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Journal of Environmental Science and Health, Part B Pesticides, Food Contaminants, and Agricultural Wastes

Publication details, including instructions for authors and subscription information:
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To cite this Article: , 'Effects of glyphosate and foliar amendments on activity of
microorganisms in the soybean rhizosphere', Journal of Environmental Science and
Health, Part B, 42:2, 125 - 132

xxxx:journal To link to this article: DOI: 10.1080/03601230601123227

URL: <http://dx.doi.org/10.1080/03601230601123227>

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Effects of glyphosate and foliar amendments on activity of microorganisms in the soybean rhizosphere

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A field study was conducted to determine the effects of glyphosate on microbial activity in the rhizosphere of glyphosate-resistant (GR) soybean and to evaluate interactions with foliar amendments. Glyphosate at 0.84 kg ae ha⁻¹ was applied GR soybean at the V4–V5 development stages. Check treatments included a conventional herbicide tank mix (2003 study only) and no herbicides (hand-weeded). Ten days after herbicide application, a commercially available biostimulant and a urea solution (21.0% N) were applied to soybean foliage at 33.5 mL ha⁻¹ and 9.2 kg ha⁻¹, respectively. Soil and plant samples were taken 0, 5, 10, 15, 20 and 25 days after herbicide application then assayed for enzyme and respiration activities. Soil respiration and enzyme activity increased with glyphosate and foliar amendment applications during the 2002 growing season; however, similar increases were not observed in 2003. Contrasting cumulative rainfall between 2002 and 2003 likely accounted for differences in soil microbial activities. Increases in soil microbial activity in 2002 suggest that adequate soil water and glyphosate application acted together to increase microbial activity. Our study suggests that general soil microbial properties including those involving C and N transformations are not sensitive enough to detect effects of glyphosate on rhizosphere microbial activity. Measurements of soil-plant-microbe relationships including specific microbial groups (i.e., root-associated *Fusarium* spp.) are likely better indicators of impacts of glyphosate on soil microbial ecology.

Keywords: Dehydrogenase activity; respiration; microbial ecology; herbicides; rhizosphere microorganisms; biostimulants.

Introduction

Glyphosate [*N*-(phosphonomethyl)glycine, Roundup[®]], a broad spectrum, nonselective herbicide for post-emergent control of a wide range of weeds,^[1] is the most widely used herbicide due to the introduction and broad acceptance of genetically-modified (GM), glyphosate-resistant (GR) crop varieties in the late 1990's. On a global basis, soybean is the most prevalent GM crop in GR cropping systems, planted on 60% of the global GM-cropped land in 2005.^[2]

Glyphosate is systemic and not readily metabolized by plants; it is translocated and may accumulate in meristematic regions including roots and nodules.^[3–5] Glyphosate that accumulates in the roots of susceptible plants is eventually released into the rhizosphere.^[6,7] Field and laboratory studies have shown that glyphosate directly increases soil bacterial and fungal populations, possibly serving as a nutrient source for microbial growth.^[8–12] Glyphosate may

also be toxic to some bacteria and fungi possibly due to inhibition of metabolic pathways.^[9,12–14] Thus, the direct net effect of glyphosate application on general microbial indicators may be confounded because selected populations may be stimulated while others are suppressed.

Glyphosate application may indirectly alter the root environment by triggering a plant response resulting in “atypical root exudations.”^[15] Atypical root exudates affect rhizosphere microbial activity. GR crops treated with glyphosate may be altered in root growth and development.^[16] Inhibition of aromatic amino acid synthesis and phytoalexin production by glyphosate may also weaken and predispose plants to pathogenic microbial invasion.^[15,17]

Foliar-applied biostimulants (products containing plant hormones and other organic and inorganic compounds) and liquid fertilizers affect soil microbial activity. Grozyme[®] (boric acid, cobalt sulfate, copper sulfate, ferric nitrate, manganese nitrate, sodium molybdate, zinc nitrate and “enzyme systems”) and PT-21[®] (21% nitrogen in urea form) are foliar amendments used to increase crop yield.^[18] These foliar amendments provide nutritional or metabolic augmentation that may affect rhizosphere microbial activity resulting in improved crop growth and productivity.^[18–21] These foliar amendments may influence plant physiology and biology and offset potential adverse

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Received June 14, 2005.

effects of glyphosate on microbial activity in the soybean rhizosphere.

