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Aerobic degradation assessment for the fungicide BAS 505 using batch and intact soil core methodologies

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AEROBIC DEGRADATION ASSESSMENT FOR THE FUNGICIDE BAS 505 USING BATCH AND INTACT SOIL CORE METHODOLOGIES

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ABSTRACT

Although registration protocol stipulates that pesticide degradation be quantified using homogeneous soil, research suggests that degradation in intact soil may give results more consistent with field data. This project examined degradation of the turf and cereal fungicide BAS 505 [*N-methyl-(E)-2-methoxyamino-2-(2-((2,5-dimethylphenoxy)methyl)phenyl) acetamide*]. Yearlong and four-month-long incubation studies compared degradation rates in intact cores and homogeneous (batch) samples of Ruston fine sandy loam (*fine-loamy, siliceous, thermic Typic Paleudults*) soil. Recovery of BAS 505 in methanol extracts was measured 12 times over the 360 d incubation by HPLC-LSC analysis, and declined to 36 % and 57 % of the amount applied in cores and batch soils, respectively, by 360 d. But degradation in cores was faster than in batch soil only after long-term incubation. Since geostatistical surveys of soil biological, chemical and physical properties at the study site revealed spatial variability, a four month incubation using soil from different landscape positions was performed to verify field-wide consistency. Recovery was measured 5 times and after 120 d decreased to 65 % and 67 % of applied in cores and batch soils (averaged across all positions), respectively. This study found no significant difference in degradation of BAS 505, either between systems for any landscape position or among positions for intact and batch soils. In both studies and systems, degradation rate decreased over time and could be described by N^{th}-order kinetics but not 1^{st}-order. Sorption BAS 505 in the Ruston soil was reversible so that sorption kinetics was likely not the cause of slowing degradation rate. Decreasing microbial activity with time (as by lack of nutrient inputs) may have occurred but this was not shown by the highly variable microbial biomass C data. A follow-up greenhouse study that compared BAS 505 degradation in packed cores of Ruston soil under bermudagrass (*Cynodon dactylon (L.) Pers.*), bare and bare but shaded found evidence that

the presence of living grass led to faster degradation of BAS 505 versus bare soil. Thus, long-term static laboratory incubations may be a poor basis for projecting environmental fate and persistence.

CHAPTER 1

INTRODUCTION

Overview

The fate of pesticides and their susceptibility to transport may affect soil and water quality. Consequently, registration of pesticides is subject to strict environmental testing. This has led to less environmentally harsh compounds than earlier mercury and chlorinated forms. Among fungicides, for example, benomyl [*1-[(butylamino)carbonyl]-1H-benzimidazol-2-yl]carbamic acid methyl* ester] and thiabendazole [*2-(4-thiazolyl)-1H-benzimidazole*] have been developed since 1970 (Marsh, 1977). Nevertheless, environmental fate parameters must be determined for all pesticides seeking registration and, if there is concern about possible negative environmental impact, pesticide fate and transport can be modeled based on these parameters.

The fate of a pesticide in soil depends on its chemical structure. Differences in structure give a range in water solubilities of pesticides ranging from largely insoluble (hydrophobic) compounds to highly soluble (hydrophilic) compounds. From one extreme to the other, environmental risks vary. While the risk of off-site transport (runoff or leaching) of hydrophobic compounds is low, these tend to be persistent. The opposite holds for hydrophilic compounds. Models for pesticide fate and transport in the soil environment, therefore, implicitly account for pesticide structure and its effect on sorption / desorption, volatilization and degradation (abiotic and biotic).

Among these phenomena, biotic degradation may be the most difficult to accurately model. While degradation data are typically generated in laboratory incubations, conditions are not the same as in the field soil. It is not surprising that laboratory data for this phenomenon

often fail to describe it in the field. Resolving such inconsistency between laboratory and field data was the impetus for this project.

In particular, whereas aerobic biodegradation of the fungicide BAS 505 [*N-methyl-(E)-2-methoxyamino-2-(2-((2,5-dimethylphenoxy)methyl)phenyl)acetamide*] in the laboratory proceeded fairly slowly (half-life ~ 90 d; Scott Jackson, BASF, personal communication), dissipation of this highly sorbed, low volatility compound in field soil was much more rapid (half-life ~ 22 d). Given various reports (discussed later) that degradation rate is typically different in intact soil than in homogeneous samples of the same soil and often faster in the intact soil, BASF funded a study for development of an intact core method for measuring aerobic degradation rate. Though degradation in homogenous soils is required for pesticide registration, results from intact soil might be included as additional, alternative data for aerobic degradation in soil.

Pesticide Registration

General

The process of registering a pesticide is a scientific, legal and administrative procedure through which EPA examines the ingredients of the pesticide, the particular site or crop on which it is to be used, the pattern of its use (amount, frequency and timing), and appropriate storage and disposal practices. There are various tests associated with pesticide fate including: (i) residue chemistry, (ii) hydrolysis, (iii) photolysis, (iv) metabolism in soil (aerobic and anaerobic), (v) mobility (leaching, adsorption / desorption and volatility), (vi) dissipation under actual field use conditions, (vii) accumulation in food supplies, (viii) hazards to target and nontarget organisms (acute, chronic, subchronic, teratogenicity and mutagenicity), and (ix) pesticide drift. These data must be provided by the manufacturer of the pesticide as a thorough examination and assurance

of product performance. Pesticides that meet federal standards to protect human health and the environment are granted a license or "registration" that permits their distribution, sale and use according to specific directions and requirements identified on the label (EPA, 2003). In assessing the degradation of BAS 505, this project dealt with the environmental fate component of this process.

Environmental Fate Studies

Table 1.1 lists environmental fate data required when applying for registration of a pesticide (EPA, 2005). This table was made in accordance with 40 CFR 158.29 which describes the types and amounts of data needed for environmental fate of a pesticide. Kinds of studies and data needed depend on whether a pesticide is applied to land or water, or used in the greenhouse, forest, domestic outdoors or indoors. Table 1.1 shows which tests are required (R), conditionally required (CR), or not usually required (-----). In general, degradation through hydrolysis and photolysis, degradation through aerobic and anaerobic metabolism, and mobility are the primary concerns in assessing pesticide safety in the environment. Conditionally required tests are performed consistent with intended use of the pesticide (e.g., photolysis on soil required for a surface-applied but not for an incorporated pesticide). A more thorough examination of how these studies support pesticide registration and the methodologies typically used are described in the following sections.

Physicochemical Degradation

Hydrolysis studies determine the potential of the pesticide to degrade in water, whereas photodegradation studies determine the potential of the pesticide to degrade in water, soil or air when exposed to sunlight. Hydrolysis studies are conducted in the dark and carried out in solutions buffered at pHs of 5, 7 and 9. Typically, a range of concentrations within the aqueous

Table 1.1. Environmental fate data requirements for registration of a pesticide.

Kind of data required	General Use Patterns								
	Terrestrial		Aquatic		Greenhouse		Forestry	Domestic outdoor	Indoor
	Food crop	Nonfood	Food crop	Nonfood	Food crop	Nonfood			
Degradation studies-lab	R[†]	R	R	R	R	R	R	R	-----
Hydrolysis……									
Photodegradation:	R	R	R	R	-----	-----	R	-----	-----
In water……...									
On soil…….	CR	-----	-----	-----	-----	-----	CR	-----	-----
In air……	CR	-----	-----	-----	-----	-----	-----	-----	-----
Metabolism studies-lab	R	R	-----	-----	R	R	R	R	-----
Aerobic soil…….									
Anaerobic aquatic……	-----	-----	R	R	-----	-----	R	-----	-----
Aerobic aquatic……	-----	-----	R	R	-----	-----	-----	-----	-----
Mobility studies	R	R	R	R	R	R	R	R	-----
Leaching and adsorption / desorption									
Volatility:	CR	-----	-----	-----	CR	CR	-----	-----	-----
Lab……..									

(Table 1.1 Continued)

Volatility: Field….....	CR	-----	-----	-----	CR	CR	-----	-----	-----
Dissipation studies-field Soil……	R	R	-----	-----	-----	-----	-----	R	-----
Aquatic-sediment……..	-----	-----	R	R	-----	-----	-----	-----	-----
Forestry…….	-----	-----	-----	-----	-----	-----	R	-----	-----
Combination and tank mixes	-----	-----	-----	-----	-----	-----	-----	-----	-----
Soil long-term…….	CR	-----	CR	-----	-----	-----	-----	-----	-----
Accumulation studies Rotational crops-Confined……..	CR	-----	CR	-----	-----	-----	-----	-----	-----
Field……..	CR	-----	CR	-----	-----	-----	-----	-----	-----
Irrigated crops……	-----	-----	CR	CR	-----	-----	-----	-----	-----
In fish……..	CR	CR	CR	CR	-----	-----	CR	-----	-----
In aquatic non-target organisms	-----	-----	-----	CR	-----	-----	CR	-----	-----

† R= Required; CR= Conditionally required; ----- = Not usually required.

solubility of the pesticide are used, including a concentration high enough (but ≤ 250 parts per million) to determine the kinetics of the reaction, and permit isolation and identification of hydrolysis products (via gas chromatography / mass spectrometry). But the duration of the test is not to exceed 30 days.

Photolysis samples are exposed to either natural or simulated ($\lambda > 290$ nm) sunlight. Aliquots for analysis are taken at four or more sampling time intervals, with at least one observation made after one-half of the test substance has degraded or after the equivalent of 30 days of natural sunlight (12 hours of light per day), whichever comes first. When sunlight is used, a record is kept of intensity of incident sunlight, time of exposure, latitude, time of year, atmospheric cover, and other major variables which affect incident light. For artificial light, the nature of source, intensity, wave length distribution, time of exposure, and relationship of light intensity to that of natural sunlight are needed (EPA, 2005).

Biological Degradation

Aerobic and anaerobic soil metabolism studies are performed to determine breakdown rate and identify products that result from biological degradation. Typically radioisotope analytical techniques are used for determination of material balance among various extractable and unextractable pools. Treated aerobic soils are maintained at a constant temperature of 18 to 30 ^{o}C and moisture content of 75 % of 1 / 3 bar moisture. Soil sampling times are typically at 1, 3, 7 and 14 days, and 1, 2, 3, 4, 6, 9, and 12 months post-treatment. Samples are extracted and parent compound half life, residues and metabolites determined. Anaerobic soil metabolism studies are performed as above but under water-logged conditions and typically for no longer than 60 days (EPA, 2005).

Mobility Studies

These studies include measures of leaching and adsorption / desorption and laboratory / field volatility. The leaching study assesses the mobility of the pesticide and its degradates through columns packed with various soils or approximations of such systems. Adsorption / desorption determines the potential of the pesticide and its degradates to bind to soils of different types, controlling mobility. Each study should include, at a minimum, four soils (e.g., sand, sandy loam, silt loam, clay loam or clay), each having a pH within 4 to 8, unless the pesticide is restricted to one particular soil type. At least one soil should have an organic matter content ≤ 1 % (sand or sandy loam). The test substance is aged under aerobic conditions for 30 days or one half-life (whichever is shorter) in the soil and treated soil should be maintained between 18 to 30 ^{o}C and at a moisture content of 75 % of 1 / 3 bar moisture content during the aging period. Leaching studies may use soil columns directly, approximate leaching using soil thin layer chromatography plates or infer leaching behavior from batch adsorption / desorption.

Volatility studies (field or laboratory) examine the adverse effects to man via inhalation exposure at sites of application or biological effects in nontarget organism at some distance from the treated site. Air samples are collected from where applied and analyzed for residues. Data include volatility, air concentrations, vapor pressure, temperature, relative humidity, soil type, and, if in the field, meteorological conditions such as wind velocity and cloud cover. The pesticide is applied at the recommended field rate, whether in the lab or field (EPA, 2005).

Field Dissipation

These studies address pesticide loss as a combined result of chemical and biological processes (e.g., hydrolysis, photolysis and microbial transformation) and physical migration (e.g., volatilization, leaching and plant uptake). Data from these studies can reduce potential

overestimation of exposure and risk and can confirm assumptions of low levels of toxic degradates. These studies should be conducted in at least two different sites which are representative of the areas where the pesticide is expected to be used. Soil samples should be taken in increments, to a maximum depth of 15 cm, provided that the pesticide does not leach past that depth. Sampling times should be at pre-application, date of application, and immediate post-application. Furthermore, the residue data should be collected until patterns of decline of the test substance and patterns of formation and decline of degradation products are established in soil or to specified times for agricultural crops and outdoor use, whichever comes first: i) field and vegetable crop, 18 months, ii) orchard crop and pasture, 12 months, iii) domestic outdoor and turf, 4 months, and iv) shelter belts and rights-of-way, 2 months. Data collected are reported as residue decline curves in tested soil, rainfall and irrigation water used, grade (slope), soil and air temperature, techniques and times of planting and harvesting, application time and method, sampling times and techniques, dates and stages of crop and pest development, and depth, weight and volume of sample taken for analysis (EPA, 2005).

The focus of this project was methodology for pesticide biodegradation and possible methodological effects on degradation rate / kinetics. Since the test compound was a fungicide, a brief review of fungicide uses, including those for BAS 505, is in order before proceeding into microbial transformations of pesticides, models for pesticide degradation and sorption, and the literature on pesticide degradation in homogeneous and intact soil.

Fungicide Uses for Turfgrass and Agricultural Crops

Since 1970, the fungicides benomyl [*1-[(butylamino)carbonyl]-1H-benzimidazol-2-yl]carbamic acid methyl ester*] and thiabendazole [*2-(4-thiazolyl)-1H-benzimidazole*] have been applied commercially for the control of turf diseases, particularly on golf course tees and greens,

and for disease control in seed production crops. Prior to 1970, many turf grass diseases were successfully controlled with mercury fungicides and chloroneb [*1,4-dichloro-2,5-dimethoxybenzene*] (Marsh, 1977). The most common turf grass diseases in the southern U.S. are: (i) dollar spot caused by *Sclerotinia homoeocarpa* F.T. Bennett, (ii) brown patch caused by *Rhizotonia solani* Kuhn, (iii) pythium or cottony blight caused by *Pythium ultimum* Trow and *Pythium aphanidermatum* (Edson) Fitzpatrick, and to a lesser extent, (iv) rust diseases.

Dollar spot is commonly found in bentgrasses (*Agrostis* spp.) and fescues (*Fescuta* spp.) in the northern U.S. and the British Isles. In warmer regions of the U.S., bermudagrass (*Cynodon dactylon* (L.) Pers.), bahiagrass (*Paspalum notatum* Flugga), and zoysiagrasses (*Zoysia* spp.) can be severely affected with the disease, especially under conditions of low fertility (Marsh, 1977; Turgeon, 1991). Brown patch is most active during periods of high relative humidity, high night temperatures, and is severe on St. Augustine grass (*Stenotaphrum secondatum* (Walt.) Kuntze) and bentgrass. Bermudagrass and bahiagrass are somewhat less susceptible but prone to attack whereas Kentucky bluegrass (*Poa pratensis* L.) is seldom damaged. Brown patch is characterized by a dark blue ring at the edges of diseased areas ranging from a few inches to several feet in diameter. Severity increases where there is excess nitrogen available in conjunction with high soil moisture (Marsh, 1977; Turgeon, 1991). Pythium or cottony blight is a disease of cool-season grasses, is the most destructive of these diseases and affects bentgrasses, fescues, and Kentucky bluegrass. During the winter months in the southern U.S., pythium blight can be a problem on golf greens and tees over-seeded with ryegrass (*Lolium multiflorum* Lam.) and difficult to control with fungicides (Marsh, 1977; Turgeon, 1991). Rusts consist of rust fungi that attack turf grasses but they rarely occur at a frequency or intensity to be of major importance on mowed lawns. However, they can be a

problem on crops grown for seed production, hay or pastures. 'Merion' variety of Kentucky bluegrass, certain fine leaved perennial ryegrasses and bermudagrass are highly susceptible to rust (Marsh, 1977; Turgeon, 1991). Fungicides are applied to agricultural crops to control the spread of leaf rust, stem rust, powdery mildew, *Fusarium* head blight, or stripe rust diseases. These diseases are widespread and infection begins on the lower leaves and spread upwards.

Uses of the Strobilurin Fungicide, BAS 505

The intended use for BAS 505 is foliar application to turf and cereal crops, including wheat. Currently, BAS 505 is registered in the European Union but is still in the process of registration in the United States. It is a member of the strobilurin group of fungicides and has the active ingredient name *dimoxystrobin.* Strobilurin fungicides were first introduced for registration in 1996. These compounds have single-site activity with one mode of action and are relatively prone to resistance (Liskey, 2002). The formulation of BAS 505 is a suspension concentrate containing dimoxystrobin and epoxiconazole (another fungicide), which provides protective and curative control of various fungal pathogens in winter wheat. Dimoxystrobin exhibits a broad spectrum of control when mixed with epoxiconazole, with excellent control of *S. nodorum*, and very good control of *S. tritici*, yellow rust, brown rust and ear blight compared to, for example, kresoxim-methyl + fenpropimorph (HGCA, 2006). The strobilurin class of fungicides could replace conventional fungicides such as mancozeb, chlorothalonil, or copper hydroxide (EPA, 2004).

When the pesticide is applied to the area or crop of interest, the compound can transform under various metabolic pathways via enzymatic reactions which can form degradates that are either more or less toxic than the parent compound

Microbial Transformation of Pesticides

There are four major possibilities for transformation / inactivation: (i) the pesticide can serve as a substrate for growth and energy; (ii) it can undergo cometabolism (i.e., microorganisms transform it, but cannot derive energy for growth from it); (iii) it or an intermediate can be conjugated with naturally occurring compounds; and (iv) it can be incorporated and accumulated within an organism, hence accumulation in biomass. In many cases, several transformations occur, not just one, when a pesticide is exposed to a whole microflora under natural conditions. Metabolism by various pathways results in many different products (Bollag, 1974).

In order to determine whether a pesticide can serve as the only carbon source needed for growth, an enrichment culture technique is usually used. If a pesticide can be used in such a way, it is degraded to compounds that can be channeled into known oxidative cycles such as the Krebs cycle, and thus the organism can derive all the necessary energy needed for metabolism. Cometabolism generally does not result in extensive degradation of a pesticide molecule but involves reduction or elimination reactions forming intermediate products with possibly decreased or increased environmental toxicity. It is carried out by bacteria, actinomycetes and fungi, and therefore can be assumed to widely occur in natural ecosystems. Enzymes involved in catalyzing the initial metabolic reaction often lack substrate specificity. In many cases, pesticides are transformed not by biodegradation but by microbially mediated polymerization or conjugation. Conjugation reactions are syntheses by which a pesticide or any intermediate products are linked with other endogenous substrates resulting in the formation of methylated (CH_3-), acetylated (CH_3CO-) or alkylated (C_nH_{n+1}-) compounds, glycosides (acetal or ketal formed from the reaction of a cyclic monosaccharide molecule with another monosaccharide) or

amino acid conjugates. The formation of a conjugate usually makes the molecule more polar and therefore more water- and less lipid-soluble. Conjugations of pesticides and other xenobiotic chemicals are common and frequent reactions in all higher organisms but they have not been found to a similar extent in microorganisms. Microbial accumulation of pesticides is attributed to a passive physical process of absorption rather than active metabolism. High accumulation in microbes occurs with chlorinated hydrocarbons, such as DDT, dieldrin, aldrin, and heptachlor and some organophosphates (Bollag, 1974; Bollag and Liu, 1990).

All microbial transformations are caused by enzymes, and since all synthetic pesticides are foreign materials, many of the enzymes catalyzing a certain reaction are induced. However, it is difficult to predict which molecular change can be expected for a specific microbe since each group of organisms can alter a particular molecular structure differently. But a certain mode of biological attack can be anticipated on the basis of the molecular structure of the pesticide (Bollag, 1974). For example, the structure of BAS 505 (Fig. 1.1) contains labile aryl methyl groups and these are oxidized in the early stage of its degradation (Scott Jackson, BASF, personal communication.)

Several reactions may take place when a pesticide undergoes detoxication (Alexander, 1999). These include (i) hydrolysis- cleavage of a bond by the addition of water, (ii) hydroxylation- the addition of -OH, (iii) dehalogenation- replacement of a halogen by H (reductive dehalogenation), by OH (hydrolytic dehalogenation) or removal of the halogen and an adjacent H (dehydrodehalogenation), (iv) demethylation or other dealkylation- the removal of methyl or other alkyl substituent, (v) methylation- the addition of methyl groups, (vi) nitro reduction- reduction of the nitro to an amino group, (vii) deamination- the removal of an amino group, (viii) ether cleavage, (ix) conversion of nitrile to amide and (x) conjugation. The

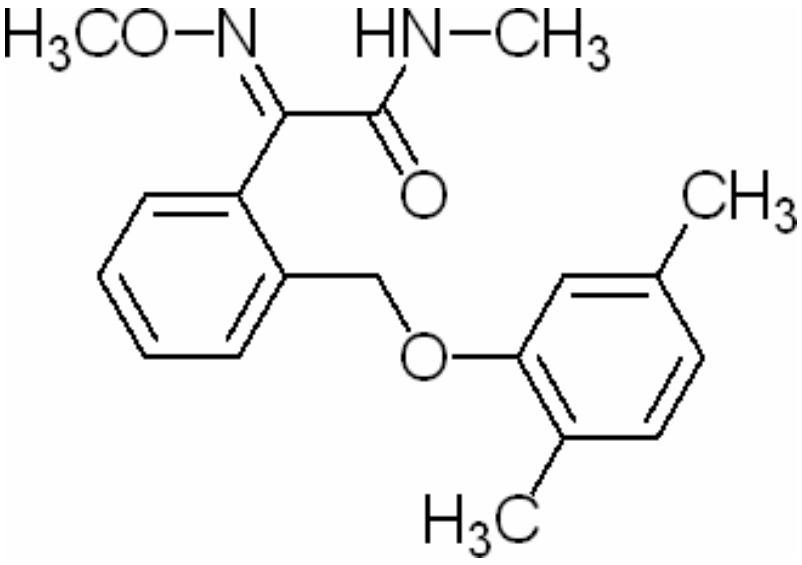

Figure 1.1 . Structure of BAS 505 [*N-Methyl-(E)-2-methoxyamino-2-(2-((2,5-dimethylphenoxy) methyl) phenyl) acetamide*].

metabolic pathway of a compound varies with the structure of each pesticide and affects the rate of parent decomposition, rates of formation and decline of degradation products and, consequently, kinetic models for these processes.

