EFFECTS OF LIGHT AND TEMPERATURE ON NITROGEN-FIXATION RATES IN ARABLE SOILS AND SEASONAL FLUCTUATIONS IN NITROGEN-FIXATION RATES IN ARABLE SOILS

by

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A very special thanks is given to my wife, Mary Jo Bailey, and children, George Eric and Jennifer Jo, for their support, patience and sacrifices during the course of this work.

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FOREWARD

This dissertation was written in the form of two papers for publication in Soil Biology and Biochemistry with the concurrence of my committee.

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PART I

EFFECTS OF LIGHT AND TEMPERATURE ON NITROGEN-FIXATION RATES IN ARABLE SOILS

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Summary--N_2-fixation rates were determined by C_2H_2 reduction in intact cores taken from 0-1 cm of two soils. The cores were moistened and incubated under varying light (0-88 $W \cdot m^{-2}$) and temperature (12-38°C) conditions. Multiple regressions were used to relate $n\underline{M}$ C_2H_4 produced with light and temperature. For each soil a higher proportion of the data was explained by the regression when $n\underline{M}$ $C_2H_4 \cdot cm^{-2}$ surface area was used as the dependent variable than when $n\underline{M}$ $C_2H_4 \cdot g^{-1}$ was used. Soil 1 produced a maximum of 3 $n\underline{M}$ $C_2H_4 \cdot cm^{-2} \cdot d^{-1}$ in the dark compared to 189 $n\underline{M}$ $C_2H_4 \cdot cm^{-2} \cdot d^{-1}$ at optimum light and temperature conditions (51 $W \cdot m^{-2}$, 30°C). Soil 2 produced a maximum of 2 $n\underline{M} \cdot cm^{-2} \cdot d^{-1}$ in the dark while 211 $n\underline{M} \cdot cm^{-2} \cdot d^{-1}$ were produced under the same optimum light intensity and temperature. Because of the influence of light, and the fact that the regression analyses indicated that the fixation was a surface area phenomenon, it was concluded that blue-green algae at the surface of the soils were responsible for most of the C_2H_2 reduction which occurred.

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INTRODUCTION

Recent shortages of both N fertilizer and the fuel required to produce the fertilizer have emphasized the need for additional study of biological fixation of atmospheric N_2 in arable soils. Considerable attention has been paid to N_2 fixation by legumes and their associated rhizobia. Many of these studies have been reviewed by Hardy, Burns and Holsten (1973). However, a variety of other N_2-fixing microorganisms also inhabit the soil in appreciable amounts, among these are the blue-green algae.

Most of the determinations of the amount of N_2 fixed and evaluations of the environmental factors regulating this fixation by blue-green algae in soils have been done on flooded rice fields or other soils that do not lend themselves to cultivation. Henriksson, Englund, Heden and Was (1973) surveyed the extent and amount of algal $N_2[C_2H_2]$-fixation in Swedish soils. Their study revealed a variation sometimes greater than 10-fold among samples from various points within a single square meter and even greater variation from soil to soil. Additionally, they were unable to show correlations between temperature and $N_2[C_2H_2]$-fixation rates in their field studies in spite of great fluctuations in temperature.

Alexander (1974), reviewing the results of the IBP Tundra Biome circumpolar N_2 fixation study, noted that in most sites blue-green algae (living free in the soil or in lichen or moss associations) were responsible for most of the $N_2[C_2H_2]$ fixed. She concluded that the *in situ* $N_2[C_2H_2]$-fixation rate in the tundra was primarily governed by

moisture and temperature (15-20°C optimal) with light also playing an important role. In a contrasting environment, Rychert and Skujins (1974) measured $N_2[C_2H_2]$-fixation rates by blue-green algae-lichen crusts in the Great Basin Desert of Utah. They found maximal N_2-fixation rates at 19-23°C, -1/3 bar pressure water content and light intensity of 200 $\mu E \cdot m^{-2} \cdot sec^{-1}$.

There are no studies of N_2-fixation rates by blue-green algae in soils under row crop cultivation. Yet, the abundance of blue-green algae can frequently be appreciated merely by observing the blue-green color at the surface of the soil. Therefore, the purpose of this study was to determine the effects of light and temperature on the $N_2[C_2H_2]$-fixation rates and to assess the importance of blue-green algae in the $N_2[C_2H_2]$-fixing capacities of two cultivated soils.

MATERIALS AND METHODS

Experiments were run on two different types of soil in the fall of 1974. Soil 1, a Greendale silt loam (Fluventic Dystrochrepts; fine loamy, siliceous, mesic) was chosen because it had acquired restricted drainage, in an area of adequate size for study, due to construction of a small road. In the growing season preceding the study American chestnut seedlings were grown there in row crop fashion. These seedlings were not fertilized and not treated with any pesticides. This soil had a pH of 5.6, available P in excess of 9 parts/10^6, organic matter content of 3.02%, 0.51% $CaCO_3$ equivalents, 0.148% total N and a cation exchange capacity of 7.9 meq/100 g soil. The composition of

displaced solution was 10.23 meq Ca^{++}, 1.87 meq Mg^{++}, 1.47 meq K^{+} and 0.09 meq Na^{+}/100 g soil. Soil 2, a Groseclose silt loam (Typic Hapludults; clayey, mixed, mesic), was chosen because of its well drained condition. The study plot remained undisturbed since the corn harvest of the previous year. The soil had a pH of 5.6, available P in excess of 27 parts/10^6, organic matter content of 4.71%, 0.45% $CaCO_3$ equivalents, 0.194% total N and a cation exchange capacity of 6.2 meq/100 g soil. The composition of displaced solution was 8.57 meq Ca^{++}, 1.66 meq Mg^{++}, 1.35 meq K^{+} and 0.05 meq Na^{+}/100 g soil.

The soils were examined at the time of C_2H_2 reduction for blue-green algae by dilution plating onto modified Goram's medium (Henriksson, Henriksson and Pejler, 1972) to which 75 mg·l^{-1} of cyclohexamid was added. Soil 1 contained 2.4 x 10^4 colonies of Nostoc commune Vaucher, 5.6 x 10^3 Schizothrix Friesii (Agardh) Gomont and 1.5 x 10^3 Microcoleus vaginatus (Vaucher) Gomont·g^{-1}. Soil 2 contained 3.6 x 10^4 colonies of N. commune, 4.6 x 10^4 S. calcicola (Agardh) Gomont and 8.0 x 10^2 M. vaginatus·g^{-1} dry weight.

