

Abiotic Reduction of Pendimethalin and Trifluralin in Controlled and Natural Systems Containing Fe(II) and Dissolved Organic Matter

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The environmental fate of dinitroaniline herbicides is poorly understood, despite their classification as Persistent Bioaccumulative Toxins by the U.S. Environmental Protection Agency. This study investigated the abiotic reduction of pendimethalin and trifluralin in controlled laboratory systems in the presence of Fe(II) and fulvic acids isolated from various surface waters and in sediment pore waters containing naturally abundant levels of dissolved Fe(II) and dissolved organic matter (DOM). It was found that Fe(II) was necessary for pendimethalin and trifluralin reduction to occur in controlled systems and that higher concentrations of DOM slowed Fe(II)-mediated reactions. Pendimethalin and trifluralin reduction in natural pore waters was roughly an order of magnitude slower compared to controlled Fe(II)–DOM solutions, indicating that the reactive Fe(II) species responsible for reduction are concentration-limited in natural pore waters relative to controlled systems. The results show that caution must be exercised when extrapolating results from controlled system reactions to natural systems and that abiotic reduction of both trifluralin and pendimethalin is observed within 3–7 days in anaerobic sedimentary pore waters containing high concentrations of both dissolved Fe(II) and DOM.

KEYWORDS: Trifluralin; pendimethalin; dinitroaniline; Fe(II); dissolved organic matter; pore water; reduction; iron; pesticide; Pony Lake fulvic acid; Suwannee River fulvic acid; Old Woman Creek fulvic acid

INTRODUCTION

The environmental fate of pesticides is of concern due to their potential long-term effects on aquatic ecosystems. Dinitroaniline herbicides, such as pendimethalin and trifluralin, are used widely throughout the United States as a pre-emergent treatment on a variety of agricultural crops (**Figure 1**) (1–3). U.S. Geological Survey agricultural pesticide use maps for 2002 show that pendimethalin application to soybean, cotton, and corn crops totaled 80% of its agricultural use (5.13, 2.62, and 2.51 million pounds, respectively) (2). Soybean and cotton crop treatments consumed a combined 60% of trifluralin agricultural use in the United States (3 and 2.7 million pounds, respectively) (3). Both pendimethalin and trifluralin are of environmental concern as they have been listed as Persistent Bioaccumulative Toxins by the U.S. Environmental Protection Agency (4).

Although pendimethalin and trifluralin generally are retained in the soil column when applied as pre-emergent pesticides (1), erosion and surface water transport of suspended solids result in widespread distribution of these herbicides in United States waterways (5, 6). Contaminated particles can settle from surface waters into wetland sediments due to the slowing of water flow, and emergent vegetation naturally present in such environments

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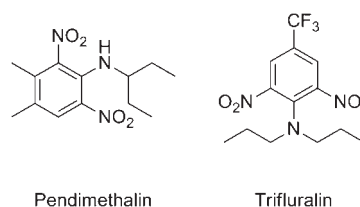


Figure 1. Structures of pendimethalin and trifluralin.

traps suspended particles (7). The fate of hydrophobic contaminants in wetland sediments is of particular interest as they are either recalcitrant or amenable to degradation by biotic and abiotic pathways.

Reduction of nitroaromatic probe compounds in anaerobic sedimentary environments was found to be a solution-phase process (8). In many freshwater systems, benthic sediment pore waters contain high concentrations of dissolved Fe(II) (in the hundreds of micromolar concentration range) and dissolved organic matter (DOM; measured as dissolved organic carbon (DOC), up to ~3 mM C) (9–11). Dissolved Fe(II) and surface water fulvic acid isolates are known to promote the abiotic reduction of pentachloronitrobenzene in controlled laboratory solutions (12), and complete reduction of pentachloronitrobenzene to pentachloroaniline has been observed abiotically in natural pore waters containing high

concentrations of Fe(II) and DOM (11). Similarly, the abiotic reduction of dinitroaniline herbicides has the potential to occur in the presence of solution-phase Fe(II) and DOM in both controlled systems and natural pore waters.

Several investigators reported abiotic pendimethalin and trifluralin reduction in highly controlled laboratory systems in the presence of aqueous Fe(II), Fe(II)/goethite, and H₂S/juglone reductant systems (13, 14). However, to date, the effect of DOM on the Fe(II)-mediated reduction of pendimethalin and trifluralin in natural systems remains unclear. In this study, pendimethalin and trifluralin reduction was studied in experiments containing solutions of Fe(II) and surface water fulvic acid isolates ("controlled systems", where the fulvic acids represent DOM) and in natural pore waters collected anoxically from freshwater wetland sediments (Old Woman Creek, Ohio). We hypothesize that dissolved Fe(II) is necessary for reduction to occur and that unique Fe(II)-DOM-mediated reduction pathways exist for dinitroaniline herbicides relative to mononitroaromatic compounds. The major goals of this study were to characterize pendimethalin and trifluralin reduction kinetics in the presence of Fe(II) and DOM isolates and to compare results from the "controlled" laboratory experiments with those conducted using natural sediment pore waters.