Pesticide Degradation Models

Soils are biologically, chemically and physically complex systems. As a result, besides the chemical structure of the pesticide, various soil conditions affect pesticide degradation and attempts to model it. These conditions include: (i) diffusional barriers within the soil that may limit or prevent contact between microbial cells and organic substrates; (ii) sorption of the pesticide to clay or humus constituents that may affect the kinetics of decomposition of the sorbed pesticide relative to the compound free in solution; (iii) presence of other organic compounds that can be metabolized by the biodegrading species, repressing or enhancing

decomposition of the pesticide; and (iv) supply of inorganic nutrients, oxygen, or growth factors that may govern the rate of transformations (Alexander and Scow, 1989).

Despite such complexities, first-order kinetics (mono-phasic or one-compartment kinetics) have often been used to describe degradation. Decline of concentration with time is proportional to the concentration at any time (Beulke and Brown, 2001),

$$dC / dt = -kC \quad (1)$$

where C is concentration ($\mu g\ g^{-1}_{soil}$), t is time (d) and k is degradation rate constant (d^{-1}). First-order kinetics assume concentration of the substrate is rate limiting (Hamaker, 1972).

However, in certain cases, degradation rate may show a bi-phasic pattern where soil residues decrease slowly after an initial rapid decline and persist at a low level thereafter (Beulke and Brown, 2001). When this occurs, an N^{th} order model may be appropriate to account for such deviation from first-order kinetics (Hamaker, 1972). The N^{th}-order model is expressed as

$$dC/dt = -kC^N \quad (2)$$

Alternatively, multiple degradation compartments and first-order rate constants may be invoked. A general formulation of a two-compartment model (Hamaker, 1972; Hill and Schaalje, 1985; Zimdahl et al, 1994; Gaston et al., 1996a) is

$$C = C_1 + C_2 \quad (3a)$$

$$dC_1 / dt = -(k_1 + \alpha)\ C_1 + \beta C_2 \quad (3b)$$

$$dC_2 / dt = -(k_2 + \beta)\ C_2 + \alpha C_1 \quad (3c)$$

where subscripts refer to the different compartments and α and β (d^{-1}) are mass transfer coefficients for movement of the compound between compartments 1 and 2. Gustafson and Holden (1990) extended the multiple compartment concept to a distribution of many compartments but without mass transfer among them.

There are also several simple degradation models that have been considered, though perhaps not widely used (Schmidt et. al, 1985). Whereas the first-order, N^{th}-order and multiple compartment models do not consider any relationship between the substrate and microorganisms acting on it, one example that does is the Michaelis-Menten relationship,

$$dC / dt = - (V_{max} C) / (K_m + C) \quad (4)$$

where C is concentration in solution, V_{max} is the maximum reaction rate (which includes contributions from extracellular soil enzyme activities and intracellular soil microbial activities catalyzing the transformation reactions) and K_m is the Michaelis constant. This equation is used when growth of microorganisms actively metabolizing a substrate occurs at the expense of another organic compound, where the rate of transformation per cell of the substrate is likely to follow some form of saturating kinetics. When the population of microorganisms is stable and $C << K_m$, however, the relationship reduces to first-order.

Another example of dependency of pesticide degradation on microbial population is Monod kinetics (Simkins and Alexander, 1984; Greer et al., 1992),

$$dC / dt = \mu_{max} C X / (K_s + C) Y \quad (5)$$

where μ_{max} (h^{-1}) is the maximum growth rate, K_s ($\mu g\ mL^{-1}$) is the half-saturation growth constant, X ($\mu g\ mL^{-1}$) is the biomass concentration, and Y is the yield (biomass produced per mass of

substrate consumed). This equation is used to describe sigmoidal kinetics of substrate disappearance resulting from microbial growth, i.e., with X increasing with time. As with Michaelis-Menten kinetics, with X constant and if $C << K_s$, Eq. 5 reduces to first-order kinetics. Successful application of Monod kinetics to the modeling of biodegradation data in aqueous systems has been previously described. However, Monod kinetics may not be directly applicable to soil (Focht and Shelton, 1987), presumably because a substantial fraction of the applied pesticide may be sorbed to soil surfaces, hence, not directly available for biodegradation.

Soil biodegradation models should account for bioavailability by incorporating terms that reflect both the total fraction sorbed and soil solution concentration (Shelton and Doherty, 1997). Without accounting for sorption, important differences in degradation rate among soil media may be obscured (Zablotowicz et al., 2000). As sorption may influence the degradation of pesticides, a review of the chemistry of pesticide sorption to soils and what methods are typically used to quantify it is presented in the next section.

Chemistry of Sorption for Organic Pesticides / Contaminants

Adsorption describes the process whereby constituents are concentrated at the interface of two phases, either the solid-liquid, liquid-liquid, gaseous-liquid, or gaseous-solid boundary. Adsorption retards mass flux by diffusion, convection and dispersion and so affects the mobility of a pesticide in a soil medium (Scheunert, 1993). *Absorption* describes the process in which a component is transferred from the bulk state of one phase into the bulk state of the other phase. *Sorption* is the term used to describe adsorption and / or absorption and may involve specific interactions between polar or charged sites and polar or charged components or nonspecific interactions between nonpolar sites and nonpolar compounds or combinations of the two processes.

Adsorption may involve (i) physical or van der Waals forces, (ii) hydrogen bonding, (iii) ion exchange or (iv) chemisorption. van der Waals interaction with the organic fraction of soils and sediments is responsible for the sorption of many compounds, particularly those that are hydrophobic. Generally, the lower the water solubility of a compound and the higher the amount of organic C in the soil or sediment, the greater is the extent to which a hydrophobic compound is sorbed. There are two views by which hydrophobic molecules are retained by organic matter. One is physical sorption by organic matter, and the other is diffusion and partitioning into organic matter (Alexander, 1999). Although the hydrophobic surfaces are primarily organic, they may also include –Si-O-Si- bonds at mineral surfaces. Regardless, hydrophobic sorption is the partitioning of nonpolar organics out of the polar aqueous phase and onto or into hydrophobic surfaces in the soil, caused by the weak interaction between the solute and the solvent (Hassett and Banwart, 1989). Large molecules may be retained on clay surfaces by hydrogen bonding as well, but for some low molecular weight organics, ion exchange (particularly, cation exchange) is important (Alexander, 1999).

Given that sorption affects biodegradation (Shelton and Doherty, 1997), clay minerals that differ in surface area and cation exchange capacity should differ in effect on biodegradation. Montmorillonite, for example, frequently sorbs potential microbial substrates because of its high cation exchange capacity and its expanding lattice structure. Many organic substrates can enter between the silicate sheets that make up this clay, and thereby become protected. Typically, if effects of clay occur on sorption, montmorillonite will have the greatest effect compared to kaolinite and illite (Alexander, 1999).

The sorption of many, if not most, nonpolar organics on soils and sediments produces linear isotherms up to solution concentrations of 60 – 80 % of the sorbing species' water

solubility. At higher solution concentrations the amount sorbed may increase little or greatly, the isotherm rapidly deviating from linearity (such as the formation of a discrete organic phase or inadequate techniques to measure adsorption). For either one, the Freundlich equation is appropriate

$$C_s = K_f C_w^N. \quad (6a)$$

In this model, C_s is the sorbed concentration ($\mu g\ g^{-1}$ or $\mu mol\ kg^{-1}$ soil), C_w is the equilibrium concentration of the sorbate ($mg\ L^{-1}$ or $\mu mol\ L^{-1}$), and K_f and N ($\neq 1$) are empirical constants. When N = 1, as with most nonpolar species, the Freundlich constant K_f becomes a partition constant and the isotherm linear (Hassett and Banwart, 1989; Evangelou, 1998; Green and Karickhoff, 1990, Alexander, 1999),

$$K_f = C_s/C_w. \quad (6b)$$

Sorption studies are commonly performed by the batch-suspension method, which consists of agitating soil and pesticide solution in a closed vessel (i.e., centrifuge tubes) for sufficient time to achieve equilibrium. Experimental variables include the solution / soil ratio, temperature, antecedent condition of the soil, type of vessel and nature of agitation. One limitation of the batch-suspension method is poor precision when sorption is low (Green and Karickhoff, 1990).

Chemical and physical properties of the compound and medium, as well as experimental conditions can alter the kinetics of biodegradation and sorption of pesticides. Various work has found differences in degradation measured in homogeneous and intact soil.

Pesticide Degradation in Homogeneous and Intact (Heterogeneous) Soil

Laboratory incubation experiments may be well suited to study the kinetics of pesticide decomposition (Richter, et al., 1992). However, several studies have found inconsistencies between pesticide degradation measured using homogeneous soil in hydrodynamically static (batch) systems and estimated using heterogeneous, intact soil cores through which soil water moves. For example, studies by Moorman and Harper (1989) and Locke and Harper (1991) on metribuzin [*4-amino-6-(1,1-dimethyl-ethyl)-3-(methylthio)-1,2,4-triazin-5(4)-one*] degradation in homogeneous soil found that degradation was best described using nonlinear kinetics. However, a subsequent study using intact soil cores, subject to intermittent simulated rainfall, suggested that metribuzin degradation followed first-order kinetics (Locke et al., 1994).

Similarly, Gaston et al. (1996a) found that bentazon [*3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3)-one 2,2 dioxide*] degradation in homogeneous soil was nonlinear and that the apparent decrease in degradation rate with time could be best described as a two-compartment process with separate first-order rate constants for each. Since degradation of bentazon is faster under aerobic conditions, these compartments may have physically corresponded to inter-aggregate (relatively well-aerated) and intra-aggregate (relatively anoxic) pore space (Gaston et al., 1996a). However, in a parallel study of bentazon degradation during unsaturated water flow through intact cores of Dundee soil, a single first-order model adequately described the disappearance of this compound (Gaston and Locke, 1996). Although there was evidence for preferential water flow through the intact cores, the rate of solute mass transfer from water-conducting to non-conducting soil matrix was very slow. Thus, since bentazon transport was restricted to only a portion of the soil matrix and this was likely better aerated than the non-conducting region, degradation was faster than expected.

In general, aeration within soil cores is poorer than in common apparatus, biometer flask (Bartha and Pramer, 1965), used to measure pesticide degradation in soil. Aeration in the former depends on gas exchange at the soil surface. The ratio of surface area for gas exchange to soil volume in cores is much smaller than the artificially large ratio that exists in flasks containing a thin layer of soil. Thus, compounds that are degraded faster under relatively anoxic conditions should exhibit faster degradation in soil cores. For example, acifluorfen [*5-[2-chlor-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid*] is thought to be degraded faster under anaerobic conditions. Gaston and Locke (2000) found nearly an order of magnitude faster degradation of this compound in soil cores than in flasks.

Gaston et al. (2001a) examined effects of tillage and cover crop on fluometuron [*N,N-dimethyl-N'-[3-(triflurormethyl)phenyl]-urea*] degradation using homogeneous samples of surface soil. Despite high microbial activity in vetch soils, fluometuron degradation was slower where vetch was planted than in either native winter annuals or wheat plots. In contrast, fluometuron degradation in intact cores of vetch soils was not suppressed relative to degradation in native or wheat soils (Gaston et al., 2003). A possible explanation was that biodegradation in closed, no-flow systems were slowed by high levels of inorganic N under vetch. However, displacement of available N from soil cores by simulated rainfall apparently stimulated fluometuron degradation. Given the mobility of nitrate-N in soil water, coupled with plant-uptake in the field, results from the intact soil cores would seem to better approximate degradation behavior in the field where high levels of inorganic N persist.

Such disparity of results leaves the pesticide fate researcher in something of a quandary. Geometric fidelity of intact soil cores to field soil suggests these are a better model than homogeneous soil in flasks. Not only is aeration status likely a better match with field

conditions and soil solution flow possible, small-scale biological and chemical spatial variability (Gaston and Locke, 2002), which may affect pesticide fate, are preserved in soil cores. On the other hand, batch methods are more convenient. A rational compromise is the use of intact cores to verify results of batch experiments.

Research Objectives

In particular, a new fungicide developed by BASF (BAS 505, *N-Methyl-(E)-2-methoxyamino-2-(2-((2,5-dimethylphenoxy) methyl) phenyl) acetamide*) showed rapid dissipation in field studies but much slower aerobic degradation in laboratory studies with homogeneous soil. Based on previous laboratory studies that compared degradation of pesticides in homogenized and intact soil, it was suggested that the slow degradation of BAS 505 in homogenized soil samples might be an artifact. Accordingly, this project undertook the following objectives:

1) Develop a convenient methodology for tracking the aerobic degradation of BAS 505 in intact soil cores and compare degradation in cores and batch soil (Chapter 2);
2) Examine the effect of spatial variability in soil properties / landscape position on BAS 505 degradation using intact cores and batch soil (Chapter 3);
3) Determine effects of sorption / desorption on BAS 505 degradation (Chapters 2 and 3) and;
4) Assess effect of living plants in soil on BAS 505 degradation (Chapter 3).

Note on the Risk and Toxicology of BAS 505

According to the preliminary MSDS, LD_{50} (rat, oral and dermal) was > 2000 mg / kg, LC_{50} (rat, inhalation for 4 hr). The compound was not irritating to rabbit skin or eyes and not a

sensitizer (guinea pig). In a range finding reproduction toxicity study in rats, signs of developmental toxicity were noted in pups at doses that induced toxicity in the parental animals. However, these pathological changes showed no dose response relationship. In a range finding prenatal developmental toxicity study in rabbits, signs of developmental toxicity were noted in pups at doses that induced toxicity in the mother. This material was found to be very highly toxic to fish (EC_{50} for daphnia at 24 h < 1 ppm) and green algae (EC_{50} at 72 h was 0.01 - 0.03 mg / L). The LC_{50} for earthworms at 14 d was 1 - 15 mg / kg_{soil} and it adversely affected earthworm reproduction. Draft results of an early life stage study in fish show the overall NOAEL (no observed adverse effect level) was 0.000316 mg / L and the lowest concentration with adverse effects (LOAEC) was 0.001 mg/L. Rainbow trout had a NOAEC = 0.01 mg / L (nominal concentration). Threshold level for compound related lethal effect and for toxic signs was > 0.01 and < 0.0316 mg / L. However, BAS 505 does not accumulate in fish and will rapidly eliminate from fish if exposure occurs, with 50 and 90% depuration for the whole fish at 0.49 hours and 1.62 hours, respectively (BASF, personal communication). Given this toxicological background, the greatest risk for BAS 505 would appear to be offsite transport into adjacent water bodies.

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CHAPTER 2

ONE YEARLONG DEGRADATION OF THE FUNGICIDE BAS 505 IN BATCH AND INTACT CORE SOIL

Introduction

Pesticide degradation in soil is studied in laboratory incubations. Homogeneous, bulk soil is typically used, making it a particularly convenient method. The results of such studies are used to estimate how persistent a pesticide may be once applied to field soil (Richter, et al., 1992). Recently, BASF developed BAS 505 [*N-methyl-(E)-2-methoxy amino-2-(2-((2,5-dimethylphenoxy)methyl) phenyl)acetamide*] for fungal disease control in turf and cereal. Environmental fate data on BAS 505 generated for registration showed rapid dissipation in the field but slower degradation using homogeneous soil in the laboratory. These results are similar to the findings of several studies in which pesticide degradation was alternatively measured using homogenous soil in batch systems and heterogeneous, intact soil cores.

For example, studies by Moorman and Harper (1989) and Locke and Harper (1991) on metribuzin [*4-amino-6-(1,1-dimethyl-ethyl)-3-(methylthio)-1,2,4-triazin-5(4)-one*] degradation in homogeneous Dundee soil (fine-silty, mixed, thermic Aeric Ochraqualfs) found that degradation was best described using nonlinear kinetics. But a later study using intact soil cores subject to simulated rainfall suggested that degradation followed first-order kinetics (Locke et al., 1994).

Similarly, Gaston et al. (1996) found that bentazon [*3-(1-methylethyl)-(1H)-2,1,3-benzo thiadiazin-4(3)-one 2,2 dioxid*e] degradation in homogenous Dundee soil was nonlinear and best described as a two-compartment process with compartments corresponding to inter-aggregate (well-aerated) and intra-aggregate (poorly-aerated) pore space. But degradation during unsaturated water flow through intact cores of Dundee soil was adequately described by a single

first-order model (Gaston and Locke, 1996) and faster than in homogeneous soil since bentazon residence was largely restricted to the better aerated, water conducting region.

In general, aeration within soil cores is poorer than biometer flasks (Bartha and Pramer, 1965) commonly used to measure pesticide degradation in soil. Aeration in the former depends on gas exchange at the soil surface, aided by infiltration of well-oxygenated water. Therefore, the ratio of surface area for gas exchange to soil volume in cores is smaller than the artificially large ratio in flasks containing 1 to 2 cm of soil. Thus, for compounds that are degraded faster under relatively anoxic conditions, degradation in soil cores may be more rapid. For example, since acifluorfen [*5-[2-chlor-4-(trifluoromethyl) phenoxy]-2-nitrobenzoic acid*] is degraded faster under anaerobic conditions (Andreoni et al., 1994), it was not surprising that Gaston and Locke (2000) found ~ 10 times faster degradation in soil cores than in flasks.

Such disparity of results poses a dilemma. The physical structure of intact soil cores makes them a much closer approximation to field soil than homogeneous soil. Aeration status is likely a better match with field conditions and soil solution flow possible. Soil cores also preserve the small-scale biological and chemical spatial variability (Gaston and Locke, 2002) which may affect pesticide fate. Furthermore, since the pesticide is applied drop-wise to the soil surface in cores but uniformly blended into a depth-averaged mix of homogeneous soil, both site and pattern of application to cores are more similar field application than in the batch system. On the other hand, batch methods are more convenient.

Thus, the primary objectives of this study were to develop a convenient methodology for tracking the aerobic degradation of BAS 505 in intact soil cores and compare degradation in cores and batch soil. Sorption / desorption of BAS 505 and the relation between its persistence and microbial biomass were also examined.

Materials

Bulk Soil and Intact Soil Cores

Surface 0 to 7.5 cm Ruston series (fine-loamy, siliceous, thermic Typic Paleudult) soil from a mowed bermudagrass (*Cynodon dactylon* (L.) Pers.) lawn at the LSU AgCenter Calhoun Research Station, Calhoun, LA was used. Random cores (7.5 cm diameter by 7.5 cm deep) were taken from a 2 x 2 m hill backslope area. Soil in cores was later combined, thoroughly mixed and stored at 4 ^{o}C pending the degradation study.

In the 2 x 2 m area, 96+ intact cores (0 to 7.5 cm) were taken. These were sealed in airtight wrapping and placed in padded boxes to avoid jarring during transport. Grass stems and thatch were later removed from the soil surface, soil protruding from the bottom of cores was cut flush and cores were weighed to determine net mass of field-moist soil. Bottoms of cores were fitted with a Teflon end disk. These were re-wrapped and stored at 4 ^{o}C pending the degradation study. Three additional cores were taken with a 5-cm diameter coring device for soil bulk density.

Test Compound

Radiolabeled (phenyl-U-^{14}C) BAS 505 [*N-methyl-(E)-2-methoxy amino-2-(2-((2,5-dimethylphenoxy)methyl) phenyl)acetamide*] (95 % purity, total activity 96.2 MBq) was provided by BASF Corp., Research Triangle Park, NC. The major impurity was the (Z) isomer of BAS 505 (Fig 1.1).

Reagent Chemicals and Solvents

All chemicals were reagent grade and chromatography solvents were HPLC grade. Scinti-Safe Plus 50 % scintillation cocktail (Fisher) was used for LSC except for combusted soil

samples (Carbo Sorb / Permafluor, Perkins Elmer) and HPLC-LSC analysis (In-Flow BD, IN/US Systems, Inc.).

General Equipment

Waters 2695 Separations Module, equipped with a Waters 996 Photodiode Array Detector and IN/US Systems, β-Ram.

Beckman Instruments, Inc. LS Analyzer, Model LS6KLL.

United Technologies Packard Model 306 oxidizer (Packard Instrument Co., Downers Grove, IL).

