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Research article

Evaluating phytochemical and microbial contributions to atrazine degradation

K.M. Hatch [a,*,1], R.N. Lerch [b], R.J. Kremer [c], C.D. Willett [d], C.A. Roberts [e], K.W. Goyne [f,2]

[a] University of Missouri, 1406 E. Rollins St., Columbia, MO, 65211, USA
[b] USDA-ARS, 1406 E. Rollins St., Columbia, MO, 65211, USA
[c] USDA-ARS, 902 S. College Ave., Columbia, MO, 65211, USA
[d] University of Arkansas, 1 University of Arkansas, Fayetteville, AK, 72701, USA
[e] University of Missouri, 1112 University Ave, Columbia, MO, 65211, USA
[f] University of Missouri, 902 S. College Ave., Columbia, MO, 65211, USA

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ABSTRACT

The inclusion of warm-season grasses, such as switchgrass (*Panicum virgatum*) and eastern gamagrass (EG) (*Tripsacum dactyloides*), in vegetated buffer strips has been shown to mitigate herbicide contamination in runoff and increase herbicide degradation in soil. The mode of action by which buffer strip rhizospheres enhance herbicide degradation remains unclear, but microorganisms and phytochemicals are believed to facilitate degradation processes. The objectives of this study were to: 1) screen root extracts from seven switchgrass cultivars for the ability to degrade the herbicide atrazine (ATZ) in solution; 2) determine sorption coefficients (K_d) of the ATZ-degrading phytochemical 2-β-D-glucopyranosyloxy-4-hydroxy-1,4-benzoxazin-3-one (DBG) to soil and Ca-montmorillonite, and investigate if DBG or ATZ sorption alters degradation processes; and 3) quantify ATZ degradation rates and soil microbial response to ATZ application in mesocosms containing soil and select warm-season grasses. Phytochemicals extracted from the roots of switchgrass cultivars degraded 44–85% of ATZ in 16-h laboratory assays, demonstrating that some switchgrass cultivars could rapidly degrade ATZ under laboratory conditions. However, attempts to isolate ATZ-degrading phytochemicals from plant roots were unsuccessful. Sorption studies revealed that DBG was strongly sorbed to soil (K_d = 87.2 L kg^{-1}) and Ca-montmorillonite (K_d = 31.7 L kg^{-1}), and DBG driven hydrolysis of ATZ was entirely inhibited when either ATZ or DBG were sorbed to Ca-montmorillonite. Atrazine degradation rates in mesocosm soils were rapid ($t_{0.5}$ = 8.2–11.2 d), but not significantly different between soils collected from the two switchgrass cultivar mesocosms, the eastern gamagrass cultivar mesocosm, and the unvegetated mesocosm (control). Significant changes in three phospholipid fatty acid biomarkers were observed among the treatments. These changes indicated that different ATZ-degrading microbial consortia resulted in equivalent ATZ degradation rates between treatments. Results demonstrated that soil microbial response was the dominant mechanism controlling ATZ degradation in the soil studied, rather than root phytochemicals.

1. Introduction

Atrazine (ATZ; 1-chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine) continues to be among the most heavily used herbicides in the world (Sharma et al., 2019), with more than 25 million kg applied to US cropland in 2018 (USDA, 2018). Atrazine's mode of action and weed control spectrum are well understood (Wessels and Van der Veen, 1956) and the herbicide has been widely reported to contaminate surface and groundwaters (Lerch and Blanchard, 2001; Krutz et al., 2010; Lerch et al., 2017; Stayner et al., 2017). Improved understanding of ATZ fate in the soil environment is necessary to prevent off-site movement and mitigate the exposure of humans and aquatic ecosystems to ATZ.

Recent studies have elucidated the importance of the soil rhizosphere

* Corresponding author.
E-mail addresses: kml6wc@umsystem.edu (K.M. Hatch), lerchr@missouri.edu (R.N. Lerch), KremerR@missorui.edu (R.J. Kremer), cammydwillett@gmail.com (C.D. Willett), RobertsCr@missouri.edu (C.A. Roberts), GoyneK@vt.edu (K.W. Goyne).
[1] Present Address: 11333 Big Bend Blvd., St. Louis, MO 63122, USA.
[2] Present Address: Virginia Tech, 310 W. Campus Dr., Blacksburg, VA 24060, USA.

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List of abbreviations

ATZ	Atrazine KAN Kanlow cultivar
BREC	Bradford Research and Extension Center K_d Sorption coefficient
Bx	Benzoxazinone
PLFA	Phospholipid fatty acid Ca-montmorillonite Calcium-saturated montmorillonite
SAT	Saturated to
CIM	Cimarron cultivar Unsaturated PLFA Ratio
DBG	2-β-D-glucopyranosyloxy-4-hydroxy-1,4-benzoxazin-3-one
EG	Eastern gamagrass
SG	Switchgrass $t_{0.5}$ Half-life
SOM	Soil Organic Matter HA Hydroxyatrazine
VBS	Vegetated buffer strip

and the direct and indirect effects of vegetation on ATZ degradation in soil (Lin et al., 2011; Willett et al., 2016). Studies have overwhelmingly shown that microbial degradation of ATZ predominates in the soil environment (De Schrijver and De Mot, 2008) but application frequency, vegetation/crop management, and rhizosphere soil quality greatly affect ATZ persistence in soil (Krutz et al., 2010; Lerch et al., 2017). In addition to microbial degradation, ATZ is vulnerable to photodegradation (Marchetti et al., 2013) and hydrolysis through interactions with soil colloids (Armstrong and Chesters, 1968) and benzoxazinone (Bx) phytochemicals (Castelfranco et al., 1961; Wenger et al., 2005; Willett et al., 2013; Willett et al., 2016). Adding to the complexity of ATZ degradation studies, chemical and microbial ATZ degradation processes can result in the formation of identical ATZ metabolites, namely hydroxyatrazine (HA) (Fig. 1).

Atrazine degradation via microbial activity is tied to specific genes that are typically stored in plasmids and transferred horizontally through the soil microbial community via lysogeny and plasmid conjugation (Devers et al., 2005; Liang et al., 2012). These transferable ATZ-degrading genes permit a variety of microbes to detoxify ATZ. The

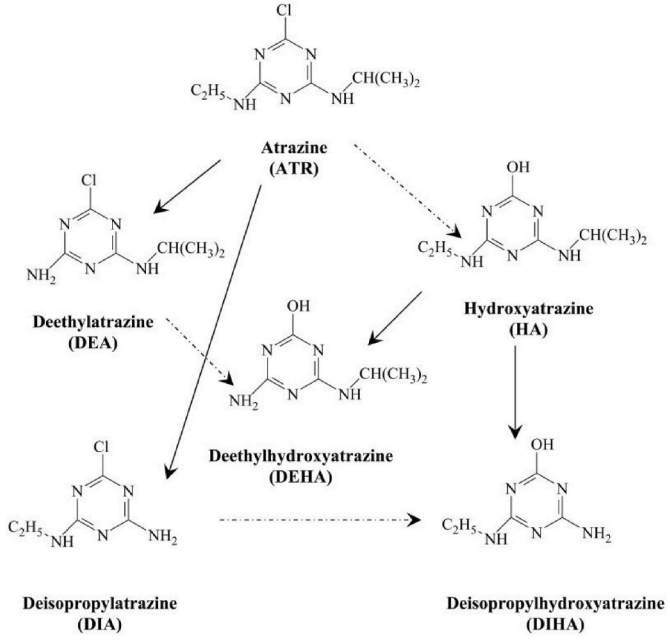

Fig. 1. Atrazine degradation and metabolite formation (Lin et al., 2008). Solid arrows indicate N-dealkylation and dashed arrows indicate hydrolysis.

transferable nature of these genes allows for ATZ degradation among differing microbial consortia and multiple microbial degradation pathways within a consortium (Liang et al., 2012). It is widely accepted that ATZ-degrading genes may remain in soil for several years but decrease to a negligible level if application is limited to every four years (Krutz et al., 2010). However, annual and biannual application of ATZ is common in the United States and the reported half-life ($t_{0.5}$) of ATZ in soil has declined as widespread adaptation of ATZ-degraders in soil has been documented (Krutz et al., 2010; Lerch et al., 2017). Wenger et al. (2005) noted the $t_{0.5}$ of ATZ ranged from 60 d to >1 yr, while more recent reports document a $t_{0.5}$ of <10 d in adapted soil (Lerch et al., 2017) and rapid ATZ degradation even in soils with no recorded application history (Sánchez et al., 2019).

