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ANL-83-100
Part III

ANL--83-100-Pt.3

DE85 008344

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ENVIRONMENTAL RESEARCH DIVISION*
ANNUAL REPORT

✓ Environmental Effects Research

January - December 1983

P. F. Gustafson, Acting Division Director

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December 1984

Preceding Report

ANL-82-65 Part III January-December 1982

*The Radiological and Environmental Research (RER) Division (A. F. Stehney, Acting Director) became part of the Environmental Research (ER) Division on the latter's formation in May 1983.

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FOREWORD

In the year 1983, there were several significant changes that affected the Ecological Sciences Section. In order to consolidate environmental activities at Argonne National Laboratory the former Radiological and Environmental Research (RER) and Environmental Impact Studies (EIS) divisions plus some parts of the Land Reclamation Program were combined into a single administrative unit and named the Environmental Research Division. With this change, ecological studies gained five staff and the Ecology Section was renamed the Environmental Effects Research Program. The ecological sciences acquired new expertise and some expansion of research facilities, namely an analytical laboratory and a new greenhouse.

During the year, the ongoing research programs maintained continuity. The Terrestrial Ecology group continued its involvement in the National Crop Loss Assessment Network, and studies of O_3 effects on winter wheat and soybeans were completed. Experiments on $O_3 \times SO_2$ interactions on soybeans were also performed. The Microcosms for Acid Rain Studies (MARS) project had its first full year of research and much information concerning acid rain impacts on soil-plant systems was collected. A study of the influence of temporal variations in rain acidity on soybean productivity was also initiated.

The aquatic radiochemistry group continued measurements of the mobility of plutonium and americium at a disposal site at Los Alamos and initiated similar work at Hanford. Laboratory tracer experiments were carried out to study the adsorptive behavior of neptunium, the solubility limits of plutonium, and the influence of rare earth concentration on the sorption and redox behavior of plutonium.

The soil-plant process group initiated several studies on the influence of mycorrhizae to host plants in disturbed and natural environments. Much of the past research has been concerned with understanding mycorrhizal fungi propagule dynamics as related to disturbances associated with energy extraction. Future research will be directed at understanding how below-ground symbiotic associations may increase the fitness of host plants. Emphasis is being placed on resource acquisition and compartmental strategies.

While 1983 did not see any dramatic changes in the magnitude of funding or the direction of research, the prospects for future years look bright. The expansion in research personnel and facilities should enhance the Environmental Effects Research Program's position in environmental research.

IMPACT OF OZONE ON YIELD OF WINTER WHEAT

L. W. Kress, H. J. Smith, and J. E. Miller

Background and Scope

Argonne National Laboratory has been a participant in the National Crop Loss Assessment Network (NCLAN) since 1980. A major objective of this program is to determine the dose-response relationships between ozone (O_3) and the yield of important agricultural crops. From 1980 through 1982, Argonne has investigated the effects of O_3 on soybean, field corn, winter wheat, and sorghum.^{1,2,3,4} Winter wheat was selected for study again in 1983.

Previous work with four cultivars of soft red winter wheat exposed to O_3 in open-top chambers indicated that yield reductions ranged from 1% to 11% at 0.06 ppm O_3 (seasonal 7-h/day mean) and 11% to 25% at 0.10 ppm O_3 compared with a 0.03 ppm control treatment.⁵ The 1982 experiments at Argonne with three different soft red winter wheat cultivars produced estimated yield reductions of 10% to 24% at 0.06 ppm O_3 and 32% to 53% at 0.10 ppm O_3 .³

The 1983 study was initiated to provide biological response data suitable for use in setting ambient air quality standards and in assessing the economic consequences of O_3 exposure. The objective was to examine the dose-response relationships of two cultivars of soft red winter wheat (both examined in 1982) grown in the Midwest under chronic exposure to a range of O_3 concentrations.

Progress in 1983

Field plantings (seed was fall sown) of two cultivars of winter wheat (Abe, Arthur-71) were exposed to various concentrations of O_3 in open-top chambers. The experimental design was a randomized complete block with three replicates of six treatments. The treatments were: AA -- no chamber; CF -- charcoal-filtered air chamber (serving as control); NF -- non-filtered air chamber with a small amount of O_3 added to provide a concentration equal to that in the AA plot; NF+0.02 -- NF with O_3 added to 0.02 ppm above ambient; NF+0.04 -- NF with O_3 added to 0.04 ppm above ambient; and NF+0.06 -- NF with O_3 added to 0.06 ppm above ambient. The chambers were placed over the plots

on 6 May, and O_3 additions began on 8 May, as the plants began spring growth. Ozone was added to the chambers 7-h/day (1000-1700 CDT). All chamber fans were turned off nightly from 2230-0630 CDT. Ozone was not added on two full days and five half days due to rain, but O_3 concentrations were recorded and included in the seasonal average. Ozone additions were terminated 30 June, when the leaves and heads were brown. Wheat heads and stems were harvested 5-10 July. Throughout the experiment the O_3 concentrations in each chamber were monitored, and O_3 and SO_2 concentrations were continuously monitored in the ambient air.

The spring of 1983 was excessively wet (18.6 cm rain in May) and the wheat plants grew 10 to 15 cm taller than normal. Three of the plots in one block (CF, NF, NF+0.06) had to be discarded because of severe lodging (the plants fell over) just after heading.

An analysis of variance indicated significant treatment effects for the yield variables kilograms/hectare (kg/ha), 100-seed weight, seed weight/head, number of seeds, and number of heads/meter row. A means comparison test of the chambered plot data only indicated a significant difference among the treatments for number of heads/meter (as had been noted in 1982), but it was not caused by the O_3 treatments. Thus, the number of seeds, kg/ha yield, and stem weight values were adjusted by covariance analysis using number of heads as the covariate. Significant yield reductions were noted at the highest O_3 treatment for Abe (kg/ha, 100-seed weight) and at the two highest O_3 treatments for Arthur-71 (100-seed weight) (Table 1). There were small increases in seed number for both cultivars as O_3 increased, which counteracted the O_3 effect on 100-seed weight and reduced the significance of the kg/ha yield reductions. The kg/ha yield reductions can be accounted for totally by seed weight reductions. (In 1982, reductions in seed number had accounted for about one-third of the overall yield reduction in the range of 15%). Grain weight was affected more than stem weight by the O_3 treatments, which could reflect previous damage to the photosynthetic capacity needed to fill the heads or the higher O_3 concentrations during head filling (7-h mean O_3 of 0.039 ppm for May and 0.057 ppm for June).

The data from the 1982 and 1983 studies have been plotted according to a Weibull regression model, which is being used in the NCLAN program because of its versatility and biological plausibility.⁶ The model may take many

Table 1. Yield responses of two cultivars of winter wheat exposed to charcoal-filtered air (CF), ambient open plot (AA), and ambient air plus O₃ (at indicated concentration in ppm) in open-top chambers (NF) for 7-h/day for 53 days during 1983.

Treatment	O ₃ Conc. (ppm)	Abe [†]					Arthur-71 [†]				
		Number Heads	Number Seeds	kg/ha	100-Seed Weight (g)	Stem Weight (g)	Number Heads	Number Seeds	kg/ha [†]	100-Seed Weight (g)	Stem Weight (g)
AA	0.045	143 b [*]	3037a	6381a	3.68a	130a	161a	3429a	6695a	3.47a	130a
CF	0.018	167ab	3080a	5808ab	3.44ab	124ab	160a	2995 b	5151 bc	3.10 b	115 bc
NF	0.044	196a	3295a	5986ab	3.45ab	126ab	143a	3271ab	5622ab	3.13 b	124ab
NF+0.02	0.062	154ab	3163a	5982ab	3.36ab	117 b	137a	3125 b	5214 bc	3.04 b	113 bc
NF+0.04	0.078	160ab	3175a	5762 b	3.26 b	119ab	157a	3252ab	4943 bc	2.73 c	107 c
NF+0.06	0.095	155ab	3042a	4939 c	2.88 c	112 b	137a	3098 b	4397 c	2.59 c	109 bc
		% of CF					% of CF				
AA		86	99	110	107	105	101	114	130	112	113
NF		117	107	103	100	102	89	109	109	101	108
NF+0.02		92	103	103	98	94	86	104	101	98	98
NF+0.04		96	103	99	95	96	98	109	96	88	93
NF+0.06		93	99	85	84	90	86	103	85	84	95

[†]Number seeds, kg/ha, and stem weight are adjusted values from covariance analysis using number heads as the covariate.

^{*}Means in a column followed by the same letter are not significantly different at P = 0.05 according to the Duncan's New Multiple Range Test.

different forms (shapes) and thus is useful in presenting dissimilar data sets in a similar format. The Weibull regression equation is given as:

$$\text{yield} = \alpha \cdot \exp [-(O_3/\text{sig})^c], \quad (1)$$

where α is the yield at 0.00 ppm O_3 ; O_3 is the dose (seasonal 7-h/day mean in ppm); sig is the O_3 concentration when yield is 0.37 α ; and c is a dimensionless shape parameter. The yield responses of the Abe and Arthur-71 cultivars were nearly identical and have been combined for each year (1982 and 1983) (Fig. 1). The different responses between the two years were not statistically different, and thus a combined curve for both cultivars over both years is also presented. The responses of both the Abe and Arthur-71 cultivars were different from the response of the Roland cultivar (used only in 1982), which was significantly more sensitive to O_3 , especially at the lower concentrations near ambient.

The O_3 sensitivity exhibited by the Abe and Arthur-71 cultivars was somewhat less in 1983 than it had been in 1982. It may be that the conditions of excess moisture and high fertility allowed the plants to overcome the O_3 stress at near ambient concentrations. Winter wheat (Abe and Arthur-71) is still to be considered as more sensitive than grain sorghum⁴, or field corn¹ and less sensitive than soybean² (Fig. 2). The cultivar Roland, however, is more sensitive than Corsoy soybean.

Prospects

These data will be included with data from five other field sites for evaluation of the effects of O_3 on important agricultural crops. This information will be important to the current reevaluation of the National Ambient Air Quality Standard for O_3 in terms of the biological response data and as input into a comprehensive economic analysis of crop yield losses induced by air pollution.

Figure 1. Weilbull model dose-response curves for winter wheat yield as a function of the 7-h seasonal mean O_3 concentration, summarizing 1982 and 1983 data.

Figure 2. Weilbull model dose-response curves for sorghum, corn, wheat, and soybean yields as a function of the 7-h seasonal mean O_3 concentration, summarizing 1980-1983 data.

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YIELD RESPONSE OF FOUR SOYBEAN CULTIVARS TO OZONE

L. W. Kress, H. J. Smith, and J. E. Miller

Background and Scope

Soybeans (Glycine max L.) are a major crop worldwide and one of the leading cash crops in the midwestern United States. They are sensitive to ozone (O_3) air pollution, and reductions in yield from O_3 exposure have been demonstrated under ambient field conditions.^{1,2}

Research performed within the National Crop Loss Assessment Network (NCLAN) has allowed development of exposure-response relationships for six soybean cultivars, and statistical comparisons have suggested that the yield responses to O_3 among all six cultivars may be homogeneous.³ (However, significant year-to-year variation has been shown for one cultivar at one site.) Predicted percent yield reductions, based on a common response model, are 12.1% at 0.05 ppm O_3 (seasonal 7-h/day mean), 17% at 0.06 ppm, and 30.7% at 0.09 ppm.³ The 0.05 ppm level is the approximate ambient average for O_3 at Argonne National Laboratory, which is the NCLAN site for the Midwest.