From each soil 360 cores 0.9 cm dia x 1 cm were randomly taken for determination of C_2H_2 reduction rates. Ten cores were placed upright and intact in each of 36 horizontally held Pyrex disposable culture tubes (16 x 125 mm). The culture tubes were divided into 6 groups of 6 tubes each. The soil cores were immediately returned to the laboratory where sufficient water was added so that they became saturated. Excess water was drained from the tubes. The tubes were then plugged with cotton and placed in the incubator to acclimate for 24 h at the lowest

temperature setting tested. In order to determine the amount of $N_2[C_2H_2]$ fixed heterotrophically, one group of six culture tubes was kept in the dark by wrapping it in aluminum foil.

After 24 h of acclimation the test tubes were capped with rubber septa and C_2H_2 was injected into them to give 0.1 atm C_2H_2. One group of 6 test tubes from each soil received no C_2H_2 to determine whether C_2H_4 was being produced in its absence by fungi and bacteria.

The soil cores were positioned in the incubator at various distances from the lights at the top of the chamber in order to provide different light intensities. The soil cores were incubated on a 14 h day, 10 h night regimen. Illumination was provided by both incandescent and cool white fluorescent lights. Light intensity was measured in the 400-750 m range.

Gas samples were taken with 3 cm^3 evacuated glass containers (Becton, Dickinson and Co.) after 24 h. After the samples were taken, the soil cores were purged with compressed air before another experiment was begun. Thus, the same soil cores were used on 5 consecutive days with higher temperatures each day to determine the effects of various light and temperature conditions on C_2H_2 reduction rates. Day 6 of incubation duplicated the conditions of the first day to determine whether there was a significant change in $N_2[C_2H_2]$-fixing capacity of the cores throughout the 6 day period.

Internal temperatures of the test tubes were measured using a thermister with bayonet probes. These probes were inserted through rubber septa into additional test tubes which also contained moistened

soil cores. Because the light heated the contents of the test tubes, those tubes positioned near the lights had wider ranges between day and night temperatures than did the test tubes positioned further from the lights.

The amount of C_2H_4 produced was determined with a Hewlett-Packard model 700 g.c. equipped with H_2 flame ionization detectors and 182 x 0.6 cm glass columns packed with Porapak T (50-80 mesh). Helium gas at a flow rate of 60 $cm^3 \cdot min^{-1}$ served as the carrier.

Data taken from the C_2H_2 reduction measurements were used in single and multiple linear regression procedures to determine which of the dependent variables (light intensity, day temperature and night temperature) best explained the independent variable (amount of C_2H_4 produced) throughout the linear portions of the curves. Therefore, non-linear portions of the data were eliminated. Data were used where light was between 0 and 51 $W \cdot m^{-2}$, and where day temperatures were below or equal to 30°C.

RESULTS

The amount of $C_2H_4 \cdot cm^{-2} \cdot d^{-1}$ produced under varying experimental conditions is shown in Table I.1 where each mean value is derived from 60 randomly selected soil cores divided equally among six replicates. These mean values for Soils 1 and 2 are plotted in Figs. I.1 and I.2, respectively. In every case, no C_2H_4 was produced by bacteria and fungi in the absence of C_2H_2.

The regression procedure indicated that a two variable model consisting of light and day temperature best explained the data for each

FIG. I.1. $N_2[C_2H_2]$ ACTIVITY OF THE TOP 0-1 cm OF SOIL 1 INCUBATED UNDER VARIOUS LIGHT AND TEMPERATURE CONDITIONS

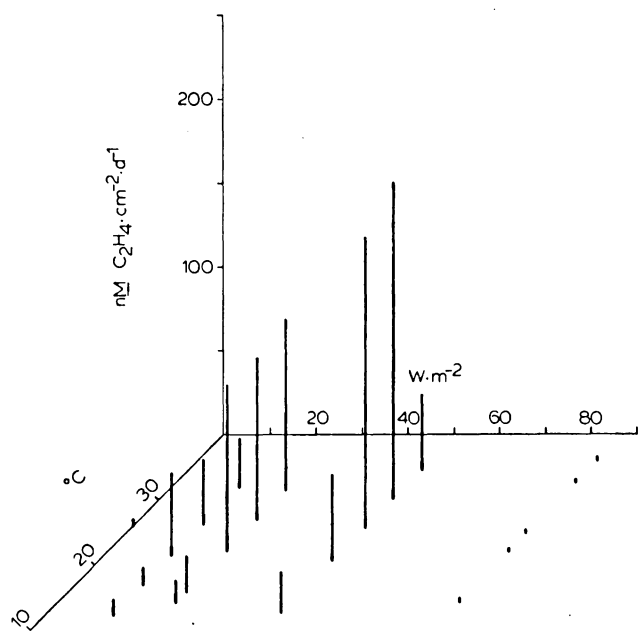
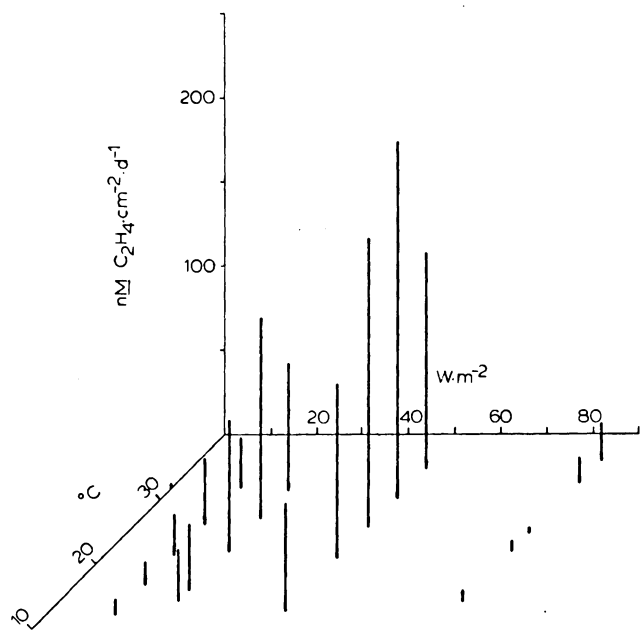