The role of Bx compounds in plant biochemistry (Fomsgaard et al., 2004; Park et al., 2004) and chloro-triazine reactivity (Castelfranco et al., 1961; Wenger et al., 2005; Rice et al., 2012; Willett et al., 2013) has been well-studied for decades. Benzoxazinones are highly reactive and frequently produced in above and below ground biomass of select members of the *Poaceae* family of grasses (Park et al., 2004; Willett et al., 2013). They are secondary metabolites produced for defensive purposes (Park et al., 2004) with insecticidal, fungicidal, and anti-microbial qualities (Fomsgaard et al., 2004). Furthermore, Bx compounds [e.g., DIMBOA (2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one), DIBOA (2,4-dihydroxy-(2H)-1,4-benzoxazin-3(4H)-one), DIMBOA-Glc (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), and DIBOA-Glc (DBG) (2-β-D-glucopyranosyloxy-4-hydroxy-1,4-benzoxazin-3-one); Fig. 2] have the ability to degrade chloro-triazines through hydrolysis reactions (Castelfranco et al., 1961; Wenger et al., 2005; Rice et al., 2012; Willett et al., 2013).

Willett et al. (2016) demonstrated that Bx-mediated ATZ hydrolysis occurs via nucleophilic attack by the hydroxamic acid moiety in which the C-2 chlorine is replaced with a hydroxyl group (Fig. 3). Atrazine reactive Bx compounds are produced by several members of the *Poaceae* family of plants including rye (*Secale cereale*), eastern gamagrass (EG) (*Tripsacum dactyloides*), and corn (*Zea mays*). While the Bx-ATZ reaction has been well studied, much of the research has focused on reactivity in solution (Castelfranco et al., 1961; Willett et al., 2013, Willett et al., 2016). Research focusing on Bx reactivity in soil revealed a substantial decrease in Bx toxicity to root-knot nematode (*Meloidogyne incognita*) and select plants (Meyer et al., 2009; Rice et al., 2012; Teasdale et al., 2012). Although not explicitly stated, the likely cause of the observed reduction in Bx reactivity was removal of Bx compounds from solution via sorption to the soil solid phase as the authors reported low levels of Bx and Bx metabolites in soil extracts (Meyer et al., 2009).

Currently, there are no available data quantifying the sorption of Bx compounds to soil colloids. As sorption can potentially affect chemical reactivity of a compound, studies evaluating Bx sorption to soil are necessary for understanding the behavior of these compounds in the environment (Harris and Sheets, 1965). Reported soil:solution partition coefficients (K_d) for ATZ are typically <10 L kg^{-1}, indicating relatively weak sorption and high mobility (Correia et al., 2006; Lerch et al., 2017). Reports of Bx extraction experiments imply that these compounds may sorb strongly to soil, as minimal leaching of Bx compounds through the soil profile has been reported (Meyer et al., 2009; Rice et al., 2012). Additionally, we are unaware of any previous studies examining the direct influence of Bx sorption on reactivity of the phytochemical with ATZ or other herbicides.

The widespread contamination of streams by ATZ has prompted the development of management practices capable of reducing ATZ transport from agricultural fields via surface runoff. These practices include herbicide incorporation into the soil, no-till combined with cover cropping, split herbicide application, reduced herbicide application, and the use of vegetated buffer strips (VBS) (Glenn and Angle, 1987; Gaynor et al., 1995; Lerch et al., 2017). Advantages of VBS for reducing ATZ transport include the relative ease of establishment, low maintenance once established, and no need for specialized equipment. Vegetative

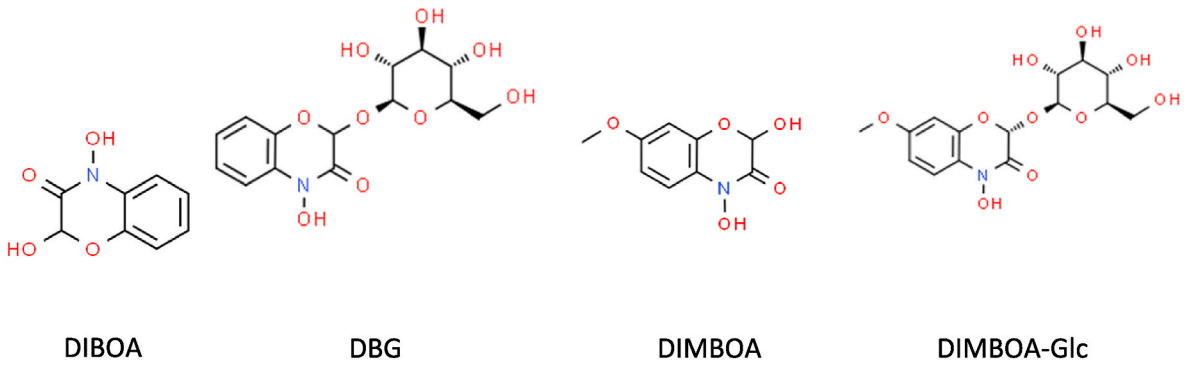

Fig. 2. Benzoxazinoid structures (www.chemspider.com).

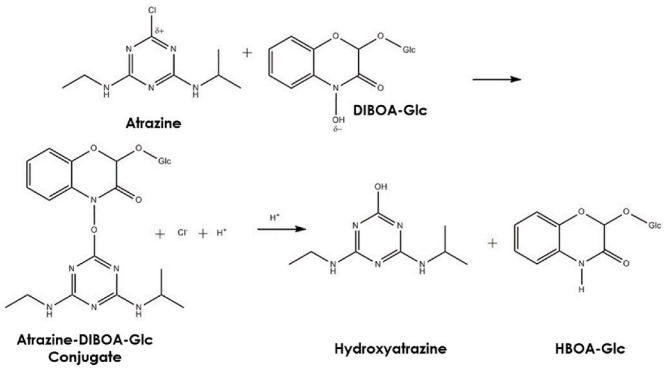

Fig. 3. Reaction mechanism of atrazine with 2-β-D-glucopyranosyloxy-4-hydroxy-1,4-benzoxazin-3-one (DIBOA-Glc or DBG) (Willett et al., 2016).

buffer strips also provide the benefits of reducing sediment and nutrient load in runoff (Patty et al., 1997; Lerch et al., 2017). The utility of VBS for herbicide load reduction in runoff is well-documented but efficacy is highly variable, ranging from 20 to 100% (Patty et al., 1997; Lerch et al., 2017).

The efficacy of VBS is influenced by buffer width, rainfall rate, soil properties, and vegetation composition (Lerch et al., 2017). Vegetation composition is a critical factor affecting VBS efficacy (Lin et al., 2008) because of differences in root structure, plant density, growth characteristics (e.g., warm-vs cool-season grasses), and resulting influences on soil quality over time (Lerch et al., 2017; Oram et al., 2017). The incorporation of Bx producing plants into VBS may further enhance triazine herbicide degradation. Several studies have shown enhanced ATZ degradation in the rhizospheres of VBS containing *Poaceae* grasses (Lin et al., 2008, 2011), but the relative contributions of microbial and phytochemical ATZ degradation were not assessed in these studies. As discussed, some *Poaceae* grasses produce ATZ degrading Bx compounds and are also known to support diverse soil microbial populations (Liang et al., 2016). Additionally, research has revealed increased microbe-driven ATZ degradation in treatments containing microbes and plants, compared to microbe only or plant only treatments (Zhang et al., 2014).

While EG and switchgrass (SG) (*Panicum virgatum*) have been shown to enhance ATZ degradation in the rhizosphere (Lin et al., 2011), SG has advantages for use in VBS. For example, several SG cultivars are adapted to a wide range of environments (Table 1) and, typically, SG is easier to establish. However, the presence of ATZ-degrading phytochemicals has yet to be detected in SG. Interestingly, there appears to be considerable variability in ATZ degradation between SG experiments (Seybold et al., 2001; Lin et al., 2008, 2011; Mersie et al., 2015). It is unclear if differing reports of ATZ degradation are an indirect result of favorable microbial environments in SG rhizospheres or, more directly, the production of unidentified ATZ-degrading phytochemical(s).

Table 1
Properties of the switchgrass cultivars studied.

Switchgrass Cultivar	Ecotype	Ploidy	MS[a]	USDA HZ[b]
Kanlow	Lowland	4×	NM	6,7
Cave-in-Rock	Upland	8×	NM	5,6,7
Shawnee	Upland	8×	M	5,6,7
Carthage	Upland	8×	NM	5,6,7
Shelter	Upland	8×	NM	4,5,6
Trailblazer	Upland	8×	M	4,5
Sunburst	Upland	8×	M	3,4,5
Pathfinder	Upland	8×	M	4,5
Alamo	Lowland	4×	NM	6,7,8,9

[a] "MS" stands for "Modification Status". Cultivars marked "NM" were "Not Modified" but were selected from a specific location and are named after their original source locations. Cultivars marked "M" were modified through breeding and selection processes. Data for this table were accumulated from Casler et al., (2015).
[b] USDA hardiness zone (HZ).

