ethylene (figure 4).

A comparison of figures 2, 3, and 4 led us to believe that most of the organisms with nitrogen fixing ability are found in or on the visible epiphyllae which consist mainly of liverworts and lichens. Microscopic examination of liverworts and lichens taken from leaves have shown that blue-green algae are often embedded in both these organisms. One of the species of Nostoc used in later acetylene tests of pure cultures was isolated from liverworts. Although the usual algal partner of an epiphyllous lichen is a green alga, blue-greens are often found also in tumor-like growths called cephalodia. The fact that the leaf scraped clean of visible epiphyllae still showed ability to reduce acetylene (figure 3) may be explained by the fact that Azotobacter could be isolated from it.

Figure 5 shows good conversion of acetylene by older Manilkara leaves with epiphyllae in a flask, while figure 6 shows very high conversion to ethylene by epiphyllae scraped from older Manilkara leaves like those in figure 5.

The data shown in figures 5 and 6 reconfirm the concept established in the experiment shown by figures 2, 3 and 4 and indicate that the nitrogen fixing ability of epiphyllae is not host specific. The same species of lichens and liverworts have been identified from a wide variety of leaves from Peru, Panama, and Colombia as well as Puerto Rico.

Leaves in plastic bags on trees (figures 7 and 8) showed reduction of acetylene to ethylene as determined in earlier ^{15}N experiments by Edmisten and Kline (1968). The acetylene reduction tests represented by figures 7 and 8 were performed on leaves of the same grapefruit tree that was used in the preliminary ^{15}N test as well as on leaves from a climax species, Manilkara. In the ^{15}N test, it was found that epiphyllae scraped from leaves had 10% of their total nitrogen as ^{15}N which had been taken up from $^{15}N_2$ during the 48 hour exposure period and incorporated into organic form. Host Citrus leaves from which epiphyllae had been scraped and washed had 1% of their total nitrogen as the stable isotope ^{15}N. When considered together, these experiments indicate that epiphyllae have the ability to fix atmospheric nitrogen and that some of the fixed nitrogen is transferred to the host leaf within a 48-hour period.

Since blue-green algae have long been known to fix atmospheric nitrogen, it was not surprising to find 4 genera on leaves that showed conversion of acetylene to ethylene. Figures 9 and 10 show the actual

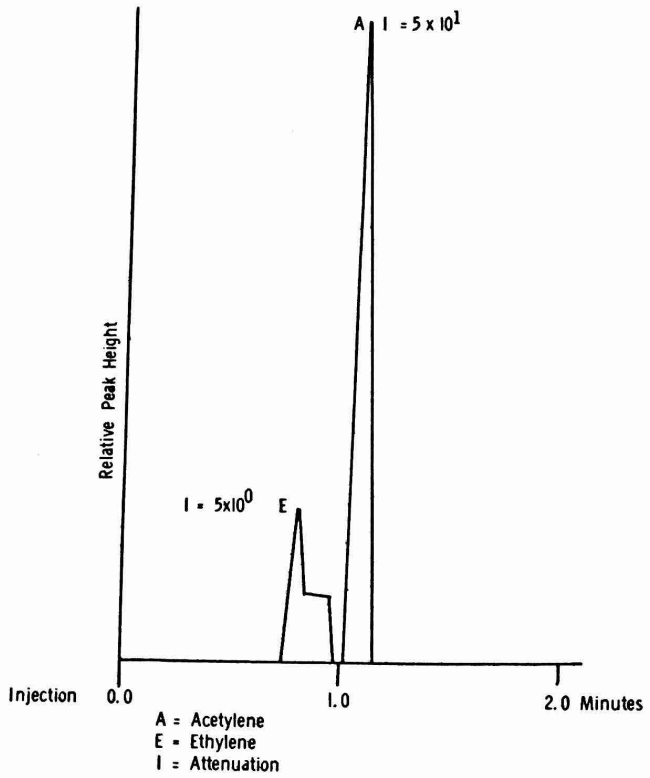

Fig. 7. Scale drawing of gas chromatograph tracings to show conversion of acetylene to ethylene by a Citrus leaf in a plastic bag on the tree.

