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Soil carbon and nitrogen mineralization as affected by atrazine and glyphosate

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Abstract Atrazine alone and atrazine plus glyphosate were added to soil to determine their effect on soil microbial activity as measured by C and N mineralization (Cmin, Nmin) and soil extractable atrazine without the use of radiolabelled isotopes. Atrazine alone was added to soils as a formulated product (Aatrex 4L) at a field rate of 2× (94 mg kg^{-1}), 4× (188 mg kg^{-1}), and 6× (282 mg kg^{-1}) with an assumed soil penetration depth of 58 mm. Glyphosate, as Roundup Ultra, was added along with atrazine to soil in equal amounts bringing the total cumulative herbicide amount to 2× (188 mg kg^{-1}), 4× (376 mg kg^{-1}) and 6× (564 mg kg^{-1}) assuming a 2-mm soil penetration depth for glyphosate. Atrazine plus glyphosate stimulated microbial activity more than atrazine alone. During 56 days of incubation, mineralized C and N were highly correlated (r^2 =0.93). In addition, the C and N added from the herbicides were correlated with the amounts of C and N mineralized above the controls and were highly correlated (r^2 =0.93 for Cmin and r^2 =0.97 for Nmin). Cmin was greatest during the first 7 days of incubation after herbicide application while Nmin was greatest during the day 14 to day 28 period indicating a possible substrate shift from glyphosate to atrazine since atrazine has more N relative to C than glyphosate. Atrazine extracted from soil at four time periods (day 7, 14, 28, and 56) showed similar degradation curves (DT_{50} = 10.5 days) for the atrazine and atrazine-glyphosate treatments for all rates, with the exception of the 6× rate after 14 days and the 2× rate after 28 days of incubation where glyphosate appeared to slightly enhance the degradation of atrazine.

Keywords C and N mineralization · Degradation · Soil microbial biomass · Soil microbial activity

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Introduction

Atrazine and glyphosate are effective and widely used herbicides. Tank mixing atrazine and glyphosate is used for immediate burndown and residual weed control especially in corn (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moensch) production. Glyphosate is a non-selective, foliar-applied herbicide used to control weeds preplant or post-emergence in tolerant crops or by using shielded sprayers in non-tolerant crops. Glyphosate's 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), and it is readily adsorbed to clay minerals and hydrous oxides (McConnell and Hossner 1985; Glass 1987). K_d values range from 33 to 660 ml g^{-1} (Glass 1987; US Department of Agriculture 1990). Glyphosate adsorption correlates with the amount of vacant phosphate sorption sites and may occur through binding of the phosphonic acid moiety (Ahrens 1994), yet glyphosate is degraded microbially in soil and water (Ahrens 1994). It has a reported field half-life of 47 days and a laboratory half-life of <25 days (Ahrens 1994). Glyphosate is usually sprayed on growing plants and is not intentionally soil applied; however, 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 nutrient and pH status, temperature, and moisture, though they differ in importance depending on the pesticide involved (Weber et al. 1993). Soil moisture 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).

Average concentrations of surface-applied herbicides to soil may be as low as 1–4 mg kg^{-1} (Moorman 1989). These concentrations, however, are usually calculated based on a 15-cm furrow slice. Concentrations based on a 15-cm soil depth may be misleading since soil penetra-

tion of glyphosate may be only a few millimeters due to its high adsorptivity (Sprankle et al. 1975). Calculations based on a 15-cm soil depth may substantially underestimate the glyphosate and other herbicide concentrations that soil microbes are exposed to in the shallow zone of herbicide penetration.

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 C, N, and P, all of which are essential nutrients to soil microorganisms, it should be readily mineralized.

Heterotrophic soil microorganisms acquire C and N for maintenance and growth by decomposing plant residues and other organic materials added to soils. Herbicides with low C:N ratios (<15) may potentially be readily mineralized, with N that is in excess of microbial demand being released in inorganic form (Alexander 1977). Glyphosate has a C:N ratio of 3:1 and may have an immediate impact on soil microbial activity.

