FATE AND DEGRADATION OF PETROLEUM HYDROCARBONS IN STORMWATER BIORETENTION CELLS

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

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August 2012

Acknowledgements

There are many people without whom this work or even arriving at this place would not have been possible.

First, there are those who provided invaluable academic support throughout my time at the University of Minnesota. I could not have done this without the support, guidance, nurture, and encouragement of my advisors, Paige Novak and Ray Hozalski. They have been very patient intellectual shepherds and allowed me to grow as a researcher. My committee members, Tim LaPara, John Nieber, and John Gulliver have taught me much in their classes, broader implications of my research, and constructive critiques. My many labmates in the Novak and Hozalski groups have provided useful laboratory instruction, constructive feedback, and hours of pleasant interaction.

Second, I must acknowledge the sources of funding which made this work and my graduate school career possible. The funding from the National Science Foundation Graduate Research Fellowship Program (GRFP) and Integrative Graduate Education and Research Traineeship (IGERT, DGE-0504195) has allowed me to pursue my own research interests and learn from those in complimentary fields. I am also indebted to the Graduate School and to the Minnesota chapter of the Achievement Rewards for College Scientists (ARCS) Foundation for their student support. Funding for our research supplies also came from the Minnesota Water Resources Center.

Finally, this work would not have been possible without the unending support of my family. Foremost, my wife Nancy-Jeanne has been there every step of the way all the while tolerating, encouraging, and supporting with unconditional love. I absolutely cannot say this enough. My parents, Bob and Cathy, have always provided the gumption, work ethic, drive, and perspective that are necessary to succeed in life. My brother Kevin has also been a source of continued sibling support during this experience. Lastly, I'd like to thank John Gierke, professor at Michigan Tech, for believing in me from day one and continuing to be a true mentor.

Abstract

This dissertation describes the investigation of the fate of hydrocarbons in stormwater bioretention areas and those mechanisms that affect hydrocarbon fate in such systems. Seventy-five samples from 58 bioretention areas were collected and analyzed to measure total petroleum hydrocarbon (TPH) residual and biodegradation functional genes. TPH residual in bioretention areas was greater than background sites but low overall (<3 µg/kg), and well below either the TPH concentration of concern or the expected concentration, assuming no losses. Bioretention areas with deep-root vegetation contained significantly greater quantites of bacterial 16S rRNA genes and two functional genes involved in hydrocarbon biodegradation. Field soils were capable of mineralizing naphthalene, a polycyclic aromatic hydrocarbon (PAH) when incubated in the laboratory. In an additional laboratory investigation, a column study was initiated to comprehensively determine naphthalene fate in a simulated bioretention cell using a ^{14}C-labeled tracer. Sorption to soil was the greatest sink of naphthalene in the columns, although biodegradation and vegetative uptake were also important loss mechanisms. Little leaching occurred following the first flush, and volatilization was insignificant. Significant enrichment of naphthalene degrading bacteria occurred over the course of the experiment as a result of naphthalene exposure. This was evident from enhanced naphthalene biodegradation kinetics (measured via batch tests), significant increases in naphthalene dioxygenase gene quantities, and a significant correlation observed between naphthalene residual and biodegradation functional genes. Vegetated columns outperformed the unplanted control column in terms of total naphthalene removal and

biodegradation kinetics. As a result of these experiments, a final study focused on why planted systems outperform unplanted systems was conducted. Plant root exudates were harvested from hydroponic setups for three types of plants. Additionally, a solution of artificial root exudates (AREs) as prepared. Exudates were digested using soil bacteria to create metabolized exudates. Raw and metabolized exudates were characterized for dissolved organic carbon, specific UV absorbance, spectral slope, florescence index, excitation-emission matrices, and surface tension. Significant differences on character were observed between the harvested exudates and the AREs, as well as between the raw and metabolized exudates. Naphthalene desorption from an aged soil was enhanced in the presence of raw exudates. The surface tension in samples containing raw harvested exudates was reduced compared to samples containing the metabolized exudates. Plant root exudates may therefore facilitate phytoremediation by enhancing contaminant desorption and improving bioavailability. Overall, this resarch concludes that heavily planted bioretention systems are a sustainable solution to mitigating stormwater hydrocarbon pollution as a result of likely enhanced contaminant desorption, and improved biodegradation and plant uptake in such systems.

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1.1 Stormwater Runoff and Pollution

Stormwater from urban runoff contains pollutants and generates unnatural flow quantities that lead to the degradation of surface water quality, presenting one of the greatest challenges to ensuring clean water for human and ecological health (1-4). The impervious surfaces that accompany urbanization (e.g., roads, parking lots, rooftops) lead to increased volumes of stormwater runoff and facilitate degradation of receiving water bodies (4-14). In addition, connectivity of impervious surfaces causes damaging surges in stormwater volume and velocity to streams (15-17). The traditional goal of stormwater management has been to prevent flooding by collecting runoff in storm sewers that discharge directly to surface water bodies. Stormwater, however, can contain a wide variety of pollutants at levels that may pose human or ecological health risks including heavy metals (18-23), nutrients (12, 15, 19, 24-31), suspended solids (12, 19, 32-38), pathogens (19, 30, 32, 39-44), pesticides (30, 45-47), and petroleum hydrocarbons (19, 30, 32, 36, 39-44, 48-61). In fact, stormwater pollutant concentrations typically exceed those in treated wastewater (2). Therefore, understanding stormwater pollution and approaches to ameliorate its impacts is necessary to protect water quality in lakes, rivers, and estuaries.

1.2 Hydrocarbon Pollution in Stormwater

Petroleum hydrocarbons are known constituents of urban stormwater (15, 19, 62), yet little research has focused on hydrocarbons compared to most other stormwater

pollutants, such as nutrients or suspended solids. Petroleum hydrocarbons are considered priority pollutants (63) and can be acutely toxic to aquatic life (64) at low concentrations (65, 66); concentrations above 0.01 to 0.1 mg/L are known to harm fisheries (67). Concentrations of hydrocarbons vary spatially and temporally and have been measured in stormwater at 0.2 to 277 mg/L (31, 67-72). Numerous sources of petroleum hydrocarbon pollution exist in urban catchments, including leaky storage tanks, parking lot and roadway runoff, automotive emissions, elicit dumping, spills, and tire particles (15, 55, 64). Petroleum hydrocarbons in stormwater, particularly oil/grease and BTEX (Benzene, toluene, ethybenzene, xylenes) compounds can be traced to transportation activities (73, 74). Indeed, petroleum hydrocarbon concentrations in watersheds correlate with automobile usage (69, 75) and highway runoff can be contaminated with substantial quantities of hydrocarbons (70, 71, 76).

Furthermore, urban stormwater runoff contains polycyclic aromatic hydrocarbons (PAHs), a class of hydrocarbon that is known for toxicity, recalcitrance in the environment, and carcinogenic potential for some compounds (77). Estimates suggest urban runoff contributes between 14 and 36% of PAHs to aquatic environments (51, 57). PAH concentrations in urban streams are up to 20 times greater during peak flow than at baseflow (78), illustrating the impact of stormwater. Most PAHs in stormwater are associated with suspended particulate matter (15, 51, 79, 80). The concentrations per mass of particle range from 10 to 70 mg/kg (80), with aqueous concentrations typically between 0.3 and 6 μg/L (51).

