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Soil Temperature and Fumigation Effects on Plant Phosphorus Uptake and Related Microbial Properties[1]

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ABSTRACT

Early season problems with growth of corn (*Zea mays* L.) under cool, wet conditions prompted a study of the effects of soil and environmental conditions on mineralization and plant uptake of phosphorus (P). Our objective was to determine the effect of soil test P, temperature, and soil fumigation on soil P availability and uptake during early corn growth. Corn was grown in growth chambers at temperatures of 14°C or 25°C. Soils were a high-P Hastings silty clay loam (fine, montmorillonitic, mesic Udic Argiustoll) and a low-P Sharpsburg clay loam (fine, montmorillonitic, mesic Typic Argiudoll). Plants grew for up to 42 d either in soil which had been fumigated with methyl bromide to reduce microbial populations or left unfumigated. We harvested whole pots for soil and plant analysis at 1, 14, 28, and 42 d after planting. Biomass carbon (C) and biomass P were lower in fumigated soils and biomass C increased with time. Fumigation increased Bray P1-extractable P at all times. Phosphatase activity and mycorrhizal colonization were both reduced

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by fumigation. Cumulative plant P uptake was highest in Hastings at 25°C. Higher temperature and higher initial P status increased plant P uptake during early growth. Plants grown in fumigated soil did not take up more P, despite greater extractable P.

INTRODUCTION

Field observations of early season stunting and purpling of corn grown on fumigated soil raised questions regarding microbial involvement in the P availability cycle (Jawson et al., 1993). Analyses of plant samples from that study confirmed lower plant P content during early growth as well as lower mycorrhizal root colonization in plants grown on fumigated soil. This was despite similarities in "available" P (Bray and Kurtz, 1945) in fumigated and non-fumigated soils.

Environmental conditions including cool, wet weather during and immediately following planting are generally acknowledged as contributing to visual P-deficiency symptoms in corn (Donahue et al., 1983). As weather warms and plants mature, these symptoms usually disappear and plants recover. During approximately the first six weeks of plant growth when P requirement is high, microbial mineralization of organic P may be a key factor in maintaining adequate P nutrition of plants (Anderson, 1980; Stevenson, 1986).

A growth chamber study was consequently conducted to determine the relationships among microbial activity, and P availability and uptake. Microbial populations were reduced in one set of pots by fumigation with methyl bromide prior to the initiation of the study. Additional experimental variables included temperature and soil series which differed in initial soil P availability.

The specific objective of the study was to determine the effect of soil test P, temperature, and soil fumigation on soil P availability and plant uptake during early corn growth.

MATERIALS AND METHODS

Soils and Soil Properties

Two soils which differed in Bray P1-extractable P were used for this study. A Sharpsburg clay loam which is low in extractable P was collected from the top 15 cm of plots located at the University of Nebraska Research and Development Center near Ithaca, Nebraska. A Hastings silty clay loam which is high in extractable P was collected from the top 15 cm of plots located at the South Central Research and Extension Center near Clay Center, Nebraska. Both plot areas had no recent history of P fertilizer inputs. After collection, soils were stored in a field moist condition at approximately 5°C. Soil chemical and physical characterization was performed on air-dried subsamples of soil which had been

passed through a 2-mm sieve. Analyses performed according to procedures described by Dahnke (1988) were as follows: organic matter content, pH (1:1 in water), Bray P1-extractable P, nitrate-nitrogen (NO_3-N), and exchangeable potassium (K). Other tests performed were cation exchange capacity (CEC) by ammonium saturation at pH 7 (Soil Conservation Service, 1984) and particle size analysis by the hydrometer method (Day, 1965). The P-adsorption coefficient (slope of the line relating P added to a soil slurry versus P adsorbed) was determined by the National Soil Survey Laboratory, Lincoln, Nebraska (Soil Conservation Service, 1984). Table 1 shows the results of these analyses.