Atrazine [2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine] is a herbicide widely used to control broad-leaved weeds and has a C:N ratio of 1.6:1. Microbial degradation is the principal mechanism of atrazine dissipation (Esser et al. 1975). Various soil microorganisms are known to degrade atrazine partially by N-dealkylation or dehalogenation reactions (Kaufman and Kearney 1970; Behki and Khan 1986; Mougin et al. 1994; Bouquard et al. 1997). Complete and rapid mineralization of the triazine ring has been shown (Gschwind 1992; Mandelbaum et al. 1993; Assaf and Turco 1994). Triazine ring mineralization may imply the development of microbial communities that can utilize the N in the triazine ring (Cook and Hutter 1981; Mandelbaum et al. 1995).

Various organic and inorganic amendments have been shown to adversely affect atrazine degradation. The simultaneous addition of mineral N and glucose retarded atrazine mineralization in atrazine-adapted (previously applied) and non-adapted soils (Abdelhafid et al. 2000a). Similarly, Alvey and Crowley (1995) reported that glucose and sodium citrate repressed atrazine degradation. They suggested that reduced atrazine mineralization was related to the direct inhibition of a specific microbial degrader's ability to mineralize atrazine or to changes in the microbial community. The organic N sources pyrazine, albumin, adenine, and arginine have also inhibited atrazine mineralization (Abdelhafid et al. 2000b), and the inhibitory effect increased with N mineralization rate.

Current literature pertaining to pesticide degradation primarily evaluates single pesticide systems. However, tank mixing pesticides and prepackaged mixtures have become common agricultural practices (Singh et al. 1990; Colvin 1993). When coupling this phenomenon with the increasing use of glyphosate-tolerant crops, concerns arise regarding the potential effect of glyphosate on the degradation of pesticides such as atrazine. The impact of post-emergence applications of glyphosate and atrazine on weed control in glyphosate-resistant crops has been evaluated (Johnson et al. 2000). However, no data is available regarding the impact of glyphosate on atrazine degradation from microbial activity in soil.

The purpose of this research was to quantify the soil microbial response to atrazine and atrazine-glyphosate mixtures in soil.

Materials and methods

The soil used was a Weswood silt loam (fine, mixed, thermic Fluventic Ustochrept) with soil pH of 8.3 (1:2 soil/water), soil organic matter content of 10.6 g kg^{-1} soil, 115 g sand kg^{-1}, 452 g silt kg^{-1}, 310 g clay kg^{-1}, and 123 g $CaCO_3$ kg^{-1}. Extractable soil P was in the very high category as determined by the Texas A&M University Agricultural Extension Service Soil Testing Laboratory. Soil was collected from unfertilized grain sorghum plots at the Texas A&M University Agricultural Research Farm near College Station, Texas at a depth of 5 cm. The plots sampled had received the same cultural practices for 16 years before sampling. Soils were passed through a 2-mm sieve with obvious roots and plant residue being discarded.

Atrazine was added to soil in the form of Aatrex 4L at field rates of 2× (94 mg kg^{-1}), 4× (188 mg kg^{-1}), and 6× (282 mg kg^{-1}) alone as well as with glyphosate in equivalent amounts. The isopropylamine salt of glyphosate as RoundUp Ultra (480 g active ingredient l^{-1}) was also added to soil at rates of 2× (94 mg kg^{-1}), 4× (188 mg kg^{-1}), and 6× (282 mg kg^{-1}). Rate calculations were based on the 1× rate being 0.84 kg ha^{-1} for glyphosate and a shallow 2-mm soil interaction depth due to glyphosate's high adsorptivity and low leachability (Sprankle et al. 1975; McConnell and Hossner 1985) and being 2.24 kg ha^{-1} with a 58-mm soil interaction depth for atrazine (Mandelbaum et al. 1995). Total C added to soil from atrazine was 42, 84, and 126 mg kg^{-1} and 63, 127, and 191 mg kg^{-1} for the mixture of atrazine and glyphosate (2×, 4×, 6×, respectively). Total N added was 31, 62 and 93 mg kg^{-1} for atrazine and 41, 82, and 124 mg kg^{-1} for the mixture of atrazine and glyphosate. C:N ratios were 1.35 for atrazine, and 1.54 for the mixture of atrazine and glyphosate. Control treatments with no herbicide addition were included to measure normal soil microbial activity.