Coal-tar based pavement sealcoats are known to release PAHs into aquatic environments (48, 49, 55, 75, 80-82). Coal-tar based sealcoats are primarily used in the

eastern/central portions of the United States due to historic industrial production and contain significantly higher levels of PAHs than the asphalt-based sealcoats used in the western US (81); coal-tar sealcoats commonly contain >50,000 parts per million (ppm) total PAHs, whereas asphalt-based sealcoats typically contain <100 ppm total PAHs (49). Indeed, coal-tar sealed parking lots exhibit 50 times the PAH flux of unsealed parking lots (55), and can be responsible for up to 84% of PAH concentrations in stream and lake sediments (82). At one study-site parking lot, total PAH concentrations in downstream sediments increased from 4 mg/kg to over 95 mg/kg following application of coal-tar sealcoats (49). Increased PAH concentrations in urban aquatic sediments in recent decades can be attributed to coal-tar sealcoats (83). Coal-tar is composed of approximately 22.5% PAHs by weight, of which naphthalene and 2-methylnaphthalene are the leading components (10 and 5.3% of total mass, respectively) (84). The environmental concerns surrounding coal-tar based sealcoats has prompted several municipalities to ban or regulate application including: Austin, TX; Washington, D.C.; Dane Co, WI; and several cities in Minnesota (49). It is clear that PAHs originating from urban sources such as coal-tar based sealcoats and transported via stormwater are accumulating in aquatic systems.

As a result of PAH-contaminated stormwater, retention pond sediments have also become contaminated with PAHs (50, 56, 85). In a study of retention ponds in the Minneapolis-Saint Paul metropolitan area of Minnesota, PAH concentrations (measured as a suite of 13 PAHs) ranged from 0.2 mg/kg to 65.8 mg/kg with an average of 11.0 mg/kg, equal to 0.19 to 7.28 benzo(a)pyrene equivalents (BaP_{eq}) (86). In half of the ponds sampled, PAH concentrations exceeded Minnesota's Level 2 Soil Reference Value

of 3 mg/kg BaP_{eq}, which can add substantially to associated disposal costs (85, 86). In a set of stormwater ponds in South Carolina, the sum of 16 PAHs was measured up to 160 mg/kg, with ponds in commercial areas containing substantially higher residual concentrations than ponds in residential areas (50). Other studies of ponds have yielded similar results (56). Thus, PAH prevalence in stormwater runoff appears ubiquitous and current research on the fate of these compounds in stormwater ponds indicates inadequate removal.

1.3 Bioretention Technology

Stormwater management practices have become a vital component of the urban landscape to prevent flooding and can improve stormwater quality (1). Examples include stormwater collection systems (*e.g.*, curb and gutter, catch basins), stormwater ponds, hydrodynamic separators, and infiltration based practices. Conventional stormwater mitigation practices, *i.e.*, retention ponds, are inadequate at addressing many ecological concerns (1, 17) or mitigating certain pollutants, including PAHs (50, 56, 87, 88). Low impact development (LID) is an alternative approach to stormwater management that entails decentralized stormwater infiltration practices to more closely emulate the predevelopment hydrologic regime (89, 90) and has been proven to reduce impacts on the watershed when properly implemented (1). Thus, LID has catalyzed an array of new best management practices (BMPs) such as vegetated greenroofs, constructed wetlands, permeable pavement, disjoining of impervious surfaces, and bioretention (91). LID stormwater management is being employed in various communities nationwide (*e.g.*, 2,

92, 93) to meet water quality regulations, provide green space, and improve the quality of life in urban environments (43, 94).

Bioretention practices, also called raingardens or bioinfiltration practices (Figure 1.1), are shallow vegetated depressions containing an engineered soil media into which stormwater from impervious surfaces is directed for infiltration (15). Bioretention is one of the most common LID BMPs because these practices are typically small in size, relatively inexpensive to install and maintain, and include aesthetic benefits (15, 19). Bioretention seeks to address the dual challenges of hydrologic and pollution control in stormwater management (15, 19, 62, 95). Current research indicates that properly designed and installed bioretention areas are effective at infiltrating the majority of small rainfall events (*i.e.*, less than 25 mm) (19, 43, 96-100). Because runoff from impervious surfaces in urban areas contains harmful contaminants, it is also important to understand the pollutant removal efficacy of bioretention. Bioretention areas have demonstrated pollutant removal capabilities in both laboratory and field studies (23, 101-106); nevertheless, pollutant fate in bioretention is not well understood. Most studies on this topic employ a "black box" approach, where fluxes of pollutants in and out are measured to determine removal efficiency. Unfortunately, results vary widely depending upon widely heterogeneous environmental conditions (88); thus, this methodology yields little understanding of the fundamental mechanisms involved in pollutant removal. Such understanding is critical if one is to predict and improve pollutant attenuation. Possible pollutant removal mechanisms include sorption, filtration, volatilization, plant update, or biotransformation / biodegradation processes. Of these, biodegradation of pollutants represents "permanent" removal because the pollutant is actually destroyed. Other

mechanisms are temporary sinks for stormwater pollutants; sorption, for example, can lead to re-mobilization of pollutants after the sorption capacity has been exhausted. A better understanding of pollutant fate and the key removal mechanisms will enhance bioretention design.

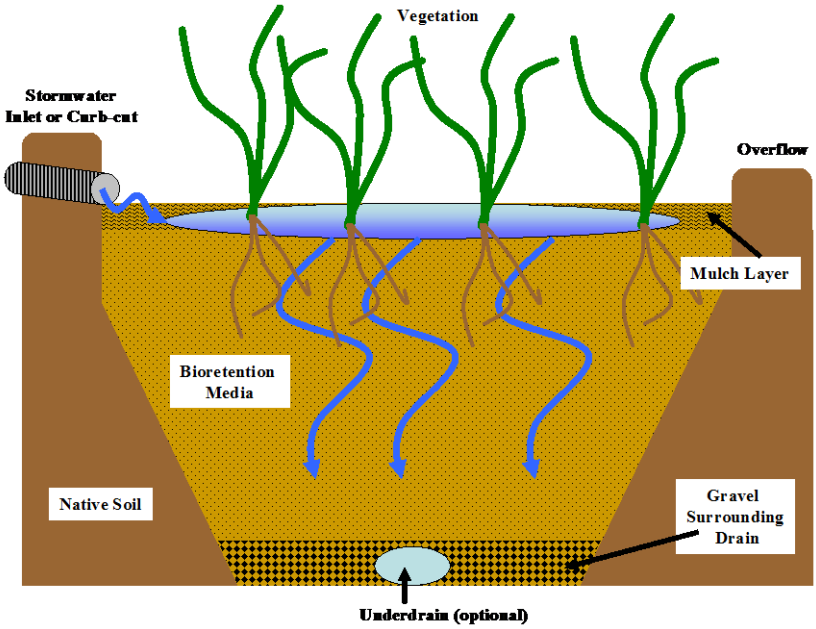

Figure 1.1: Cross-section diagram of bioretention cell [top, adapted from (62)]. Installed bioretention area in residential neighborhood [below, photo: LeFevre].

