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Relationships between microbial biomass and dissipation of 2,4-D and dicamba in soil

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Abstract A study was conducted to evaluate relationships between microbial biomass and the dissipation of 2,4-D (2,4-dichlorophenoxy acetic acid) and dicamba (2-methoxy-3,6-dichlorobenzoic acid) in soil. We hypothesized that the size of the microbial biomass should be a strong predictor of the pesticide degradation capacity of a particular soil. Soils with a high microbial biomass should have relatively high levels of general microbial activity and should support a diversity of degradation pathways. In this study, we quantified the degradation of 2,4-D and dicamba in a range of soils with different concentrations of microbial biomass. The herbicides 2,4-D and dicamba were added to similar soils collected from five different land use types (home lawn, cornfield, upland hardwood forest, wetland forest, and aquifer material) and incubated for 80 days under laboratory conditions. Herbicide residue and microbial biomass (C and N) analyses were performed 5, 10, 20, 40, and 80 days following herbicide application. Microbial biomass-C and -N and soil organic matter content were positively correlated with dissipation of 2,4-D and dicamba. The results suggest that there are relationships between the size of the soil microbial biomass and the herbicide degradation capacity of an ecosystem. These relationships may be useful for developing approaches for evaluating and predicting the fate of pesticides in different ecosystems.

Key words 2,4-D · Dicamba · Microbial biomass · Wetland · Forest

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Introduction

The herbicides 2,4-D (2,4-dichlorophenoxy acetic acid) and dicamba (2-methoxy-3,6-dichlorobenzoic acid) are among the most widely used weed control agents in the United States. They are used in agriculture, home lawn, forestry, and road and railroad maintenance programs. These herbicides are among the most mobile pesticides used (Helling and Turner 1968) and have been found to leach into surface and groundwater (Bailey and White 1970; Gold et al. 1988).

Microbial degradation of pesticides in soil is a key factor controlling the movement of these compounds into water and air. Considerable research over the past 30 years has shown that 2,4-D is degraded readily by soil microorganisms in many cases (McCall et al. 1981, Parker and Doxtader 1983; Ou 1984; Smith and Aubin 1991), but there is less information available for dicamba. Many studies have demonstrated the ability of microbial populations to develop an ability to degrade pesticides over time (Loos 1975; Spain and Van Veld 1983; Ou 1984; Hickman and Novak 1989). However, widely used compounds such as 2,4-D and dicamba are often applied to soils once, or infrequently, in forestry, road and railroad maintenance and residential uses. The factors that control the ability of soils to degrade these compounds in these situations are unknown (McCall et al. 1981). Understanding these factors is thus essential for predicting the fate and transport of these compounds in many landscapes (Torstensson 1980).

We hypothesized that the size of the microbial biomass should be a strong predictor of the pesticide degradation capacity of a particular soil. Soils with a high microbial biomass should have relatively high levels of general microbial activity and should support a diversity of degradation pathways (Smith and Paul 1990; Levanon et al. 1994). In this study, we quantified the degradation of 2,4-D and dicamba in a range of soils with different levels of microbial biomass. Our objective was to determine if there is a relationship between the size of the microbial biomass and the herbicide degradation capacity of different soils.

Table 1 Characteristics of soils taken from different land use types for herbicide dissipation study

Land use	pH[a]	Moisture by mass at field capacity (g kg^{-1})	Organic matter (g kg^{-1})	Classification
Aquifer (AQ)	4.98	191	<5.0[b]	Sand and gravel (no classification)
Corn (CORN)	5.61	192	35[c]	Enfield silt loam (coarse-silty over sandy or sandy skeletal, mixed mesic Typic Dystrochrepts)
Hardwood (HDW)	4.57	258	88[c]	Merrimac sandy loam (sandy, mixed, mesic Typic Dystrochrepts)
Sod (SOD)	5.94	242	20[d]	Merrimac sandy loam (sandy, mixed, mesic Typic Dystrochrepts)
Wetland (WTL)	3.51	1720	15[c]	Walpole sandy loam (sandy, mixed, mesic Typic Endoaquepts)