MATERIALS AND METHODS

Chemicals and Dissolved Organic Matter. Trifluralin, 99.1% purity 2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl)benzenamine (Riedel-de Haën, Seelze, Germany), and pendimethalin, 98.4% purity *N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitroaniline (Riedel-de Haën), were used as received and made into separate stock solutions in HPLC-grade methanol (Fisher Scientific, Pittsburgh, PA). HPLC-grade methanol and 18.2 MΩ Milli-Q UV Plus water (Millipore, Billerica, MA) were used as solvents. MOPS, 99% purity 3-[*N*-morpholino]propanesulfonic acid (Sigma-Aldrich, St. Louis, MO), FeCl₂·4H₂O certified grade (Fisher Scientific; Alfa Aesar, Ward Hill, MA), Fe(NH₄)₂(SO₄)₂·6H₂O certified ACS grade (Fisher Scientific), ammonium acetate (Jenneile Enterprises, Cincinnati, OH; Fisher Scientific), 1,10-phenanthroline monohydrate certified ACS grade (Fisher Scientific), glacial acetic acid certified ACS Plus grade (Fisher Scientific), concentrated HCl certified ACS grade (Fisher Scientific), concentrated H₂SO₄ certified ACS grade (Fisher Scientific), and solid NaOH (Mallinckrodt AR, Phillipsburg, NJ; Fisher Scientific) were used as received. Fulvic acid isolates, used as the DOM source in controlled system experiments, were prepared by us using the XAD-8 method (15) or purchased from the International Humic Substances Society. We used Pony Lake, Antarctica (Pony Lake fulvic acid), and Suwannee River, Georgia (Suwannee River fulvic acid), International Humic Substances Society standards, whereas we isolated the fulvic acid from Old Woman Creek, Ohio (Old Woman Creek fulvic acid). Pony Lake fulvic acid represents organic matter derived from surface waters with primarily microbial and algal organic matter inputs, whereas Suwannee River fulvic acid is primarily derived from higher plant material precursors (16). Old Woman Creek fulvic acid represents surface water fulvic acids from a combination of both microbial and higher plant sources (16) and was isolated from the surface waters overlying the wetland sediments used for pore water extractions in this study.

Preparation of Controlled System Reaction Media. All controlled system reactions were performed in a glovebox equipped with a Pd-catalyzed O₂ scrubber under a 95:5 v/v nitrogen/hydrogen atmosphere. All solutions prepared outside the glovebox were purged with Ar gas for 1 min per milliliter of solution prior to transport into the glovebox and use in experiments. Controlled system reaction media were prepared using aqueous MOPS stock solution diluted with Milli-Q (target [MOPS] = 28 mM). For reactions containing DOM, an aqueous stock solution prepared with the fulvic acid isolate was added to the reaction medium. FeCl₂·4H₂O was added to the reaction medium either as a solid or as a pH ~3 stock solution for reactions containing Fe(II). The solution pH was adjusted with 2 N NaOH inside the glovebox, and the pH was measured using a 240 pH/temperature meter (Beckman, Brea, CA) and an Aqua Pro pH probe (Thermo Orion, Beverly, MA). All solutions were filtered with Milli-Q-rinsed IC Acrodisc 0.45 μm × 25 mm

diameter Supor polyethylene sulfone membrane filters (Pall Life Sciences, Ann Arbor, MI) and allowed to equilibrate for a minimum of ~30 min. Previous work showed that nitroaromatic reactivity was unaffected by reaction medium equilibration time (12, 17). After equilibration, each solution was drawn into a ground glass syringe. Solutions used for filter-sterilized control reactions were filtered through a sterile 0.2 μm × 25 mm diameter Acrodisc membrane filter (Pall Life Sciences) using a sterile three-port valve (Medex Technologies, Monsey, NY) into an autoclaved ground glass syringe.

Sediment Coring and Pore Water Collection. Sediment cores were collected during August 2007 from Old Woman Creek National Estuarine Research Reserve, a federally protected wetland located adjacent to Lake Erie in Huron, OH. Intact sediment cores were collected from the lower wetland using a punch-core sampling technique and transported to the Old Woman Creek laboratory. At the facility sediment cores were hydraulically transferred into core squeezers (18) equipped with 16 extraction ports spaced 1–2 cm apart with depth and fitted with 3/4 in. screw-to-female luer fittings and 70 μm Porex rod filters (Interstate Specialty Products, Sutton, MA). The top and bottom of the squeezer were sealed with Teflon plungers and mounted on Unistrut beam supports. Each screw-to-female luer fitting was sealed with a three-port valve, which was fitted with a 10 or 20 cm³ ground glass syringe. The three-port valves and syringes were evacuated with 95:5 nitrogen/hydrogen v/v gas to remove O₂. Cores were pressurized by forcing the bottom plunger into the core, which subsequently forced pore waters from the sediments into the ground glass syringes.