Prior to 1983, only one cultivar of soybean (Corsoy-79) had been examined at Argonne. Since soybeans are more important in the Midwest than elsewhere in the country, tests were conducted at Argonne in 1983 to determine the effects of four concentrations of daily O_3 exposures on the yield of four soybean cultivars--Williams-79, Pella, Corsoy-79, and Amsoy-71. Williams-79 was the cultivar most commonly planted on Illinois farms in 1983 (9% of total acreage). Pella was a relatively new cultivar rapidly gaining popularity (3%). Corsoy-79 ranked seventh in the state (2%), but first in the northeastern Illinois counties (9%). Amsoy-71 ranked tenth in Illinois (1%), and has been noted as sensitive to acid precipitation effects.⁴

Progress in 1983

Seeds of four cultivars of soybean were sown in alternate rows on a 76-cm row spacing and a 3.5-cm plant spacing in mid-July 1983. The late planting caused senescence to occur later in the fall (mid-October) and probably reduced overall seed yield, but relative response to the O_3 stress may not have been affected. Single 2-m rows of each cultivar were paired by

maturation rate (Corsoy-79 and Amsoy-71; Pella and Williams-79) in adjacent plots of similar treatments. There were three blocks (replications) of five treatments. Each soybean pair occurred in each treatment and each block. The treatments consisted of ambient O_3 in open plots (0.046 ppm, 7-h/day seasonal mean), and four concentrations of O_3 in open-top chamber enclosures (0.022, 0.046, 0.071, 0.094 ppm). The O_3 concentrations in the chambers were determined by charcoal-filtration, no filtration, or addition of a constant amount of O_3 to the ambient O_3 . Ozone treatments were administered from 28 July to 18 October and were characterized as the 7-h/day (0900-1600 CST) mean seasonal concentration.

A split-plot analysis of variance of the chambered plot data indicated significant treatment and cultivar effects for yield (kg/ha), but only significant cultivar effects for individual seed weight (weight of 100 seeds, or 100 swt). (However, there was a significant $O_3 \times$ block interaction that may have confounded the O_3 effect for 100 swt.) Separate analyses of variance for each cultivar indicated significant ($P = 0.05$) O_3 effects for Corsoy-79, Pella, and Williams-79 cultivars, but not for Amsoy-71 (Prob. $> F = 0.118$). The percentage yield reductions with increasing O_3 concentrations, however, were rather similar for all four cultivars (Table 1). At the highest O_3 concentration, reductions in individual seed weight accounted for about half of the overall yield (kg/ha) reduction, as has been noted previously.^{1,5}

Data for each of the four cultivars have been plotted according to a Weibull regression model in Figure 1. (The model is explained elsewhere in this report.⁵) The regressions for each cultivar are significant ($P = 0.05$). The curves appear to be somewhat dissimilar, but a statistical test for homogeneity (performed by partitioning the $O_3 \times$ cultivar sum of squares into three types of Weibull models) indicates that the responses of the four cultivars to O_3 are homogeneous and that a common model (with an R^2 of 0.65) can be used to describe the responses of all four cultivars. Based on the model, the predicted yield reductions as compared with a control of 0.025 ppm O_3 are 12.6% at 0.05 ppm O_3 , 17.2% at 0.06 ppm, and 29.9% at 0.09 ppm.

These loss estimates are nearly identical to those produced from the model of previous soybean data sets--12.1% at 0.05 ppm O_3 , 17% at 0.06 ppm, and 30.7% at 0.09 ppm.³ These data indicate that in terms of yield reduction, different soybean cultivars exposed to O_3 in different regions and in different years may be responding to the O_3 in a similar fashion.

Table 1. Yield response of four soybean cultivars exposed to O_3 in open-top chambers for 83 days.

O_3 [†] (ppm)	Amsoy-71		Corsoy-79		Pella		Williams-79	
	kg/ha [‡]	100 swt [*]	kg/ha	100 swt	kg/ha	100 swt	kg/ha	100 swt
0.022	1978	15.9	2096	15.2	2744	19.7	2536	17.8
0.046	1793	15.1	1671	14.5	2103	18.1	1887	16.7
0.071	1609	13.8	1780	12.4	2300	17.1	1764	14.1
0.094	1231	13.1	1139	11.1	1863	17.0	1735	14.7
0.046 (AA) [§]	1967	14.7	1936	13.9	2109	17.3	2078	15.8
Percent Reduction from 0.22 ppm Treatment								
0.046	9	5	20	5	23	8	26	6
0.071	19	13	15	18	16	13	30	21
0.094	38	18	46	27	32	14	32	17
0.046 (AA)	1	8	8	9	23	12	18	11

[†]7-h/day seasonal mean concentrations.

[‡]kg/ha = kilograms per hectare.

^{*}100 swt = weight of 100 seeds.

[§]AA = open plot.

Figure 1. Weibull model regression curves indicating yield as a function of the 7-h/day seasonal mean O_3 concentration for four soybean cultivars exposed in open-top field chambers for 83 days.

Prospects

The 1983 data will be included with the other data generated in the NCLAN program to evaluate the effects of O_3 on crop yield. Future research will attempt to quantify the confounding effects of soil moisture stress on soybean sensitivity to O_3 .

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EFFECTS OF OZONE AND SULFUR DIOXIDE ON SOYBEAN YIELD

L. W. Kress, R. J. Smith, and J. E. Miller

Background and Scope

Because of their agricultural importance in the Midwest, and indeed worldwide, soybeans (Glycine max L.) have been the subject of several investigations seeking to determine the effects of air pollutants on crops. Reduction of soybean yields as a result of exposure to ozone (O_3) in the air has been demonstrated under ambient field conditions.^{1,2} Additionally, soybeans have been shown to be sensitive to elevated sulfur dioxide (SO_2) concentrations, such as might be found near a point source.³

The effects of O_3 and SO_2 combinations on soybean yield have been examined in some recent field studies. Additive yield reductions were noted for soybeans (Dare cultivar) exposed for 133 days to 0.10 ppm O_3 + 0.10 ppm SO_2 for 6-h/day in closed-top field chambers.⁴ Mixtures of SO_2 and O_3 dispensed with a linear gradient field exposure system caused decreased yield of Hark soybeans, but the interactive effects were not evaluated.⁵ In a relevant study of snap beans, the yield loss from exposure to SO_2 was greater in open-top chambers with nonfiltered air than in chambers with charcoal-filtered air, indicating a possible synergistic effect of SO_2 and ambient O_3 .⁶

There previously has been only one field study with seasonal exposures designed to evaluate the dose-response relationship between soybean yield and both O_3 and SO_2 .⁷ In that study, a 7-h/day seasonal mean O_3 concentration of 0.055 ppm caused a 20% yield reduction for Davis cultivar soybeans; SO_2 had no effect except at concentrations above those known to exist regionally (> 0.026 ppm 4-h/day seasonal mean). Neither pollutant altered the dose-response relationships for the other except at high concentrations (0.125 ppm O_3 and 0.367 ppm SO_2), in which case the effects were less than the additive effects of the pollutants singly.

The study reported here was initiated to evaluate the effects of daily O_3 and periodic SO_2 exposures on the yields of two soybean cultivars. This experimental exposure regime was designed to simulate SO_2 exposures from a point source superimposed on a regional O_3 stress.

Progress in 1983

Seed of two cultivars of soybean (Corsoy-79 and Amsoy-71) were sown in alternate rows on a 76-cm row spacing and a 3.5-cm plant spacing in mid-July 1983. Two 1-m rows of each cultivar were exposed to O_3 and/or SO_2 in each of 18 plots. There were four O_3 treatments (0.023, 0.046, 0.066, and 0.092 ppm, 7-h/day seasonal mean) and four SO_2 treatments (0.020, 0.089, 0.295, and 0.564 ppm, 4-h/day, 3 days/week for 11 weeks). There were also two ambient air plots. This 4 x 4 experimental design required 16 open-top chambers and was unreplicated. Pollutant exposures were from 28 July to 14 October.

A split-plot (cultivars were the subplots) analysis of variance using only the chambered plot data indicated significant ($P = 0.05$) O_3 , SO_2 , and cultivar effects on overall yield (kg/ha), number of seeds, seed weight/plant, and individual seed weight (weight of 100 seeds, or 100 swt). There were no significant O_3 x cultivar or SO_2 x cultivar effects. Linear and quadratic models indicated no significant multiplicative interaction between O_3 and SO_2 . The overall yield (kg/ha) effect of O_3 averaged across all SO_2 treatments was similar for both soybean cultivars (Table 1). The cultivar effect noted in the split-plot analysis of variance was due to greater yield for Amsoy-71 at all O_3 treatment levels. As O_3 increased, the individual seed weight (100 swt) was reduced more for Corsoy-79 than for Amsoy-71, but seed number was reduced more for Amsoy-71 than for Corsoy-79. The overall yield (kg/ha) effect of SO_2 averaged across all O_3 treatment levels was also similar for both cultivars (Table 2). Most of the yield reduction appeared to be due to reductions in seed number rather than in seed weight.

The data are plotted according to a Weibull regression model for O_3 (Fig. 1) and SO_2 (Fig. 2) effect on overall yield (kg/ha). The Weibull model has been adopted for use in the National Crop Loss Assessment Network (NCLAN) because of its versatility. The Weibull regression equation is given as:

$$\text{yield} = \alpha \cdot \exp [-(O_3/\text{sig})^c], \quad (1)$$

where α is the yield at 0.00 ppm O_3 (or SO_2) concentration; O_3 is the O_3 dose [seasonal 7-h/day mean O_3 (or for SO_2 the SO_2 dose--4-h/day, 3 day/week mean) concentration in ppm]; sig is the O_3 (or SO_2) concentration at which

Table 1. Effects of seasonal mean O₃ treatments on the yield of two soybean cultivars exposed for 7-h/day in open-top chambers for 79 days. Yields were averaged over four SO₂ treatments (0.02, 0.09, 0.30, 0.56 ppm, 4-h/day, 3 days/week).

O ₃ Treatment (ppm)	AMSOY-71 [†]				CORSOY-79 [†]			
	kg/ha	Number Seeds	100 swt	sw/p	kg/ha	Number Seeds	100 swt	sw/p
0.023	1786	844	16.1	5.17	1487	712	15.9	4.14
0.046	1768	909	14.9	5.06	1431	761	14.8	4.09
0.066	1422	723	14.1	3.91	1281	726	12.9	3.29
0.092	1216	684	13.6	3.44	988	634	11.7	2.66
Percent Reduction from 0.023 ppm Treatment								
0.046	1	(+7)	7	2	1	(+7)	7	1
0.066	20	14	12	24	18	(+2)	19	21
0.092	32	19	16	33	34	11	26	36

[†]kg/ha = kilograms per hectare.

100 swt = weight of 100 seeds.

sw/p = seed weight per plant.

Table 2. Effects of seasonal mean SO₂ treatments on the yield of two soybean cultivars exposed for 4-h/day, 3 days/week in open-top chambers for 79 days. Yields are averaged over four O₃ treatments (0.023, 0.046, 0.066, 0.092 ppm, 7-h/day, 7 days/week).

SO ₂ Treatment (ppm)	AMSOY-71 [†]				CORSOY-79 [†]			
	kg/ha	Number Seeds	100 swt	sw/p	kg/ha	Number Seeds	100 swt	sw/p
0.02	1810	860	16.2	5.21	1549	803	14.9	4.22
0.09	1566	872	13.8	4.34	1379	790	13.2	3.67
0.30	1586	841	14.1	4.39	1229	692	13.2	3.42
0.56	1228	637	14.7	3.63	1016	548	13.9	2.88
Percent Reduction from 0.02 ppm Treatment								
0.09	13	(+1)	15	17	11	2	11	13
0.30	12	2	13	16	21	14	11	19
0.56	32	26	9	30	34	32	7	32

[†]kg/ha = kilograms per hectare.

100 swt = weight of 100 seeds.

sw/p = seed weight per plant.

Figure 1. Weibull regression models for the yield of two soybean cultivars as a function of the 7-h/day seasonal mean O_3 concentration (79 days). The yield is averaged over four SO_2 treatments (0.02, 0.09, 0.30, 0.56 ppm, 4-h/day, 3 days/week).

Figure 2. Weibull regression models for the yield of two soybean cultivars as a function of the 4-h/day, 3 days/week, 11 week seasonal mean SO_2 concentration. The yield is averaged over four O_3 treatments (0.023, 0.046, 0.066, 0.092 ppm, 7-h/day, 79 day seasonal mean).

yield is 0.37 alpha; and c is a dimensionless shape parameter. A combined bivariate ($O_3 + SO_2$) model was also developed by multiplying the O_3 term by the SO_2 term. Based on the bivariate model, there was no indication of cultivar heterogeneity in yield response to the pollutants. At a given SO_2 concentration, yield reductions averaged across both cultivars would be predicted to be 12% at 0.06 ppm O_3 and 42% at 0.10 ppm O_3 compared with a control of 0.025 ppm O_3 .

