# Bioremediation and Biodegradation

## Effect of Roundup Ultra on Microbial Activity and Biomass from Selected Soils

R. L. Haney, S. A. Senseman,* and F. M. Hons

### ABSTRACT

**Herbicides applied to soils potentially affect soil microbial activity. The quantity and frequency of Roundup Ultra [RU; *N*-(phosphonomethyl)glycine; Monsanto, St. Louis, MO] applications have escalated with the advent of Roundup-tolerant crops. The objective of this study was to determine the effect of Roundup Ultra on soil microbial biomass and activity across a range of soils varying in fertility. The isoproplyamine salt of glyphosate was applied in the form of RU at a rate of 234 mg active ingredient $kg^{-1}$ soil based on an assumed 2-mm glyphosate–soil interaction depth. Roundup Ultra significantly stimulated soil microbial activity as measured by C and N mineralization, as well as soil microbial biomass. Cumulative C mineralization as well as mineralization rate increased above background levels for all soils tested with addition of RU. There were strong linear relationships between C and N mineralized, as well as between soil microbial C and N ($r^2 = 0.96$ and 0.95, respectively). The slopes of the relationships with RU addition approximated three. Since the isopropylamine salt of glyphosate has a C to N ratio of 3:1, the data strongly suggest that RU was the direct cause of the enhanced microbial activity. An increase in the C mineralization rate occurred the first day following RU addition and continued for 14 d. Roundup Ultra appeared to be rapidly degraded by soil microbes regardless of soil type or organic matter content, even at high application rates, without adversely affecting microbial activity.**

The increasing use of Roundup-tolerant crops has increased concerns regarding the potential environmental impact of glyphosate. Roundup Ultra (RU) is a nonselective, foliar-applied herbicide used to control weeds preplant or postemergence in tolerant crops or by using shielded sprayers in nontolerant crops. The active ingredient of RU is glyphosate, or more accurately, the isopropylamine salt of glyphosate, and its mode of action is inhibition of 5-enolpyruvylshikimate-3-phosphate synthase, resulting in the depletion of essential aromatic amino acids needed for plant survival (Ahrens, 1994). Glyphosate is readily adsorbed by clay minerals and hydrous oxides (Glass, 1987; McConnell and Hossner, 1985). The $K_d$ values have been reported to range from 33 to 660 L $kg^{-1}$ (Glass, 1987; USDA, 1990). Glyphosate adsorption correlates with the amount of vacant phosphate sorption sites and may occur through binding of the phosphonic acid moiety (Ahrens, 1994). Glyphosate is microbially degraded in soil and water and has a reported field half-life of 47 d and a laboratory half-life of <25 d (Ahrens, 1994). However, it is not known what effect the product-grade RU (which includes surfactant and other inert products) has on indigenous microbial populations and activities across a range of soils varying in fertility.

Although glyphosate is not intentionally soil applied, a significant concentration of material may reach the soil surface during broadcasted preplant or early-season applications. The amount of herbicide available to soil microorganisms depends on various factors, including available nutrients, pH, temperature, and moisture, though they differ in importance depending on the pesticide involved (Weber et al., 1993). Soil water and temperature directly affect many biological processes, including plant metabolism and microbial degradation, and thereby influence bioactivity and persistence of the chemicals (Weber et al., 1993).

Dick and Quinn (1995) investigated 26 bacterial strains from sites without prior addition of glyphosate and found that all 26 could metabolize glyphosate via the initial cleavage of its carbon–phosphorus bond. Since glyphosate contains carbon, nitrogen, and phosphorus, all of which are essential nutrients for soil microorganisms, it should be readily mineralizable.

In our previous work, we found that increasing rates of RU resulted in linear responses of C and N mineralization while no response was observed in soil microbial biomass (SMB) C and N (Haney et al., 2000) when incubated at 25°C. Soil microbial biomass C and N were determined 14 d after addition of RU instead of 3 d, allowing more time for RU-derived C and N to be incorporated into microbial cells. Since heterotrophic soil microorganisms acquire C and N for maintenance and growth by decomposing plant residues and other organic materials in soils, herbicides with low C to N ratios (<15) may potentially be readily mineralized, with N that is in excess of microbial demand being released in the inorganic form (Alexander, 1977). Work done by Forlani et al. (1999) showed evidence of unculturable microorganisms when exposed to glyphosate. We chose C and N mineralization and soil microbial biomass C and N as indicators of microbial activity since these methods are usually sensitive to low C to N ratio substrates and allow for a more realistic study of the effect of RU on field soils since most laboratory studies are conducted using analytical glyphosate, which farmers do not use (Haney et al., 2000).