This work aims to clarify plant contributions to ATZ degradation by contrasting direct phytochemical contributions to ATZ degradation with indirect contributions from ATZ-degrading rhizosphere microbiota. The objectives of this work were to: 1) screen root extracts from seven SG cultivars for the ability to degrade ATZ in solution; 2) determine sorption coefficients (K_d) of the ATZ-degrading phytochemical DBG to soil and Ca-montmorillonite, and investigate if DBG or ATZ sorption alters degradation processes; and 3) quantify ATZ degradation rates and soil microbial response to ATZ application in mesocosms containing soil and select warm-season grasses.

2. Materials and methods

2.1. Chemicals and materials

Analytical standards of atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine, CAS No: 1912-24-9), deethylatrazine (DEA) (2-chloro-4-amino-6-isopropylamino-1,3,5-triazine, CAS No: 6190-65-4), deisopropylatrazine (DIA) 2-chloro-4-ethylamino-6-amino-1,3,5-triazine, CAS No: 1007-28-9), hydroxyatrazine (HA) (2-hydroxy-4-ethylamino-6-isopropylamino-1,3,5-triazine didealkylatrazine (6-chloro-1,3,5-triazine-2,4-diamine, CAS No: 1216850-337), ammeline (2-hydroxy-4,6-diamino-1,3,5-triazine, CAS No: 645-92-1), and deethylhydroxyatrazine (DEHA) (2-hydroxy-4-amino-6-isopropylamino-1,3,5-triazine, CAS No. 19,988-24-0) were ≥97% purity and obtained from Chem Service Inc. (West Chester, PA, USA). All solvents and reagents used for working standards, root and soil extractions, and HPLC mobile phases including methanol (CH_3OH), acetonitrile (CH_3CN), dichloromethane (CH_2Cl_2), ethyl acetate ($CH_3COOC_2H_5$), and concentrated phosphoric acid (H_3PO_4), were chromatography grade and obtained

from Fisher Scientific (Pittsburgh, PA, USA). 2-β-D-glucopyranosyloxy-4-hydroxy-1,4-benzoxazin-3-one (DBG) (~100% purity, 4.58 mM in 80% aq. CH_3OH) was isolated and purified from EG roots (*Tripsacum dactyloides*, Pete cultivar) harvested from the University of Missouri Bradford Research and Extension Center using methods described by Willett et al. (2014). Sodium azide (NaN_3, 99.99+%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultra-pure (18 MΩ) water was used to prepare all aqueous reagents and experimental samples. Ca-montmorillonite was obtained from the clay mineral society (Chantilly, VA).

2.2. Establishment of SG cultivars and root harvesting

Seven cultivars of SG were planted in January 2016 (Fig. 5) and permitted to grow for 9–11 months. Seeds were obtained from the NRCS Plant Materials Center, in Elsberry, MO, USA. All plants were started in 25-well trays in water-saturated Pro-Mix potting soil containing pearlite (https://www.promixgardening.com/en). There were 25 plants for each cultivar. Seedling plugs were transplanted to larger pots as required. Plants were grown in a greenhouse under ambient light until May when all plants were moved to an outdoor location. Greenhouse temperatures ranged from 23 to 29 °C. Plants were harvested from October 5, 2016, to November 21, 2016. Immediately following harvest, roots were rinsed with tap water, followed by ultra-pure water, and frozen at -4 °C. Root extracts from these plants were then used to compare reactivity with ATZ across cultivars (Fig. 5).

2.3. Phytochemical extraction method

Switchgrass root extracts were prepared using a modified method described by Willett et al. (2013). Roots were separated from above ground biomass and rinsed with ultra-pure water to remove soil. In a porcelain mortar, root material was combined with liquid nitrogen and broken into pieces of homogeneous size with a porcelain pestle. New mortars and pestles were purchased for this procedure and assigned to each cultivar of SG to prevent cross contamination. Twenty-five grams (dry weight equivalent) of root material was weighed into 250 mL polypropylene bottles and combined with 100 mL of 90% CH_3OH in water. Samples were agitated for 18 h on an end-to-end shaker to facilitate phytochemical extraction. Each cultivar was extracted in triplicate. Samples were centrifuged, filtered, and concentrated to 0.5 mL in a water bath (40 °C) under ultra-pure N_2 gas (Caliper LifeScience TurboVap II). Samples were brought to a final volume of 5.0 mL in 25% aqueous CH_3OH to prevent microbial decay. Each 5.0 mL sample was filtered through 0.7 and 0.45 μm nylon syringe filters in tandem and stored at -4 °C.

2.4. ATZ degradation assay

Similar to Willett et al. (2013), a standard assay was developed to test, in triplicate, seven SG cultivars for their ability to degrade ATZ. In 5.0 mL, conical, glass centrifuge tubes, 1.0 mL of root extract was combined with 0.2 mL ATZ solution (10.0 mg L^{-1} ATZ in 25% CH_3OH), resulting in a final volume of 1.2 mL and final concentration of 1.667 μg mL^{-1} ATZ in each replicate. Control samples, consisting of 1.0 mL of 25% CH_3OH and 0.2 mL ATZ solution (10.0 mgL^{-1} ATZ in 25% CH_3OH), were incubated concurrently. PTFE-lined lids were secured with Parafilm and each sample was vortexed for 5 s and sonicated for 20 min. Samples were wrapped in aluminum foil and reacted in the dark for 16 h at ambient temperature (22–25 °C). After the incubation period, remaining ATZ was extracted from each sample by liquid-liquid extraction. Each sample received 1.0 mL of CH_2Cl_2 and samples were vortexed for 5 s. The CH_2Cl_2 was removed using Pasteur pipets and placed into a new centrifuge tube. Liquid-liquid extraction of each sample was repeated two additional times. The total volume of CH_2Cl_2 reacted with each sample was evaporated to dryness under N_2 gas, and the residue was re-dissolved in 1.0 mL of 40% aqueous CH_3CN prior to analysis. Atrazine concentration was determined using HPLC with a diode array detector (HPLC-DAD) (Shimadzu Nexera XR 20 A). The following column and conditions were used for HPLC analysis: Agilent Zorbax Eclipse XDB C18 column (3.5 μm, 2.1 × 250 mm); flow rate, 0.5 mL min^{-1}; mobile phase composition, 40% CH_3CN: 60%, 0.1% aqueous H_3PO_4; column oven temperature, 40 °C; injection volume, 2.0 μL; detection at 220 nm; retention time, 4.9 min.

2.5. Sorption of DBG and ATZ

Three sorbents were reacted with ATZ and DBG in aqueous solution to determine single-point partition coefficients (K_d) of the herbicide and phytochemical. The sorbents investigated were: 1) Mexico silt loam soil (A horizon) from the University of Missouri Bradford Research and Extension Center (BREC), located approximately 5 miles east of Columbia, Missouri, USA; 2) Ca-saturated montmorillonite; and, 3) a 40:60 mix of sand and Mexico silt loam that resulted in a loam-textured soil. Equilibration experiments were carried out prior to K_d determination for each substrate and chemical combination using methods described by Chu et al. (2013). Descriptions of the soils, preparation of Ca-montmorillonite, and preliminary equilibration experiments are described in the Supplemental Materials (S1.1 Experimental Details for DBG and ATZ Sorption).

To determine K_d values for ATZ specific to each of the three solid substrates, 2.0 g of solid were combined with 4.0 mL of ATZ solution (1 mg L^{-1} ATZ in a background electrolyte solution of 0.01 M $CaCl_2$ and 0.0015 M NaN_3). Sodium azide was included in the background solution to inhibit microbial degradation of ATZ and DBG. The use of NaN_3 to prevent microbial degradation is common, however it must be used at low concentrations, as was done in this study, to avoid NaN_3 reaction with ATZ (Chefetz et al., 2006). The K_d values for DBG specific to each of the three solid substrates were evaluated by reacting 2.0 g of solid with 30.0 mL of DBG solution (30.0 mg L^{-1} DBG in a background electrolyte solution of 0.01 M $CaCl_2$ and 0.0015 M NaN_3). All sorption experiments utilized 50.0 mL fluorinated ethylene propylene (FEP) round bottom centrifuge tubes as reaction vessels, and each sorbent by chemical combination was replicated in triplicate. Samples were agitated on an end-to-end shaker until equilibrium was achieved (24 h for ATZ to all solids, and DBG to clay, but 9 d for DBG to soil and sand:soil mix), followed by centrifugation at 7500 rpm for 12 min. Supernatant (0.5 mL aliquot) was analyzed using HPLC-DAD to quantify concentrations of ATZ and DBG remaining in solution. Preliminary testing revealed negligible sorption of ATZ or DBG (<5%) to the reaction vessels, thus the mass of ATZ and DBG sorbed to each solid (q) was calculated based on mass lost from solution. The K_d values were calculated by dividing q by the equilibrium concentration of ATZ or DBG remaining in solution after reaction (c_{eq}) (Essington, 2004). The concentration of ATZ remaining in solution was quantified using the HPLC-DAD method described in Section 2.4. The concentration of DBG remaining in solution was measured using the following HPLC-DAD method: Agilent Zorbax Eclipse XDB C18 column (3.5 μm, 2.1 × 250 mm); flow rate, 0.7 mL min^{-1}; mobile phase composition, 20% CH_3OH: 80%, 0.1% phosphoric acid (H_3PO_4); column oven temperature, 40 °C; injection volume, 2.0 μL; detection at 254 nm; retention time, 4.5 min.