The actual and predicted O_3 -induced yield reductions in this study are nearly identical to those noted for the Corsoy-79 cultivar in 1980.¹ These results are also similar to those of Heagle et al.⁷ for O_3 and the interaction of O_3 and SO_2 . Our SO_2 exposure regime of somewhat higher concentrations in intermittent fumigations appeared to have had less effect (about 45% less) on yield than their daily exposure regime.⁷ Our results indicating that O_3 affected seed weight (100 swt) as much as or greater than seed number, while SO_2 affected primarily seed number, are also similar to the results of Heagle et al.⁷

Prospects

The results presented here confirm the O_3 sensitivity of soybean. They also suggest that even low SO_2 concentrations (0.10 ppm) near a point source could cause substantial (7% to 10%) yield reduction. These data will be included with those from five other field sites for evaluation of the effects of O_3 on important agricultural crops. In areas with the potential for the occurrence of both O_3 and SO_2 , these data may be particularly useful.

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RELEASE OF CARBON DIOXIDE FROM TWO SOILS EXPOSED TO VARIOUS LEVELS OF ACIDITY IN RAIN

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Background and Scope

Although there is much speculation regarding the possibility for harmful effects of acid rain on the productivity of poorly buffered soils, there is a paucity of research data on the topic. Changes in soil fertility, soil-plant relationships and soil-water relationships as a result of acid rain have been hypothesized. The biological processes occurring in the soil are significant factors in the functioning of the entire ecosystem, and thus direct or indirect effects of acid rain on the soil biota would influence the total system dynamics. The limited data available in this area must be interpreted cautiously because comprehensive experiments with frequent sampling at realistic rain pH levels have not been conducted.

In order to obtain dose-response information on seasonal effects of acid rain on soil biota, measurements of soil respiration rates were included in our field studies¹ of plant/soil systems exposed to various levels of simulated rainfall acidity.

Progress in 1982

Net heterotrophic activity (bacterial, fungal, faunal, and root) is commonly described as soil respiration and determined by measuring CO₂ evolution. In our studies the rates of CO₂ release per unit soil surface area were determined for plots that had been treated with varying levels of acidity in simulated rain. Our procedure involved titrating a strong alkali that had

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been placed over the soil (vegetation removed) and sealed with a chamber pushed into the top 2 cm of soil for a 24-h period. Soil respiration rates in the two soils (Alvin and LaHogue) used in the study were measured a total of 12 times in the summer and fall. There were three replicates per treatment for each of the measurement dates. Measurements were made either shortly after a simulated rain event while the soil was still moist or after at least three "dry" days when the surface soil was dry.

Results from the summer measurements indicated that the plots treated with pH 4.2 rain simulant had higher respiration rates than did plots treated with more (pH 3.5 and 3.2) or less (pH 4.5 and 5.6) acidic rain when the measurement was made following a rain event. Both soils responded in this manner (Figs. 1 and 2). The differences were statistically significant ($p < 0.05$) for the 21 July measurement. A possible explanation for the observed response is that the soil microorganisms were adapted to the ambient level of rainfall acidity (pH $\approx$ 4.2), and an increase or decrease from that level resulted in reduced microbial activity. Further study is needed to confirm this hypothesis.

Respiration measurements made three months later during the fall were very similar among all acidity treatments in the Alvin soil, although respiration for the control treatment (pH 5.6, no strong acids) was generally lower than for the acid rain treatments (Fig. 3). Statistically significant differences among treatments occurred in the LaHogue soil only on the first measurement, where the pH 4.2 and pH 3.2 treatments resulted in higher respiration rates than did the control (pH 5.6) (Fig. 4). As with the Alvin soil, the LaHogue control plots usually had lower rates of CO_2 release than did the acid-treated plots. When the data from all the measurements made in the fall are averaged, the differences between the control and acid-treated plots are obvious (Fig. 5).

Our data on the net heterotrophic activity of the soil indicate that microorganisms may be adapted to ambient levels of rain acidity. However, the response (in terms of changes in respiration rate) to the treatments appears to be different in the fall compared to the summer. This may be due to the cumulative effect of the treatments or to the fact that the principal soil organisms vary seasonally.

Figure 1. Rate of CO_2 release (respiration) in Alvin soil as a function of simulated rain acidity during July and August; $n = 3$.

Figure 2. Rate of CO_2 release (respiration) in LaHogue soil as a function of simulated rain acidity during July and August; $n = 3$.

Figure 3. Rate of CO₂ release (respiration) in Alvin soil as a function of simulated rain acidity during October and November; n = 3.

Figure 4. Rate of CO₂ release (respiration) in LaHogue soil as a function of simulated rain acidity during October and November; n = 3.

Figure 5. Means for October and November respiration rates in soils exposed to various levels of acidity in rain, n = 18.

Prospects

These studies will be continued in 1984 so that seasonal differences and cumulative impacts may be measured.

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IMPACT OF RAINFALL ACIDITY ON NITRIFICATION RATES AND pH OF TWO SOILS

P. M. Irving, J. C. Inman,* and T. H. Gibbons†

Background and Scope

In unmanaged ecosystems, a very fine balance usually exists between vegetative and soil nutrient pools. For this reason, even small disturbances to such systems may result in important changes in nutrient transformations. Since most nitrogen (N) is in the organic state in soils, the availability of inorganic N to plants is controlled by the rates of mineralization of organic N and nitrification of mineralized N by the soil microbial community. Acid precipitation is an agent that could potentially affect N cycling through direct input of nitrogen or through impacts on the microbial and vegetative communities from acidity or from sulfur and nitrogen inputs.

Results from a study¹ of three forest soils indicated that simulated acid rain (pH 3.5 - 4.1) partially inhibited nitrate production when the soil was amended with ammonium, but had no effect or was stimulatory in unamended soil. Dry deposition of SO₂ and NO₂ gases inhibited nitrification in certain soils, although the mechanism for the inhibition was not determined.²

The research reported here is part of an on-going, large-scale field microcosm study³ to examine the effects of acid precipitation on two plant/soil systems. The soils are classified as a loam (LaHogue) and a sandy loam (Alvin); the vegetative cover is a grass-legume mixture.

Progress in 1983

Surface soil samples (2-cm depth) were removed in late July from each of the microcosms which had been treated with simulated rain having average pH values of 5.6, 4.2, or 3.2. There were three replicate plots per treatment.

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After being air-dried, the soils were amended with one of the following: (1) deionized water to bring soil moisture to 70% of field capacity; (2) deionized water (as in #1) and 100 μg $\text{NH}_4\text{Cl-N}$ per gram of soil to provide an ammonium source; (3) deionized water and an ammonium source (as in #2) with 10 μg nitrotyrin per gram of soil to inhibit autotrophic nitrification; and (4) deionized water and 10 μg dried, ground red clover per gram of soil to provide an organic carbon and nitrogen source. The amended soil samples were incubated at 24°C for 30 days. They were sampled periodically during incubation for determination of inorganic-N using steam distillation and titration techniques. Soil pH was measured from samples removed from field plots treated with simulated rain having pH values of 5.6, 4.5, 4.2, 3.5, and 3.2. One set of control (pH 5.6) was also sheltered from ambient snow. Soils were sampled in mid-summer and early fall, and pH was determined using a 1:1 soil-to-water ratio.

In July 1983, after simulation of 28 cm of rain beginning in September 1982, the acidity of the LaHogue surface soil was significantly higher ($p < 0.05$) for the pH 3.2 treatments than for the 4.2 or 5.6 treatments. The Alvin soil also had a higher acidity in the 3.2 pH rain treatment, although the data were not statistically significant. When sampled again in November, after 55 cm of simulated rain, acidity of both soils was significantly higher ($p < 0.05$) in plots treated with simulated pH 3.2 rain (Fig. 1).

In the nitrotyrin-amended soils, nitrification ($\text{NO}_3\text{-N}$ production) was inhibited and mineralization ($\text{NH}_4\text{-N}$ production) occurred at approximately the same rate for both soils and for all treatments. Thus, any decreases in accumulation of ammonium observed in the unamended, ammonia-amended, or clover-amended soils may be assumed to have resulted from nitrification or immobilization.

A decrease in the rate of nitrate production occurred after 10 days of incubation for the Alvin soil treated with the most acidic (pH 3.2) rain simulant and amended with ammonium (Fig. 2a). This was probably not due to greater immobilization (microbial assimilation) in this soil because the rate of NH_4 depletion was not as rapid either (Fig. 2b). In unamended and clover-amended soils, however, nitrification was similar for plots treated with acidic rain and non-acidic rain, although overall rates were about one-half those of the NH_4 -amended soil. Since nitrification is itself an acidifying

Figure 1. Surface (2 cm) soil acidity in Alvin and LaHogue soils exposed to simulated rain - October 1983. Bars with a common letter are not significantly different according to Duncan's Multiple Range Test, $p \leq 0.05$.

Figure 2. Nitrification in ammonium-amended Alvin soil exposed to simulated rain: (A) nitrate production, and (B) ammonium depletion.

process, the higher initial rate of nitrification that occurred in the ammonia-amended soil could have resulted in further acidification of the already-acidified soil treated with the pH 3.2 rain, to the point that microbial activity was inhibited in the later stages of the incubation period.

In the LaHogue soil, initial measurements indicated that $\text{NO}_3\text{-N}$ was about 50% lower in the plots treated with the pH 4.2 rain simulant (Fig. 3a, day 0). Nitrification was rapid in the pH 4.2 plots, and was balanced by $\text{NH}_4\text{-N}$ consumption (Fig. 3b); however, there were no changes in NO_3 or NH_4 levels during incubation of soils from the other plots. The trends were similar for both the NH_4 -amended, clover-amended, and unamended soils. Soil respiration measurements suggest that the low initial levels of $\text{NO}_3\text{-N}$ in the pH 4.2-treated LaHogue soil were the result of immobilization due to greater microbial activity in these plots.

Figure 3. Nitrification in ammonium-amended LaHogue soil exposed to simulated rain: (A) nitrate production, and (B) ammonium depletion.

In the Alvin plots amended with a carbon source (clover), ammonia accumulated for about the first 15 days during the incubation period. After this period, sufficient quantities of ammonium were presumably produced through decomposition of the clover to provide adequate substrate for

nitrification, resulting in rapid accumulation of $\text{NO}_3\text{-N}$ in these plots (Fig. 4). In the LaHogue soil, mineralization ($\text{NH}_4\text{-N}$ production) was rapid in all plots of the clover-amended soils. Nitrate nitrogen was initially lost from these plots, possibly as a result of immobilization by decomposers. After about 12 days of incubation, $\text{NO}_3\text{-N}$ began accumulating in all LaHogue plots, with the greatest rate in the pH 4.2 plots (Fig. 5).

Evaluation of the data indicates that acid rain may have an effect on N cycling in the Alvin soil when more than $60\text{ }\mu\text{g NH}_4\text{-N}$ are oxidized, possibly by influencing microbial activity through acidification of surface soil. The greater rate of vegetative growth in the Alvin soil compared to the LaHogue soil⁵ is a likely explanation for the lower initial (day 0) soil $\text{NO}_3\text{-N}$ levels in the Alvin soil plots. Data from the unamended soils indicate that mineralization and nitrification in all the Alvin soil plots is substrate limited.

The rain pH 4.2-treated LaHogue plots also had lower soil $\text{NO}_3\text{-N}$ on day 0 and exhibited mineralization and nitrification responses similar to the Alvin soil, although the rates were slightly lower. It is unclear why N was mineralized but nitrification was inhibited in the rain pH 5.6- and 3.2-treated LaHogue plots, although initial soil $\text{NO}_3\text{-N}$ levels may have had an influence by affecting the carbon-to-nitrogen (C:N) ratio. Some nitrification was observed when these plots were amended with a carbon source.

Prospects

The numerous and often subtle interconversions of soil N that occur make it extremely difficult to study the action of a potential stress agent, such as acid rain, on N transformations in plant/soil systems. The studies reported here will be expanded to include sulfur cycling and will be continued in order to advance our understanding of basic soil processes and the long-term effects of acid rain on these processes.