The objective of this study was to determine whether RU application that reaches the soil has a detrimental or favorable effect on the microbial biomass and activities as measured by C and N mineralization or soil

Department of Soil & Crop Sciences, Texas Agricultural Experiment Station, Texas A&M Univ., College Station, TX 77843-2474. Received 2 Apr. 2001. *Corresponding author (s-senseman@tamu.edu).

Published in J. Environ. Qual. 31:730–735 (2002).

**Abbreviations:** RU, Roundup Ultra; SMB, soil microbial biomass.

microbial biomass from a range of soils covering a wide array of soil characteristics.

## MATERIALS AND METHODS

Nine soils from Georgia and Texas were used in this study. The soils varied in soil pH (4.7 to 8.2), soil organic C (4.1 to 52.3 g C $kg^{-1}$ soil), and clay content (6 to 45%) (Table 1). Land management of these soils ranged from row crop production to permanent pasture. The isopropylamine salt of glyphosate as Roundup Ultra (480 g active ingredient $L^{-1}$) was added to soil at a rate of 234 mg $kg^{-1}$. This amount was based on the recommended rate of RU being 0.84 kg $ha^{-1}$ and a shallow 2-mm soil interaction depth due to glyphosate's high adsorptivity and low leachability (McConnell and Hossner, 1985; Sprankle et al., 1975; Haney et al., 2000). A control treatment with no RU was included for each soil; therefore, comparison with RU additions to each soil would measure the positive or negative influences on microbial biomass and activity for each soil.

### Carbon Mineralization

Soil samples were initially dried at 40°C for 24 h to ensure homogeneity of soil moisture content. Samples (100 g) were subsequently wetted and preincubated for 7 d and incubated at 30°C prior to RU addition; we incubated at 30°C to allow increased activity from a higher incubation temperature than our previous work at 25°C (Haney et al., 2000). Wetting amounts are calculated using the 90% of clay content technique. For example, if a soil contains 10% clay then 10% × 0.9 = 9 mL water per 100 g soil; if only 50 g are used, then 4.5 mL water is added to the soil sample. The 7-d incubation period before RU addition allowed microbial respiration to reach a baseline level after the initial flush of activity from soil drying and rewetting. Franzluebbers et al. (1996) showed that dried and rewetted soils exhibited similar microbial biomass and activities as continuously moist samples after an incubation period of 5 to 10 d. Roundup Ultra was added in 5 mL of distilled water to soil samples, increasing the final moisture content to 20% w/w (approximately 60% water filled pore space). Soils were placed in gas-tight 1-L glass containers along with a vial containing 10 mL of 1 *M* KOH to trap evolved $CO_2$ and a vial of water to maintain humidity. Soils were incubated at 30°C with KOH traps replaced daily until 7 d and then at Days 14, 24, 28, and 50. Unreacted alkali in the KOH traps was titrated with 1 *M* HCl to determine $CO_2$–C (Anderson, 1982).

### Nitrogen Mineralization

Nitrogen mineralization was determined by subtracting the initial inorganic N concentration of nonincubated soil samples from soil N extracted after 50 d of incubation. Inorganic N was extracted from 7-g soil subsamples using 28 mL of 2 *M* KCl. Samples were shaken for 30 min on a reciprocating shaker and filtered, and the extracts were analyzed for $NH_4$–N and $NO_2$– plus $NO_3$–N using an autoanalyzer (Technicon Industrial Systems, 1977a,b). The sum of the above N forms was designated inorganic N.

### Soil Microbial Biomass Carbon and Nitrogen

Soil microbial biomass C and N were determined 14 d after RU addition for each soil. Soil microbial biomass C was determined by fumigation–incubation by exposing 40 g of soil to alcohol-free $CHCl_3$ vapor for 24 h (Jenkinson and Powlson, 1976). Following evacuation and removal of vapors, soil was incubated in 1-L gas-tight glass containers for 10 d at 30°C. Carbon dioxide evolved during the 10-d incubation period following fumigation was trapped in 1 *M* KOH and determined as described previously. The quantity of evolved $CO_2$–C was divided by an efficiency factor of 0.41 to estimate microbial biomass C (Voroney and Paul, 1984).

Soil microbial biomass N was determined by analyzing soil $NH_4$–N concentrations of fumigated samples following the 10-d incubation period minus initial $NH_4$–N prior to fumigation, divided by an efficiency factor of 0.41 (Carter and Rennie, 1982). Extraction and analysis of $NH_4$–N was accomplished as previously described.

