PHOSPHORUS RUNOFF LOSSES FROM ALFALFA [1/]

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Abstract

Phosphorus (P) released from plants after freezing at the end of the growing season may be a contributor to P in runoff from agricultural landscapes. We evaluated P release from alfalfa (*Medicago sativa* L.) and mixed grass species [mainly quackgrass (*Agropyron repens*)] after freezing or drying in laboratory and field studies. Freezing released 18-30 % of the total P as dissolved reactive P (DRP) and 11-25 % of the total P as soluble P. Drying released more water soluble P than freezing with 30 and 74% of the total plant P released as DRP and total soluble P respectively. Freezing plants in the laboratory or in-field treatment with the herbicide paraquat (1,1'-dimethyl-4, 4'-bipyridinium ion) greatly increased water-extractable P, with more P extracted from grasses than from alfalfa. Alfalfa grown on soils with excessive P soil test levels released more P after freezing than plants grown on soils with optimum P levels. A strong correlation (R^2 = 0.97) was found between soluble P released by freezing and total P in alfalfa collected from fields with soil test P levels ranging from 35 to179 ppm. Runoff from paraquat-treated alfalfa or grass field plots subjected to simulated rainfall contained higher P concentrations than runoff from untreated plants. The effects of natural freezing of alfalfa on P losses in runoff were evaluated by collecting runoff from alfalfa and control plots during the October through May over winter period. Although laboratory and simulated rainfall experiments showed the potential for P losses after plant freezing, alfalfa treatments did not affect P or sediment loads in natural runoff in one year of measurement. Climatic conditions including the timing and extent of plant freezing and drying and of precipitation events after freezing likely influence the potential for P losses in natural runoff.

Introduction

Phosphorus (P) in runoff from cropland is an environmental concern because this P can contribute to eutrophication of natural waters and stimulate weed and algae growth in lakes and streams (Carpenter et al. 1998). Wendt and Corey (1980) found substantial amounts of phosphorus in runoff from alfalfa (*Medicago sativa* L.) after plants were frozen at the end of the growing season. This suggests that soluble phosphorus release from plants following the onset of freezing temperatures could be a contributor of non-point source P losses from cropland. Interestingly, Timmons et al. (1970) also observed that freezing bluegrass (*Poa pratensis* L.) followed by thawing increased soluble nutrients in a subsequent leaching by about the same amount as drying the plant material.

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They noted that freezing and thawing followed by drying released 80% of the total plant P from bluegrass in a water-soluble form. Muir et al. (1973) found a significant correlation (r = 0.45) between P concentration stream water samples and the extent of legume production in the region represented by the surface water sample. They speculated that the higher P concentrations could be due to leaching of P from alfalfa during the time period when alfalfa is dormant. Muir et al. (1973) also noted that higher livestock density coincides with areas of intensive legume production.

In a study of P leached from growing crops [cotton (*Gossypium hirsutum*), sorghum (*Sorghum sudanense*), and soybean (*Glycine max*.)] by simulated rainfall, Sharpley (1981) showed that a substantial portion (14 to 94%) of the soluble P in runoff could be attributed to plant leachate P. The plant contribution decreased with increasing rates of P fertilization and with plant age and soil-water stress. Interestingly, a period of only one day between rainfall events was needed for leachable P to re-accumulate on the leaf surface.

Residues of cereal crops harvested for grain are also potential sources of soluble P in runoff from cropland (Schreiber, 1999). Schreiber showed that significant amounts of soluble P were leached from corn residues left on the soil surface after grain harvest when these residues were subjected to simulated rainfall. Runoff P concentrations and losses were greater at lower rainfall intensities and higher residue loading rates.