Because of the current widespread use of glyphosate-resistant (GR) cropping systems, concern has developed over potential impacts of glyphosate on rhizosphere microbial activity and subsequent effects on crop productivity. Our research objective was to characterize the effects of glyphosate on rhizosphere microorganisms by monitoring enzymatic and respiration activities in field-grown soybean. Interactions of glyphosate with a biostimulant (Grozyme[®]) and foliar applied liquid fertilizer (PT-21[®]) on microbial activity were also investigated.

Materials and methods

Experimental design

The field experiment was conducted in 2002 and repeated in 2003 at the University of Missouri Bradford Research and Extension Center (38°53'N, 92°12'W) located 17 km east of Columbia, MO. GR soybean ('DeKalb DKB38-52') was planted in 76-cm rows on a disked Mexico silt loam (fine, smectitic, mesic Aeric Vertic Epiaqualfs) fertilized and managed consistent with recommended practices.^[22] Individual main plots were 6.1 m × 3.7 m. The experimental design was a repeated-measure (6 sample dates) split-plot arrangement with 2 herbicides (main plots): glyphosate (Roundup Ultra Max[®]) and a tank-mix of clethodim (Reflex 2LC[®]) and fomesafen (Select 2EC[®]) in 2003. Subplots consisted of four foliar amendments (subplots): urea solution at 21% nitrogen (PT-21[®]), a biostimulant consisting of boric acid, cobalt sulfate, copper sulfate, ferric nitrate, manganese nitrate, sodium molybdate, zinc nitrate and enzyme systems (Grozyme Z-93[®]), a combination of urea solution and biostimulant; and no foliar amendment. Therefore, a total of eight treatments with four field replications were investigated. Herbicides were applied to appropriate block strips at a 130 L ha⁻¹ spray volume at pressure of 138 Kpa using 11003 nozzles (Spraying Systems¹). Glyphosate at 0.84 kg a.e. ha⁻¹ was applied to appropriate block strips when soybeans were at the V4-V5 growth stage.^[23] Main plots not receiving glyphosate were not treated in 2002; in 2003, plots received a post-emergence application of clethodim (0.42 a.i. kg ha⁻¹) + fomesafen (0.175 a.i. kg ha⁻¹) with 1.101 L crop oil concentrate as a surfactant. At 10-days post-herbicide application, urea solution (9.2 kg ha⁻¹), biostimulant (33.5 mL ha⁻¹) and a combination of urea solution + biostimulant were foliar-applied to appropriate experimental units with a backpack sprayer.

Rhizosphere soil samples were obtained by excavating plant roots and associated soil from the outer rows of each plot. Soil adhering to the roots was removed by shaking and used for respiration and enzyme assays. Samples were taken

0, 5, 10, 15, 20 and 25 days after herbicide application. Three samples per experimental unit were taken and thoroughly mixed to generate a composite sample. Soil moistures were determined gravimetrically and all results are expressed on an oven dry weight basis.