2.6. Effect of sorption on DBG hydrolysis of ATZ

Ca-montmorillonite containing sorbed ATZ (System A in Fig. 4) and sorbed DBG (System B in Fig. 4) were used to explore the effect of sorption on ATZ hydrolysis by DBG (Fig. 3). The initial steps of sorbing ATZ or DBG to Ca-montmorillonite followed protocols described previously (see section 2.5 Sorption of ATZ and DBG). After centrifugation and analysis of the supernatant solution by HPLC-DAD to quantify the mass of ATZ or DBG lost from solution, clay pellets were resuspended and rinsed in a solution containing 60% supernatant and 40%

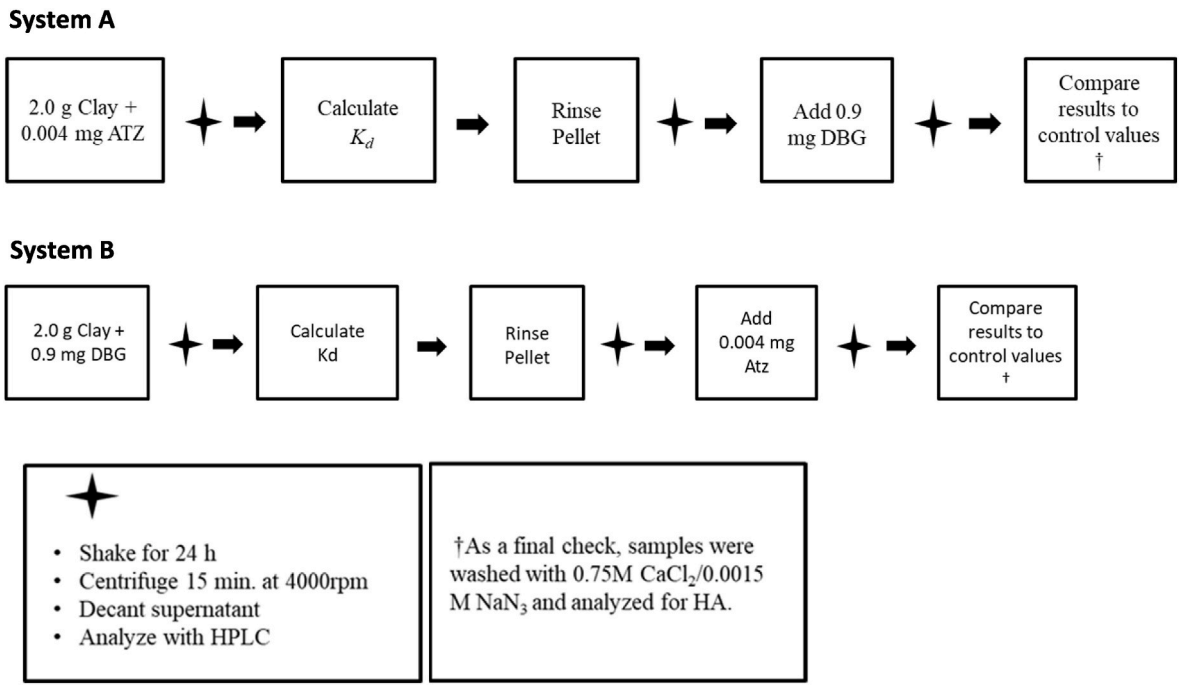

Fig. 4. Illustration of K_d and atrazine (ATZ) degradation experiments.

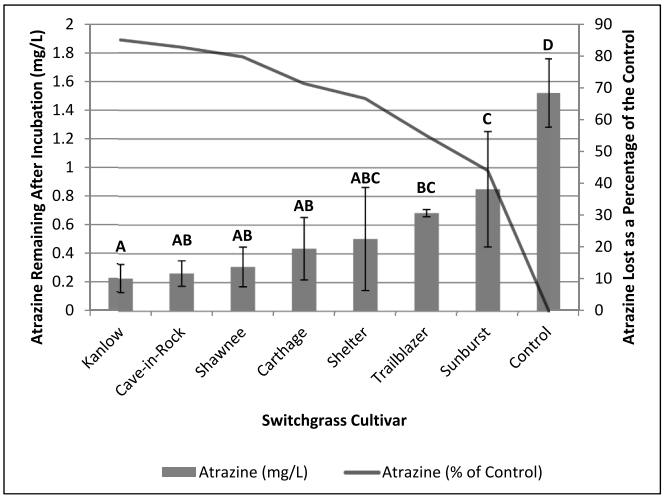

Fig. 5. Comparison of atrazine (ATZ) degraded as a percentage of controls and remaining ATZ concentration (mg L^{-1}) in seven switchgrass root extracts after a 16 h incubation. Control samples contained no root extracts. Error bars indicate standard deviation.

background electrolyte solution (0.01 M $CaCl_2$ and 0.0015 M NaN_3). This step was included to reduce entrained compounds while minimizing desorption (Shechter et al., 2006). During the rinse step, samples were placed on an end-to-end shaker for 24 h and centrifuged at 7500 rpm for 12 min. The supernatant was again analyzed to quantify the mass of compound in solution and then discarded. The desorbed mass of ATZ or DBG was then subtracted from the mass of compound sorbed after the initial reaction step, thus providing an accurate reflection of ATZ or DBG on clay surfaces prior to subsequent steps.

To demonstrate sorption effects on DBG hydrolysis of ATZ, Ca-montmorillonite equilibrated with ATZ (System A in Fig. 4) was reacted with 0.9 mg DBG dissolved in 4.0 mL of background electrolyte solution (0.01 M $CaCl_2$ and 0.0015 M NaN_3). Clay equilibrated with DBG (System B in Fig. 4) was reacted with 0.004 mg ATZ dissolved in 4.0 mL of background electrolyte solution. Systems A and B were each tested in triplicate and 50.0 mL FEP round bottom centrifuge tubes were used as reaction vessels. All samples were allowed to react for 24 h. After 24 h, samples were centrifuged at 7500 rpm for 12 min and supernatant was analyzed for ATZ, DBG, and HA (the reaction product of ATZ and DBG) (Fig. 3) using HPLC-DAD. To determine sorbed HA to clay at the end of the reaction period, samples were resuspended in 4.0 mL of 0.75 M $CaCl_2$ with 0.0015 M NaN_3 and agitated on and end-to-end shaker for 24 h. Samples were centrifuged for 12 min at 7500 rpm and the supernatant was analyzed for HA. The methods used for quantification of Atrazine and DBG with HPLC-DAD were described in sections 2.4 and 2.5 respectively. Hydroxyatrazine was quantified using the following column and conditions for HPLC-DAD analysis: Agilent Zorbax Eclipse XDB C18 column (3.5 μm, 2.1 × 250 mm); flow rate, 0.5 mL min^{-1}; mobile phase composition, 25% CH_3OH: 75%, 0.1% aqueous H_3PO_4; column oven temperature, 40 °C; injection volume, 2.0 μL; detection at 240 nm; retention time, 2.9 min.

Control samples for each system (A and B) were assembled in triplicate to examine the DBG-ATZ reaction in absence of Ca-montmorillonite. Reactions were conducted in 50.0 mL FEP round bottom centrifuge tubes and contained the amounts of ATZ and DBG remaining in the clay system after equilibration, but before reaction with the second compound. The control for system A contained 0.0016 mg ATZ and 0.9 mg DBG dissolved in 0.01 M $CaCl_2$ with 0.0015 M NaN_3. Control samples for System B contained 0.004 mg ATZ and 0.587 mg DBG dissolved in 0.01 M $CaCl_2$ with 0.0015 M NaN_3. Each control treatment maintained the same amount of solution as the corresponding test samples, including entrained solution. The total mass of entrained solution in System An exceeded that of entrained solution in System B by 0.83 g, a result of the sorptive properties of the ATZ present in system A, which is further discussed in section 3.2.