1.4 Fate of Petroleum Hydrocarbons in Bioretention Areas

There are several reports in the peer-reviewed literature regarding the effectiveness of bioretention systems at removing petroleum hydrocarbons from stormwater. Based on current research, bioretention shows promise for the removal of petroleum hydrocarbons from stormwater infiltrate. For example, removal of hydrocarbons in laboratory tests of simulated bioretention areas was >90% (74, 103) with results for full-scale bioretention cells also indicating >85% removal (51, 107). The results from three separate studies involving stormwater infiltration basins in France, Germany, and Maryland suggest that most of the hydrocarbons removed from the stormwater were retained (i.e., sorbed) in the first few centimeters of soil (51, 72, 76). Nevertheless, research on bioretention cells has generally not examined contaminant fate. The majority of the work conducted in this area has focused on hydrocarbon removal by examining mass flux across a laboratory-scale column or a full-scale bioretention cell. A comprehensive understanding of the ultimate fate of stormwater pollutants within bioretention is necessary to ascertain if these contaminants are *degraded* or simply *retained* within the bioretention matrix. Because of their prevalence in urban runoff and their accumulation in stormwater retention ponds (56), the fate of hydrocarbons in bioretention areas requires further investigation. This will provide the scientific basis for enhanced bioretention design and facilitate effective pollutant removal to ensure the long-term sustainability of these LID stormwater treatment systems.

1.5 Objectives

The objectives of this research were to 1) determine if hydrocarbon residuals accumulate in stormwater bioretention areas, 2) determine if biodegradation occurs in bioretention areas, 3) comprehensively examine the fate of a representative hydrocarbon in a laboratory-scale bioretention area, and 4) examine the effect of plant root exudates on enhanced contaminant desorption and biodegradation. Descriptions of each chapter are briefly summarized below.

Chapter 3: The Role of Biodegradation in Limiting the Accumulation of Petroleum Hydrocarbons in Raingarden Soils

A field study of bioretention areas (75 samples from 58 raingardens and 4 upland sites) was conducted to examine accumulation of petroleum hydrocarbons and the potential for biodegradation. Total petroleum hydrocarbon (TPH) residual on the soil was low overall (< 3 µg/kg) and at least three orders of magnitude less than expected based upon predicted loading, indicating that hydrocarbon loss mechanisms are active within these systems. TPH residual in raingarden soils was not different between catchment land uses, but was significantly higher than the upland control sites. Bacterial DNA was also extracted from the soils and 16S rRNA genes and two biodegradation functional genes (phenol monooxygenase and naphthalene dioxygenase) were quantified. The number of copies of 16S rRNA genes and functional genes were greater in the sites planted with deeply-rooted natives and cultivars than in raingardens containing simply turf grass or mulch, suggesting that planted raingardens may be better able to assimilate hydrocarbon inputs. Soils from selected field sites (n=8) representing a range of planting and land use types were used to inoculate batch degradation flasks. All were able to

degrade naphthalene, a representative hydrocarbon, and the initial mineralization rate correlated to the initial quantity of 16S rRNA genes in the soil. This study is the first to investigate the prevalence of stormwater hydrocarbon residual in bioretention areas and to employ quantitative polymerase chain reaction to assess biodegradation potential.

Chapter 4: Fate of Naphthalene in Laboratory-Scale Bioretention Cells: Implications for Sustainable Stormwater Management

A comprehensive study was conducted on the fate of ^{14}C-naphthalene, a representative hydrocarbon, within controlled laboratory bioretention columns. Three column setups were used: one planted with a legume, one with grass, and one unplanted (*i.e.*, control) to examine the influence of vegetation. Overall naphthalene removal efficiencies for the bioretention cells were 93% for the two planted cells and 78% for the unplanted cell, with the majority of the leaching immediately following the "first flush." Adsorption to soil was the dominant naphthalene removal mechanism (56-73% of added naphthalene), although mineralization (12-18%) and plant uptake (2-23%) were also important. Volatilization was negligible (<0.1%). Two biodegradation functional genes, naphthalene dioxygenase and phenol monooxygenase, correlated with ^{14}C-residual as a function of depth. Enrichment of naphthalene degrading bacteria was evident from enhanced biodegradation activity in batch tests using column soils as inoculum. Vegetated samples and those with previous naphthalene exposure exhibited reduced biodegradation lag times. Furthermore, significant increases in naphthalene dioxygenase functional gene quantities were observed, approaching levels observed at field sites. This was the first comprehensive study of the fate of hydrocarbons in bioretention media or to examine the influence of vegetation on stormwater hydrocarbon uptake and biodegradation.

Chapter 5: Root Exudate Impacts on Contaminant Desorption and Biodegradation

This work examined enhanced contaminant desorption as a mechanism for increased degradation in rhizosphere (root zone) soils. The rhizosphere effect, *i.e.*, increased contaminant degradation rates and extents, has been commonly observed yet is poorly understood. Three plant species representing diverse families employed during phytoremediation and at bioretention sites were grown hydroponically to harvest root exudates. Artificial root exudates (AREs) were also created using a literature recipe. Harvested and artificial exudates were digested using mixed culture soil bacteria to form metabolized exudates. All raw and metabolized exudates were characterized measuring the following: dissolved organic carbon, specific ultra violet absorbance, spectral slope, surface tension, and florescence (florescence index and excitation emission matrices). As expected, the organic carbon character of the metabolized exudates differed markedly from the raw exudates; however, an unexpected finding was that the ARE character was substantially different from the harvested exudates. The raw and metabolized exudates were tested for changes in contaminant desorption from an aged soil. Raw exudates significantly increased naphthalene desorption compared to metabolized exudates. This may be due to surface-active compounds within the raw exudates, as evidenced by a reduction in surface tension. Raw exudates acted to supress naphthalene biodegradation, likely by providing a competing labile carbon source; nevertheless, the literature suggests that this effect is overshadowed in the environment by increasing total microbial biomass in the rhizosphere. This research demonstrated that enhanced desorption from root exudate may be a contributing mechanism to the rhizosphere effect.

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Chapter 2: Literature Review

Bioretention is being implemented throughout the United States and abroad to help reduce transport of pollutants and excess stormwater volume across impervious areas to surface waters. Research to remove specific pollutants from stormwater is dependent on an understanding of physical, biological, and chemical processes. This review examines current research concerning the likely mechanisms governing the fate of hydrocarbons in stormwater bioretention areas. Additionally, this section provides background relating to biodegradation of hydrocarbons, including biodegradation pathways, the influence of vegetation, and the bioavailability of pollutants.

2.1 Potential Hydrocarbon Removal Mechanisms in Bioretention Areas

Possible removal mechanisms for stormwater petroleum hydrocarbons in bioretention areas include: sorption, filtration, volatilization, plant uptake, and biodegradation (photolysis is ignored due to design for rapid infiltration) (Figure 2.1). A brief description of how these removal mechanisms relate to bioretention is discussed below.

