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Soil Microbial Activity Is Affected by Roundup WeatherMax and Pesticides Applied to Cotton (*Gossypium hirsutum*)

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Adoption of glyphosate-based weed control systems has led to increased use of the herbicide with continued use of additional pesticides. Combinations of pesticides may affect soil microbial activity differently than pesticides applied alone. Research was conducted to evaluate the influence of glyphosate-based cotton pest management systems on soil microbial activity. Soil was treated with commercial formulations of trifluralin, aldicarb, and mefenoxam + pentachloronitrobenzene (PCNB) with or without glyphosate (applied as Roundup WeatherMax). The soil microbial activity was measured by quantifying C and N mineralization. Soil microbial biomass was determined using the chloroform fumigation−incubation method. Soils treated with glyphosate alone exhibited greater cumulative C mineralization 30 days after treatment than all other treatments, which were similar to the untreated control. The addition of Roundup WeatherMax reduced C mineralization in soils treated with fluometuron, aldicarb, or mefenoxam + PCNB formulations. These results indicate that glyphosate-based herbicides alter the soil microbial response to other pesticides.

KEYWORDS: C mineralization; cotton; glyphosate; *Gossypium hirsutum*; microbial activity; N mineralization; pesticides; soil microbial biomass

INTRODUCTION

Cotton production typically requires intensive chemical management of weeds (*1*), insects (*2*), and diseases (*3*). However, varieties of cotton and other crops have recently been genetically engineered to reduce reliance on pesticides that pose environmental or health risks, specifically herbicides (*4*) and insecticides (*5*). The introduction of glyphosate-tolerant crops in 1996 (*6*) had a tremendous impact on weed management (*7*). From an environmental perspective, the use of glyphosate {*N*-(phosphonomethyl)glycine} would be preferred over other herbicides that exhibit greater soil mobilities, half-lives, or mammalian toxicities. However, glyphosate-based weed control systems have resulted in extensive use of the herbicide, with multiple applications in a single growing season becoming common (*8*).

Glyphosate may increase microbial activity because the herbicide's low carbon:nitrogen ratio represents a desirable balance of those nutrients for soil microbes (*9*). Glyphosate is rapidly degraded by soil microbes, presumably by cometabolism (*10*, *11*), resulting in increased microbial biomass and activity (*9*, *12*). Additionally, glyphosate application coincidental to soil-active pesticides may modify pesticide behavior, particularly with regard to microbial degradation. For example, when glyphosate and atrazine {6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-1,3,5-triazine-2,4-diamine} were applied simultaneously to soil samples, microbial activity was generally enhanced, although atrazine degradation rates varied (*13*). Atrazine degradation was reduced by the presence of glyphosate 8 and 12 days after herbicide application (*13*). However, after 14−28 days, atrazine degradation was enhanced when glyphosate was present in the soil (*14*).

In cotton production systems, the herbicides trifluralin [2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine] or fluometuron [*N*,*N*-dimethyl-*N*'-{3-(trifluoromethyl)phenyl}urea], the fungicides PCNB (pentachloronitrobenzene), and mefenoxam {methyl *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)-D-alaninate}, and the insecticide−nematicide aldicarb [2-methyl-2-(methylthio)propanal *O*-{(methylamino)carbonyl}oxime] may be present in the soil at the time of glyphosate application. These products are metabolized, to varying degrees, by soil microbes (*15*−*17*) and may alter the activity of the soil microbial community. The effect of a commercially available glyphosate formulation on the response of carbon and nitrogen mineralization and soil microbial biomass to trifluralin, fluometuron, PCNB, mefenoxam, and aldicarb was evaluated.

MATERIALS AND METHODS

Soil. The soil used in this study was a Weswood clay loam (fine-silty, mixed superactive, thermic Udifluventic Haplustept). Bulk soil was collected from a fallow field that was previously planted with cotton

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Table 1. Selected Properties of Soils Used in the Study[a]

soil[a]	clay (%)	pH	O.M. (%)	μg mL^{-1} NO_3	P
pasture	34	7.6	6.2	4	52
cultivated field	32	8.0	1.4	20	38

[a] Soil was Weswood clay loam collected from a bermuda grass pasture and a fallow field previously planted with cotton.

