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Diffusion Limitation for Atrazine Biodegradation in Soil

Teresa A. Johnson[1], Timothy R. Ellsworth[2], Robert J. M. Hudson[2], Gerald K. Sims[3*]
[1]Younkin Success Center, Ohio State University, Columbus, USA
[2]Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, USA
[3]Department of Entomology Plant Pathology and Weed Science, New Mexico State University, Las Cruces, USA
Email: *gksims@nmsu.edu

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ABSTRACT

Effects of sub-millimeter scale heterogeneity in chemical and microbial distributions on atrazine degradation were examined using *Pseudomonas* sp. strain ADP introduced into soil at a population mimicking atrazine-adapted soils (~2000 cells/g), and employing a range of soil water pressures (−100, −300, −500 kPa). Heterogeneous cell distribution was employed in all treatments whereas uniformity of distribution was a variable for atrazine introduction. Two methods of initially distributing atrazine in soil were examined. Proximally-applied atrazine (PAA) was intended to yield elevated atrazine concentrations in the vicinity of the degraders. Dispersed atrazine (DA) was introduced to distribute the chemical uniformly as compared to the distribution of degraders. Both rate and extent of degradation were greater than PAA, regardless of water content, presumably due to proximity of atrazine to degraders. Biodegradation decreased with decreasing water content for both application methods, attributed to decreases in atrazine's effective diffusion. Mineralization of nearly 100% of DA in soils receiving a heterogeneous inoculum with a greater cell density (~10^7 cells/g) indicates that biodegradation was limited by the distance atrazine had to diffuse. Results support the hypothesis that enhanced populations of atrazine degraders, as reported elsewhere for atrazine-adapted soils, though heterogeneously distributed, may overcome bioavailability limitations.

Keywords: Biodegradation; Environmental; Agriculture; Degradation; Soil; Atrazine; *Pseudomonas* sp.

1. Introduction

The herbicide, atrazine (2-Chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine) has been used for more than fifty years [1], and remains among the most widely used herbicides globally, though recently banned in the European Union in 2004 [2]. Atrazine is considered somewhat persistent, with a reference $t_{1/2}$ of 60 d [3], however, in the case of atrazine-adapted soils, the $t_{1/2}$ can be as short as 3.5 days [4-6]. The ecology of atrazine degraders has been examined to use a variety of approaches [7,8], including most recently, stable isotope probing [9]. Though theoretically feasible [10], applications of ^{15}N-DNA-stable isotope probing to examine organisms causing enhanced degradation of atrazine at field rates provided equivocal results, owing to bioavailability limitations [6]. Using RNA-based ^{13}C-stable isotope probing, it was shown that the diversity of atrazine degraders varies over millimeter scales in soil [9], though it was necessary to use ^{13}C-atrazine at three orders of magnitude beyond the solubility limit to detect these relationships.

*Corresponding author.

Bioavailability and active degrader populations are ranked among the most influential factors in biodegradation of organic substrates [11]. Adsorbed substrates are typically unavailable to microorganisms, even in aqueous suspensions [12], and soil sorption can similarly reduce availability of herbicides to target weeds [13], reducing effectiveness. Diffusion within micro-porous (impenetrable by bacteria) soil aggregates can further slow sorption kinetics and biodegradation [14-17]. Atrazine degradation can be limited by bioavailability, though the herbicide is less hydrophobic than many herbicides, such as trifluralin [18]. Only 50% to 80% of atrazine applied to soil becomes sorbed [19], however detection of the herbicide in drainage water several years following its last application [20,21], suggests atrazine residues exhibit limited bioavailability despite modest sorption, possibly due to physical inaccessibility.

