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PHYSIOLOGICAL RESPONSES OF SWITCHGRASS (*PANICUM VIRGATUM* L.) TO ORGANIC AND INORGANIC AMENDED HEAVY-METAL CONTAMINATED CHAT TAILINGS

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ABSTRACT

Study plots established at the Galena subsite of the Cherokee County Superfund Site in southeastern Kansas by the U.S. Bureau of Mines in 1990 were examined during the summer of 1996 to determine whether physiological criteria could be used to determine suitability of switchgrass for remediation of heavy-metal contaminated substrates. Switchgrass was chosen because it was the most frequently encountered species on these plots. Treatment plots included a treatment control, an organic residue treatment of 89.6 Mg ha^{-1} composted cattle manure, and two inorganic fertilizer treatments recommended for either native grass or grass/legume mixtures. Plant response variables were photosynthetic rate, leaf conductance to water vapor, internal concentration of carbon dioxide in leaves, foliar transpiration rate, leaf water-use efficiency, predawn leaf xylem water potential, and midday leaf xylem water potential. Predawn and midday xylem water potentials were higher for grass/legume inorganic treatment than for the other inorganic treatments. Leaf conductances were lower for organically treated plots than those plots not organically amended and both photosynthesis and transpiration were lower for organically treated plots. Leaf conductances and transpiration were higher for grass/legume treated plots than for plots lacking inorganic treatment. Water-use-efficiency was higher for native grass-treated plots than for other inorganic treatments. Grass/legume inorganic treatment in absence of organic treatment increased predawn and midday xylem water potential, leaf conductance, photosynthesis, and transpiration. Organic amendment in absence of inorganic amendment increased predawn and midday xylem water potential.

Key words: *heavy-metal contamination; switchgrass, remediation*

INTRODUCTION

Solid waste produced by the mineral industry accounts for 80% of non-agricultural land-disposed solid waste in the U.S., potentially producing widespread human health and environmental risks (Veith et al., 1988). Although a number of options have been proposed for restoration of areas impacted by these wastes (Palmer, 1990), phytoremediation may present the most cost-effective means of restoring these areas. An abandoned mineral lands superfund site in Cherokee County in southeastern Kansas provides an opportunity to examine the establishment of vegetation on chat tailings produced by lead and zinc mining and smelting operations. Comparing chat tailings at this site with a typical soil for the region, Norland et al. (1990) found that chat tailings had 54% less organic matter and 26% less available phosphorus than did the native soil, suggesting that the addition of organic and inorganic amendment might aid the establishment of vegetation on chat tailings. Norland (1994a) determined plant density and coverage data on vegetation he established on plots amended with a variety of organic and inorganic treatments. Among the treatments, he found that composted cattle manure applied at the rate of 89.6 Mg ha^{-1} produced the highest total cover of warm-season and cool-season grasses and legumes and an inorganic fertilizer treatment recommended for grass/ legume mixtures produced a higher cover value than a fertilizer recommended for native grass establishment but not higher than unamended plots.

The purpose of the present study is to provide plant water potential and gas exchange data in addition to the vegetation data provided by Norland. The organic increase vegetative coverage resulting from the addition of organic amendments, could be the result of increased water potential and consequently the rates of photosynthesis, particularly under conditions of drought stress.

PROCEDURES

Study Plots

The study plots were established at Galena in Cherokee County, Kansas, in 1990 on an unremediated portion of a 285 km^2 Superfund Site on the National Priorities List. Results presented in this study are based on physiological measurement of plants sampled from a subset of plots included in a larger factorial experiment designed by Norland to investigate organic and inorganic amendment to chat tailings at this site. The subset used in this study consisted of six 2.5 X4.0 m plots comprising all combinations of two levels of organic amendment (none and composted cattle manure at the rate of 89.6 Mg ha^{-1}dry weight basis) and three levels of inorganic amendment (none, a fertilizer recommended for establishing native grasses [22.4 kg ha^{-1} nitrogen, 67.2 kg ha-1 phosphorus, and 89.6 kg ha^{-1} potassium] and a fertilizer treatment recommended for the establishment of grass/legume mixtures [44.8 kg ha^{-1} nitrogen, 112.9 kg ha^{-1} phosphorus, and 156.8 kg ha^{-1} potassium]) were selected. The plots had been seeded with a mixture of two cool season grasses, four warm season grasses and four leguminous forbs. One of the warm season grasses, *Panicum virgatum* was present in all plots and was used in this study. Soil moisture was monitored on selected plots by means of soil psychrometers (Wescor, Inc., Model PCT-55-15) and physiological data were collected following the period between July 25 and August 16, 1996, during which there was 5.6 mm of rainfall.