Experimental Design

The experiment was set up in a split-split plot design replicated over time. Each replicate consisted of two growth chambers. Chambers were identical except for temperature which was either 14°C or 25°C. Humidity was 60±3% and photon flux density (measured at table top height) was approximately 1500 $\mu mol \cdot m\text{-}2 \cdot s^{-1}$ with 16/8 h light/dark periods in both chambers. Each growth chamber was divided

TABLE 1. Chemical and physical properties of Sharpsburg and Hastings soils (surface 15 cm, air dried, <2 mm).

Property	Sharpsburg	Hastings
Textural class	clay loam	silty clay loam
Sand (%)	23.15	17.63
Coarse silt (%)	25.73	32.67
Fine silt (%)	18.52	18.38
Very fine silt (%)	3.09	2.55
Clay (%)	29.51	28.77
pH	6.92	6.59
Exchangeable K (mg kg^{-1} soil)	312	558
Extractable P (mg kg^{-1} soil)	10	31
NO_3-N (mg kg^{-1} soil)	49.8	26.1
Organic matter (%)	3.69	2.48
CEC ($cmol_c$ kg^{-1} soil)	25.2	18.4
P adsorption coefficient (mg kg^{-1} soil)	2.9	3.0

into two blocks and sample units (i.e., pots) were randomly assigned to blocks within each growth chamber. Within each block were 16 pots representing the two soil series (Hastings or Sharpsburg), two fumigation treatments (fumigated or not fumigated), and four sampling times (1, 14, 28, and 42 d after planting). The experiment was replicated four times.

The split-split plot design was analyzed statistically as follows. The main plot effect was temperature. Soil series was the first split and fumigation treatment was the second split. Since samples were taken over time within each replication, it was assumed that sampling times were correlated with each other, and therefore would confound analysis of main effects of the experiment. To eliminate variance due to time, repeated measures analysis of variance (Federer, 1955) was used. All statistical analyses were performed using procedures of the Statistical Analysis System, Version 5 (SAS Institute, 1985). The 5% confidence level was used to denote statistical significance.

Fumigation Procedure

All soils were sieved (2-mm) at field moisture content and an approximately 10 kg subsample of each soil was placed on 0.15-mm thick black plastic sheets. A max-min thermometer was placed on top of the soil to verify that temperatures during fumigation were greater than the vaporization temperature of methyl bromide, thus promoting effective fumigation. The plastic was then folded and sealed with duct tape. Soil was fumigated using one 0.45 kg can of methyl bromide (Meth-O-Gas) per soil bag at a rate of 45 g methyl bromide kg^{-1} soil, equivalent to 277 g methyl bromide m^{-2} in the field. Bags were opened after approximately 3 d and allowed to air for one day before sample pot preparation.

Sample Unit Processing

Sample pots were prepared by placing fumigated or non-fumigated soil in 3.8-L containers lined with clear plastic bags. Three Asgrow Rx 717 corn seeds were planted in each pot. Soils were initially watered with approximately 100 mL tap water to promote seed germination. Pots were then watered every two to three days during the experiment to maintain gravimetric water content of approximately 0.27, corresponding to -33 kPa water potential for each soil.

At 14, 28, and 42 d after planting, any plant material in a pot to be sampled was harvested by clipping the plant at the soil surface. Plants were placed in labeled paper bags, dried in an oven at approximately 80°C, and subsequently crushed and analyzed. At 1, 14, 28, and 42 d after planting, soil from one set of pots was removed in the plastic bag liners and stored at 4°C.

At the time of plant harvest, root material was hand separated from soils, washed, and stored in vials of 1:1 (v:v) ethanol and water at 4°C for subsequent vesicular arbuscular mycorrhizae (VAM) colonization observations. The day following

sampling, representative subsamples from bags containing the soil were brought to room temperature and used for microbial analyses. At the same time another subsample of soil was set out to air-dry at room temperature for chemical analyses.