C mineralization was determined from soil samples that were dried at 40°C to ensure homogeneity of soil moisture content. Samples of 100 g were subsequently rewetted to approximately 15% moisture and incubated at 25°C for 7 days before herbicide addition. The 7-day incubation period prior to herbicide 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 5–10 days. Herbicides were added in 5 ml of distilled water to soil samples, increasing the final moisture content to 20% (around 60% water-filled pore space). Soils were placed in gas-tight 1-l glass containers along with a vial containing 10 ml 1 M KOH to trap evolved CO_2 and a vial of water to maintain humidity. Soils were incubated at 25°C with KOH traps replaced at 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 42, and 56 days. Unreacted alkali in the KOH traps was titrated with 1 M HCl to determine CO_2-C (Anderson 1982).

N mineralization was determined by subtracting the initial inorganic N concentration of non-incubated soil samples from soil N extracted after 7, 14, 28 and 56 days of incubation. Inorganic N was extracted from 7-g soil subsamples using 28 ml 2 M KCl. Samples were shaken for 30 min on a reciprocal shaker, filtered and the extracts analyzed for NH_4-N and NO_2- plus NO_3-N using an autoanalyzer (Technicon 1977a, b). The sum of the above N forms was designated inorganic N.

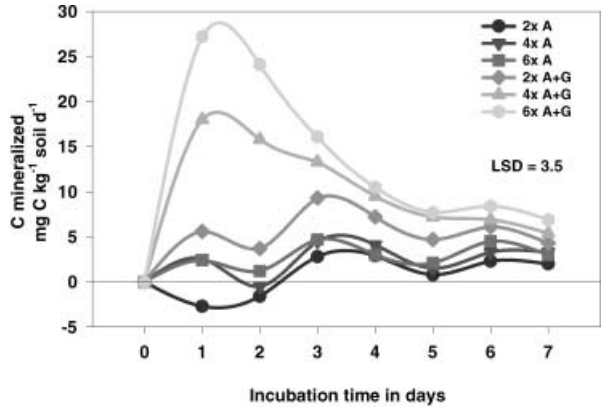

Fig. 1 Soil C mineralization over time as affected by herbicide addition (*2×, 4×, 6×* rate of herbicide additions, *A* atrazine, *A + G* atrazine plus glyphosate). The "0" line on the *y*-axis represents the control with no herbicide addition, which has been subtracted from each data point. The LSD of 3.5 represents the least significant difference between treatments at a given time interval

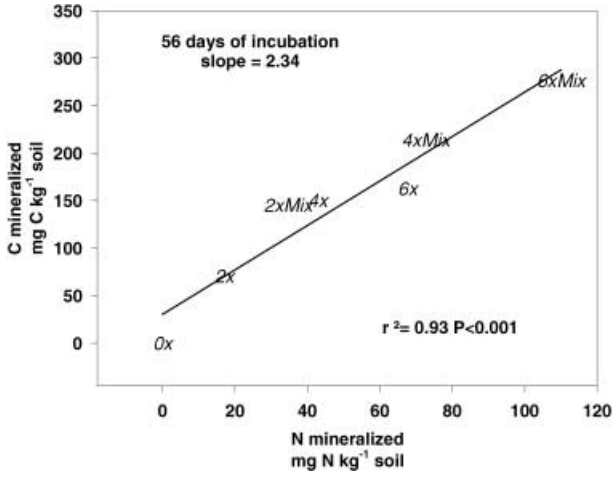

Fig. 2 N versus C mineralized during 56 days of incubation. Control data have been subtracted to show treatment effects on soil microbial activity

