Stimulated Rhizodegradation of Atrazine by Selected Plant Species

Chung-Ho Lin,* Robert N. Lerch, Robert J. Kremer, and Harold E. Garrett

The efficacy of vegetative buffer strips (VBS) in removing herbicides deposited from surface runoff is related to the ability of plant species to promote rapid herbicide degradation. A growth chamber study was conducted to compare ^{14}C–atrazine (ATR) degradation profiles in soil rhizospheres from different forage grasses and correlate ATR degradation rates and profiles with microbial activity using three soil enzymes. The plant treatments included: (i) orchardgrass (*Dactylis glomerata* L.), (ii) smooth bromegrass (*Bromus inermis* Leyss.), (iii) tall fescue (*Festuca arundinacea* Schreb.), (iv) Illinois bundle flower (*Desmanthus illinoensis*), (v) perennial ryegrass (*Lolium perenne* L.), (vi) switchgrass (*Panicum virgatum* L.), and (vii) eastern gamagrass (*Tripsacum dactyloides*). Soil without plants was used as the control. The results suggested that all plant species significantly enhanced ATR degradation by 84 to 260% compared with the control, but eastern gamagrass showed the highest capability for promoting biodegradation of ATR in the rhizosphere. More than 90% of ATR was degraded in the eastern gamagrass rhizosphere compared with 24% in the control. Dealkylation of atrazine strongly correlated with increased enzymatic activities of β-glucosidase (GLU) (r = 0.96), dehydrogenase (DHG) (r = 0.842), and fluorescein diacetate (FDA) hydrolysis (r = 0.702). The incorporation of forage species, particularly eastern gamagrass, into VBS designs will significantly promote the degradation of ATR transported into the VBS by surface runoff. Microbial parameters widely used for assessment of soil quality, e.g., DHG and GLU activities, are promising tools for evaluating the overall degradation potential of various vegetative buffer designs for ATR remediation.

Atrazine (ATR) is one of the most widely applied herbicides in the United States with an estimated 36.3 million kg of ATR applied to more than 66% of all United States corn (*Zea mays* L.) acreage in 2006 (USDA, 2007). Contamination of surface and groundwater by ATR and its chlorinated metabolites has been linked to public health risks and ecological impacts (Hayes et al., 2002; Stanko et al., 2010; Swan et al., 2003).

Multispecies vegetative riparian buffer strips (VBS) have been recommended as a potentially cost-effective conservation practice to reduce nonpoint-source pollution of adjacent waterways (Krutz et al., 2003; Schultz et al., 2000). Previous research has shown that VBS used in agroecosystems reduce the transport of and encourage the deposition of both dissolved and sediment-bound ATR in surface runoff (Arora et al., 1996; Cole et al., 1997; Hoffman et al., 1995; Lin et al., 2007; Lowrance et al., 1997; Schultz et al., 1995; Schultz et al., 1991). Following the deposition of ATR into the VBS, only small fractions of deposited ATR in VBS were taken up by plants or leached into the leachates. Rhizodegradation is considered the primary biotic mechanism for ATR dissipation in VBS (Henderson et al., 2007; Lin et al., 2008; Schroll and Sabine, 2004; Singh et al., 2004). More than 15 ATR degradation products have been identified through either physiochemical or biochemical processes (Cook, 1987; Jensen, 1982; Sadowsky et al., 1998) (Fig. 1). The major ATR degradation products include N-dealkylated metabolites, deethylatrazine (DEA), and deisopropylatrazine (DIA), and the hydroxylated metabolites, hydroxyatrazine (HA), deethylhydroxyatrazine (DEHA), and deisopropylhydroxyatrazine (DIHA) (Fig. 1).

Microbial degradation of ATR in rhizospheres usually requires a group or "consortium" of microorganisms (Mandelbaum et al., 1993; Sadowsky et al., 1997). The associated biochemical processes involve a wide spectrum of enzymatic reactions, e.g., dealkylation, deesterification, hydroxylation, dehalogenation, and oxidization (Ambus, 1993; Bollag and Liu, 1990; Mandelbaum et al., 1993). Previous findings have indicated that some soil enzymatic parameters widely used to assess soil quality in the past might be useful as indicators to evaluate the overall potential

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Abbreviations: ATR, atrazine; C_3, cool-season; C_4, warm-season; DDA, didealkylatrazine; DEA, deethylatrazine; DEHA, deethylhydroxyatrazine; DHG, dehydrogenase; DIA, deisopropylatrazine; DIHA, deisopropylhydroxyatrazine; EG, eastern gamagrass; FDA, fluorescein diacetate; GLU, β-glucosidase; HA, hydroxyatrazine; HPLC, high-performance liquid chromatography; IB, Illinois bundle flower; OG, orchardgrass; RYE, perennial ryegrass; SM, smooth bromegrass; SW, switchgrass; TALL, tall fescue; THAM, tris-hydroxymethyl-aminomethane; TOC, total organic carbon; TPF, triphenyl formazan; TTC, triphenyltetrazolium chloride; VBS, vegetative buffer strips.