### Clay Content, Soil Organic Carbon, and pH

Soil pH was estimated with 2:1 water to soil. Clay content was determined by the hydrometer method and soil organic C from the modified Mebius method (Thomas, 1996).

### Statistical Analysis

All treatments were replicated three times. Analysis of variance was used for generation of means and for determination

**Table 1. Characteristics of soils.**

| Location | Series | Texture† | USDA soil classification | Soil pH (water) | Clay content | Organic C | Depth of sampling | Land management‡ |
|---|---|---|---|---|---|---|---|---|
| | | | | | % | g $kg^{-1}$ | cm | |
| Waynesboro, GA | Lakeland | S | thermic, coated Typic Quartzipsamment | 4.7 | 10.1 | 5.9 | 30–60 | cropped (cotton, soybean) |
| Watkinsville, GA | Cecil | SL | fine, kaolinitic, thermic Typic Kanhapludult | 4.8 | 12.8 | 15.0 | 0–15 | pasture (bermudagrass) |
| Amarillo, TX | Pullman | SCL | fine, mixed, superactive, thermic Torrertic Paleustoll | 5.7 | 28.7 | 11.6 | 0–7.5 | cropped (sorghum, wheat) |
| Overton, TX | Bowie | fSL | fine-loamy, siliceous, semiactive, thermic Plinthic Paleudult | 6.3 | 6.0 | 4.1 | 0–7.5 | pasture (bermudagrass) |
| Stephenville, TX | Windthorst | fSL | fine, mixed, thermic Udic Paleustalf | 6.4 | 13.0 | 18.3 | 0–7.5 | pasture (bermudagrass) |
| Watkinsville, GA | Pacolet | SCL | fine, kaolinitic, thermic Typic Kanhapludult | 6.6 | 26.9 | 52.3 | 0–5 | pasture (tall fescue) |
| Malone, TX | Houston Black | C | very-fine, smectitic, thermic Oxyaquic Hapludert | 7.8 | 45.0 | 13.7 | 0–10 | cropped (sorghum, wheat) |
| College Station, TX | Weswood | SiCL | fine-silty, mixed, superactive, thermic Udifluventic Ustochrept | 8.0 | 28.4 | 23.7 | 0–7.5 | pasture (bermudagrass) |
| Weslaco, TX | Hildalgo | SCL | fine-loamy, mixed, hyperthermic Typic Calciustoll | 8.2 | 22.3 | 9.8 | 0–7.5 | cropped (cotton, corn) |

† S, sand; SL, sandy loam; SCL, sandy clay loam; fSL, fine sandy loam; C, clay; SiCL, silty clay loam.
‡ Cotton, *Gossypium hirsutum* L.; soybean, *Glycine max* (L.) Merr.; bermudagrass, *Cynodon dactylon* (L.) Pers.; sorghum, *Sorghum bicolor* (L.) Moench; wheat, *Triticum aestivum* L.; tall fescue, *Festuca arundinacea* Schreb.; corn, *Zea mays* L.

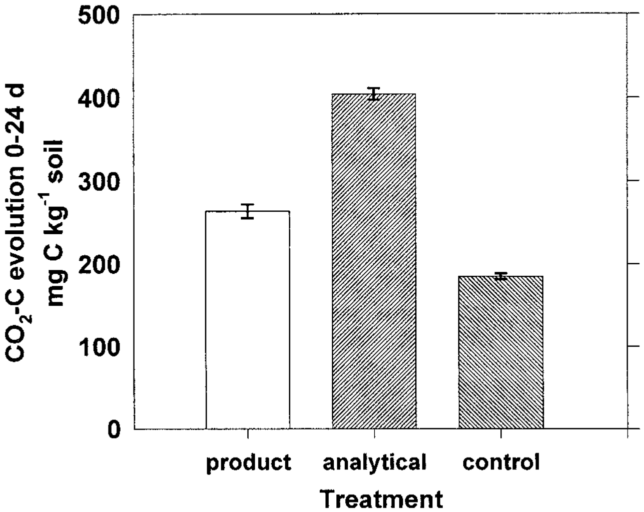


**Fig. 1. Carbon dioxide evolution from soil after applications of Roundup Ultra (product; applied at 3 × field rate) and analytical-grade glyphosate, and a control.**

of standard error terms. Linear regression was used to assess relationships among variables. Model adequacy was based on residual plot analysis. Treatment means within each incubation interval were separated using Tukey's test at the 5% level of significance (SPSS, 1997).