200
100
nM $C_2H_4 \cdot cm^{-2} \cdot d^{-1}$
$W \cdot m^{-2}$
20
40
60
80
°C
30
20
10

FIG. I.2. $N_2[C_2H_2]$ ACTIVITY OF THE TOP 0-1 cm OF SOIL 2 INCUBATED UNDER VARIOUS LIGHT AND TEMPERATURE CONDITIONS


200
100
nM C_2H_4.cm^{-2}.d^{-1}
W.m^{-2}
20
40
60
80
°C
30
20
10

of the soils. Eighty-five percent of the data of the poorly drained soil (Soil 1) was explained by the equation log $n\underline{M}\ C_2H_4 \cdot cm^{-2} \cdot d^{-1}$ = 0.119 + 0.095 W light$\cdot m^{-2}$ + 0.0578 day temp. Eighty-three percent of the data for the well-drained cornfield soil (Soil 2) was best explained by the equation log $n\underline{M}\ C_2H_4 \cdot cm^{-2} \cdot d^{-1}$ = 0.327 + 0.0163 W light$\cdot m^{-2}$+ 0.4204 day temp. The equation for Soil 1 was not different from the equation for Soil 2 at the 0.01 level of significance.

A higher proportion of the data was explained by each regression when surface area of the tops of the cores ($n\underline{M}\ C_2H_4 \cdot cm^{-2} \cdot d^{-1}$) was used as the dependent variable than when weight of the soil cores ($n\underline{M}\ C_2H_4 \cdot g^{-1} \cdot d^{-1}$) was used. In addition, Table I.1 shows that Soil 1 in the dark produced a maximum of only 3.7 $n\underline{M}\ C_2H_4 \cdot cm^{-2} \cdot d^{-1}$ while 189 $n\underline{M}$ were produced under optimum light and temperature conditions. The effect of light on Soil 2 was equally impressive: in the dark a maximum of only 2.3 $n\underline{M}\ C_2H_4 \cdot cm^{-2} \cdot d^{-1}$ were produced while 211 $n\underline{M}$ were produced under optimum light and temperature conditions. Figures I.1 and I.2 further illustrate the magnitude of the effect of light on $N_2[C_2H_2]$-fixation rates in these soils.

DISCUSSION

Because of the influence of light, and the fact that the regression analyses indicated that the fixation was a surface area phenomenon, it was thought that blue-green algae at the surface of the soil were responsible for most of the $N_2[C_2H_2]$ fixation which occurred. Inspections of the Figures indicate that these organisms fix significant

TABLE I.1. NITROGENASE ACTIVITY (nM $C_2H_4 \cdot cm^{-2} \cdot d^{-1}$) FROM THE TOP 0-1 cm OF TWO SOILS INCUBATED UNDER DIFFERENT LIGHT AND TEMPERATURE CONDITIONS.

			Soil 1		Soil 2	
$W \cdot m^{-2}$	°C (Day)	°C (Night)	nM C_2H_4*	s†	nM C_2H_4*	s†
0	12.0	7.8	0.1	0.3	0	0
0	16.7	12.8	0	0	0.2	0.3
0	21.1	17.2	0.3	0.1	0.4	0.4
0	26.0	21.8	3.4	2.5	0.8	0.5
0	31.1	27.2	0.2	0.3	2.3	2.2
15.2	12.7	7.8	10.0	3.8	9.2	3.3
15.2	16.7	12.8	11.8	4.4	13.5	5.9
15.2	21.1	17.2	50.4	16.7	25.2	8.5
15.2	26.0	21.8	38.5	5.7	40.4	14.8
15.2	31.1	27.2	33.4	16.7	31.5	15.7
22.8	13.9	7.5	14.5	10.8	31.7	26.1
22.8	15.6	12.8	22.7	14.2	40.5	20.5
22.8	21.7	17.2	98.8	69.7	78.2	57.1
22.8	26.7	21.8	96.4	23.0	119.6	58.8
22.8	31.1	27.2	101.9	66.8	76.3	35.6
51.2	12.5	7.2	30.0	16.0	64.9	43.5
51.2	20.6	12.8	51.1	16.1	104.5	29.1
51.2	25.6	17.2	147.3	29.6	171.0	46.0
51.2	30.0	21.8	188.8	30.8	211.1	70.9
51.2	34.4	27.5	44.5	17.5	128.5	44.2
87.5	14.0	7.2	0.9	1.0	6.9	6.0
87.5	21.7	13.3	0.4	0	6.2	7.7
87.5	24.4	16.7	0.1	0.1	3.1	5.4
87.5	32.2	22.2	1.0	1.1	14.8	11.7
87.5	35.6	27.2	1.0	0.8	22.0	15.5

*Mean value of nitrogenase activity; in each case n = 6.

†Sample standard deviation.

amounts of $N_2[C_2H_2]$ only over a narrow range of light and temperature conditions.

The data indicated that the 30°C day temperature optimum in these Virginia soils is higher than that normally found in the tundra (Alexander, 1974), but in close agreement with desert soil crusts from Utah (Rychert and Skujins, 1974), and less than that of the lichen Peltigera refescens (Weiss) Humb. from Scotland (Hitch and Stewart, 1973.

The 51 $W \cdot m^{-2}$ light optimum observed here is in reasonable agreement with the optimum light intensity for blue-green algae in desert soils of Utah (Rychert and Skujins, 1974).

When the theoretical conversion factor of 3 M C_2H_2 reduced to 1 M N_2 fixed (Hardy et al, 1973) was used it was found that, under optimal laboratory conditions, Soil 1 fixed 0.16 kg $N_2 \cdot ha^{-1} \cdot d^{-1}$ while Soil 2 fixed 0.17 kg $N_2 \cdot ha^{-1} \cdot d^{-1}$. Field incubations were abandoned because the high intensity of sunlight shining through the glass vessels produced excessively high temperatures in the soil cores. Elimination or reduction of the sunlight would alter the $N_2[C_2H_2]$-fixing rates of the blue-green algae and thereby defeat the purpose of a field study.