[a] Measured with a glass electrode in 20 g soil: 20 ml deionized water
[b] Data from University of Rhode Island Soil Testing Laboratory
[c] Data from Groffman et al. (1991)
[d] Data from Gold et al. (1988)

Materials and methods

Five different soils were used in a laboratory microcosm study. Included were soils from a freshwater aquifer (AQ), a cornfield (CORN), an undisturbed hardwood forest (HDW) dominated by oak (*Quercus* sp.), a home lawn (SOD), and a freshwater wetland (WTL) dominated by red maple (*Acer rubrum*). Soil samples were collected in bulk from the top 25 cm of the profile at each site excluding recognizable surface litter and root materials (Table 1). The aquifer material was collected 60 cm below the water table and 160 cm below the soil surface from a previously excavated site. The aquifer was open to atmospheric conditions at the time of collection. The sites were all located at the University of Rhode Island Peckham Farm, in Kingston, RI, USA.

Following collection, the SOD, HDW, and CORN soils were passed through a 2-mm wire mesh screen to remove non-soil materials. The WTL soil and AQ material were too moist to pass through the screen and it was deemed too destructive to the integrity of the samples to dry them prior to screening (Powlson and Jenkinson 1976). Soil moisture was determined by the pressure membrane technique (Klute 1986) except for the AQ material, which was too unconsolidated, and for which percentage moisture was determined by mass difference after drying (Table 1). One-hundred-gram portions (oven-dry weight) of each material were placed in loosely covered 300-ml beakers, adjusted to field capacity (-0.01 MPa), and placed in an incubator for 7 days at 21°C) prior to the start of the experiment. This was done to allow the samples to acclimate for a short period because it has been found that disturbance of soil aggregates kills some soil organisms and also exposes some previously inaccessible substrates to microbial attack (Jenkinson and Powlson 1976). Water contents were checked gravimetrically on day 1 and every 5 days subsequent to the initiation of the experiment and brought to field capacity as required. In order to better simulate field conditions, the temperature levels within the incubator were adjusted every 10 days to reflect the 30-year (1957–1987) average temperatures recorded at the Kingston, Rhode Island, weather station. The study began with an incubation temperature of 21°C and the temperature was decreased 1°C every 10 days thereafter.

Thirty microcosms were created for each of the five materials. Herbicides were applied to 15 microcosms per material and 15 were retained as untreated control samples. The herbicide applied to the treated samples was a commercially available mixture: Trimec Classic (PBI-Gordon Corp., Kansas City, KS). Trimec Classic contains: 25.93% 2,4-D (dimethylamine salt); 13.85% 2(2-methyl-4-chlorophenoxy) propionic acid (MCPP); and 2.76% dicamba (dimethylamine salt) as active ingredients. The treated samples were amended with 25 mg 2,4-D kg^{-1} soil (ODW), and 2.59 mg dicamba kg^{-1} soil (ODW) on day 1 of the study. This rate of application was greater than most field application rates, which average about 7.5 mg 2,4-D kg^{-1} soil in the top 1 cm of soil, but there is rarely even distribution of herbicide when applied in field situations, and concentrations of 2,4-D of 25 mg kg^{-1} soil at the soil surface are not uncommon (Parker and Doxtader 1983).

Herbicide levels were measured at 5, 10, 20, 40, and 80 days following application by destructively sampling three treated microcosms per material per sampling date. Herbicide residues were extracted from the soil samples and concentrations of 2,4-D and dicamba were quantified using the extraction and high-performance liquid chromatography (HPLC) methods described by Voos et al. (1994). Briefly, soil samples were extracted with a methanol:water solution (1:1 v/v), shaken, vacuum filtered, and the herbicides isolated from the filtered solutions using 3-ml amino-bonded solid-phase extraction columns. The herbicide residues were quantified using HPLC equipped with a variable-wavelength UV detector and a C_{18}-bonded phase column.