Several studies indicate that soluble P losses from vegetation and plant residues in natural landscapes after freezing or drying can contribute to P in runoff. For example, White (1973) investigated the effects of freezing and drying on nutrient loss from native vegetation in South Dakota prairies. He reported that freezing increased nutrient release (including P) from vegetation if it was actively growing when frozen. White (1973) concluded that release of nutrients to runoff water after freezing was probably important if the vegetation is growing when frozen. Nutrient release from dried or mature vegetation after freezing was less important because cell rupture and release of soluble nutrients was limited by the lower water content of the plant cells when frozen. White and Williamson (1973) showed that P in runoff from cultivated, fertilized plots cropped with oat, corn, and alfalfa or fallowed was similar to runoff P content from native prairie. They concluded that P losses from native prairie that was periodically burned were similar to losses from cropland. Singer and Rust (1975) found that soluble P in runoff from a deciduous forest in Minnesota was highest during spring snowmelt and when soils were frozen. The source of the soluble P was most likely from leaf litter on the soil surface, which had been exposed to many freeze-thaw cycles resulting in cell rupture and soluble P release. The authors noted that runoff containing sufficient P to impact water quality in streams and downstream lakes occurs from forest systems. The amounts of P lost from forest (0.07 kg/ha/yr) are similar to those reported for a fertilized agricultural watershed (Gburek and Heald, 1974). Since alfalfa occupies about one million ha in Wisconsin (WDATCP, 2001), and grass pastures and roadsides also represent substantial areas, investigation of this potential source of P in runoff deserves research attention.

The overall objective of this study was to determine the extent of P losses from alfalfa and grasses after freezing or drying in laboratory and field experiments. This study also included work to determine the extent of P losses from alfalfa in natural runoff subjected to various management treatments over the winter.

Materials and Methods

Laboratory Experiments

Preliminary experiments to determine soluble P release from alfalfa were performed with fresh alfalfa plants harvested from greenhouse flats or from field experiments at the early bloom stage of plant development. Samples of approximately 150 g fresh weight were subjected to several treatments before extraction with deionized water (DI) to determine soluble P release. These treatments were: fresh plant extraction, extraction after freezing (24 hr at -5^{o} C), extraction after freezing and thawing (24 h at room temperature after freezing and before extraction), and extraction after drying in a forced air dryer at 70^{o} C for 48 h. Separate alfalfa samples were dried, ground to pass a 20-mm screen, and analyzed for total P by inductively coupled plasma optical emission spectrometry (ICP-OES). The fresh and dry weights of these samples were also used to determine dry matter concentration in the fresh alfalfa plant tissue.

The amounts of soluble P released by the pretreatments were determined by shaking the alfalfa samples with 1300 mL of DI water in 1500-mL wide mouth jars for 1 hr and filtering the extracts through Whatman no. 5 filter paper. The filtered extracts were analyzed for dissolved reactive P (DRP) using the ascorbic acid method (Murphy and Riley, 1962) and for total soluble P using an ammonium persulfate-sulfuric acid digestion method (USEPA, 1993).

To evaluate the influence of soil test P on the amounts of p released from alfalfa after freezing, four alfalfa production fields with a range of soil test P values (35 to 179 ppm) were identified at the University of Wisconsin Agricultural Research Station at Arlington, WI. Soil samples (0-15 cm) and alfalfa plants were collected from replicated (four) areas within each field. Alfalfa plants were harvested at the early bloom stage of development and were subjected to laboratory freezing and extraction as described above. Alfalfa samples were also extracted fresh (no freezing). Total P concentration in the alfalfa was determined by ICP-OES, and the Bray P-1 soil test (Frank et al., 1998) was performed on the soil samples.

Simulated Rainfall Experiments

Phosphorus losses in runoff from alfalfa and mixed grasses species [mainly quackgrass (*Agropyron repens*)], following simulated rainfall were determined in field experiments using a randomized complete block design with four replications. The herbicide paraquat (1,1'-dimethyl-4, 4'-bipyridinium ion) was used to simulate freezing. The mode of action of paraquat is to rupture cell membranes (WSSA, 1994), which is generally similar to the effect of freezing. Treatments evaluated in the alfalfa study included: 1) Untreated alfalfa at early bloom stage (control); 2) Alfalfa removed by cutting at ground level; 3) Alfalfa treated with paraquat 3 days before simulated rain; 4)

Alfalfa treated with paraquat 3 days before simulated rain, with 1.25 cm of rainfall applied before the simulated rainfall treatment; and 5) Alfalfa treated with paraquat 10 days before simulated rain. Treatments in the mixed grass experiment were: 1) Untreated grass (control); and 2) Grass treated with paraquat 3 days before simulated rain.