After collection, pore waters were immediately transferred to a glovebox under 95:5 v/v nitrogen/hydrogen equipped with a Pd-catalyzed O₂ scrubber and filtered through a 0.45 μm membrane filter directly into glass serum vials. The glass serum vials were sealed with rubber septa in the glovebox at Old Woman Creek and then were transported on ice to our laboratory in Columbus, OH. At our Columbus laboratory, the serum vials were transferred to the glovebox and stored under 95:5 v/v nitrogen/hydrogen with a Pd-catalyzed O₂ scrubber. Pore waters were drawn into ground glass syringes immediately before reaction with pendimethalin and trifluralin.

Measurement of pH, Fe(II), and DOM. Prior to reaction with pendimethalin and trifluralin, aliquots of the reaction medium were separated to measure pH and [Fe(II)] for all controlled and pore water reaction samples and to determine DOM concentrations (for the pore water as dissolved organic carbon, DOC). The [Fe(II)] was measured by reacting acidified sample aliquots with 1,10-phenanthroline and measuring the complex absorbance at 508 nm using a UV-vis spectrophotometer, as described in ref 11. The DOM concentrations were measured as DOC using a Shimadzu TOC 5000 (Shimadzu, Columbia, MD). For controlled systems, [DOC] values for fulvic acid stock solutions were used to calculate [DOC] for the reaction solution. Pore water [DOC] was measured directly from acidified pore water aliquots.

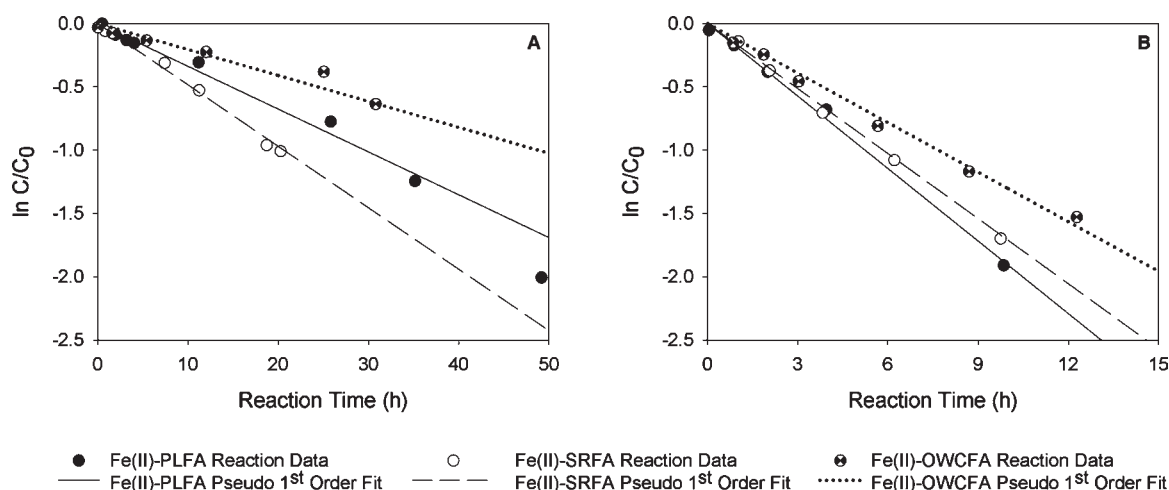
Kinetics Studies. Ar-purged trifluralin and pendimethalin methanol-based stock solutions were injected directly into the reaction medium- or pore water-filled ground glass syringes using a glass-tip micropipet, with a 0.1% v/v methanol composition in solution. Herbicide stock solutions were added in low-volume, methanol-based amounts to reach the target initial herbicide concentration of 1 μM without changing the volume and chemical composition of the synthetic or natural pore water, per prior studies with nitroaromatic compounds (13, 17). Prior work showed that this low volume of methanol in solution did not influence experimental results (13, 17). The target starting concentration of ~1 μM herbicide in the reaction solutions was used to measure kinetic behavior at concentrations within the detection limits of our instrument and to be comparable to the range of natural concentrations measured for pendimethalin (0.06–0.33 μmol/kg) and trifluralin (0.05–0.36 μmol/kg) in suspended aquatic sediments (6).

Kinetic time points were collected by adding a ~2 mL aliquot of the reacted solution to a borosilicate glass autosampler vial preloaded with ~20 μL of 2 N HCl. Reaction vials were sealed with screw caps equipped with Teflon-lined septa. Reaction aliquots were analyzed by HPLC using a Shimadzu SCL-10AT pump, an SIL-10A autosampler, a DGU-14A degasser, an SCL-10A system controller, and an SPD-10A UV-vis detector (Shimadzu). The column used was a 150 mm × 4.6 mm i.d., 5 μm, Pinnacle II C18, with a 10 mm × 4 mm i.d. guard column of the same

Table 1. Fe(II)- and DOC-Normalized Pseudo-First-Order Rate Constants (k_{obs}) for Pendimethalin and Trifluralin Reduction in Controlled Fe(II)–DOM Reaction Media, Filter-Sterilized Reactions, and Natural Pore Water Reactions^a