Methods

Soil Characterization

Triplicate sub-samples of bulk soil were used to determine field gravimetric water content, pH (2:1, water:soil), organic C (Nelson and Sommers, 1982), CEC (sum of basic and acidic cations; 1 N NH_4OAc and $BaCl_2$-TEA extractions, respectively), texture (Gee and Bauder, 1986) and microbial biomass C (Vance et al., 1987). Volumetric water content at field capacity (1/3 bar) was estimated using measured texture and bulk density via pedotransfer function parameters given in Wosten and van Genuchten (1988). Soil characterization data are presented in Table 2.1.

Preparation of BAS 505 Application Solution

Application Rates

The target application rate was 0.28 kg ai ha^{-1}. Given the cross sectional area of cores, 0.004560 m^2, BAS 505 was applied at 0.1277 mg ai $core^{-1}$. For batch soil (20 g oven-dry equivalent) in the biometer flasks, corresponding surface area was calculated from known bulk density and sampling depth. In particular, surface area (mass / ρ_B) / 7.5 cm was 1.93 cm^2. Therefore, the application rate for biometer flask soil was 0.005405 mg ai $flask^{-1}$.

Table 2.1. Characterization data for the Ruston soil.

Textural Class	Sandy Loam
% Sand	62.6
% Silt	26.8
% Clay	10.6
Bulk Density (g cm^{-3})	1.36 ± 0.01
pH (1:2, soil: H_2O)	5.38 ± 0.05
Organic C (g kg^{-1})	15.1 ± 0.8
CEC (cmol (+) kg^{-1})	7.91 ± 0.48
Moisture (1/3 bar, g H_2O / 100 g)	14.9
Microbial Biomass C (μg g^{-1})	285 ± 54

†Field capacity was calculated as per Wosten and van Genuchten (1988).
‡Measurements other than for texture were done in triplicate.

Dilution of Stock Solution

The radiolabeled BAS 505 was reconstituted in 40.00 ml of acetonitrile to give a concentration = 20.30 mg ai / 40.00 mL = 0.5075 mg ai mL^{-1}. Thus, for the biometer flasks, to deliver the required 0.005405 mg ai $flask^{-1}$ ai to flasks in 100 μL, the stock solution was diluted by a factor of (0.05405 / 0.5075) = 0.1065 with acetonitrile. This solution was also used for the cores, but it was applied in (0.1277 mg ai $core^{-1}$ / 0.005405 mg ai $flask^{-1}$) * 0.100 mL $flask^{-1}$ = 2.363 mL $core^{-1}$. Two hundred mL of input solution were prepared by diluting 21.30 mL of stock solution into 200 mL with acetonitrile.

Biometer Flask Experiment

Twenty g (oven-dry equivalent mass) of soil were transferred to each of 33 biometer flasks and three 250-mL Nalgene centrifuge bottles. One hundred μL of 54.04 μg mL^{-1} BAS 505 were applied drop-wise to the soil, followed by sufficient water to achieve 75 % of field capacity, and the soil thoroughly mixed with a stainless steel spatula. Weights of flasks with treated soil were recorded. The dosed soil in centrifuge bottles was immediately extracted as described below to determine recovery of ^{14}C and BAS 505. Ten mL of 1 N NaOH was added to flask sidearms, flasks stoppered and set in a dark, temperature-controlled room (23 ^{o}C).

Extraction Methodology and LSC for ^{14}C

Soil in biometer flasks was removed after 2, 5, 7, 14, 30, 60, 90, 120, 180, 270 and 360 d incubation and transferred to 250 mL Nalgene centrifuge bottles for extraction (Day 0 samples were in centrifuge bottles from outset). Samples were extracted with MeOH (~30 mL x 2 times), followed by MeOH:water, 1:1 volume basis (~30 mL x 2 times; for recovery of more polar degradation products). Soil suspensions were shaken (325 rpm) at room temperature in the dark for one hour, centrifuged and supernatants decanted into glass bottles (Teflon-lined caps; separate bottles for MeOH and MeOH:water extracts). Mass of extract and entrained MeOH:water solution were recorded. The latter was evaporated, dry soil removed from the centrifuge bottle and ground prior to analysis for unextractable ^{14}C (combustion / LSC, described below). Duplicate 1 mL aliquots of MeOH and MeOH:water extracts were analyzed by LSC. The MeOH extracts were concentrated by rotary evaporation. The MeOH:water extracts were similarly concentrated to remove MeOH, then diluted with 0.01 N $CaCl_2$ to ~ 100 mL and this solution passed through conditioned C_{18} solid phase extraction (SPE) columns (Waters Associates, Milford, MA). The BAS 505 and any metabolites retained were eluted with 2.5 mL

of MeOH. Duplicate 0.25 mL aliquots of concentrated extracts were analyzed by LSC for possible losses during processing. Also, 1 mL aliquots of effluent from the C_{18} columns were analyzed to verify negligible loss in this step. All concentrated extracts were analyzed by HPLC-LSC as described below.

Mineralization and Maintenance

One mL aliquots of NaOH in biometer flask sidearms were removed at 2, 5, 7 d and weekly thereafter for LSC analysis of evolved $^{14}CO_2$. The NaOH was removed, weights of flasks measured and water added as needed to restore 75 % field capacity. Fresh NaOH was added, flasks stoppered and incubation continued. To ensure aerobic conditions, flasks were also briefly unstoppered at midweek.

Intact Core Experiment, Full

A randomly selected set of 36 cores (of 96+ taken; three replicates for each of the 12 incubation periods) was assigned to this experiment. Each was dosed with 2.36 mL of 54.04 μg mL^{-1} BAS 505 to give an aerial application rate of 0.28 kg ha^{-1}. Sufficient water was then applied to the surface of each core to bring its average water content to 75 % field capacity and mass of cores recorded. Thirty-three cores were then placed in a 5 L capacity airtight container into which was also placed an open vial containing 20 mL of 1 N NaOH as a $^{14}CO_2$ trap. Containers were sealed and incubated in the dark at 23 ^{o}C. The remaining three cores (day 0 replicates) were sampled as described below immediately following application of BAS 505 and water.

After prescribed incubation, cores were removed from containers and two sub-samples (1.6 cm diameter cores to the depth of soil in cores) removed, transferred to 250 mL Nalgene centrifuge bottles and extracted as described for biometer flasks (except larger volumes of

MeOH and MeOH:water, proportional to the greater mass of soil in the sub-cores). Processing of extracts and LSC analyses were as described above. Additional core sub-samples were taken for microbial biomass C (below). Remaining soil was crumbled, mixed and preserved for ^{14}C analysis by combustion. Mineralization was tracked as described above. Cores were watered as indicated by loss of mass. Containers were briefly opened midweek for re-aeration.

Microbial Biomass C and Intact Cores, Untreated Controls

Sub-core soil for microbial biomass C was crumbled, roots or other apparent organic debris removed, material mixed thoroughly and 10 g (moist weight) sub-samples transferred to two 50 mL Erlenmeyer flasks for microbial biomass C analysis by the chloroform fumigation method (Vance et al., 1987).

An additional 36 randomly assigned cores were amended with 2.36 mL BAS 505-free ACN and maintained identically to the above triplicate intact core series with respect to replenishing lost water and ventilating containers twice weekly. Sub-cores were taken when treated cores were sampled and these analyzed for microbial biomass C.

Intact Core Experiment, Abridged

This parallel experiment was conducted identically to and concurrent with the triplicate intact core experiment described above, however, it included only two replicates for each sampling time (24 randomly assigned cores).

Sorption / Desorption

Five-g (oven-dry equivalent) samples of air-dry 0 to 7.5 cm depth soil were placed in 50-mL Pyrex centrifuge tubes. Fifteen-mL of 0.1, 1, 2, 5 or 10 μM solutions of ^{14}C BAS 505 in 0.01 N $CaCl_2$ background (< 1 % ACN) were added to centrifuge tubes in triplicate. Another

triplicate set of these solutions was added to tubes without soil to measure sorption, if any, onto tubes. Suspensions and blanks were shaken 24 h. Soil solution was separated from suspension by centrifuging (10 min at 3,000 g). Sorption was calculated from change in solution concentration of ^{14}C, corrected for blank sorption.

The reversibility of sorption was determined by measuring release of BAS 505 sorbed from 10 μM input samples after 24 h equilibration by sequential extractions with 0.01 N $CaCl_2$ for 1.5, 4.5 then 18 h shaking times.

Analyses

HPLC

Concentrated MeOH and MeOH:water extract (SPE) samples were analyzed by HPLC, with LSC detection (IN/US Systems, β-Ram). The HPLC column used was a Phenomenex Ultracarb 5ODS (30), 250 mm x 4.6 mm i.d. Solvents were: i) water with 0.05% formic acid and ii) ACN with 0.05% formic acid. Each run consisted of a linear gradient from 95:5, water:ACN to 5:95 water:ACN over 60 min, followed by constant 5:95, water:ACN for 5 min, then return to 95:5 water:ACN over 10 min for 75 min run time. The column temperature was 30 ˚C. Preliminary quantification was performed using β-Ram detector software. Background noise was subtracted and fraction of total radioactivity attributable to BAS 505 determined. In turn, this fraction was multiplied by radioactivity in the sample determined by separate LSC analysis. Radioactivity due to any other discernable peaks was similarly quantified.

Combustion of Samples for LSC

Duplicate 0.3 g sub-samples of residual soil from extraction bottles, with added 0.3 g of cellulose, were oxidized on United Technologies Packard Model 306 oxidizer (Packard

Instrument Co., Downers Grove, IL) to quantify unextractable ^{14}C. Liberated ^{14}C was trapped and analyzed by LSC (Davidson et al., 1970).

Estimation of ^{14}C Recoveries from Biometer Flask Soil and Intact Cores

Total recovery of ^{14}C for biometer flask soil was the sum of radioactivity in MeOH extract, MeOH:water extract, liberated on combustion and evolved as $^{14}CO_2$. Total recovery for core soil was the sum of ^{14}C in the extraction sub-sample (that in MeOH extract, MeOH:water extract and liberated on combustion) + that estimated for the microbial biomass C sub-sample (assumed proportional to recovery from the extraction sub-sample) + radioactivity in remaining soil (determined by combustion of triplicate samples) + evolved $^{14}CO_2$ trapped in NaOH.

Modeling and Statistical Analyses

The degradation rate of BAS 505 was modeled by first-order degradation kinetics, $M = M_o \exp(-kt)$, where M is mass of BAS 505 recovered (μmole), M_o is mass applied, t is time (d) and k is degradation rate constant (d^{-1}). The power rate or N^{th}-order kinetic model (Hamaker, 1972), $M = [M_o^{(1-N)} + (N-1)kt]^{(1/[1-N])}$, where N is order (dimensionless), was also considered.

Sorption was described with Freundlich and linear models. The Freundlich is $S = K_f C^N$, where S is sorbed concentration (μmol kg^{-1}), C is solution concentration (μmol L^{-1}), and K_f and N are constants. The linear model is $S = K_d C$, where K_d (L kg^{-1}) is the sorption coefficient.

The ANOVAs and curve fitting were done with SAS (SAS Institute, Inc., 1996).

Results and Discussion

Sorption / Desorption

BAS 505 sorption data (Fig. 2.1) were described using a Freundlich isotherm with best-fit estimates of $K_f = 4.89 \pm 0.08$ and $N = 0.96 \pm 0.02$. Thus, BAS 505 was sorbed essentially in direct proportion to its solution concentration (N = 1). The best-fit linear model gave an R^2 of

0.999 with $K_d = 4.74 \pm 0.16$. Since for any mass of BAS 505 in the Ruston soil at 0.75 of field capacity (= 0.149, Table 2.1), a K_d of this magnitude leaves only about 0.03 of the BAS 505 in solution and labile to biological degradation. Such large extent of sorption might affect BAS 505 degradation rate if there is a difference in degradation rates between solution and sorbed phases, and desorption was slow (Zablotowicz et al., 2000). However, data for desorption of BAS 505 by sequential extraction with 0.01 N $CaCl_2$ (Table 2.2) showed little kinetic limitation. Desorbed BAS 505 nearly equaled that predicted assuming instantaneous equilibrium described by K_d.

The sorption / desorption results are strictly applicable to the batch soil. Somewhat greater sorption in the surface soil of cores might occur due to higher organic C content than averaged over the 0 to 7.5 cm depth. However, if desorption of BAS 505 in the surface soil exhibited slow kinetics, this behavior, though less pronounced due to admixture with sub-surface soil, still should have been evident.

Microbial Biomass

A reasonable hypothesis was that soil treated with BAS 505 would have significantly lower microbial biomass than untreated soil, considering its fungitoxic nature. However, variability among replicates was large and differences in biomass C were not statistically significant (Fig. 2.2). Also, the apparent decrease with time in microbial biomass C in the treated soil was not significant.

Extractable, Unextractable, Mineralized and Total Recoveries of ^{14}C

Total recoveries of ^{14}C, based on recoveries in the various fractions (MeOH and MeOH: water extracts, $^{14}CO_2$ mineralized and as unextractable, UnEx, ^{14}C) are summarized in Table 2.3. Recoveries in the extractable and unextractable pools were determined by taking the proportions recovered as ^{14}C from sub-samples to represent the situation for the whole core, then multiplying

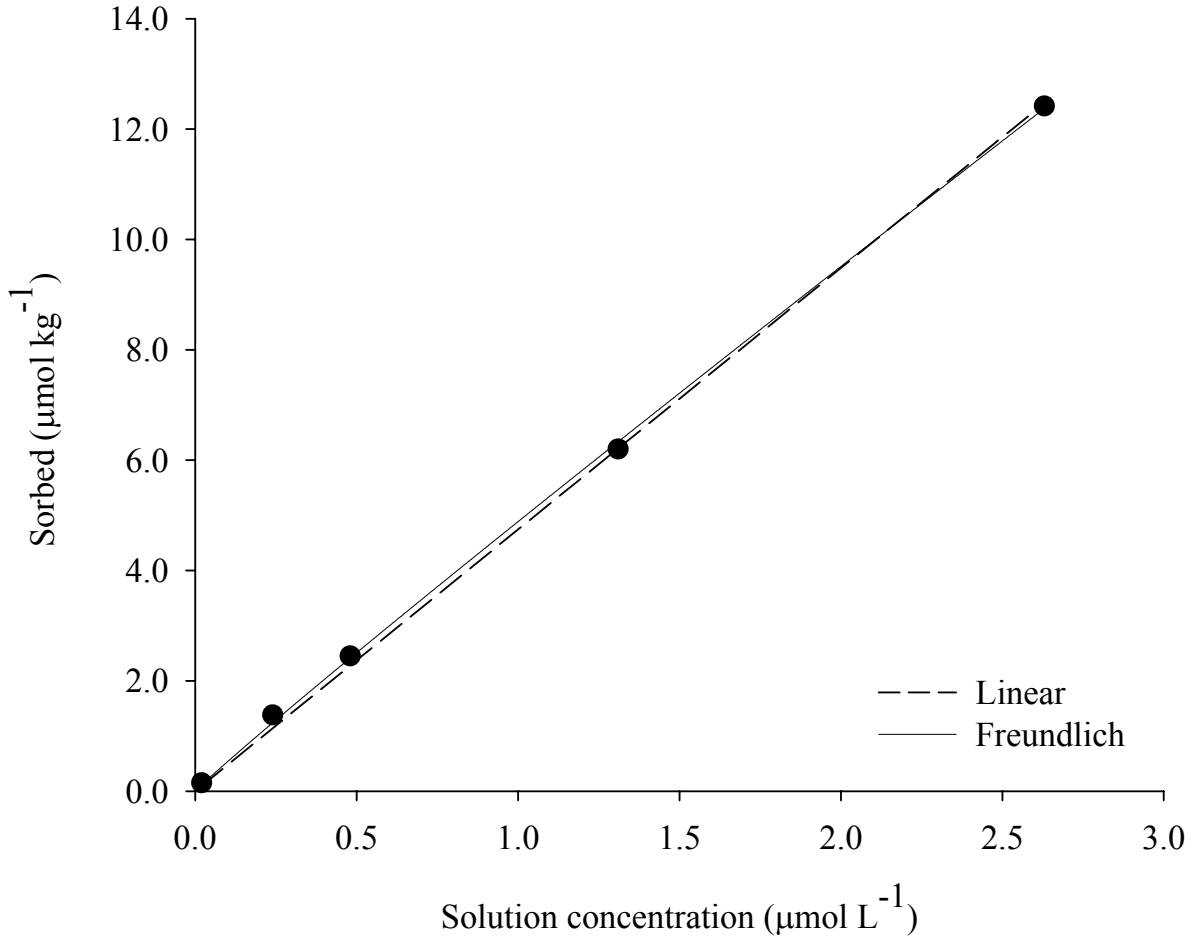

Figure 2.1. BAS 505 sorption described by the Freundlich and linear models. The Freundlich model is $S = 4.89\ C^{0.961}$ ($R^2 = 0.999$) and the linear model is $S = 4.74\ C$ ($R^2 = 0.999$).

Table 2.2. Desorption of BAS 505 that was sorbed during a 24 h equilibration.

Extraction		Fraction Desorbed		
#	Time (h)	Per Extraction	Cumulative	Predicted
1	1.5	0.55 ± 0.02	0.55 ± 0.02	0.62
2	4.5	0.28 ± 0.01	0.83 ± 0.03	0.87
3	18.0	0.14 ± 0.01	0.97 ± 0.03	0.97

†Stepwise and cumulative fractions desorbed by sequential extractions, and predicted desorbed (linear, instantaneous equilibrium) are given.

by the total ^{14}C recovered, less that evolved as $^{14}CO_2$, then dividing by the total ^{14}C applied. Total recoveries of ^{14}C were acceptably high, averaging 92 % and 93 % for cores and biometer flasks, respectively, with no trend toward decreasing recovery with time. Methanol extraction accounted for ~ 87 % to 90 % recovery of the radiolabel at day 0 for both batch and core soils, decreasing to ~ 66 % and ~ 46% for batch and cores soils, respectively, at day 360. A subsequent MeOH:water extraction accounted for an additional 3 % to 6 % for batch and 5 % to 10 % for core soils throughout the study.

Mineralization rates of BAS 505 from batch and intact soils are shown in Fig. 2.3. The rate of mineralization is the fraction of total applied ^{14}C that was evolved as $^{14}CO_2$ during any sampling interval, divided by the length of that interval. Data are averages for all experimental units at any time (i.e., decreasing from 33 biometer flasks at day 2 to 3 flasks beyond day 270; or decreasing from 57 cores to 5 cores, the full and abridged sets combined). The initial rate over

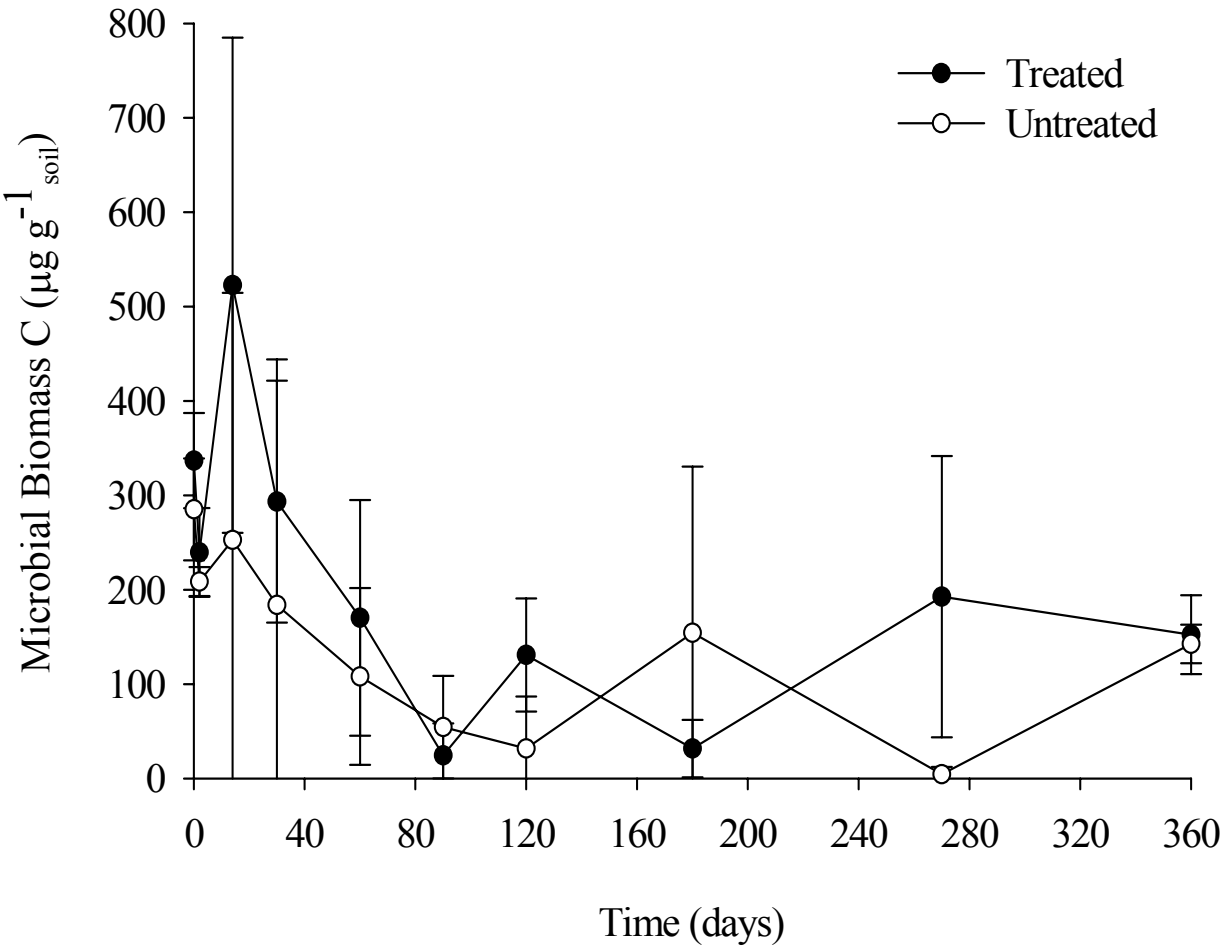

Figure 2.2. Comparison of microbial biomass C in cores of Ruston surface soil with and without treatment with BAS 505 (0.28 kg ha^{-1}). Means for five treated and three untreated cores are shown.

the 0 to 2 d incubation for the batch soil (0.00144 μg mineralized d^{-1} / μg applied) was nearly five times that for the cores (0.00030 μg mineralized d^{-1} / μg applied). This contrasting behavior has been seen before (Gaston and Locke, 1996 and 2000) and was likely due to the comparatively large surface area to mass ratio for soil in the biometer flasks compared to the soil cores. Mineralization rate for the batch soil decreased sharply at first and then gradually over the course of the study (Fig. 2.3), whereas that for cores decreased sharply at first but then increased to a nearly constant rate of $^{14}CO_2$ loss that was much greater than for the batch soil. At day 360, batch soil had a mineralization rate of 0.00009 d^{-1} compared to 0.00068 d^{-1}. However, integration of the curves (Fig. 2.3) over the study gave similar fractions of applied ^{14}C mineralized (~ 8 % for batch soil and ~ 11 % for cores). Inasmuch as mineralization is complete degradation, a higher rate of mineralization in cores than batch soil suggests different kinetics and more complete degradation in the cores.