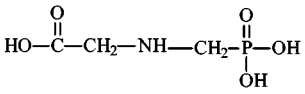
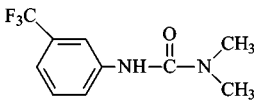
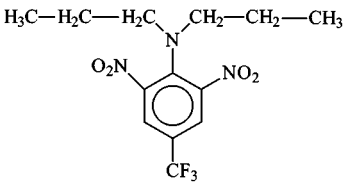
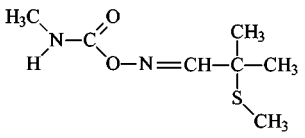
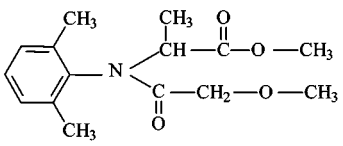
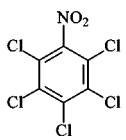
and from a bermuda grass pasture. Soil characteristics are presented in **Table 1**. Soils were air-dried and passed through a 2 mm sieve prior to the beginning of the experiment.

Chemicals. Commercial formulations of glyphosate (Roundup WeatherMAX, Monsanto Co., St. Louis, MO), trifluralin (Treflan HFP, Dow AgroSciences, Indianapolis, IN), fluometuron (Cotoran 4L, Griffin L.L.C., Valdosta, GA), PCNB + mefenoxam (Ridomil Gold PC GR, Syngenta Crop Protection, Inc., Greensboro, NC), and aldicarb (Temik brand 15G, Bayer CropScience, Research Triangle Park, NC) were used (**Table 2**). Pesticides will be referred to by their common names throughout this paper. Reagents, except HCl, were obtained from Fisher Scientific (Fair Lawn, NJ). Hydrochloric acid was obtained from LabChem, Inc. (Pittsburgh, PA).

Sample Preparation. Fifty-five grams of dry weight equivalent soil was placed in each gastight container containing 1 M KOH to trap evolved CO_2, and soils were rewetted to approximately 50% water-filled pore space (20% gravimetric water content). Samples were incubated in the dark at 30 °C for 7 days to allow stabilization of the soil microbial biomass following the initial flush of activity after rewetting (*18*). Potassium hydroxide traps were then removed, and pesticide treatments were applied. Pesticides were applied in water to bring the final gravimetric water content to 25%. Rates of application were consistent with recommended rates and adjusted for effective soil interaction depths of 1.5 mm for glyphosate (*14*) and 37.5 mm for fluometuron (*19*), trifluralin (*19*), aldicarb (*15*), and mefenoxam + PCNB (*20*) (**Table 3**). Factors included two soils, two glyphosate rates, and five pesticide treatments. Pesticide treatments and rates are listed in **Table 2**. Treatments were replicated three times in a split-plot experimental design with soil as the main plot and pesticide treatments as the subplot.

Microbial Activity. The microbial activity was determined by measuring C and N mineralization (*21*). Potassium hydroxide traps were replaced at 1, 2, 3, 4, 10, and 30 days after treatment to determine the amount of CO_2 evolved during that period. The amount of CO_2 absorbed was determined by titrating the remaining base with 1 N HCl, and C mineralized was calculated as described by Zibilske (*21*). Nitrogen mineralization was determined by comparing inorganic N (NO_3 and NH_4) concentration in 10 g soil subsamples at 2, 10, and 30 days after treatment to the initial inorganic N concentration. Nitrogen analysis was completed using a Rapid Flow Analyzer (OI Analytical, College Station, TX).

Table 2. Solubility, Half-Life, Organic Carbon Partitioning Coefficient, and Chemical Structure of Pesticides Used in the Study[a]

Active ingredient	S_w[b] g/L	$T_{1/2}$[b] (d)	Log K_{ow}[b]	Structure
glyphosate	12.0	3 to 174	-3.40	
fluometuron	0.08	10 to 100	2.38	
trifluralin	0.004	57 to 126	4.83	
aldicarb	6.0	20 to 361	0.053	
mefenoxam	7.1	19 to 730	1.75	
pentacholoronitrobenzene	0.0001	120 to 300	5.10	

[a] Information adapted from refs *39* and *40*. [b] Abbreviations: S_w, water solubility; $T_{1/2}$, half-life; and K_{ow}, octanol:water partioning coefficient.