Figure 4. Nitrification in clover-amended Alvin soil exposed to simulated rain: (A) nitrate production, and (B) ammonium depletion.

Figure 5. Nitrification in clover-amended LaHogue soil exposed to simulated rain: (A) nitrate production, and (B) ammonium depletion.

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BIOMASS PRODUCTION OF A GRASS-LEGUME MIXTURE AS A FUNCTION OF THE ACIDITY OF SIMULATED RAIN

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Background and Scope

The effects of acidic rain on vegetation have been studied for a relatively short-time (<10 years) as compared to ozone (>20 years) and sulfur dioxide (>100 years). For this reason, the techniques, methodologies, and approaches used to study the impacts of acidic rain are only now becoming sufficiently sophisticated to allow cautious preliminary conclusions. Interpretations of currently available data suggest that acidic deposition probably does not cause the overwhelming stress to vegetation that has been hypothesized. One explanation is that nutrient inputs from acidic rain may sometimes counteract the stress from acidity, but supportive research evidence is weak. The potential for acidic precipitation effects on the long-term productivity of soils is also unknown.

Forage systems (i.e., grass-legume mixtures used for hay or livestock feed) are an important vegetative group that is potentially sensitive to acidic rain because it is frequently unmanaged and grown on marginal soils. For these reasons, a timothy-clover mixture was selected as the vegetative cover for the two soils under investigation in our large-scale field project to examine the effects of acidic deposition on plant/soil systems.¹ The grass-legume mixture allows us to compare interspecies competition between two different important plant families, and because timothy and clover are herbaceous perennials, cumulative effects from one season to the next may be observed. The following is a summary of plant productivity data after one year of rainfall simulation.

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Progress in 1983

Timothy (cv. FS954) and red clover (cv. Arlington) were broadcast seeded at rates of 8.4 and 21.9 kg per ha, respectively, on 6 May 1983. Two soils were used in the study--Alvin, a sandy loam, and LaHogue, a loam. There were five rain simulant treatments (pH 5.6, 4.5, 4.2, 3.5, 3.2) with three replicate plots per treatment. Plot size was 2.4 x 1.2 m. All plots received ambient snowfall during the 1982-83 winter except for one set of controls (pH 5.6). Characteristics of the rainfall simulation are described in Table 1. The experimental design and treatments are further described in a previous report.¹

Table 1. Characteristics of rainfall simulation.

Background ions ($\mu\text{eq/l}$)	$\text{Ca}^{+2} = 8.2$, $\text{Mg}^{+2} = 2.9$, $\text{K}^{+} = .79$, $\text{Na}^{+} = 7.3$, $\text{NH}_4^{+} = 14.3$, $\text{Cl}^{-} = 3.0$, $\text{NO}_3^{-} = 26.1$, $\text{SO}_4^{-2} = 39.9$
$\text{SO}_4:\text{NO}_3$ (by weight)	2.45
Frequency	1-4 wk^{-1}
Duration	1-195 min event ⁻¹
Intensity	0.06 - 1.0 cm h^{-1}
Total number of events	63
Total H_2O	52.3 cm

Vegetation on the Alvin soil was harvested twice during the growing season, on 7 July and 10 September. Plant productivity response to rain pH was similar for both harvests - total dry weight (timothy + clover) increased with increasing acidity (Fig. 1). The timothy-to-clover dry weight ratio decreased with acidity (Fig. 2) because the stimulation of yield resulting from the rain acidity was entirely due to increased growth of clover (timothy was unaffected by the acid treatments).

Regression analysis of the cumulative data for the effect of pH on clover dry weight indicated a significant ($p = 0.007$) linear relationship (Table 2). Because the block effect was significant, the mean square for pH x block interaction term was used as the error term for calculation of the F probability values.

Figure 1. Vegetative productivity in Alvin soil as a function of rain acidity - 1983 (n = 3 plots per treatment).

Figure 2. Timothy to clover dry weight ratio in Alvin soil as a function of rain acidity - 1983.

Table 2. Analysis of variance for the effect of rain acidity on clover yield in Alvin soil.

Source	DF	SS	F value	PR > F
Block	2	121135	13.0	0.001
pH	1	53418	11.5	0.005
pH x Block	2	1866	0.2	0.820
pH†	1	53417	57.3	0.007

† Mean square for pH x block used as error term since the block effect was significant.

Although seed germination appeared to be equal in both soils, crop growth rate for LaHogue was well below that for Alvin. Therefore, the LaHogue plots were harvested only in September 1983. Regrowth after harvest appears to be excellent for both soils.

Analysis of variance indicated no differences in timothy or clover growth in the LaHogue soil as a result of rain acidity; however, variation among replicates was great (Fig. 3). The timothy-clover ratio was also unaffected in this soil.

Photosynthetic rates of the red clover were determined in early August through measurements of CO₂ uptake. Rainfall acidity had no statistically significant effect on the rate of CO₂ uptake on the basis of leaf surface area for plants in either of the soils. Photosynthetic rates of clover were more than 30% lower in the LaHogue soil than in the Alvin soil, and leaf size in the LaHogue soil was approximately 13% lower than in the Alvin.

Prospects

Although statistically significant differences in vegetative productivity were not observed in the LaHogue soil, the variation in plot replicates should be reduced in future harvests since renewed growth appears more uniform.

The stimulation of clover growth in the Alvin soil by acid rain affected the plant population ratio; therefore, continued investigation of the long-term impact on plant competition is warranted. Sulfur requirements are usually higher for plants that are nitrogen fixers (such as red clover)

Figure 3. Vegetative productivity in LaHogue soil as a function of rain acidity - 1983 (n = 3 plots per treatment).

because sulfur and nitrogen nutrition is interrelated. Our investigations have also indicated that nitrogen cycling may be affected by rain acidity.² These effects, which may be quite subtle initially, require continued investigation so that the long-term productivity of the systems may be assessed. The studies are thus being maintained without interruption so that cumulative effects may be measured.

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RESPONSE OF SOYBEANS TO TEMPORAL VARIATIONS IN RAINFALL ACIDITY

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Background and Scope

Research on the effects of acidic rain is relatively "young", and thus research methodology is in developmental stages. Accordingly, we have initiated a series of experiments to identify those properties of rain that may be important in influencing biological response so that rain may be simulated properly in controlled studies.

Nearly all experimental studies of the effects of acidic rain on crops have been performed using rain simulants with constant chemistry from event to event. Such an approach may result in misleading information because of the absence in the experiments of the type of fluctuations and acidity peaks that occur naturally (Fig. 1). Variation in rainfall acidity may have important biological consequences. For example, a particular level of hydrogen ion deposition received by a plant in a few high-acidity rain events may be more damaging than the same deposition received in more events with lower acidity levels. Conversely, a particularly stressful (high acidity) event may result in morphological changes (e.g., thickening of cuticle) that will prevent further injury during a more sensitive stage.

As an example of such consequences, results from a previous greenhouse study¹ indicated that the marketable yield of radish was reduced by simulated rain having a mean pH of 3.8 when the pH of the individual events varied from 3.0 to 5.6, while the yield was unaffected by an identical amount of rainfall simulant having a constant pH of 3.8.

Information obtained in studies such as that reported here, will aid in interpreting the results of past work and in designing future experiments.

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Figure 1. Acidity of rainfall events at Argonne National Laboratory, June-October 1980.

Progress in 1983

Field-grown soybeans (Glycine max cv. Amsoy 71) were exposed to simulated rain in treatment groups with: (1) temporal variations in acidity among events, and (2) no variation in acidity from event to event. The plants within each treatment group were exposed to one of four mean rain acidity levels with average pH values of 5.6 (no strong acids), 4.2, 3.7, and 3.4. Each treatment was replicated six times, resulting in a total of 48 experimental plots, each with 12 harvested plants. Other experimental characteristics are described in Table 1.

The soybean life cycle was divided into three stages - vegetative, flowering, and reproductive - and the variable pH treatment regime was replicated within each of the three stages. There was one peak acidity event within each stage.

The soybean plants were examined after each peak acidity event for symptoms of visible injury. The simulated rain treatments with temporal variations in acidity resulted in a greater number of leaf lesions at the 3.4 and 3.7 pH levels than did simulated rain with the same total H^+ deposition but without variations in pH from event to event (Table 2).

Table 1. Experimental characteristics.

<u>Simulated Rain</u>	
Frequency	2 events/wk
Duration	1 h/event
Intensity	1 cm/h
Total events	22
Total rain amount	21.3 cm
SO ₄ :NO ₃ (by weight)	2.45
<u>Soil</u>	
Name	LaHogue
Texture	loam
CEC	14.4

Table 2. Percentage of plants exhibiting visible injury following acidity peaks within constant and variable treatment groups.

Stage	<u>Constant</u>		<u>Variable</u>	
	pH	%	pH	%
Vegetative	3.4	14	2.92	38
	3.7	7	3.24	10
	4.2	0	3.74	0
	5.6	0	5.62	0
Flowering	3.4	18	2.97	43
	3.7	4	3.28	21
	4.2	0	3.76	0
	5.6	0	5.70	0

Photosynthetic rate, as determined through measurements of CO₂ uptake, was higher in the variable treatment plots compared to constant and higher in the acidic treatments compared to control (Fig. 2). Because of the high degree of variability among plants within each treatment, the differences in photosynthetic rate between treatments were not statistically significant.

Multiple regression analyses indicated differences between treatment groups (constant vs variable) for seed weight per plant ($p = 0.058$) (Fig. 3) and number of pods per plant ($p = 0.017$) (Fig. 4). There was a significant interaction between treatment (constant vs variable) and pH relative to number

Figure 2. Rate of CO₂ uptake in soybeans treated with variable and constant rain acidity, n = 12.

Figure 3. Seed weight per soybean plant as a function of rainfall acidity: (A) Constant acidity treatments, and (B) Variable acidity treatments.

Figure 4. Number of pods per plant in soybeans treated with constant and variable rain acidity.

of pods per plant ($p = 0.046$). A linear regression (Eq. 1) was significant for pod number per plant as a function of pH for the variable acidity treatments:

$$y = \beta_0 + \beta_1 (\text{pH}) \quad (1)$$

where:

y = number of pods per plant
 pH = rain simulant mean pH
 β_0 = 19.2 ± 2.5
 β_1 = -1.18 ± 0.58

model:

$\text{PR} = 0.050$
 $F = 4.18$
 $\text{C.V.} = 16.2$
 $R^2 = 0.16$

The slope of the line was positive with respect to acidity. Linear, quadratic, and cubic regressions were not significant for any of the dependent variables tested for the constant acidity treatment.

Prospects

Although treatment (constant vs variable pH) differences were statistically significant for some of our yield parameters in this study, experimental protocol requires that field experiments be repeated at least once before conclusions are finalized. Accordingly, the experiment will be performed again in 1984.

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LIMITS OF PLUTONIUM SOLUBILITY IN NATURAL WATERS

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Background and Scope

Plutonium in aqueous solution can form some very insoluble hydrolysis products and other compounds, and, consequently, its solubility in natural waters has in the past been assumed to be quite low. However, no unambiguous measurement of its solubility limit in any natural water has ever been made. Last year we reported on a series of experiments, using water from both Lake Michigan and a small pond on the Argonne National Laboratory site, that showed no detectable change in plutonium speciation at concentrations below about 10^{-8} moles/liter (the highest concentration used).¹ Those experiments were done in the presence of a natural sediment and used measurements of the oxidation state distribution of the Pu and its adsorption to the sediment as indicators of speciation changes. As long as no change in speciation occurred, we felt confident in concluding that the solubility limit had not been exceeded. Other experiments done in particle-free water indicated a plutonium-bearing precipitate did form at total Pu concentrations of 10^{-6} moles/liter. The solubility limit for Pu in these waters was, therefore, at least 10^{-8} moles/liter, but probably not more than 10^{-6} moles/liter.

Progress in 1983

Based on last year's studies we concluded that experiments conducted in the presence of natural particles produced results that were more representative of the expected behavior of Pu in natural systems, as well as more reproducible, and hence were preferable to experiments conducted under particle-free conditions. We have now extended such experiments to higher Pu concentrations (up to 10^{-5} moles/liter of added Pu) using the same equilibration procedure as last year. The results of these new experiments are presented in Table 1, which includes last year's data for comparison. For each water, last year's two highest concentrations (10^{-8} and 10^{-7} moles/liter) were repeated. The last four entries for each water are the new data.