Gas Exchange Measurements

Photosynthesis, transpiration, stomatal conductance, and internal leaf CO_2 concentrations were determined by a Portable Photosynthesis System (LI-COR, Inc, Model 6200) operated in closed mode with a one-quarter liter chamber. In each of the six plots, four plants were randomly chosen. For each plant, three replicate sets of gas exchange data were collected for a pair of intact leaves (with a combined mean leaf area of 4.72 cm^2) to give a sample size of 12 for each plot. The measurement endpoint was reached for each replicate when a 5 ppm reduction in chamber CO_2 concentration had occurred. Water-use-efficiency was determined by dividing the rate of photosynthesis (mmol CO_2 m^{-2} s^{-1}) by the rate of transpiration (mmol H_2O m^{-2} s^{-1}).

Xylem Water Potential Measurements

Predawn and midday xylem water potential was determined by a Plant Water Status Console (Soilmoisture Equipment Corp., Model 3000). In each plot, one leaf for each of six randomly selected plants was excised for the determination of predawn xylem water potential and one leaf from each of six plants was excised for determination of midday xylem water potential. Time between excision and

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determination was approximately two minutes. Water loss was minimized by enclosing the excised leaf blade in a plastic bag containing moist paper towel. Differences between predawn and midday xylem water potential were also determined.

Statistical Analysis

Statistical analysis was conducted using the Statistical Analysis System (SAS Institute, Inc.,1982). All physiological responses were analyzed as a 2X3 factorial ANOVA with organic and inorganic treatments as the main factors. Interactions between main factors were also examined. In those cases in which a main factor was determined to be significant (probability level of $p<$ or $= 0.05$), the Fishers LSD test was used for multiple comparisons among treatment levels.

RESULTS

Predawn and midday xylem water potentials (Figure 1) were not significantly different for manured compared to non-manured treatments. Grass/legume inorganic treatments (Figure 2) produced higher (less negative) predawn and midday water potentials than the other inorganic treatments (Table 1). Differences between midday and predawn xylem water potentials (MD-PD) were not significant for any of the treatment comparisons. All treatments were at or below permanent wilting percentage of -1.5 MPa, indicating that plants in all six treatments were being subjected to drought stress.

Leaf conductances were higher for non-manured than for manured treatments (Figure 1) and the grass/legume inorganic treatment (Figure 2) had higher conductances than for those that were not amended with inorganic fertilizer (Table 1). Rates of photosynthesis were higher for non-manured than manured treatments but were not different among inorganic treatments. Internal carbon dioxide concentrations were higher for manured than non-manured treatments and higher for grass/legume inorganic treatment than for other inorganic treatments. Transpiration rates were higher for non-manured than manured treatments and higher for the grass/legume inorganic treatment than for other inorganic treatments. There were no differences in water-use efficiency (Figure 1) between manured and non-manured treatments, but among inorganic treatments (Figure 2) the native grass treatment produced a higher water-use efficiency than other inorganic treatments.

In addition to main factor comparisons, the 2X3 factorial ANOVA indicated significant interactions between organic and inorganic treatments for photosynthesis, transpiration, conductance, predawn xylem water potential, and mid-day xylem water potential (Table 1 and Figure 3). For all measured variables that were significant, responses to the two organic treatments by plants receiving native inorganic amendment were different. Plants receiving organic amendments had lower (more negative) predawn and midday xylem water potentials, lower conductances, and lower rates of photosynthesis and transpiration than those without organic amendment. In addition, those plants without inorganic amendment but with organic amendment had higher (less negative) predawn and midday xylem water potentials than those lacking both inorganic and organic amendments.