Table 3. Pesticide Treatments Applied to Pasture and Cultivated Field Soil[a]

pesticide[b]	interaction depth (mm)	concentration[c,d] (μg active ingredient/kg soil)
fluometuron	37.5	4.1
trifluralin	37.5	2.0
aldicarb	37.5	10.2
mefenoxam + PCNB	37.5	8.3 + 0.42
glyphosate	1.5	152.7
glyphosate + fluometuron	1.5 + 37.5	152.7 + 4.1
glyphosate + trifluralin	1.5 + 37.5	152.7 + 2.0
glyphosate + aldicarb	1.5 + 37.5	152.7 + 10.2
glyphosate + mefenoxam + PCNB	1.5 + 37.5	152.7 + 8.3 + 0.42
untreated		0

[a] Soil was Weswood clay loam collected from a bermuda grass pasture and a fallow field previously planted with cotton. [b] Pesticides were applied as formulated products: glyphosate, Roundup WeatherMax; fluometuron, Cotoran 4L; trifluralin, Treflan HFP; aldicarb, Temik 15G; and mefenoxam + pentachloronitrobenzene (PCNB), Ridomil Gold PC GR. [c] Rate of application of formulated product: Cotoran 4L, 4.67 L/ha; Treflan HFP, 2.34 L/ha; Temik 15G, 5.62 kg/ha; Ridomil Gold OC GR, 11.23 kg/ha; and Roundup WeatherMax, 7.01 L/ha. [d] Calculations of concentration assume that the mass of a 15 cm furrow slice is 2200000 kg.

Microbial Biomass. Soil microbial biomasses of C and N were determined using the fumigation−incubation method (*22*). After 10 days of incubation, soil subsamples were fumigated with chloroform ($CHCl_3$) and incubated in the dark at room temperature overnight. Soil samples were then placed in airtight containers with 10 mL of KOH and incubated at 30 °C for an additional 10 days, after which C and N mineralized were determined as previously described. The amounts of mineralized C and N were divided by a *k* factor of 0.41, representing the fraction of biomass mineralized (*23*, *24*).

Data Analysis. All data were analyzed using Statistical Analysis Systems version 9.1 (SAS Institute, Inc., Cary, NC). The influence of glyphosate on daily rates of C and N mineralization was analyzed in the mixed model with replicates as a random effect and all other effects fixed. Pairwise contrasts of each pesticide with and without glyphosate were evaluated. Cumulative C and N mineralized were analyzed using the general linear model ($\alpha = 0.10$) to compare the slopes of pairwise contrasts of each pesticide treatment with or without glyphosate. Total C and N mineralized as well as soil microbial biomass C and soil microbial biomass N were subjected to analysis of variance using the mixed model ($\alpha = 0.10$).

RESULTS AND DISCUSSION

For all variables measured, pasture soil exhibited greater microbial activity and biomass than cultivated soil, likely due to differences in soil disturbance and management. However, soil characteristics did not interact with other factors. Therefore, data were combined across soils. Where an interaction of glyphosate with other pesticides occurred, data are presented by treatment. Other data are presented by the appropriate main effects.

Carbon and Nitrogen Mineralization. Rates of CO_2 accumulation for each pesticide applied with and without glyphosate are shown in **Figure 1**. Significant model terms used for slope analysis of contrasts included day, day*treatment, day*soil, soil, and day*treatment*soil. Soils treated with glyphosate exhibited an enhanced rate of C mineralization relative to untreated soils (**Figure 1A**). Other researchers have reported enhanced C mineralization following application of both analytical grade (*25*) and formulated (*9*) glyphosate. Cumulative C mineralization after 30 days was greater in soils treated only with glyphosate as compared to all other treatments. However, cumulative C mineralized in the remaining treatments was similar to untreated soils.