Analyses

Microbial and Biochemical

Microbial biomass C was determined using the initial substrate induced respiratory response method (Anderson and Domsch, 1978) as modified by Smith et al. (1985). Microbia-l-biomass P was determined following Brookes et al. (1984) with no correction for P fixation as P-adsorption coefficient analysis indicated fixation was not significant for these soils (Table 1). Acid phosphatase enzyme activity, an index of the potential for organic P mineralization, was measured by the method of Tabatabai and Bremner (1969) using p-nitrophenyl phosphate as a substrate. All biological analyses were performed in duplicate and calculated on a dry-weight basis.

Soil and Plant Composition

Bray P1-extractable P (Dahnke, 1988) was determined on air-dried soil samples. Plant samples were dried, ground in a Wiley mill, and prepared for total P analysis using the dry ashing method (Jackson, 1958).

Vesicular Arbuscular Mycorrhizae (VAM) Fungi Colonization

Roots for VAM analysis were washed, prepared, and stained with trypan blue following the method of Phillips and Hayman, (1970). Percent root colonization by VAM fungi was estimated under a light microscope using the gridline intersect method of Giovannetti and Mosse (1980).

RESULTS AND DISCUSSION

Plant Phosphorus Uptake

Temperature and soil series significantly affected plant P uptake. Plants grown on the Hastings soil at 25°C had the highest cumulative P uptake followed by Sharpsburg at 25°C (Figure 1). Fumigation did not affect plant P uptake or plant weight at harvest (data not shown), although plants grown in fumigated soil at 25°C did show the purpling typical of P deficiency or a striped chlorosis at approximately 28 d. Visual symptoms appeared later or in some cases did not appear at all on plants grown at 14°C, probably because lower temperature reduced their overall development and consequently reduced their P demand. At the same time, lower temperature also may have suppressed the ability of plants growing in P-deficient soils to alter their root morphologies and make more efficient use of what limited soil P is present (Atkinson, 1990).

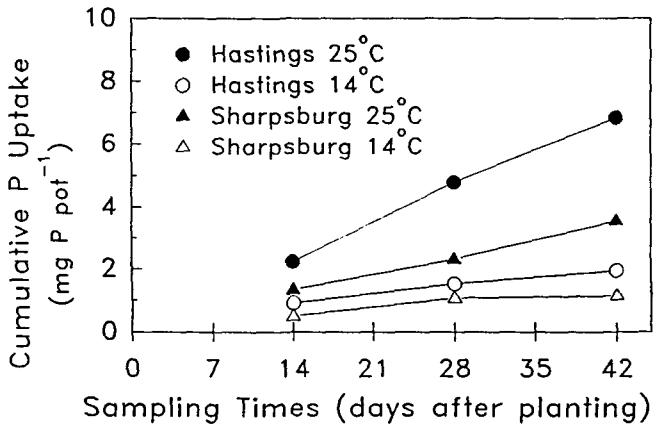

FIGURE 1. Cumulative plant phosphorus uptake as affected by soil and temperature. Points are mean values of blocks, fumigations, and replicates (n=16).

It had been expected that fumigation would cause differences in plant growth or P uptake. In the field study which motivated the experiments described here, corn plants grown on fumigated soil were stunted early in the season and had lower plant P concentrations than their non-fumigated counterparts (Jawson et al., 1993). In this experiment, the lack of fumigation effect on plant weight or P uptake may have been due to inherent differences between conditions in the field versus those in the growth chamber-for example, limited soil volume or controlled light intensity. This resulted in soil series and temperature dominating plant P uptake in the growth chamber.

Biomass Carbon and Phosphorus

Biomass C was measured to confirm the effectiveness of fumigation. For both soils, fumigated samples had significantly less biomass C than non-fumigated, indicating that fumigation successfully reduced the size of the microbial population (Figure 2). There was no effect of temperature on biomass C. The Sharpsburg soil had higher biomass C values for both fumigated and non-fumigated samples compared with their Hastings soil counterparts. This difference was expected as Sharpsburg soil had more organic matter to support microbial activity than Hastings soil. Fumigated soils exhibited an increase in biomass C over time of the study indicating recovery and regrowth of the microbial population. Biomass, however, did not recover to pre-fumigation levels during this time period.