	Fe(II)–Pony Lake fulvic acid	Fe(II)–Suwannee River fulvic acid	Fe(II)–Old Woman Creek fulvic acid	Fe(II)–Pony Lake fulvic acid, nonsterile	Fe(II)–Pony Lake fulvic acid, filter-sterilized	Old Woman Creek sediment pore water reaction
Pendimethalin						
normalized k_{obs} (h/ $\mu\text{M Fe(II)}$ / $\mu\text{M DOC}$)	$(2.97 \pm 0.40) \times 10^{-8}$	$(3.79 \pm 0.43) \times 10^{-8}$	$(3.00 \pm 0.37) \times 10^{-8}$	$(7.79 \pm 1.61) \times 10^{-9}$	$(5.50 \pm 1.18) \times 10^{-9}$	$(2.86 \pm 0.65) \times 10^{-9}$
R^2	0.9874	0.9910	0.9796	0.9461	0.9487	0.9756
pH	7.80	7.75	7.81	7.39	7.39	7.77
[Fe(II)] (μM)	670	640	340	780	800	380
[DOC] (mM-C)	1.7	2.0	2.0	1.3	1.3	3.2
Trifluralin						
normalized k_{obs} (h/ $\mu\text{M Fe(II)}$ / $\mu\text{M DOC}$)	$(2.34 \pm 0.16) \times 10^{-7}$	$(3.13 \pm 0.10) \times 10^{-7}$	$(1.91 \pm 0.22) \times 10^{-7}$	$(2.34 \pm 0.16) \times 10^{-7}$	$(1.38 \pm 0.16) \times 10^{-7}$	$(2.14 \pm 0.27) \times 10^{-8}$
R^2	0.9964	0.9987	0.9837	0.9964	0.9833	0.9804
pH	7.80	7.79	7.81	7.80	7.80	7.77
[Fe(II)] (μM)	680	660	340	680	650	380
[DOC] (mM-C)	1.2	0.8	2.0	1.2	1.2	3.2

^a Error is reported as the 95% confidence interval for the kinetic rate fit.

**Figure 2.** Reduction of pendimethalin (A) and trifluralin (B) in controlled systems containing Fe(II) and DOM fulvic acid isolates. PLFA, Pony Lake fulvic acid; SRFA, Suwannee River fulvic acid; OWCFA, Old Woman Creek fulvic acid.

material (Restek, Bellefonte, PA). Both pendimethalin and trifluralin were monitored at λ 225 nm with a 1.0 mL/min flow rate and 250 μL injection volume. The mobile phase consisted of an isocratic 77:23 methanol/water v/v mobile phase acidified to pH \sim 3 with 12 N HCl. Calibration curves for pendimethalin and trifluralin were generated for each reaction series by plotting the HPLC-derived peak area of serially diluted calibration standard solutions versus the concentration of herbicide in the calibration standard. The calibration curve concentrations ranged from 0.05 to 2.5 μM . Concentrations of pendimethalin and trifluralin in the reaction aliquots were quantified by relating peak retention times to the calibration standards and peak areas to the calibration curves. Reactions were considered to be complete when the calculated concentration was below the lowest calibration standard tested, which also coincided with the lower end detection limit of the instrument (\sim 0.05 μM).

Pseudo-first-order rate constants (k_{obs}) were calculated from natural log concentration versus time data. Initial concentrations of the two herbicides were calculated during the rate fit, as the true initial concentration could not be determined due to immediate reaction of pendimethalin and trifluralin upon addition to the reaction medium. All reaction kinetics for the Fe(II)–DOM controlled systems and natural pore waters were modeled using a pseudo-first-order model, as most reactions performed to 3 half-lives in controlled systems showed kinetic rate fits with $R^2 \geq 0.98$.

RESULTS AND DISCUSSION

Pendimethalin and trifluralin were both reduced in controlled systems containing Fe(II) and DOM fulvic acid isolates at pH, [Fe(II)], and [DOC] levels similar to those present in natural freshwater wetland sediment pore waters (Table 1; Figure 2). Starting herbicide concentrations for these reactions ranged from 0.8 to 1.6 μM herbicide. For both pendimethalin and trifluralin, the Fe(II)–Suwannee River fulvic acid reactions are faster than the Fe(II)–Pony Lake fulvic acid reactions (Table 1). The Fe(II)–Old Woman Creek fulvic acid reaction appears to be slower than the other Fe(II)–DOM reactions; however, this may result from a lower [Fe(II)] in this experiment relative to the other Fe(II)–DOM solutions (Table 1). Overall, the trifluralin reduction rate is faster than pendimethalin reduction, and this may be due to differences in their structures; trifluralin contains two nitro groups that may be subject to reduction, compared to the one nitro group of pendimethalin (the other nitro group is sterically hindered) (Figure 1). Furthermore, trifluralin contains other structural components that can be abiotically converted by Fe(II) as observed in ref 13.