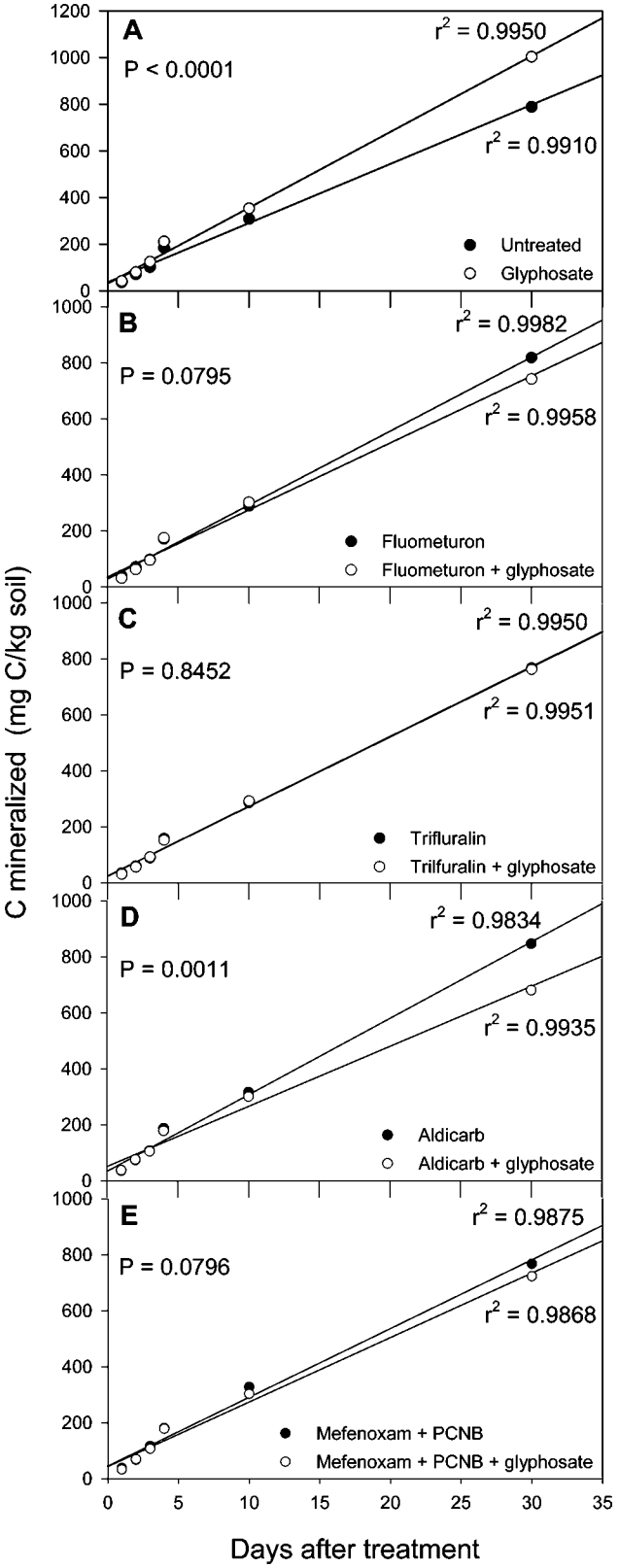


Figure 1. Effect of glyphosate on cumulative C mineralization in soil between 1 and 30 days after treatment. *P* values indicate significance of tests for different slopes ($\alpha = 0.10$). (**A**) No herbicide and glyphosate, (**B**) fluometuron and fluometuron + glyphosate, (**C**) trifluralin and trifluralin + glyphosate, (**D**) aldicarb and aldicarb + glyphosate, and (**E**) mefenoxam + pentachloronitrobenzene (PCNB) and mefenoxam + PCNB + glyphosate.

When fluometuron was applied in combination with glyphosate, mineralized C accumulated at a slower rate relative to when fluometuron was applied alone (**Figure 1B**). This is contradic-

tory to data reported by Bozarth and Funderburk (*26*) showing that the addition of glucose enhanced CO_2 evolution in soils treated with analytical grade fluometuron. It was expected that the addition of glyphosate should have served as a source of nutrients, thereby enhancing microbial growth and pesticide degradation. It should be noted, however, that glyphosate was applied as the formulated product in this experiment and there is evidence of microbial suppression caused by surfactants in the formulation (*12*). Furthermore, other researchers concluded that complete degradation of phenylurea herbicides, such as fluometuron, requires multiple, interdependent bacterial species (*27*). It is possible that fluometuron was toxic to the microorganisms that were the primary metabolizing agents of glyphosate, yet allowed fluometuron-metabolizing microorganisms to function, albeit at a slower rate than microorganisms metabolizing glyphosate applied alone.