Table 1. Plutonium concentrations, oxidation state distributions, and K_D values at various concentrations of added plutonium.

Sample Source	Plutonium Concentration (moles/L)		Percent of Dissolved Pu Present as Pu(III,IV)	K_D Value for Pu(III,IV) (L/kg)
	Total Added	In Solution		
Lake Michigan Water	1×10^{-13}	0.19×10^{-13}	14	0.55×10^6
	1×10^{-12}	0.14×10^{-12}	22	0.50×10^6
	1×10^{-11}	0.16×10^{-11}	20	0.50×10^6
	1×10^{-10}	0.14×10^{-10}	27	0.40×10^6
	1×10^{-9}	0.16×10^{-9}	16	0.52×10^6
	1×10^{-8}	0.17×10^{-8}	15	0.61×10^6
	1×10^{-7}	0.16×10^{-7}	18	0.53×10^6
	1×10^{-8}	0.15×10^{-8}	21	0.44×10^6
	1×10^{-7}	0.14×10^{-7}	19	0.51×10^6
	1×10^{-6}	0.08×10^{-6}	28	0.65×10^6
	1×10^{-5}	0.04×10^{-5}	20	1.90×10^6
ANL Pond Water	1×10^{-13}	0.18×10^{-13}	75	0.12×10^6
	1×10^{-12}	0.21×10^{-12}	74	0.10×10^6
	1×10^{-11}	0.19×10^{-11}	79	0.11×10^6
	1×10^{-10}	0.26×10^{-10}	81	0.07×10^6
	1×10^{-9}	0.20×10^{-9}	83	0.09×10^6
	1×10^{-8}	0.21×10^{-8}	81	0.09×10^6
	1×10^{-7}	0.19×10^{-7}	83	0.10×10^6
	1×10^{-8}	0.24×10^{-8}	36	0.14×10^6
	1×10^{-7}	0.22×10^{-7}	31	0.18×10^6
	1×10^{-6}	0.23×10^{-6}	60	0.09×10^6
	1×10^{-5}	0.06×10^{-5}	36	0.70×10^6

In Lake Michigan water, the experiments at 10^{-8} and 10^{-7} moles/liter show no change from the previous data for fractional retention in solution, oxidation state distribution or K_D (where K_D = concentration per kilogram of sediment divided by concentration per liter of water). The next higher concentration, 10^{-6} mole/liter added, shows little change in oxidation state or K_D , but the fraction remaining in solution is slightly depressed. At 10^{-5} moles/liter the fraction remaining in solution is depressed still further and the K_D has definitely increased, although the oxidation state distribution has not changed. We feel this shows that a plutonium compound has precipitated

and the concentration remaining in solution (4×10^{-7} moles/liter) probably marks the upper limit to Pu solubility in this water. The fact that there was no change in oxidation state of the dissolved Pu shows that polymerization reactions (yielding dissolved species) were not important. If polymers had formed and had yielded dissolved species, the reduced form would have become increasingly abundant, which did not happen.

The results of the experiments in pond water are generally similar to those in Lake Michigan water. Both oxidation state distribution and K_D show the expected differences due to the increased concentrations of dissolved organics in pond water. But the concentration where precipitation begins is approximately the same in the two waters. Only the highest concentration tested (10^{-5} moles/liter) shows a decrease in the fraction of Pu that remains in solution and an increase in K_D . The concentration remaining in solution in that sample (6×10^{-7} moles/liter) is only slightly higher than the corresponding concentration in Lake Michigan water (4×10^{-7} moles/liter). We have no explanation for the difference in oxidation state distribution in the replicate samples except to note that they were different samples of water and hence may have had slightly different water chemistry.

In addition to their use in measuring the solubility limit of Pu, these experiments can be used to estimate the number of sorption sites on the sediment surface that are available to plutonium. Since the K_D value did not decrease as the Pu concentration was increased, it appears that only a single type of site was utilized up to the point where a precipitate formed. The number of these sites must at least equal the number of Pu atoms sorbed at the highest concentrations where Pu was still sorption limited. For the two experiments using 10^{-6} moles/liter total plutonium, this amounted to 15×10^{-6} and 13×10^{-6} moles/g. Hence, the sorption capacity of this sediment must exceed 3 mg of Pu per gram of sediment.

Prospects

We feel that many important aspects of Pu behavior can only be addressed through experiments conducted at elevated Pu concentrations and that the experiments discussed here are only a beginning. In order to measure true association constants for Pu with natural materials (e.g., sediments or organic colloids), the effective equivalent weights of these substances must be determined. The most straightforward method of determining these

equivalent weights is to titrate the system to an equivalence point by increasing the Pu (or other metal) concentration as was done in these experiments. Because a Pu precipitate formed at Pu concentrations lower than the equivalence point, indicating the adsorber had not become saturated, it appears that the direct approach is not feasible. We are, therefore, exploring the possibility of using other elements, initially rare earths, to saturate the binding sites. If it can be shown that Pu and the rare earths utilize the same binding sites, then such experiments could reveal the equivalent weights of adsorbers because of the much higher solubility limits of the rare earths. Early experiments are very encouraging and will be continued. Because adsorption and precipitation are both potentially important regulators of Pu behavior at moderate Pu concentrations, we feel it is necessary to have a good understanding of both processes to truly understand the limits of Pu solubility in natural waters.

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$^{239,240}\text{Pu}$, ^{137}Cs , AND ^{90}Sr IN TRIBUTARIES OF LAKE MICHIGAN

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Background and Scope

Long-lived radionuclides that were introduced to the environment in the 1950s and 1960s as a result of the atmospheric testing of nuclear weapons make excellent tracers of many natural processes. Because their environmental inputs were well documented and were reasonably uniform over broad areas, these radionuclides are particularly well suited to studies of the processes that redistribute substances after deposition. For example, measurements of the concentrations of these radionuclides in rivers can be used to estimate loss rates from watersheds or input rates to the receiving water bodies.

We have measured the concentrations of $^{239,240}\text{Pu}$, ^{90}Sr , and ^{137}Cs in Lake Michigan periodically over the past decade and have used the measurements to estimate the rates at which these elements are transferred from the lake water into sediments. Such calculations involve the overall material balance for the element of interest, including the rates at which it is transferred into the lake from the watersheds via rivers. These transfer rates are assumed to be small for $^{239,240}\text{Pu}$, ^{90}Sr , and ^{137}Cs , based on measurements made in some other rivers; however, no consistent set of measurements is available for any of the rivers flowing into Lake Michigan.

Progress in 1983

We measured the concentrations of $^{239,240}\text{Pu}$, ^{90}Sr , and ^{137}Cs in four major Lake Michigan tributaries--the Menominee, Manistee, Grand, and St. Joseph rivers. Each river was sampled four times over a one year-period, and all three elements were measured in both dissolved and particle-associated fractions. Samples were collected near the river mouths, stored in black polyethylene bottles, and filtered through 0.45- μm membrane filters (Millipore HAWP) within four days of collection. The filters were ashed at 500°C, weighed, and spiked with appropriate internal standards. The individual isotopes in the ash were isolated and counted using the methods of Nelson et al.¹ with several modifications noted below.

Fifty-liter aliquots of the filtrates were used to determine the concentrations and oxidation states of $^{239,240}\text{Pu}$ using the rare-earth fluoride precipitation method of Lovett and Nelson.² The rest of the filtrate (~100 L) was spiked with ^{85}Sr and stable Cs, acidified, and evaporated to a volume of ~100 mL. ^{90}Sr and ^{137}Cs were then isolated and counted using the methods of Nelson et al.¹ The presence of sulfate in the waters necessitated performing a carbonate precipitation prior to the usual strontium nitrate precipitate so as to avoid the formation of insoluble strontium sulfate. Low levels (0.1 to 1 pCi per sample) of strontium in the particulate matter necessitated additional precipitation to remove interference from natural decay chain beta emitters. Ferric hydroxide, barium chromate, and lead sulfide were used for this purpose.³ A strontium carbonate precipitation was used as an intermediate in the transition from acetic acid-ammonium acetate buffered chromate solution to the acidic sulfide solution. A second strontium carbonate precipitation was performed prior to the yttrium hydroxide precipitation. Dissolved organic carbon (DOC) concentrations in filtered water were measured with a Barnsted Photochem DOC analyzer, pH with a glass electrode, conductivity with a YSI model 33, and alkalinity by titration to pH 4.3 end point.

Results of the non-radiometric measurements are presented in Table 1. Concentrations of DOC were fairly uniform, with only two samples from the Menominee being out of the range of 4-9 ppm. A distinct difference in color was noted among the rivers. The Menominee was much browner than the others; the Manistee was nearly colorless. No significant variation in pH was observed--all measurements were in the range of 7-8. The Menominee had significantly lower alkalinity and conductivity than the other rivers, reflecting the fact that the watershed of the Menominee is primarily on the Canadian Shield. The two northern rivers, the Menominee and the Manistee, usually had lower suspended loads than the Grand and St. Joseph, indicative of the higher level of agricultural activity in the watersheds of the latter two rivers.

Plutonium concentrations in solution and on the suspended particles, as well as the corresponding distribution ratios (K_D = concentration per kg of solids divided by concentration per liter of water), are presented in Table 2. The highest Pu concentrations on particles were found in the

Table 1. Water quality characteristics of the four rivers.

River/ Date	Dissolved Organic Carbon (mg/L)	pH	Alkalinity [†] (mg/L)	Suspended [‡] Load (mg/L)	Conductivity (μmho/cm)
<u>Menominee</u>					
11-1-82	12.5	7.20	80	3.0	150
1-31-83	14.3	7.00	92	0.73	150
5-4-83	7.3	----	76	2.2	155
8-13-83	9.2	7.8	92	2.2	177
<u>Manistee</u>					
11-2-82	5.7	7.7	140	5.7	308
2-1-83	4.0	7.62	142	4.9	286
5-5-83	5.0	----	130	5.0	322
8-14-83	5.6	7.72	152	5.9	350
<u>Grand</u>					
11-3-82	6.5	7.45	206	30.9	490
2-1-83	5.7	7.8	214	4.2	312
5-5-83	8.2	----	148	51.6	390
8-14-83	8.2	7.65	162	17.6	500
<u>St. Joseph</u>					
11-3-82	5.7	7.6	208	31.1	330
2-1-83	5.7	7.8	214	4.0	312
5-5-83	7.1	----	162	43.4	393
8-14-83	9.0	8.0	180	14.0	410

[†]As CaCO₃.

[‡]As an ash weight basis.

Table 2. Concentrations of $^{239,240}\text{Pu}$ in dissolved and particulate forms in the four rivers.