Nostoc commune was the only heterocyst-producing blue-green alga found in these two soils. Because of the importance of heterocysts in N_2 fixation (Weare and Benemann, 1973) this species may have been responsible for all of the photosynthetic $N_2[C_2H_2]$ fixation which occurred. The abundance of this species, estimated by the dilution plate method, was approximately the same in both soils, which would

account for the fact that the soils showed similar responses to light and temperature and had nearly identical maximum fixation rates.

While the amount of $N_2[C_2H_2]$ fixed by blue-green algae in the two soils was small, the data suggested that they fixed more $N_2[C_2H_2]$ than any of the other organisms. The low pH of the soils was not conducive to growth of blue-green algae (e.g. Shields and Durrell, 1964; Reddy and Giddens, 1975), nor was the frequent desiccation of the surface layers of the bare soil (Shields and Drouet, 1962). Liming these soils and placing them under irrigation or a no till system would produce a more favorable habitat for growth of blue-green algae and subsequent N_2 fixation.

Blue-green algae are unique in that they are the only aerobic N_2-fixing organisms that can supply the necessary energy, reductant and carbon skeletons to which the reduced nitrogen is attached by the process of photosynthesis (Hardy et al, 1973; Donze, Raat and van Gorkom, 1974). Hence, none of these essential ingredients is directly tied to the supply of readily available organic carbon in the soil. This unique combination of physiological characteristics makes blue-green algae candidates as the most important N_2-fixing organisms in cultivated fields. The results confirmed the importance of blue-green algae and indicated that they can fix significant amounts of nitrogen.

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PART II

SEASONAL FLUCTUATIONS IN NITROGEN-FIXATION RATES IN ARABLE SOILS

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Summary--N_2-fixation rates were determined by C_2H_2 reduction in intact cores taken from 0-1 cm of two soils. Moistened cores were incubated under varying environmental conditions at four different times of the year to determine the effects of day temperature, night temperature and light intensity on $N_2[C_2H_2]$-fixation rates. The responses of the two soils to light and day temperature varied widely among the four sampling dates indicating that it was not possible to predict amounts of $N_2[C_2H_2]$ fixed annually based on responses at one time of the year. One soil fixed virtually no $N_2[C_2H_2]$ under any of the environmental conditions tested at two of the sampling dates. For the remaining data, the influence of light was strong indicating that blue-green algae were responsible for most of the $N_2[C_2H_2]$ which was fixed. Day temperature also influenced the $N_2[C_2H_2]$-fixation rates but night temperature had no measurable effect. $N_2[C_2H_2]$ fixation rates of the soils did not appear to be related to the abundance of blue-green algae as estimated by the dilution plate method.

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INTRODUCTION

Previously, Bailey (In Review) has described the effects of light and temperature on $N_2[C_2H_2]$-ase activities of two arable soils. In these soils, free-living blue-green algae were shown to be the most important $N_2[C_2H_2]$-fixing organisms. While the data reported were taken in the fall, it seemed logical that the $N_2[C_2H_2]$-ase activities of these soils would respond differently to light and temperature at different times of the year. Variations in response could be brought about by (1) variations in the physiological conditions of the N_2-fixing organisms, (2) changes in the quantity of N_2-ase available in the soil, and (3) changes in the species composition of the N_2-fixing population.

Earlier studies have described *in situ* $N_2[C_2H_2]$-ase activities of blue-green algae in soil (*e.g.* Stutz and Bliss, 1975; Henriksson, Englund, Heden and Was, 1972; Granhall and Selander, 1973). Rychert and Skujins (1974) carried soil samples to the laboratory where they were able to evaluate the effects of various light intensities and temperatures on soil samples with algal crusts. These studies, too, were all performed at a single time of the growing season or over a period of several months. There are no studies dealing with blue-green algae in soil which have evaluated $N_2[C_2H_2]$-fixing kinetics at various seasons of the year as functions of abiotic environmental conditions. With this in mind, the data reported here were collected to determine the magnitude of change of the $N_2[C_2H_2]$ fixation rates at several sampling dates throughout a year.

MATERIALS AND METHODS

The soils and methods reported here were the same as those previously described for the fall (September 28) of 1974 (Bailey, In Review). Additional data reported here were taken from soil cores collected January 9, May 3, and June 14, 1975. Soil 2 remained uncultivated throughout the entire study period. However, Soil 1 was plowed shortly before the May collection date. No other mechanical or chemical treatments were performed on this soil throughout the remainder of the study period.

Ethylene produced during the May and June studies was measured on a Beckman GC 4 g.c. with H_2 flame ionization detectors and a Sargent model SR recorder. Glass columns (182.9 x 0.64 cm) packed with Porapak T (50-80 mesh) were employed. Helium gas at a flow rate of 86 $cm^3 \cdot min^{-1}$ served as the carrier.

The January, May and June studies employed 6 groups of 5 test tubes from each soil rather than 6 groups of 6 tubes each as reported for September.

The series of experiments from the June collection were begun in a controlled environment chamber which failed to operate properly midway in the series of experiments. The soil cores were transferred from this chamber to a properly functioning chamber and equilibrated for 24 h before the experiments were continued. Light intensities in the two chambers were not the same; hence more light intensities are reported for the June experiments. In order to insure that the soil cores collected for the June experiments were not held in the chamber

any longer than previous experiments, collection of data was terminated without testing as many temperature regimens.

RESULTS

The amounts of $C_2H_4 \cdot cm^{-2} \cdot d^{-1}$ produced under varying experimental conditions for each of the four sampling dates are shown for Soil 1 in Fig. II.1. Comparable data for Soil 2 are displayed in Fig. II.2. Each line of the September data represents a mean value derived from 60 soil cores divided equally among 6 test tubes. For the remaining data, each line represents a mean value derived from 50 soil cores divided equally among 5 test tubes. Counts of algae for each season are given in Table II.1. While the fall data were presented previously (Bailey, In Review), they are reproduced here to facilitate comparison.

Soil 1 showed declining $N_2[C_2H_2]$-fixation rates at comparable levels of light and temperature from September to January to May with a significant increase from May to June (Fig. II.1). Soil 2 showed a decline in $N_2[C_2H_2]$-fixation of approximately 95% between September and January with virtually no $N_2[C_2H_2]$ fixation at the May and June measurements. Comparison of Figs. II.1 and II.2 with Table II.1 reveals that there was no correlation between counts of blue-green algae and $N_2[C_2H_2]$-fixation rates.