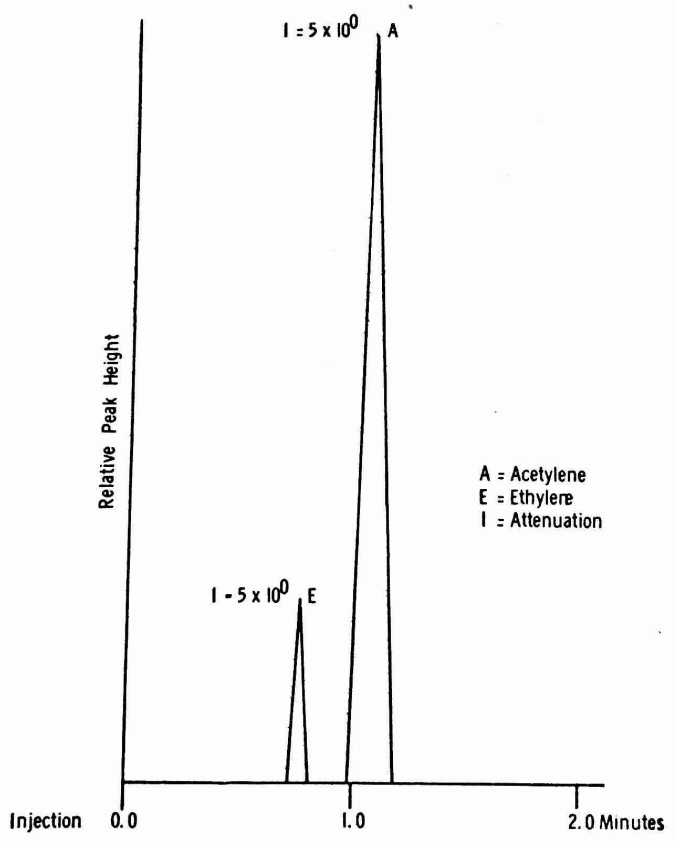

Fig. 8. Scale drawing of gas chromatograph tracings to show conversion of acetylene to ethylene by a Manilkara leaf in a plastic bag on tree.

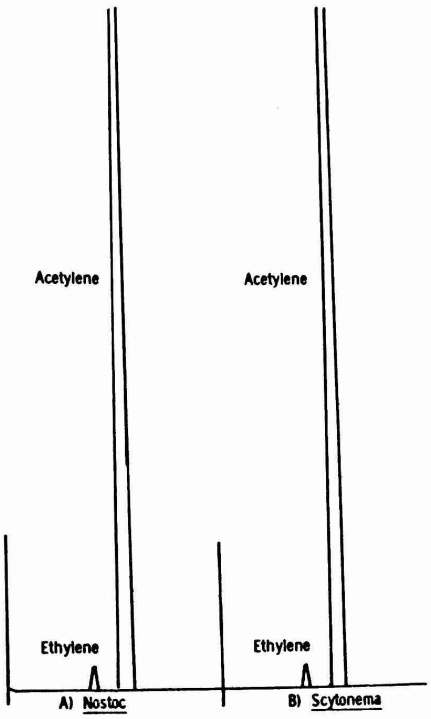

Fig. 9. Gas chromatograph tracings to show conversion of acetylene to ethylene by blue-green algae.

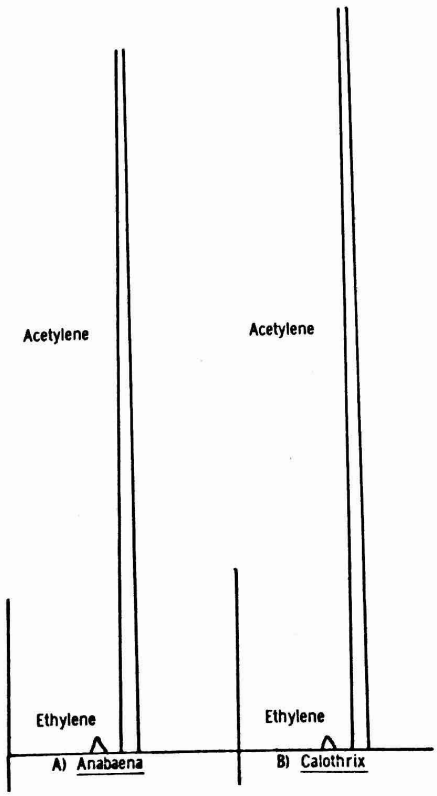

Fig. 10. Gas chromatograph tracings to show converion of acetylene to ethylene by blue-green algae.

tracings of the gas chromatograph for the four blue-green algae Nostoc, Scytonema, Anabaena and Calothrix from leaves at El Verde.

The blue-green algae used in the tests illustrated by figures 9 and 10 were isolated from Citrus leaves taken from the El Verde forest and were grown in Chu's nitrogen-free media. They were transferred with sterile technique four times before being tested for the ability to fix nitrogen in order to help assure their being in pure culture.

Conclusions and Implications

Mixed populations of leaf epiphytes have been shown by two separate methods to have the ability to fix atmospheric nitrogen. The principal organisms thought to be responsible for the fixation have been shown to be various blue-green algae and free-living aerobic bacteria which live in and on leaf lichens and liverworts as well as on the bare leaf during early stages of successional coverage of a new leaf.