Unextractable ^{14}C for intact cores (UnEx, Table 2.3) increased from approximately 1 % to 21 % over the year compared to < 1 % to 18 % for batch soils. Unlike patterns in mineralization rates, there was no statistical difference between batch and intact cores in development of unextractable ^{14}C.

Degradation of BAS 505

There was little difference in variability among replicates from the triplicate and duplicate core series in recovery of BAS 505 (Table 2.4). Across all cores, recovery averaged 92% on Day 0. After one year, recovery averaged 36 %. For the batch soil, BAS 505 recovery decreased from 93 % to 57 %. Comparison of extractable ^{14}C (Table 2.3) with recovered parent (Table 2.4) shows that from Day 2, an increasing proportion of extractable ^{14}C was due to compounds other than parent. However, distinct peaks in HPLC chromatograms other than due

Table 2.3. Percentage recoveries of ^{14}C (as fraction of applied) from cores and batch soil in different pools.

Extraction Time (Days)	Experimental System									
	Cores[†]	Batch	Cores	Batch	Cores	Batch	Cores	Batch	Cores	Batch
	MeOH	MeOH	MeOH: water	MeOH: water	UnEx	UnEx	$^{14}CO_2$	$^{14}CO_2$	Total ^{14}C	Total ^{14}C
0	86 ± 7 a[‡]	90 ± 2 a	5 ± 2 a	3 ± 0 a	1 ± 0 b	0.3 ± 0.1 a	-----	-----	93 ± 8 a	93 ± 2 a
2	83 ± 6 a	87 ± 1 a	7 ± 0 b	4 ± 0 a	2 ± 0 a	2 ± 0.2 a	0.1 ± 0 b	0.3 ± 0 a	92 ± 7 a	93 ± 1 a
5	83 ± 13 a	83 ± 2 a	6 ± 2 a	5 ± 1 a	2 ± 1 a	3 ± 1 a	0.1 ± 0 b	0.5 ± 0 a	92 ± 14 a	90 ± 2 a
7	78 ± 3 a	82 ± 4 a	9 ± 1 b	3 ± 0 a	4 ± 1 a	3 ± 1 a	0.2 ± 0 b	0.6 ± 0 a	91 ± 3 a	88 ± 5 a
14	86 ± 12 a	83 ± 0 a	8 ± 2 b	3 ± 0 a	5 ± 2 a	5 ± 0.2 a	0.3 ± 0 b	1 ± 0 a	99 ± 15 a	92 ± 0 a
30	78 ± 5 a	77 ± 2 a	7 ± 2 a	5 ± 0 a	6 ± 1 b	8 ± 1 a	0.4 ± 0.1 b	2 ± 0 a	91 ± 4 a	92 ± 1 a
60	68 ± 2 a	68 ± 1 a	11 ± 3 b	6 ± 0 a	9 ± 2 b	14 ± 1 a	0.6 ± 0.1 b	4 ± 0.3 a	88 ± 4 a	92 ± 1 a
90	76 ± 5 a	69 ± 4 a	8 ± 1 b	5 ± 1 a	9 ± 2 b	14 ± 3 a	1 ± 0.2 b	5 ± 1 a	94 ± 7 a	93 ± 1 a
120	63 ± 11 a	61 ± 1 a	9 ± 2 b	5 ± 1 a	14 ± 4 a	18 ± 3 a	2 ± 1 b	7 ± 1 a	88 ± 16 a	91 ± 4 a
180	65 ± 8 a	64 ± 8 a	8 ± 2 a	6 ± 1 a	17 ± 3 a	19 ± 4 a	3 ± 1 b	7 ± 3 a	93 ± 11 a	96 ± 0 a
270	52 ± 11 a	56 ± 4 a	8 ± 2 a	6 ± 1 a	22 ± 5 a	20 ± 4 a	10 ± 6 a	12 ± 4 a	93 ± 5 a	94 ± 6 a
360	46 ± 5 b	66 ± 10 a	6 ± 1 a	5 ± 1 a	21 ± 4 a	18 ± 7 a	11 ± 2 a	7 ± 6 a	84 ± 7 b	96 ± 3 a

[†]Combined mean for triplicate and duplicate intact core series.
[‡] At any time, means for same ^{14}C pool (cores and batch) followed by the same letter are not significantly different (LSD $\alpha = 0.05$).

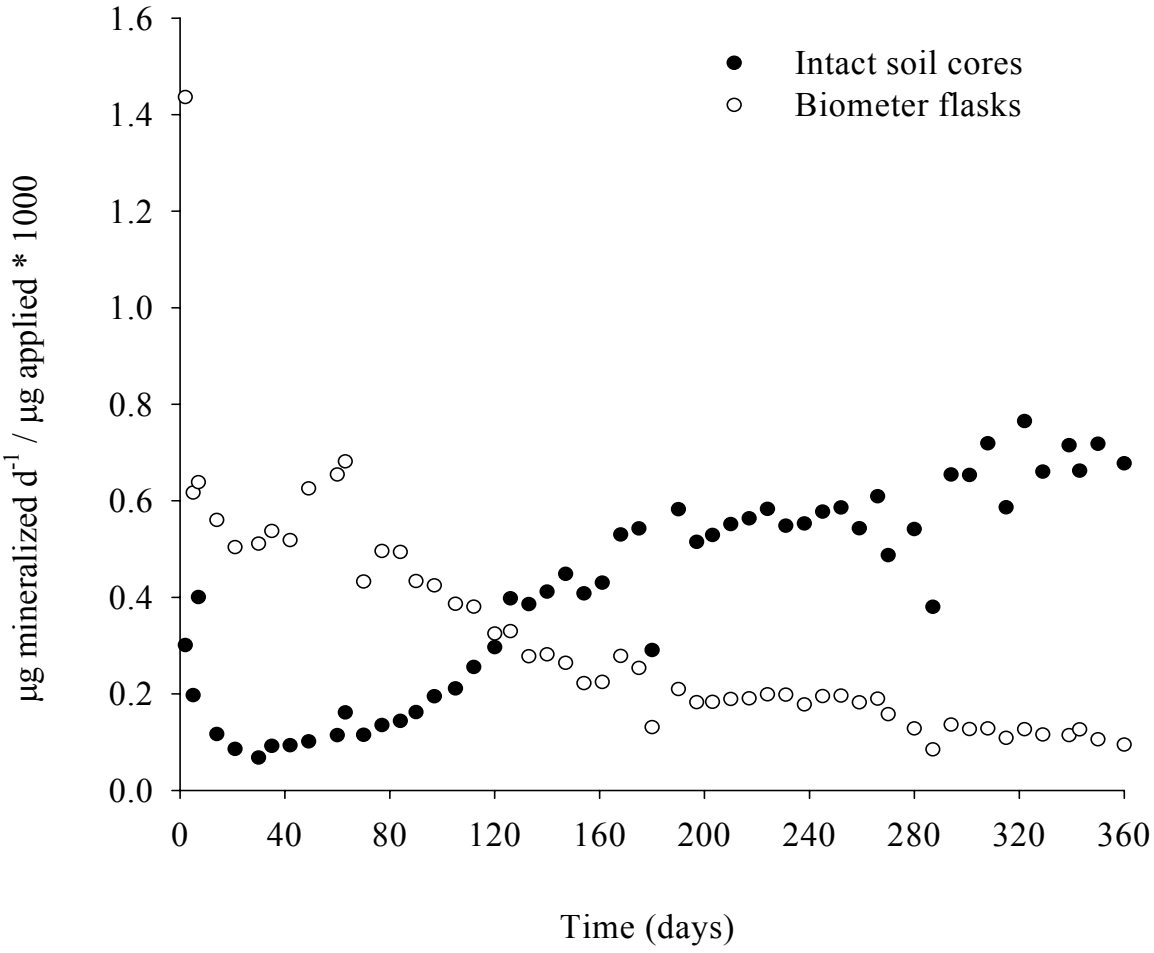

Figure 2.3. Mineralization rate of BAS 505 in batch and intact soils (triplicate and duplicate series combined).

Table 2.4. Percent recoveries of BAS 505 (μg recovered /applied) from cores and batch soil in MeOH and MeOH: water extracts (data combined).

Time (days)	Triplicate Cores	Duplicate Cores	All Cores[†]	Batch Soils
0	94 ± 9	87 ± 7	92 ± 8 a[‡]	93 ± 2 a
2	93 ± 7	85 ± 1	90 ± 7 a	87 ± 1 a
5	85 ± 16	76 ± 1	82 ± 12 a	78 ± 1 a
7	76 ± 2	73 ± 0	75 ± 3 a	77 ± 3 a
14	78 ± 2	76 ± 21	77 ± 11 a	78 ± 1 a
30	66 ± 6	72 ± 4	69 ± 6 a	73 ± 1 a
60	58 ± 2	55 ± 1	57 ± 2 a	58 ± 1 a
90	64 ± 2	62 ± 3	63 ± 2 a	63 ± 6 a
120	51 ± 10	60 ± 3	56 ± 8 a	54 ± 2 a
180	51 ± 7	57 ± 4	53 ± 7 a	52 ± 10 a
270	44 ± 10	39 ± 13	42 ± 10 a	53 ± 5 a
360	37 ± 7	36 ± 2	36 ± 4 b	57 ± 10 a

[†]Combined mean for triplicate and duplicate intact core series.
[‡]Within a row, means followed by the same letter are not significantly different (LSD $\alpha = 0.05$).

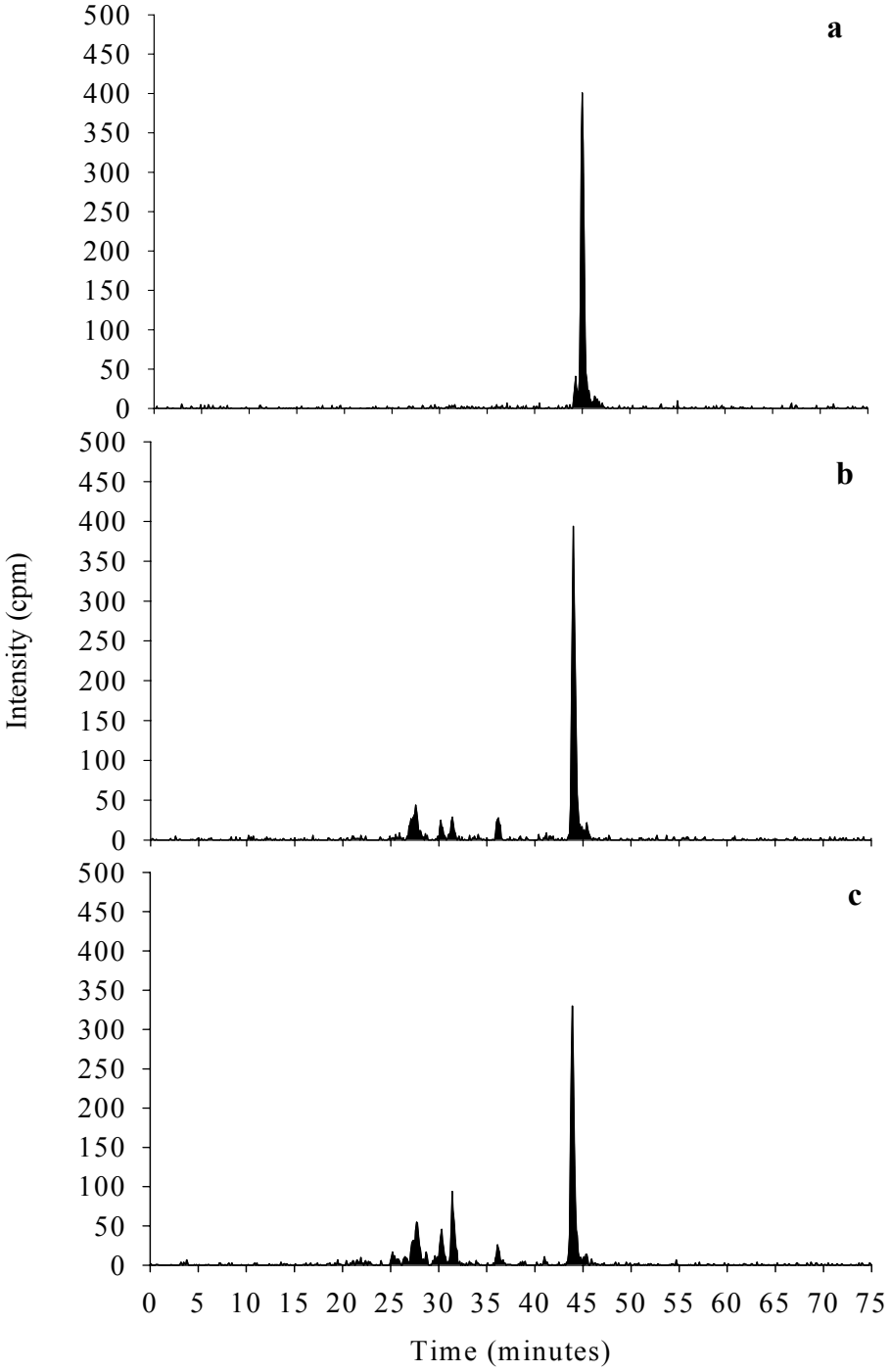

Figure 2.4. HPLC chromatograms for intact core series: (a) Methanol extract-Day 0, (b) Methanol extract-Day 360 and (c) Methanol:water extract-Day 360.

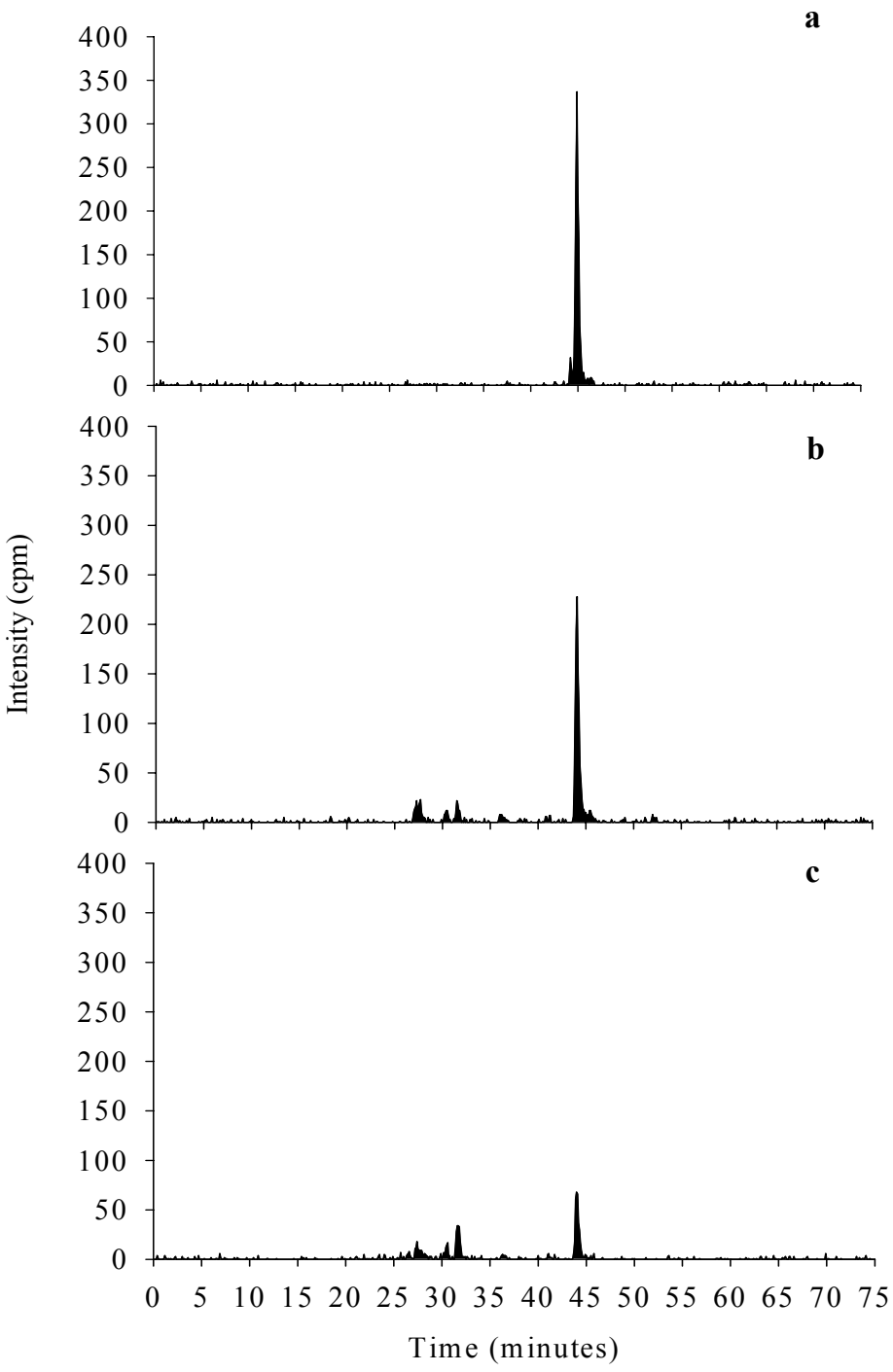

Figure 2.5. HPLC chromatograms for batch soil series: (a) Methanol extract-Day 0, (b) Methanol extract-Day 360 and (c) Methanol:water extract-Day 360.

to BAS 505 were not observed until after 120 d incubation. Figures 2.4 and 2.5 show LSC chromatograms of BAS 505 and its metabolites for intact and batch soils after extraction with MeOH at day 0, and MeOH and MeOH:water at day 360 of the study. Peaks included those between 29 and 32 min that correspond to retention times of BF 505-7 [*(E)-o-[(2-hydroxycarbonyl-5-methyl)phenoxymethyl]-2-methoxyimino-N-methylphenylacetamide*] and BF 505-8 [*(E)-o-[(5-hydroxycarbonyl-2-methyl)phenoxymethyl]-2-methoxyimino-N-methyl phenylacetamide*], and at ~ 27 - 28 min and ~ 36 - 37 min. The latter coelutes with methyl salicylate (which would form upon ether cleavage followed by oxidation and decarboxylation of BAS 505).

Based upon recovery data, the degradation rate of BAS 505 in cores was significantly greater than that in batch soil only by Day 360 (Table 2.4). Also, Fig. 2.6 shows that rather than first-order kinetics, N^{th}-order kinetics clearly better described BAS 505 degradation over the full course of the study for both intact core and batch soils (Table 2.5 gives model parameters). Data from the first 60 days, however, were consistent with those generated in registration studies. Deviation from first-order kinetics / slow overall degradation and faster degradation in cores than batch soil by Day 360 warrant further consideration.