## RESULTS AND DISCUSSION

A preliminary study was conducted on the effect of the product-grade glyphosate in the form of RU on $CO_2$ evolution compared with analytical-grade glyphosate. The results indicate an inhibition of $CO_2$ evolution after 24 d of incubation when using RU, although $CO_2$ evolution is still significantly higher from RU than the control but significantly less than analytical-grade glyphosate (Fig. 1). We therefore concluded that RU increases microbial activity in spite of the slightly inhibitory effects due to the surfactant or other inert products that soil microbes may or may not completely overcome.

The first day after RU addition, C mineralization for all soils was significantly different from the control (Fig. 2). The 30°C incubation temperature appeared to enhance microbial activity and resulted in no apparent lag phase after RU addition when compared with a similar study using Weswood soil (fine-silty, mixed, superactive, thermic Udifluventic Ustrochrept), and conducted at 25°C (Haney et al., 2000). The stimulation of soil microbial activity appeared to be unique to each soil, as soils lower in clay content and soil organic C tended to reach their peak mineralization rate a few days later than the soils with higher clay and organic matter content (Fig. 2). Carbon mineralization rates for the higher organic C soils tended to peak earlier and then dropped significantly in contrast to the lower organic C soils that may have first had to increase their microbial biomass in response to an easily mineralizable substrate. The flush of C mineralization was greater in the higher organic C soils possibly due to a greater population of microbial biomass, which mineralized the added substrate more quickly. Of the nine soils tested, two reached their peak on Day 2, five by Day 3, one on Day 4, and one on Day 5. By Day 7, only the Lakeland soil (thermic, coated Typic Quartzipsamment) had yet to release more C as $CO_2$ than was added as RU. By 14 d of incubation, however, all soils had evolved more C as $CO_2$ than was added as RU.

Two different aspects of soil microbial activity are depicted in Fig. 2 and 3. Figure 2 shows when the peak mineralization flush occurred for each soil after RU addition, while Fig. 3 identifies the soils that released the most $CO_2$ during the first 7 d of incubation. Soils differed greatly in organic C content (Table 1). Some soils responded similarly (Fig. 2B,C) to RU addition at the end of 7 d while others did not (Fig. 2A). These data showed that generally as soil organic carbon (SOC) increased, C mineralization did also; however, when SOC, soil pH, and clay content are regressed with the flush in $CO_2$ (the difference between RU and no RU after 14 d) the relationships are poor ($r^2$ = 0.02, 0.57, and 0.41 respectively, data not shown). Therefore, SOC,

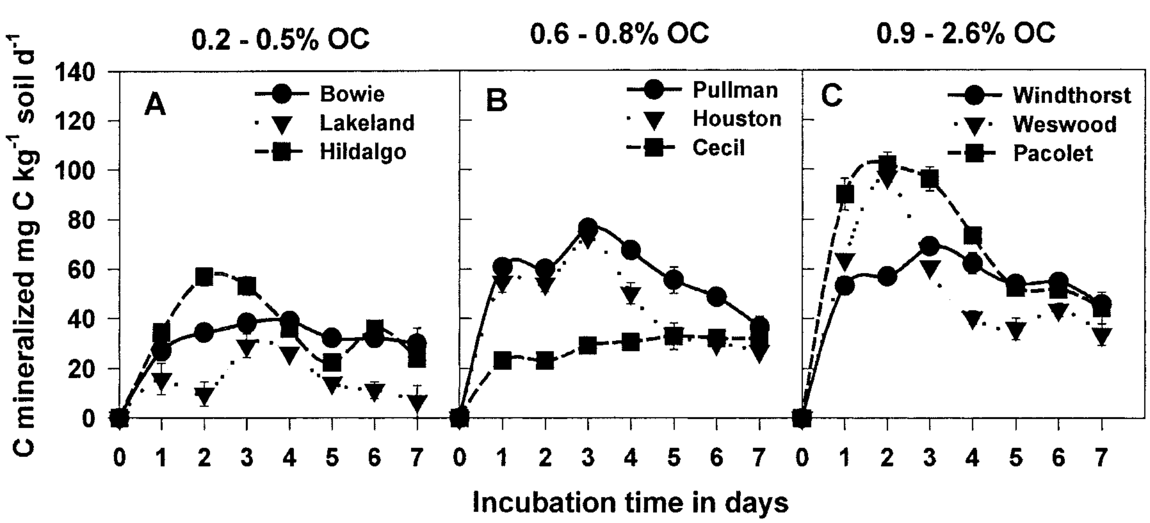


**Fig. 2. Effect of Roundup Ultra on daily carbon mineralization between 1 and 7 d from different soils with (*A*) 0.2 to 0.5% organic C, (*B*) 0.6 to 0.8% organic C, and (*C*) 0.9 to 2.6% organic C. Error bars indicate one standard deviation.**