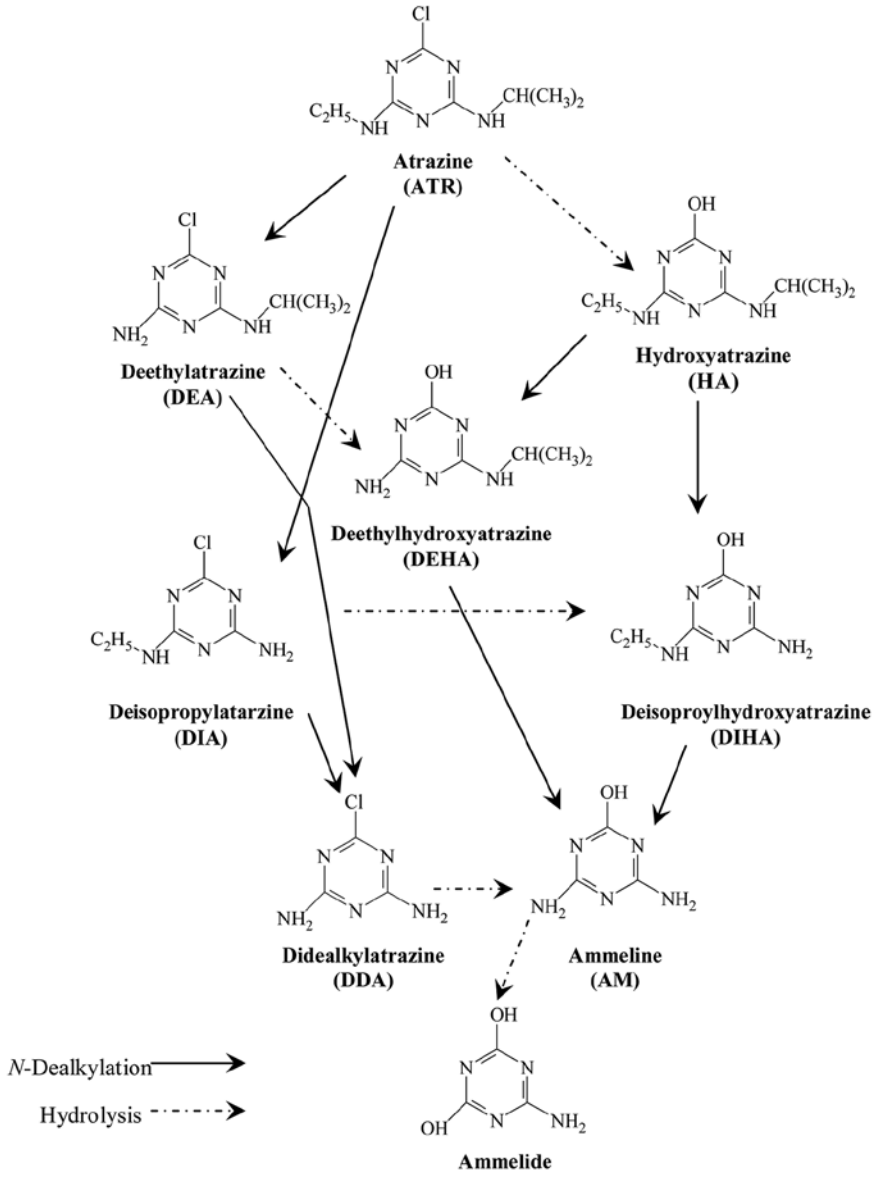

Fig. 1. The major atrazine degradation pathway (Cook, 1987; Jensen, 1982; Sadowsky et al., 1998).

for herbicide biodegradation in VBS (Bergstrom et al., 1998; Zablotowicz et al., 2000). For instance, the fluorescein diacetate (FDA) hydrolytic enzyme activities, commonly used to estimate total soil microbial activity, were highly correlated with the deesterification of fenoxaprop–ethyl (Zablotowicz et al., 2000). The dehydrogenase, which has been used as a valid biomarker of soil quality under different agronomic practices, is usually required to catalyze the oxidation and dehalogenation of several herbicides and other organic compounds (Beller et al., 1996; Waarrde et al., 1993).

Various VBS designs have been prescribed in the United States to encourage deposition of dissolved and sediment-bound ATR in surface runoff (Lin et al., 2007; Tim and Jolly, 1994; Udawatta et al., 2004). However, to date, few studies have compared the effectiveness of different plant rhizospheres in enhancing degradation of ATR. Results from a field lysimeter study have shown stimulated dealkylation and hydroxylation rates of ATR in rhizospheres of selected grass species, including warm-season and cool-season species (Lin et al., 2008). In the same study, the increased degradation rates of ATR in these grass treatments were found strongly correlated with increased microbial biomass carbon. The objectives of this study were to investigate the effects of these plant species with additional species commonly found near Midwest regions on the activities of the soil microbial enzymes and their association with the fate of isotope-labeled ATR in rhizospheres. The use of isotope-labeled ATR allows us to quantify the mineralized ATR and a wide spectrum of ATR degradation products. The goals of the study are (i) to compare ATR degradation rates supported by soil rhizospheres from different forage grasses and (ii) to correlate ATR degradation profiles with microbial activities of soil enzymes, specifically activities of the β-glucosidase (GLU), dehydrogenase (DHG), and FDA hydrolysis.

Materials and Methods

Experimental Design

The experiment was conducted in an environment-controlled growth chamber (Environmental Growth Chambers GC72 walk-in unit, Chagrin Falls, OH) with triplicate replications of seven forage species: (i) orchardgrass (OR), (ii) smooth bromegrass (SM), (iii) tall fescue (TALL), (iv) Illinois bundle flower (IB), (v) perennial ryegrass (RYE), (vi) switchgrass (SW), and (vii) eastern gamagrass (EG). A control treatment with no plants was also included and maintained with the same irrigation and fertilization regime as other treatments. The forage treatments included five cool-season (C_3) species (OR, SM, TALL, IB, and RYE) and two warm-season (C_4) species (SW and EG). Environmental conditions were: light intensity of 1400 μmol photons m^{-2} s^{-1}, light/dark period of 15/9 h, humidity of 50%, and temperature at 25°C (light)/20°C (dark). Two-month-old seedlings were transplanted into 1-L, wide-mouth, polypropylene jars containing 1000 g of soil to allow growing for 100 d in a mixture of 60% sand and 40% Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualfs) collected from the A horizon of the soil profile. The soils were collected from a site without previous history of ATR application (were not previously exposed to ATR). The soils were sterilized in an autoclave for 60 min and exposed to ambient air in a greenhouse for 2 wk before the experiment. Soil characterization, nitrogen content, and total organic carbon (TOC) analyses were completed by the University of Missouri Soil Characterization Laboratory using standard methods of analysis (Burt, 2010). The soil particle size consisted of 108 g kg^{-1} clay, 338 g kg^{-1} silt, and 557 g kg^{-1} sand (sandy loam texture). Cation exchange capacity was 8.1 cmol kg^{-1}, soil pH 7.2, and initial organic carbon content 6 g kg^{-1}. The plants and control