River/ Date	Dissolved			Particulate		$K_D \times 10^{-5}$	
	Pu(III,IV) (fCi/L)	Pu(V,VI) (fCi/L)	Sum (fCi/L)	(fCi/L)	(fCi/g)	(L/Kg)	
<u>Menominee</u>							
11-1-82	0.76 ± 0.05	0.06 ± 0.02	0.82 ± 0.05	0.25 ± 0.02	97 ± 8	1.2 ± 0.1	
1-31-83	0.44 ± 0.03	0.017 ± 0.006	0.46 ± 0.03	0.09 ± 0.01	125 ± 18	2.7 ± 0.4	
5-4-83	0.27 ± 0.02	0.07 ± 0.01	0.34 ± 0.02	0.19 ± 0.01	90 ± 6	2.6 ± 0.2	
8-13-83	0.09 ± 0.01	0.041 ± 0.007	0.13 ± 0.01	0.14 ± 0.02	60 ± 10	4.6 ± 0.8	
<u>Manistee</u>							
11-2-82	0.042 ± 0.006	0.035 ± 0.006	0.077 ± 0.008	0.053 ± 0.008	10 ± 1	1.3 ± 0.1	
2-1-83	0.035 ± 0.008	0.038 ± 0.009	0.07 ± 0.01	0.059 ± 0.009	12 ± 2	1.7 ± 0.3	
5-5-83	0.041 ± 0.006	0.016 ± 0.009	0.06 ± 0.01	0.079 ± 0.009	16 ± 2	2.7 ± 0.3	
8-14-83	0.054 ± 0.007	0.0134 ± 0.004	0.067 ± 0.008	--Sample lost in analysis--			
<u>Grand</u>							
11-3-82	0.04 ± 0.01	0.46 ± 0.03	0.50 ± 0.03	0.26 ± 0.02	9.0 ± 0.8	0.18 ± 0.02	
2-1-83	0.040 ± 0.009	0.18 ± 0.02	0.22 ± 0.02	0.055 ± 0.008	13 ± 2	0.59 ± 0.09	
5-5-83	0.041 ± 0.007	0.018 ± 0.004	0.059 ± 0.008	0.50 ± 0.04	9.6 ± 0.8	1.6 ± 0.1	
8-14-83	0.017 ± 0.003	0.022 ± 0.004	0.039 ± 0.005	0.13 ± 0.01	7.3 ± 0.7	1.9 ± 0.2	
<u>St. Joseph</u>							
11-3-82	0.06 ± 0.01	0.065 ± 0.008	0.125 ± 0.013	0.32 ± 0.02	11.0 ± 0.8	0.88 ± 0.06	
2-1-83	0.020 ± 0.006	0.08 ± 0.01	0.10 ± 0.01	0.054 ± 0.008	13 ± 2	1.3 ± 0.2	
5-5-83	0.063 ± 0.008	0.016 ± 0.004	0.079 ± 0.009	0.24 ± 0.04	5.5 ± 1	0.7 ± 0.1	
8-14-83	0.017 ± 0.004	0.021 ± 0.004	0.038 ± 0.006	0.11 ± 0.01	5.4 ± .07	1.4 ± 0.2	

Menominee River, with an average of 93 fCi/g, compared with an average of 10.2 fCi/g for the other three rivers. Agricultural soils in the Midwest should contain about 9 fCi/g, assuming that an average local Pu deposition of 200 fCi/cm² is mixed uniformly to a plow depth of 17 cm in soil having a bulk density of 1.33 g/cm³. Concentrations found in the three rivers not on the Canadian Shield are therefore consistent with a nearly direct transfer of agricultural soil into the river, and are comparable to concentrations found in other eastern North American rivers--9.5 fCi/g in the Mississippi River and its major tributaries,⁴ 14 fCi/g in the Miami River (Ohio),⁵ and 20 fCi/g in the Hudson River.⁶ The higher concentrations found in the Menominee can not result from a difference in deposition and so must reflect, on average, a dilution of the input into less soil per unit area. This is consistent with the generally thinner soils on the Canadian Shield and with the less intensive agricultural land use there. Because the plutonium concentrations (per gram) are relatively constant for each river, the concentrations of particle-bound plutonium (per liter) are highly dependent on the suspended loads in the rivers.

Dissolved Pu concentrations, in general, reflect the concentrations on the solids, with the Menominee again being the highest. Most of the dissolved Pu in the Menominee is in the reduced form, Pu(III,IV). Based on our experience with other waters, this indicates significant complexation with DOC. Total Pu concentrations are substantially lower in the Manistee and St. Joseph, and are more equally distributed between the two oxidation state groups. The Grand would be very similar to these two rivers were it not for the anomalous November 1982 and February 1983 samplings. Our experience indicates that the comparatively high proportion of Pu present in the oxidized form in these samples is unusual for a water having about 6 ppm DOC. This suggests that these unreplicated samples may have been contaminated during collection or analysis. The concentrations of total dissolved Pu in these rivers are similar to those previously measured in other rivers--0.22 fCi/L in the Mississippi and its tributaries,⁴ 0.15 fCi/L in the Miami (Ohio),⁵ and 0.3 in the Hudson.⁶ The K_D values for this set of river samples are relatively constant and average about 10⁵ L/kg, a value typical of many other natural systems.

The effectiveness of the natural DOC compounds in these rivers as complexing agents for Pu(III,IV) was investigated using a previously described technique.⁷ In this method, the colloidal-sized DOC (designated as COC) is concentrated about tenfold using a stirred ultrafiltration cell and a nominal 1000 molecular weight cutoff membrane. The organic-rich concentrate and the corresponding ultrafiltrate are later recombined in varying proportions to yield waters having a range of COC concentrations. Each of these waters is then spiked with ²³⁷Pu tracer, an aliquot of Lake Michigan sediment is added (61 mg/L), and the water is stirred for one week. The water is then filtered, the Pu in the filtrate is separated into reduced and oxidized fractions, and the characteristic ²³⁷Pu radioactivity is measured in all fractions.

The results of these experiments for all four rivers are presented graphically in Figure 1, which shows the dependence of K_D on COC concentration. At low COC concentrations, the K_D values are uniformly high and do not vary with COC, indicating insignificant association of Pu and COC. Above a critical COC concentration (higher than a few ppm), the K_D values decrease dramatically for each river, showing that the COC has become an effective competitor for Pu(III,IV). These curves are very similar to others we have generated for a variety of lakes, which suggests that the association between Pu and COC is similar in each case. Since the values of K_D begin to decrease at concentrations of COC slightly lower than the ambient values in all waters, we conclude that COC complexes are important factors regulating Pu(III,IV) adsorption in all these rivers. Fluctuations in COC concentration would therefore be expected to cause corresponding fluctuations in the concentrations of dissolved, reduced Pu.

Measurements of ¹³⁷Cs and ⁹⁰Sr are summarized in Table 3. The concentrations of ¹³⁷Cs in both dissolved and particulate fractions were near the lower limits of detectability. As with ^{239,240}Pu, the ¹³⁷Cs and ⁹⁰Sr concentrations in the Menominee were slightly higher than those in the other three rivers. No seasonal trends in ¹³⁷Cs concentration were discernible in any river. Both dissolved and particulate phases contributed significantly to transport during periods of low suspended load, with the particulate phase dominating during periods of high suspended load.

Comparison of the ¹³⁷Cs and ^{239,240}Pu concentrations in both phases suggests that the rates at which the two radionuclides are being transferred

Figure 1. Variation in K_D values of reduced plutonium as a function of colloidal organic carbon concentration (COC) in laboratory experiments for the four rivers. Arrows indicate the ambient concentration of COC.

Table 3. Concentrations of ^{137}Cs and ^{90}Sr in dissolved and particulate forms in the four rivers.

River/ Date	¹³⁷ Cs				⁹⁰ Sr			
	Dissolved (fCi/L)	Particulate		K _d × 10 ⁻⁵ (L/kg)	Dissolved (fCi/L)	Particulate		K _d × 10 ⁻³ (Lg/kg)
		(fCi/L)	(fCi/g)			(fCi/L)	(fCi/g)	
<u>Menominee</u>								
11-1-82	14 ± 3	6 ± 4	2400 ± 1700	1.7 ± 0.4	401 ± 4	2.7 ± 0.6	1280 ± 270	3.2 ± 0.7
1-31-83	13 ± 2	4 ± 1	6000 ± 1600	5 ± 1	214 ± 5	2.4 ± 0.9	3400 ± 1200	16 ± 6
5-4-83	-----	-0.5 ± 3	-220 ± 1500	-----	359 ± 7	6 ± 0.6	3000 ± 300	8.4 ± 0.9
8-13-83	12 ± 19	3 ± 8	1300 ± 3500	1 ± 2	485 ± 15	3.6 ± 0.7	1600 ± 300	3.3 ± 0.6
<u>Manistee</u>								
11-2-82	7 ± 7	3 ± 3	560 ± 640	0.8 ± 0.8	69 ± 2	1.5 ± 0.6	380 ± 160	6 ± 2
2-1-83	2 ± 1	4 ± 2	850 ± 320	4 ± 2	72 ± 5	1.4 ± 0.9	300 ± 200	4 ± 3
5-5-83	3 ± 8	2 ± 3	340 ± 670	1 ± 3	156 ± 6	8 ± 2	1500 ± 400	10 ± 3
8-14-83	5 ± 3	-----	-----	-----	119 ± 14	-----	-----	-----
<u>Grand</u>								
11-3-82	9 ± 4	8 ± 6	290 ± 190	0.3 ± 0.1	220 ± 10	5.77 ± 0.08	263 ± 4	1.20 ± 0.06
2-1-83	7 ± 5	5 ± 3	1100 ± 650	2 ± 1	144 ± 5	2.8 ± 0.8	650 ± 230	5 ± 2
5-5-83	5 ± 4	39 ± 1	760 ± 24	1.5 ± 1.0	360 ± 10	13 ± 2	160 ± 30	0.44 ± 0.08
8-14-83	7 ± 3	3 ± 4	160 ± 220	0.2 ± 0.1	280 ± 10	3.1 ± 0.6	180 ± 40	0.50 ± 0.08
<u>St. Joseph</u>								
11-3-82	9 ± 4	14 ± 2	500 ± 73	0.6 ± 0.2	117 ± 2	1.9 ± 0.6	85 ± 27	0.7 ± 0.2
2-1-83	3 ± 3	4 ± 1	950 ± 250	3 ± 3	140 ± 4	3.8 ± 0.9	930 ± 220	7 ± 2
5-5-83	3 ± 1	40 ± 2	930 ± 50	3 ± 1	170 ± 10	16 ± 2	380 ± 40	2.2 ± 0.3
8-14-83	4 ± 2	5 ± 2	850 ± 400	2 ± 1	170 ± 6	6.2 ± 0.7	310 ± 30	1.8 ± 0.2

from the watersheds into the lake are approximately equal. The decay-corrected ratio of ^{137}Cs to $^{239,240}\text{Pu}$ in deposited fallout is about 50:1, not unlike the ratios seen in these samples. Overall similarity in transfer rates is also consistent with the observation that the K_d values for both elements are similar ($\sim 10^5$).

Unlike ^{137}Cs and $^{239,240}\text{Pu}$, the ^{90}Sr in these rivers is transported primarily in solution. Dissolved concentrations were 10 to more than 100 times higher than the corresponding particulate-associated concentrations. Concentrations of ^{90}Sr on the particles (on a per gram basis) were only slightly lower than the corresponding concentrations of ^{137}Cs , consistent with the fallout ratio of about 0.7 ($^{90}\text{Sr}/^{137}\text{Cs}$). The comparatively high concentrations of dissolved ^{90}Sr are therefore not derived from leaching of particles after they enter the rivers, but rather must come from particles not being actively transported.

The concentrations found for all three isotopes were generally lower than the corresponding concentrations found in Lake Michigan. Current Lake Michigan concentrations in solution are 0.26 fCi/L for $^{239,240}\text{Pu}$, 38 fCi/L for ^{137}Cs , and 670 fCi/L for ^{90}Sr . Only for $^{239,240}\text{Pu}$ in the Menominee River does the dissolved river concentration exceed the lake concentration. A similar situation holds for concentrations on particles, where in no case does the average river concentration exceed the lake concentration. Current concentrations on suspended solids in Lake Michigan are 150 fCi/g for $^{239,240}\text{Pu}$, 20,000 fCi/g for ^{137}Cs , and 3,400 fCi/g for ^{90}Sr . River inputs to Lake Michigan are therefore reducing overall lake concentrations via dilution with less active materials.

Prospects

We feel this set of measurements adequately answers the pertinent questions of radionuclide transport from Lake Michigan watersheds into the lake and need not be repeated unless some unforeseen event occurs. They will now be incorporated into a general model describing trace substance cycling in the Great Lakes.

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REDUCTION OF PLUTONIUM(V) BY FRESH SEDIMENTS

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Background and Scope

The binding of plutonium to sediments and suspended particles is controlled by a number of factors, such as pH and humic acid concentration. Oxidation state is another such factor, since the reduced forms of plutonium [Pu(III) and (IV)] bind much more tightly to particles than does the predominant oxidized form, Pu(V). The relative concentrations of Pu(III,IV) and Pu(V) vary widely among natural waters, from 90% Pu(V) in Lake Michigan, to 50% in ocean waters, to 0-10% in bogs and eutrophic waters.