Although this study was not designed to be quantitative but rather qualitative, preliminary calculations based on the areas below the ethylene and acetylene peaks of figures 1 through 10 indicate that the rates of nitrogen fixation would range between .05 Kg/acre/day to .15 Kg/acre/day.

The biomass of epiphyllae in tropical rain forests has not been established, but the presence of heavy populations of epiphyllae has been noted on leaves of all synusia of the El Verde forest except the exposed leaves of the upper canopy. When one realizes that there are between 5 and 15 acres of leaves over each acre of ground in El Verde, the potential nitrogen input by epiphyllae becomes an important factor to be considered in the nitrogen budget of any moist tropical forest.

The results of these experiments suggest a new way of adding nitrogen fertilizers to crop plants. It would appear feasible to isolate and grow blue-green algae and bacteria from leaves and select the ones with high ability to live on leaves and fix atmospheric nitrogen. Such known "fixers" could be sprayed on crops such as Citrus, pineapple or sugar cane in irrigation water with certain chemicals added to facilitate the adhesion of micro-organisms to leaves.

If man could effectively copy this symbiosis on his crop plants, the nitrogen fixed would become available to the crop plants directly through the leaves and from leachate in rain and irrigation water.

Finally, this experiment has demonstrated that the quick, inexpensive acetylene reduction test for the ability to fix nitrogen is a reliable tool as shown by the independent ^{15}N experiment. The acetylene

reduction test was also performed on well-nodulated, hemoglobin-containing root masses from six species of legumes from El Verde with strongly positive results. The six were Inga vera, Inga laurina, Andira inermis, Neorudalphia volubilis, Ormosia krugii and a successional species of Desmodium.

A series of acetylene reduction tests should be performed to quantitatively establish the rates and extent of all nitrogen fixation in the El Verde forest and thus establish a nitrogen budget for a tropical rain forest. Puerto Rico Nuclear Center should support studies in which various nitrogen fixing epiphyllae are grown on citrus, pineapple and sugar cane with their crop yields and nitrogen contents compared to untreated control crops.

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TERMITES AT EL VERDE: 1968 RECHECK

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P. Murphy and R. Wiegert made preliminary surveys of Nasutitermes costalis nests at El Verde, beginning in 1965, and Wiegert's subsequent studies have been concerned mainly with their metabolism. McMahan continued and expanded the survey studies, making a complete census of nest condition and tunnel occupancy during the summers of 1966, 1967, and 1968. The chief aim of the studies was to examine the effects of the 92-day (Spring 1965) exposure of a gamma source (^{137}Cs) in the Radiation Center.

Methods

N. costalis nests within 80 m of point zero in the Radiation Center and in the South Contro Center has been mapped originally by Wiegert, with later additions by McMahan. At each survey period the areas were scoured for new nests, and each old nest was examined to see if it was still active.

A tunnel survey was also made each summer. Every tree (dead or alive) of one-inch diameter or greater within 30 m of point zero in the Radiation, South Control, and North Cut Centers was carefully examined for evidence of termite tunnels. If a tunnel was found it was checked for occupancy and by which species (Usually N. costalis or Parvitermes discolor; once Glyptotermes was found inside a stub on which were P. discolor tunnels).

Results

Nests

In the summer of 1966 there were 11 active Nasutitermes nests in the Radiation Center, 11 in the S. Control Center, and an undetermined number in the N. Cut Center (none within 30 m of point zero in the latter).

By July 1967 five of the Radiation Center nests had been abandoned (14,15,19,20,21), while only one (7) was newly empty in the S. Control Center. That year a new nest (26) was found at about 26 m from point zero in the Control Center.

At the 1968 survey (July 9-21) nest 18 in the Radiation Center had been abandoned and nest 12 was barely active - only two soldiers were ever seen to emerge to investigate disturbance of the nest surface.

But two more nests were found to be abandoned also in the S. Control Center: Nests 2 and 9. A new nest (27) was found in the Radiation Center, only about 8 m NNE (behind the big Cyrilla tree) from point zero. Figure 1 shows the position and states of nests in the two centers in July 1968.

Tunnels

The 1966 and 1967 studies had shown that about 10% of the trees in the Centers had tunnels or tunnel fragment on them. This was also true for 1968. Table 1 gives the percentages of tunnel occupancy for the three years. It shows that in 1966 the percentage of occupied tunnels in the Radiation Center was much less than that for the two Control Centers and that this percentage had actually decreased in 1967.