Simulated rain applications (76 mm/h for 60 min) were made as described by Bundy et al. (2001). Runoff collected from each plot was analyzed for sediment, DRP, and total P as described by Bundy et al. (2001). Before applying the simulated rain treatments, all alfalfa from the 0.83 m^2 harvest area in treatment 2 was weighed to determine dry matter yield, and subsamples of the harvested alfalfa were subjected to freezing and drying treatments followed by extraction to determine water soluble P release (See *Laboratory Experiments* above). Similarly, alfalfa plant samples were collected from border areas of the paraquat-treated plots (treatment 3) for determination of water-extractable P.

Natural Runoff Experiment

Natural runoff collectors were installed in an established alfalfa stand (45 cm height growth) in September 2001. Plot frames were used to create a known harvest area of 1 m^2. The steel plot frames were pushed into the soil to a 15-cm depth, and an aluminum runoff collection trough was placed across the entire down slope edge of the runoff plot. The collection trough was fitted with a drain and tubing leading to a 115-L galvanized garbage pail containing an 8-L polyethylene bucket for collection of the runoff. The garbage pail was installed into the soil so that only the top edge and lid remained above the soil surface. This provides sufficient gradient for runoff to flow from the collection trough to the runoff collection container inside the garbage pail. Screening was used to cover the runoff collection trough and was placed over the end of the delivery tube entering the collection pail to screen out plant residues and insects. The following treatments were imposed on the plots in a randomized complete block design with four replications. 1) Untreated alfalfa (control); 2) Alfalfa removed by cutting at ground level; and 3) Alfalfa cut to 20 cm height. Alfalfa harvested in treatment 2 was weighed to determine dry matter yield and subsampled for total P analysis. Natural runoff was collected at 7 to 10 day intervals or immediately following major precipitation events during 10/15/01 through 5/01/02. Runoff at each sampling date was analyzed for sediment, DRP, and total P as described by Bundy et al. (2001).

Results and Discussion

Phosphorus Released from Alfalfa by Laboratory Freezing and Drying Treatments

Freezing and drying significantly increased the amount of soluble and total P released from alfalfa plants. Freezing released 18-30 % of the total P as DRP and 11-25 % of the total P as soluble P (Table 1). Drying released more water soluble P than freezing with 30 and 74% of the total plant P released as DRP and total soluble P, respectively. Fresh treatment alfalfa released the lowest amount of soluble P; however, 10 and 14% of total plant P was extracted from fresh alfalfa as DRP and total soluble P, respectively (Table 1). Sharpley (1981) found that 7 to 9 % of the P leached from fresh plants was soluble organic P which is generally similar to the comparable values found in the current study. Frozen treatment alfalfa released significantly more soluble P than fresh treatment alfalfa. Freeze-thaw treatment alfalfa and Dry treatment alfalfa were not significantly different from each other, but were both significantly higher than the Fresh and the frozen treatment alfalfa. There was no significant difference in total soluble P released between fresh treatment alfalfa and frozen treatment alfalfa. There was significantly more total P released in the freeze-thaw treatment than in both the fresh and frozen treatment alfalfa. There was significantly more total soluble P released from the dry treatment than from the freeze-thaw, frozen, and fresh treatments. In previous work, Timmons et al. (1970) reported that freezing bluegrass followed by thawing greatly increased soluble P that could be extracted relative to fresh vegetation and released about the same amount of soluble P as drying the plant material.

Freezing and drying also markedly increased the amount of water soluble P extracted from field-grown alfalfa (Table 2), but the percentage of total plant P released was usually less than that found with greenhouse grown alfalfa. Freezing released 6-9% of the total P as DRP and 8-13% of the total P as soluble P. Drying released more water soluble P than freezing with 18 and 32% of the total plant P released as DRP and total soluble P, respectively. Frozen treatment alfalfa released significantly more soluble P than fresh treatment. The Freeze-thaw treatment released significantly more soluble P than frozen and fresh treatment alfalfa. Dry treatment alfalfa released significantly more soluble P than fresh, frozen, and freeze-thaw treatment alfalfa. Total soluble P released from alfalfa by the freezing and drying treatments followed an identical pattern to that observed for soluble P. Previous work has either measured increased P losses from vegetation after freezing (Wendt and Corey, 1980; Timmons et al, 1970; White 1973; Singer and Rust, 1975) or suggested that this might be a source of P in runoff (Muir et al., 1973). Results from our study confirm that soluble P released from plants by freezing or drying is a potential source of P in runoff from cropland.