Atrazine extraction and determination using high performance liquid chromatography (HPLC) were conducted on 5-g soil subsamples collected at 7, 14, 28 and 56 days after the experiment began and stored at –10°C. The subsamples were air-dried, passed through a 2-mm sieve, and thoroughly mixed. Subsamples were placed in 125-mm × 20-mm screw cap test tubes and shaken for 10 min in 10 ml 9:1 $CH_3CN:H_2O$. After centrifugation for 3 min at 1,400 rpm, 5 ml of extract were added to 25 ml 1% AcOH and drawn through a 3-ml sulfonic acid cartridge (Varian, Harbor City, Ill.). Atrazine was removed from the sulfonic acid cartridge with 4 ml 3:7 CH_3CN:0.1 M K_2HPO_4. The eluent was mixed and an aliquot removed for HPLC analysis on a Waters RP8 Symmetry Shield C8 column with a Waters instrument equipped with a UV detector at 225 nm. The mobile phase was 3:7 $CH_3CN:H_2O$ with a flow rate of 2 ml min^{-1} and the injected volume was 20 µl. Atrazine extraction efficiency was 96% ±5, and samples were corrected for percent recovery.

All treatments were replicated three times. Analysis of variance was used for generation of means and for determination 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 the Fisher LSD test at the 5% level of significance (SPSS 1997).

Results and discussion

Both herbicide treatments stimulated microbial activity as indicated by C mineralization with the exception of the 2× atrazine treatment, which was below the control sample until the third day of incubation (Fig. 1). Results for control samples (no glyphosate or atrazine added) were subtracted to show the effect of the herbicides on soil microbial activity. The daily rate of CO_2 evolution after the addition of herbicides was not significantly different from the control for all treatments at 28 days of incubation with the exceptions of 4× A + G (atrazine plus glyphosate), 6× A, and 6× A + G, which were different from the controls (LSD =3.5). After 56 days of incubation, all treatments had returned to background levels (Fig. 1). After subtracting controls, the ratio of C mineralized to N mineralized after 56 days of incubation was 2.34:1 based on linear regression and the results were highly correlated (Fig. 2). The C:N mineralization ratio was 9.2:1 for the non-amended control soil (data not shown). These results indicate a significant increase in the rate of C and N mineralization due to the addition of these herbicides.

The amount of C and N added as atrazine alone and atrazine plus glyphosate and the quantities mineralized are compared in Tables 1 and 2. The 2× rate of atrazine mineralized 67% more C than the amount of C added as atrazine. The 4× rate of atrazine mineralized 78% more and the 6× rate mineralized 28% more C than the amount of C added as atrazine. The addition of glyphosate with atrazine significantly increased C mineralization in all treatments compared with atrazine alone. The addition of glyphosate with atrazine (mix) resulted in a 244% increase in C mineralized at the 2× rate. This large increase represents the contribution of glyphosate to microbial respiration compared with atrazine alone. This response may influence the degradation of other chemicals as suggested from earlier work (Haney et al. 2000). The 4× mix of atrazine with glyphosate and 6× mix resulted in a 46% and 76% increase in C mineralized, respectively. These lower values for the 4× and 6× mix may be indicative of an increase in Cl^- toxicity from mineralization of atrazine to the microbial population and therefore a lower mineralization rate.

N mineralization showed trends similar to the C data when comparing N added vs N mineralized. For atrazine alone only 55% of the N added was mineralized after 56 days of incubation for the 2× rate. The 4× rate mineralized 67% and the 6× rate mineralized 73% of the N added as atrazine. In comparison, the atrazine plus glyphosate treatment for the 2× rate mineralized 83% more N. The 4× rate had a 88% increase and the 6× rate showed a 89% increase in N mineralized above the N added from the herbicide mixture (Table 2, Fig. 3b). The apparent lower mineralization rates for N vs C may be

Table 1 C added and C mineralized above the control over 56 days for the atrazine only and the atrazine plus glyphosate treatments. Units are expressed as mg C kg^{-1} soil