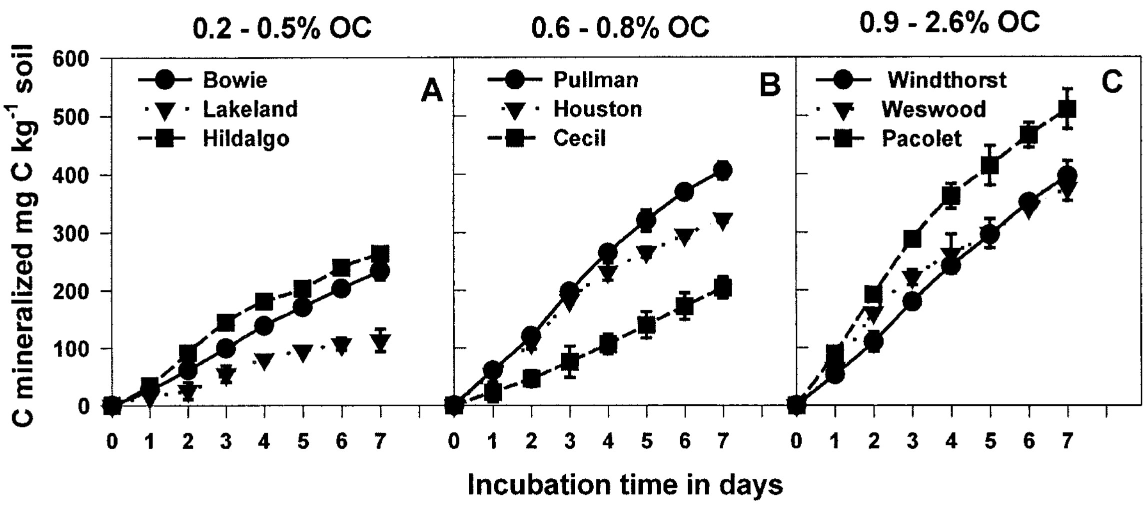


**Fig. 3. Effect of Roundup Ultra on cumulative carbon mineralization between 1 and 7 d from nine soils with (*A*) 0.2 to 0.5% organic C, (*B*) 0.6 to 0.8% organic C, and (*C*) 0.9 to 2.6% organic C. Error bars indicate one standard deviation.**

soil pH, and clay content do not necessarily indicate how rapidly RU will be mineralized in a given soil.

Fourteen days after RU addition, all soils returned to background $CO_2$ levels. Soil microbial biomass C and N (SMBC, SMBN) were determined at this time, as the added RU had apparently been completely mineralized. Five of the nine soils showed significant increases in SMBC with RU addition (Fig. 4A). However, all nine soils exhibited significantly increased SMBN with RU addition (Fig. 4B). Roundup Ultra addition to soil appeared to affect the microbial N content to a greater extent than microbial C, thereby effectively lowering the microbial biomass C to N ratio and releasing N to the soil. The average increase in SMBC due to the addition of RU was 17% compared with 76% for SMBN. This result may indicate an enhanced ability to mineralize N that is in excess of microbial demand from RU degradation.

After 50 d of incubation following RU addition, five of the nine soils tested had significantly greater C mineralization and eight of nine soils showed significantly greater N mineralization (Fig. 5). The average increase for all soils for C mineralization due to RU addition was 18% compared with 108% for N mineralization. After 50 d of incubation all soils had released more C above the control than was added by RU. Roundup Ultra appeared to be readily mineralized regardless of soil type, clay content, pH, or soil organic C content.