Slow desorption of the highly sorbed BAS 505 was likely not the cause of deviation from first-order kinetics and slow overall degradation (see Table 2.2). Another possible explanation is decreasing microbial activity, particularly activity of BAS 505 degraders. But no trend in microbial biomass C with time could be established due to variability in the data (and organisms with a specific capacity to degrade BAS 505 were not identified or enumerated). Nevertheless, lack of nutrient input through the soil surface with infiltrating water (absent in static systems) or

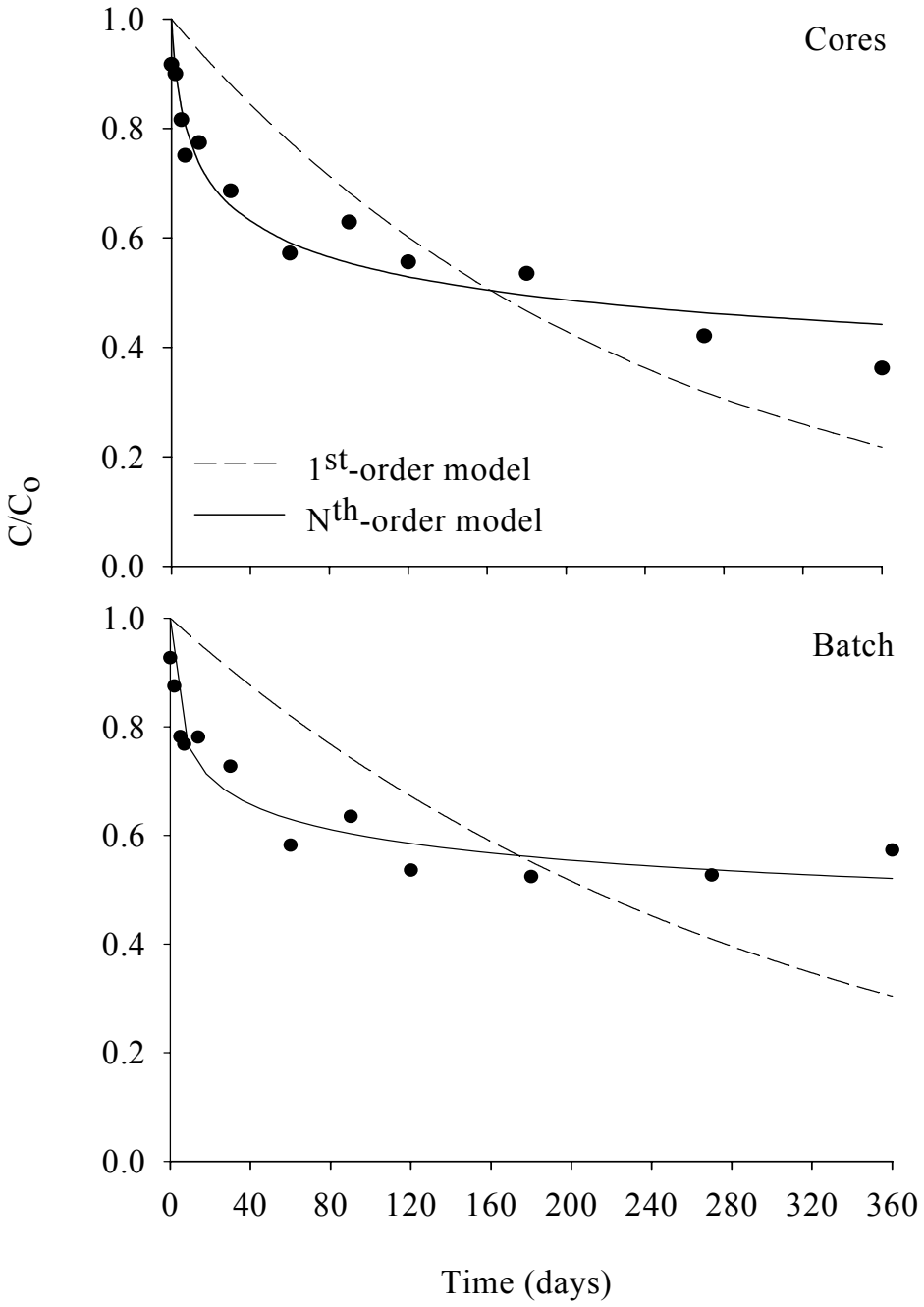

Figure 2.6. Degradation of BAS 505 described by 1st- and Nth -order models for intact cores (top, triplicate and duplicate series combined) and batch soil (bottom).

Table 2.5. First- and N^{th}-order degradation model parameters for 360 d incubation and 1^{st}-order rate constants for 60 d incubation.

System	Incubation (d)	Model	k (d^{-1})	N
Batch	360	1^{st}-order	0.003 ± 0.001	-----
	360	N^{th}-order	0.134 ± 0.064	10.38 ± 1.40
	60	1^{st}-order	0.012 ± 0.003	-----
Cores†	360	1^{st}-order	0.004 ± 0.001	-----
	360	N^{th}-order	0.063 ± 0.024	7.04 ± 0.99
	60	1^{st}-order	0.012 ± 0.003	-----

† Triplicate and duplicate series combined.

to the living rhizosphere may have had a negative effect on microbial activity in these long-term laboratory incubations. On the other hand, decreasing degradation rate may simply reflect small-scale spatial variability in degradation rate (Gustafson and Holden, 1990), for which a continuum of spatially variable first-order rate constants generates the type of nonlinearity seen in Fig. 2.6.

Regardless of why degradation of BAS 505 slowed over time, it was ultimately faster in cores than batch soil –an important result with respect to the objective of the study. Faster degradation in cores by Day 360 may be related to differences in microbial populations and organic matter substrates between cores and batch soil. Coarse organic matter (roots) was removed from the batch soil, depleting its C pool. But the physical disruption of the soil may have exposed otherwise protected substrate (Franzluebbers and

Arshad, 1997). More importantly, BAS 505 was applied to the surface of cores, where both microbial populations and substrate levels were likely highest, but it was applied to homogenous 0 to 7.5 cm depth, batch soil. Whatever advantage better aeration of the batch soil initially offered, lower average microbial populations and substrate levels in it may have limited the degradation of BAS 505 at longer times. In contrast, the highly sorbed BAS 505 remained near the soil surface in cores.

Summary

In this study, an intact core methodology for measuring aerobic pesticide degradation was developed and the degradation rates of BAS 505 determined using this and conventional (batch) methodologies were compared. The extent of core replication needed for adequate precision was concurrently examined. Also, the effect of BAS 505 on microbial biomass was tracked over this year-long project.

Recommendations

Results led to several recommendations on intact core methodology for measuring aerobic degradation. With respect to the trial core apparatus, unless the soil is apparently structured, a smaller diameter core than that used (7.5 cm) is recommended, if collecting sub-samples as sub-cores. While this would decrease the extent of short-range soil variability captured by the core sample, the smaller mass of soil would result in proportionally larger extraction sub-samples and likely decrease experimental error among replicates. Alternatively, the entire soil core (7.5 cm) should be removed from its stainless steel casing, thoroughly mixed and a sub-sample then collected.

Beyond methodological details, long-term incubations seem undesirable because experimental artifacts may be exaggerated. Microbial populations in surface field soils

are in flux due to positive and negative environmental influences. While the intent of controlled conditions (temperature and water content) is to increase uniformity over time, lack of nutrient input via organic matter added as surface litter (and transport into the soil with infiltrating water, absent in static systems) or to the rhizosphere, for example, is a possible negative effect in laboratory incubations. The longer the incubation, the more pronounced the negative effect. Thus results from long-term degradation studies, particularly in wholly artificial systems like homogeneous soil in biometer flasks, may be poor approximations to and substantially underestimate biodegradation rate in the field.

Reducing the number of replicates per sampling time to two is not recommended because precision suffered. Sub-sampling of homogenized soil cores may compensate but if the number of sampling times were reduced (shorter total incubation time), more replicates would be needed to ensure low uncertainty in estimates of rate constants.

Microbial biomass C data showed high variability despite best efforts to exclude extraneous C sources (e.g, thatch and roots). Therefore, sub-samples from intact cores (especially of turf soil) may not be well-suited to this assay but sub-sampling of homogenized soil from cores might improve precision.

Experimental Conclusions

Based on BAS 505 recoveries, there was no difference in degradation rate between core (triplicate, duplicate or combined) and batch systems until Day 360. Exposure of BAS 505 to likely higher microbial populations in cores (surface soil) compared with batch (homogenized 0 to 7.5 cm depth) soil is a possible explanation for long-term faster degradation in cores. Regardless of system, the degradation rate of BAS 505 in Ruston soil decreased with time. This nonlinearity, described by N^{th}-order

kinetics, may be an artifact of (static) incubation without nutrient re-supply to soil microorganisms. However, there was high variability in microbial biomass C data and no significant trend toward decreasing biomass with time.

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CHAPTER 3

DEGRADATION OF BAS 505 IN BULK SOIL AND INTACT CORES FROM DIFFERENT LANDSCAPE POSITIONS

Introduction

Soil properties vary spatially in vertical and lateral directions. Such variability may affect the efficacy and fate of pesticides on and in the soil. For example, the extent of field-scale soil lateral variability demonstrated in Staddon et al. (2004), Farenhorst et al. (2001), Gaston et al. (2001) or Novak et al. (1999) would be expected to influence pesticide degradation rate, directly or indirectly through sorption effects. Thus, varied rates of pesticide application may be necessary to achieve optimal pest control but minimal off-site pesticide loss (Gaston et al., 2001).

Such variability should be taken into account when assessing pesticide fate for whatever purpose, including product registration. Typically, however, fate studies use homogenized, composite soil samples. Although this test medium may capture the range of many relevant soil properties (e.g., texture, OC, pH and microbial activity / population density), the effects of soil structure on aeration / drainage and short-range variability in biological and chemical properties are lost. Clearly, the effect of field-scale lateral variability is lost to averaging.

A comparison of BAS 505 [*N-methyl-(E)-2-methoxyamino-2-(2-((2,5-dimethylphenoxy)methyl)phenyl)acetamide*] degradation in batch and intact core soil showed faster degradation in cores, but only after a year incubation (Chapter 2). Regardless of experimental system, degradation of this turf / cereal fungicide was slow (nearly ½ of applied remaining after a year). Questions raised in the yearlong degradation of BAS 505 (Chapter 2) led to the current study.

In particular, given the spatial variability in soil biological, chemical and physical properties at the study site, it was uncertain whether either lack of short-term differences between the degradation of BAS 505 in batch and core systems or overall slow degradation were typical at the field-scale. Little difference in degradation between systems may have been a site-specific result. Also, slow degradation in the laboratory compared to field dissipation (Scott Jackson, BASF, personal communication) may have been site-specific or a methodological artifact. Laboratory incubation studies are static, without natural recharge of soil fertility by nutrient cycling or inputs from living root biomass to the rhizosphere. Waning fertility may not have an impact on microbial dynamics in short-term incubations but may in long-term studies such as in Chapter 2. Thus, the major objectives of this study with BAS 505 were to: 1) determine the spatially variable range in degradation rate; 2) compare degradation in batch and core soil taken from different locations in the original study site, thereby confirming that the two media present equivalent degradation environments; and 3) compare degradation rate in the presence and absence of living turf grass.

The study site, a coastal plain soil that was cleared of forest for cotton [*Gossypium hirsutum*] production and much later converted to pasture, was expected to exhibit evidence of erosion, with surface texture increasing in sand going down slope (Walthall and Nolfe, 1998). The site was mapped for biological, chemical and physical properties that may affect pesticide degradation and soil from shoulder, backslope and toeslope positions (USDA-NRCS, 2005) was used in the study.

Materials

Study Site and Soil Sampling

The study site, located at the LSU AgCenter Calhoun Research Station, Calhoun LA, has Ruston series (fine-loamy, siliceous, thermic Typic Paleudult) soil planted with bermudagrass (*Cynodon dactylon* (L.) Pers.). It is bounded by forest on two sides, a tilled field and an intermittent stream on the others. A 40 m x 80 m sub-area of the site was grid sampled to 7.5 cm on a regular 10 m grid (three sub-samples per grid node). Samples were sealed in air-tight bags and kept chilled during return to the laboratory, where these were used to measure biological, chemical and physical properties.

Core (7.5 cm diameter by 7.5 cm deep) samples in stainless steel sleeves were taken from 2 x 2 m areas on hill shoulder, backslope, and toeslope positions within the grid-sampled area, thereby giving samples representative of the range of soil properties at the site. These were sealed in air-tight wrapping, padded to avoid jarring and kept chilled during transport to the laboratory, where 18 cores per landscape position were randomly selected as experimental intact core units and soil in remaining cores was combined to give bulk soil samples. Three additional cores from each position were taken for soil bulk density using a 5-cm diameter corer. Grass stems and thatch were later removed from the surface of intact cores, soil protruding from the bottom was cut away flush, and cores were weighed to determine net mass of field-moist soil. Bottoms of cores were fitted with Teflon end disks. These were re-wrapped and stored at 4 ^{o}C pending the degradation study. Bulk samples were thoroughly mixed and stored at 4 ^{o}C pending the degradation study.

Test Compound, Reagent Chemicals and Solvents

Radiolabeled (phenyl-U-^{14}C) BAS 505 (95 % purity, total activity 96.2 MBq) was provided by BASF Corp., Research Triangle Park, NC. The major impurity was the (Z) isomer of BAS 505 (Fig 1.1). All chemicals were reagent grade and chromatography solvents were HPLC grade. Scinti-Safe Plus 50 % scintillation cocktail (Fisher) was used for LSC except for combusted soil samples (Carbo Sorb / Permafluor, Perkins Elmer) and HPLC-LSC analysis (In-Flow BD, IN/US Systems, Inc.).

Methods

Soil Characterization

Grid samples were mixed and sub-samples analyzed for moisture content. Field moist sub-samples were adjusted to uniform water content and assayed for flourescein diacetate (FDA) hydrolytic activity (Schnürer and Rosswall, 1982). Remaining soil was air-dried, ground, sieved (< 2 mm) and analyzed for organic C (Nelson and Sommers, 1982), pH (2:1, water:soil) and texture (Gee and Bauder, 1986).

Field-moist sub-samples of bulk soil from each landscape position were assayed for biomass C (Vance et al., 1987) but not FDA hydrolysis, and sub-samples were air-dried, ground, sieved and analyzed for the above parameters plus CEC (sum of basic and acidic cations; 1 N NH_4OAc and $BaCl_2$-TEA extractions, respectively, USDA-NRCS, 2004). All analyses were in triplicate except texture. Volumetric water content at 75 % field capacity (1 / 3 bar) for bulk soils was estimated using measured texture and bulk density via pedotransfer function parameters given in Wosten and van Genuchten (1988).

Preparation of BAS 505 Application Solution

Radiolabeled material was reconstituted in 40.0 mL of acetonitrile. Activity consistent with BASF data was confirmed using LSC (Beckman Instruments, Inc. LS Analyzer, Model LS6KLL). A portion of the reconstituted BAS 505 was diluted in more acetonitrile to produce 54.04 μg mL^{-1}, which if applied in 100 μL to 20 g soil in the biometer flasks would equal a rate of 0.28 kg a.i. ha^{-1}. This concentration assumed that 20.0 g of soil to 7.5 cm depth represented a field surface area of 1.93 cm^3 (based on measured bulk density).

Batch Methodology

Twenty g (oven-dry equivalent) of soil were transferred to each of 36 biometer flasks (Bartha and Pramer, 1965) and nine 250-mL Nalgene centrifuge bottles. One hundred μL of the 54.04 μg mL^{-1} BAS 505 were applied to the soil, thoroughly mixed with a stainless steel spatula, followed by sufficient water to achieve 75 % of field capacity. Weights of flasks with treated soil were recorded. The dosed soil in centrifuge bottles was immediately extracted as described below to determine recovery of ^{14}C and BAS 505. Ten mL of 1 N NaOH was added to flask sidearms, flasks securely stoppered and set in a dark, temperature-controlled room (23 ^oC).

Soil in biometer flasks was removed after 14, 30, 60, and 120 d incubation and transferred to 250 mL Nalgene centrifuge bottles for extraction. Samples were extracted with MeOH (~30 mL x 3 times). Soil suspensions were shaken (325 rpm) at room temperature in the dark for one hour. Methanol supernatants were decanted into separate glass bottles (Teflon-lined caps), extraction repeated and supernatants combined. Mass of extract and entrained MeOH were recorded. The latter was evaporated, dry soil removed from the centrifuge bottle and ground prior to analysis for unextractable ^{14}C (combustion / LSC, described below). Duplicate 1

mL aliquots of MeOH extracts were analyzed by LSC. The MeOH extracts were concentrated by rotary evaporation. All concentrated extracts were analyzed by HPLC-LSC as described below.

One mL aliquots of NaOH in biometer flask sidearms were removed weekly for LSC analysis of evolved $^{14}CO_2$. Remaining NaOH was removed, weights of flasks measured and water added as needed to restore 75 % field capacity. Fresh NaOH was added, flasks stoppered and incubation continued. To ensure aerobic conditions, flasks were also briefly unstoppered at midweek.

Intact Core Methodology

A randomly selected set of 15 cores from shoulder, backslope, and toeslope (total of 45 cores) were used. Each was dosed with 2.36 mL of 54.04 μg mL^{-1} BAS 505 to give an aerial application rate of 0.28 kg ha^{-1}. Sufficient water was then applied to the surface of each core to bring its average water content to 75 % field capacity and mass of cores recorded. Thirty-six cores were then placed in 5 L capacity airtight containers and into each was also placed an open vial containing 20 mL of 1 N NaOH as a $^{14}CO_2$ trap. Containers were sealed and incubated in the dark at 23 ^{o}C. The remaining nine cores (day 0 replicates) were sampled as described below immediately following application of BAS 505 and water.

After prescribed incubation, cores were removed from containers, soil emptied from cores, thoroughly mixed and 50 g oven-dry equivalent sub-samples were obtained for extraction. Sub-samples were transferred to 250 mL Nalgene centrifuge bottles and extracted as described for biometer flasks (except larger volumes of MeOH, proportional to the greater mass of soil). Processing of extracts and LSC analyses were as above. Remaining soil was crumbled, mixed

and preserved for ^{14}C analysis by combustion. Mineralization was tracked as above. Cores were watered as indicated by loss of mass. Containers were briefly opened midweek for re-aeration.

Sorption Equilibrium Study

Inasmuch as sorption may influence degradation (Zablotowicz et al., 2000), retention of BAS 505 by shoulder, backslope and toeslope soils was also determined. Five-g (oven-dry equivalent) samples of these soils were placed in 50-mL Pyrex centrifuge tubes. Fifteen-mL of 0.1, 1, 2, 5 or 10 μM solutions of ^{14}C BAS-505 were added to centrifuge tubes in triplicate. Another triplicate set of these solutions was added to tubes without soil to measure sorption, if any, onto tubes. Suspensions and blanks were shaken 24 h. Soil solution was separated from suspension by centrifuging (10 min at 3,000 g). Sorption was calculated from change in solution concentration of ^{14}C, corrected for blank sorption.

Influence of Bermudagrass on BAS 505 Degradation

Eighteen PVC cores (10.0 cm diameter by 10.0 cm long) were fitted with end-caps and packed with Ruston soil (equal mix of that from slope positions) to a bulk density of 1.35 g cm^{-3}. Cores were wet to 75 % field capacity and six cores seeded with bermudagrass. Half of the remaining cores were loosely covered with Al foil (giving three treatments, grass + sun, bare + sun and bare – sun) and all were set in the greenhouse. Mass of cores was monitored and evaporative / transpirational losses replaced daily. When grass was well-established, unlabeled BAS 505 was applied to soil beneath grass or to bare cores at a rate = 3.24 kg ha^{-1} (= 12 x prescribed rate so as to ensure recoveries quantifiable by HPLC / UV detection), shown in Fig 3.1. A layer of thatch (0.2 g / core) was also added to soil beneath grass. As previously,

Figure 3.1. Example of seeded and bare bermudagrass cores.

soil was maintained at 75 % field capacity and at six and 12 wk, three replicate cores of each treatment were emptied, thoroughly mixed and 50 g extracted, concentrated as previously described and 1 mL samples filtered into HPLC vials for analysis of BAS 505 recovery.

Chemical Analyses

Concentrated MeOH samples were analyzed by HPLC, with LSC (IN/US Systems, β-Ram) and UV detection. The HPLC column was a Phenomenex Ultracarb 5ODS (30), 250 mm x 4.6 mm i.d. Solvents were: i) HPLC grade water with 0.05% formic acid and ii) acetonitrile with 0.05% formic acid. Each run consisted of a linear gradient from 95:5, water:acetonitrile to 5:95 water:acetonitrile over 60 min, followed by constant 5:95, water:acetonitrile for 5 min, then return to 95:5 water: acetonitrile over 10 min for 75 min run time. The column temperature was

30 ˚C. Preliminary quantification was performed using β-Ram detector software. In particular, background noise was subtracted and fraction of total radioactivity attributable to BAS 505 determined. In turn, this fraction was multiplied by radioactivity in the sample determined by separate LSC analysis. Radioactivity due to any other discernable peaks was similarly quantified. Detection of unlabeled BAS 505 was by UV.

Duplicate 0.3 g sub-samples of residual soil from extraction bottles or of residual soil, plus 0.3 g of cellulose, were oxidized (Packard Oxidizer 306, United Technologies Packard). The liberated ^{14}C was trapped and analyzed by LSC (Davidson et al., 1970).

^{14}C Recoveries

Total recovery of ^{14}C for biometer flask soil was the sum of radioactivity in MeOH extract, liberated on combustion and evolved as $^{14}CO_2$. Total recovery for core soil was the sum of ^{14}C in the extraction sub-sample (that in MeOH extract and liberated on combustion) + radio-activity in remaining soil (determined by combustion of triplicate samples) + evolved $^{14}CO_2$ trapped in NaOH. The distribution of ^{14}C between extractable and unextractable in sub-samples was assumed to hold for the entire core.