Fumigation and soil series significantly affected biomass P effect of measurements while temperature did not (Figure 3), paralleling the effect of

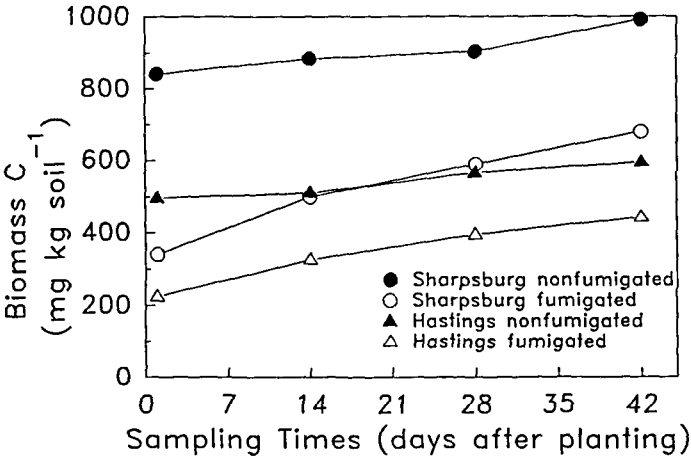

FIGURE 2. *Changes in soil biomass carbon with time as affected by soil and fumigation.* Points are mean values of block, temperatures, and replicates (n=16).

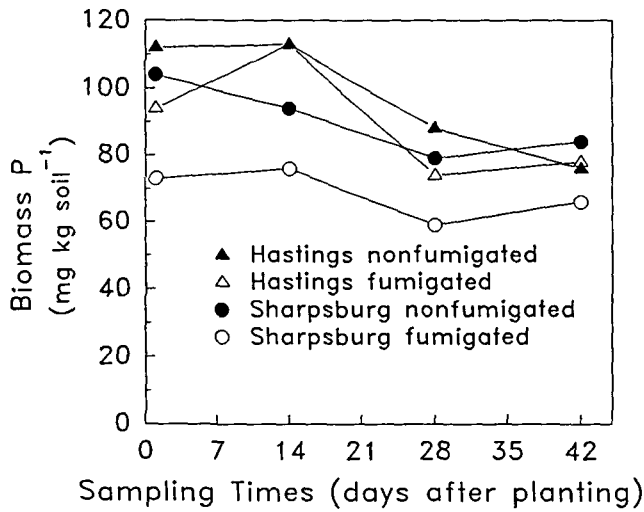

FIGURE 3. Changes in soil biomass phosphorus with time as affected by soil and fumigation. Points are mean values of block, temperatures, and replicates (n=16).

TABLE 2. Biomass C/biomass P ratios of fumigated and non-fumigated soils over time.

Soil	Treatment	Sampling time (days after planting)			
		1	14	28	42
Sharpsburg	Fumigated	4.6	6.5	10	10.3
Sharpsburg	Non-fumigated	8.1	9.4	11.4	11.8
Hastings	Fumigated	2.4	2.9	5.4	5.7
Hastings	Non-fumigated	4.4	4.5	6.4	7.8

treatments on biomass C. All soil-treatment combinations showed only slight fluctuations over time. The ratio of biomass C/biomass P also increased with time of sampling, mainly because of the increase in biomass C related to the buildup of microbial cell structural components in the recovering population (Table 2). Brookes et al. (1984) found slightly higher biomass C/P ratios than in this study, although their measurements were not made on previously fumigated soils or over time.

The method used to determine biomass P potentially complicates data interpretation, especially for the initial sampling. The biomass P determination involves fumigating half of a soil sample with chloroform, extracting P from fumigated and non-fumigated halves and calculating biomass P by difference. The initial methyl bromide fumigation combined with the chloroform fumigation may have resulted in somewhat lower biomass P values. It is also likely that the recovering microbial population of the methyl bromide-fumigated soils is qualitatively as well as quantitatively different from that of the non-fumigated soils. Thus comparison of biomass P, biomass C, or any indirect indicator of microbial activity, between the two groups of treated soils would have to be undertaken with care (Jenkinson and Ladd, 1981).