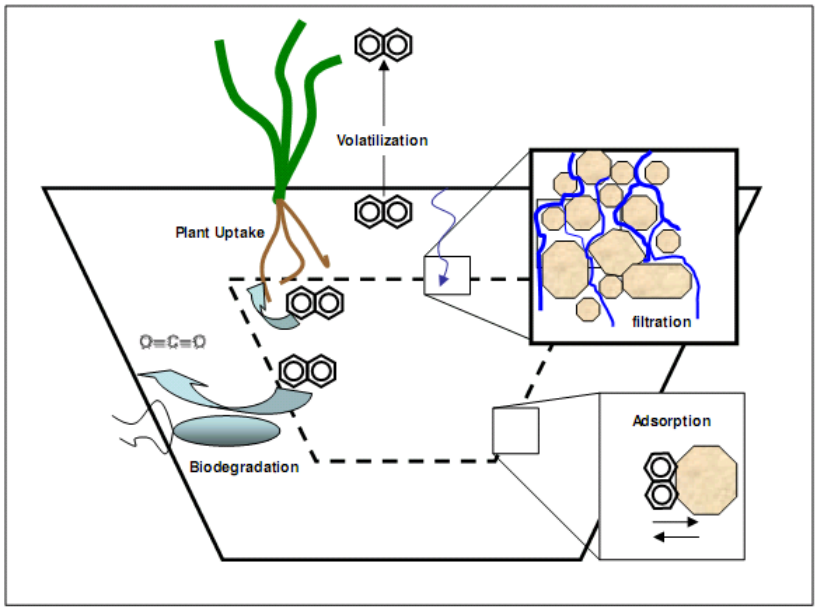

Figure 2.1: Conceptual diagram of removal mechanisms for stormwater petroleum hydrocarbons in bioretention cells.

Sorption is presumed to be the dominant hydrocarbon removal process in bioretention (1-3). Adsorption is a physical-chemical phenomenon in which a substance accumulates at a solid-liquid surface to achieve a lower thermodynamic energy state (4, 5). Sorption is an equilibrium process and is typically reversible (4), which is important for bioretention applications because contaminants could be released if sorption capacity is exhausted. The tendency of hydrocarbons to sorb is influenced by the type of hydrocarbon; less soluble PAHs and heavier molecular weight hydrocarbons tend to adsorb more readily (6). The properties of selected hydrocarbons are listed in Table 2.1, including the solubility and octanol-water portioning constant (K_{ow}). Using linear free energy relationships (LFERs) for specific classes of compounds (Equation 2.1), the organic carbon partitioning constant (K_{oc}) can be estimated from the K_{ow} for PAHs (5).

Equation 2.1 $$\log K_{oc} = 0.98 \log K_{ow} - 0.32$$

The sorption equilibrium constant of organic compounds to soils (K_d) is typically estimated to be directly proportional to the product of the K_{oc} and the fraction of organic carbon in the soil (f_{oc}) (Equation 2.2) (5).

Equation 2.2 $$K_d = f_{oc} K_{oc}$$

Nevertheless, a sub-set of organic carbon in soil (i.e., pyrogenic black carbon) may actually control the bioavailability of some recalcitrant PAHs (7, 8). Black carbon comprises approximately 4% of total soil carbon but exceeds the sorption capacity of amorphous organic carbon by 10-100 times (particularly for planar molecules) and may not be practically reversible (8). In bioretention areas, sorption to organic matter not only directly removes hydrocarbons, but also facilitates other removal mechanisms. For example, hydrocarbon sorption followed by desoprtion can also allow for subsequent biodegradation, and hydrocarbon sorption to particles allows improved filtration efficiency.

Many petroleum hydrocarbons present in stormwater are adsorbed to particles (3, 9-12), often making sorption and filtration interrelated removal mechanisms for hydrocarbons. In fact, virtually all hydrocarbon contamination is found in the first few centimeters of bioretention media in the field, and PAH removal is proportional to particle removal (2, 3, 6, 13). Various bioretention soil media (e.g., sandy media, loamy soil, and media with mulch) appear to perform similarly (14), although pure sand filters are generally ineffective in the removal of dissolved hydrocarbons (15). Unfortunately, particles can eventually clog the bioretention media, resulting in poor stormwater infiltration if the bioretention area is not properly maintained or combined with particle pretreatment (16, 17).

Volatilization is also a possible mechanism for hydrocarbon loss, but often it is assumed to be of minor importance (1) because bioretention cells are designed for rapid infiltration. Most PAHs have a fairly low air-water partition constant (Table 2.1) and instead tend to sorb to soil organic matter (5). Light aromatic BTEX compounds found in gasoline may readily volatilize from stormwater.

Table 2.1: Properties of selected hydrocarbons. Data from (5).

Hydrocarbon Category	Compound Name	Molecular Formula	Molecular Mass ($g\ mol^{-1}$)	-log Air-Water Partition Constant	Solubility ($mg\ L^{-1}$, 25° C)	log Octanol-Water Partition Constant ($log\ K_{ow}$)
BTEX Compounds	Benzene	C_6H_6	78.1	0.65	1748	2.17
	Toluene	C_7H_8	92.2	0.60	556	2.69
	Ethylbenzene	C_8H_{10}	106.2	0.50	168	3.20
	1,2-Dimthylbenzene (o-xylene)	C_8H_{10}	106.2	0.69	189	3.16
PAHs	Naphthalene	$C_{10}H_8$	118.2	1.74	32.2	3.33
	Acenaphthalene	$C_{12}H_{10}$	154.2	2.29	3.96	4.20
	Phenanthrene	$C_{14}H_{10}$	178.2	2.85	1.12	4.57
	Anthracene	$C_{14}H_{10}$	178.2	2.80	0.0447	4.68
	Benzo(a)pyrene	$C_{20}H_{12}$	252.3	4.79	0.00183	6.13
n-Alkanes	Methane	CH_4	16.0	-1.43	24.2	1.09
	n-Hexane	C_6H_{14}	86.2	-1.74	12.75	4.00
	n-Octane	C_8H_{18}	114.2	-2.07	0.720	5.15

Plant uptake may also contribute to hydrocarbon removal from stormwater in bioretention systems (18-21). Soluble hydrocarbons may be taken up, directly transpired through vegetation, and then evaporated at the plant stomata; this strategy has been applied at multiple phytoremediation study sites (18, 19, 22). The propensity for volatile organic compounds to be volatilized by vegetation is a function of the K_{ow} and can be estimated by Equation 2.3, where TSCF is the transpiration stream concentration factor, which is the ratio of the concentration in the transpiration stream to that of the bulk liquid in contact with the roots.

Equation 2.3 $$\log TSCF = 0.756e^{\left(\frac{-(\log K_{ow}-2.50)^2}{2.58}\right)} \quad (23)$$

Contaminants taken up into vegetation can be metabolized to less toxic substances by enzymes within the plant cell, the so-called "green liver" concept (24-27). Contaminants and/or their metabolites, however, can also accumulate in plant tissue (21, 25, 28-30). Nevertheless, contaminants taken up and sequestered into plant biomass are not typically bioavailable to wildlife that may ingest the vegetation as a result of incorporation into indigestible plant components (25). Thus, incorporation of stormwater hydrocarbons into plant biomass should be considered a desirable, benign removal mechanism. Although plants may directly take up petroleum hydrocarbons, the phytoremediation literature indicates that the largest impact of plants in contaminated soils is through the stimulation of microbiological activity in the rhizosphere (28, 31-36). In bioretention, the influence of vegetation on the attenuation of stormwater pollutants has been largely ignored (9).