Enzymological analyses

Dehydrogenase activity based on the reduction 2,3,5-triphenyltetrazolium chloride (TTC) was used to estimate respiration of viable microorganisms based on the method of Casida.^[24] Soil β -glucosaminidase activity was assessed as an indicator of soil N mineralization according to the method developed by Parham and Deng.^[25] Soil β -glucosidase was assessed as an indicator of soil C mineralization according to the method of Tabatabai.^[26]

Soil respiration

Substrate induced respiration (SIR) is an indicator of potential soil microbial respiration.^[27] For our study, 5 g soil adjusted to 25% water content in Hungate tubes was amended with 1.0 ml of 25% glucose solution, incubated at 25°C, and evolved CO₂ from the headspace was quantified at 3 and 7 days using a gas chromatograph (Buck Scientific Model 910), with He carrier gas at a flow rate of 14 mL L⁻¹ through a silica gel column at 50°C and a retention time of 6 minutes. Total CO₂ evolved over the 7-day incubation periods was determined from known calibration standards.^[28]

Statistical analysis

Data presented are mean values of four independent field replicates. A General Linear Model was used to analyze the data. Analysis of Variance (ANOVA) and mean separations [Fisher's Protected least significant difference (LSD)] were performed using Statistical Analysis System (SAS) software.

Results

Because non-glyphosate treated plots in 2002 were infested with dense weed stands, a post-emergence conventional herbicide was applied for weed control in 2003. We assumed this practice would have little effect on rhizosphere microbial activity based on previous studies that showed other non-glyphosate herbicides did not affect microbial populations in the soybean rhizosphere.^[29] Differences in weather conditions between 2002 and 2003 likely contributed to microbial activity differences observed between years. Reduced precipitation in 2003 (7.5 cm during the growing season) coupled with higher precipitation in 2002 (21 cm) during the sample period partly explain the variable trends in microbial activity observed across years.

Dehydrogenase activity

Rhizosphere soil of plants treated with glyphosate had greater dehydrogenase activity 25 days after application

¹Trade names are used for clarity and do not represent endorsement by USDA-ARS or the University of Missouri.