Bray P1-Extractable Phosphorus

Sharpsburg soil is significantly lower in Bray P1-extractable P than is Hastings soil (Figure 4). Fumigation increased extractable soil P at each sampling time for all soils and temperatures, with the exception of the Hastings soil at 25°C.

Bray P1-extractable P did not fluctuate greatly during the 42 d period even though there was a statistically significant time effect. No P inputs were made and plant P uptake increased with plant growth over time. This would suggest

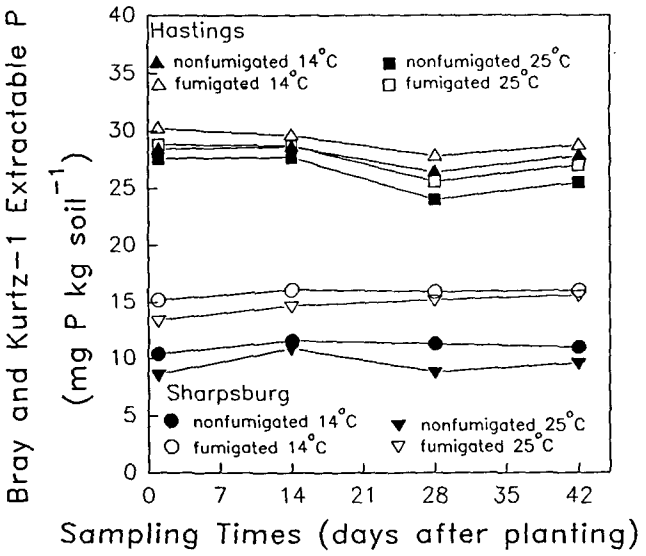

FIGURE 4. Changes in Bray and Kurtz-1 extractable P as affected by soil, fumigation, and temperature. Points are mean values of blocks and replicates (n=8).

that the Bray P1 pool was being replenished. The fumigated soils had dead biomass P, at least part of which may be extracted with the Bray P1-P fraction. Recovery of microbial biomass in fumigated soil would increase mineralization and immobilization activity over time.

Observations and measurement of lower plant P in fumigated soils (Jawson, 1993) and purpling seen at 25°C in this study do not seem to be related to plant-available P as measured by Bray P1. This implies that apparent plant P deficiency is due to plant uptake rather than to soil availability.

Acid Phosphatase Enzyme Activity

Acid phosphatase enzyme activity was consistently higher in non-fumigated than in fumigated soils, but did not differ between the two soil series or temperatures, therefore these results are averaged (Figure 5). Acid phosphatase enzyme activity had the same pattern for fumigated soil as for non-fumigated soil. An activity peak at 28d may have been due to exudation of the enzyme by plant roots at a time when plant demand was high. In contrast, Hedley et al. (1982) found increasing acid phosphatase activity through a 35 d study but used a smaller soil volume and higher planting density than in our study.

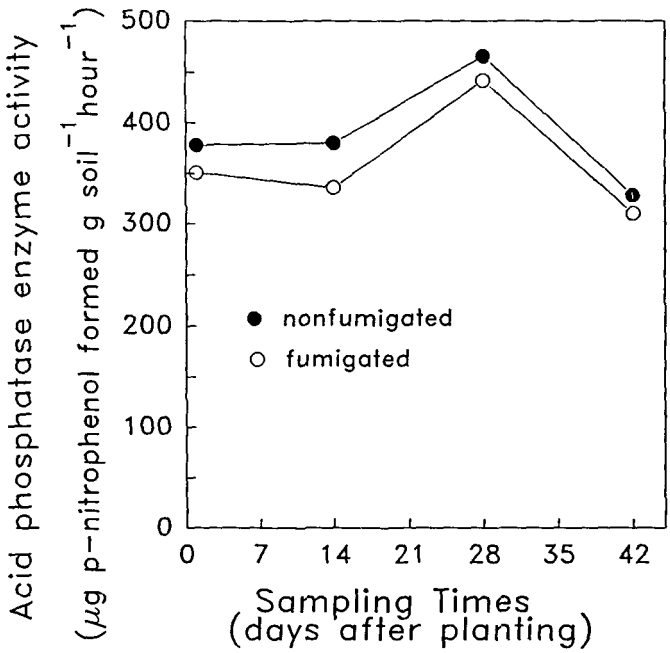

FIGURE 5. Changes in acid phosphatase enzyme activity with time as affected by fumigation. Points are mean values of soil series, temperatures, and replicates (n=32).