2.7. Mesocosm study

A mesocosm study was conducted to investigate ATZ degradation rate as a function of grass treatment and rhizosphere microbial community structure. Mesocosms consisted of three grass treatments and one unvegetated control treatment grown in soil. Each treatment was

replicated in quadruplicate. The grass treatments consisted of two lowland, SG cultivars [Cimarron (CIM) and Kanlow (KAN)] and one EG cultivar (Pete). While the EG cultivar selected for investigation is known to produce DBG (Willett et al., 2013, 2014), it was unknown if the SG cultivars produced phytochemicals capable of enhancing ATZ degradation. However, preliminary testing of root extracts from these two SG cultivars using the ATZ assay described in Section 2.4 indicated that KAN, but not CIM, enhanced ATZ degradation (see the Supplemental Materials S1.2 Experimental Details for ATZ Degradation by Mesocosm SW Root Extracts).

All grass treatments were established using live plants collected from plots located at BREC that were at least five years old. Upon collection from the field, grasses were planted into 13.2 L plastic buckets containing a growth medium of 40% by mass sand:soil (A horizon of Mexico silt loam soil) to ensure sufficient water infiltration and root aeration. Each grass treatment contained two to three plants of 12–15 live stems in each mesocosm. The Mexico silt loam soil was collected from a location at BREC that had not experienced ATZ application for at least five years.

Prior to planting, the sand:soil mixture was sieved to 2.0 mm and plant material was removed. Sand and soil were homogenized using a concrete mixer and air-dried. All mesocosms were packed to a bulk density of 1.2 g cm^{-3} (15.9 kg oven-dry sand:soil mix per container). To prevent loss of ATZ from the system, mesocosms were not equipped with drainage holes. Each treatment contained a combined soil moisture/temperature probe (CS625, Campbell Scientific, Logan, UT, USA) and datalogger (CR200×; Campbell Scientific, Logan, UT, USA) to record volumetric soil water content and soil temperature at 10 min intervals. Volumetric water content during the study ranged from 9.0 to 35.0%, while typical soil temperature was measured between 25 and 26 °C.

After planting, mesocosms were transferred to a greenhouse where the grasses were permitted to mature. Treatments received weekly applications of 325 mL of liquid fertilizer for 4 weeks. Fertilizer was N, P, and K (20:20:20) and prepared according to the manufacturer's instructions (103.5 mL per 13.25 L water). All treatments received 14 h of light per d using supplemental light as needed. Plants were permitted to mature for 23 weeks, at which time laboratory grade ATZ in water with trace amounts of CH_3OH (0.005% CH_3OH to enhance ATZ solubility) was applied at a rate of 0.2 mg kg^{-1} soil.

Soil samples were collected from each mesocosm at 0, 3, 7, 14, 39, 56, and 112 d after ATZ application using a 2.5-cm diameter soil probe that permitted sampling over the entire depth of soil. Each individual core was well-homogenized prior to ATZ extraction and analyzed as described by Lerch et al. (2015). In brief, ATZ was extracted from 5.0 g of soil (dry weight) in 50 mL polypropylene centrifuge tubes containing 5.0 mL 18 MΩ Ultrapure water and 5.0 mL water-saturated toluene ($C_6H_5CH_3$). Samples were shaken on an end-to-end shaker on high speed for 2 h and centrifuged for 20 min at 4000 rpm. Triplicate 1 mL aliquots of solution were removed from each sample and placed into HPLC vials, evaporated to dryness, and re-dissolved in 1.0 mL ethyl acetate ($CH_3COOC_2H_5$). Analysis of ATZ was performed by gas chromatography/ion-trap mass spectrometry as described in detail by Lerch et al. (2015) and in the Supplemental Materials (S1.3 Experimental Details for ATZ Degradation in Mesocosms). On a soil basis, the ATZ limit of detection was 0.22 μg kg^{-1}.

The remaining sample from each homogenized soil core was vacuum sealed, frozen, and stored in BPA-free, plastic bags at -4 °C. Samples collected at 0, 14, 39, 56, and 112 d after ATZ application were analyzed for PLFA by the University of Missouri Soil Health Assessment Center (Columbia, Missouri, USA) using methods by Buyer and Sasser (2012). Analysis of PLFA data was performed with Sherlock Software version 6.0 (MIDI Corp, Newark, NJ). The biomarkers used for PLFA analysis are identified in the Supplemental Materials (S1.4 Biomarkers for Phospholipid Fatty Acid Analysis). Data from this analysis were used to estimate total nmol PLFA g^{-1} soil and mol% of soil microorganisms including Gram-positive and Gram-negative bacteria, anaerobic bacteria, actinobacteria, eukaryotes, fungi, and arbuscular mycorrhizae (AM) fungi. Ratios of PLFA biomarkers (nmol g^{-1} soil) were also calculated for mono:poly saturated, saturated:unsaturated, and fungal:bacterial.

2.8. Statistical analyses

All data sets were checked for normality using the Shapiro-Wilk test ($\alpha = 0.05$) to determine the appropriate statistical analyses. Measurements of ATZ degradation from assays of the seven SG cultivars were normally distributed and differences among cultivars were determined by one-way analysis of variance (ANOVA) ($\alpha = 0.05$) using the SAS GLM least squares procedure and Tukey's multiple comparison test ($\alpha = 0.05$).

Atrazine degradation data in the mesocosm study were found to best fit first-order models which were obtained by non-linear regression analyses with Sigma Plot 12.3 (Systat Software, Inc., San Jose, CA) using the two parameter exponential decay model ($[A] = [A]_o e^{-kt}$). Atrazine $t_{0.5}$ values were also computed from first-order models applied to individual replications of all treatments.

If PLFA datasets were normally distributed or could be transformed to achieve normality, analysis of variance (ANOVA) and Tukey's multiple comparisons of means were performed using R 3.2.4. Data sets were transformed according to the results of Box-Cox tests applied to each data set and specified in the Results section. If data could not be adequately transformed, the Kruskal-Wallis test (H-test) and Tukey's multiple comparison of means were performed to determine differences between treatments using SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA). The significance level for all tests was $\alpha = 0.05$. Kendall's rank correlation test was used to assess correlations between soil ATZ concentration and total PLFA, mol % actinobacteria, mol % Gram-negative, mol % Fungi, and mol % arbuscular mycorrhizal fungi biomarkers, using R 3.2.4.

3. Results

3.1. Atrazine degradation among SG cultivars

The degradation rate of ATZ in root extract assays showed significant differences among the seven SG cultivars compared to controls without root extract (Fig. 5). Root extracts from the SG cultivars showed wide variation in the ability to degrade ATZ, ranging from 44% (Sunburst) to 85% (KAN) of control values (Fig. 5). Kanlow extracts degraded significantly more ATZ than those from Trailblazer, Sunburst or the control ($p = 0.024$, 0.003, and <0.001 respectively) (Fig. 5).

After examining several traits across SG cultivars, it was noted that KAN, Cave-in-Rock, and Shawnee, cultivars that exhibited the greatest ATZ degradation potential, are best suited for hardiness zones (HZ) 5–7 (Table 1). This research was conducted in Columbia, Missouri, USA which occupies HZ 6. Switchgrass cultivars that degraded the least ATZ in this experiment (Sunburst and Trailblazer) are suitable for HZ 4 or lower. Additionally, preliminary testing of Alamo cultivar, suitable for HZ 6–9, showed this cultivar did not significantly degrade ATZ (data not shown). Therefore, we postulate that SG cultivars best suited for moderate HZ are capable of degrading ATZ, while those suitable for more extreme environments fail to enhance ATZ degradation. Alternatively, it is possible that SG cultivars must be grown within their optimal HZ to maximize production of ATZ degrading phytochemicals.

3.2. Sorption of DBG and its effect on ATZ hydrolysis

The partition coefficients (K_d) for ATZ were 2.89 L kg^{-1} to field soil, 1.72 L kg^{-1} to the sand:soil mix, and 2.38 L kg^{-1} to Ca-montmorillonite (Table 2). These K_d values are consistent with those previously reported for ATZ and demonstrate its relatively weak sorption to soil colloids (Correia et al., 2006; Lerch et al., 2017). Sorption of DBG showed high

Table 2
Atrazine (ATZ) and 2-β-D-glucopyranosyloxy-4-hydroxy-1,4-benzoxazin-3-one (DBG) sorption to soil, a 40:60 sand:soil mix, and Ca-montmorillonite ± standard deviation.