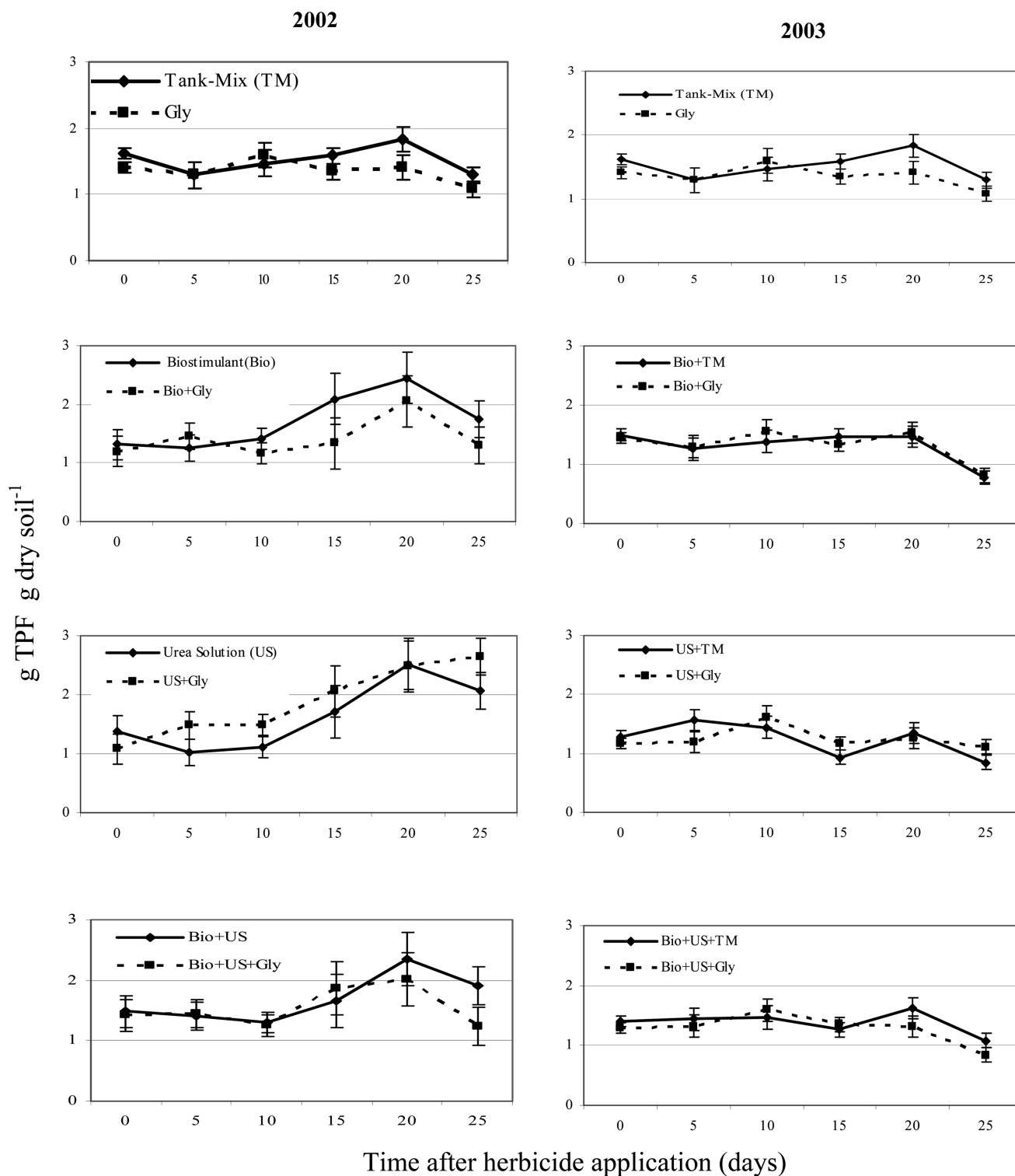


Fig. 1. Dehydrogenase activity in rhizosphere soils of soybean following herbicide and foliar amendment applications. Foliar amendments were applied 10 days after herbicide application. Bars indicate least significant difference (LSD) ($p < 0.05$).

compared to no herbicide application in 2002 (Fig. 1). In 2003 no differences in dehydrogenase activity were detected between the herbicide treatments. In 2002, dehydrogenase activity increased in rhizospheres of plants treated with urea solution and biostimulant compared with plants receiving

no herbicide or either foliar amendment. Plants treated with glyphosate followed by urea solution had greater dehydrogenase activity at 25 days after glyphosate application; however, the biostimulant decreased activity when combined with glyphosate.

β -Glucosaminidase

In 2002, rhizosphere soil of plants treated with glyphosate had greater β -glucosaminidase activity than rhizosphere soil of plants with no herbicide applied (Fig. 2). No

consistent differences were detected between the foliar amendments across years. No differences between herbicide treatments in β -glucosaminidase activity were observed in 2003.