Modeling and Statistical Analyses

The degradation rate of BAS 505 was modeled by first-order degradation kinetics, $M = M_o \exp(-kt)$, where M is mass of BAS 505 recovered (μmole), M_o is mass applied, t is time (d) and k is degradation rate constant (d^{-1}). Applicability of the N^{th}-order kinetic model (Hamaker, 1972), $M = [M_o^{(1-N)} + (N-1)\ kt]^{(1\ /\ [1-N])}$, where N is the order (dimensionless), was also considered.

Sorption was described with Freundlich and linear models. The Freundlich model is $S = K_f C^N$, where S is sorbed concentration (μmol kg^{-1}), C is solution concentration (μmol L^{-1}), and

K_f and N are constants. The linear model is $S = K_dC$, where K_d (L kg^{-1}) is the sorption coefficient.

Variograms were generated from grid node data, these described by appropriate models and spatial distributions of texture, OC, pH and FDA hydrolysis developed by block-kriging (David, 1977). The ANOVAs and curve fitting were by SAS (SAS Institute, Inc., 1996).

Results and Discussion

Soil Characterization

Figure 3.2 shows change in elevation at the study site. The average slope was 3.8 % (or an angle of 2.2 °). Examples of semivariograms for the mapped properties are plotted in Fig 3.3, together with appropriate variogram models (parameters given in Table 3.1). Variograms for texture were linear for distances set by the dimensions of the sub-area, whereas those for FDA hydrolysis, organic C and pH were spherical. Among the latter, the range was greatest for FDA hydrolysis and least for organic C, with organic C showing only short-range spatial dependence among samples. Linearity of clay and sand variograms to 80 m is consistent with other studies that found long-range spatial dependence (Trangmar et al., 1987; Gaston et al., 2001), extending even for kilometers (Ovalles and Collins, 1988). The fairly short-range spatial dependence in organic C and pH falls with the scatter of findings in the literature. For example, Trangmar et al. (1987) found variogram ranges of only a few meters for both properties, and Campbell (1978) and Staddon et al. (2004) found that pH values were spatially dependent only for about 10 or 20 m, respectively. But Gaston et al. (2001) found spatial dependence in organic C and pH to over 300 m, and Yost et al. (1982) and Trangmar et al. (1986) found long-range dependence in soil pH.

Figure 3.4 shows spatial distributions of clay, organic C and FDA hydrolysis rate generated using variogram model parameters (Table 3.1). Clay content generally decreased and sand content increased (not shown) going to lower positions, consistent with an eroded coastal plain landscape (Walthall and Nolfe, 1998). To some degree, organic C and FDA hydrolysis rate followed this pattern, with lowest values in the sandy toeslope, however, relationships to texture and elevation were weak. There was no clear pattern in pH with respect to texture and elevation (not shown). Regardless, the shoulder, backslope and toeslope sampling areas (Fig. 3.4) cover the range in texture and organic C found at the study site so that potential affects of spatially variable soil properties on BAS 505 degradation should be captured by samples from these positions. Characterization data for the shoulder, backslope and toeslope samples are given in Table 3.2.

Sorption Study

BAS 505 sorption data (Fig. 3.5) were described using a Freundlich isotherm with best-fit estimates for shoulder, backslope, and toeslope of $K_f = 5.45 \pm 0.30$ and $N = 0.99 \pm 0.05$, $K_f = 4.66 \pm 0.38$ and $N = 0.86 \pm 0.06$, and $K_f = 4.29 \pm 0.04$ and $N = 0.91 \pm 0.01$, respectively. Greatest nonlinearity occurred for the backslope soil as was evident in lowest R^2 value when the linear model was fit to the data (R^2 = 0.997, 0.985 and 0.997 for shoulder, backslope and toeslope soils, for linear K_ds = 5.37, 3.92 and 3.84, respectively). Comparison of K_ds with clay and OC data (Figure 3.4 and Table 3.2) show consistency –greatest sorption by shoulder and least by toeslope soils. Such large extent of sorption might affect BAS 505 degradation rate if rates were different in solution and sorbed phases, and desorption was slow (Zablotowicz et al., 2000).

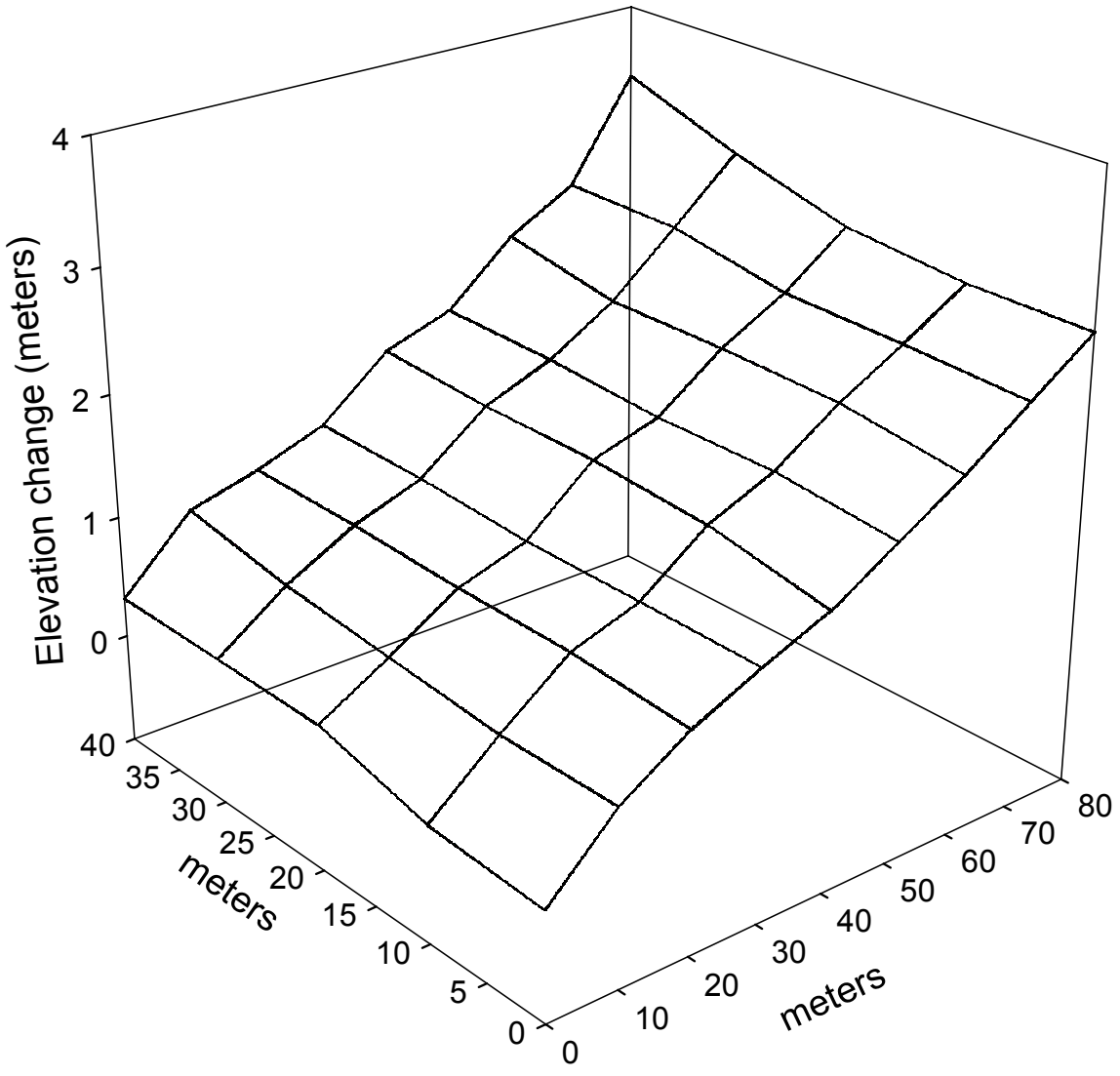

Figure 3.2. Increase in elevation (meters) at the 80 x 40 m sub-area relative to the lowest position.

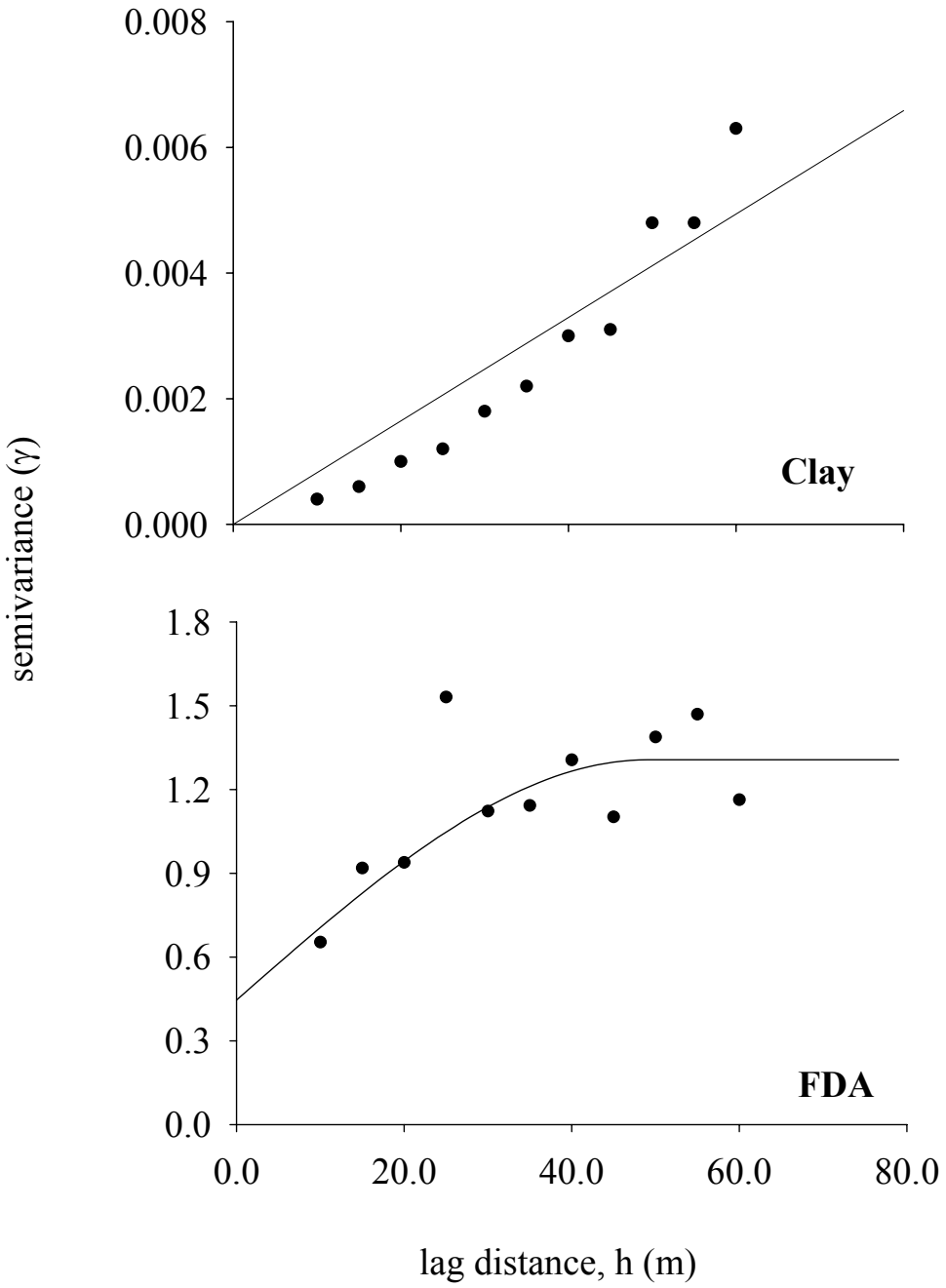

Figure 3.3. Variograms of clay and FDA hydrolysis from surface 0 – 7.5 cm soil.

Table 3.1. Variogram model parameters for properties of surface 0 – 7.5 cm soil at the study site.

Parameter	Nugget, C_o	Sill, $C_o + C$	Range, A (m)	Slope, B	Model
Clay	0.000	-----	-----	0.0008	Linear[H]
Sand	0.000	-----	-----	0.0016	Linear
OC	0.018	0.070	16	-----	Spherical[I]
FDA hydrolysis	0.857	1.224	49	-----	Spherical
pH	0.001	0.135	32	-----	Spherical

[H] $\gamma(h) = C_o + Bh$

[I] $\gamma(h) = C_o + C\,[(1.5)(h/A) - (0.5)(h/A)^3]$, $h < A$; and $\gamma(h) = C_o + C$, $h \geq A$, where γ is semivariance and h is lag distance (m) (David, 1977).

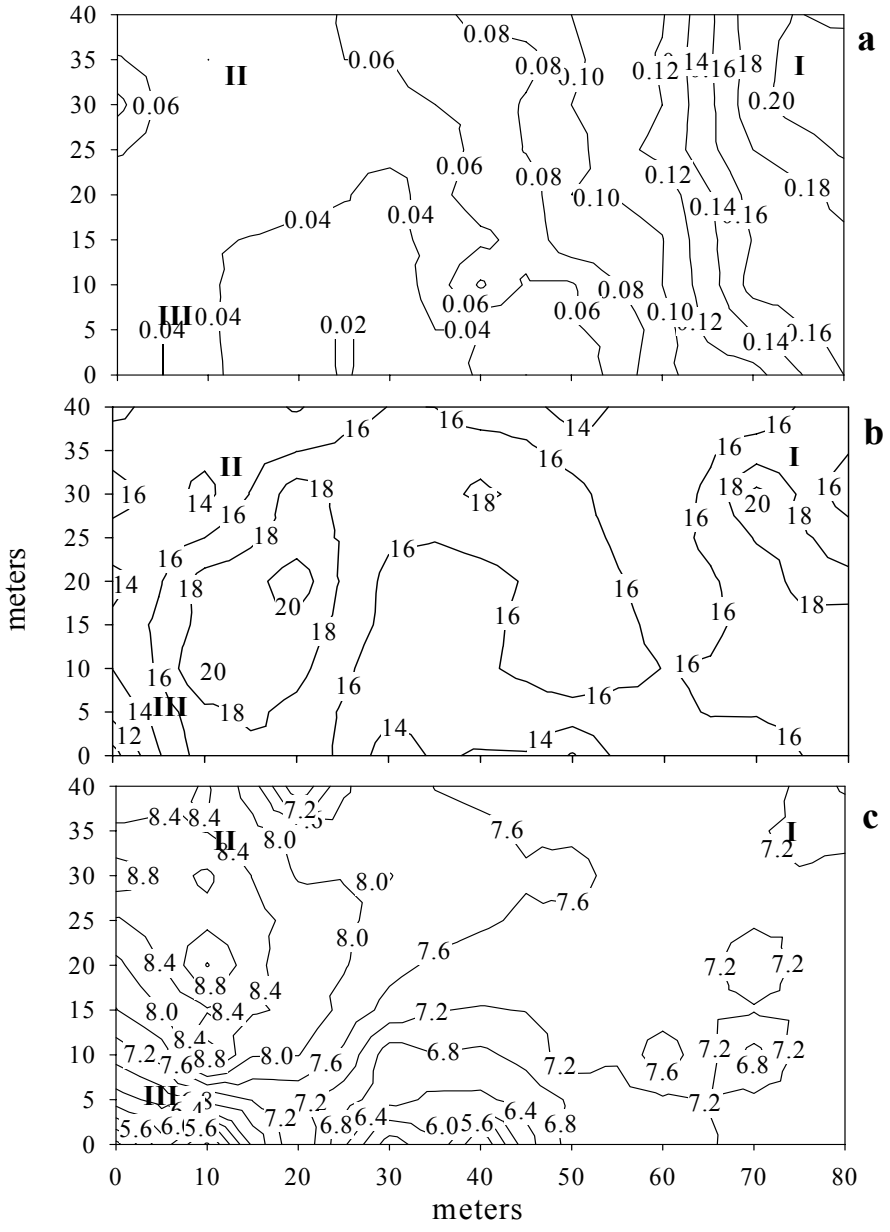

Figure 3.4. Distribution of soil properties in an 80 x 40 m sub-area of the study site: (a) clay fraction, (b) soil organic C (g / kg) and (c) FDA hydrolysis rate (nmol g^{-1} h^{-1}) (Schnürer and Rosswall, 1982). Roman numerals: I = shoulder, II = backslope, and III = toeslope are the three locales for the study..

Table 3.2. Characterization data for bulk 0 – 7.5 cm soil from the three landscape positions.

Property	Position		
	Shoulder	Backslope	Toeslope
Clay (%)	20.0	5.2	4.2
Sand (%)	56.8	74.7	79.6
Bulk Density (g cm^{-3})	1.39	1.30	1.35
pH; 1:2, soil: H_2O	4.50 ± 0.07	4.45 ± 0.05	4.29 ± 0.02
Organic C (g / kg)	16.4 ± 0.1	14.1 ± 0.1	12.3 ± 0.1
CEC (cmol (+) / kg)	8.0 ± 2.0	5.7 ± 1.2	2.7 ± 1.2
Moisture at -33 kPa (g H_2O / 100 g)	14.0	14.9	14.4
Microbial Biomass C (μg g^{-1})	225 ± 66	193 ± 51	126 ± 21

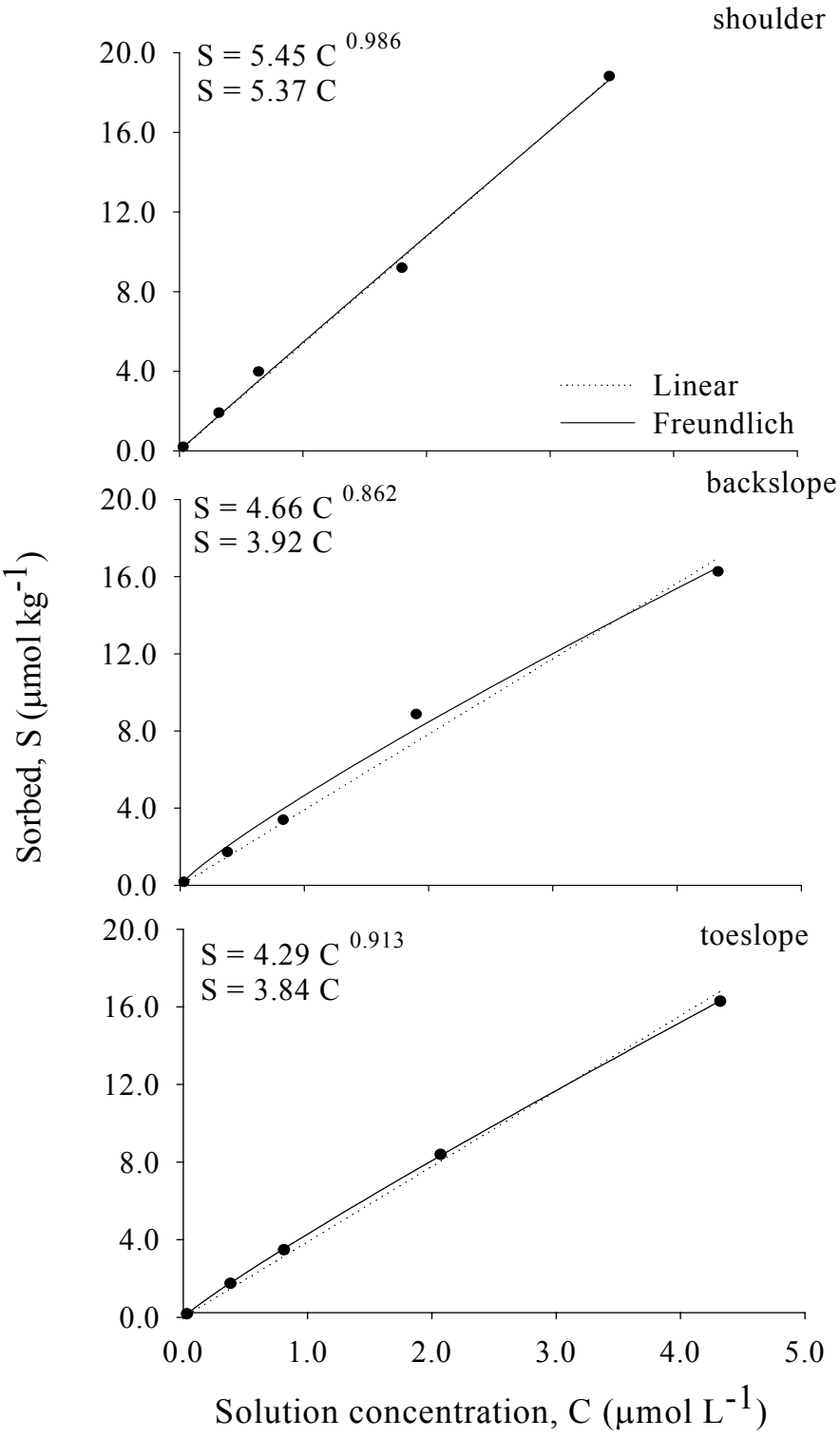

Figure 3.5. BAS 505 sorption described by Freundlich and linear models.

Sorption of BAS 505 in surface soil (down slope from the shoulder position) determined in the yearlong study (Chapter 2) was consistent with data for the shoulder and backslope positions. Earlier, a K_d of 4.74 was found, intermediate to higher and lower K_ds found higher and lower in the landscape.