treatments were irrigated with 100 mL of water once every other day with a micro-dripping irrigation system. The fertilizer solution containing N, P, K, and micronutrients (Peters 20:20:20; Micromax Micronutrients, The Scotts Company, Marysville, OH) was applied to each jar every week only during the first 3-wk establishment phase. After 3 mo (100 d) of plant growth, rhizosphere soil was separated from each plant and soil moisture content was adjusted to 20%. Atrazine solution prepared with 0.1 μCi of ^{14}C–ATR with a specific activity of 18.1 mCi/mmol (atrazine-ring-UL-^{14}C, purity >95%; Sigma-Aldrich, St. Louis, MO) and nonradioactive ATR was then added to 50 g (dry weight equivalent) of soil to achieve a final concentration of 100 μg kg^{-1}. Before ^{14}C–ATR application, a subsample of the rhizosphere soil was collected to determine β-glucosidase, dehydrogenase, and fluorescein diacetate hydrolytic enzyme activities. Measurement of GLU, DHG, and FDA activities were chosen as these enzymes have been commonly used in the assessment of microbial activity and overall soil quality. The herbicide-treated soil was then incubated in sealed 500-mL Mason jars for 115 d at 25°C in the dark. Atrazine-mineralized $^{14}CO_2$ was trapped using alkali traps (10 mL of 2 M NaOH) placed in Mason jars and the traps were periodically replaced on the first, second, fourth, sixth, tenth, and sixteenth weeks throughout the incubation period. After end of experiment, roots and shoots were collected. The biomass dry weight was recorded.

Chemical Analysis

After 115 days incubation, ^{14}C–ATR and its degradation products were extracted with 250 mL of 90% MeOH followed by 1 h of sonication. The extract was centrifuged for approximately 5 min at approximately 3000 rpm and the supernatant was collected. The extraction was repeated and supernatants were combined. The extract was concentrated to 10 mL using a Savant concentrator (Holbrook, NY). The ^{14}C-labeled 2,4-dichloro-phenoxyacetic acid was added to each sample as an internal standard for final volume correction. The final extracts were concentrated to 200 μL under N_2 flow and injected into an SCL–10Avp high-performance liquid chromatography system (HPLC) (Shimadzu, Columbia, MD). The ^{14}C–ATR and its degradation products were separated using a silica-based Columbus C8 column (4.6 mm by 250 mm, 5 μm; Phenomenex, Torrance, CA). The radioactivity was detected by an inline IN/US ScinFlow β-Ram Model 3 (Tampa, FL) flow scintillation analyzer (HPLC–FSA). Injection volume was 10 μL and mobile phase flow rate was 1 mL min^{-1}. The ^{14}C–ATR and its metabolites were eluted with a two-part mobile phase gradient. Mobile phase A consisted of 0.1% H_3PO_4 buffer (pH = 2.1) and mobile phase B was 100% acetonitrile. The gradient started at 10% and ramped linearly to 40% at 30 min, 75% at 40 min, 10% at 45 min, and held at 10% for 14 min. Metabolites were identified by comparing the retention times of unlabeled standards based on HPLC–UV detection at 220nm (Fig. 2A and B). The standards including ATR, DEA, DIA, HA, DIHA, DEHA, didealkylatrazine (DDA), ammeline, and ammelide were purchased through ChemService (West Chester, PA). To further confirm the presence of parent ATR and its metabolites, the same set of standards and soil extracts were reinjected and eluted with the same stepwise gradient but with a basic buffer consisting of 5mM KH_2PO_4 (pH = 7.7) as the mobile phase A (Fig. 3A and B). The ^{14}C–ATR mineralization was determined by measuring the radio activities of the evolved $^{14}CO_2$ using Beckman LS600 liquid scintillation counter (Beckman, Fullerton, CA). The extraction efficiencies of soil ATR, DEA, and DIA were >95% and >75% of the applied ^{14}C was recovered from this study.