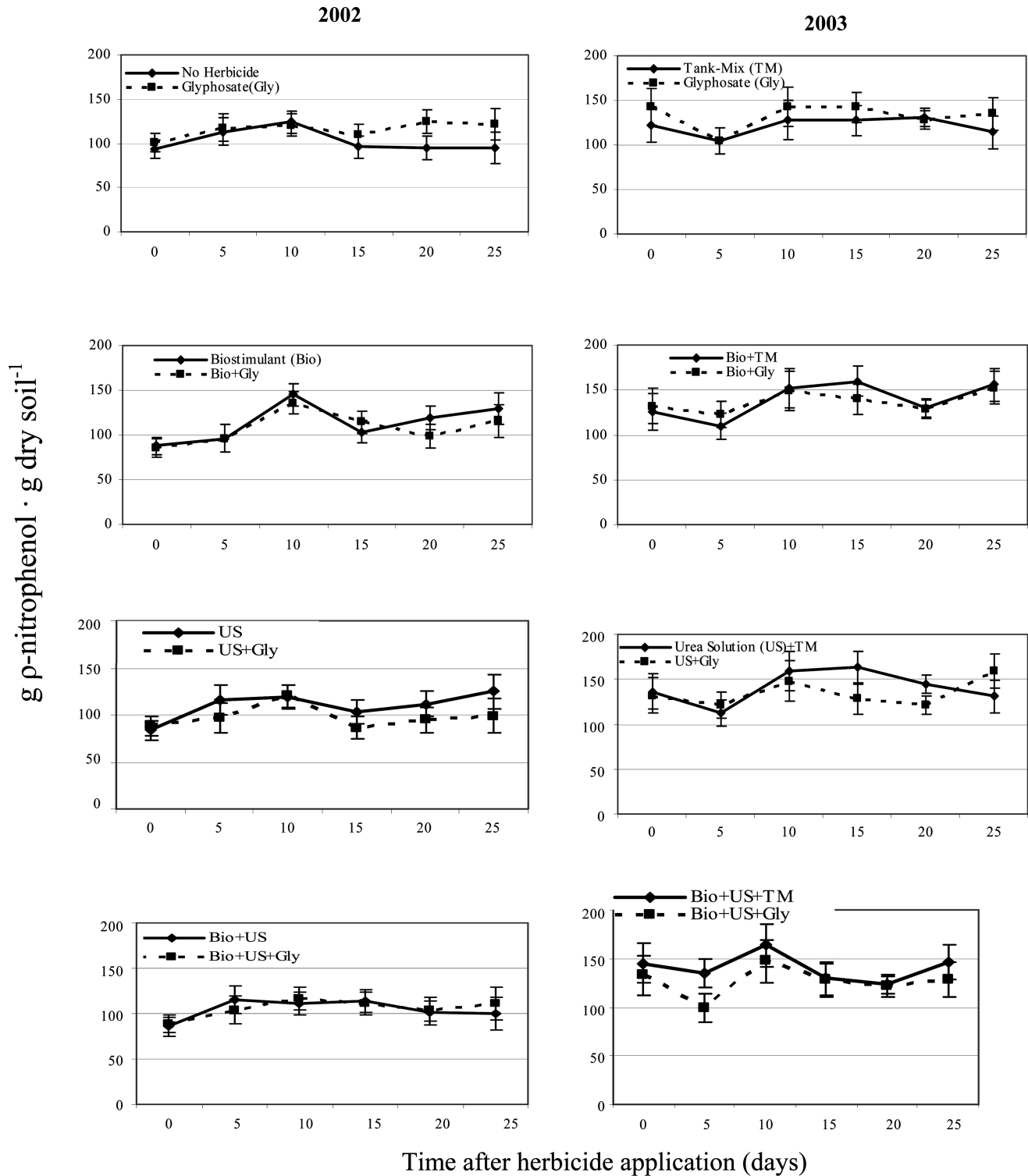


Fig. 2. β -Glucosaminidase activity in rhizosphere soils of soybean following herbicide and foliar amendment applications. Foliar amendments were applied 10 days after herbicide application. Bars indicate least significant difference (LSD) ($p < 0.05$).