Table II.2 gives the regression equations with the highest correlation coefficients for the two soils at the four sampling dates. Because a multiple linear regression technique was used to model the data, those data above the optimum conditions were eliminated from the regression equations. Limits of light intensities and day temperatures

FIG. II.1. $N_2[C_2H_2]$-FIXATION RATES IN SOIL 1 VERSUS LIGHT AND DAY TEMPERATURE FOR EACH OF THE FOUR SAMPLING DATES

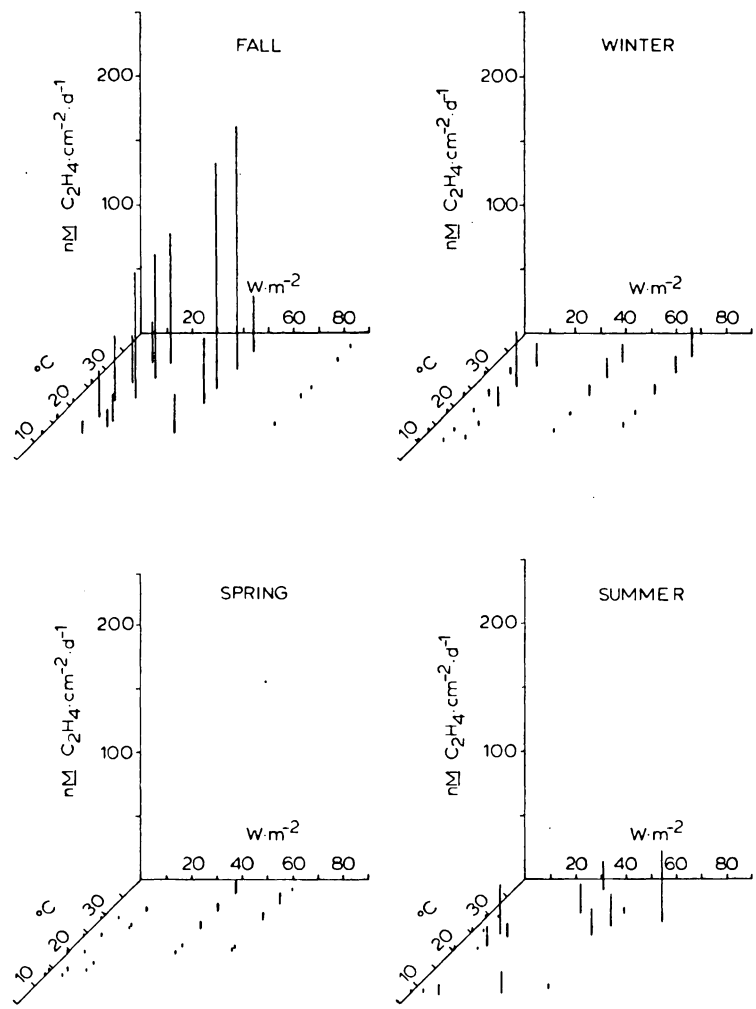
FALL
WINTER
SPRING
SUMMER
nM C2H4·cm-2·d-1
200
100
W·m-2
20
40
60
80
°C
10
20
30

FIG. II.2. $N_2[C_2H_2]$-FIXATION RATES IN SOIL 2 VERSUS LIGHT AND DAY TEMPERATURE FOR EACH OF THE FOUR SAMPLING DATES

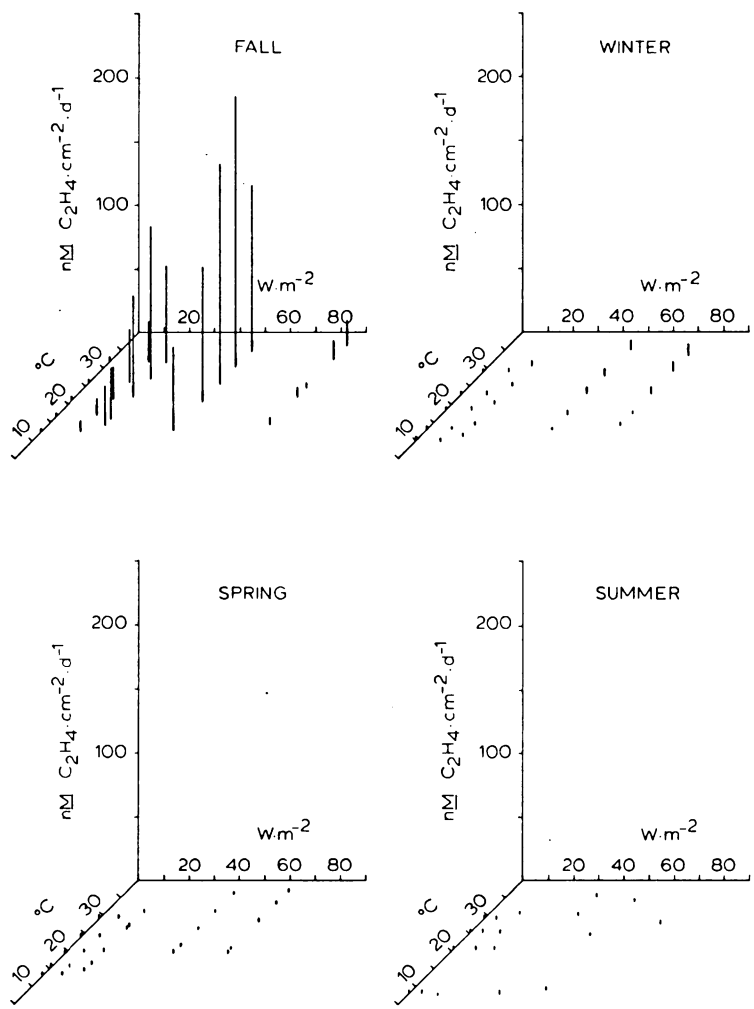
FALL
WINTER
SPRING
SUMMER
nM C2H4.cm-2.d-1
200
100
W.m-2
20
40
60
80
°C
10
20
30

TABLE II.1. COLONIES OF BLUE-GREEN ALGAE BY THE DILUTION PLATE METHOD FOR SOILS 1 AND 2 AT EACH OF THE SAMPLING DATES.