Both pendimethalin and trifluralin are reduced in controlled reactions containing Fe(II) and MOPS buffer (indicated

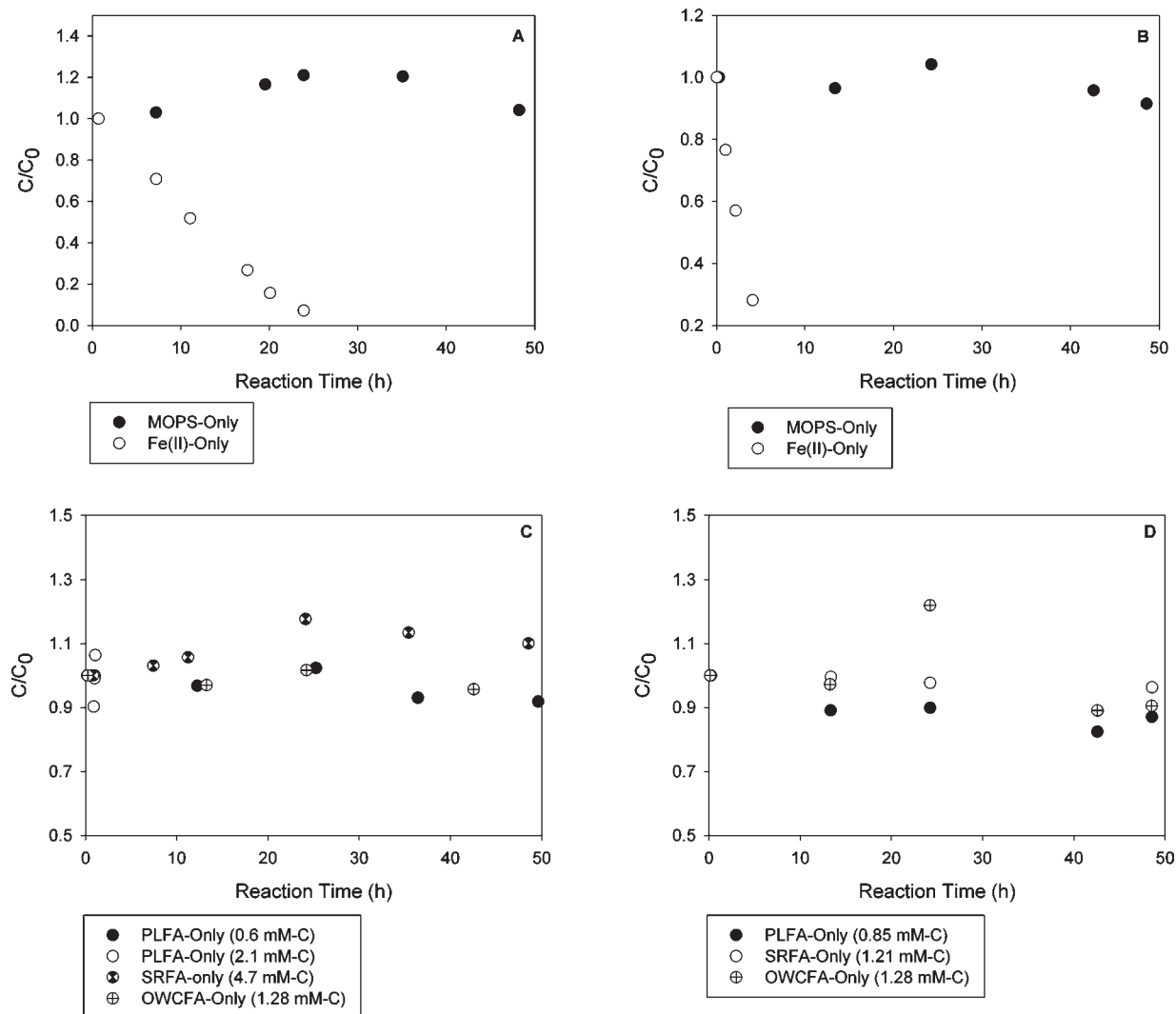


Figure 3. Pendimethalin (A) and trifluralin (B) reactivity in Fe(II)-only and MOPS-only solutions; DOM-only control reactions for pendimethalin (C) and trifluralin (D). PLFA, Pony Lake fulvic acid; SRFA, Suwannee River fulvic acid; OWCFA, Old Woman Creek fulvic acid.

as Fe(II)-only in the figure legend) (Figure 3A,B), but are not reduced in our Fe(II)-free MOPS-only (MOPS-only reactions indicated in the figure legend) (Figure 3A,B) or Fe(II)-free fulvic acid and MOPS control reactions (DOM-only reactions indicated in the figure legend) (Figure 3C,D), demonstrating that Fe(II) is necessary for reduction to occur. Starting herbicide concentrations for these reactions ranged from 0.7 to 1.6 μM . We further probed the effects of pH and [Fe(II)] on reduction kinetics using pendimethalin, as only one nitro functional group is reduced and side reactions involving other ring substituents are not expected (14), thereby simplifying the interpretation of our results. We observed faster pendimethalin reduction rate constants in experiments with increased [Fe(II)] at constant pH and [DOC] and increased pH at constant [Fe(II)] and [DOC] (Figure 4). The starting concentrations for pendimethalin ranged from 0.7 to 2.1 μM for the experimental results presented in Figure 4.