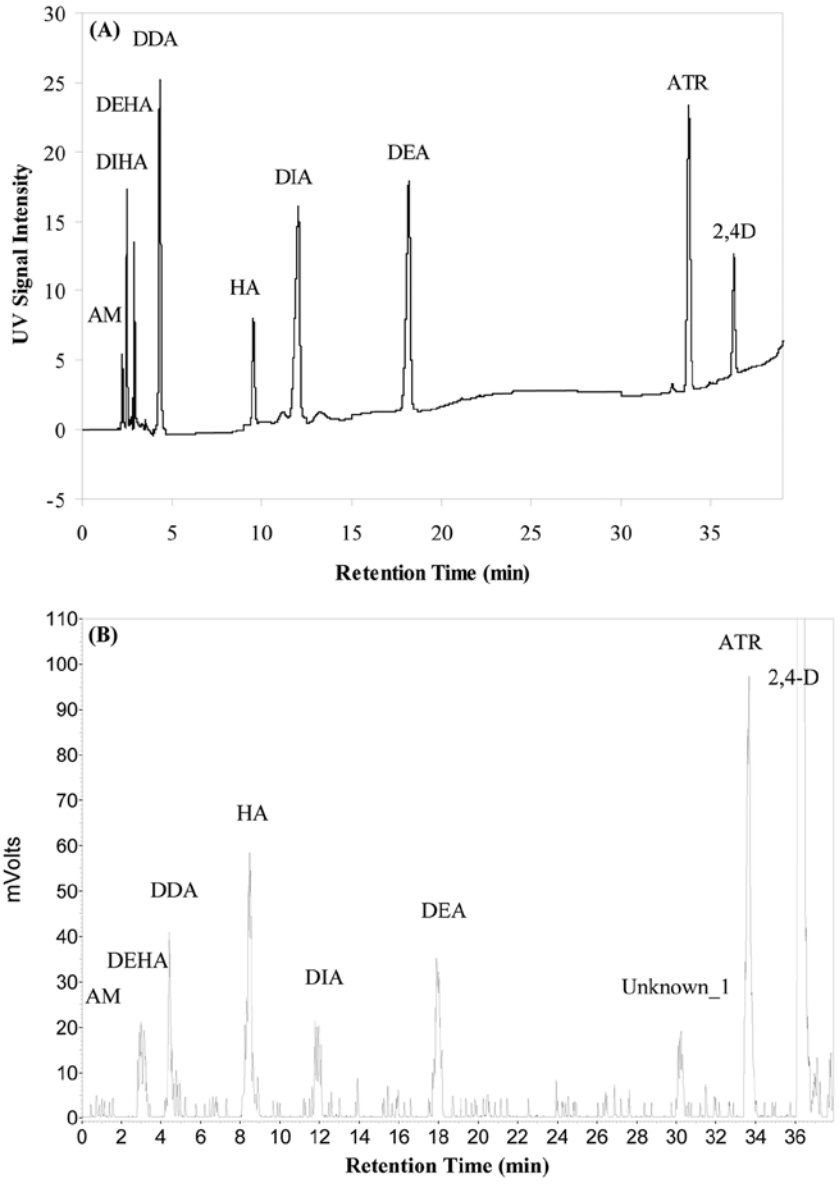

Fig. 2. Retention times of atrazine and its metabolites eluted with acidic mobile phase with 0.1% H_3PO_4 buffer (pH 2.1) by HPLC–UV (A: standards) and HPLC–FSA (B: soils collected from smooth bromegrass) analysis. Acronyms of the atrazine and its metabolites were described as in Fig. 1.

Microbial Enzyme Activity

The soil moisture content was determined before the analysis and soil moisture content was adjusted to 20% before analysis. Dehydrogenase activity was measured following the procedure described by Gerba and Brendecke (1995). Briefly, this procedure uses 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-triphenyltetrazolium chloride (TTC) as the electron acceptor, which is reduced to the red-colored, methanol-soluble, triphenylformazan (TPF). Six grams of moist soil from each sample was placed in a test tube. Samples were incubated statically with 1 mL of 3% TTC and 3 mL of 0.2 M $CaCO_3$ buffer solution for 24 h at 37°C. This reaction was terminated with 10 mL of methanol and TPF was extracted with 30 mL of additional methanol. The final extract was filtered with Whatman #42 filter paper and TPF concentration was determined spectrophotometrically at 485 nm.

To assess microbial carbon utilization efficiency, GLU activity was quantified according to procedures described by Tabatabai (1994). The method is based on colorimetric measurement of *p*-nitrophenol released by GLU when soil is incubated with buffered (pH 6.0) *p*-nitrophenyl-β-D-glucoside solution. One gram of moist soil was sampled and incubated with solution containing 0.25 mL of toluene and 4 mL of 8 mM tris-hydroxymethyl-aminomethane buffer (THAM; pH 6) for 1 h at 37°C. Then, 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.1 M THAM (pH 12) solution were added. This solution was vortexed and filtered through Whatman #2 filter paper. The absorbance was measured at 410 nm.

Fluorescein diacetate hydrolytic activity was determined by the enzymatic assay procedures described by Bandick and Dick (1999). One gram (dry weight equivalent) of moist soil from each sample was placed into a 50-mL Erlenmeyer flask. Twenty mL of 60 mM sodium phosphate buffer (pH 7.6) and 100 μL of 4.8 mM FDA were added to the flask. The flask was then capped with parafilm. Samples were incubated for 105 min on a reciprocal shaker at room temperature. After 105 min, 10 mL of acetone was added to terminate the hydrolysis reaction. Samples were then filtered through Whatman #4 filter paper and centrifuged for 5 min. Fluorescein (the product of FDA hydrolysis) concentration was determined spectrophotometrically at 490 nm. Pairwise correlation analysis was performed to determine the relationships between the ^{14}C–ATR degradation and activities of soil enzymes.