Based on viable counting methods, atrazine degraders are apparently few in number in non-adapted agricultural soils (in some cases undetectable) whereas populations increase to >10^3 degraders g-soil^{-1} in atrazine-adapted soils [5,22]. Gonod, *et al.* [23], showed that 2,4-D de-

graders (typically present at similar or greater numbers than atrazine degraders) are heterogeneously distributed on scales of a few millimeters. At small (~one millimeter) scales, cells would be restricted to the pores of sufficient size to satisfy life support requirements. An inhabitable (to a bacterium) water-filled pore could be assumed to be a spherical volume with a radius of >1-μm [15], while most pores in silt loam soils are <100-μm in size [24] with up to 50% of the pore volume occurring in pores of <1-μm [15], indicating many unoccupied pores. If we approximate the >1-μm pores as spheres with an average diameter of 10-μm, then one g of an atrazine-adapted silt loam soil (30% porosity and 10^3 degraders) would have 10^6 spherical pores and assuming organisms are uniformly distributed, at most 0.07% of the pores would contain degraders. In unsaturated soils, larger pores are relatively dry while smaller pores may remain filled, and this network of pores is connected by tortuous paths through water films or pore throats [25]. For a given spatial separation, diffusion of solutes between adjacent pores is slower than within pores due to tortuosity. Thus, degradation of substrates is likely hindered by tortuous diffusion between unoccupied and occupied (by degraders) pores in unsaturated soils, resulting in isolation of a considerable fraction of soluble pesticide from degraders [26,27]. Constraints on diffusion imposed by compartmentalization thus limit competition among microbial populations, contributing to the diversity and functional redundancy [28]. Though these relationships are clear from the existing literature, it remains unclear the degree to which heterogeneity on this small scale affects biodegradation kinetics.

Impact of soil compartmentalization on microbial processes also depends on the fraction of water-filled pore space. As water content decreases, diffusion of non-volatile solutes decreases, thus xenobiotic degradation also tends to decrease with decreasing soil water content [29-31]. Harris [32] concluded that solute diffusion was more likely than osmotic stress to limit microbial processes at low water content. The characteristic spatial scales (L) of concentration gradients are related to the time scales (T) over which they may form or break down and to the effective diffusion coefficient (D_{eff}) by a scaling relationship of the form [15]:

$$T \approx L^2 / D_{\text{eff}} \tag{1}$$

This suggests that atrazine persistence may be linked to slow diffusion at relevant spatial scales. The observed persistence of atrazine for time scales considerably longer than a month requires a concentration gradient with a length scale greater than 0.2 - 1 mm. Spatial variability at this scale and larger likely arise due to non-uniform pesticide application and low degrader populations.

Models of pesticide fate in soil generally assume that there exists a local continuum scale, which is treated as being the same for each of the physical continua, as well as for each "site" or sorptive region within a continuum. Microbial cell density and other variables of interest (substrate concentration, soil water pressure, volumetric water content, etc.) are assumed to be "well-mixed" properties within the local continuum scale. This local continuum scale is commonly referred to as a Representative Elementary Volume, or REV [33]. Biodegradation rates (R) may be expressed as mass per unit time as second-order functions of a local scale degrader population (N_{degrader}) and a local scale average bulk concentration of soluble pesticide (C_{bulk}) [15,34]:

$$R = k_{\text{apparent}} \times N_{\text{degrader}} \times C_{\text{bulk}} \tag{2}$$

The apparent rate coefficient (k_{apparent}) depends on the Michaelis-Menten parameters for degradation ($V_{\max}$ and K_m) and the ratio of xenobiotic concentrations at the cell surface (C_{cell}) to that in bulk solution (C_{bulk}):

$$k_{\text{apparent}} = \frac{V_{\max}}{K_m} \times \frac{C_{\text{cell}}}{C_{\text{bulk}}} \tag{3}$$

The latter ratio is typically assumed to be unity, since diffusion limitation is not commonly considered as a factor. Thus, this expression assumes the existence of a local spatial volume, in which the influence of variations in the chemical concentration at microbial cell surfaces, with respect to the average solution concentration within the volume, is assumed negligible. Whether or not this is justified it is difficult to address, as diffusion kinetics are difficult to measure at small scales within soils, and are often inferred from experimental systems in which only overall mass transfer rates are measurable. Despite experimental difficulties, accounting for pesticide diffusion limitation at scales relevant to processes controlling microbial access have potential to improve our understanding of both persistence and enhanced degradation of pesticides [35-37], and may provide insight into the drivers of microbial functional redundancy in soils.