Substrate + Chemical	K_d (L kg^{-1})
Soil + ATZ	$2.89+^{0.14}$
40:60 Mix + ATZ	$1.72+^{0.103}$
Clay + ATZ	$2.38+^{0.19}$
Soil + DBG	$87.2+^{13.7}$
40:60 Mix + DBG	$35.5+^{12.1}$
Clay + DBG	$31.7+^{0.197}$

affinity to the sorbents studied with K_d values of 87.2 L kg^{-1} to field soil, 35.5 L kg^{-1} to the sand:soil mixture, and 31.7 L kg^{-1} to Ca-montmorillonite. Notably, DBG sorption to the field soil was greater than sorption to the other two sorbents, the variability of DBG K_d values were greater for the field soil and sand:soil mix than clay, and the time for DBG sorption to equilibrate with the field soil and sand:soil mix was 9 d compared to only 24 h for Ca-montmorillonite.

These results suggest that soil organic matter (SOM) may exert a strong influence on DBG sorption. The slow equilibration, high sorption intensity, and highly polar nature of the DBG molecule suggest that multiple weak electrostatic bonding mechanisms were likely occurring between DBG and soil colloids. Compounds sharing structural similarities with Bx compounds, like benzoic acid, have been shown to reversibly bond to soil colloids via van der Waal or hydrogen bonding (Dalton, 1999). However, SOM appears to create opportunities for irreversible binding of these comparatively simplistic structures as noted by Inderjit and Bhowmik, (2004). The opportunity for several types of weak electrostatic bonds to form between DBG and SOM, as indicated by the structure of DBG (Fig. 2), implies the potential for strong bonding of DBG molecules with SOM via the additive effect of numerous bonds. The structurally diverse nature of SOM, and resulting microenvironments within, create highly tortuous diffusion pathways for reactive compounds like DBG (Weber et al., 1990), resulting in the observed disproportionate equilibration times for DBG in soil versus Ca-montmorillonite. Lastly, the K_d for DBG in the sand:soil mix was similar to that of DBG in Ca-montmorillonite, but the equilibration time for DBG in the sand:soil mix was 9 d, the same as that for DBG in field soil. These results indicated that overall sorption intensity of DBG at equilibrium was very similar between the sand:soil mix and Ca-montmorillonite treatments, but the presence of even diluted levels of SOM in the sand:soil treatment substantially reduced the rate of DBG sorption. While research has indicated that lower soil pH may decrease sorption rate of some compounds (Chen et al., 2020), the pH of clay was 5.5 and the soil pH was 6.65. Therefore, clay systems should have produced slower sorption rates if pH, rather than SOM, was controlling sorption rate.

Experiments testing the effect of sorption on the reaction between DBG and ATZ were conducted on Ca-montmorillonite clay equilibrated with either DBG or ATZ following a rinse step to reduce the amount of entrained compound. Atrazine and DBG equilibrated clay lost 7.23 and 2.11% of the compound, respectively, through desorption in the rinse step, leaving a total of 0.0025 mg ATZ in system A and 0.600 mg DBG in system B (Fig. 4). These experiments showed that when either compound was equilibrated with Ca-montmorillonite, no ATZ degradation occurred (Table 3). No detectable levels of the reaction product HA were present in ATZ or DBG equilibrated samples. However, in control samples without clay 25–36% of added ATZ was converted to HA within 24 h. To ensure HA was not formed in equilibrated samples and then sorbed, all samples containing clay were washed with a solution of 0.75 M $CaCl_2$ and 0.0015 M NaN_3 post reaction. The supernatant was analyzed for HA but none was detected in solution after 24 h of agitation. Approximately 11% of ATZ in ATZ equilibrated control samples was lost from solution and expected to have sorbed to the container. Sorption of ATZ to container sides was previously observed in low quantities (<5%)

Table 3
Fate of atrazine (ATZ) in the presence and absence of Ca-montmorillonite saturated with either ATZ or 2-β-D-glucopyranosyloxy-4-hydroxy-1,4-benzoxazin-3-one (DBG) ± standard deviation.

Sample ID[†]	% Atz Sorbed	% Atz in Solution	% Atz Degraded to HA
ATZ Eq Clay + DBG	$44.0+^{3.72}$	$56.0+^{3.72}$	0.00
ATZ + DBG Control[a]	$11.3+^{6.02}$	$63.4+^{0.95}$	$25.3+^{6.39}$
DBG Eq Clay + ATZ	$48.6+^{0.180}$	$51.4+^{0.180}$	0.00
DBG + ATZ Control[b]	$0.0+^{2.95}$	$67.5+^{4.17}$	$36.7+^{1.22}$

Samples were equilibrated (Eq) with ATZ or DBG and then received the opposite chemical in solution. This table shows the mass balance, on a molar basis, of ATZ in solution, degraded to the ATZ metabolite hydroxyatrazine (HA), and sorbed to solid surfaces. The amount of ATZ sorbed was determined by subtracting the amount of ATZ in solution and the amount of ATZ degraded to HA from the amount of ATZ in the system prior to reaction with DBG. Negative values were rounded to zero.

[a] Control samples contained the amount of ATZ, DBG, and volume present in the ATZ equilibrated clay samples.

[b] Control samples contain the amount of ATZ, DBG, and volume present in the DBG equilibrated clay samples.

and variability among samples was low (standard deviation of 2.68) when only ATZ was present in solution, however, variability was much higher in reaction systems with DBG and ATZ present (standard deviation of 6.02), which may have contributed to the increase in calculated ATZ sorbed to containers of reacted samples.

These results demonstrated that when ATZ in solution was added to a system containing sorbed DBG, the reaction between DBG and ATZ was completely inhibited. The reaction was also inhibited when DBG in solution was added to a system containing sorbed ATZ. These results are best explained by the sorption mechanisms affiliated with each compound. Sorption of ATZ to smectitic clays has been shown to occur primarily between the mineral layers (Aggarwal et al., 2006). The relatively large size of DBG, with a molecular weight of 343 g mol^{-1}, compared to ATZ (215 g mol^{-1}), suggests that DBG sorption would likely occur on exterior surfaces or edges of clay minerals. This scheme was supported by the observed increase in entrained solution for the sorbed ATZ treatment, which contained 0.83 g more solution compared to sorbed DBG treatment, (as noted in section 2.6). Atrazine in the mineral interlayer would enhance mineral expansion and permit the entrance of more water molecules than samples without sorbed ATZ (Aggarwal et al., 2006). Interlayer sorption of ATZ is likely to shield sorbed ATZ from interactions with other compounds such as DBG. Additionally, DBG molecules are capable of conformational change, indicating sorption may interfere with the reactivity of the hydroxamic acid moiety upon sorption. Overall, sorption of either DBG or ATZ completely inhibited Bx induced ATZ hydrolysis.

3.3. ATZ degradation in soil mesocosms

Among all treatments, ATZ degradation was rapid, with $t_{0.5}$ of 8.8 d in the control and 8.2 d, 11.2 d, and 9.4 d in mesocosms planted to CIM, KAN, and EG, respectively. There were no significant differences between the treatments with regard to $t_{0.5}$ ($p = 0.793$) (Fig. 6). These results were similar to those of Lerch et al. (2017) who also reported ATZ degradation rates in unvegetated control that were as rapid as grass treatments. However, previous work by Lin et al. (2008, 2011) consistently showed that ATZ degraded much faster in grass rhizospheres compared to unvegetated control. Furthermore, Bx compounds produced by EG (Willett et al., 2013, 2016) and unidentified phytochemicals from SG roots (Fig. 5) have been shown to degrade ATZ in solution, and laboratory experiments indicated substantial ATZ degradation in KAN root extracts but not in CIM root extract. Results from the mesocosm experiment are in direct contrast to the aforementioned work. Similar rates of ATZ degradation between all mesocosm treatments