Pu(V) is probably the thermodynamically stable dissolved form in oxygenated, neutral waters. In the presence of particles, the binding of Pu(III,V) tends to draw the equilibrium in the direction of the reduced form. Since Pu(V) is stable indefinitely in the absence of particles, it is of interest to know what factors control (drive or catalyze) the interconversions of oxidation state. Similar interconversions have been demonstrated in situ and in cultures of bacteria for arsenic, iron, manganese, iodine, and other elements. We conducted the experiments summarized here to investigate the possible involvement of sediment-bound microorganisms in redox interconversions of plutonium.

Progress in 1983

Fresh surface sediment was collected from a small stream that originates on the Argonne National Laboratory property. The sediment was washed by shaking it in stream water and was coarsely hydrograded to remove large particles. Sediment particles that remained suspended after 40 seconds were centrifuged. A sample of sediment was resuspended to a known concentration in deionized water.

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Experiments were normally conducted in stream water from which all material larger than 1000 MW had been removed by ultrafiltration. A typical incubation consisted of 100 mL ultrafiltered river water, 20 mg sediment, and a tracer amount of $^{237}\text{Pu(V)}$. After 15 minutes of stirred incubation, the sediment was filtered off for counting. Dissolved Pu(III,IV) and Pu(V) were determined in the filtrate by LaF_3 fractionation. Normally, dissolved Pu(IV) was a negligible fraction of the total plutonium, and the amount of the element reduced was calculated entirely from the amount adhering to the sediment. Reduction of Pu(V) was linear for at least 30 minutes [Figure 1, curve (c)], permitting reasonable kinetic measurements.

Figure 1. Binding of Pu(IV) and Pu(V) to fresh, dried, and ashed sediments. Curve (a): Pu(IV) and fresh sediment; (b) Pu(IV) and dried sediment; (c) Pu(V) and wet sediment; (d) Pu(IV) and ashed sediment; (e) and (f) Pu(V) and dried or ashed sediment.

Sediments were treated by drying at 105°C or by ashing at 500°C . Drying would be expected to at least temporarily disable microorganisms present, and ashing would be expected to remove all organic matter. Sediments treated either way no longer reduced Pu(V) [Figure 1, curves (e) and (f)].

When Pu(IV) was substituted for the Pu(V) tracer, very rapid binding occurred to both fresh and dried sediments [Figure 1, curves (a) and (b)]. The initial rate of Pu(IV) binding was so rapid that it was clear that the reduction of Pu(V), and not the binding, was rate-limiting. Binding to ashed sediments [curve (d)] was somewhat slower and apparently more limited in capacity.

If the Pu(V) reduction were catalyzed by microorganisms, the reaction would be expected to have a pH optimum not far from pH 7, and the reducing activity should be drastically inhibited by prior heat treatment of the sediments.

A pH curve was constructed, using carbonate, tris, and MES buffers. Reduction was drastically inhibited through the alkaline range, with a sharp upturn at about pH 8 (Figure 2). This would be an expected reaction from a typical microbial community. The rate of reduction, however, remained high down to pH 5.3, which is not typical.

Figure 2. pH curve for Pu(V) reduction by fresh sediment. The dashed curve used MES buffer and the solid curve carbonate. The curves were adjusted to meet at pH 8.2.

Heat treatment for 3 minutes at a series of increasing temperatures did not reveal any temperature-sensitivity of the reaction up to and including 100°C. Thus, a microbial agent was not involved in the reduction process; the reducing agent was heat-resistant, non-living, and insoluble.

The reducing power of stirred sediment suspensions showed a slow decline over a period of hours. We suspected that this was due to reaction of the reducing agent with oxygen. Two suspensions were prepared and stirred, one in open air and one under a nitrogen atmosphere. At intervals, samples were collected and the rate of Pu(V) reduction was measured. The exclusion of oxygen prevented the decline of reducing power (Figure 3). If the air-stirred suspension was placed under nitrogen, however, the reducing power did not recover, even after 24 hours.

Figure 3. Loss of reducing power of fresh sediment on stirring in air. Samples were taken at intervals and reduction was measured for 15 min. In the control, air was replaced by nitrogen.

Even after air-oxidation of the sediment suspensions had gone to completion, some residual reducing power remained. The rate of this reaction was very slow, but remained constant for at least 24 hours. Once more, a control using sediment that had been heated to 100°C showed little difference (Figure 4).

Figure 4. Effect of heat treatment on the reducing power of fresh sediments. A suspension was heated to 100°C for 10 min. before reduction rate was measured.

In summary, (a) fresh sediments have a strong but labile ability to reduce Pu(V) to Pu(III,IV); (b) they also have a weaker, nonlabile reducing ability; (c) the reduction is not biologically mediated, although the reducing equivalents may originally have been generated by microbiological agencies in the sediments; (d) the pH curve is consistent with the possibility that a metal, with a redox potential that changes drastically near pH 8 (e.g., Fe or Mn), may be the direct agent of reduction.

Prospects

Understanding the control of oxidation state of trace elements (including actinides) is of crucial importance in determining the fates of those substances in aquatic as well as all natural systems. In only a few cases has the mechanism of reduction or oxidation been directly characterized. This work may have observed a new dimension to the problem: redox reactions that are not directly attributable to microorganisms, but are mediated by abiotic agents.

Work will be continued in an attempt to clarify the nature of the sediment-bound reducing agent for both rapid and slow phases of the reaction. The same phenomenon in suspended particles will also be investigated.

PLUTONIUM IN VOLCANIC MATERIALS

K. A. Orlandini

Background and Scope

Within the past ten years, two publications^{1,2} have reported the discovery of plutonium in volcanic effluents at concentrations up to four orders of magnitude higher than typically found in uranium minerals (e.g., 10^{-14} g/g). Naturally derived plutonium at such concentrations is unexpected. In order to substantiate this remarkable observation, we measured the plutonium content of material from the same sources and from similar strata.

Progress in 1983

Volcanic materials are readily available, but field collections made after the mid-1940s may have been contaminated with fallout plutonium. For our measurements, the possibility of contamination was avoided by using samples from Chicago's Field Museum of Natural History collection that have remained sealed since 1925 and thus were never exposed to atmospheric fallout containing artificially produced radionuclides.

Using a standard isotopic dilution technique, we measured the plutonium content of volcanic materials from Katmai (ash, 1912), Vesuvius (ash, 1906), Kilauea (lava, 1890), and Mt. St. Helens (ash, 1980) (Table 1). The Mt. St. Helens ash, collected at Portland, was included to compare a contemporary collection to the sealed and uncompromised museum samples. The plutonium values found in the secured samples approached the values typical for plutonium in uranium minerals; these were more than two orders of magnitude lower than plutonium values reported by Meier et al.¹ for similar volcanic strata that were field sampled about 1970 or later. Our measurements indicated that the plutonium content of Mt. St. Helens material was nearly 50 times lower than that reported by Sakuragi et al.² for similar material from the same location and same eruption.

Table 1. Concentration of plutonium in certain volcanic materials.

Sample	^{239}Pu (g/g)
Kilauea [†] (lava, pre-1890)	
Sample 677	$< 0.3 \times 10^{-16}$
Sample 678	$< 0.5 \times 10^{-16}$
Sample 2565	$< 0.6 \times 10^{-16}$
Sample 2571	$< 1 \times 10^{-16}$
Sample 2581	$< 3 \times 10^{-16}$
Mt. St. Helens (ash, 1980)	36×10^{-16}
Katmai (ash, 1912)	13×10^{-16}
Vesuvius (ash, 1906)	3×10^{-16}

[†]Kilauea samples are identified by museum numbers.

Prospects

The transuranic program focuses on the environmental behavior and fate of plutonium released to the environment from weapons tests and the nuclear industry. The finding of very old natural deposits of plutonium would provide an invaluable tool for the study of very-long-term behavior of manmade transuranium elements. However, the results reported here refute other claims that such deposits exist.

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ISOLATION AND CHARACTERIZATION OF ECOTYPIC VARIANTS OF MYCORRHIZAL FUNGI FROM DISTURBED AND NATIVE ECOSYSTEMS

A.-C. McGraw and R. M. Miller

Background and Scope

Vesicular-arbuscular mycorrhizal fungi increase the vegetative and reproductive productivity of plants, particularly under conditions in which nutrient availability becomes limiting to plant growth.¹ The mechanism for growth promotion generally is taken to be improved phosphorus (PO_4) uptake as a consequence of an expanded root absorption surface area created by an extended root-fungus hyphae network.¹ Positive growth effects associated with mycorrhizal fungi have been described repeatedly, especially under controlled environmental conditions and occasionally under natural conditions. Researchers investigating such effects have, however, relied heavily on a few "favorite" strains of the mycorrhizal fungi passed around among themselves. In addition, there are several reports of specificity to the fungus-induced plant growth response.² When such a reliance on so few strains is found, interpretations of the function and structure of natural communities of mycorrhizal fungi and their interrelationships with the associative plant communities can be erroneous, or at best, inadequate.

In spite of evidence that mycorrhizal fungi vary considerably in their effects on host plants, speciation of these fungi is based primarily on spore morphology. There is a complete absence of research into the range of either morphologic or genetic variability of mycorrhizal fungi. If a study of such variability is to be meaningful at the population level, then fungal variants need to be collected from a population of mycorrhizal fungi in the field. The objectives of this study were to isolate successfully morphological variants from field sites in Wyoming and Illinois and then characterize the genotypes of the mycorrhizal fungi.

Progress in 1983

Soil samples were collected from undisturbed and disturbed sites at the Jim Bridger Coal Mine in Wyoming (WYO), from an Illinois sand-prairie ecosystem (IL-P), and from two Illinois pastureland sites (JAAP and ANL).

Cultures of mycorrhizal fungi were initiated by collecting spores from soil sievings from each of the four sites, concentrating spores in a sucrose gradient, and using the spores to inoculate a grass hybrid host. Before the inoculation step, spores were separated into species or variants of species based on morphological characteristics observable with a dissecting microscope. Plants were grown under greenhouse conditions, and three months after establishment of plant-fungus combinations, roots from each variant x plant combination were evaluated for the presence of the fungi in the root cortex. The frequencies of successful isolations from each of four field sites are presented in Table 1.

Four isolates representing two species (Glomus monosporum [GMONO], G. mosseae [GMOSS]) of mycorrhizal fungi were selected for a controlled-environment study of their effects on the growth of three plant species native to the Wyoming study site (Experiment 1). Additionally, a regime was imposed to evaluate the response of the same plant-fungus system to increasing levels of PO_4 . In a second study (Experiment 2), spores from the same collections used in Experiment 1 were used to evaluate the success rate of single-spore inoculations and to generate genotypes for use in studying inheritance of select "marker" characteristics in mycorrhizal fungi (Table 2).

Initial above-ground growth measurements taken after seven weeks of growth of Agropyron smithii and Linum lewisii were subjected to statistical analysis to identify differences between fungal variants within PO_4 levels (Tables 3 and 4). The effects of fungal isolates on plant growth suggest an interaction between PO_4 level and isolates, where plant growth (especially in terms of the number of tillers and leaf surface area) was improved by GMONO-1 and GMOSS-1 relative to uninoculated plants at the low PO_4 level, but not at the higher PO_4 level. Differences were detected between fungal isolates for the effects on plant growth at all PO_4 levels studied. Because the fungal isolate- PO_4 level response may change with time, further evaluations are scheduled at two additional harvest dates.

Prospects

Variants of a population of mycorrhizal fungi were isolated successfully from several field sites, with selected genotypes subsequently used to evaluate variability of the growth response for several plant species native

Table 1. Frequencies of successful isolations of mycorrhizal fungi from several sites in Wyoming and Illinois.

Fungus Species	Site [†]				Total
	WYO	IL-P	JAAP	ANL	
<u>Acaulospora gerdemannii</u>	0/3	---	---	---	0/3
<u>Acaulospora spp.</u>	1/1	---	---	---	1/1
<u>Gigaspora gigantea</u>	---	0/1	---	---	0/1
<u>Glomus claroideum</u>	1/3	---	---	---	1/3
<u>G. constrictum</u>	2/4	---	---	---	2/4
<u>G. fasciculatum</u>	2/13	0/1	---	0/1	2/15
<u>G. macrocarpum</u>	1/9	0/1	2/2	1/2	4/14
<u>G. monosporum</u>	4/9	---	1/1	---	5/10
<u>G. mosseae</u>	2/2	---	---	---	2/2

[†]WYO: Representing both disturbed and undisturbed land in Jim Bridger Mine, Wyoming.