In the present research, experiments were conducted in which the batch scale ratio $C_{\text{cell}}/C_{\text{bulk}}$ was purposefully manipulated to indirectly evaluate whether diffusion at cellular-to millimeter-scales influences atrazine degradation. Two nearly opposite initial conditions were created. In one, soil was saturated with a methanol solution containing atrazine, in order to minimize inter-aggregate heterogeneity by bathing aggregates in a common pool of delivery solvent with limited sorptive retardation (*Lee et al.*, 1993). In this case, the ratio $C_{\text{cell}}/C_{\text{bulk}}$ approached unity when the degrader-containing suspension was subsequently introduced (after solvent had evaporated). In the second method, atrazine was added with degraders, in a volume of water that occupied only 10% of the total soil pore space, enhancing proximity between substrate

and degraders. In this case, the ratio C_{cell}/C_{bulk} would initially be greater than unity. Initial degradation rates should reflect these differences in the ratio C_{cell}/C_{bulk}, whereas the subsequent degradation rates should reflect the rate of atrazine diffusion and redistribution. Complete degradation of the pesticide would require it to diffuse from uninhabited to inhabited pores, driven by concentration gradients created by biodegradation.

2. Materials and Methods

2.1. Soil

Cisne silt loam soil (fine, montmorillonitic, mesic, Mollic Albaqualf) was obtained from the surface of an agricultural field in Brownstown, Illinois, USA. The Cisne is a deep, poorly drained, slowly permeable soil formed in loess on glacial till plains. After air-drying, soil was sieved to obtain 0.4 to 2.0 mm diameter aggregates, typical at the surface of the site. The soil had a particle density of approximately 2.6 $g \cdot cm^{-3}$, and within the incubation vessels, settled to a bulk density of 1.3 $g \cdot cm^{-3}$, yielding a total porosity of 0.51. The average bulk density of individual Cisne soil aggregates within this size class is approximately ~1.7 $g \cdot cm^{-3}$ (K. Olson, pers. comm.), yielding an intra-aggregate porosity of 0.36. The soil had a pH of 6.0, an organic carbon content of 1.1%, and a cation exchange capacity of 8.5 cmolc kg-$soil^{-1}$. Extractable NH_4^+-N and NH_3^--N concentrations were 6.1 and 14.9 $mg \cdot kg^{-1}$ respectively, as determined by the micro-scale Berthelot method as modified by Sims [38].

2.2. Chemicals

Atrazine (>99% purity) was acquired from Chem Service, West Chester, PA. Radiolabeled atrazine [UL-14C] (specific activity 6.3×10^8 $Bq \cdot mmol^{-1}$; >99% radiochemical purity) was purchased from Sigma Chemical Co., St. Louis, MO. Water and methanol were Optima™ grade (Fisher Scientific, Pittsburgh, PA). All other chemicals were ACS reagent grade and purchased from either Fisher or EM Science (Gibbstown, NJ).

2.3. Microorganism and Culture Conditions

We chose to use *Pseudomonas* sp. strain ADP (*P*. ADP, provided by the University of Minnesota, St. Paul) as it has been shown to degrade atrazine constitutively [39], with rates unaffected by exogenous soil inorganic N sources [40], which can otherwise inhibit atrazine degradation in some soils [41]. The organism has been shown to succeed as a soil inoculum [39,40], and mineralizes the atrazine ring with insignificant metabolite production in soil [40]. The organism was cultured at 22˚C - 27˚C in a defined medium containing glucose (0.5 $g \cdot L^{-1}$) and atrazine (8.1 mg $N \cdot L^{-1}$) as sole N-source [42] with agitation at 90 $rev \cdot min^{-1}$. Prior to each experiment, cells were harvested by centrifugation and washed once with sterile growth medium without a C, or N source.