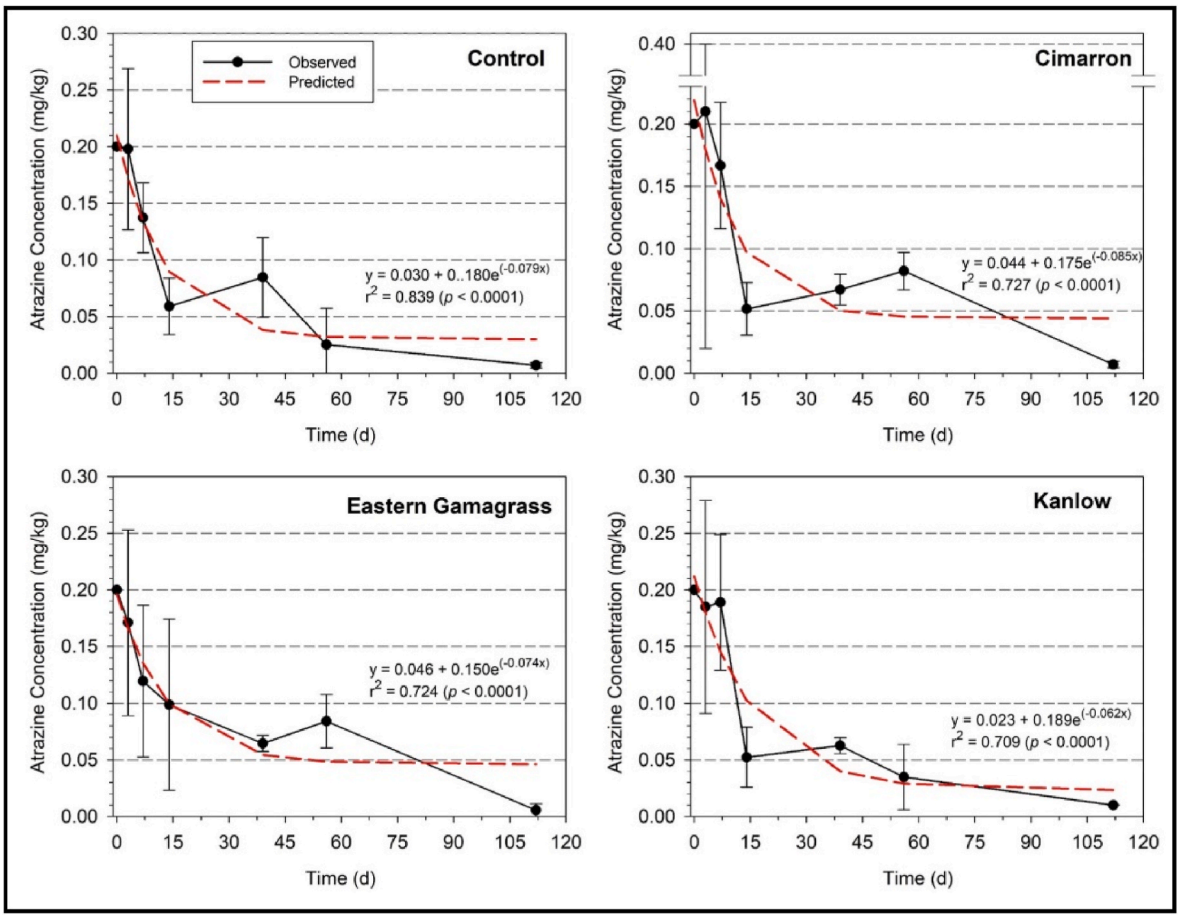

Fig. 6. Atrazine degradation in soil mesocosms by vegetation treatment.

further supported the results of laboratory sorption experiments illustrating the phytochemical-ATZ reaction was inhibited in a soil system.

The short ATZ $t_{0.5}$ of the control treatment suggested that a significant population of ATZ degrading microorganisms remained in soil years after the cessation of ATZ application. It has been shown that ATZ degrading genes can be transferred horizontally within the soil microbial community (Devers et al., 2005); however, infrequent ATZ application (i.e., less than once every 4 yr) has been reported to reduce ATZ degrading gene abundance in soils (Krutz et al., 2010). There are three likely explanations for the continued presence of ATZ degrading genes in the microbial communities of this study. First, spray drift from neighboring fields was possibly sufficient to sustain the presence of ATZ degrading genes via annual low-level exposure. Second, atmospheric deposition of ATZ degrading microorganisms from neighboring treated fields could have sustained a population of ATZ degrading microorganisms (Griffin, 2007; Krutz et al., 2010). Third, the potential for lysogenic horizontal gene transfer in soil of the *trz*N gene responsible for dechlorination of triazines in bacteria has been documented (Ghosh et al., 2008). It is possible soil bacteriophages serve as a reservoir for ATZ degrading bacterial genes in soil, maintaining their prevalence regardless of ATZ application rate. The rapid rate of ATZ loss observed in this study was apparently due to the combination of ATZ degrading soil microorganisms and the favorable temperature and moisture conditions of the experiment.

3.4. Phospholipid fatty acids response to ATZ

To investigate microbial contributions to ATZ degradation in mesocosms, we examined changes in total PLFA, several PLFA biomarker groups, and three ratios of select biomarker groups for all treatments (Table 4). Results of PLFA groups exhibiting significant changes between treatments sampled at the same time or changes over time within treatment are presented here, while comparisons outside this criteria were not reported. The ANOVA test indicated that two biomarkers showed significant treatment differences and eight biomarkers significantly varied over time (Table 4). Thorough examination of Tukey's multiple comparisons of means revealed that total PLFA, % actinobacteria, and % arbuscular mycorrhizal fungi exhibited the most informative microbial changes. Tukey's comparisons of saturated to unsaturated PLFA ratio (SAT:UNSAT) revealed significant differences only between the control at 56 days post ATZ application compared to CIM and EG at day 0. No significant differences between treatments

Table 4
Statistical analyses of total phospholipid fatty acid (PLFA) (nmol g^{-1} soil), PLFA biomarker groups (mol% g^{-1} soil) and biomarker ratios, with associated *p*-values for ANOVA of treatment, time, and treatment × time interactions.

Groupings of PLFA	Treatment	Time	Treatment X Time
Total PLFA	**<0.001**	**0.001**	0.086
Actinobacteria	0.066	**<0.001**	**0.021**
Gram-pos bacteria	0.208	**<0.001**	**0.014**
Gram-neg bacteria	0.090	0.358	**0.011**
Fungi	0.411	0.082	**0.002**
AM Fungi	0.446	**<0.001**	0.239
Eukaryote[a]	0.634	**0.003**	NA
Mono:Poly[a]	0.612	**<0.001**	NA
Sat:Unsat	**<0.001**	**0.002**	0.928
Fungi:Bacteria	0.071	**<0.001**	0.086

[a] Data could not be transformed and was non-normal; analyzed using Kruskal-Wallis test.

within sampling period or within treatment at different sampling periods were observed within the SAT:UNSAT data (data not shown).

3.4.1. Total PLFA

Total PLFA biomarkers showed the control treatment contained significantly less total PLFA than some grass treatments over the study period. Following ATZ application, the control treatment had significantly less total PLFA than CIM treatment at 14 and 112 d after application, and significantly less total PLFA than CIM and EG at 56 d (Fig. 7). While the control showed a steady decline in total PLFA over the study, grass treatments generally maintained total PLFA levels throughout the experiment at or above those at time 0. This indicated overall greater robustness of the soil microbial community under these warm-season grasses, compared to the unvegetated control. While the ANOVA analysis did not indicate significant differences in total PLFA between grass treatments, CIM had the shortest ATZ $t_{0.5}$ (8.2 d) and consistently had the greatest total PLFA levels, suggesting that total PLFA may have been related to the observed rapid ATZ degradation.

3.4.2. Percent actinobacteria biomarkers

Actinobacteria biomarkers showed an evident treatment response ($p = 0.066$, Table 4) and examination of Tukey's comparisons of means demonstrated a sustained increase in percent actinobacteria in control treatment over time (Fig. 8). The percentage of actinobacteria also appeared to be increasing over time in KAN treatment but differences were only significant when comparing measurements from 14 to 112 d post ATZ application. Prior research has indicated several actinobacteria are capable of efficiently degrading triazine herbicides (De Schrijver and De Mot, 2008), and they are among the more stress resilient soil microorganisms (Alvarez et al., 2017). The actinobacteria *Nocardia* sp. has been reported to initiate ATZ degradation in soils (Smith et al., 2005) and the actinobacteria *Arthrobacter, Rhodococcus, Sterptomyces, Frankia,* and *Kokuria* have also been reported to degrade s-triazines (Alvarez et al., 2017).

3.4.3. Arbuscular mycorrhizal fungal biomarkers

While ANOVA results for AM Fungi yielded no significant differences among treatments, the significance of time was substantial ($p < 1 \times 10^{-7}$) for each treatment. The percentage of AM Fungi in the CIM treatment showed a significant increase by 112 d post ATZ application when compared to initial measurements of percent AM fungi in CIM and control treatments. This change was not observed in any other treatment (Fig. 9). An increase in AM fungi may be an indication that many of the microorganisms in this system capable of enhancing ATZ degradation were AM fungi, or were affiliated with the presence of AM fungi, especially in the CIM treatment. It has been shown that warm season grasses (Göransson et al., 2008), and SG in particular (Liang et al., 2016), form

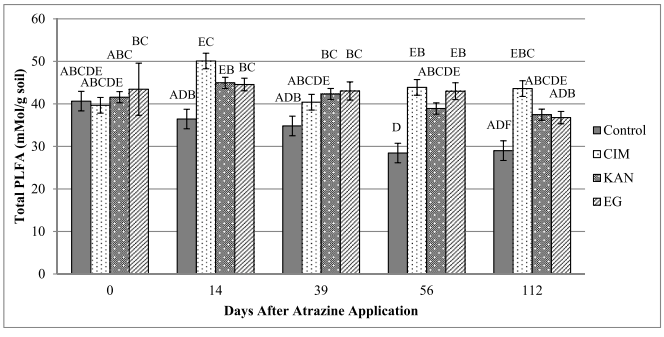

Fig. 7. Total phospholipid fatty acid (PLFA) response to atrazine (ATZ) application in soil mesocosms by treatment. Control = unvegetated; CIM = Cimarron switchgrass; KAN = Kanlow switchgrass; and EG. Bars with the same letter are not significantly different. Data presented above are not transformed. Error bars indicate standard deviation.