All microcosms sampled at each date were analyzed for microbial biomass carbon (biomass-C) and nitrogen (biomass-N), and mineral-N (NH_4^+-N and NO_3^--N). Biomass-C and -N were determined using the chloroform fumigation-incubation method (Jenkinson and Powlson 1976). Though this method is not recommended for highly acidic soils, we were more interested in gaining a relative index of microbial biomass in the various soils, not in quantifying absolute biomass numbers. Carbon dioxide was measured on an SRI 8610 gas chromatograph (SRI, Redondo Beach, CA) equipped with a methanizer and a flame ionization detector. Mineral-N was extracted from soil samples with 2 *M* KCl. The soil/KCl (1:4) mixture was shaken for 1 h on an orbital action shaker, filtered through Whatman #42 filter paper and quantified colorimetrically using an Alpkem RFA 300 Rapid Flow Analyzer (ALPKEM Corporation 1986). A proportionality constant for C (0.41) was used to calculate biomass-C from the CO_2 flush (Voroney and Paul 1984). Biomass-N was calculated from the formula proposed by Voroney and Paul (1984).

Results

2,4-D was completely dissipated by day 80 in three of the five materials used: HDW, SOD, and WTL (Fig. 1). The mean concentration of 2,4-D remaininng in the CORN soil on day 80 was 0.19 mg kg^{-1} and in the AQ material 1.13 mg kg^{-1}, but these mean concentrations were not significantly different ($P<0.05$) from zero. Measurable concentrations of 2,4-D were present in four of the five soils

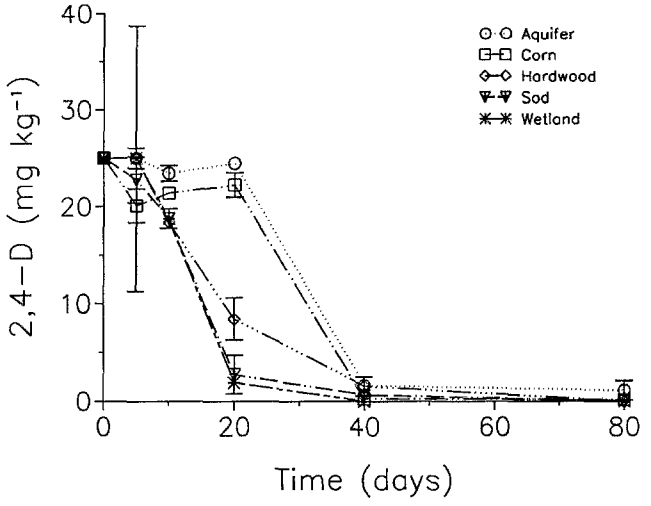

Fig. 1 Mean concentration of 2,4-D in soils from five different land use types sampled 5 times during an 80-day study (*bars* indicate±SE)

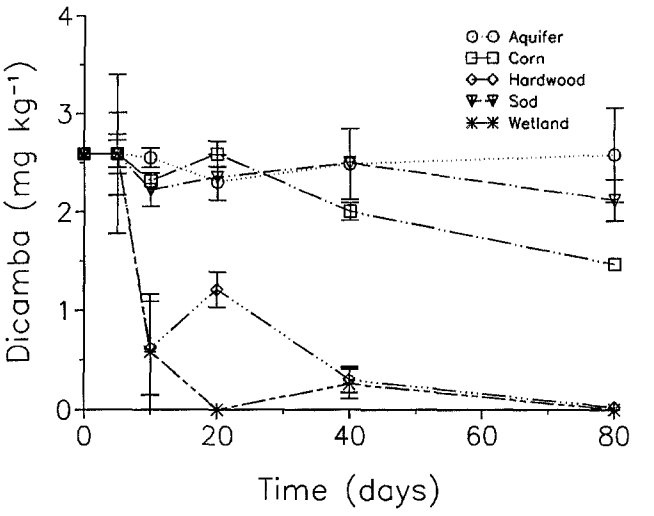

Fig. 2 Mean concentration of dicamba in soils from five different land use types sampled 5 times during an 80-day study (*bars* indicate±SE)