DISCUSSION AND CONCLUSIONS

Norland (1991) indicated for the first year of his study at Galena that composted cattle manure applied at the rate of 89.6 Mg ha-1 increased total density and cover of all species compared to control plots with *P. virgatum* having the highest density of all species encountered. He found no difference in total density or cover among fertilizer treatments, however. Approximately 3.5 years after establishment of the plots, Norland (1994a) showed that this rate of amendment with cattle manure continued to have higher total cover. Native grass fertilizer treatment produced higher total cover than grass/legume fertilizer-treated plots but neither were different from unfertilized plots. Norland (1994a) showed that tailings, organically amended at 89.6 Mg ha^{-1}, had reduced levels of bioavailable Cd, Pb, and Zn and increased levels of non-bioavailable fractions of these same metals.. Addition of organic residues to chat tailings had no effect on total nitrogen or phosphorus levels but increased levels of potassium (Norland, 1991).

In the current study, effects of organic and inorganic amendments were examined as main factors among plots receiving all possible combinations of two levels of organic and three levels of inorganic amendments. Data indicate that the organic amendment does not improve xylem water potential for switchgrass. Leaf conductances for organically amended plots were lower than for unamended plots, providing a means of reducing water loss and drought stress but at the cost of reduced rates of photosynthesis. Higher internal carbon dioxide concentrations reflected the reduced rates of photosynthesis.

The above observation is not consistent with Norland's 1994 observations that 89.6 Mg ha^{-1} increased total cover, suggesting that organic amendment would increase photosynthesis and productivity. One factor that may have been different from the period of Norland's observation of these plots is the conditions of drought that occurred during the present study. Although the amount of precipitation that occurred during the current study was approximately the same as the average for the years of Norland's observation (1220 mm compared to 1234 mm), the 23 days from July 25 to August 16, 1996, when physiological measurements were made, saw 5.6 mm precipitation and leaf xylem water potentials were below -1.5 MPa, the permanent wilting percentage for most vascular plants. Coverage and density values are more of an indication of overall conditions for a longer period (1990-1993).

Among inorganic amendments, the grass/legume treatment produced higher (less negative) predawn and midday xylem water potentials than other inorganic treatments. This was not consistent with higher total coverage of all species for native grass treatments reported by Norland (1994a). Leaf conductances were higher for grass/legume treated plots than untreated plots which translated into higher rates of transpiration but not photosynthesis. Curiously, internal carbon dioxide concentrations were higher for the grass/legume inorganic treatments though there were no differences in rates of photosynthesis among inorganic treatments. Of all inorganic treatments, fertilizer recommended for native grass provided higher water-use efficiency than did either of the other inorganic treatments.

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Higher water-use efficiency may have accounted for the higher total coverage for native grass-treated plots reported by Norland (1994a).

The 2X3 Factorial ANOVA provided an opportunity to examine interactions among main factors. Native grass fertilizer in the absence of organic amendment increased predawn and midday xylem water potentials. Higher xylem water potentials were responsible for higher leaf conductances and consequently, higher rates of transpiration and photosynthesis without a change in water-use efficiency. Based on Norland's analyses, I had anticipated an increase in survival on organically amended soils. However, the present study indicates that in the absence of fertilizer xylem, water potential was higher (less negative) in organically amended plots but also higher in native grass-fertilized plots in the absence of organic amendment. Although there was little difference in gas exchange between organic treatments in absence of fertilizer, the application of native grass fertilizer increased rates of gas exchange in absence of organic amendment.

Norland (1994) found no significant interactions between composted cattle manure applied at the rate of 89.6 Mg ha^{-1} and any of the three fertilizer treatments based on total cover. He did find, however, that at an application rate of 44.8 Mg ha^{-1}, there was an interaction between grass/legume fertilizer and organic amendment with a reduction in total cover compared with native grass fertilizer or no fertilizer.