Biodegradation is a key performance parameter for the sustainability of bioretention systems. Unlike sorption capacity which can be exhausted, biodegradation is effective so

long as the loading rate does not exceed the assimilation capacity. In addition, pollutants are degraded and destroyed rather than simply retained. Although biodegradation is a presumed hydrocarbon removal mechanism in bioretention systems (9), only one study explicitly has examined biodegradation in a simulated bioretention system mulch-layer (1) and no research has been conducted on biodegradation of hydrocarbons in bioretention media (37). It is therefore unclear whether biodegradation is occurring in full-scale raingardens and how the effects of raingarden design (*e.g.*, planted or unplanted) and environmental conditions (*e.g.*, pollutant loading or land use) influence hydrocarbon biodegradation.

2.2 Biodegradation of Petroleum Hydrocarbons

Biodegradation of petroleum hydrocarbons is a well-studied subject within the field of bioremediation. Microorganisms can metabolize and use petroleum hydrocarbons as an energy source, breaking down and eventually mineralizing complex hydrocarbons to carbon dioxide and water (38). Biodegradation of petroleum hydrocarbons can be stimulated by adding nutrients, a carbon source, or electron acceptors (39). Biodegradation of petroleum hydrocarbons also can occur without addition of amendments via natural attenuation, albeit over an increased time horizon. To be effective in raingarden applications, natural attenuation is likely the only practical biodegradation option; if effective, it is likely to be a key performance parameter in petroleum hydrocarbon removal from stormwater. Maximizing the effectiveness of bioremediation requires a basic understanding of microbial enzymes and biodegradation pathways, as well as the bioavailability of pollutants.

2.3 Microbial Degradation Pathways

Microorganisms use enzymes to degrade complex chemical structures. To metabolize aromatic compounds such as BTEX compounds or PAHs, an aromatic ring must be cleaved. Bacteria that aerobically degrade BTEX compounds or PAHs use oxygenase enzymes to insert molecular oxygen into the aromatic ring; this is generally the rate limiting step in hydrocarbon degradation (38, 40). Typically, mono- or dioxygenases introduce oxygen atoms into the aromatic ring; further oxygen addition to the ring causes cleavage (39). Ring cleavage (Figure 2.2) may occur via ortho fission (oxygen addition between the two carbon atoms connected to the hydroxyl group) or meta fission (oxygen addition at one of the adjacent double bonds). A diverse array of potential biodegradation pathways often exist for a single compound. Although some bacteria may be able to completely mineralize complex organic pollutants, individual strains often do not possess full degradation pathways (28). In addition, a consortium of bacteria is typically more efficient at mineralizing pollutants than a single strain possessing a complete pathway (41). Because the byproducts of aromatic ring cleavage are relatively labile and can be degraded by a wide variety of microorganisms (42), greater total quantities of bacteria generally facilitate faster rates of mineralization (28, 43-45).

Biodegradation of petroleum hydrocarbons, including BTEX, PAH, and aliphatic compounds, may occur under both aerobic and anaerobic conditions, although the rates under the latter tend to be much slower and some PAHs may be only partially oxidized via co-metabolism rather than supporting growth (39, 46-55). Alternative electron acceptors under anaerobic conditions may include nitrate, iron, sulfate, manganese, or

carbon dioxide (54). An aerobic environment is more conducive for a rapid and complete degradation of PAHs and is generally more desirable for remediation (38).

The ability of a bacterium to produce oxygenase enzymes is encoded by specific functional genes, often found on plasmids (small circular non-chromosomal double-stranded DNA molecules) (38, 39). These genes can be measured using techniques such as quantitative polymerase chain reaction (qPCR) using specific primers that target catabolic functional genes. Measurement of specific genes is useful because there can be a positive correlation between the relative quantities of biodegradation functional genes and the potential contaminant remediation (56). The primers used to target portions of the microbial genome vary depending upon the purpose of the analysis. Targeting conserved portions of the 16S rRNA gene has been used as an indicator of total *Bacteria* (57); nevertheless, this value does not necessarily reflect true cell numbers because 16S rRNA gene copies may vary between bacterial species (57). Bioremediation indicator genes generally should be substrate specific, conserved, and target important metabolic pathways (57, 58). For example, dioxygenase primers have been developed for monitoring sites undergoing natural attenuation (40) and quantifying the BTEX degradation potential in environmental samples (58). Enzymes used in biodegradation may not be completely substrate specific; naphthalene dioxygenase, for example, catalyzes the initial reaction with oxygen and many PAHs (59). DeBruyn et al. used primers targeting the alpha dioxygenase subunit (three target genes: *nidA*, *nagAc*, and *nahAc*) via qPCR to compare PAH mineralization (naphthalene and pyrene) to functional gene quantities at a coal-tar contaminated superfund site (60). It was observed that mineralization rates for multiple PAHs were positively correlated with quantities of

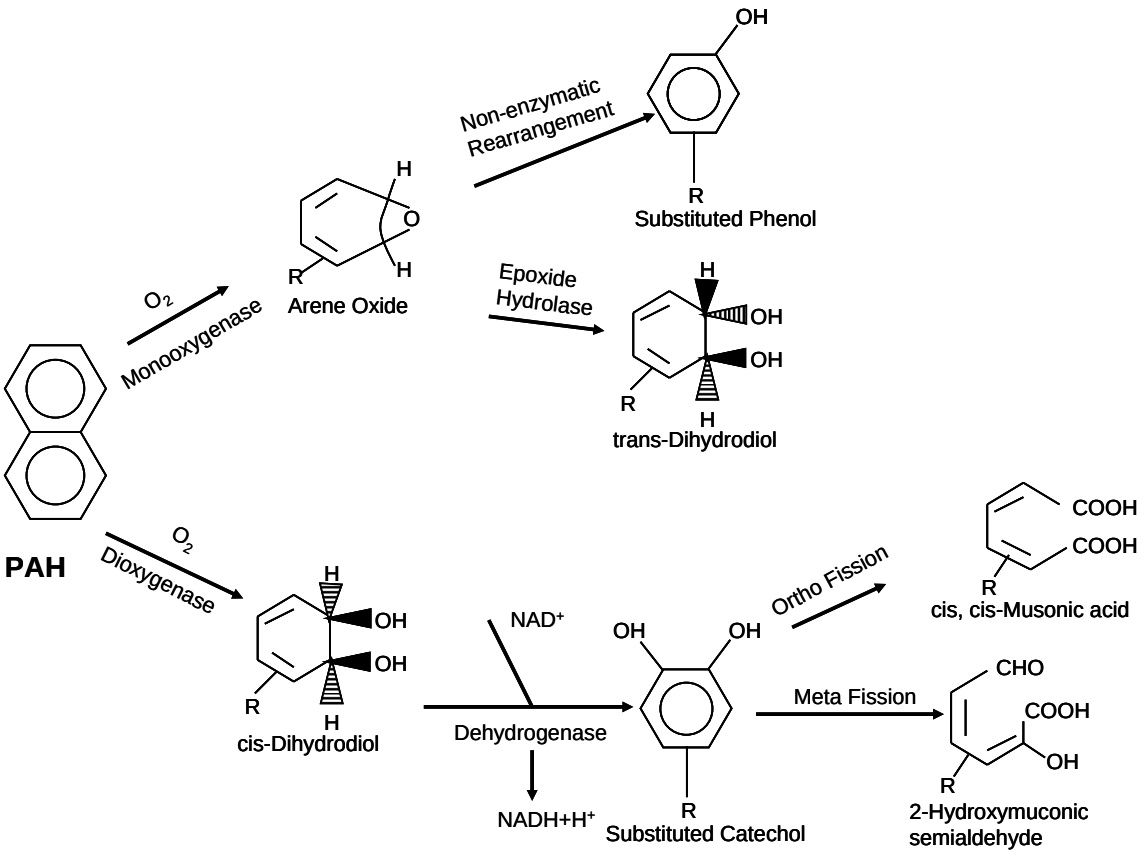

Figure 2.2: Several potential biodegradation pathway of PAHs. Adapted from (38).