Some studies have shown that DOM is able to directly reduce organic compounds (8, 19–21). For example, ref. 21 showed that reduction of the nitro moiety of methyl parathion occurs in the presence of electrochemically reduced DOM analogues, for example, anthraquinone-2,6-disulfonic acid, and the absence of Fe(II). We did not, however, investigate the possibility that reduced DOM (by microbes, electrochemically or abiotically) could be actively involved in the reduction of pendimethalin and

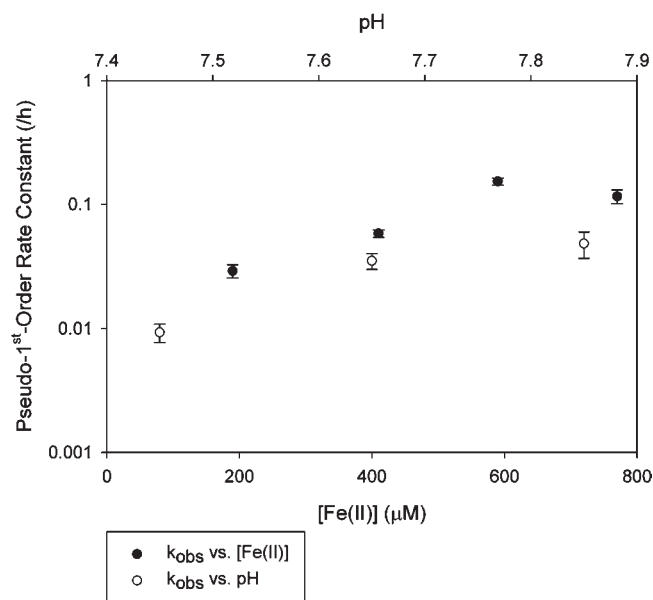


Figure 4. Pendimethalin pseudo-first-order rate constants (k_{obs}) versus [Fe(II)] (solid symbols, bottom x-axis) and versus pH (open symbols, top x-axis) in Fe(II)–Pony Lake fulvic acid reaction media.

Table 2. Fe(II)- and DOC-Normalized Pseudo-First-Order Rate Constants (k_{obs}) for Pendimethalin and Trifluralin Reduction in Fe(II)–DOM Reaction Media with Varied [DOC]^a

	pH	[Fe(II)] (μM)	[DOC] (mM-C)	k_{obs} (h/ μM Fe(II)/ μM DOC)	R^2
Pendimethalin					
Fe(II)–Pony Lake fulvic acid	7.77 \pm 0.04	640 \pm 50	1.3	$(2.00 \pm 0.13) \times 10^{-7}$	0.9975
			1.7	$(4.86 \pm 0.71) \times 10^{-8}$	0.9919
			2.0	$(6.04 \pm 0.50) \times 10^{-8}$	0.9946
Fe(II)–Suwannee River fulvic acid	7.77 \pm 0.04	640 \pm 50	1.0	$(1.58 \pm 0.06) \times 10^{-7}$	0.9991
			2.0	$(3.79 \pm 0.43) \times 10^{-8}$	0.9910
			3.0	$(6.03 \pm 0.16) \times 10^{-8}$	0.9997
Trifluralin					
Fe(II)–Pony Lake fulvic acid	7.81 \pm 0.01	660 \pm 20	0.9	$(2.79 \pm 0.15) \times 10^{-7}$	0.9970
			1.2	$(2.34 \pm 0.16) \times 10^{-7}$	0.9964

^a Error is reported as the 95% confidence interval for the kinetic rate fit.

trifluralin. It is possible that some fractions of the fulvic acid isolates, even if exposed to O₂, are incompletely oxidized (22–25). However, on the basis of the results of our experiments, we believe that reaction pathways involving Fe(II) are the main contributors to pendimethalin and trifluralin reduction in systems with elevated Fe(II) and DOM.

Filter-sterilized control reactions show that pendimethalin reduction rates are the same (within the 95% confidence interval for the rate fit) in both nonsterile and sterile reaction media (Table 1). Trifluralin reduction rates are slightly slower in the sterile reaction media compared to the nonsterile solution (Table 1). This difference was observed only for the trifluralin reaction media, and we are confident that our kinetics data truly represent a faster abiotic pathway relative to slower microbially mediated pathways. For example, trifluralin reduction mediated by anaerobic microorganisms in moist soils amended with an organic substrate was found to have half-lives on the order of 4 days (26). In all of the experiments presented here, trifluralin was removed by at least 70% within 2.5 days. We suspect that the difference in trifluralin reduction kinetics between our sterile and nonsterile systems is related to a specific abiotic trifluralin reductant that is partially removed from solution by the filter sterilization process. An example of such a reductant would be a reactive Fe(II)–DOM fraction removed during the filter sterilization process that otherwise would interact with trifluralin.

Our controlled experimental results for pendimethalin and trifluralin complement our prior work in which we observed abiotic pentachloronitrobenzene reduction in controlled system reaction media containing Fe(II) and DOM, where increased [Fe(II)] resulted in faster pentachloronitrobenzene reduction (12). However, in contrast to our pendimethalin and trifluralin results, pentachloronitrobenzene reduction in both Fe(II)–Pony Lake fulvic acid and Fe(II)–Suwannee River fulvic acid 0.45 μM filtered solutions was faster than in Fe(II)-only reaction media (12). In our pendimethalin and trifluralin reactions, Fe(II)-only controlled system reactions were faster than any of the Fe(II)–DOM reactions. For example, we observed pendimethalin and trifluralin reduction to <30% of the initial concentration within 30 h (Figure 3A) and 10 h (Figure 3B), respectively, in Fe(II)-only solutions, whereas the same extent of removal for both compounds in Fe(II)–DOM solutions required roughly 50 h (pendimethalin) and 20 h (trifluralin) (Figure 2).