The difference between non-fumigated and fumigated soils may be attributed to the difference in microbial population or activity. It is not clear, however, why there was little difference in acid phosphatase activity between soil series. Despite the increase in biomass C/biomass P with time, microorganisms apparently did not respond by producing more acid phosphatase. While enzyme activity contributes to overall P availability, it appears that chemical factors or microbial factors other than phosphatase activity dominate the supply of Bray P1-extractable P in these soils.

The presence of residual phosphatase enzyme from both plant and microbial sources may help explain the apparent lack of temperature effect. The enzyme is extracellular and, once exuded by plant roots or microorganisms, can persist in a soil. Thus the phosphatase activity shown in Figure 5 is greatly influenced by the enzyme history, of these soils and less by their incubation temperature during the time of this study.

Vesicular Arbuscular Mycorrhizae (VAM) Colonization

Only fumigation affected VAM colonization rates of corn roots at 25°C (data not shown). Roots from non-fumigated soil at 25°C consistently had higher colonization rates (25-40%) than those in fumigated soil (10-25%). These observations are similar to those of Haas et al. (1987). Plants grown at 14°C so lagged in development behind those grown at 25°C that sufficient root material for analysis was recovered from the lower temperature treatments only at 42 d. At that time, Sharpsburg soil had 37% VAM colonization and Hastings soil had 9% VAM colonization. VAM colonization decreases as available soil P increases (Raju et al., 1990) which fits our observation because Hastings soil had higher Bray P1-extractable P than did Sharpsburg soil (Table 1). As noted earlier, visual P-deficiency symptoms were observed in the fumigated soils even though they had higher Bray P1 concentrations. The only difference between fumigated and non-fumigated soils that could account for this was mycorrhizal colonization rate. Mycorrhizae may well be important in initial P uptake by corn even where there is adequate soil P as measured by standard soil tests.

A slightly different experimental design could better assess the influence of microbial activity on P supply processes. While fumigation does decrease microbial population, it does not necessarily decrease microbial activity in the same proportion. Once the fumigant is dissipated, the surviving microorganisms are quite active. A treatment that both decreases the microbial population and continually suppresses its activity would be needed.

CONCLUSIONS

Plants grown in both soils had higher P uptake at 25°C, with the Hastings soil higher than the Sharpsburg soil at corresponding temperatures. Mycorrhizae colonization appears to assist plant uptake of available P. Fumigation decreased biomass C at all times and biomass P at most times within each soil. Sharpsburg soil consistently had more biomass C than did Hastings. Biomass C/Biomass P increased with time, implying lower microbial P uptake with time relative to microbial C. The Hastings soil had the higher Bray P1-extractable P of the two soils. Fumigation generally increased Bray P1-extractable P but without producing a parallel increase in plant P uptake. Thus observed P-deficiency symptoms are not due simply to soil P availability. While no measurements of root growth were taken, overall adaptation by the plants to P status of the different treatments was not observed. The increase in Bray P1-extractable P with fumigation was greater in the Sharpsburg soil at both temperatures. Fumigation decreased acid phosphatase enzyme activity and mycorrhizae colonization.

Future research will be directed to the interactions of microbial activity and soil P availability, e.g., which chemical pools are used by mycorrhizae versus other soil microorganisms and plants as well as the environmental effects (largely

temperature and soil water content) which govern the rates of transformations between pools. Controls on root growth and morphology in response to soil P status will have to be maintained to the extent possible. This will help to determine whether chemical fractionation can be used to assess biologically-available P.

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