The rate of C mineralization in soils treated with trifluralin was not influenced by the addition of glyphosate (**Figure 1C**). Trifluralin is a dinitroanaline herbicide that inhibits mitosis in susceptible species (*28*) and is susceptible to biodegradation (*29*). Previous research (*30*, *31*) indicates that formulations of pendimethalin, another dinitroanaline herbicide, have negative effects on the growth of fungal species. It is likely that the herbicide would negatively impact the activity of glyphosate-metabolizing microorganisms, reducing their activity.

Carbon was mineralized at a slower rate in soils that were treated with glyphosate and aldicarb relative to soils treated only with aldicarb (**Figure 1D**). Jones and Norris (*32*) reported that microbially mediated oxidation of aldicarb begins immediately upon application to soil. Other reports (*33*) indicate that repeated application of formulated aldicarb results in increased bacterial populations with decreased diversity. Increases in one segment of the bacterial population at the expense of species diversity may reduce biodegradation of compounds such as glyphosate that are not utilized by the enhanced group of bacterial microorganisms.

Accumulation of mineralized C in soils treated with mefenoxam + PCNB decreased in response to glyphosate addition (**Figure 1E**). It has been reported that a mefenoxam-based fungicide increased the bacterial population of soil (*34*). Furthermore, Lièvremont et al. (*35*) suggested that metabolism of analytical grade PCNB by unaffected fungal species may be greater under C-limited conditions. This may account for greater microbial activity in soils treated with mefenoxam + PCNB alone. The reduction in microbial activity in soils treated with mefenoxam + PCNB and glyphosate relative to soils treated with glyphosate alone is likely due to the microbial selection caused by these pesticides.

Cumulative N mineralization 30 days after treatment is shown in **Figure 2**. Nitrogen mineralization was greater in soils that had been treated with applications that included glyphosate as compared with soils that were not treated with glyphosate. This result is in agreement with Haney et al. (*12*), who reported enhanced N mineralization following the addition of glyphosate in eight of nine soils evaluated. No differences in N mineralization rates were observed when pairwise contrasts were evaluated (data not shown).

Cumulative C and N mineralized were well-correlated in treatments containing glyphosate but were not in treatments that did not contain glyphosate (**Figure 3**). This result indicated the strong influence of glyphosate on microbial activity, possibly due to the availability of C and N in the herbicide. Haney et al. (*12*) reported an increase in C and N mineralization equal to the amounts of C and N added as glyphosate when several soils were studied. The increased mineralization observed in these studies was not of equal magnitude; however, the addition of other pesticides is likely to have altered activity of the soil microfauna.

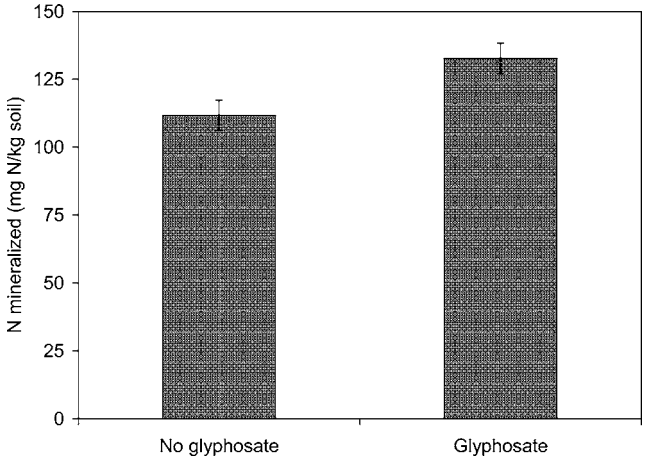


Figure 2. Nitrogen mineralized during 30 days of incubation in all pesticide treatments with and without glyphosate. Bars represent the least significant difference ($\alpha = 0.10$) of 5.6 ($P = 0.0114$).