The literature suggests that the methods for SMBC and SMBN usually do not separate differences between herbicide treatments (Olson and Lindwall, 1991; Hart and Brookes, 1996). In our study, SMBC was highly correlated to SMBN with and without RU addition (Fig. 6A). Carbon and N mineralized were also highly correlated (Fig. 6B). It is interesting to note that the slopes (C to N ratio) of the regression of both graphs were

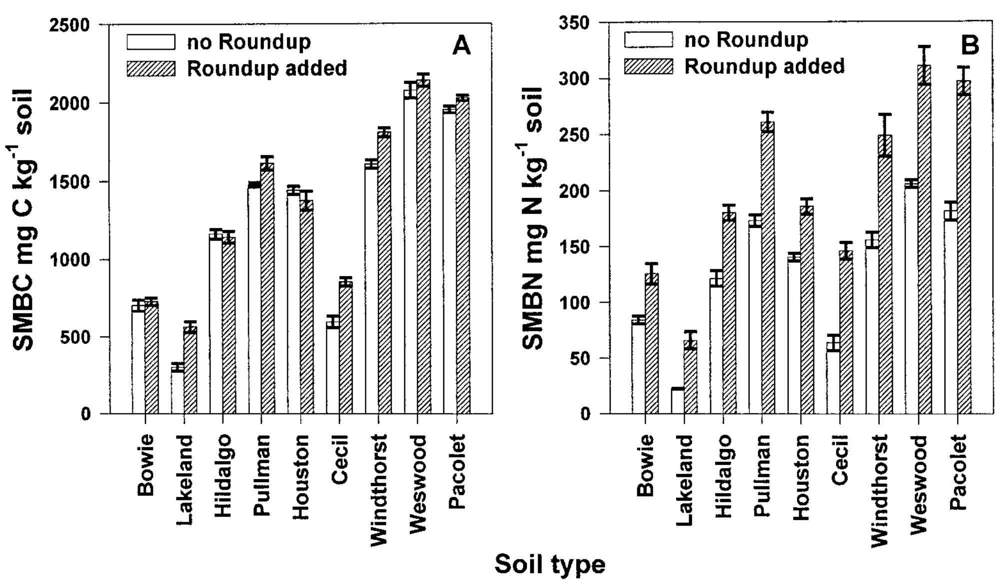


**Fig. 4. Soil microbial biomass (*A*) carbon (SMBC) and (*B*) nitrogen (SMBN) with and without Roundup Ultra addition. Error bars indicate one standard deviation.**

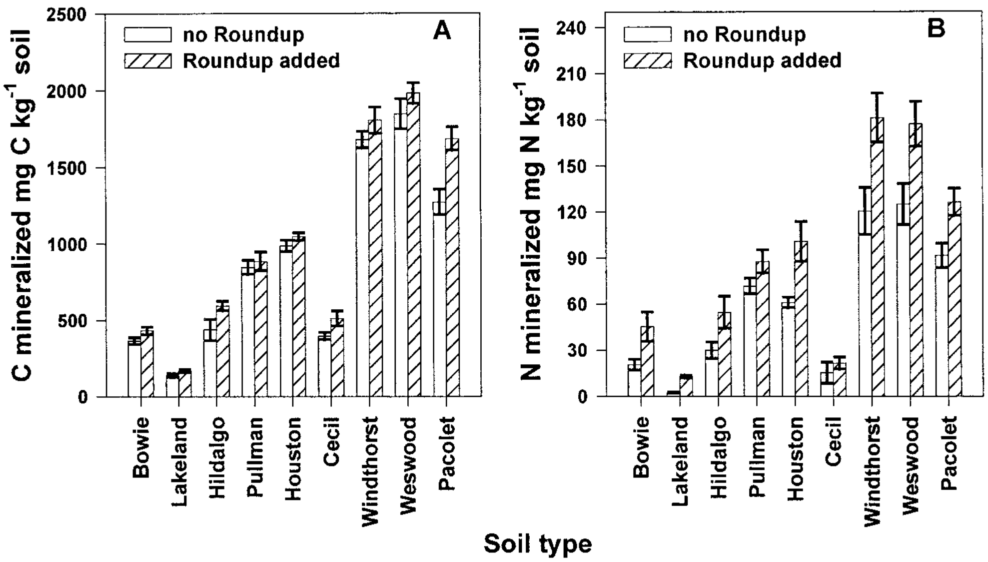


**Fig. 5. Mineralization for (*A*) carbon and (*B*) nitrogen, after 50 d of incubation for nine soils. Error bars indicate one standard deviation.**

reduced by roughly three with the addition of RU. Since the C to N ratio of the isopropylamine of glyphosate is 3:1, these data support the hypothesis that the addition of isopropylamine of glyphosate in RU was directly responsible for the increase in SMBC, SMBN, and C and N mineralization. These results support the earlier work done on Weswood soil (Haney et al., 2000).

Soil microbial biomass C was estimated on the 14th day after addition of RU and was highly related to the amount of C mineralized at both 7 and 14 d (Fig. 7). The relationships were not as strong on Day 7 as on Day 14, which may indicate that soil microbial respiration is coupled to the size of the population when substrate is not limiting. In both instances, RU addition increased C mineralized per unit of microbial biomass.