Herbicide treatment	C added by atrazine	C added by glyphosate	C added by atrazine plus glyphosate	C mineralized from atrazine	C mineralized from atrazine plus glyphosate	Increase in C mineralized by addition of glyphosate
2×	42.3	21.6	63.9	70.8	145.2	74.4
4×	84.6	43.2	127.8	150.4	213.8	63.4
6×	126.9	64.9	191.8	162.3	276.5	114.2

Table 2 N added and N mineralized above the control over 56 days for the atrazine only and the atrazine plus glyphosate treatments. Units are expressed as mg N kg^{-1} soil

Herbicide treatment	N added by atrazine	N added by glyphosate	N added by atrazine plus glyphosate	N mineralized from atrazine	N mineralized from atrazine plus glyphosate	Increase in N mineralized by addition of glyphosate
2×	31.0	10.4	41.4	17.1	34.7	17.6
4×	64.0	18.7	82.7	42.9	72.7	29.8
6×	93.0	31.1	124.1	67.7	110.1	42.4

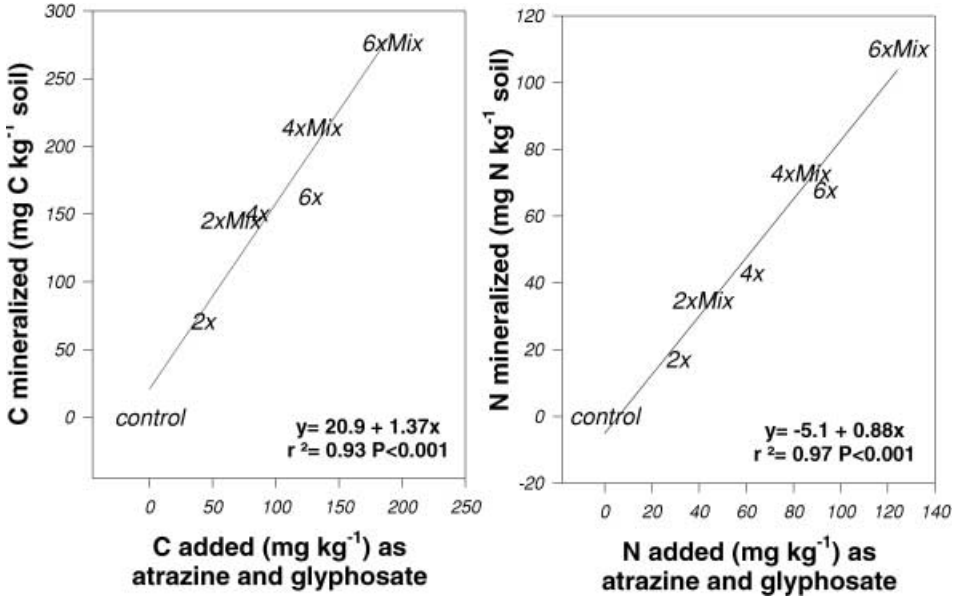

Fig. 3 C and N added as atrazine only and atrazine plus glyphosate versus C mineralized in 56 days (**a**), and N mineralized in 56 days (**b**)

due to N immobilization in microbial biomass; since soil microbial C and N are not as sensitive a measurement as C and N mineralization, they were not included in the analysis (Franzluebbers et al. 1999).

C mineralization was significantly greater than the C added from both the atrazine only and atrazine-glyphosate treatments. However, for N mineralization, some of the N may have been assimilated into the microbial biomass and therefore rendered unavailable for extraction. When regressed across all treatments, approximately 137% of the C added from the herbicides was mineralized in 56 days, while only 88% of he herbicide N was mineralized.

When rates of C and N mineralization were analyzed during the 56-day incubation, flushes of C and N were evident at the intervals 0–7 and 14–28 days (Figs. 4 and 5). These data suggest a microbial preference for the substrate glyphosate early in the incubation period. Peaks at 0–7 days indicate C and N mineralization primarily from glyphosate while peaks at 14–28 days may predominantly be from atrazine. This is possibly due to complete mineralization of glyphosate by day 14 (Haney et al. 2000).