Soil	Species	Colonies·g^{-1} dry wt. of soil			
		Sept.	Jan.	May	June
1	Nostoc commune Vaucher	2.4×10^4	5.3×10^4	1.5×10^4	1.8×10^5
1	Schizothrix Friesii (Agardh) Gomont	5.6×10^4	1.2×10^4	1.6×10^4	2.0×10^5
1	Microcoleus vaginatus (Vaucher) Gomont	1.5×10^4	1.0×10^4	trace	7.8×10^5
1	Cylindrospermum musicola Kuetz	0	0	trace	trace
2	N. commune	3.6×10^4	1.1×10^4	9.6×10^5	7.8×10^5
2	S. calcicola (Agardh) Gomont	4.6×10^4	2.6×10^4	1.4×10^4	1.5×10^5
2	M. vaginatus	8.0×10^2	4.4×10^4	6.0×10^3	1.2×10^4
2	Oscillatoria submembranaceae (Vaucher) Gomont	0	0	trace	0
2	unidentified unicell	0	0	trace	0

TABLE II.2 REGRESSION EQUATIONS WITH THE HIGHEST CORRELATION COEFFICIENTS FOR SOILS 1 and 2 AT EACH SAMPLING DATE.

Soil	Month	Equation	Limits	Correlation Coefficient	n
1	Sept.	$\log n\underline{M}\ C_2H_4 \cdot cm^{-2} \cdot d^{-1} = 0.119 + 0.00985\ W \cdot m^{-2} + 0.0518°C$	$15.2 \leq W \cdot m^{-2} \leq 51.2$ $12.0 \leq °C \leq 30.0$	.85	72
2	Sept.	$\log n\underline{M}\ C_2H_4 \cdot cm^{-2} \cdot d^{-1} = 0.327 + 0.0163\ W \cdot m^{-2} + 0.0420°C$	$15.2 \leq W \cdot m^{-2} \leq 51.2$ $12.0 \leq °C \leq 30.0$	.83	72
1	Jan.	$\log n\underline{M}\ C_2H_4 \cdot cm^{-2} \cdot d^{-1} = -2.09 + 0.0680\ W \cdot m^{-2} + 0.0827°C$	$9.4 \leq W \cdot m^{-2} \leq 16.9$ $10.0 \leq °C \leq 28.9$	.81	45
1	Jan.	$\ln n\underline{M}\ C_2H_4 \cdot cm^{-2} \cdot d^{-1} = -3.13 + 0.202°C$	$49.5 \leq W \cdot m^{-2} \leq 75.0$ $12.8 \leq °C \leq 33.3$	.92	50
2	Jan.	$\log n\underline{M}\ C_2H_4 \cdot cm^{-2} \cdot d^{-1} = -1.45 + 0.00824\ W \cdot m^{-2} + 0.0593°C$	$9.4 \leq W \cdot m^{-2} \leq 49.5$ $10.0 \leq °C \leq 28.9$	.80	70
1	May	$\log n\underline{M}\ C_2H_2 \cdot cm^{-2} \cdot d^{-1} = -1.97 + 0.0159\ W \cdot m^{-2} + 0.0592°C$	$6.9 \leq W \cdot m^{-2} \leq 63.9$ $14.0 \leq °C \leq 31.0$	.88	65
2	May	n.d.			
1	June	$\log n\underline{M}\ C_2H_2 \cdot cm^{-2} \cdot d^{-1} = -0.126 + 0.0287\ W \cdot m^{-2} + 0.0152°C$	$4.1 \leq W \cdot m^{-2} \leq 48.3$ $8.0 \leq °C \leq 30.5$	.59	55
2	June	n.d.			

from which the regression equations were derived are also given in Table II.2. The log of C_2H_4 production described significantly more data in every case than did the antilog. Soil 1 in the January experiment showed a marked decline in C_2H_4 production at the higher light intensities studied (49.5 and 75.0 $W \cdot m^{-2}$). Separate regression equations for the two higher light intensities were not different at the 0.01 level of significance so the data were combined and a regression equation was derived using day temperature as the only independent variable. $N_2[C_2H_2]$-fixation rates were not modeled for Soil 2 for the May and June experiments because the amount of C_2H_4 produced was at the same order of magnitude as the error in gas chromatography.

Less than 5% of the measurements of C_2H_4 production in the absence of C_2H_2 showed any trace of C_2H_4. When C_2H_4 was found it was not produced at rates any higher than 0.3 $n\underline{M} \cdot cm^{-2} \cdot d^{-1}$. Therefore, it was not subtracted from the amounts of C_2H_4 measured in the $N_2[C_2H_2]$-reduction experiments.

Measurement of $N_2[C_2H_2]$-fixation rates after the experiments were finished (at light and temperature regimens comparable to those of the first day of the experiments) revealed that there was no significant change in $N_2[C_2H_2]$-fixing capacities of the soil cores during the period of time in which they were held in the incubator.

DISCUSSION

The previously reported similarities in $N_2[C_2H_2]$-fixation rates for Soils 1 and 2 (Bailey, In Review) did not persist after the

September sampling date. These similarities were originally suspected to be due to similar amounts of Nostoc commune Vaucher. However, increases of colony forming units of N. commune were later found when $N_2[C_2H_2]$-fixation rates decreased.

$N_2[C_2H_2]$-fixation rates in Soil 1 showed strong responses to light at each sampling date with an increase of at least 25-fold from maximum fixation rate in the dark to maximum fixation rate in the light. Likewise, $N_2[C_2H_2]$-fixation rates in Soil 2 responded strongly to light in September and January. This confirms an earlier report (Bailey, In Review) that blue-green algae at the surface of these soils are the most important $N_2[C_2H_2]$-fixing organisms.

The responses of Soil 1 in January (Table II.2) were particularly odd: at low light intensities, increases in light resulted in increases in the amount of $N_2[C_2H_2]$ fixed. However, $N_2[C_2H_2]$-fixation rates were significantly reduced at 45.9 and 75.0 $W \cdot m^{-2}$. Here, an increase in light intensity from 49.5 to 75.0 $W \cdot m^{-2}$ had no measurable effect. The single independent variable (day temperature) regressed against ln of $N_2[C_2H_2]$ explained more of the data than any of the other models. This is especially interesting considering the fact that log of $N_2[C_2H_2]$ production was superior to ln in all of the other models. While the amounts of $N_2[C_2H_2]$ fixed at comparable temperatures were not different for the two light intensities, light still played an important role. This is evident from the fact that only 0.16 n$\underline{M}$ $C_2H_4 \cdot cm^{-2} \cdot d^{-1}$ were fixed in the dark at 28.9°C while 12.16 n$\underline{M}$ were fixed at 28.9°C and 75 $W \cdot m^{-2}$. Perhaps the separate regression

equations for Soil 1 in January are due to separate species or at least to separate physiological processes operating under the different sets of environmental conditions.