Table 2 Mean 2,4-D concentrations at day 20, mean dicamba concentrations at day 80 and 0–80 day mean concentrations of microbial biomass-C and -N in soils from five different land use types (*BDL* below detection limit)

Land use	2,4-D (mg kg^{-1})	Dicamba (mg kg^{-1})	Microbial biomass N (m kg^{-1})	Microbial biomass C (mg kg^{-1})
Aquifer	24.5 a[a]	2.59 a	BDL	103 b
Corn	22.3 a	1.47 b	30 c	106 b
Hardwood	8.6 b	0.02 c	76 b	172 b
Sod	2.7 c	2.13 ab	22 c	95 b
Wetland	1.9 c	BDL	256 a	656 a

[a] Values followed by the same letter are not significantly different at $P<0.05$

Table 3 Pearson product moment or Spearman (nonparametric) correlation coefficients (whichever was higher) among 2,4-D and dicamba remaining and soil organic mater content and microbial biomass-C and -N in soils from five different land use types

	Dicamba[a]	2,4-D[b]	2,4-D (w/o Sod)[c]
Microbal biomass C	–0.78***	–0.54*	–0.83***
Microbial biomass N	–0.97**	–0.69**	–0.88***
Soil organic matter content[d]	–0.96*	NS	–0.98**

[a] Dicamba correlations were done with values from day 80 because differences in herbicide concentrations between soils were greatest at this date. $N=10$ because only two of the three replicates at each sampling date were analyzed for both microbial biomass and herbicide concentration

[b] 2,4-D correlations were done with values from day 20 because differences in herbicide concentrations between soils were greatest at this date. $N=10$ because only two of the three replicates at each sampling date were analyzed for both microbial biomass and herbicide concentration

[c] Sod soil removed from analysis because it had been previously treated with 2,4-D

[d] $N=5$ for these correlations because soil organic matter content and herbicide concentrations were not measured on the same samples at the same time

* Correlation significant at $P<0.10$

** Correlation significant at $P<0.05$

***Correlation significant at $P<0.01$

at day 40, but none of the mean concentrations were significantly ($P<0.05$) different from zero at this date. There were significant ($P<0.05$) differences in 2,4-D concentrations between several of the soils on day 20, when they graphically appear to differ the most (Fig. 1, Table 2). The kinetics of herbicide disappearance were not investigated during this study. There were too few data points, especially during the early stages of the experiment, to provide a substantive kinetics analysis.

There were significant ($P<0.05$) differences in the amount of dicamba present on day 80 among several of the soils (Fig. 2, Table 2). Dicamba was totally or nearly dissipated by day 80 in two of the soils: HDW and WTL. The AQ material and SOD and CORN soils had mean dicamba levels ranging from 2.59 mg kg^{-1} to 1.48 mg kg^{-1} on day 80 [all significantly ($P<0.05$) different from zero]. Mean concentrations of dicamba in the HDW and WTL soils were not significantly ($P<0.05$) different from zero at day 40.

Concentrations of microbial biomass-C and -N varied significantly ($P<0.05$) among the different materials (Table 2), but there were no significant differences between herbicide-treated and control samples (data not presented). Biomass-C and -N increased in all treatments over the first 20 days of the incubation and were relatively stable between days 20 and 80 (data not presented).

There were significant ($P<0.05$) correlations between microbial biomass-C and -N and soil organic matter content and 2,4-D and dicamba dissipation (Table 3). Correlations were done using data from day 20 for 2,4-D and day 80 for dicamba, the dates when herbicide concentrations were most different among the soils. The correlations between 2,4-D and microbial biomass-C and -N were much

stronger when the sod soil was removed from the analysis. This may be due to the fact that the SOD soil had previously been treated with 2,4-D (see "Discussion" below).