A smaller C flush was noted at 0–7 days for atrazine only, but a larger flush was noted at 28 days for the 4× and 6× rates. It should be noted that treatments with atrazine plus glyphosate resulted in greater C mineralized from the 0- to 7- and 14- to 28-day intervals as compared to atrazine alone. Stimulation by the atrazine/glyphosate mixture may have resulted in greater atrazine degradation later in the incubation.

The largest flush of carbon occurred during the 0- to 7-day incubation period, while the predominant flush of N occurred during the 14- to 28-day interval. This may be expected because atrazine has a lower C:N ratio (1.4:1) than glyphosate (3:1). Therefore, a greater C

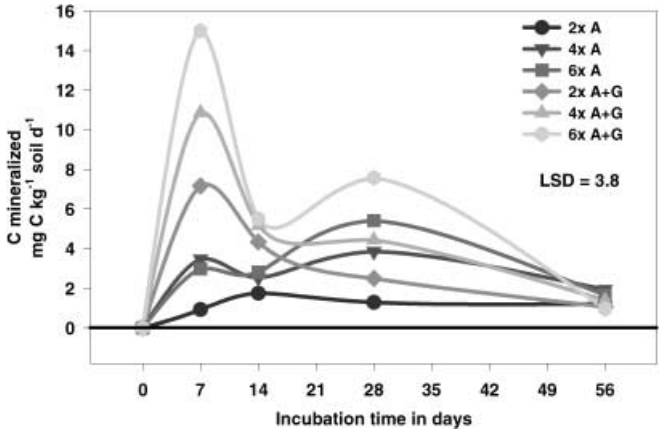

Fig. 4 Soil C mineralization over time. (*2×, 4×, 6×* rate of herbicide additions, *A* atrazine, *A + G* atrazine plus glyphosate). The "0" line on the *y*-axis represents the control with no herbicide added and has been subtracted from all treatments. The LSD of 3.8 represents the least significant differences between treatments at a given time interval

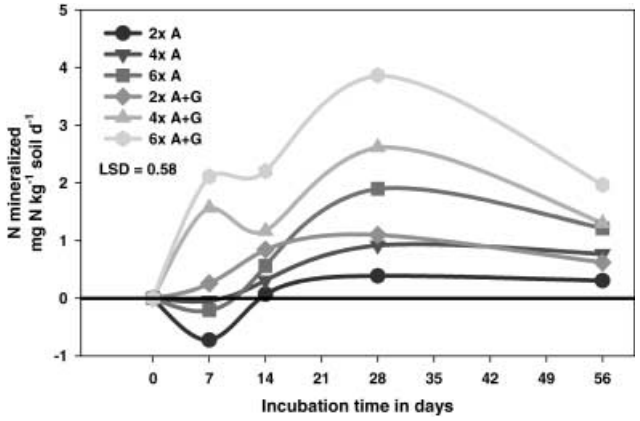

Fig. 5 N mineralized over time. (*2×, 4×, 6×* rate of herbicide additions, *A* atrazine, *A + G* atrazine plus glyphosate. The "0" line on the *y*-axis represents the control with no herbicide added and has been subtracted from all treatments. The LSD of 0.58 represents the least significant differences between treatments at a given time interval

flush is possible when glyphosate is the primary substrate. Consequently, a greater flush of N would occur when atrazine is the predominant substrate (Figs. 4, 5).

Extractable atrazine remaining at various periods of the incubation is shown in Fig. 6. A significant decrease in atrazine remaining with glyphosate addition at the 6× rate was noted at 14 days of incubation compared with atrazine alone. A similar trend was noted for the 2× treatment at 28 days of incubation. Comparing the dissipation times (DT_{50}) for all treatments, the average DT_{50} was 10.5 days and was not statistically different across treatments (P <0.05; data not shown).