In conclusion, results of this study do not suggest a reason for the higher cover of *Panicum virgatum* on organically amended chat tailings. Under conditions of drought stress, there was an observable physiological advantage of higher (less negative) predawn and midday xylem water potentials provided by organic amendment only if inorganic amendment was not present. A greater advantage was provided by the use of native grass fertilizer which provided an even greater increase in xylem water potential, both predawn and midday xylem water potential accompanied also by increased leaf conductance and rates of photosynthesis and transpiration.

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Figure 1. Physiological Responses of *Panicum virgatum* to Organic Amendments. Each bar represents the mean of 36 gas-exchange measurements or 18 xylem water potential determinations. Unlike letters at the top of each group of bars indicate significant differences (p< or =0.05).

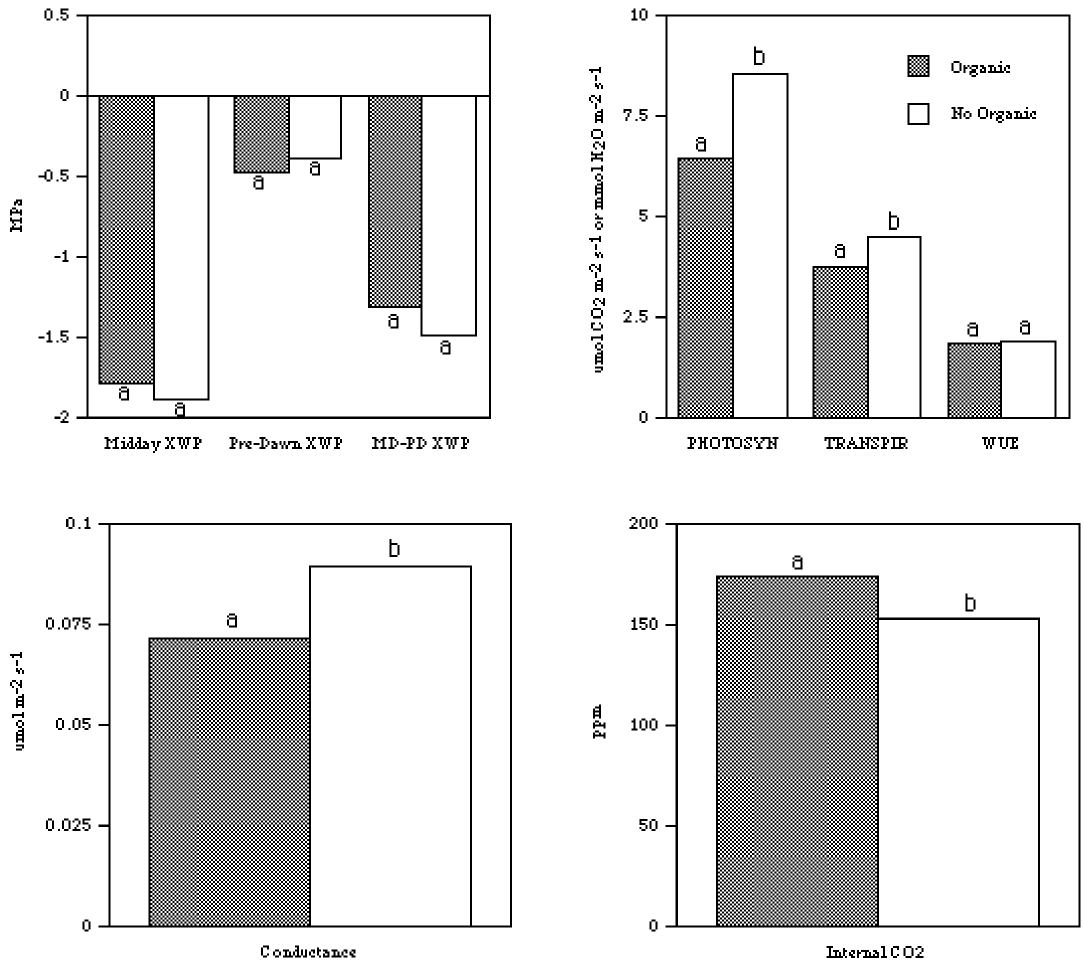

Figure 2. Physiological Responses of *Panicum virgatum* to Inorganic Amendments. Each bar represents the mean of 24 gas-exchange measurements or 12 xylem water potential determinations. Unlike letters at the top of each group of bars indicate significant differences (p< or =0.05).