The 1968 survey showed for the first time that reinvasion of Radiation Center by termites had begun. The new Nasutitermed nest in this center has already been mentioned, and the occupied tunnels were probably, in large part, from this nest. While the percentage of occupancy was till not as great as in the Control Centers, the probability seems good that by 1969 it will more nearly equal them.

TABLE 1

Percentage of Tunnel Occupancy for the Three Centers

Year	Radiation Center	S. Control Center	N. Cut Center
1966	12	51	38
1967	8	45	44
1968	23.6	44.7	42.6

Discussion

Three years after removal of the gamma source from the Radiation Center, effects of the irradiation in terms of nest abandonment by Nasutitermes costalis seem to be still appearing. Twice as many nests in this center as in the Control Center were abandoned in 1968. The unusual amount of nest abandonment may be attributable to sterilization of reproductives and the consequent lack of normal colony growth which would offset natural attrition.

It seems surprising that three years were required for evidence of refaunation of the irradiated area, and this evidence of reinvasion was contributed solely by N. costalis. Nasutitermes-occupied tunnels are naturally more numerous in the vicinity of nests (Parvitermes discolor constructs no discrete nests), and the new nest in the Radiation Center probably explains the increase. The lack of increase in P. discolor occupancy of tunnels to more nearly approximate the Parvitermes densities of the Control Centers may reflect the slowness of termite refaunation of an irradiated area.

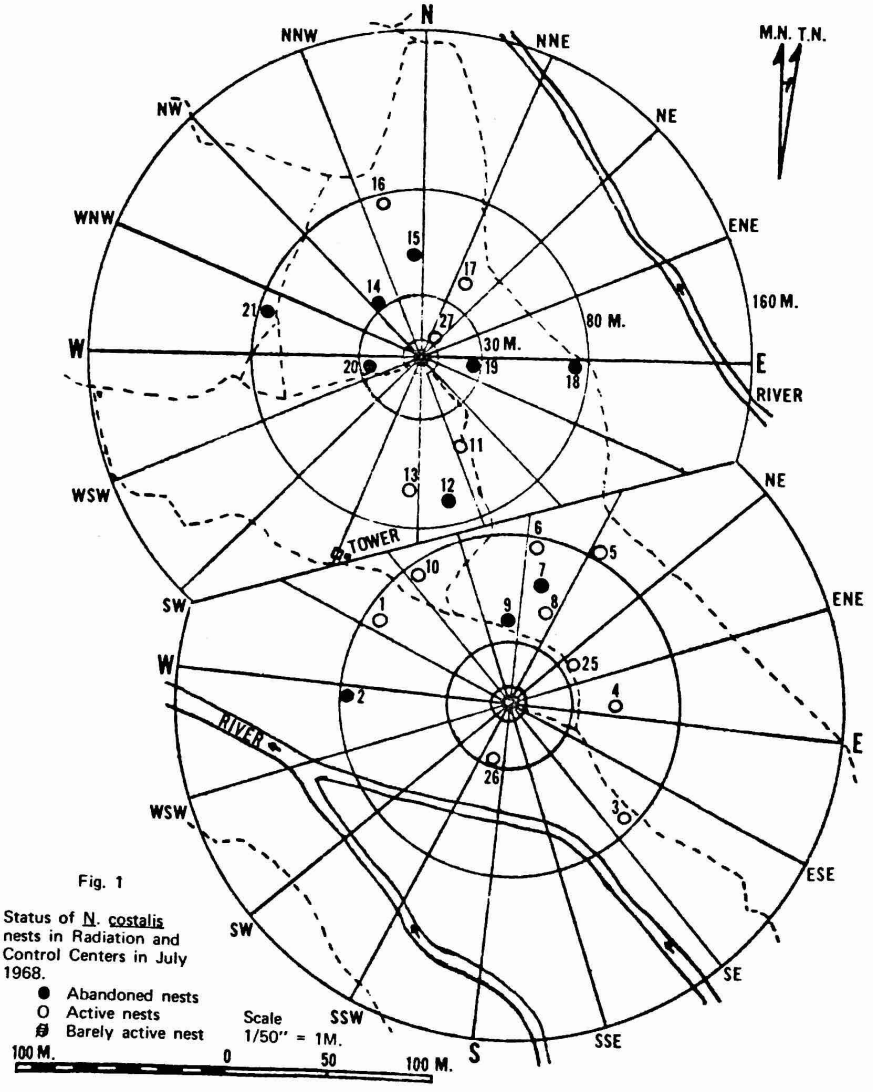

Fig. 1

Status of N. costalis nests in Radiation and Control Centers in July 1968.

● Abandoned nests
O Active nests
Barely active nest