Others (13) reported slow trifluralin reduction in Fe(II)-only reaction media, which they attributed to the presence of trace colloids. However, we previously showed that our controlled reactions using 0.45 μM filtered Fe(II)–DOM media are essentially colloid-free (12). Additionally, a prior study (14) reported

rapid reduction of both pendimethalin and trifluralin in Fe(II)-only reaction media, with pseudo-first-order rate constants of $0.81 \pm 0.31/\text{h}$ for pendimethalin and $3.40 \pm 0.71/\text{h}$ for trifluralin at pH 7.8 and [Fe(II)] = 1 mM. Fitting our Fe(II)-only reaction data to a pseudo-first-order kinetic model results in pseudo-first-order rate constants of $0.08 \pm 0.02/\text{h}$ for pendimethalin at pH 7.75 and [Fe(II)] = 790 μM and $0.31 \pm 0.04/\text{h}$ for trifluralin at pH 7.83 and [Fe(II)] = 720 μM . The important comparison in this study is that pendimethalin and trifluralin reduction in our Fe(II)-only solutions, and those independently performed by others (14), occurs more rapidly than the Fe(II)–DOM reactions reported here.

At [DOC] > 1.7 mM C, reduction rate constants for pendimethalin remain generally constant (Table 2). Starting herbicide concentrations for these experiments ranged from 0.6 to 1.7 μM . These data, and results presented above, show that DOM inhibits Fe(II)-mediated reduction pathways for both pendimethalin and trifluralin in controlled experiments. The different results for Fe(II)–DOM-mediated reduction of pentachloronitrobenzene (12) versus the pendimethalin and trifluralin results presented here may be due to differences in structure and chemical behavior among the compounds. In a study on hydroquinone and iron porphyrin-mediated reduction of a suite of substituted mononitroaromatic compounds, second-order reduction rate constants for mononitroaromatics of a particular base structure differed due to the location of ring substituents relative to the nitro group being reduced (27). The presence of electron-withdrawing substituents also increased the mononitroaromatic reduction rate (27). Another study (14) proposed that the mechanism for dinitroaniline reduction differs from that for mononitroaromatic reduction due to differences in the linear free energy relationship for these classes of compounds in Fe(II)/goethite systems. They explained that, although the one-electron reduction potentials for substituted nitrobenzenes and dinitroaniline herbicides are within a similar range, structural differences between the herbicide classes (such as the effect of multiple substituents on the dinitroanilines) can affect the compound-specific reduction mechanisms (14).

Slower reaction kinetics in Fe(II)–DOM reaction media relative to Fe(II)-only reaction media also may result from complexation of Fe(II) with DOM ligands that affect Fe(II) reductive capacity. We suspect that the concentration of inorganic Fe(II) species would be too low to be an important reductant in reactions containing DOM (both in controlled systems and in natural pore waters) at the ambient Old Woman Creek sediment pore water pH levels observed by us (pH 7.77). For example, others (28) calculated the speciation of Fe(II) from pH 1 to 10 in the presence of excess oxalate in a simple electrolyte media and found that roughly 80% of the Fe(II) across the pH range from 7 to 8 was organically

complexed. Given that Fe(II) ligands that are presumably stronger than oxalate are present in pore water DOM, we suspect that the amount of complexed iron is significantly >80%, both in our controlled Fe(II)–DOM reaction media and in natural pore waters. In some cases, these Fe(II)–DOM complexes may be highly reactive, as shown by others with 4-chloronitrobenzene and explosive nitrosamines in the presence of Fe(II) and catechol or thiol ligands (29–31). Alternatively, Fe(II)–DOM complexes may form that are less reactive toward pendimethalin and trifluralin, such as those observed previously for pentachloronitrobenzene in Old Woman Creek pore waters (11).

Pendimethalin and trifluralin reduction in the presence of both Fe(II) and DOM also may be affected by interactions between the herbicides and DOM. The logarithms of the octanol–water partition coefficients constants ($\log K_{ow}$) for pendimethalin and trifluralin are 5.18 and 5.34, respectively (32–34), which makes both compounds ideal candidates for forming hydrophobic complexes with DOM. For example, the pendimethalin octanol–water partition coefficient is nearly identical to pyrene's K_{ow} value. Using data from ref 35, the estimated DOM–pyrene binding constant (K_{doc}) for Suwannee River fulvic acid is approximately 10290 L/kg. The amount of analyte bound to DOM can be determined by

$$\% \text{ bound} = [\text{OC}]K_{doc}/(1 + [\text{OC}]K_{doc}) \quad (1)$$

where [OC] is the DOM concentration as organic carbon in kg/L. Using an [OC] of 3.8×10^{-5} kg/L (equivalent to the 3.2 mM C DOM concentration in our pore water), we estimate that approximately 28% of the pendimethalin is bound to the DOM phase. Thus, this fraction could be rendered unavailable for reaction by the reductants present in our system. Trifluralin's K_{ow} is only slightly higher than pendimethalin's value, and trifluralin would presumably bind to the DOM phase in a similar fashion.