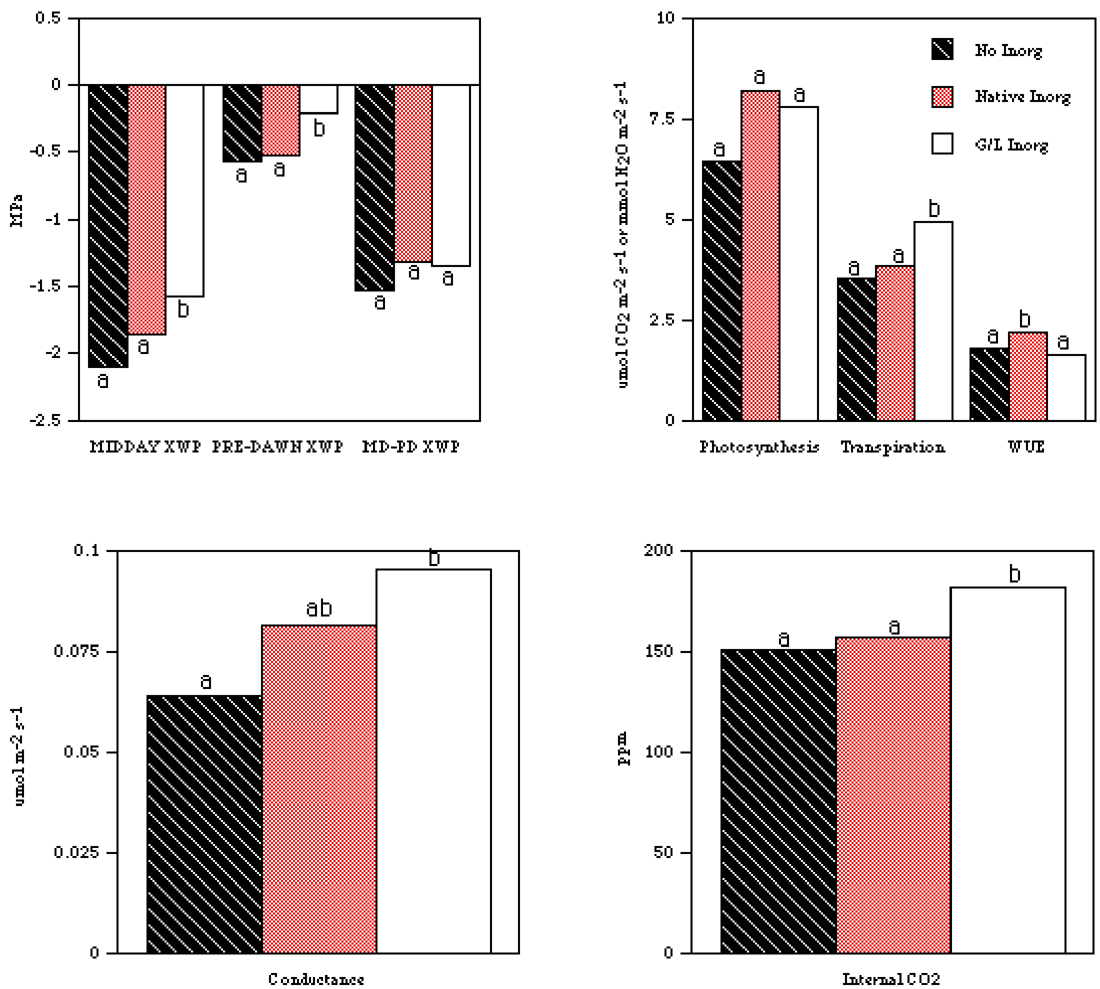

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Figure 3. Physiological Responses of *Panicum virgatum* Showing Interactions Between Organic and Inorganic Amendments. Each sample point represents the mean of 18 gas-exchange measurements or 9 xylem water potential determinations.