BAS 505 Degradation Behavior

Recoveries from ^{14}C Pools

Total recoveries of ^{14}C based on recoveries in the various fractions (MeOH, unextractable and $^{14}CO_2$) by method and landscape position are summarized in Table 3.3. Average total recoveries were > 90 % for cores and batch soils, but total recovery dipped to < 90 % for cores by day 120. Methanol extraction accounted for ~ 97 % recovery of the radiolabel at day 0 from cores, decreasing to ~ 72, 72 and 73 % for shoulder, back- and toeslope positions at day 120, respectively. This fraction accounted for ~ 100 % recovery from batch soils at day 0, decreasing to ~ 81, 79 and 76 %, respectively, at day 120. Comparing methanol recoveries to the year study (Chapter 2), there was slightly greater recovery with methanol in this study.

Unextractable ^{14}C for intact cores increased from 2 % to 12, 10 and 18 % at day 120 compared to 1 % to 15, 22 and 31 % for batch soils from shoulder, back- and toeslope, respectively. For cores, there was significantly more unextractable ^{14}C in toeslope soil by day 30 than in shoulder or backslope soil. This was also true for batch soils by day 14 and by day 60 there was more unextractable ^{14}C in backslope than shoulder soil. Comparing systems, there was greater accumulation of unextractable ^{14}C in batch soils from back- and toeslope positions by day 60 than for corresponding cores. When comparing unextractable recoveries from this study to those from the yearlong study (Chapter 2; batch and core soil down slope from shoulder position), results agree reasonably well for the backslope soil.

Table 3.3. Percentage recoveries of ^{14}C (as fraction of applied) from cores and batch soil from different landscape positions and pools.

Position	Pool	Cores					Batch Soil				
		Day 0	Day 14	Day 30	Day 60	Day 120	Day 0	Day 14	Day 30	Day 60	Day 120
Shoulder	MeOH	94 ± 11	92 ± 6	83 ± 10	75 ± 3	72 ± 4	100 ± 1	85 ± 1	90 ± 3	89 ± 3	81 ± 1
	UnEx	2 ± 1	4 ± 0	6 ± 1	12 ± 2	12 ± 1	1 ± 0	2 ± 0	6 ± 0	12 ± 1	15 ± 3
	CO_2	-----	0.2 ± 0	0.3 ± 0	0.5 ± 0	0.6 ± 0	-----	0.9 ± 0	1.1 ± 0	1.3 ± 0	1.8 ± 1
	Total	96 ± 12	96 ± 6	89 ± 11	88 ± 5	85 ± 5	101 ± 1	88 ± 1	97 ± 3	102 ± 4	98 ± 5
Backslope	MeOH	98 ± 12	103 ± 17	82 ± 5	83 ± 16	72 ± 6	100 ± 2	93 ± 2	90 ± 1	87 ± 1	79 ± 6
	UnEx	2 ± 0	4 ± 1	10 ± 3	11 ± 2	10 ± 1	1 ± 0	3 ± 0	6 ± 1	16 ± 1	22 ± 2
	CO_2	-----	0.2 ± 0	0.4 ± 0	0.4 ± 0	0.4 ± 0	-----	1.2 ± 0	1.3 ± 0	1.7 ± 0	3.3 ± 1
	Total	100 ± 12	107 ± 18	92 ± 8	94 ± 18	82 ± 7	101 ± 2	97 ± 2	97 ± 2	104 ± 2	104 ± 9
Toeslope	MeOH	101 ± 5	91 ± 3	75 ± 5	73 ± 6	73 ± 6	101 ± 1	89 ± 2	90 ± 3	86 ± 2	76 ± 4
	UnEx	2 ± 1	7 ± 1	14 ± 3	17 ± 3	18 ± 4	1 ± 0	5 ± 1	17 ± 1	23 ± 2	31 ± 2
	CO_2	-----	0.1 ± 0	0.3 ± 0	0.5 ± 0	0.6 ± 0	-----	1.4 ± 0	1.7 ± 0	2.2 ± 0	3.9 ± 0
	Total	103 ± 6	98 ± 4	89 ± 8	90 ± 9	92 ± 10	102 ± 1	95 ± 3	109 ± 3	111 ± 4	111 ± 6

Upon completion of the study, mineralization was ~ 2 - 4 % for batch soils and 0.6 % for cores, with greatest mineralization in toeslope batch soils. For cores, there was no trend in differences among landscape positions but for batch soils, mineralization generally followed, toeslope > backslope > shoulder. Throughout the study, more ^{14}C was mineralized in batch soils than cores (all positions) as can also be seen from mineralization rates (Fig. 3.6).

The rate of mineralization is the fraction of total applied ^{14}C that was evolved as $^{14}CO_2$ during any sampling interval. Data are averages for all experimental units at any time (i.e., decreasing from 12 biometer flasks and cores at day 14 to 3 flasks and cores after day 60 for each position). The initial rate over the 0 to 7 d incubation for the batch soil from shoulder, backslope, and toeslope positions (0.0011, 0.0014 and 0.0018 μg mineralized d^{-1} / μg applied, respectively) was about ten times that for the cores (0.00014, 0.00013 and 0.00013 μg mineralized d^{-1} / μg applied, respectively). Faster initial mineralization in batch soil also occurred in the yearlong study (Chapter 2) and, again, was likely due to the comparatively large surface area to mass ratio for soil in the biometer flasks compared to the soil cores (Gaston and Locke, 1996 and 2000). Mineralization rate sharply decreased in batch soils, then slowly increased beyond 42 d to a secondary maximum at about 90 d. Mineralization rate in the batch soils was generally greater than that in cores throughout the study, and at day 120, batch shoulder, backslope and toeslope soils had mineralization rates of 0.00010, 0.00022 and 0.00024 d^{-1} compared to 0.00004, 0.00004 and 0.00003 d^{-1} for respective cores. Differences in ^{14}C recoveries in unextractable and mineralized pools among landscape positions and between systems, therefore, suggest possible spatial and methodological differences in degradation dynamics.

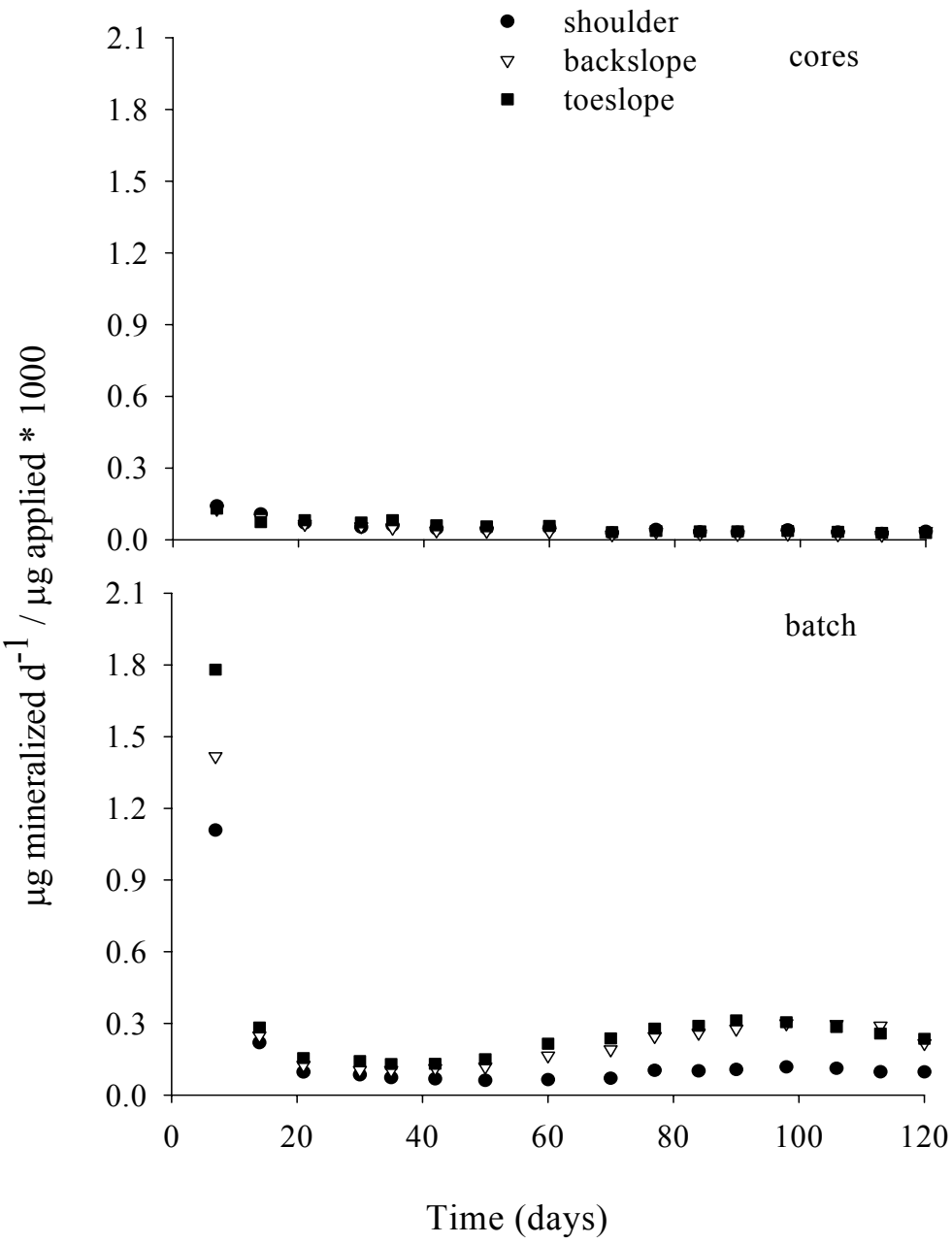

Figure 3.6. Mineralization rate of BAS 505 by intact cores and batch soils from shoulder, backslope and toeslope positions.

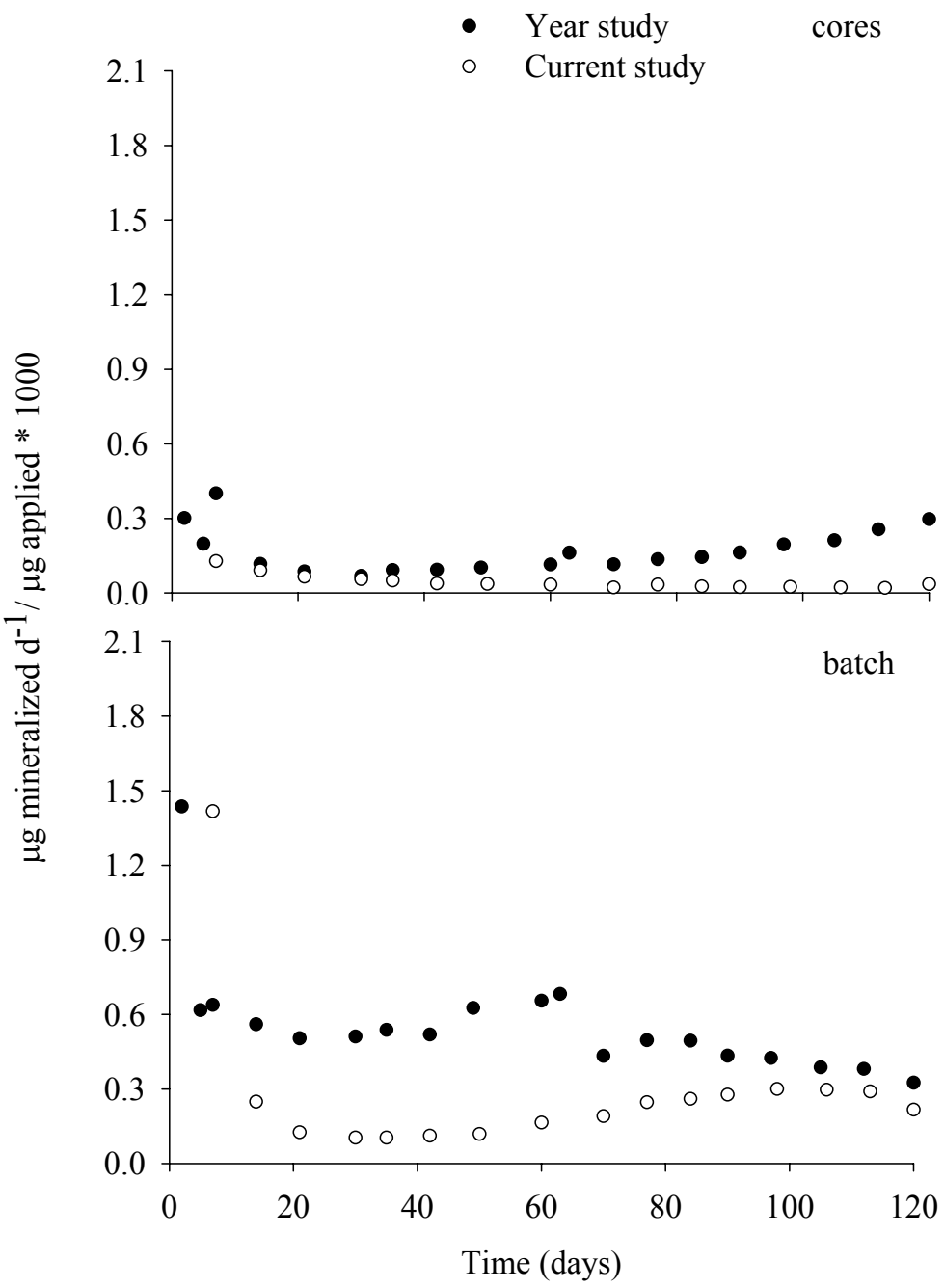

Figure 3.7. Comparison of mineralization rate between yearlong (Chapter 2) and current studies - soil cores (top) and batch soils (bottom).

The initial decrease in batch mineralization rate was similar but more pronounced than in the yearlong study (Fig. 3.7). Although less BAS 505 was mineralized by 120 d in the current study, by day 120 rates were nearly the same. In cores, the initial rate slightly decreased to low steady rates of $^{14}CO_2$ loss and did not subsequently increase as in the yearlong study (Fig. 3.7).

Degradation of BAS 505

Recoveries of BAS 505 from cores and batch soils from the three landscape positions after 0, 14, 30, 60 and 120 d incubation are shown in Table 3.4. Within either system, landscape position had no significant effect on BAS 505 recovery, hence, degradation rate. Recoveries decreased from ~ 97 % on d 0 to 67, 68 and 73 % on d 120 in cores from shoulder, backslope and toeslope positions, and from ~ 100 % on d 0 to 67, 60 and 63 % in batch soils.

Furthermore, there were no significant differences in recoveries between core and batch systems for shoulder and backslope soils at any incubation time (comparisons not shown in Table 3.4) and differences between systems for the toeslope soils were not consistent – significantly lower recoveries from cores than batch soils after 30 and 60 d incubation, but neither earlier or later. Thus, degradation rate for BAS 505 was not significantly different in cores or batch soils for any landscape position.

Nor were there apparent differences due to landscape position or system in extractable metabolites. Fig. 3.8 through 3.10 show recovery chromatograms for BAS 505 and its metabolites for intact and batch soils after extraction with MeOH at day 0 and 120 of the study from shoulder, sideslope and toeslope positions. As in the year-long study (Chapter 2), peaks included those between 29 and 32 min, corresponding to BF 505-7 [*(E)-o-[(2-hydroxy carbonyl-5-methyl)phenoxymethyl]-2-methoxyimino-N-methylphenylacetamide*] and BF 505-8 [*(E)-o-[(5-*

Table 3.4. Fractional recoveries of BAS 505.

Time (d)	Position	Cores	Batch
0	Shoulder	0.937 ± 0.038a[†]	0.997 ± 0.012a
14		0.809 ± 0.080a	0.763 ± 0.011a
30		0.790 ± 0.048a	0.796 ± 0.026a
60		0.665 ± 0.050a	0.751 ± 0.034a
120		0.647 ± 0.020a	0.670 ± 0.022a
0	Backslope	0.975 ± 0.115a	1.004 ± 0.020a
14		0.933 ± 0.153a	0.806 ± 0.018a
30		0.742 ± 0.049a	0.783 ± 0.004a
60		0.772 ± 0.166a	0.712 ± 0.014a
120		0.685 ± 0.055a	0.604 ± 0.100a
0	Toeslope	1.008 ± 0.048a	1.015 ± 0.005a
14		0.814 ± 0.013a	0.798 ± 0.021a
30		0.726 ± 0.040b	0.840 ± 0.019a
60		0.622 ± 0.038b	0.749 ± 0.007a
120		0.730 ± 0.085a	0.629 ± 0.033a

[†]Within a row, means followed by same letter are not significantly different (LSD $\alpha = 0.05$). Parent recoveries (μg recovered/applied) in combined methanol extracts.

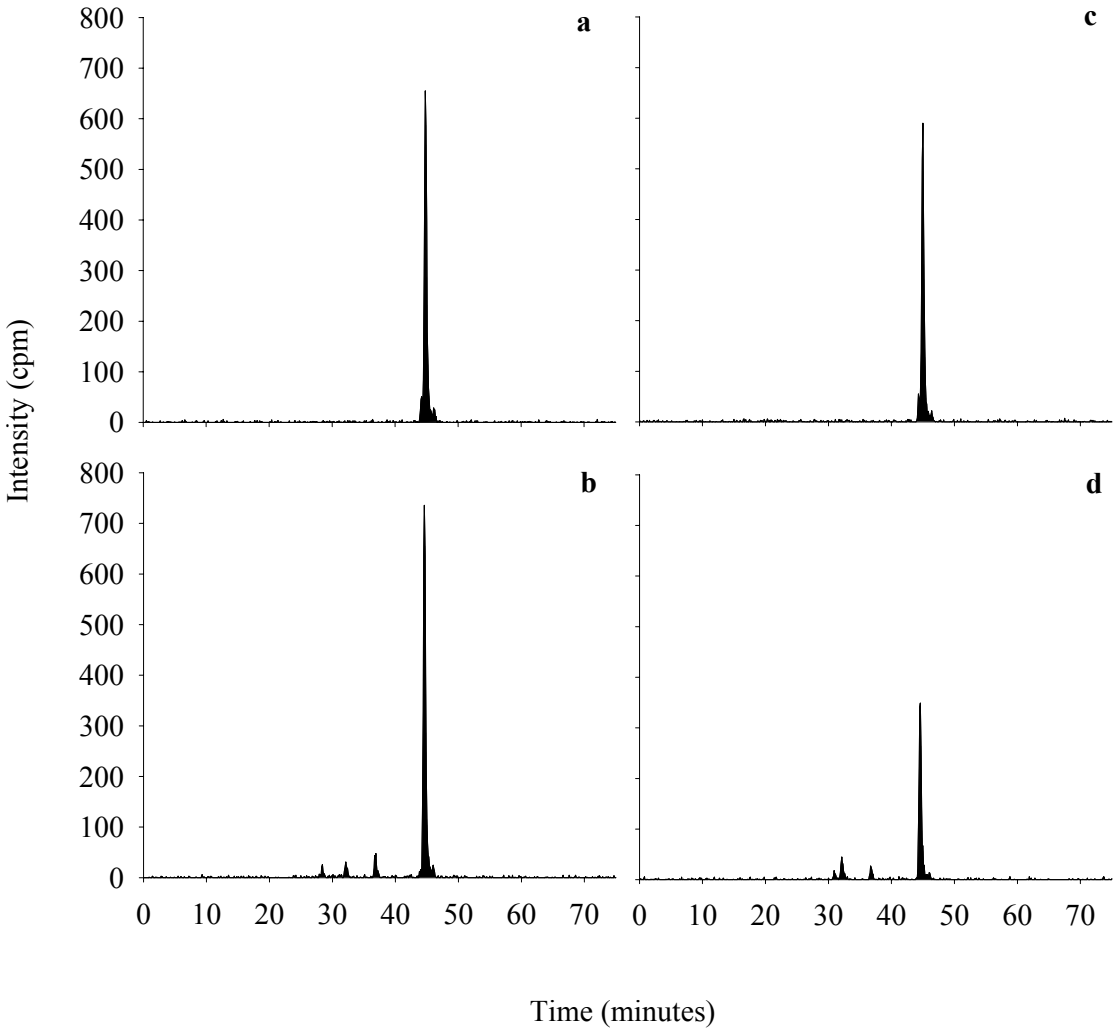

Figure 3.8. HPLC chromatograms derived from ^{14}C parent and its metabolites- Shoulder a) Methanol extract-Day 0 (cores), b) Methanol extract-Day 120 (cores), c) Methanol extract-Day 0 (batch), and d) Methanol extract-Day 120 (batch).