The C mineralization results were compared with soil extractable atrazine (Fig. 7). Increasing glyphosate concentration increased C mineralization and may account for the differences in extractable atrazine at day 28 of the 2× rate and day 14 of the 6× rate (Fig. 6). Extraction data

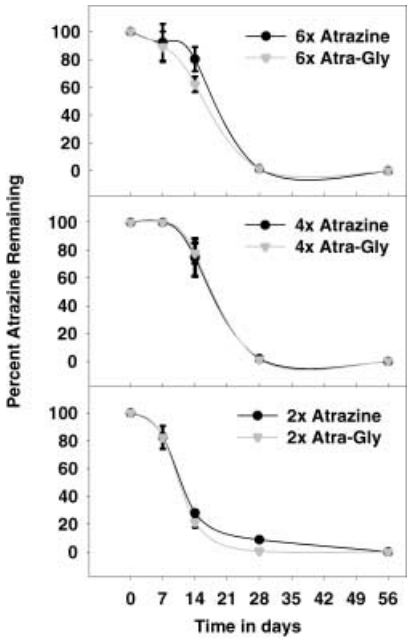

Fig. 6 Extractable atrazine during 56 days of incubation. *Error bars* indicate one standard deviation (*Atra-Gly* atrazine-glyphosate)

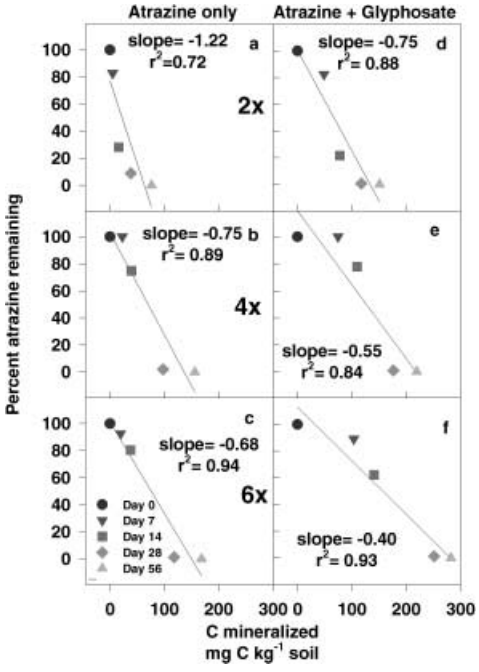

Fig. 7a–f Percentage of atrazine remaining versus C mineralized during 56 days of incubation. **a**–**c** Atrazine only and **d**–**f** atrazine plus glyphosate. Controls have been subtracted for the C mineralized data

indicated that no atrazine was present by 56 days of incubation for either the atrazine only treatment or the atrazine plus glyphosate treatment. For atrazine only, the slopes from regression were significantly different between the 2× and 4× rates. There was also a significant difference in regression slopes between the 2× and the

6× rate (P <0.05; Fig. 7). For the mixture of atrazine and glyphosate none of the slopes from regression were significantly different (Fig. 7d–f). Within rates, only the 2× and the 2× mix were different at P =0.056. Only the 6× rate of herbicide treatment at 14 days of incubation and the 2× rate at day 28 were significantly different at P <0.1 for atrazine only vs atrazine plus glyphosate (Fig. 6). From these data, it appears that the mixture of atrazine with glyphosate may slightly enhance the degradation of atrazine.

Conclusions

Soil C and N mineralization was sensitive to the addition of atrazine as well as atrazine mixed with glyphosate. The addition of low C:N ratio herbicides stimulates microbial activity and enhances the eventual mineralization of these compounds. The addition of glyphosate with atrazine appears to provide sufficient microbially available N to overcome the initial immobilization observed with atrazine alone. Herbicide addition may also stimulate mineralization of native organic matter. It is interesting to note that soil microbes appeared to prefer glyphosate to atrazine as a substrate, but that as the glyphosate was depleted, atrazine was quickly degraded. C mineralization appeared to be closely and inversely related to the percentage of atrazine remaining in the soil over time. These results imply that C mineralization may be an effective preliminary mechanism for estimating herbicide degradation in soil.

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