IL-P: Illinois sand-prairie ecosystem.

JAAP: Illinois pastureland.

ANL: Illinois (Argonne National Laboratory) pastureland.

Table 2. Treatment origin of mycorrhizal fungus isolates from Wyoming used in Experiments 1 and 2.

Isolate [†]	Experiment	Land Type [#]
GMONO-1	1,2	Undisturbed
GMONO-2	1,2	Disturbed
GMOSS-1	1,2	Undisturbed
GMOSS-2	1	Disturbed

[†]GMONO-1 and GMONO-2 (Glomus monosporum, isolates 1 and 2, respectively), or GMOSS-1, and GMOSS-2 (G. mosseae, isolates 1 and 2, respectively).

[#]Undisturbed (native land) or disturbed (topsoil treatment) soil treatment.

Table 3. Effects of phosphorus levels and isolates of mycorrhizal fungi on above-ground plant growth of Agropyron smithii (7 weeks growth)

PO ₄ † Level	Fungus Treatment‡	Shoot Height (mm)	Tillers (no.)	Blades (no.)	Blade Width (mm)	Blade Length (mm)	Leaf surface Area (mm ²)*	Rhizomes (no.)
Low	GMONO-1	193.3 b**	2.3 b	6.8 bc	3.1 cde	153.4 bcd	3003 c	0.1 c
	GMONO-2	171.3 c	2.8 b	8.6 ab	2.7 e	132.8 e	2861 c	0.1 c
	GMOSS-1	188.0 bc	3.2 ab	8.8 ab	3.1 cde	149.5 bcde	3684 bc	0.2 bc
	GMOSS-2	196.3 b	2.6 b	8.1 bc	3.1 cde	158.3 b	3577 bc	0.1 c
	Uninoculated	195.0 b	2.4 b	6.8 bc	3.3 bcd	153.8 b	3155 c	0.0 c
Medium	GMONO-1	202.7 ab	2.7 b	8.3 bc	3.4 abcd	157.8 b	3858 bc	0.9 a
	GMONO-2	206.0 ab	2.4 b	6.8 bc	3.5 abcd	154.8 bc	3372 c	0.3 bc
	GMOSS-1	188.3 bc	2.8 b	8.8 ab	3.3 bc	138.9 cde	3644 bc	0.3 bc
	GMOSS-2	195.5 b	2.2 b	6.6 bc	3.3 bc	152.8 b	3206 c	0.3 bc
	Uninoculated	203.9 ab	2.6 b	7.1 bc	3.5 abcd	161.2 ab	3829 bc	0.3 bc
High	GMONO-1	202.5 ab	2.4 b	7.7 bc	3.8 a	160.7 b	4076 bc	0.3 bc
	GMONO-2	206.3 ab	2.4 b	7.0 bc	3.8 a	160.1 ab	3843 c	0.4 ab
	GMOSS-1	200.7 ab	3.0 ab	7.9 bc	3.9 a	167.1 ab	4637 ab	0.5 ab
	GMOSS-2	220.5 a	2.1 b	5.9 c	3.6 ab	178.2 a	3398 bc	0.4 ab
	Uninoculated	198.3 b	4.1 a	10.7 a	3.5 abc	154.4 bcd	5155 a	0.5 ab

†PO₄ (phosphate) not added to sand:soil (2:1,v/v) mixture (Low), or added to achieve 15 or 31 µg PO₄/g soil (Medium or High, respectively).

‡GMONO-1, GMONO-2 (Glomus monosporum, isolates 1 and 2, respectively) and GMOSS-1, GMOSS-2 (G. mosseae, isolates 1 and 2, respectively).

*Calculated by (no. blades [primary plant] x blade width (mm) x blade length (mm) x 0.905 [surface area constant]).

**Values in a column followed by different letters differ significantly (Probability < 0.05) by a protected Least Significant Difference procedure.

Table 4. Effects of phosphorus and mycorrhizal fungus species on above-ground plant growth of flax seven weeks after inoculation.

PO ₄ Level†	Fungus Treatment‡	Shoot Height (mm)	Number of Branches
Low	GMONO-1	44.3 b*	1.8 b
	GMOSS-1	27.3 b	1.2 b
	Uninoculated	33.8 b	1.4 b
High	GMONO-1	79.8 a	6.0 a
	GMOSS-2	85.3 a	5.3 a
	Uninoculated	96.5 a	5.3 a

†PO₄ (phosphate) not added to sand:soil (2:1, v/v) mixture (Low), or added to achieve 31 µg PO₄/g soil (High).

‡GMONO-1 (*Glomus monosporum*) or GMOSS-1 (*G. mosseae*).

*Values in a column followed by different letters differ significantly (Probability < 0.05) by a protected Least Significant Difference procedure.

to the Red Desert of Wyoming. Although only one of three evaluation dates for measuring above-ground plant growth has occurred, it is evident that fungal isolates vary in their effects on the plants studied. Growth of inoculated plants was not always superior to that of uninoculated plants. Growth depression associated with mycorrhizae in conjunction with high internal PO₄ levels in the plant and with early stages in plant growth has occasionally been reported in the literature.

Additional data will be collected from the ongoing experiments to evaluate tissue-PO₄ levels and age as factors affecting the plant response (particularly photosynthesis and carbon partitioning) to isolates of mycorrhizal fungi. An understanding of the ecophysiology of the plant-fungus system may allow manipulation of the system to favor improved plant establishment, survival, and productivity, particularly in disturbed ecosystems.

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EDAPHIC FACTORS AND MYCORRHIZAL DISTRIBUTION IN SAGEBRUSH-SHADSCALE
COMMUNITIES OF THE RED DESERT, WYOMING

R. M. Miller

Background and Scope

The reestablishment of functional soil ecosystems is critically important to the successful restoration of disturbed areas in the arid and semiarid West. Of major concern in these environments are the effects of various types of disturbances on vesicular-arbuscular mycorrhizal fungi, an important component of below-ground ecosystems.

Mycorrhizal fungi are soil-borne organisms that invade the roots of vascular plants and establish a symbiotic relationship. The hyphae of the fungi extend to the plant roots and through the soil, increasing the effective surface area available to the roots for absorption of mineral ions and water. In the symbiosis, the fungus provides the plant with nutrients, and the plant provides the fungus with a favorable growth milieu and carbohydrates for metabolism. This relationship is particularly important in arid western ecosystems where nutrient availability and water stress are limiting to plant growth.

An initial characterization of mycorrhizal fungi for plants that occur in the Red Desert of Wyoming was carried out during the period 1977 through 1979. This site is in a region where large tracts of surface-extractable coal reserves exist or are currently being mined. We found that the density of mycorrhizal plants was correlated with the degree of disturbance and harshness of a site, with nonmycorrhizal chenopods apparently dominating disturbed areas, and mycorrhizal species characteristic of later successional stages occupying undisturbed areas.¹ In recent studies, we have found indications that in some plant species, the ability to possess mycorrhiza may be related to the type of neighboring plant associates.²

To better document the community dynamics of mycorrhiza in this ecosystem, we initiated a study to determine the edaphic factors in conjunction with a mycorrhizal bioassay by which infection potentials and spore numbers for several undisturbed communities were determined.³

Progress in 1983

A contiguous grid system composed of 15 cells was placed over an undisturbed area in the Red Desert in 1978. The cells were 20 x 15 m in size and arranged in a 3 x 5 matrix. Soil samples were collected from the cells during the growing seasons of 1978, 1980, 1981, and 1983. Each sample was composed of four pooled soil cores collected with a bucket auger placed to a depth of 10 cm within each cell. These samples were returned to the laboratory for bioassay, spore counts, and physical and chemical analysis.

We found that the edaphic factors associated with high spore numbers and infection potential are related to soil/plant moisture characteristics and soil aeration (Table 1). A positive association for sand and negative associations for water-retaining ability (available soil water x bulk density) and for silt were found both for infection potential and spore numbers. Porosity was also significantly correlated with spore numbers, but not with infection potential. An osmotic effect, as measured by electrical conductivity, was also evident for infection potential and spore numbers; with increasing soil salinity, there was a corresponding decrease in mycorrhizal propagules (Table 1).

A stepwise multiple regression analysis using a maximum R^2 (MAXR) selection procedure⁴ was run for the undisturbed community edaphic factors, where infection potential was the dependent (y) variable and soil physical and chemical characteristics, as well as plant community type, were independent (x) variables. Because the chances were high for multicollinearity and because independence of soil factors is not possible, care was taken to avoid variables that were a measure of each other. A model developed to predict mycorrhizal fungi inoculum potential (Table 2) indicates that water availability and nutrient status of the soil are the primary factors associated with distribution of mycorrhizal fungi.

If the edaphic factors of disturbed areas are considered in comparison with data from the undisturbed plant communities, the inoculum potential of a soil is influenced most by the plant community type present, with this factor coming first in the model having an $R^2 = 0.43$. Also important in determining inoculum potential are the salinity level, porosity, pH, and water-retaining capacity of the soils. Preliminary data indicate that similar factors are responsible for mycorrhizal distribution whether soils are disturbed or

Table 1. Correlation coefficients for edaphic factors and mycorrhizal propagule density in native undisturbed community soils of the Red Desert (n = 39).

Factors	Correlation Coeff. (r) [†]	
	Infection	Spores ($\sqrt{n}$)
Physical		
Water-retaining capacity	-0.62***	-0.51***
Sand	+0.60***	+0.43**
Silt	-0.52***	-0.32*
Clay	-0.34*	-0.35*
-0.033 MPa tension	-0.58***	-0.40**
Porosity	n.s.	+0.50***
EC (saturation extract)	-0.43**	-0.33*
Bulk density	n.s.	-0.54***
Stones	n.s.	-0.34*
Field moisture percentage	n.s.	-0.37*
Chemical		
Exchangable magnesium	-0.40**	-0.47***
Exchangable sodium	-0.32**	-0.32*
Carbon-to-phosphorus ratio	+0.46***	+0.44**
Total Kjeldahl nitrogen	+0.28*	+0.36**
Organic carbon	+0.34**	+0.41**
Carbon-to-nitrogen ratio	+0.30*	+0.30**
Bicarbonate-extractable phosphorus	n.s.	+0.31*
pH (saturated paste)	-0.32**	-0.42**

[†] * = P<0.05; ** = P<0.01; *** = P<0.001, n.s. = not significant.

Table 2. Stepwise multiple regression (MAXR) analysis of soil factors on mycorrhiza inoculum potential at the Red Desert research site (n = 39).

Model	β Value	F	P > F	R ²
Best Single-Factor Model:		18.50	0.0001	0.33
Intercept	58.97			
Water-retaining ability	-2.70	18.50	0.0001	
Best Two-Factor Model:		19.52	0.0001	0.52
Intercept	2.67			
Carbon-to-phosphorus ratio	0.45	16.67	0.0002	
Exchangeable potassium	-71.51	29.58	0.0001	
Best Three-Factor Model:		22.65	0.0001	0.66
Intercept	200.60			
Bulk density	-82.93	35.79	0.0001	
Nitrate-nitrogen ($\log_{10}$)	-31.72	16.41	0.0003	
Exchangeable potassium	-57.86	24.46	0.0001	
Best Four-Factor Model:		19.74	0.0001	0.70
Intercept	-10.82			
Porosity	212.46	26.22	0.0001	
Nitrate-nitrogen ($\log_{10}$)	-26.85	9.49	0.0041	
Exchangeable potassium	-47.81	26.18	0.0001	
Exchangeable magnesium ($\log_{10}$)	-21.16	5.80	0.0216	

virgin; however, the disturbed plant community expresses itself as nonmycorrhizal Halogeton, while the undisturbed community is composed of sagebrush, shadescale, or saltbush.

Prospects

With a better understanding of those factors associated with the occurrence of mycorrhizal fungi, the role of this symbiosis can be better elucidated. This information also will permit identification of relationships between plants, soils, and fungus distribution. With this information, in turn, better capabilities can be developed to predict the consequences of disturbance to such systems.

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