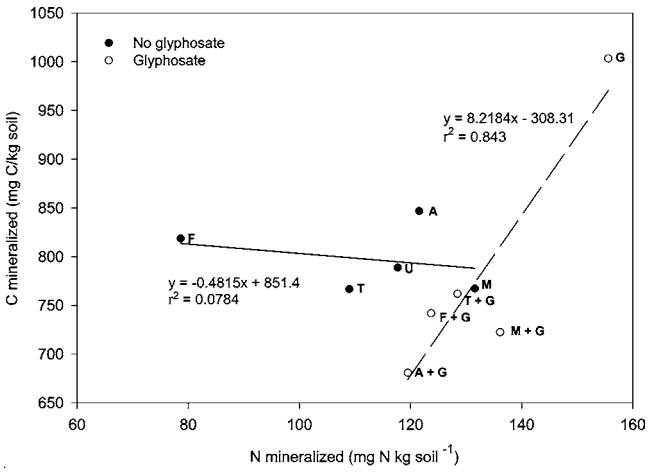


Figure 3. Cumulative C and N mineralized in 30 days after application of pesticides (A, aldicarb; F, fluometuron; M, mefenoxam + pentachloronitrobenzene (PCNB); T, trifluralin; and U, untreated) alone or with glyphosate (G).

Soil Microbial Biomass. The addition of glyphosate did not affect the response of soil microbial biomass C to any pesticide. However, soil microbial biomass C increased relative to nontreated soils when glyphosate was applied alone (**Figure 4**). Haney et al. (*12*) found that soil microbial biomass C increased due to the addition of Roundup Ultra in five of nine soils evaluated. Soil microbial biomass N was not affected by any pesticide treatment (**Figure 5**). This is similar to results following the application of Roundup Ultra reported by Haney et al. (*9*), who hypothesized that soil microbial biomass measurements using the fumigation−incubation method are less sensitive than C and N mineralization measurements for detecting the influence of microbial activity (*9*). Slow-growing bacteria have been implicated in glyphosate metabolism (*36*), preventing potential changes in soil microbial biomass from being observed during the incubation time used in these studies.

The data presented herein suggest that the response of the soil microbial community is altered when glyphosate application coincides with the presence of fluometuron, aldicarb, or mefenoxam + PCNB in the soil relative to when these pesticides

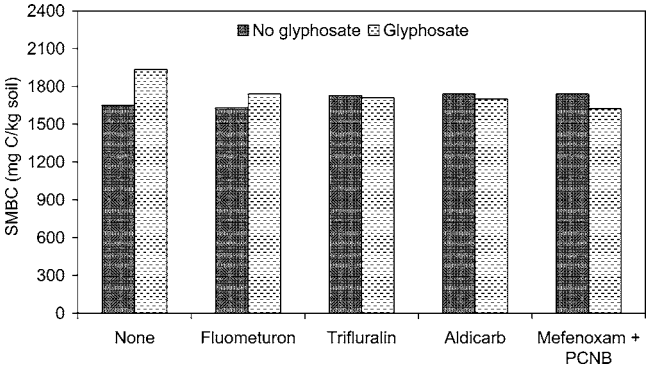


Figure 4. Sol microbial biomass C as influenced by pesticide treatments. Standard error, 107.7 ($P = 0.0983$).

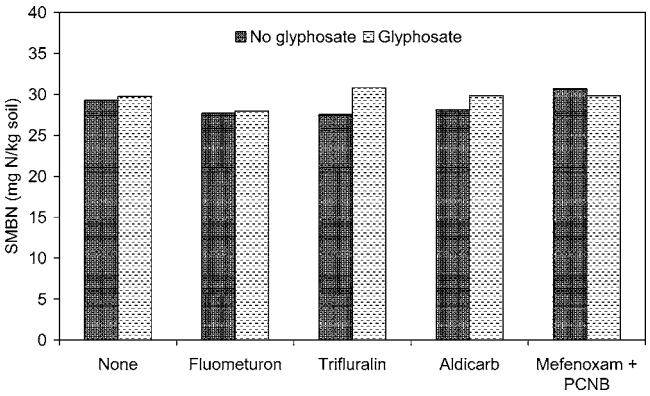


Figure 5. Soil microbial biomass N as influenced by pesticide treatments. Standard error, 2.1 ($P = 0.672$).

are applied singly. This is not surprising given that glyphosate (*10*, *11*) and many other pesticides (*37*) are degraded via cometabolism, suggesting that the presence of other energy sources will influence the activity of the microbial community. Glyphosate is a desirable substrate for soil microfauna because, in addition to C and N, it also contains phosphorus, which may result in enhanced degradation due to microbial requirements (*38*). Conversely, the presence of adjuvants in commercial formulations may adversely affect the microbial community (*12*). Additional research is needed to elucidate the effects of pesticide combinations on pesticide degradation and soil microbial community structure and function.

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