naphthalene dioxygenase functional genes, illustrating the broad utility of these genes. Although molecular techniques such as qPCR have been employed in an array of applications, they have not been used to assess degradation potential in bioretention facilities.

There are both advantages and limitations to employing molecular techniques such as PCR for assessing biodegradation potential and efficacy. Molecular techniques are relatively rapid and inexpensive, produce quantitative results, and avoid problems associated with attempting to culture microorganisms (56, 57, 61, 62). Alternatively, PCR does not distinguish between viable and dead organisms, and determining if the

target gene is being actively expressed is often very difficult in environmental media. In addition, there may be bias in extracting DNA, and degenerate primers, non-specific binding, or attributes of the environmental matrix may effect amplification efficiency (39, 56, 57, 61). Although quantifying gene expression [via RNA (messenger RNA) extraction and reverse transcription to a DNA compliment (cDNA)] provides information regarding the activity of the target gene, accurate RNA recovery from environmental media can be challenging due to the fragile, unstable nature of the mRNA molecule (61, 63). If molecular tools are used appropriately and within their limitations, they can provide valuable information relating to the microbial community and activity at a given site.

2.4 Influence of Vegetation on In Situ Hydrocarbon Degradation

Observations in the literature demonstrate that hydrocarbons are degraded more quickly in vegetated than in non-vegetated soils (20, 28, 32, 33, 36, 64-71). Although plant uptake and pollutant metabolism occurs during phytoremediation (18, 19, 19, 22, 23, 25), the primary benefit of vegetation in phytoremediation is the stimulation of contaminant-degrading bacteria in the rhizosphere (28, 31-36). The rhizosphere is the area around the plant root that has been physically, chemically, or biologically modified by the presence of the plant (34, 69). Microbial biomass concentrations are typically one to four orders of magnitude greater in the rhizosphere than in soils outside of the root zone (28, 33, 35, 36), which is presumed to help protect the plant against pathogens (72) and harmful chemicals (36).

Although the positive influence of vegetation on petroleum degradation has been demonstrated, reasons for this effect are poorly understood. Plant roots deliver oxygen (73) and release exudates into the rhizosphere (Table 2.2), the latter of which provide a supply of labile carbohydrates and substances that also may act as contaminant analogues (64, 74). Root exudates can be natural analogues to anthropogenic contaminants such as polychlorinated biphenyls (PCBs) or PAHs (20, 28, 75), and the enzymes to degrade these compounds (*e.g.*, dehalogenases, dioxygenases) are often present within the rhizosphere microbial community, perhaps preconditioning the microbial community to anthropogenic contaminant degradation (28, 75). Exudates may make contaminants more bioavailable in the rhizosphere, and therefore more easily degraded (64, 76, 77). Conversely, the enhanced degradation potential may be due to the simple increase in the total population of bacteria present in the rhizosphere (20, 28, 78, 79); greater numbers and diversity of bacteria increase degradation efficiency through use of various intermediates in the mineralization pathway (28, 41). In fact, one study discovered that although root-derived substrates significantly increased degradation, the addition of these substrates actually repressed the expression of naphthalene dioxygenase genes (44, 45). Thus, although the positive impact of vegetation on hydrocarbon degradation has been demonstrated, the exact mechanisms for this stimulus are not fully understood.

Table 2.2 Types of compounds observed in root exudates (adapted from (45)).

Compound Class	Examples
Carbohydrates / Sugars	glucose, fructose, sucrose, maltose, galactose
Amino Acids	glycine, serine, lysine, arginine
Aromatic Compounds	phenols, salicylate, flavones, xanthones, naphthaquinones
Organic Acids	acetic acid, malic acid, propionic acid, citric acid, lactic acid
Vitamins	thiamine, niacin, riboflavin, pyridoxine
Enzymes	dehydrogenase, peroxidase, dehalogenase, nitroreductase, phosphatase, nitrilase
Volatile Organic Compounds	ethanol, methanol, acetone, acetaldehyde

Plant type may substantially influence remediation in the rhizosphere. Leguminous plants foster a symbiotic relationship between mycorrhizal fungi and rhizosphere bacteria (72, 80-82), including the nitrogen-fixing family of bacteria *Rhizobea* (80). This could enhance the quantity and/or activity of contaminant degraders (83). The nodule forming bacteria-plant interaction is a complex process, but may result in the growth of strains that are more competitive or use a wider variety of carbon sources (80). Indeed, PAH dissipation occurs at a greater rate in mycorrhizal roots than around nonmycorrhizal roots (34), and legumes have higher rates of mycorrhizal root colonization (69). This may be why leguminous plants are less affected by oil contamination compared to grasses or cereals (84, 85). Joner and Leyval (2003) suggest several explanations for the increased degradation capacity: mycorrhiza enhances the concentration of hydrogen peroxide leading to oxidation of PAHs, and mycorrhiza colonization results in altered root exudation and thus a changed microbial community, perhaps one that is better able to metabolize PAHs (34). Some plants, including legumes, are also known to release phenols (74, 86, 87), which could act as contaminant analogues to induce biodegradation of xenobiotic chemicals (88). Plant type impacts the success of phytoremediation, and could therefore be an important design element in bioretention areas.

2.5 Bioavailability of Organic Pollutants

Bioavailability refers to the extent to which contaminants can come into contact with living agents (89). From a remediation perspective, bioavailability is an important parameter because only the bioavailable fraction of a pollutant can be readily degraded by bacteria. A greater number of aromatic rings on a PAH generally makes it more

sorptive and less bioavailable (38). In classical theory, sorption is a reversible equilibrium process where the tendency for an organic contaminant to adsorb to given soil is a function of the chemical hydrophobicity (4). Thus, biodegradation of the available contaminant fraction provides a concentration gradient that drives further desorption.

Bacteria have evolved approaches to make energy-yielding substrates more bioavailable, some of which are target pollutants. One such strategy is synthesis of biosurfactants to reduce the surface tension of the solvent (90-92). The change in the organic carbon partitioning coefficient (K_{oc}) in the presence of cosolvents can be approximated by Equation 2.4 where σ^c is the cosolvancy power, and fv is the volume fraction of cosolvent.