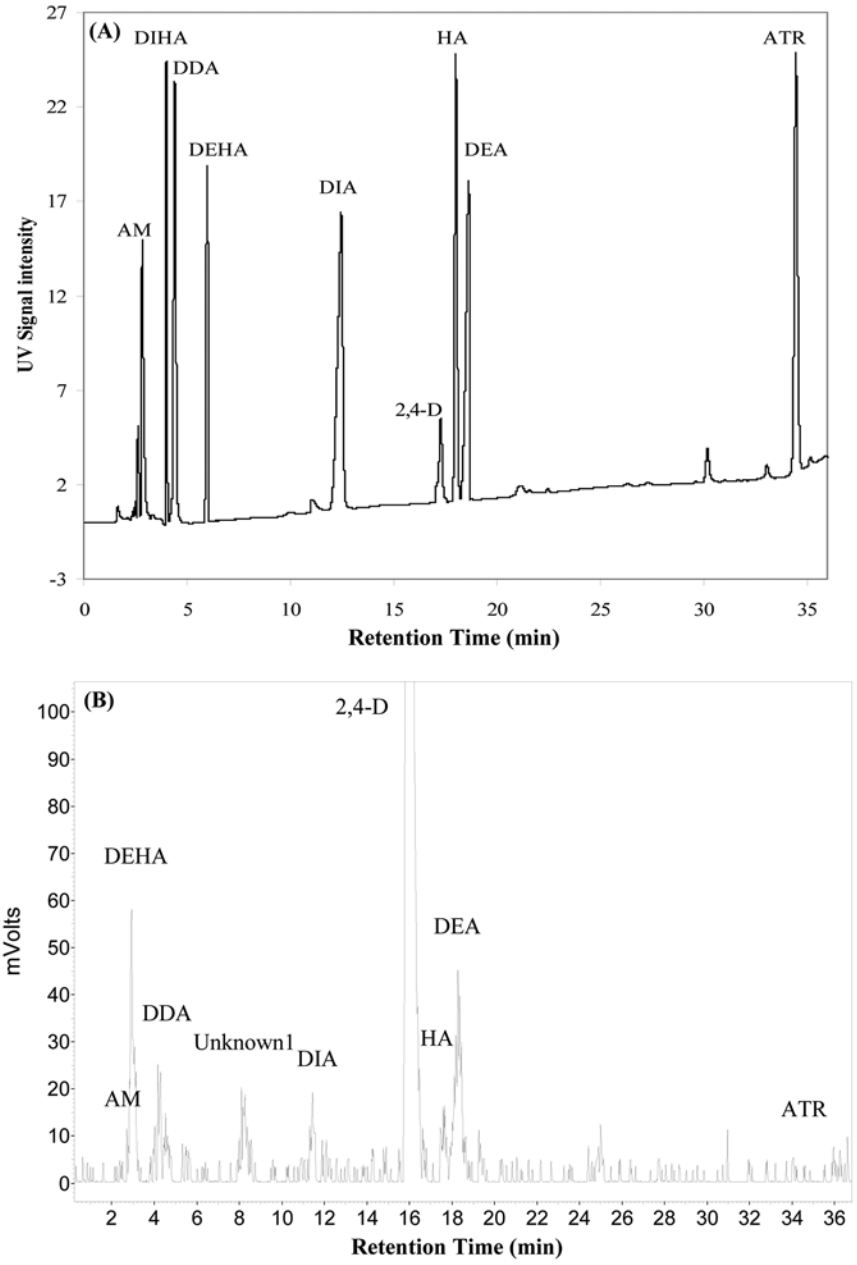

Fig. 3. Retention times of atrazine and its metabolites eluted with basic mobile phase with 5mM KH_2PO_4 buffer (pH 7.7) in HPLC–UV (A: standards) and HPLC–FSA (B: soils collected from eastern gamagrass) analysis. Acronyms of the atrazine and its metabolites were described as in Fig. 1.

Results and Discussion

Rhizodegradation of Atrazine

Compared with the plant-free control, the extent of ATR degradation in the plant rhizospheres was significantly enhanced by 84 to 260% ($p < 0.01$), through both N-dealkylation and hydroxylation reactions (Fig. 4A and B). Among the plant species tested, EG showed the highest capability for promoting ATR degradation. More than 90% of ATR in the rhizosphere of EG was degraded to less toxic metabolites (Stoker et al., 2002; Stratton, 1984), as compared with 24% in the control. The N-dealkylation was the primary detoxification pathway in the EG treatments, accounting for 58% of the extractable ATR degradation products (Fig. 4B). The DDA and DEA were two of the most predominate degradation products found in the EG rhizospheres, accounting for 28 and 23% of the total extracted ^{14}C

activities, respectively. Hydroxylated metabolites, including DEHA (12%), DIHA (2.4%), and HA (8.8%), accounted for >23% of ATR degradation in EG rhizospheres. In the other grass treatments, the rates of ATR degradation ranged from 46 to 65% of the applied ATR. Atrazine degradation rate was similar among rhizospheres of OR, SM, and SW ($p > 0.05$), where >60% of the applied ^{14}C–ATR was degraded. The similar proportion of hydroxylated metabolites across all grass treatments ($p > 0.01$) indicated a similar degree by which hydroxylation was stimulated in the plant rhizospheres. The N-dealkylation, on the other hand, showed a broad range from <10 to almost 60% of total extractable ^{14}C activity, suggesting either different degrees of enhancement or different modes of stimulation. The amount of ^{14}C–ATR mineralization was also significantly enhanced by presence of EG ($p < 0.001$). However, the mineralized ^{14}C–ATR was limited to <7% of applied ^{14}C–ATR (Fig. 5). Comparable results from a field lysimeter study by Lin et al. (2008) showed similarly enhanced microbial dealkylation and hydroxylation of ATR in soil by several of the same forage species tested. However, the extent of ATR degradation in the SW rhizospheres in the incubation study reported here were considerably lower than observed under field conditions (Lin et al., 2008; Lin et al., 2005).

The use of HPLC–FSA allowed for quantification of seven known and three unknown ATR metabolites. One of the advantages of HPLC–FSA was that it allowed us to distinguish and quantify the hydroxylated metabolites (e.g., DEHA, DIHA, and ammeline) that have generally not been reported in other studies. Our results suggested that DDA and DEA were the predominant dealkylated metabolites and HA and DEHA were the major hydroxylated metabolites found in the grass rhizospheres. The relative concentrations of the major degradation products (DDA, DEA, and HA) identified in this study were similar to that reported by Henderson et al. (2007). The three major unknown metabolites detected in this study have retention times of 19, 26, and 30 min, respectively. Based on these retention times, the polarities of these unknown metabolic products are between ATR and DEA. The percentage of these unidentified metabolites accounts for 5 to 18% of the total extracted ^{14}C activities. Given their intermediate polarity, these unknowns may be amide intermediates (Verstraeten et al., 2002) that may further degrade to DEA and DIA or glutathione conjugates of ATR (Clay and Koskinen, 1990).