While the $N_2[C_2H_2]$-fixation rate for Soil 2 dropped to almost zero in May and June, this soil remained undisturbed. Soil 1, which was plowed before the May sampling date, showed a decline in $N_2[C_2H_2]$-fixation rates in the May experiments and an increase of about 10-fold in $N_2[C_2H_2]$-fixation rates from May to June.

The high sample standard deviations reported here (Tables II.3-II.5) doubtless reflect a high variability of $N_2[C_2H_2]$-ase activity from core to core even in the small homogeneous plots which were employed. This phenomenon has been encountered earlier by Henriksson et al (1972). The magnitude of this variability may best be appreciated when one considers that each mean value reported in Tables II.3-II.5 represents data derived from 50 soil cores divided equally among 5 test tubes.

The magnitude of the seasonal changes in $N_2[C_2H_2]$-ase activity is best illustrated by using the equations in Table II.2 to calculate the $N_2[C_2H_2]$-fixation rates under standard conditions (50 $W \cdot m^{-2}$, 30°C) at each sampling date for the two soils. These calculated rates for Soil 1 are 222 nM $C_2H_2 \cdot cm^{-2} \cdot d^{-1}$ in September, 18.7 nM in January, 4.0 nM in May and 58.2 nM in June. For Soil 2 the calculated rates are 252 nM in September and only 5.5 nM in January. Thus, comparable amounts of $N_2[C_2H_2]$ were not fixed under the same conditions of light and day temperature throughout the year.

TABLE II.3. NITROGENASE ACTIVITY ($n\underline{M}\ C_2H_4 \cdot cm^{-2} \cdot d^{-1}$) FROM THE TOP 0-1 cm OF SOILS COLLECTED IN JANUARY AND INCUBATED UNDER DIFFERENT LIGHT AND TEMPERATURE CONDITIONS.

			Soil 1		Soil 2	
$W \cdot m^{-2}$	^oC (Day)	^oC (Night)	$n\underline{M}\ C_2H_4$*	s†	$n\underline{M}\ C_2H_4$*	s†
0	10.0	3.3	0.15	0.05	0.12	0.00
0	12.8	7.2	0.16	0.05	0.13	0.00
0	18.3	11.0	0.16	0.06	0.20	0.11
0	23.9	16.7	0.27	0.01	0.13	0.00
0	28.9	23.3	0.16	0.06	0.10	0.06
9.4	10.0	3.3	0.28	0.34	0.20	0.11
9.4	12.8	7.2	0.99	1.18	0.31	0.07
9.4	18.3	11.0	1.35	0.48	0.45	0.14
9.4	23.9	16.7	5.12	3.16	0.81	0.33
9.4	28.9	23.3	5.71	4.04	0.80	0.27
16.9	11.0	3.3	0.86	0.86	0.20	0.07
16.9	14.4	7.2	2.73	2.43	0.42	0.14
16.9	20.0	13.5	14.75	22.42	1.46	1.06
16.9	25.0	18.3	44.90	78.22	4.67	4.00
16.9	30.6	23.9	18.07	15.99	3.83	2.45
49.5	12.8	3.3	0.55	0.19	0.37	0.36
49.5	17.2	7.2	1.83	0.78	1.35	1.42
49.5	22.8	13.5	8.67	3.67	4.16	3.01
49.5	27.8	18.3	13.10	4.46	6.59	5.26
49.5	32.2	23.9	14.24	5.15	6.86	5.64
75.0	13.9	3.3	0.70	0.34	0.52	0.32
75.0	17.2	7.2	2.33	0.87	1.54	1.13
75.0	22.8	16.0	8.91	3.01	4.84	3.13
75.0	28.9	18.3	12.16	4.74	7.91	5.03
75.0	33.3	23.9	23.07	3.24	9.33	5.24

*Mean value of $N_2[C_2H_2]$-ase activity; in each case n = 6.

†Sample standard deviation.

TABLE II.4. NITROGENASE ACTIVITY (nM $C_2H_4 \cdot cm^{-2} \cdot d^{-1}$) FROM THE TOP 0-1 cm OF SOILS COLLECTED IN MAY AND INCUBATED UNDER DIFFERENT LIGHT AND TEMPERATURE CONDITIONS.

			Soil 1		Soil 2	
$W \cdot m^{-2}$	°C (Day)	°C (Night)	nM C_2H_4*	s†	nM C_2H_4*	s†
0	13.0	2.0	0.12	0.00	0.05	0.04
0	14.0	7.2	0.24	0.10	0.09	0.06
0	19.5	12.7	0.09	0.13	0.10	0.06
0	24.0	18.3	0.01	0.07	0.04	0.04
0	29.0	23.9	0.18	0.11	0.10	0.06
6.9	13.0	2.0	0.22	0.22	0.09	0.33
6.9	14.0	7.2	0.18	0.12	0.10	0.06
6.9	19.5	12.7	0.31	0.12	0.12	0.07
6.9	24.0	18.3	0.31	0.21	0.15	0.09
6.9	29.0	23.9	0.57	0.37	0.11	0.09
14.2	14.0	2.0	0.22	0.05	0.13	0.05
14.2	16.0	7.2	0.10	0.02	0.09	0.09
14.2	26.0	12.7	0.51	0.20	0.13	0.00
14.2	26.5	18.3	0.90	0.33	0.11	0.00
14.2	31.0	23.9	2.00	0.25	0.10	0.06
42.8	19.0	2.0	0.83	0.70	0.04	0.05
42.8	21.0	7.2	0.49	0.45	0.09	0.11
42.8	26.0	12.7	4.45	3.31	0.20	0.09
42.8	31.0	18.3	6.10	7.18	0.15	0.10
42.8	36.0	23.9	12.01	9.64	0.26	0.13
63.9	19.5	2.0	0.21	0.30	0.91	1.24
63.9	20.0	7.2	2.97	2.54	0.13	0.00
63.9	28.0	12.7	5.84	3.48	0.20	0.11
63.9	33.0	18.3	7.23	5.00	0.28	0.21
63.9	36.5	23.9	0.82	0.20	0.18	0.07

*Mean value of $N_2[C_2H_2]$-ase activity; in each case n = 6.