## SUMMARY

Roundup Ultra was readily mineralized by indigenous soil microbes and increased their population and activity. Soils with higher organic matter tended to mineralize RU more quickly initially than soils with lower organic C, possibly due to a greater microbial biomass. Soils with less organic C tended to mineralize RU at a slower rate, while their microbial biomass increased in response to the added substrate. Soil organic C, soil pH, and clay content do not necessarily indicate a soil's ability to mineralize RU. Available N may have been more limiting than substrate C in the studied soils as RU application significantly increased SMBN in all soils, but increased SMBC in only five soils. The amount of C mineralized was more highly correlated to SMBC at

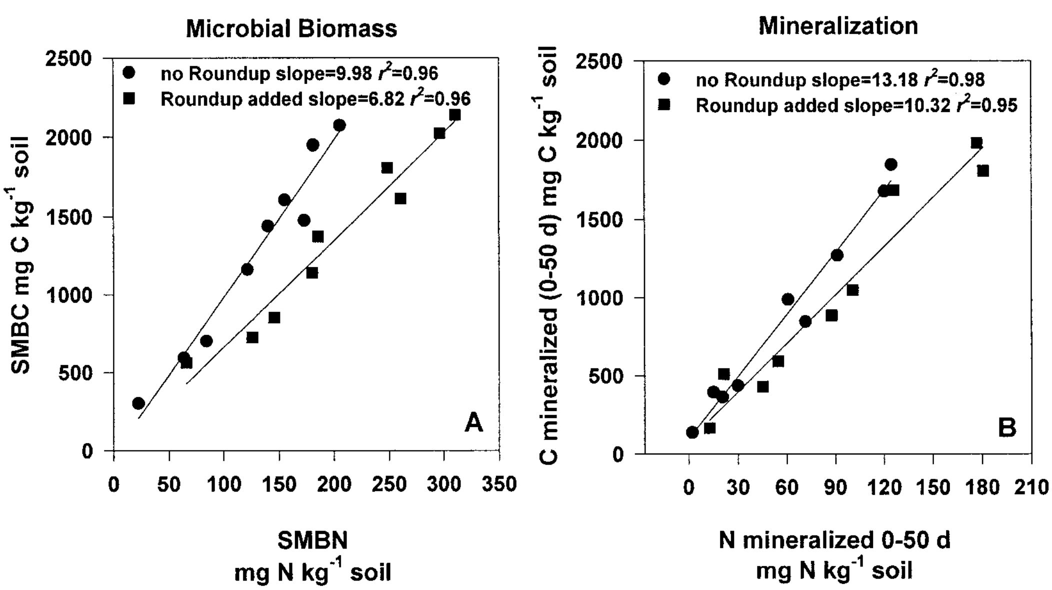


**Fig. 6. Regression analysis for (*A*) soil microbial biomass nitrogen (SMBN) vs. soil microbial biomass carbon (SMBC) with and without Roundup Ultra addition and (*B*) N mineralized vs. C mineralized in 50 d with and without Roundup Ultra addition.**

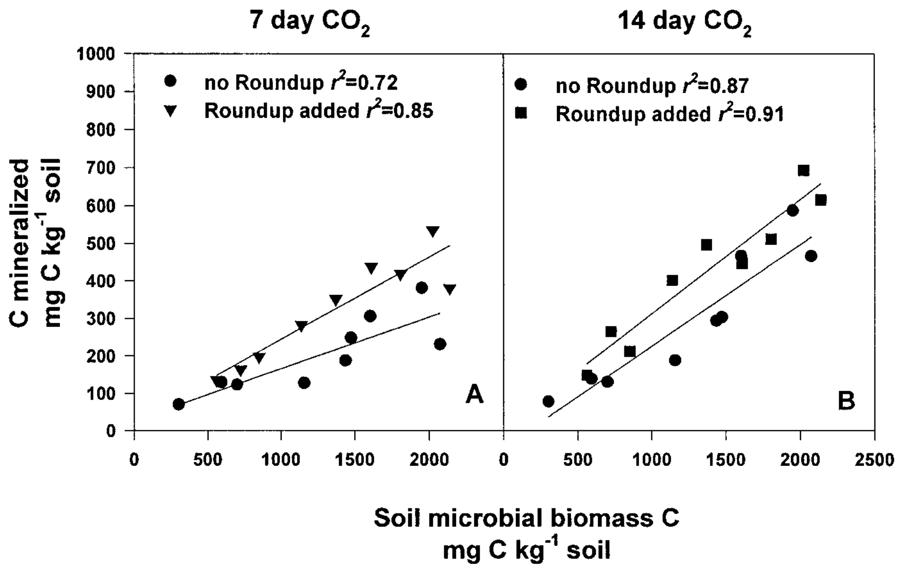


**Fig. 7. Regression analysis of soil microbial biomass carbon (SMBC) determined at 14 d with (*A*) SMBC vs. 7-d C mineralization and (*B*) SMBC vs. 14-d C mineralization.**