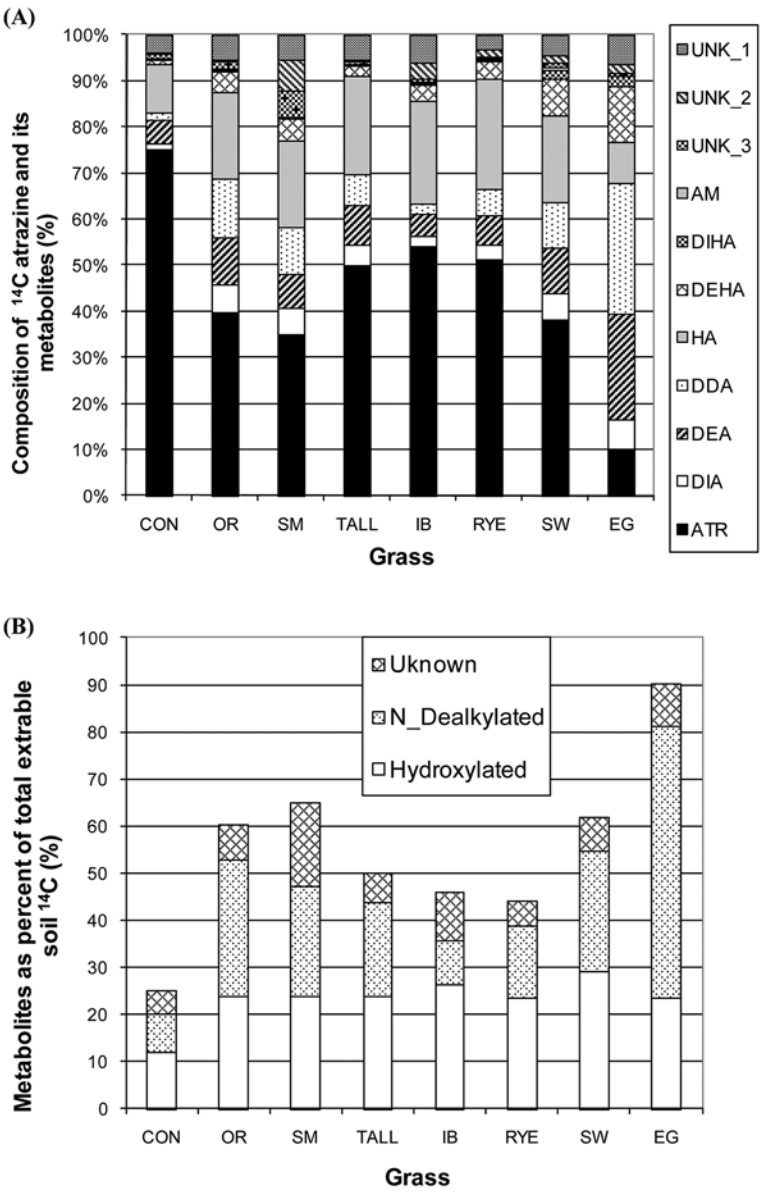

Fig. 4. Distribution of extractable ^{14}C–atrazine and its metabolites in the soils collected from rhizospheres after 115 days incubation (A). Acronyms of the atrazine and its metabolites were described as in Fig. 1. Degradation products were categorized into parent, hydroxylated (HA, DEHA, and DIHA), N-dealkylated (DEA, DIA, and DDA), and unknown metabolites (B). Plant-free control (CON), orchardgrass (OR), smooth bromegrass (SM), tall fescue (TALL), Illinois bundle flower (IB), perennial ryegrass (RYE), switchgrass (SW), and eastern gamagrass (EG) ($n = 3$).

Enzyme Activities and Atrazine Degradation

Microbial enzymatic activities were significantly higher in plant rhizospheres than in the plant-free control treatment (Fig. 5). When compared with the enzyme activities in the control, the FDA, GLU, and DHG activities in the plant rhizospheres were enhanced by 29 to 913%, 37 to 600%, and 32 to 853%, respectively. The highest GLU and DHG activities were found in the EG treatments and the highest FDA activities were found in the SW treatments. As expected, enzyme activities in the plant rhizospheres were correlated with increased mineralization of ring-labeled ATR (Fig. 5). Although ATR mineralization was significantly elevated in EG rhizospheres ($p < 0.001$), <7% of the total applied ATR was mineralized. The ATR mineralization results were positively correlated with GLU ($r = 0.571$, $p = 0.004$) and DHG ($r = 0.383$, $p = 0.065$) but poorly correlated with FDA ($r = 0.277$, $p = 0.19$) (Fig. 5). The stimulated but limited ATR mineralization in planted versus bulk soils (i.e., no plants) has also been reported in similar studies (Marchand et al., 2002). It appears that low ATR mineralization is mainly due to the lack of microbial enzymes capable of catalyzing the cleavage of the triazine ring (Mandelbaum

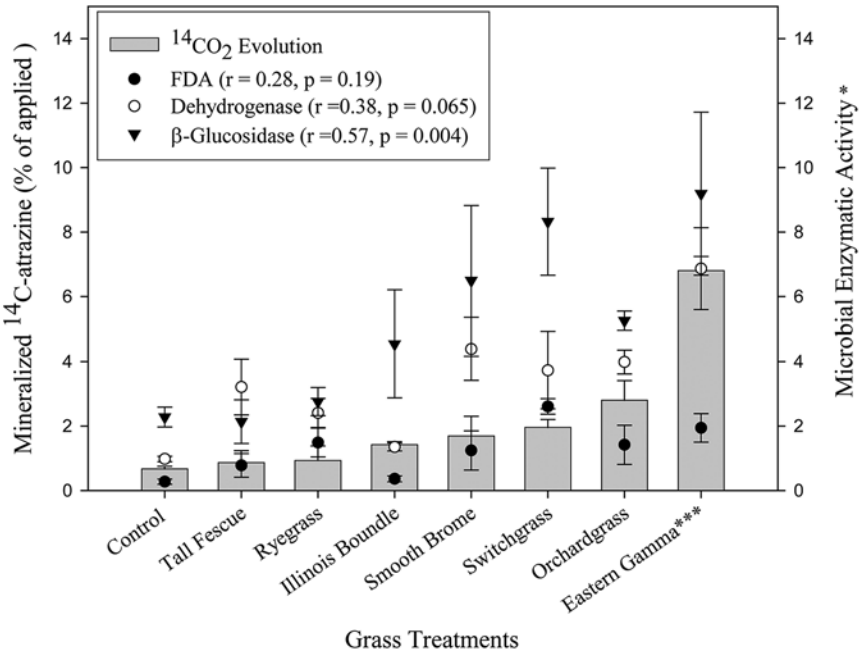

Fig. 5. Total mineralization of atrazine and microbial enzyme activities in soils with forage treatments. Control treatment is bare ground. Error bar represents the standard error of the mean (n = 3). Values in parentheses represent the Pearson coefficient of correlation between mineralization of ^{14}C–atrazine and enzymatic activity. *Dehydrogenase and β-glucosidase activities are expressed in μmol·g-1·h-1. The FDA hydrolytic activity is expressed in (nmol·g-1·h-1) ·0.01. The total mineralization of atrazine was significantly enhanced by eastern gamagrass (p < 0.001). The differences in mineralization among control and other species treatments were not significant.

et al., 1993). However, a few degraders, such as *Pseudomonas* sp. strain atrazine-degrading Pseudomonas, isolated from sites heavily contaminated with ATR, have evolved to use ATR and its metabolites as C and N sources (Mandelbaum et al., 1993). These degraders possess a series of *atz* (e.g., *atz*A–F) or trz genes (e.g., *trz*N) encoded on a self-transmissible plasmid responsible for various processes of ATR degradation and ring cleavage, resulting in rapid mineralization of ATR to carbon dioxide, ammonia, and water. The introduction of such degraders into the rhizospheres is expected to further stimulate the ATR mineralization process (Lin et al., 2009).