Effect of Soil P Levels on P Uptake by Alfalfa and P Released after Freezing

Results from our evaluation of soil test P effects on P accumulation in alfalfa and its release on freezing and drying showed that total plant P and water soluble P released by freezing increased significantly with increasing soil test P (Table 3). Soil test P in the four fields sampled ranged from 35 to 179 ppm. Total P concentration in alfalfa and soil test P were significantly higher in field 121 than in the other three fields. Strong correlations (R^2 = 0.87 to 0.79) were found between soluble P released on freezing and drying and total P concentrations in alfalfa tissues collected from fields with various soil P levels. Similarly, R^2 values show a strong correlation between total P concentrations in alfalfa tissues and soil test P levels. This observation is in contrast to the widely held view that alfalfa does not accumulate P beyond the level needed to maximize dry matter production. Therefore, results show that increasing soil test P levels increases the amount of P uptake in alfalfa and the amount of soluble P released when plants are frozen. Lowering soil test P could help minimize the potential contribution of plant P to non-point source pollution.

Simulated Rainfall Experiments: P Released from Alfalfa and Grasses by Laboratory Freezing, Drying, and Paraquat Treatments

The effects of laboratory freezing and drying treatments and in-field paraquat treatment on the forms and amounts of P released from alfalfa and grasses by extraction with deionized water are summarized in Table 4. In all cases, plant samples were collected from the appropriate treatments in the simulated rainfall experiments conducted with alfalfa and grasses at the Arlington Agricultural research Station in July 2001. As previously found with greenhouse and field grown alfalfa, laboratory freezing and drying markedly increased soluble P release from both alfalfa and grasses compared with the amounts released from fresh plant material (Table 1). For both alfalfa and grasses, freezing released significantly more soluble P (DRP) and total soluble P than was found with fresh plant tissue. Drying and paraquat treatment released significantly more soluble P than fresh plant materials or those subjected to freezing alone, and the amounts of P released by these two treatments were not significantly different. Freezing released a substantially higher percentage of the total plant P as soluble P from grasses than from alfalfa with 5% and 34% of total plant P released from alfalfa and grasses, respectively. This was also true for drying and paraquat treatments, where 35 to 42 % of total plant P was released as soluble P from alfalfa compared with 58 to 82% of total plant P released from grasses by these treatments. These results are generally similar to those of Timmons et al. (1970) who found that freezing and thawing, followed by drying released up to 80% of the total plant P in bluegrass as soluble P. Paraquat treatment appears to give a reasonable simulation of the effects of freezing and drying. Alfalfa treated with paraquat released significantly more DRP and total soluble P than fresh untreated alfalfa and released similar amounts of water soluble P as drying.

Paraquat-treated grasses also released similar amounts of water soluble P as drying (Table 4). Both dry treatment and paraquat treated grass had significantly more DRP and total soluble P than the frozen and fresh treatment grass. In general, grasses released more soluble P than alfalfa after freezing, drying, or paraquat treatment. Drying and paraquat treatment released 60 to 80% of the total P from grasses compared with 35 to 40% of the total P released from alfalfa.

Simulated Rainfall Experiments: P Levels in Runoff from Alfalfa and Grass Plots

Phosphorus concentrations and loads in runoff following simulated rainfall were significantly increased by paraquat treatment of alfalfa (Table 5). Increasing the time between paraquat treatment and simulated rainfall or including an additional rainfall event had little or no effect on P in runoff. Removing alfalfa increased soluble and total P loads in runoff, likely due to greater runoff volume and extraction of soil P. Sediment concentrations and loads in runoff were increased by paraquat treatments. Paraquat treatment of grasses increased total P concentrations in runoff, but did not influence other runoff parameters (Table 6). Although there was a higher concentration of soluble (DRP) and total soluble P in the runoff, the amounts were only significantly different for total P concentrations. Also, there were no significant differences found in sediment concentrations and loads due to paraquat treatment. Runoff volumes and P loads in runoff were much lower in grass than in alfalfa due to higher infiltration in grasses.