2.4. Soil Degradation Experiments

Mineralization of ^{14}C-labeled atrazine by *P*. ADP was measured in replicate, soil biometers treated as described below. Prior to each experiment, 150-g quantities of air-dried soil were immersed in a methanol pretreatment for 24-h. Next, the methanol was allowed to evaporate (approximately 24-h) in a sterile environment and 5-g subsamples of soil were aseptically transferred into sterile 20-mL scintillation vials (24-mm ID × 8.5-mm HT). After this methanol pre-treatment, the dehydrated soil was sterile and contained only 0.7% (w/w) water. To start the experiments, 100 µL (14 drops) of aqueous buffer containing *P*. ADP, to deliver 2000 cells $g \cdot soil^{-1}$ were applied drop-wise onto the surface of the soil sub-samples, filling approximately 5% of the total pore space within and between aggregates, or 10% of the intra-aggregate pore space (**Table 1**). After the inoculum droplets were rapidly drawn into the dry aggregates that they first contacted (roughly 10% of the aggregates present), the sample was inverted with a spatula to expose dry soil. Immediately thereafter, enough additional water to bring the soil to the desired water content was added (**Table 1**) and the soil aggregates were then mixed. The vials were sealed and incubated at 22˚C - 27˚C, with aeration at each sampling event. The mixing procedure above ensured heterogeneity of *P*. ADP distribution and minimized aggregate destruction.

2.5. Atrazine Introduction

During the above sequence, ^{14}C-labeled atrazine (ring UL) was introduced at 1 µg/gram soil (oven dry equivalent) (6.3×10^8 $Bq \cdot mmol^{-1}$), using two different procedures to create distinctly different initial distributions of atrazine relative to degraders. In one treatment, herein denoted as dispersed atrazine (DA), atrazine was dissolved in the methanol in which the soil was immersed during the sterilization pretreatment and deposited on the soil particles as the methanol evaporated. Methanol has sufficient surface tension (23 dynes/cm) to wet mineral surfaces, and exhibits Hansen solubility parameters (Van der Waals forces, hydrogen bonds) more similar to water than most solvents [43]. Methanol would be expected to deposit atrazine in both polar and non-polar environments closely resembling the distribution expected for introduction with water [44]. An additional DA treatment was included in which the initial *P*. ADP population was ~10^7 $cells \cdot g^{-1}$. In the second treatment, referred to as proximally applied atrazine (PAA), atrazine was absent from the methanol sterilization treatment, but rather was dis-

Table 1. Water content of soils used in experiments.

Treatment	Vol. water content (θ)[1]	Centrifuge extracted water (v/v)[2]	H_2O pressure (kPa)[3]
Hi-θ	0.22	0.06	−100
Me-θ	0.16	0.02	−300
Lo-θ	0.11	0	−500

[1]Computed using gravimetric water content and bulk density of 1.3 g·cm^{-3}; [2]Water recovered by centrifugation (see methods); [3]Values derived from moisture retention curve determined using standard methods [57].

solved in the 100 µL aqueous inoculum solution that was applied to each soil sample (see above).

Mineralization of atrazine was measured by liquid scintillation spectrometry (LSS) of $^{14}CO_2$ trapped on filter paper (treated with 200 µL 0.2 M NaOH) suspended from the cap of each vial. A time series was obtained by repeatedly removing and replacing traps over 15 - 61 days. On days 15 and 60, atrazine in soil solution was obtained by placing replicate 5-g samples in 10-mL syringes containing a stainless steel frit (2-µm pore), which were centrifuged at 17,200 g for 20-min and recovered pore water collected for analysis. To measure reversibly-sorbed atrazine, centrifuged soil solids were extracted sequentially with 0.01 M $CaCl_2$ (4 mL) and methanol (4 mL) and ^{14}C in the extracts analyzed using LSS [45]. The chemical form of detected radioactivity was confirmed as atrazine in extra replicate samples using HPLC analysis (radioactivity detection) as described by Bichat *et al*. [40]. The term sorbed atrazine is defined herein as the sum of these two extracts. Unextractable label (bound residue) was quantified by measuring $^{14}CO_2$ released during combustion of extracted soil samples (Biological Oxidizer-OX500, R. J. Harvey).