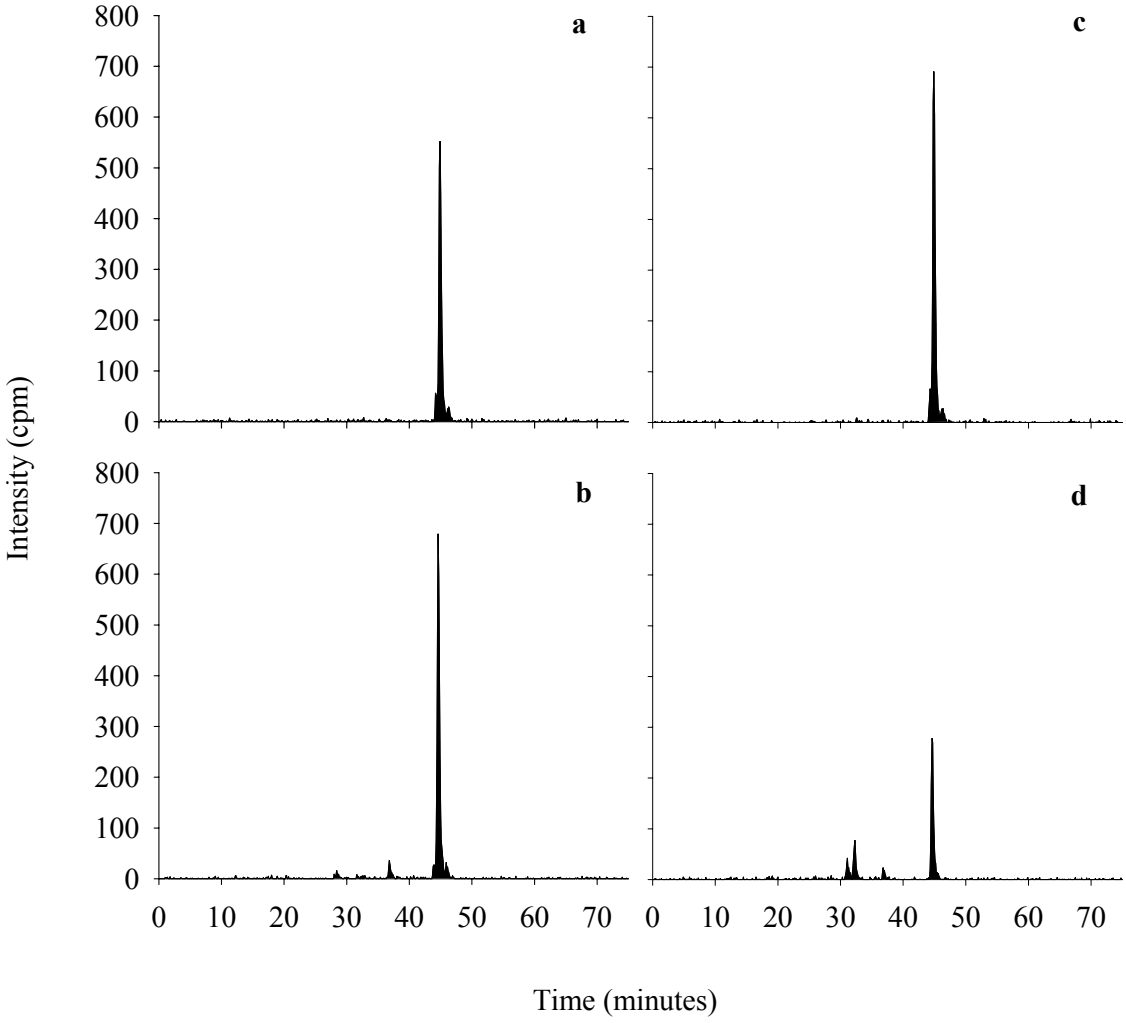

Figure 3.9. HPLC chromatograms derived from ^{14}C parent and its metabolites- Backslope a) Methanol extract-Day 0 (cores), b) Methanol extract-Day 120 (cores), c) Methanol extract-Day 0 (batch), and d) Methanol extract-Day 120 (batch).

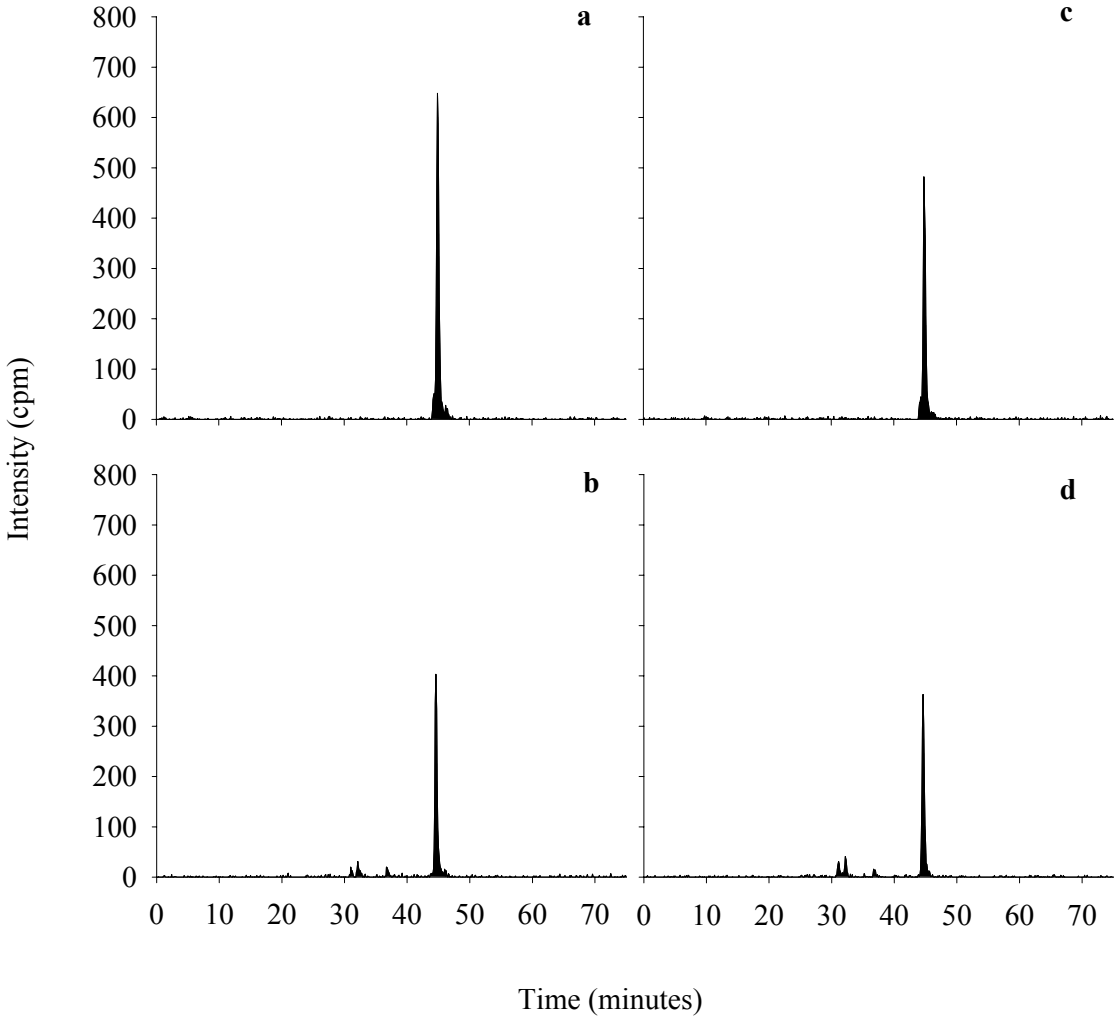

Figure 3.10. HPLC chromatograms derived from ^{14}C parent and its metabolites- Toeslope a) Methanol extract-Day 0 (cores), b) Methanol extract-Day 120 (cores), c) Methanol extract-Day 0 (batch), and d) Methanol extract-Day 120 (batch).

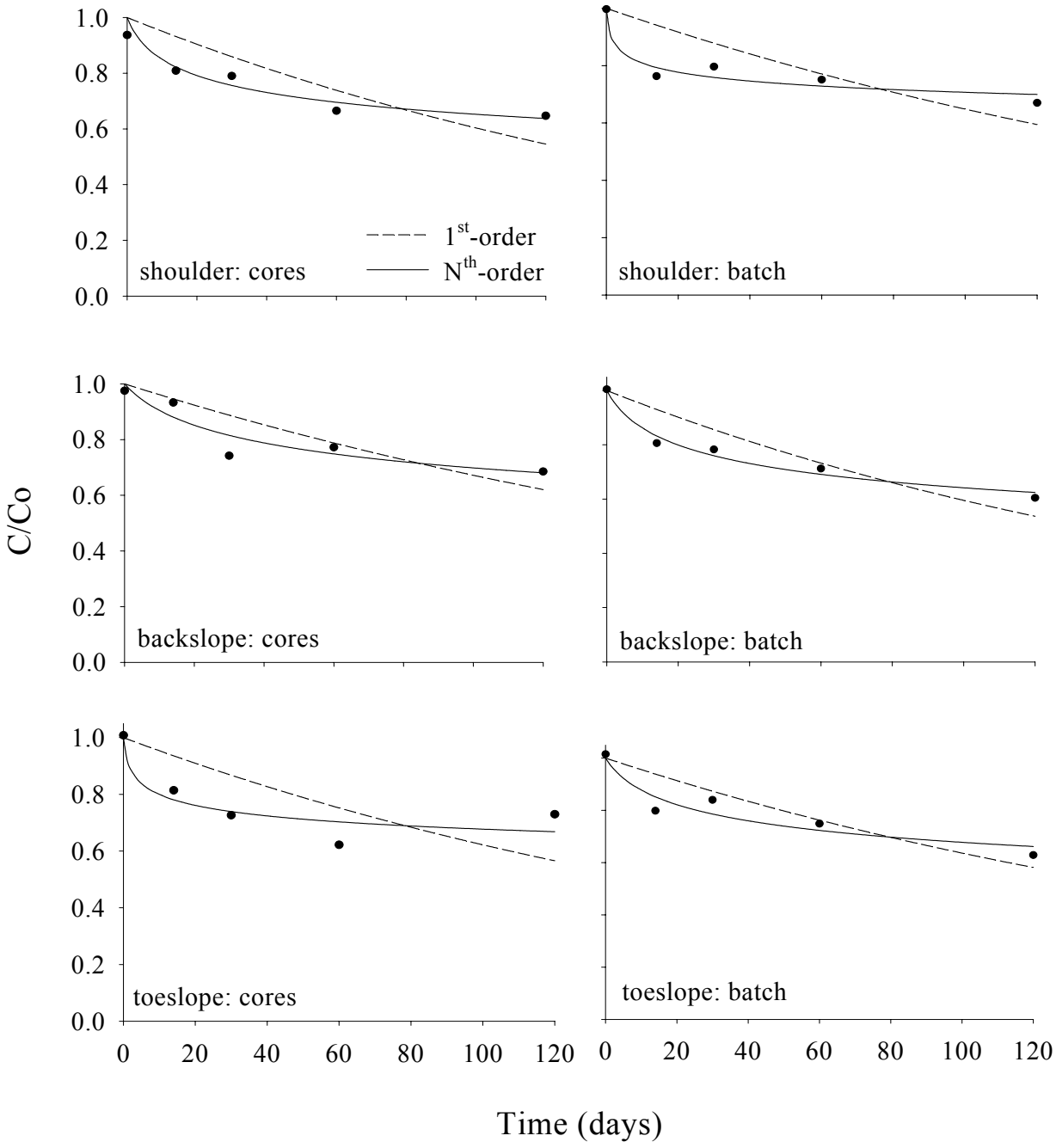

Figure 3.11. Degradation of BAS 505 described by 1st- and Nth -order models from shoulder, backslope, and toeslope positions by intact core and batch soil methodologies.

Table 3.5. First- and N^{th}-order degradation model parameters.

System	Position	1^{st}-order	N^{th}-order	
		k (d^{-1})	k (d^{-1})	N
Cores	Shoulder	0.005 ± 0.001[H]	0.032 ± 0.026	8.6 ± 3.5
Batch		0.004 ± 0.001	0.208 ± 0.442	17.9 ± 8.9
Cores	Backslope	0.004 ± 0.001	0.014 ± 0.012	7.4 ± 4.4
Batch		0.005 ± 0.001	0.025 ± 0.010	7.4 ± 1.7
Cores	Toeslope	0.005 ± 0.002	0.148 ± 0.417	14.6 ± 10.9
Batch		0.004 ± 0.001	0.022 ± 0.018	8.2 ± 4.0

[H] Standard error.

hydroxycarbonyl-2-methyl)phenoxymethyl]-2-methoxyimino-N-methylphenylacetamide], and at ~ 27 - 28 min and ~ 36 - 37 min, the latter corresponding to methyl salicylate.

The data in Table 3.4 are plotted in Fig. 3.11 along with best-fits of the first- and N^{th}-order models. The N^{th}-order model clearly better described BAS 505 degradation kinetics than did the first-order model for cores and batch soils from all landscape positions (Table 3.5 gives model parameters). The decreasing rate of BAS 505 degradation with time is consistent with results from Chapter 2. A possible explanation for deviation from first-order kinetics is decreasing microbial activity of BAS 505 degraders, but this was not substantiated. Also, lack of nutrient input through the soil surface with infiltrating water (absent in static systems) or to the

living rhizosphere (again, absent in laboratory incubations) may have had a negative effect on microbial activity.

Influence of Bermudagrass on BAS 505 Degradation

Table 3.6 gives recoveries of BAS 505 from soil cores that were planted with bermudagrass, left bare and left bare but shaded from sunlight after 6 and 12 wk incubation in the greenhouse. Due to variability among replicates, differences among treatments was significant only at $P = 0.10$ after 6 wk incubation. By 12 wks differences were significant at $P < 0.05$. But contrary to the hypothesis that infiltration through a grass canopy / thatch layer and presence of a living root system would enhance degradation of BAS 505, the difference between the bermudagrass and bare cores exposed to sunlight was not significant. While exposure of cores to sunlight clearly enhanced BAS 505 degradation, soil under a bermudagrass canopy and layer of thatch was shaded relative to bare soil. Although light intensity at the soil surface in the bermudagrass cores was not determined, modeling BAS 505 recovery from the three treatments as a linear function of presence of grass (bermudagrass cores = 1 and bare cores = 0) and assumed level of sunlight (shaded cores = 0, bare cores = 1 and bermudagrass cores = range from 1 to 0.5), that is, recovery = A x grass + B x sunlight, was highly significant for all levels of sunlight. Furthermore, the grass term increased in significance from $P = 0.26$ at full sunlight to $P< 0.01$ at half the intensity of sunlight reaching the bare soil surface. At a light intensity ~ 80%, presence of grass was significant at $P = 0.05$. Thus, presence of grass may have enhanced BAS 505 degradation, perhaps directly due to nutrient recharge via infiltration through thatch and inputs to the rhizosphere. Competition of grass and microorganisms for available N also may have stimulated the latter to use BAS 505 as a N source. For example, Gan et al. (1996) found

Table 3.6. Recoveries of BAS 505 from soil cores in greenhouse study.

Time (wk)	Bermudagrass	Bare	Shaded
6	0.796 ± 0.001 a	0.821 ± 0.064 a	0.958 ± 0.130 b
12	0.540 ± 0.072 a	0.617 ± 0.066 a	0.883 ± 0.089 b

†Within a row, means followed by same letter are not significantly different (LSD $\alpha = 0.05$).

that addition of NH_4^+ slowed atrazine degradation. Regardless of underlying reason, static conditions under which the laboratory degradation study was conducted may have inhibited degradation and led to decreasing rate of degradation relative to first-order kinetics, both in this 120 d incubation study and in the 360 d incubation study as in Chapter 2.

Conclusions

Clay content ranged from ~ 0.20 to < 0.05, organic C from ~ 20 to < 15 g kg^{-1}, pH from ~ 5 to 4 and general microbial activity (FDA hydrolysis rate) two-fold across the Ruston soil study site. Given such ranges in properties, spatial variability in the degradation rate of the fungicide BAS 505 might be expected. Sorption of BAS 505 did vary among soil taken from the upper 7.5 cm of shoulder (highest clay content), backslope and toeslope (lowest clay) landscape positions, consistent with soil properties, and in all cases was well-described by the Freundlich model. However, its degradation rate in batch soil and cores from these positions did not show any effect of differing soil properties nor were there consistent differences for any landscape position between the two soil media. Thus, for this loamy sand to sandy loam soil, the simpler, conventional batch method for measuring degradation rate was adequate and no special consideration of spatially variable soil properties was warranted.

In all cases, BAS 505 degradation was better described by N^{th}-order than first-order kinetics. The slowing rate of degradation may be due to its high extent of sorption (Zablotowicz et al., 2000), but since there is evidence for fairly rapid desorption of BAS 505 (Chapter 2), this would seem at most only a partial explanation. Another, lack of nutrient recharge in the static incubation units, was examined in a greenhouse study that compared BAS 505 degradation in soil planted with bermudagrass and left bare. When corrected for reduced sunlight under the grass canopy and thatch, the presence of grass was significantly related to BAS 505 degradation rate. Thus, the laboratory incubation methodology may itself have led to an artificially slow rate of degradation.

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CHAPTER 4

SUMMARY

Apparatus and protocol for tracking the degradation of an organic agrichemical in soil were developed and the degradation of BAS 505 was followed for one year using intact cores and homogeneous (batch) samples of surface 0 – 7.5 cm Ruston soil. Based on the previous research of others, it was presumed that degradation rates would be different, particularly, that degradation in cores might be faster than in batch soil. Although mineralization rates over time in the two systems were different, recovery of BAS 505 generally was not.

In both cores and batch soil, the degradation of BAS 505 deviated from first-order kinetics, showing a decreasing rate of degradation over time. This behavior was well-described by non-linear, N^{th}-order kinetics. There may have been several causes for such bi-phasic degradation kinetics, including high sorption and slow desorption kinetics, coupled with limited degradation in the sorbed phase. Although BAS 505 was highly sorbed in the Ruston soil, it was fairly quickly desorbed. Decreasing microbial populations / activities with time may also have slowed degradation rate. Although microbial biomass appeared to decrease from a relatively high initial level, variability in these data was large and the trend was non-significant. Given the length of this static (no plant growth and no recharge of substrates to the soil through organic matter deposition at the soil surface and release to the rhizosphere) incubation, the question arose whether laboratory incubations and data from them could be meaningfully extrapolated to the field.

Furthermore, the single, site-specific (all soil from a 2 m x 2 m area) comparison of results from intact cores and batch soils seemed a weak basis to claim that the two systems were

equivalent media for measuring pesticide degradation rate. Spatial variability in biological, chemical and physical properties at the study site might be sufficiently great that the original supposition, different and possibly faster degradation in cores, held elsewhere or even commonly across the field. A survey of texture, organic C, pH and FDA hydrolysis rate revealed trends in these properties. Therefore, a follow-up study was conducted in which intact core and batch soil from three different landscape positions (shoulder, backslope and toeslope; together largely encompassing the range in variability of these properties) were used as media for BAS 505 degradation.

Sorption of BAS 505 varied by landscape position, generally consistent with differences in texture and organic C. As in the yearlong study, although there were differences between systems in mineralization rate and development of unextractable residue at each landscape position, there were no consistent differences in recovery of BAS 505. Thus, the range in field-scale spatial variability of properties in this Ruston soil was not large enough to affect degradation rate among core samples or among batch soil samples. No difference between systems at any position confirmed that results from the yearlong study were applicable throughout the site.

Although there was a range in soil properties at the study site, other locations (as along a golf course fairway or across a wheat production field, either larger in area than the study site) might encompass an even wider range in soil properties. Thus, while data from this project failed to demonstrate differences in the rate of BAS 505 degradation with soil properties / landscape position or between systems, this may not be the case universally and further study might be warranted. However, the phenomenon of overall slow degradation of BAS 505 was especially compelling.

As in the yearlong study, degradation rate in soils from each landscape position decreased over time (again well-described by N^{th}-order kinetics). Field-wide consistency in this behavior reinforced earlier concern that results from laboratory incubations may be methodological artifacts and prompted further examination of bi-phasic kinetics. The lingering question of possibly negative effect of no nutrient recharge due to living plants was addressed in a greenhouse study using mixed soil from the three landscape positions. Recovery of BAS 505 from packed cores either planted with bermudagrass, left bare or left bare but shaded from sunlight showed a highly significant and positive effect of sunlight on degradation rate. Among the three treatments, recovery from cores with bermudagrass was numerically least, though not significantly less than from bare cores exposed to sunlight. However, correcting for the reduced level of sunlight reaching the soil surface under the bermudagrass canopy and thatch layer suggested that the presence of living plants did increase the rate of BAS 505 degradation (the presence of bermudagrass would have been significant at about 80 % of the light intensity reaching bare soil). Thus, there is evidence that pesticide degradation data obtained from static laboratory incubations, particularly long-term incubations, are a poor basis for projecting environmental fate and persistence.

Given that new crop protection chemicals promise economic benefits to producers and consumers, if the work done in this project furthers the registration and safe marketing of BAS 505 (or that of other crop protection chemicals), it will have been of more than just academic value.

VITA

Jason Allan McDonald was born in Valley Stream, New York, on July 28, 1978. He is the oldest son of Grace McDonald and has two sisters, Crystal and Heather. He graduated June 21, 1996, from Sewanhaka High School in Floral Park, New York. On May 21, 2000, he received his bachelor of science degree in environmental studies with minors in physics and philosophy from St. John's University in Jamaica, New York. He was offered an environmental studies internship at Brookhaven National Laboratory in Upton, New York, in January of 2000 and continued his research until August of 2000. He was offered a graduate research assistantship by the Department of Environmental Studies, Louisiana State University, and began his graduate studies in environmental toxicology in August of 2000 under Dr. Ralph Portier. On December 21, 2001, he had received a master of science degree in environmental sciences at Louisiana State University. Upon completion of his master's degree, he was offered a graduate research assistantship by the Department of Agronomy and Environmental Management, Louisiana State University, and continued his graduate studies in soil science with emphasis of environmental soil chemistry in January of 2002 under Dr. Lewis Gaston. He is presently a candidate for the degree of Doctor of Philosophy.