Over Winter P Losses in Natural Runoff from Alfalfa

A comparison of laboratory and natural freezing effects on P released from alfalfa is shown in Table 7. While, natural freezing significantly increased the amount of water soluble P released from alfalfa relative to unfrozen plants, the amount of soluble P released from alfalfa by natural freezing on two dates was significantly less than that released by lab simulated freezing. Visual observations of the effects of natural freezing in the field indicated that only part of the vegetation was actually frozen by these events. This is in contrast to the complete freezing accomplished with the laboratory freezing procedure, and could account for the lower release of P where plants were frozen naturally.

Although laboratory and simulated rainfall experiments showed the potential for P losses after plant freezing, alfalfa treatments did not affect P or sediment loads in natural runoff (Table 8). The absence of natural P runoff response to various alfalfa treatments seems to conflict with the findings of Wendt and Corey (1980). However, the difference in measurement techniques between simulated rainfall used by Wendt and Corey (1980) and the natural runoff collection method used in this study could account for the difference in findings. Climatic conditions including the timing and extent of plants freezing and drying and of precipitation events after freezing likely influence the potential for P losses in natural runoff.

Conclusions

- Soluble P released from plants by freezing or drying is a potential source of P in runoff from cropland.
- Freezing, freeze-thaw, and drying treatments greatly increased water soluble P extracted from alfalfa.
- Potential P losses from alfalfa increase with increasing soil test P.
- Phosphorus losses from grasses after freezing or drying are as great as from alfalfa.
- Paraquat treatment increased soluble P losses in runoff following simulated rainfall.
- Over winter natural runoff measurements do not show increased P losses due to alfalfa in one year of measurement.
- Climatic conditions including the timing and extent of plants freezing and drying and of precipitation events after freezing likely influence the potential for P losses in natural runoff.

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Table 1. Laboratory Freezing and Drying Effects on Water Soluble Phosphorus Extracted from Greenhouse Grown Alfalfa University of Wisconsin-Madison, 2001

Treatment	Soluble P (DRP)	% of Total Plant P	Total soluble P	% of Total Plant P
	(mg P/kg dry wt.)		(mg P/kg dry wt)	
Fresh	444c	10c	621c	14c
Frozen	780b	18b	482c	11c
Freeze Thaw	1274a	30a	1081b	25b
Dry	1297a	30a	3213a	74a
P>F	<0.001	<0.001	<0.001	<0.001
CV(%)	17	17	21	21

* Values within each column followed by the same letter are not significantly different at the 0.05 probability level.

* Total Plant P = 4300 mg P/kg

Table 2. Laboratory Freezing and Drying Effects on Water Soluble Phosphorus Extracted from Field*** Grown Alfalfa, University of Wisconsin-Madison, 2001

Treatment	Soluble P (DRP)	% of Total Plant P	Total Soluble P	% of Total Plant P
	(mg P/kg dry wt.)		(mg P/kg dry wt)	
Fresh	7d	0.2d	8d	0.2d
Frozen	288b	9b	434b	13b
Freeze/Thaw	213c	6c	255c	8c
Dry	607a	18a	1035a	32a
P>F	<0.001	<0.001	<0.001	<0.001
CV(%)	17	17	21	21

*Values within each column followed by the same letter are not significantly different at the 0.05 probability level.

**Total Plant P = 3300 mg P/kg

***Field samples taken from a field south of the Wisconsin Alumni Research Foundation building on the University of Wisconsin-Madison campus on May 23, 2001.

Table 3. Soil Test P Effect on Water Soluble P Extracted from Alfalfa before after Freezing Arlington Research Station, Arlington, WI, 2001

Field	Soil Test P	P Extracted Fresh	P Extracted Frozen	Total Plant P
	---------- mg P/kg ----------			
405	35c	13b	159c	3760c
307	63c	6b	160c	3570c
330	95b	13b	440b	4200b
121	179a	74a	839a	5300a
P>F	<0.001	<0.001	<0.001	<0.001
CV(%)	26	73	27	8

*Values within each column followed by the same letter are not significantly different at the 0.05 probability level.
**Soil test P = Bray P-1 method on 0-15 cm samples

Table 4. Freezing, Drying, and Paraquat Effects on Phosphorus Extracted from Alfalfa and Grasses Arlington Research Station, Arlington, WI, 2001