Water loss, determined gravimetrically after day-61, did not exceed 5% of the total water content. In one experiment, water content was increased to 0.24 mL·g^{-1} soil on day-18 by adding water without mixing. Aseptic technique was employed throughout the experiments.

2.6. Statistical Analyses

Statistical analyses of cumulative mineralization and biodegradation rates consisted of analysis of variance using multivariate repeated-measures and mean comparisons via the SAS system (SAS Institute, Cary, NC), and the data analysis tools in Microsoft Excel. Analyses were run separately for time points before and after addition of water to the treatments at 18 d.

3. Results and Discussion

No metabolites were detected, thus mineralization and bound residues were assumed the only degradation products. $^{14}CO_2$ evolution data presented for the dispersed atrazine treatments in **Figure 1** show a profound effect of initial *P*. ADP population on degradation kinetics. Nearly complete degradation of dispersed DA atrazine (up to 98% in 15 d) when elevated populations of *P*. ADP. (10^7 cells·g^{-1}) were introduced into −100 kPa soil indicates that DA atrazine was immediately bioavailable. Degradation kinetics for the −100 and −300 kPa treatments were initially identical, but diverged after about 45% of the atrazine was degraded in the elevated *P*. ADP treatments (**Figure 1**). The −300 kPa treatment contained about 75% as much total water and about 40% as much centrifuge extracted water as the −100kPa treatment (**Table 1**), and would be expected to exhibit reduced solute transport. These findings were attributed to rapid depletion of atrazine near the cells creating a concentration gradient driving atrazine movement toward the organisms.

Herein, when the initial *P*. ADP population was 2000 cells·g-soil^{-1}, whether atrazine was dispersed (DA) with the methanol or applied in the inoculum (PAA), cumulative mineralization curves at a water content of −100 kPa were S-shaped (**Figure 2**), with rates increasing for the first ~15 days and then slowing (effectively ceasing) between days 40 - 50. No degradation was observed in uninoculated soil. In both application methods, most of the atrazine was not degraded (88% in the DA versus 76% in the PAA) implying that either the atrazine was no longer available to the *P*. ADP, or another factor decreased activity of the degrader. Bound residues accounted for 40% - 50% of the applied atrazine, which is consistent with published results for soils exhibiting limited atrazine degradation [46]. Approximately 30% of the initial atrazine remained bioavailable in dissolved or reversibly sorbed forms on day-61 (**Table 2**). Measurements of bulk solution atrazine concentrations decreased ~3-fold between days 18 and 61 while the degradation rates decreased ~10-fold (**Table 2**), suggesting the supply rate to the degraders was controlled by a localized pool of atrazine that decreased more than the bulk solution.

A comparison of atrazine degradation in PAA and DA treatments at water contents of −300 kPa and −500 kPa during 18 d incubations also indicated approximately 2 fold greater degradation in the PAA treatments, regardless of water content (**Figures 2(b)** and **(c)**). These additional incubations further confirm that the local supply of atrazine was greater in the PAA treatments. At water contents of −300 kPa and −500 kPa, approximately three- to eight-fold less atrazine respectively was mineralized than during the comparable 18-d period at −100 kPa in both the DA and PAA treatments (**Figure 2**), and in contrast to the −100 kPa treatments, degradation rates remained relatively linear. Differences in cumulative degradation among the water levels were all significant at the $P < 0.01$ level. The effect of water content on degra-

Table 2. Distribution of ^{14}C among phases as measured during experiments.

Atrazine application	Water pressure (kPa)†	Water content (ml/g)†	Day 18 of incubation				Day 61 of incubation				
			$^{14}CO_2$ (% of initial)	C_w[a] (mg/L)	C_s[a] (mg/kg)	K_d[b] (L/kg)	$^{14}CO_2$ (% of initial)	C_w[c] (mg/L)	C_s[c] (mg/kg)	$^{14}C_{Bound}$ (% of initial)	K_d[b] (L/kg)
PAA	−100	0.17	12.22	0.28	0.40	1.39	24.35	0.11	0.15	39.0	1.41
PAA	−300	0.12	2.78	0.39	0.62	1.58	15.51	0.19	0.22	44.0	1.18
PAA	−500	0.08	1.42	(0.44)[d]	0.65		14.29	(0.17)[d]	(0.25)[d]	41.2	
DA	−100	0.17	5.83	0.41	0.59	1.46	12.70	0.13	0.24	49.5	1.88
DA	−300	0.12	1.72	0.40	0.60	1.49	5.79	0.18	0.27	43.7	1.47
DA	−500	0.08	0.78	(0.37)[d]	0.54		6.30	(0.21)[d]	(0.31)[d]	40.9	