14 d after addition of RU than at 7 d. Since the method for SMBC was estimated at 14 d, it appeared that activity was coupled to population when substrate was not limiting. These data suggest that RU actually enhances microbial activity and biomass and does not adversely affect the soil microbial community.

## REFERENCES

Ahrens, W.H. 1994. Herbicide handbook. 7th ed. Weed Sci. Soc. Am., Champaign, IL.

Alexander, M. 1977. Introduction to soil microbiology. 2nd ed. John Wiley & Sons, New York.

Anderson, J.P.E. 1982. Soil respiration. p. 837–871. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Carter, M.R., and D.A. Rennie. 1982. Changes in soil quality under zero tillage farming systems: Distribution of microbial biomass and mineralizable C and N potentials. Can. J. Soil Sci. 62:587–597.

Dick, R.E., and J.P. Quinn. 1995. Glyphosate-degrading isolates from environmental samples: Occurrence and pathways of degradation. Appl. Microbiol. Biotechnol. 43:545–550.

Forlani, G., A. Mangiagalli, E. Nielsen, and C.M. Suardi. 1999. Degradation of the phosphonate herbicide Roundup in soil: Evidence for a possible involvement of unculturable microorganisms. Soil Biol. Biochem. 31:991–997.

Franzluebbers, A.J., R.L. Haney, F.M. Hons, and D.A. Zuberer. 1996. Determination of microbial biomass and nitrogen mineralization following rewetting of dried soil. Soil Sci. Soc. Am. J. 60:1133–1139.

Glass, R.L. 1987. Adsorption of Roundup by soils and clay minerals. J. Agric. Food Chem. 35:497–500.

Haney, R.L., S.A. Senseman, F.M. Hons, and D.A. Zuberer. 2000. Effect of Roundup on soil microbial activity and biomass. Weed Sci. 48:89–93.

Hart, M.R., and P.C. Brookes. 1996. Soil microbial biomass and mineralization of soil organic matter after 19 years of cumulative field applications of pesticides. Soil Biol. Biochem. 28:1641–1649.

Jenkinson, D.S., and D.S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol. Biochem. 8:209–213.

McConnell, J.S., and L.R. Hossner. 1985. pH-dependent adsorption isotherms of Roundup. J. Agric. Food Chem. 33:1075–1078.

Olson, B.M., and C.W. Lindwall. 1991. Soil microbial activity under chemical fallow conditions: Effects of 2,4-D and Roundup. Soil Biol. Biochem. 23:1071–1075.

Sprankle, P.W., F. Meggitt, and D. Penner. 1975. Adsorption, mobility, and microbial degradation of Roundup in soil. Weed Sci. 23:229–234.

SPSS. 1997. SigmaStat for Windows. Version 2.03. SPSS, Chicago, IL.

Technicon Industrial Systems. 1977a. Determination of nitrogen in BS digests. Technicon Industrial Method 334-74W/B. Technicon Industrial Systems, Tarrytown, NY.

Technicon Industrial Systems. 1977b. Nitrate and nitrite in soil extracts. Technicon Industrial Method 487-77A. Technicon Industrial Systems, Tarrytown, NY.

Thomas, G.W. 1996. Soil pH and soil acidity. p. 475–490. *In* D.L. Sparks (ed.) Methods of soil analysis. Part 3. SSSA Book Ser. 5. SSSA, Madison, WI.

USDA. 1990. Agricultural Research Service pesticide properties database. Systems Res. Lab., Beltsville, MD.

Voroney, R.P., and E.H. Paul. 1984. Determination of $K_C$ and $K_N$ in situ for calibration of the chloroform fumigation–incubation method. Soil Biol. Biochem. 16:9–14.

Weber, J.B., J.A. Best, and J.U. Gonese. 1993. Bioavailability and bioactivity of sorbed organic chemicals. p. 153–196. *In* D.M. Linn et al. (ed.) Sorption and degradation of pesticides and organic chemicals in soil. ASA and SSSA, Madison, WI.