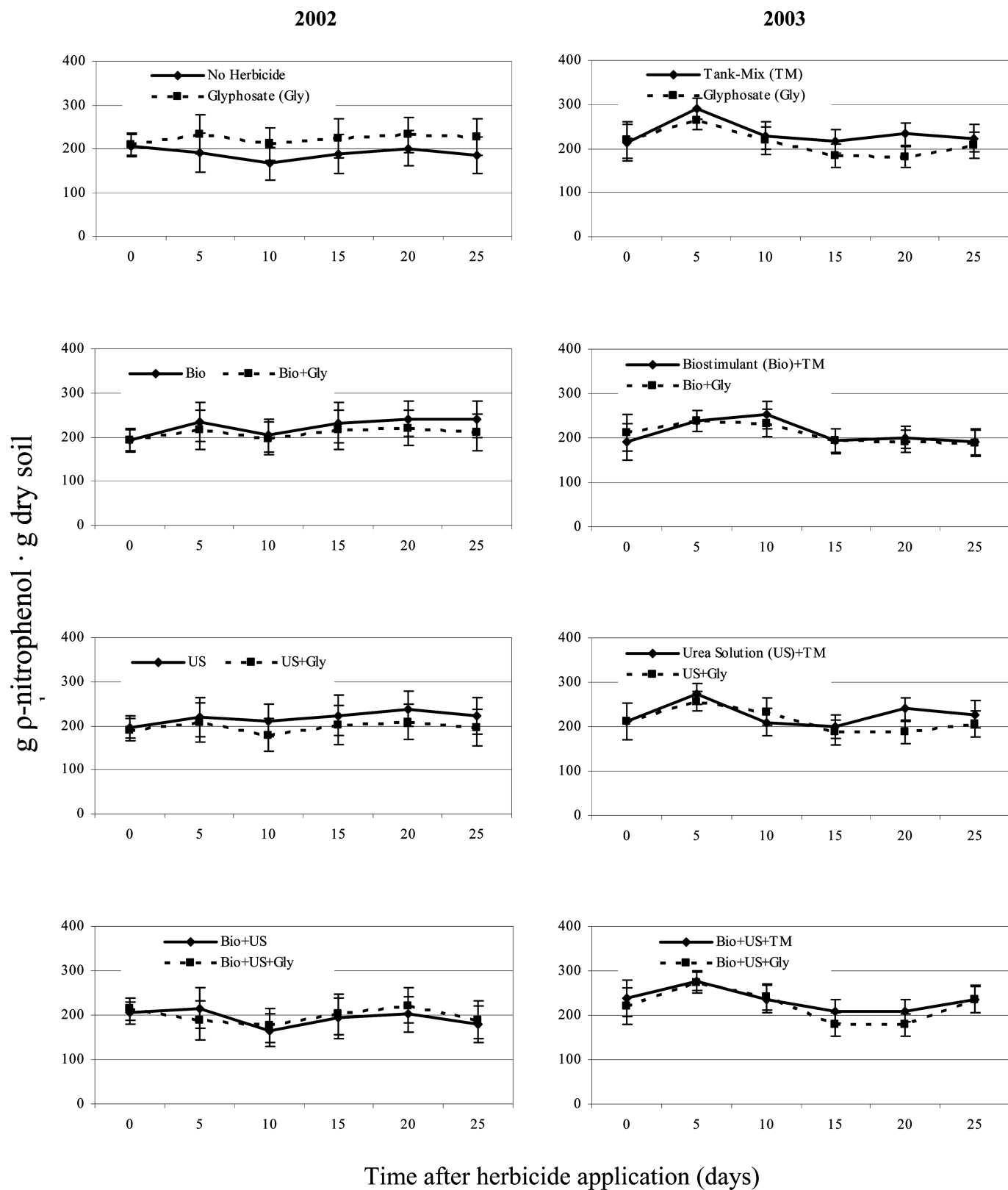


Fig. 3. β -Glucosidase activity in rhizosphere soils of soybean following herbicide and foliar amendment applications. Foliar amendments were applied 10 days after herbicide application. Bars indicate least significant difference (LSD) ($p < 0.05$).

β -Glucosidase

In 2002, β -glucosidase activity in rhizosphere soils did not differ among treatments (Fig. 3). In 2003, β -glucosidase activity in soils from plants treated with the tank-mix herbicides was higher compared with glyphosate application at 20 days after herbicide application. No consistent trends or differences due to urea solution, biostimulant, or urea + biostimulant were noted in 2002 or 2003.