†Sample standard deviation.

TABLE II.5. NITROGENASE ACTIVITY ($n\underline{M}\ C_2H_4 \cdot cm^{-2} \cdot d^{-1}$) FROM THE TOP 0-1 cm OF SOILS COLLECTED IN JUNE AND INCUBATED UNDER DIFFERENT LIGHT AND TEMPERATURE CONDITIONS.

			Soil 1		Soil 2	
$W \cdot m^{-2}$	^oC (Day)	^oC (Night)	$n\underline{M}\ C_2H_4$*	s†	$n\underline{M}\ C_2H_4$*	s†
0	8.0	1.9	0.22	0.06	0.07	0.07
0	20.3	18.5	2.13	2.54	0.20	0.13
0	25.0	19.5	0.31	0.07	0.23	0.06
0	29.0	23.9	0.51	0.14	0.17	0.07
4.1	8.0	1.9	0.48	0.37	0.11	0.07
7.5	20.3	18.5	1.74	2.14	0.21	0.08
4.1	25.0	19.5	0.82	0.72	0.13	0.00
4.1	29.0	23.9	1.65	1.62	0.30	0.07
11.5	7.5	1.9	7.85	1.79	0.35	0.16
16.0	20.3	18.5	9.62	5.23	0.24	0.08
11.5	25.0	19.5	16.89	6.30	0.13	0.00
11.5	30.5	23.9	39.42	7.48	0.25	0.09
35.6	8.0	1.9	17.35	6.96	0.56	0.07
48.3	24.4	18.5	19.69	14.21	0.35	0.08
35.6	30.0	19.5	23.79	16.94	0.49	0.14
35.6	35.5	23.9	24.13	11.16	0.32	0.14
52.4	9.0	2.0	2.12	1.41	0.42	0.07
71.3	27.6	18.5	58.25	20.23	0.38	0.08
52.4	34.0	19.5	25.36	13.06	0.49	0.14
52.4	39.5	23.9	4.20	1.91	0.37	0.09

*Mean value of $N_2[C_2H_2]$-ase activity; in each case n = 6.

†Sample standard deviation.

The differences in amounts of $N_2[C_2H_2]$ fixed at each sampling date under these standard conditions may have been due, in small part, to changes in threshold limits of light and day temperature below which no $N_2[C_2H_2]$ fixation occurred. This phenomenon cannot be evaluated with models of the log of $n\underline{M}$ $C_2H_4 \cdot cm^{-2} \cdot d^{-1}$ because 0 $n\underline{M}$ C_2H_4 has no real logarithm.

Much of the change in amounts of $N_2[C_2H_2]$ fixed under the standard conditions noted above was due to seasonal changes in acceleration of $N_2[C_2H_2]$-fixation rates with increases in light or temperature. Seasonal changes of the response of $N_2[C_2H_2]$-fixation rates to temperature are illustrated by using the equations given in Table II.2. At 50 $W \cdot m^{-2}$ an increase of 10°C in day temperature (from 20-30°C) results in 3.78-, 7.55-, 3.91- and 1.42-fold increases in $N_2[C_2H_2]$-fixation rate for Soil 1 at the September, January, May and June sampling dates, respectively. The same increase in temperature results in 2.63- and 3.91-fold increases in $N_2[C_2H_2]$-fixation rates for Soil 2 in September and January. Seasonal changes in responses of the $N_2[C_2H_2]$-fixation rates to light may be evaluated with the same equations and holding day temperature at 25°C while using a 10 $W \cdot m^{-2}$ increase in light intensity (40-50 $W \cdot m^{-2}$). For Soil 2 this 10 $W \cdot m^{-2}$ increase results in a 1.25-fold increase in $N_2[C_2H_2]$-fixation rate in September, no increase in January, a 1.44-fold increase in May and a 1.94-fold increase in June. For Soil 2 it results in a 1.46-fold increase in September and a 1.21-fold increase in January.

From the above discussion it is obvious that the $N_2[C_2H_2]$-ase activities of these soils change with respect to light and day temperature throughout the year. Because of these changes it is not possible to calculate amounts of $N_2[C_2H_2]$ fixed annually based on responses of $N_2[C_2H_2]$-ase activities to light and day temperature at one sampling date or at a series of sampling dates in one season. Further, the data from the four sampling dates reported here show wide fluctuations from season to season and are barely sufficient to show trends for the two soils which were studied.

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EFFECTS OF LIGHT AND TEMPERATURE ON NITROGEN-FIXATION RATES IN ARABLE SOILS AND SEASONAL FLUCTUATIONS IN NITROGEN-FIXATION RATES IN ARABLE SOILS

by

Donald George Bailey

(ABSTRACT)

Nitrogen-fixation rates were determined by C_2H_2 reduction in intact cores taken from the top 0-1 cm of two soils. The cores were moistened and incubated under varying light (0-88 $W \cdot m^{-2}$) and temperature (7.5-39.5°C) conditions at four different times of the year to determine the effects of day temperature, night temperature and light intensity on $N_2[C_2H_2]$-fixation rates. Multiple regression equations were derived for each season to relate $n\underline{M}$ C_2H_4 produced with light and temperature.

A greater portion of the data was explained by each regression equation when nM $C_2H_4 \cdot cm^{-2}$ surface area was used as the dependent variable than when $n\underline{M}$ $C_2H_4 \cdot g^{-1}$ was used. The influence of light was pronounced with increasing light intensities producing increasing $N_2[C_2H_2]$-fixation rates. Therefore, it was concluded that the fixation was a surface area phenomenon with blue-green algae being responsible for most of the $N_2[C_2H_2]$-fixation which occurred.

The responses of the two soils to light and day temperature varied widely among the four sampling dates indicating that it was not possible to predict amounts of nitrogen fixed annually based on responses at one

time of the year.

$N_2[C_2H_2]$-fixation rates did not appear to be related to night temperature or to the abundance of blue-green algae as extimated by the dilution plate method.