Pairwise comparison between ATR metabolites and soil enzyme activities revealed that the overall ATR degradation and N-dealkylation were strongly (p < 0.05) correlated to the activities of all three enzymes, but the activity of GLU consistently showed the highest correlation coefficients, particularly with respect to the formation of N-dealkylated metabolites (Fig. 6). The enhanced ATR degradation rates associated with stimulated microbial enzymatic activities in the presence of grasses were consistent with previous studies investigating the fate of ATR in plant microcosms (Henderson et al., 2007; Lin et al., 2008; Smith et al., 2008). Similar, positive correlations between enhanced ATR N-dealkylation rates and increased microbial biomass carbon in the rhizospheres have been observed in a field study by Lin et al. (2008). In contrast, the formation of hydroxylated metabolites was not significantly related to any of the enzyme activities (p > 0.05). The strong correlation between the enzyme activities and N-dealkylation and overall ATR degradation suggested that any of the enzymes that have been widely used for assessment of soil quality, particularly GLU, are very useful indicators of overall ATR degradation potential in VBS soils. In contrast, the weaker correlation between enzyme activities and ATR hydroxylation found in this work suggested that measured enzymes like GLU could be good indicators of N-dealkylation but poor indicators of hydroxylation of ATR.

Our finding suggested that EG provided more favorable rhizosphere conditions to sustain higher ATR biodegradation activities and support higher numbers of ATR degraders than C_3 plants to promote various biochemical degradation processes to occur (Bollag and Liu, 1990; Mahmood and Renuka, 1990; Qian et al., 1997). The observed differences in enzymatic activities and ATR degradation potential among the forage species may be a result of the differences in their morphological, physiological, and biochemical characteristics. Our finding has supported other field and laboratory studies suggesting equivalent or higher degradation rates of ATR and other herbicides in soils planted with warm-season species compared with cool-season species (Belden and Coats, 2004; Lin et al., 2008; Smith et al., 2008). Warm-season species, including EG and SW (both C4 species), partition more carbohydrates to roots as compared with C3 cool-season grasses. (Dhawan and Goyal, 2004; Jiang et al., 2002). Greater partitioning of carbohydrates to the root system likely resulted in higher concentrations of root-derived, bioavailable organic carbon and other nutrients in the rhizospheres of the C_4 species. It should be noted that both perennial C_4 EG and SW plants in our study were not allowed to achieve full maturity during the 100-d growing period. In this study, EG partitioned >87% of the carbohydrates to the root biomass—similar to OR and SM. However, due to the possible root-derived N and higher N utilization in C_4 EG as compared with other treatments, particularly the C_3 grass treatments (Oaks, 1994), the concentrations of total N in EG rhizosphere were found to be 42% higher than in the control and 50.1% higher than C_3 grass treatments. The high nitrogen use efficiency of C_4 plants may be related to biological nitrification inhibition Subbarao et al. (2009). Biological nitrification inhibition is the result of plant-induced alterations of the rhizosphere chemical and microbial conditions through the release of root exudates.

Although the link between soil herbicide dissipation rates and root biomass have been established by Smith et al. (2008), the same relationship between root biomass and ATR degradation was not found in this study (r = 0.45 p = 0.31). For example, EG has a significantly lower root biomass compared with OR and SM (Table 1), but the ATR degradation rates in EG rhizosphere were significantly higher (p < 0.005). We have also carefully examined the levels of soil TOC among grass and other treatments, and they were not significantly different (0.7–0.9%). This finding led us to believe that the difference in organic carbon composition and carbohydrates constitutes

in the rhizospheres may also influence ATR degradation profiles. Future studies, including speciation of the carbohydrate constituents (e.g., sugars speciation) in the EG root exudates, are needed to test this hypothesis.

In addition, many oxidative and hydrolytic secondary metabolite compounds contained in root exudates of C_4 species, such as hydroxamic acids, have been shown to directly hydrolyze ATR and its chlorinated metabolites, resulting in rapid formation of the hydroxylated metabolites (Martin-Neto et al., 1994; Wenger et al., 2005). For instance, the ATR-degrading benzoxazinones, DIMBOA (3,4-dihydro-2,4-hydroxy-7-methoxy-2H-1,4-benzoxazin-3-one), and its analogs