For trifluralin, additional complexities arise due to complicated reaction pathways (13) and unknown redox potentials of trifluralin reduction intermediates relative to reactive solution-phase reductant in Fe(II)–DOM solutions. Due to the heterogeneous nature of DOM and complexities inherent in interpreting Fe(II)–DOM-mediated electron transfer reactions, we are unable to provide a specific reduction pathway for pendimethalin and trifluralin reduction in controlled Fe(II)–DOM solutions at this time. Additional controlled clean system studies may aid in elucidating the chemical reductant responsible for dinitroaniline herbicide reduction in systems containing high [Fe(II)] and [DOC].

Both trifluralin and pendimethalin are reduced in natural pore waters that contain dissolved Fe(II) in the hundreds of micromolar range and high [DOC] (Figure 5; Table 1). The starting herbicide concentrations for these experiments ranged from 0.9 to 1.1 μM . Trifluralin is reduced more quickly than pendimethalin, and these data are consistent with our observations in controlled Fe(II)–DOM systems presented above. We were very limited with pore water volumes available for kinetics studies and, therefore, were unable to perform sterile and Fe(II)-free control experiments.

Trifluralin and pendimethalin reduction in the natural pore waters is an order of magnitude slower than in controlled Fe(II)–Old Woman Creek fulvic acid reaction media (Table 1). Although our Old Woman Creek controlled and natural system experiments contain different [DOC] (2.0 and 3.2 mM C, respectively), we observed that reduction rate constants remained generally stable in Fe(II)–DOM solutions where [DOC] > 1.7 mM C in our controlled system experiments (Table 2). The order

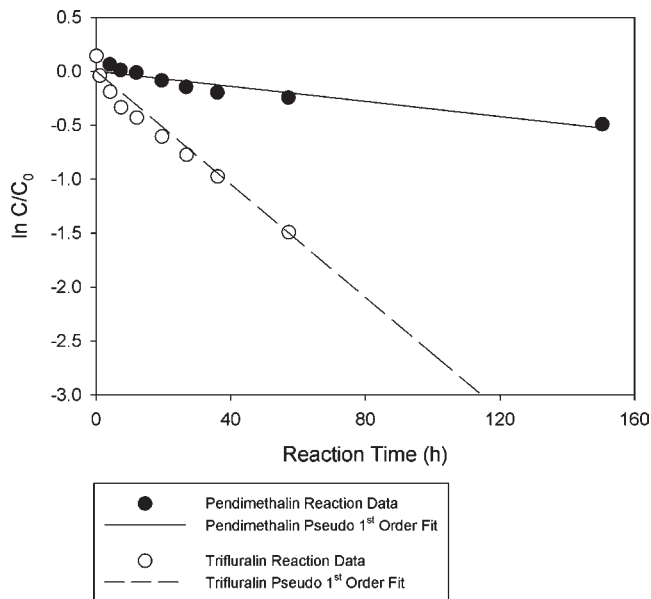


Figure 5. Pendimethalin and trifluralin reduction in natural pore waters from Old Woman Creek.

of magnitude difference in reaction rate constants between the Old Woman Creek controlled and natural pore water reactions suggests that different processes, reductants, or concentrations of reductants are responsible for this observation. Trifluralin reduction proceeded to 2 half-lives within 3 days, and pendimethalin reduction proceeded to nearly 1 half-life within 7 days in natural Old Woman Creek pore waters (Figure 5; Table 1).

Our study shows that dinitroaniline reduction occurs in solutions containing both Fe(II) and DOM and in Fe(II)-bearing natural pore waters. Slower dinitroaniline reaction kinetics observed in natural pore waters relative to controlled Fe(II)–DOM systems indicates that the primary Fe(II) reductant is concentration-limited in anaerobic sediment pore waters, and additional interferences may arise due to other DOM fractions present in natural systems (that are not captured during the fulvic acid isolation process). We hypothesize that specific Fe(II) species play an important role during the abiotic reduction of dinitroaniline herbicides in natural systems. Accurate prediction of trifluralin and pendimethalin transformation pathways in the presence of both Fe(II) and DOM requires an understanding of the specific role of Fe(II) species during reduction, and caution needs to be exercised when extrapolating controlled system experimental results (even those conducted with actual DOM or humic substances as opposed to chemical analogues) to natural waters. Future work regarding a detailed mechanistic understanding of reactive Fe(II)–organic complexes and DOM electron shuttles in natural systems is necessary to accurately predict the fate of pendimethalin and trifluralin in natural systems.

ABBREVIATIONS USED

DOC, dissolved organic carbon; DOM, dissolved organic matter; MOPS, 3-[*N*-morpholino]propanesulfonic acid; PLFA, Pony Lake fulvic acid; SRFA, Suwannee River fulvic acid; OWCFA, Old Woman Creek fulvic acid.

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