Substrate induced respiration

Substrate induced respiration (SIR) gradually increased during the first 20 days of sampling before leveling off in 2002 (Fig. 4). Rhizosphere soils from plants treated with glyphosate exhibited increased respiration levels compared with soils of plants not treated with herbicides 20 days after herbicide application in 2002. Biostimulant and urea applied at 10 days post-glyphosate application had no effect

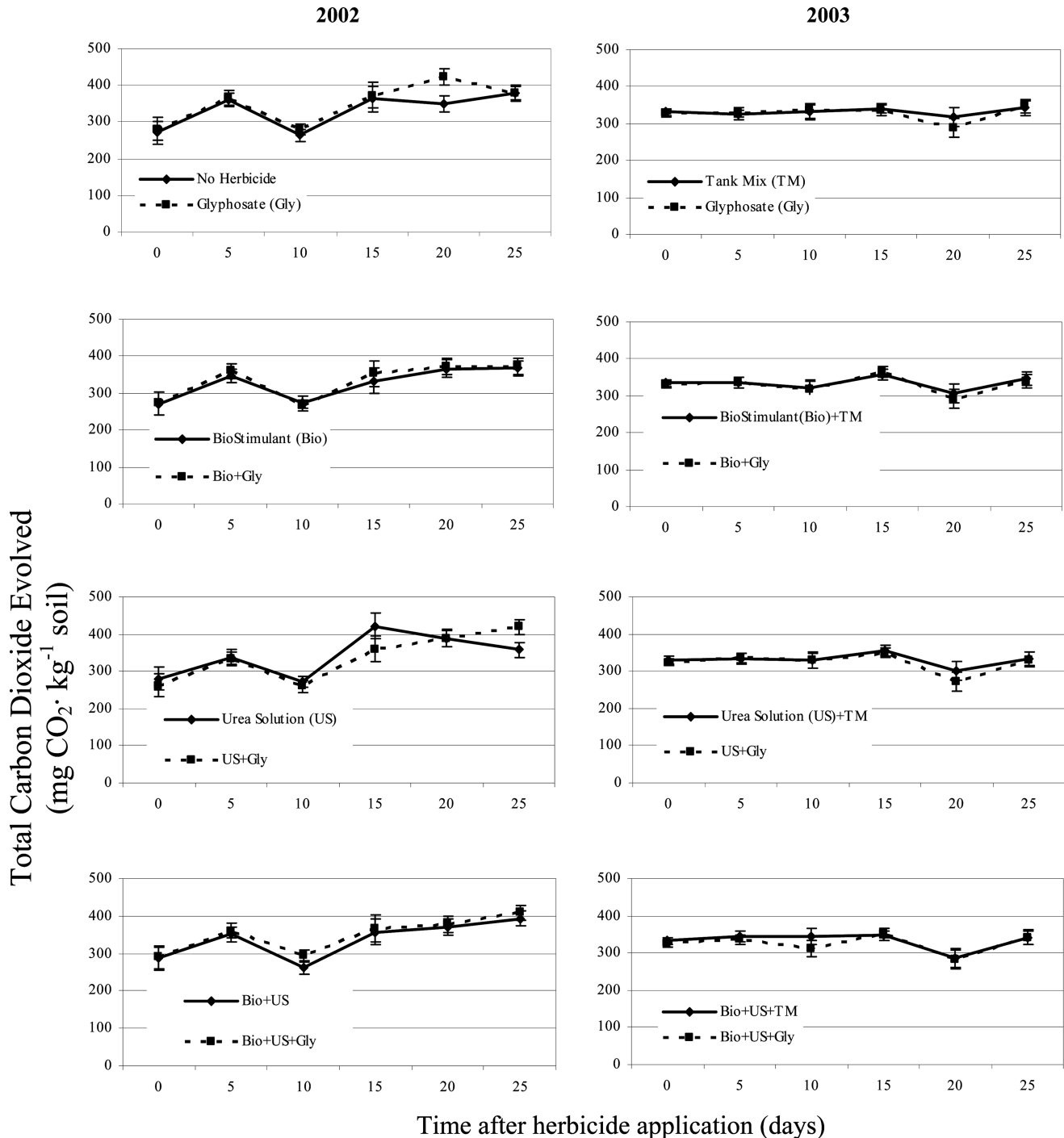


Fig. 4. Substrate induced respiration activity in rhizosphere soils of soybean following herbicide and foliar amendment applications. Foliar amendments were applied 10 days after herbicide application. Bars indicate least significant difference (LSD) ($p < 0.050$).

on SIR. There were no consistent differences between treatments in 2003.