Plant	Treatment	Soluble P		Total Soluble P	
		(mg P/ kg dry wt.)	(% of total Plant P)	(mg P/ kg dry wt.)	(% of total Plant P)
Alfalfa	Fresh	19c	1c	34c	1c
	Freeze	109b	4b	137b	5b
	Dry	787a	25a	1248a	42a
	Paraquat	707a	22a	1146a	35a
P>F		<0.001	<0.001	<0.001	<0.001
CV(%)		17	16	32	31
Grass	Fresh	91c	2c	100c	3c
	Freeze	778b	21b	1281b	34b
	Dry	1172a	39a	2178a	58a
	Paraquat	1482a	39a	3110a	82a
P>F		0.002	0.002	0.005	0.005
CV(%)		17	18	26	26

* Soil test P = 83 mg P/kg (alfalfa); 203 mg P/kg (grass)
** Values within each column followed by the same letter are not significantly different at the 0.05 probability level.
*** Total plant P = 2950 mg P/kg (alfalfa) and 3750 mg P/kg (grass).

Table 5. Treatment Effects on Soluble and Total Phosphorus in Runoff from Alfalfa after Simulated Rainfall Arlington Research Station, Arlington, WI, 2001

Treatment	Runoff				Sediment	
	Soluble P		Total P			
	Conc. (mg P/L)	Load (g P/ha)	Conc. (mg P/L)	Load (g P/ha)	Conc. (g/L)	Load (g P/ha)
Control	0.09b	10b	0.20b	22b	0.05b	6c
Removed	0.20b	77a	1.34a	513a	0.03b	11c
Paraquat + 3d	0.40a	85a	1.45a	310a	0.39a	71b
Paraquat + 3d*	0.41a	68a	1.50a	249a	0.36a	51bc
Paraquat + 10d	0.29a	100a	1.80a	613a	0.39a	106a
P>F	<0.001	0.015	0.003	0.028	<0.001	0.003
CV (%)	30	44	37	40	24	55

* 1.3 cm water applied before simulated rain
** Values within each column followed by the same letter are not significantly different at the 0.05 probability level.
*** Bray-1 soil test P (0-2 cm) = 83 mg P/kg

Table 6. Treatment Effects on Soluble and Total Phosphorus in Runoff from Grasses after Simulated Rainfall Arlington Research Station, Arlington, WI, 2001

Treatment	Runoff				Sediment	
	Soluble P		Total P			
	Conc. (mg P/L)	Load (g P/ha)	Conc. (mg P/L)	Load (g P/ha)	Conc. (g/L)	Load (g P/ha)
Control	0.09 NS	2 NS	0.71b	16 NS	0.26 NS	6 NS
Paraquat + 3d	1.10	13	1.42a	17	0.52	5
P>F	0.12	0.18	0.04	0.83	0.08	0.85
CV (%)	113	116	29	89	36	95

* Values within each column followed by the same letter are not significantly different at the 0.05 probability level.
** Bray-1 soil test P (0-2 cm) = 203 mg P/kg
*** NS = No significant Difference

Table 7. Treatment Effects on Soluble P Levels in Natural and Lab Freezing Arlington Research Station, Arlington, WI, 2001

Treatment	Soluble P	% of Total
	mg P/kg dry wt.	
Lab Freezing		
Fresh	10e	0.38e
Frozen	463a	18a
Freeze-Thaw	508a	19a
Natural Freezing		
Frozen October 17, 2001	338cd	13cd
Frozen December 10, 2001	159bc	6bc
P>F	<0.001	<0.001
CV(%)	36	36
LSD	151	6

* Total P=2625 mg P/kg
** Values within each column followed by the same letter are not significantly different at the 0.05 probability level.
*** Average Bray-1 soil test P (0-2 cm) = 135 mg P/kg

Table 8. Treatment Effects on Cumulative Natural Runoff and Phosphorus Losses from Alfalfa Arlington Research Station, Arlington, WI, 2001

	Soluble P Load	Total P Load	Runoff Volume	Sediment Load
Alfalfa	(g/ha)	(g/ha)	(L)	(kg/ha)
Cut to ground	24 NS	37 NS	40 NS	3 NS
Cut 20cm	18	27	30	4
Not cut	8	15	26	3
P>F	0.33	0.35	0.68	0.84
CV (%)	86	78	73	110

* NS = No Significant Difference
** Runoff collected at two-week intervals from 10/15/01 through 5/01/02
*** Average Bray-1 soil test P (0-2 cm) = 135 mg P/kg