[a]Atrazine concentrations, C_w, (soil solution) and C_s (sorbed) are means of 9 replicates; [b]$K_d = C_s/C_w$. C_s includes reversibly-sorbed (exchangeable) species only. In abiotic sorption studies on the same soil, a K_d of 1.9 ± 0.7 L/kg was obtained; [c]Means of 6 replicates for −100 kPa and 3 replicates for −300 and −500 kPa; [d]Dissolved concentration calculated from average K_d and C_s for this experiment. †Analyses performed by A & L laboratories, Ft. Wayne, IN. performed as described by Dane and Hopmans [57]. $P_b \approx 2.6$ g·cm^{-3}, Aggregate porosity ≈ 0.32, bulk porosity ≈ 0.51.

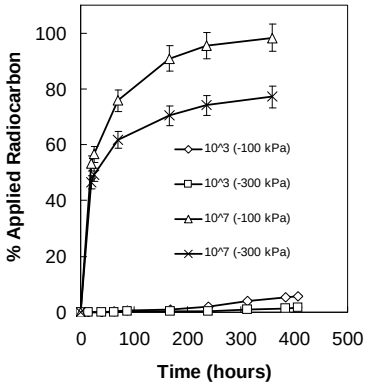

Figure 1. Cumulative mineralization of atrazine using an inoculation density of 10^3 or 10^7 viable cells g-soil^{-1}. Atrazine was dispersed (DA) with methanol and soils adjusted to specified water pressures: −100 kPa (0.24 θ), −300 kPa (0.18 θ). Error bars indicate σ. If there are no error bars shown, bars are smaller than symbol.

dation was not caused by differences in bulk dissolved atrazine concentrations (Cw), which fell within a 10% range as expected for linear partitioning with the sorbed mass predominant (**Table 2**).

The difference in degradation rates (D, µg/d) between −500 kPa and −100 kPa incubations ($D_{-500\ kPa} - D_{-100\ kPa}$) is reported in **Figure 3** for the PAA and DA application methods. Since degradation rates were initially accelerating in −100kPa treatments, $D_{-500\ kPa} - D_{-100\ kPa}$ decreases over time. When water was added (without stirring) to achieve −100 kPa on day 18 in the −500 kPa incubations, the degradation rate increased ($D_{-500\ kPa} - D_{-100\ kPa}$ ceased to decrease). Cumulative extents of atrazine degradation in the two PAA treatments after water addition were nearly identical and the rates immediately after wetting were 2- to 5-fold higher than beforehand. Response of degradation rate to rewetting was much more subtle in the case of the DA application method (**Figure 3(b)**), likely owing to greater distances required