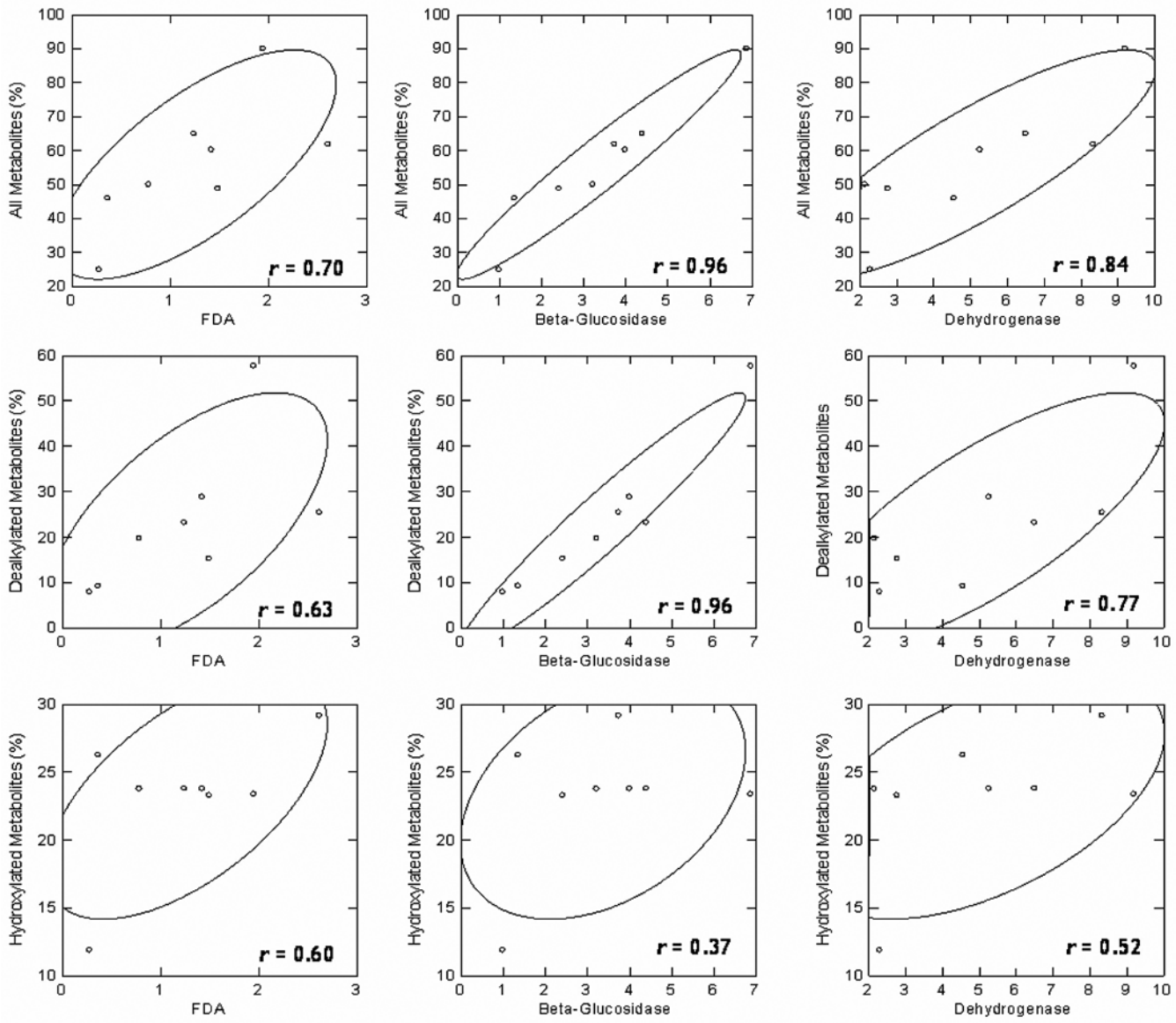

Fig. 6. Pairwise comparison between atrazine metabolites and soil enzyme activities. The level of confidence ellipse = 0.68.

Table 1. Average dry weight (g) of biomass of root and shoot biomass and the partition of carbohydrates (g). The same letters within the column were not significantly ($P < 0.05$) different (least significant difference test, $n = 3$)

Treatments	Root	Shoot	Root/shoot ratios	Partitioning of carbohydrates to the root
	g			%
OR†	378.4a	37.3b	10.2a	91.0a
SM	400.1a	37.9b	10.6a	91.4a
TALL	295.0b	52.5a	5.6b	84.9ab
IB	97.1c	25.5c	3.8c	79.2c
RYE	130.3c	24.6c	5.3ab	84.1ab
SW	242.8b	58.5a	4.1c	80.6c
EG	272.3b	37.9b	7.2ab	87.8ab

† OR = orchardgrass; SM = smooth bromegrass; TALL = tall fescue; IB = Illinois bundle flower; RYE = perennial ryegrass; SW = switchgrass; EG = eastern gamagrass.

have been found to be abundant in the C_4 ATR-tolerant plants (Wenger et al., 2005). In a separate study, we also observed a rapid chemical hydrolysis of ATR when ATR reacted with root extract from EG root tissues (data are not shown).

Although a satisfactory relationship of soil quality with quantitative pesticide biodegradation does not exist (Locke and Zablotowicz, 2004), some indirect relationships based on microbial activity, specifically enzyme activity, can be made based on assessments of soils and pesticide application. For example, FDA hydrolase may be a potential indicator for biodegradative activity of complex organic molecules, including soil-applied pesticides (Zablotowicz et al., 2000). Staddon et al. (2001) concluded that increased levels of this enzyme observed in VBS soils partly contributed to enhanced degradation of the herbicide metolachlor. Therefore, agroecosystems that include continuous vegetation are associated with high soil quality because an optimal functional soil system that develops can also enhance other beneficial activities, including biodegradation of introduced pesticides (Karlen et al., 1999; Locke and Zablotowicz, 2004). Similar to our finding, previous soil quality studies also demonstrated that microbial activity, based on activities of enzymes including FDA hydrolase, DHG, and GLU, increased considerably more under grassland vegetation than soils under intensive cultivation (Acosta-Martinez et al., 2008; Kremer and Li, 2003). Therefore, soil managed under VBS not only improves its physical status (e.g., improved aggregate stability and infiltration rates), but it also improves the biological status of the soil via stimulated microbial activity and enhanced organic matter accumulation, leading to an overall increase in soil quality and higher bioremediation potential for degradation of organic herbicides.

The work reported here identified EG as an outstanding candidate to be incorporated into VBS for mitigating the off-site transport of ATR via its ability to facilitate ATR degradation in the rhizosphere. A field rainfall stimulation study conducted on a claypan soil landscape also demonstrated the superior performance of an EG buffer over other cool-season grass buffers in reducing both dissolved and sediment-bound ATR transport in surface runoff (Lin et al., 2007). Approximately 75 to 80% of the dissolved and sediment-bound ATR transported in surface runoff was removed by 4 m of EG buffer. Thus, the implementation of buffers using EG could effectively improve both deposition and subsequent degradation of ATR with less land taken out of production. Among the other treatments, SW, OR, and SM also showed greatly enhanced degradation of ATR in this study and in a field lysimeter study (discussed above). While the warm-season grasses have superior ability to facilitate ATR degradation in soils, their establishment is not always practical, timely, or economical. Thus, it is important to recognize that cool-season species, particularly OR and SM, also facilitate enhanced degradation and they are easier and more economical to establish.

Conclusions

All plant species tested significantly enhanced ATR degradation by creating conditions that enhanced microbial enzyme activity in the rhizosphere. The ATR mineralization was significantly correlated to GLU and DHG activities in the rhizosphere of the grass treatments, although mineralization accounted for no more than 7% of the applied ^{14}C–ATR. All three enzymes significantly correlated to the formation of N-dealkylated metabolites and overall ATR degradation, but they were not significantly correlated to the formation of hydroxylated metabolites, suggesting that measured enzymes like GLU could be good indicators of overall ATR degradation and N-dealkylation but poor indicators of hydroxylation. The incorporation of EG into VBS designs is highly recommended to promote dissipation of ATR transported from croplands and deposited in the VBS. However, cool-season grasses, particularly OR and SM, also significantly enhanced ATR degradation and they are more readily established than the C_4 species. The results from this study also showed that the microbial enzyme activities widely used for assessment of soil quality, particularly GLU activity, are promising as indicators for evaluating the overall ATR degradation potential of various vegetative buffer designs.

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