Equation 2.4

$$K_{OC_{Solvent,Water}} = K_{OC} \cdot 10^{-\sigma^c \cdot fv} \quad (5)$$

Biosurfactants (Table 2.3, Figure 2.3) are surface-active compounds produced by microorganisms (93) composed of lipopeptides, glycolipids, neutral lipids, and fatty acids (92). Biosurfactants have proven effective at increasing the bioavailability of sorbed PAHs (94-96) and are attractive alternatives to synthetic surfactants at remediation sites as a result of their nontoxic nature, low adsorption on to soil, and good solubilization efficiency (97). In fact, several studies have indicated that biosurfactants are more effective for PAH desorption than xenophobic surfactants (95, 98-100). Biosurfactants have also improved phytoremediation performance in some cases (94). Nevertheless, the challenge of large-scale production, as well as limited experience in implementation,have restricted the use of biosurfactants in remediation (90). Much remains unknown

regarding the mechanisms of in situ biosurfactant production and the synergistic effects of biosurfactants produced during phytoremediation.

Table 2.3: Examples of biosurfactant types and the microorganisms that produce them (adapted from (93)).

Type of Biosurfactant	Microorganisms Responsible for Biosurfactant Creation
Rhamnolipids	*Pseudomonas aeruginosa*, *Pseudomomas* species., *Serratia rubidea*
Surfactin	*Bacillus subtilis*, *Bacillus pumilus*
Viscosin	*Pseudomonas fluorescens*
Sulfonylipids	*T. thiooxidans*, *Corynebacterium alkanolyticum*
Sophorose lipids	*Candida apicola*, *Candida bombicola*, *Candida lipolytica*, *Candida bogoriensis*
Trehalose lipids	*Arthrobacter paraffineus*, *Corynebacterium* species., *Mycobacterium* species, *Rhodococus erythropolis*, *Nocardia* species

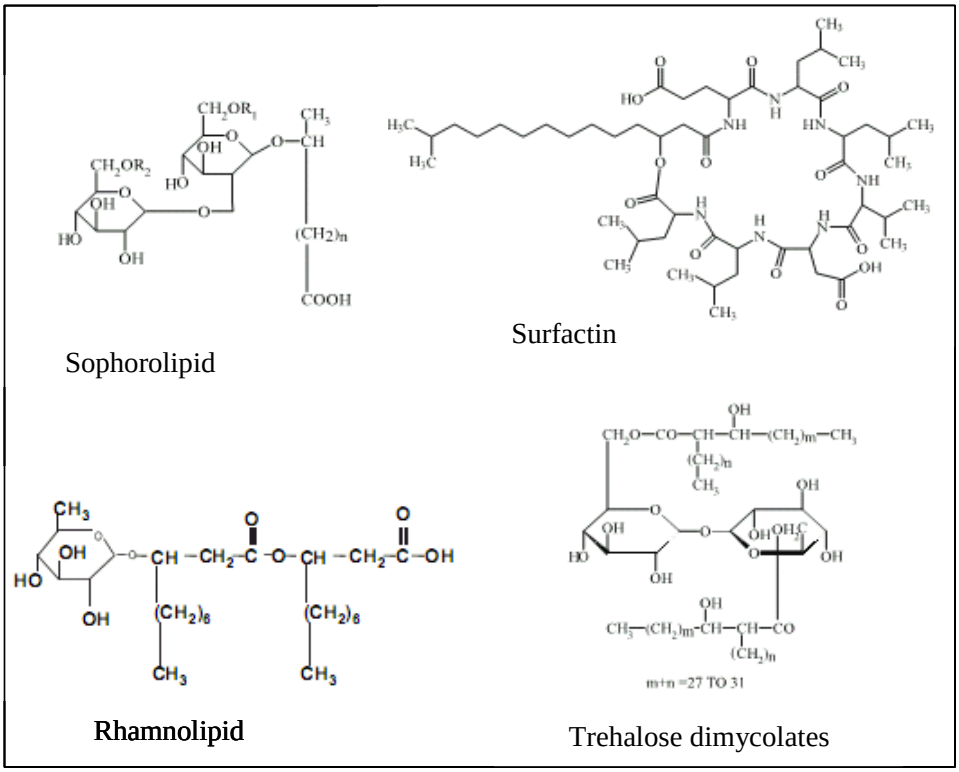

Figure 2.3: Chemical structures of selected biosurfactants. Adapted from (93, 101).

2.6 Summary and Research Needs

In summary, our understanding of the fate of hydrocarbons in stormwater bioretention areas must be enhanced. First, the extent to which hydrocarbons contaminate bioretention areas must be established to assess the impact on the urban environment. Important parameters, such as land use and vegetative cover, need to be investigated regarding their impact on hydrocarbon residual in bioretention areas. Biodegradation has been a presumed hydrocarbon removal mechanism within stormwater bioretention areas; nevertheless, degradation capacity in bioretention media has not yet been explored. Comprehensively determining the relative importance of potential loss mechanisms in bioretention areas such as sorption, volatilization, plant uptake, and biodegradation will elucidate the ultimate fate of stormwater hydrocarbons in bioretention areas. Furthermore, because bioretention sites are typically vegetated, plant presence and type may substantially impact the fate, biodegradation, and bioavailability of stormwater hydrocarbons. These insights will be vital to successfully integrating natural treatment systems into water infrastructure, but also broadly applicable to understanding contaminant fate and remediation.

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Chapter 3: The Role of Biodegradation in Limiting the Accumulation of Petroleum Hydrocarbons in Raingarden Soils

This chapter has been published in the journal *Water Research* and is cited as:

LeFevre, G.H.; Hozalski, R.M.; Novak, P.J. The role of biodegradation in limiting the accumulation of petroleum hydrocarbons in raingarden soils. *Water Res*. **2012**. DOI: 10.1016/j.watres.2011.12.040.

Previous studies have indicated that raingardens are effective at removing petroleum hydrocarbons from stormwater. There are concerns, however, that petroleum hydrocarbons could accumulate in raingarden soil, potentially resulting in liability for the site owner. In this work, 75 soil samples were collected from 58 raingardens and 4 upland (i.e., control) sites in the Minneapolis, Minnesota area, representing a range of raingarden ages and catchment land uses. Total petroleum hydrocarbon (TPH) concentrations in the samples were quantified, as were 16S rRNA genes for Bacteria and two functional genes that encode for enzymes used in the degradation of petroleum hydrocarbons. TPH levels in all of the raingarden soil samples were low (< 3 μg/kg) and not significantly different from one another. The TPH concentration in raingarden soil samples was, however, significantly greater ($p \leq 0.002$) than TPH levels in upland sites. In addition, the number of copies of Bacteria 16S rRNA genes and functional genes were greater in the raingardens planted with deeply-rooted natives and cultivars than in raingardens containing simply turf grass or mulch ($p < 0.036$), suggesting that planted raingardens may be better able to assimilate TPH inputs. The ability of microorganisms present in the soil samples to degrade a representative petroleum hydrocarbon (naphthalene) was also investigated in batch experiments. A sub-set of the field sites was selected for re-sampling, and all soil samples tested (n=8) were able to mineralize naphthalene. In these experiments the initial mineralization rate correlated with the number of copies of Bacteria 16S rRNA genes present.