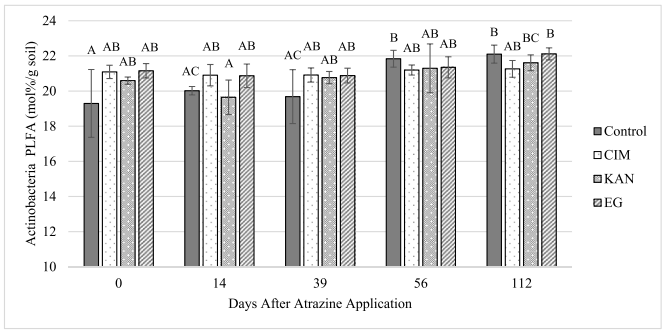

Fig. 8. Percent actinobacteria phospholipid fatty acid (PLFA) response to atrazine (ATZ) application in soil mesocosms. Control = unvegetated; CIM = Cimarron switchgrass; KAN = Kanlow switchgrass; and EG = eastern gamagrass. Bars with the same letter are not significantly different. Data were transformed exponentially ($1/y^5$, y = original data). Data presented above are not transformed. Error bars indicate standard deviation.

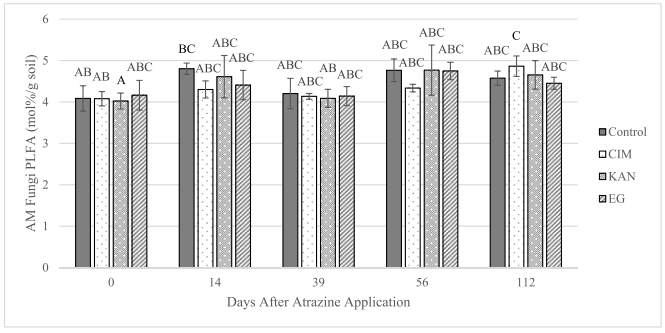

Fig. 9. Percent arbuscular mycorrhizal (AM) fungi phospholipid fatty acid (PLFA) response to atrazine (ATZ) application in soil mesocosms by treatment. Control = unvegetated; CIM = Cimarron switchgrass; KAN = Kanlow switchgrass; and EG = eastern gamagrass. Bars with the same letter are not significantly different. Data presented above are not transformed. Error bars indicate standard deviation.

relationships with AM fungi populations in soil. Research has also shown enhanced ATZ degradation in the presence of mycorrhizal fungi compared to plant roots alone, even on the same plant (Huang et al., 2009).

3.4.4. ATZ degradation and microbial community

Correlation analyses of pooled data showed ATZ concentration in soil was significantly and inversely related to percentage of actinobacteria and AM fungi biomarkers, and positively correlated to total PLFA and percent Gram-positive biomarkers (Table 5). However, all significant correlations were low, with $\tau < |0.4|$, and correlations were non-significant for percent eukaryote, fungi, and Gram-negative PLFA biomarkers. It is important to note, the biomarkers designated to each PLFA group do not overlap, and therefore, were independent variables. Hence,

Table 5
Correlation of phospholipid fatty acid (PLFA) markers and atrazine (ATZ) concentration in soil mesocosms.

PLFA Group	Correlation Coefficient[a]	*p*-value
Total PLFA	0.189	**0.013**
% Actinobacteria	−0.293	**<0.001**
% AM Fungi	−0.350	**<0.001**
% Eukaryote	−0.051	0.504
% Fungi	−0.008	0.914
% Gram-Neg	0.018	0.816
% Gram-Pos	0.253	**<0.001**

[a] Kendall's rank correlation coefficients (τ).

the fungi group did not include AM fungi, just as the Gram-positive group did not include actinobacteria.

Total PLFA was positively correlated to ATZ soil concentration, with overall decreasing total PLFA levels and ATZ concentration over time (Table 5; Fig. 7). Vryzas et al. (2012) reported no correlation between ATZ degradation and total PLFA but Nordenholt et al. (2016) had similar results to those presented here, illustrating a decrease in total PLFA that persisted for months after ATZ application. The percent of Gram-positive biomarkers was also positively correlated with ATZ content, indicating this group of bacteria was negatively impacted by ATZ application. Actinobacteria and AM Fungi contributed most to ATZ degradation in all treatments as they increased significantly with diminishing ATZ content, indicating a potentially significant role as ATZ degraders in this study.

In summation, the percentage of actinobacteria and AM fungi increased in the mesocosm study as ATZ was degraded and soil ATZ content was depleted, even as total PLFA decreased. This is compelling evidence that as ATZ was degraded into subsequently simpler structures, those microorganisms able to utilize ATZ, or ATZ metabolites, as an energy source had an advantage over those unable to utilize structurally bound ATZ carbon and nitrogen. The compilation of this data indicates the presence of efficient ATZ degrading soil microorganisms in all treatments of our mesocosm study. In the case of the CIM treatment, a substantial population of ATZ degrading microorganisms may have been supported by the actively growing SG, as indicated by the initial increase, and consistently high amounts of total PFLA (in contrast to the control) and the very rapid rate of ATZ degradation, despite the absence of ATZ degrading phytochemicals in this SG cultivar.

4. Conclusions

Root extracts of seven SG cultivars exhibited enhanced ATZ degradation and significant differences between cultivars in solution assays. However, DBG was shown to strongly sorb to soil and Ca-montmorillonite, resulting in complete inhibition of ATZ hydrolysis. Similarly, sorbed ATZ also inhibited hydrolysis by DBG. Thus, if the ATZ-degrading phytochemicals present in the SG cultivars were similar in structure to Bx compounds, they would likely sorb to soil, and phytochemically induced ATZ hydrolysis would be a negligible degradation pathway in the rhizosphere. This synopsis was supported by the similarities between ATZ degradation rates in the mesocosm study ($t_{0.5}$ = 8.2–11.2 d), which indicated both the DBG produced by EG and the unknown phytochemical(s) produced by SG had very little effect on ATZ degradation when compared to unvegetated control treatment. Instead, microbial degradation of ATZ was the primary mechanism responsible for the rapid degradation in all treatments. The material influence of microbial ATZ degradation was supported by significant changes in several PLFA biomarkers and significant correlations of some biomarkers to ATZ concentration in soil. Despite no ATZ application to the soil source site within 5 yr of collection, microbial adaption to ATZ was common in all treatments. Although warm season grasses have been shown to greatly enhance ATZ degradation, the relative contribution of root phytochemicals and microorganisms to ATZ degradation in grass rhizospheres had not been previously addressed. Results of PLFA analysis, combined with those demonstrating sorption inhibition of ATZ hydrolysis by DBG, strongly support the conclusion that ATZ degradation in these grass treatments primarily resulted from microbial degradation rather than any direct effect of phytochemical reactivity.

Future work to identify and isolate the ATZ reactive compound produced by some SG cultivars would significantly enhance overall understanding of, and permit quantification of, soil sorption effects on the SG phytochemical-ATZ reaction. Current knowledge only permits speculation with respect to the SG phytochemical mechanisms of reactivity with ATZ and soil colloids. Inclusion of metagenomics analysis of SW rhizospheres would definitively identify ATZ degrading genes contributing to ATZ degradation and potentially indicate differences in degradation pathways relative to SW cultivar. Additionally, it was demonstrated that DBG has a strong affinity for clay and soil colloids; however, Fourier transform infrared (FTIR) spectroscopy of DBG sorbed to clay could elucidate the mechanisms contributing to its sorption.

Credit author statement

Kathleen Hatch: Conceptualization, Methodology, Project administration, Formal analysis, Investigation, Writing-Original draft preparation and Review & Editing. **Robert N. Lerch:** Conceptualization, Methodology, Resources, Formal analysis, Writing-Review & Editing, Supervision, Funding acquisition. **Robert J. Kremer:** Conceptualization, Methodology, Resources, Writing-Review & Editing. **Cammy D. Willett:** Methodology, Resources, Writing- Review & Editing. **Craig A. Roberts:** Resources, Writing- Review & Editing. **Keith W. Goyne:** Conceptualization, Methodology, Resources, Writing-Review & Editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2022.115840.

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