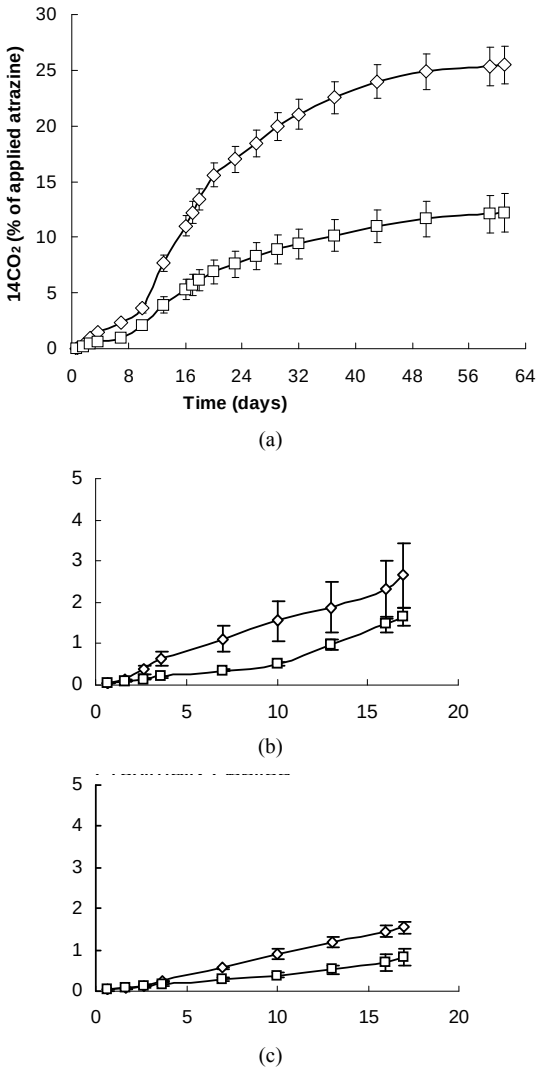

Figure 2. Cumulative mineralization of atrazine (◊ proximally applied, □ dispersed) at inoculation density of 10^3 cells g-soil^{-1}. (a) −100 kPa (0.24 θ), (b) −300 kPa (0.18 θ), (c) −500 kPa (0.12 θ). Error bars indicate σ.

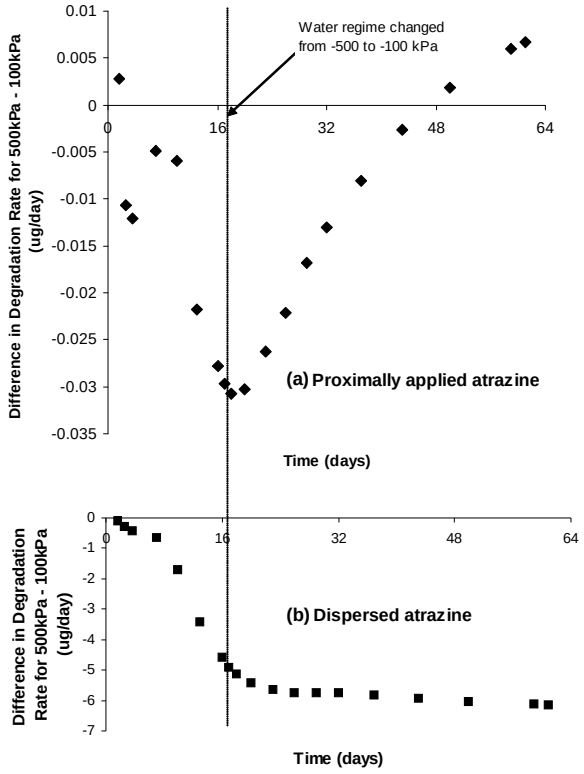

Figure 3. Impact of water regime on atrazine mineralization rate with an initial microbial population of 5 × 10^3 cells g-soil^{-1}. Data are for difference in degradation rates ($D_{-500\ kPa}$ - $D_{-100\ kPa}$ expressed in µg atrazine/day) when soil was incubated initially at −100 kPa ($D_{-100\ kPa}$) versus initially incubated at −500 kPa and adjusted to −100 kPa at day 18 (D_{-500} kPa). Panel (a) shows results for proximally applied atrazine and panel (b) shows results for dispersed atrazine.

for atrazine movement to the cells compared to the PAA treatment. Increases in degradation rates upon water addition to the PAA treatment are consistent with increases in net diffusion.