Discussion

The limited increases in rhizosphere enzymatic and respiration rates by foliar treatments (herbicides and amendments) may reflect only slightly increased microbial activity.^[26,30] Increased rhizosphere microbial activity may be beneficial or detrimental toward plant growth, soil microbial ecology, and soil quality. Beneficial effects include optimum plant growth and production due to greater availability of nutrients, resulting from mineralization mediated by rhizosphere microorganisms.^[19,21,31] Increased microbial activity and high microbial populations may also sequester plant nutrients in microbial biomass, decrease crop growth and yields, and increase susceptibility to diseases and pests.^[30] Future studies should clarify further if changes in microbial activity and populations due to glyphosate and foliar amendments are beneficial or detrimental for crop productivity and soil ecology by monitoring specific plant-microbe-soil interactions (i.e., legume nodulation by rhizobia) rather than individual general activities (i.e., microbial respiration).

Rhizosphere microbial activity may increase due to increased root exudation or altered chemical composition of the exudates. Herbicides that alter root growth and morphology subsequently affect rhizosphere microbial activity.^[15,32] Physiological plant responses associated with herbicides or foliar amendments combined with the translocation and release of herbicide components into the rhizosphere may synergistically increase rhizosphere microbial activity. This might explain general increases in microbial activity by glyphosate alone in 2002. However, inadequate precipitation during 2003 likely reduced soybean root growth and rhizosphere microbial activity and obscured treatment effects like those detected in 2002.^[33–35]

In 2002, increased microbial activity in the rhizosphere of soybeans treated with glyphosate was similar to results of previous studies that showed glyphosate, translocated and released into the rhizospheres of susceptible plants, was subsequently available to microorganisms for metabolism.^[12,36] Kremer et al.^[37] found that exudation of glyphosate into the rhizosphere by GR soybean coincided with release of high carbohydrate concentrations after application of glyphosate. Thus, a combination of glyphosate and carbohydrates released by glyphosate-treated GR soybean may explain the transient increases in enzyme activity observed in 2002 rhizosphere samples.

Conclusions

Our research confirms that foliar-applied herbicides and amendments may alter soybean rhizosphere microbial activity; however, detectable effects are greatly influenced by

other conditions such as cumulative seasonal rainfall. Altered rhizosphere microbial activity may also be related to plant physiological effects relative to translocation and release of herbicides and other compounds into the rhizosphere. Enzyme and SIR activity levels suggest that foliar application of glyphosate increases rhizosphere microbial activity under optimum field conditions. Kremer et al.^[37] suggested that the release of glyphosate into the rhizosphere could shift microbial populations to those that utilize glyphosate as a nutrient source. Foliar amendments, as applied in our study, seemed to have transient increases on rhizosphere microbial activity and interactions with the herbicide treatments. Further studies are necessary to determine the precise mechanism for the increases: altered root morphology, translocation and release of specific compounds, or a combination of mechanisms. Further, the assays used in this study are for indications of general or overall soil microbial activity. Such general microbial measurements were recently found to be similar in both GR and conventional soybean cropping systems,^[38] suggesting these general parameters are not sensitive to treatment-specific effects. Therefore, future studies should be designed to investigate plant-microbe-soil interactions rather than individual general activities to detect effects of GR soybean on specific microbial components or functions within the overall microbial community. For example, the relationship of glyphosate released into the rhizosphere by GR soybean on root colonization by *Fusarium* spp. could be an informative specific indicator.^[37] This information could then be applied in improving management of GR crops to minimize potential adverse effects on crop production and the soil ecosystem.

Acknowledgments

This research was supported by Ag Spectrum Co. Dewitt, Iowa and USDA Special Grant Number 2003-06156. The authors are grateful to Tim Reinbott, Heidi Lewis, Atim Enyenihi, and Michael Atkinson for technical assistance.

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