Discussion

Both herbicides were dissipated faster and/or more completely in the soils with high biomass-C and -N, suggesting that microbial biomass can be viewed as a regulator of the degradation capacity of different soils. There was a particularly strong relationship between the residence time of dicamba in the soils and the size of the microbial biomass and soil organic matter content. Comfort et al. (1992) suggested that dicamba degradation would be lessened in subsoils due to a lower microbial population in the mineral horizon soils as opposed to surface layer soils with higher organic matter content. In this study, both 2,4-D and dicamba degraded most slowly in the aquifer material, which had low microbial biomass and organic matter content. Veeh et al. (1996) also found a decrease in 2,4-D degradation capacity, soil organic matter content, and microbial biomass with depth. McCall et al. (1981) and Levanon et al. (1994) suggested that variation in the decomposition rates for chemicals among different soils appears to represent the basic ability of a given soil microbial community to degrade the compounds. In contrast, Entry et al. (1994) and Ghani et al. (1996) found no correlation between active or total microbial biomass with pesticide degradation.

Interestingly, correlations between 2,4-D present on day 20 and biomass-C or -N and soil organic matter content were much stronger when the SOD soil was removed from the correlation analysis. This may be due to the fact that the SOD soil had previously been treated with 2,4-D, though no 2,4-D had been applied for the 3 years prior to this experiment. Several studies (Torstensson et al. 1975; Roeth 1986; Smith et al. 1989; Smith and Aubin 1991) have found that 2,4-D mineralization was significantly enhanced in previously treated soils when compared to previously untreated soils, but there is considerable uncertainty as to how long this effect lasts after the cessation of 2,4-D applications. Holben et al. (1992) observed that soils with a previous history of 2,4-D treatment did not support higher numbers of 2,4-D-degrading bacteria than previously untreated soils several months after the last application of 2,4-D. In contrast, Smith and Aubin (1991) found that previously treated soils maintained their ability to degrade 2,4-D more rapidly than untreated soils 48 weeks after the last application.

Given the results from the SOD soil (i.e., rapid dissipation with low biomass), we can conclude that microbial biomass is a regulator only of the residence time of compounds applied to soils for the first time. It is possible that soils with high microbial biomass will develop populations capable of degrading specific compounds more rapidly than soils with low microbial biomass, but this idea requires further testing. Moreover, variation in the composition of the microbial community, which may or may not be related to its biomass, may also be a key regulator of inherent or induced herbicide degradation capacity (Entry et al. 1995; Boyle and Shann 1995).

It is important to point out that the microbial populations in our laboratory microcosms were actively growing during the early phases of the incubation. The soils used in the microcosms were sieved and disturbed during microcosm preparation, resulting in a likely loss of microbial biomass (Jenkinson and Powlson 1976). The increases in biomass-C and -N during the early stages of the laboratory experiment may have been due to increased microbial utilization of the nutrients released from organisms killed by the disturbance. A disturbed, actively growing microbial population may have different degradation capacities than a relatively stable population. The status of microbial biomass should be considered whenever microcosms are used in pesticide degradation studies.

The existence of relationships between pesticide dissipation and microbial biomass may be useful for developing approaches for evaluating and predicting the fate of pesticides in different ecosystems. The size of the microbial biomass is usually highly correlated with ecosystem net primary productivity and soil organic matter levels (Paul and Clark 1989). Vegetation influences microbial biomass dynamics through competition with microorganisms for nutrients and by providing C through senescence and litter fall (Smith and Paul, 1990). Each ecosystem has a distinctive level of microbial biomass, which is the integrated product of soil properties, vegetation type, and productivity (Myrold et al. 1989; Gregorich et al. 1991; Zak et al. 1994) and should, therefore, also have a distinctive pesticide degradation capacity. Further analysis of relationships between ecosystem properties, the size and composition of the microbial biomass, and pesticide degradation capacity may be useful for assessment of ecosystem and landscape scale dynamics of these compounds.

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