The general decrease in rates at low water pressures can be readily explained by reduced diffusion. Atrazine diffusion coefficients in these soil systems (D_{eff}) were estimated using a semi-empirical solution-phase diffusivity for atrazine [34], the modified Millington-Quirk tortuosity relation, and standard retardation factor to account for reversible adsorption [47], yielding D_{eff} ranging between 4 × 10^{-9} cm^2·s^{-1} at –100 kPa to 1 × 10^{-10} at –500 kPa. We calculated diffusion-limited initial degradation rates assuming cellular boundary layers are independent and regions containing degraders do not have depleted atrazine concentrations relative to bulk soil. For PAA/–500 kPa, the observed rate of ~0.4 ng·g-soil^{-1}·d^{-1} was approximately 10-fold greater than the calculated diffusion-limited rate of 0.03 ng·g-soil^{-1}·d^{-1}. Similarly, the observed initial degradation rates in the PAA/–300 kPa and PAA/–100 kPa treatments were 3- and 1.5-fold above the theoretical maximum rates. Most likely, the D_{eff} are too low, especially for the PAA/–500 kPa treatment. Bulk empirical D_{eff} measurements for other triazine herbicides have been reported in the range of 8 × 10^{-9} cm^2·s^{-1} to 7 × 10^{-8} cm^2·s^{-1} in soils with similar volumetric water content and physical properties [48].

Degradation kinetics observed herein using a population (2000 cells·g^{-1}) meant to simulate an atrazine-adapted soil were slower than observed at known adapted sites estimated to harbor 10^3 to 10^4 degraders·g^{-1}, using mineralization of 14C-ring labeled atrazine for MPN detection [22,49]. Slower kinetics observed here may be due partly to limited ability of *P*. ADP to adapt to the Cisne soil, or to underestimation of populations by MPN methods used in published literature. Using *P*. ADP as a model for predicting populations at adapted sites ($t_{1/2}$ < 10 days), approximately 10^4 to 10^5 degraders would be expected per gram, about one or two orders of magnitude greater than values commonly reported from ^{14}C-ring labeled atrazine MPN studies, and about one order of magnitude greater than studies using ^{14}C-ethyl-labeled atrazine [22]. Counting efficiencies of most MPN methods are reported to underestimate indigenous microbial populations by one or more orders of magnitude, based on activity measurements [50,51], and failed to detect degraders in some non-adapted soils that exhibited significant degradation [22]. Thus, the discrepancies between activity and estimates of microbial counts at atrazine-adapted sites is consistent with the use of viable counting methods. Based on activity, populations in the range of 10^4 - 10^5 cells·g^{-1} at atrazine-adapted sites would be expected to yield MPN data comparable to that reported in the literature, and are in agreement with estimates using quantitative PCR [52]. Increasing the population to 10^7 g^{-1} herein produced faster initial degradation (0.013 µg·h^{-1}) than reported for natural soil populations, suggesting the two population sizes used here bracket in situ cell densities expected at adapted sites. Results herein support the hypothesis that relatively modest population increases reported for adapted sites [22] are sufficient to overcome diffusion limitations which have been suggested to be important with degrader populations less than 10^4 cells·g-soil^{-1} [53], and may explain the loss of herbicidal effectiveness when atrazine is used repeatedly over a period of many years.

4. Conclusion

Soils are often conceptualized as liquid cultures of bacteria, comprised of water contained in habitable pores that surround aggregates containing inaccessible micropores [17], and dissolved xenobiotic outside the micropores is considered bioavailable and homogeneously distributed [54]. Our work suggests that because of tortuous diffusion in unsaturated soils, microorganisms themselves create submillimeter-scale zones depleted of xenobiotic causing

biodegradation rates to fall below those predicted from bulk concentrations, and the slowness of diffusion limitations effectively isolate a fraction of the xenobiotic from degraders. The occurrence of millimeter-scale isolated regions of pore space is apparently a function of degrader population density. Microbial population effects observed here support the hypothesis that increasing degrader populations to ~10^5 cells·g^{-1} can be sufficient to overcome diffusion barriers, and may result in strong enhancement of biodegradation, as reported elsewhere for atrazine-adapted sites. Among common herbicides, atrazine is relatively mobile in soil, having a diffusion coefficient only an order of magnitude lower than that reported for one of the most mobile herbicides, 2,4-D [55]. Many herbicides are far less mobile than atrazine (e.g., dinitroanilines exhibit D_{eff} up to six orders of magnitude lower than atrazine [56]), thus it is expected that submillimeter scale diffusion will limit biodegradation of most soil applied herbicides.

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