3.1 INTRODUCTION

Pollution from urban stormwater runoff is a significant source of surface water impairment and presents one of the greatest water quality challenges of the coming century (1-5). Common pollutants detected in stormwater runoff include nutrients such as phosphorus and nitrogen, heavy metals, pathogens, and petroleum hydrocarbons (6). Petroleum hydrocarbons are washed from impervious surfaces such as parking lots and streets by rainfall, with reported concentrations in stormwater ranging from 0.2 to 277 mg/L (7-11). The petroleum hydrocarbons on such surfaces often result from leaks or spills of fuel, oils, and greases that are associated with motor vehicle use (5). The impervious surfaces themselves can also serve as sources of petroleum hydrocarbons, as coal tar-based pavement seal coats are believed to be a significant source of polycyclic aromatic hydrocarbons (PAHs) to the environment (12-16). Petroleum hydrocarbons are of concern because some compounds under this rather broad class of chemicals are toxic to aquatic life and some are known or suspected human carcinogens (17, 18). Unfortunately, little research has been conducted on the removal of petroleum hydrocarbons from stormwater by best management practices (BMPs) and the ultimate fate of the removed hydrocarbons.

Increasingly, infiltration-based stormwater BMPs, including vegetated swales, infiltration trenches, and raingardens, are being implemented to reduce total runoff volume and ameliorate the environmental effects of stormwater through a variety of physical, chemical, and biological mechanisms (6, 19). Raingardens, also known as bioinfiltration or bioretention practices, are shallow vegetated depressions with an engineered soil media to which stormwater from impervious surfaces is directed for

infiltration (5, 20). In particular, raingardens have grown in popularity throughout the United States as a component of low impact development (LID). Because stormwater runoff contains petroleum hydrocarbons and other contaminants, it is important to determine the fate of these contaminants in raingardens.

Few studies have explicitly examined petroleum hydrocarbon removal in raingardens. Hsieh and Davis (21) observed greater than 96% decrease of oils and grease in laboratory-scale raingardens and nearly 100% in full-scale systems. Hong et al. (22) observed approximately 90%decrease of petroleum hydrocarbons in laboratory columns. DiBlasi et al. (23) examined PAH removal at a bioretention field site and reported an average 87% mass load reduction. Based on the available evidence, bioretention appears to be successful in removing hydrocarbons from infiltrated stormwater (6, 24). Nonetheless, we are unaware of any research performed to investigate the ultimate fate of hydrocarbons in raingardens. Removal is presumed to be a combination of sorption and biodegradation (22), but these two mechanisms and their relative importance in raingardens have not been adequately explored.

Biodegradation of petroleum hydrocarbons is an oxidative process initiated by enzymes that can result in hydrocarbon mineralization; biodegradation has been observed in a wide array of environments, including under both aerobic and anaerobic conditions (25). The genes that encode for oxygenases or other relevant enzymes can be enumerated using techniques such as quantitative polymerase chain reaction (qPCR). Molecular methods have the advantage of being rapid and specific, and can be used to target specific functional genes such as those encoding for oxygenase enzymes. Although the mere presence of a specific functional gene does not indicate that the reaction catalyzed by that

enzyme is actually occurring, such molecular methods appear to be useful for assessing bioremediation activity or potential. For example, qPCR was employed to evaluate the progress of monitored natural attenuation at gasoline-contaminated sites (26, 27). qPCR, however, has not yet been used to assess contaminant biodegradation capacity in bioretention systems.

Although the removal of petroleum hydrocarbons from runoff prior to discharge to a receiving water is beneficial, the potential for accumulation of pollutants in raingarden soils is a significant concern (28). For example, PAH accumulation in the sediments of stormwater retention ponds has been reported (13, 15, 29), and can lead to prohibitively high disposal costs for the dredging spoils. If such "toxic depots" are created in raingardens, there is potential environmental liability to the site owner, which could decrease the rate of implementation of these beneficial LID technologies. Therefore, it is important to establish if petroleum hydrocarbons accumulate to unsafe levels in raingarden soils.

Biodegradation of petroleum hydrocarbons occurs readily under aerobic conditions when other criteria such as pH, temperature, and nutrient levels do not limit microbial growth (30, 31). Thus, it appears that petroleum hydrocarbon degraders are ubiquitous in the environment (25, 32). As aerobic conditions are typical in shallow soil environments, it is expected that petroleum hydrocarbons would be readily biodegradable in raingardens. Nonetheless, raingarden media is designed to promote rapid infiltration, resulting in short hydraulic residence times that may be insufficient to promote biodegradation. Furthermore, the organic matter in raingarden media from the addition of compost will likely limit the bioavailability of petroleum hydrocarbons. Raingardens

commonly experience variable pollutant loading and inconsistent soil moisture conditions (5) which could inhibit the accumulation of petroleum hydrocarbon degraders. Therefore, this research was performed to examine the concentration of petroleum hydrocarbons in raingarden soils at field sites, to evaluate the potential for petroleum hydrocarbon biodegradation in raingarden soils, and to investigate what factors influence petroleum hydrocarbon residual. Finally, qPCR was investigated as a rapid method to evaluate the biodegradation potential of bioretention soils.

3.2 MATERIALS AND METHODS

Field Sampling and Site Classification. A total of 75 soil samples were collected from May to August, 2008 in the Minneapolis-St. Paul metropolitan area (Minnesota, USA); 71 of the samples were from raingarden sites and 4 additional upland samples served as controls. Fifty-eight raingardens were sampled, with one soil sample collected from 49 of the raingarden sites and multiple soil samples (up to 4) collected from 9 of the raingardens. Soil samples (approximately 10 g each) were collected just below the ground surface (or below the mulch layer, if present) at the raingarden entrance (e.g., curbcut) and/or near the lowest elevation of the raingarden. Samples were collected using sterilized scoops and were placed in autoclaved glass bottles. The raingarden sites were all infiltration-based urban stormwater best management practices and represented a wide array of sizes, vegetation types, catchment land uses, and catchment areas. In addition to the samples collected from within the raingardens, 4 additional samples were collected in a similar manner from land adjacent to, but outside of, four of the sampled raingardens. These 'upland' sites, unlike the raingarden sites, did not receive runoff from

impervious surfaces and served as controls to account for any possible direct wet or dry deposition of hydrocarbons.

Each raingarden was classified into one of four categories based on dominant catchment area land use: residential street (24 raingardens, 25 samples), residential roof (12 raingardens, 13 samples), commercial parking lot (including businesses and car pool lots; 11 raingardens, 15 samples), and light-use parking lot (which include parks, municipal buildings, schools, etc.; 11 raingardens, 18 samples). The dominant vegetation type present in each raingarden was recorded and classified into four categories: native plantings (27 raingardens, 34 samples), decorative cultivars (24 raingardens, 26 samples), turf grass/lawn (3 raingardens, 4 samples), and mulch/unplanted (4 raingardens, 7 samples).The raingarden and contributing impervious surface dimensions were measured using a surveyor's tape measure to determine the plan areas of the raingarden and the catchment or roof, respectively. Raingardens ranged in size from small (14 sq ft; 1.3 m^2) to large (20,000 sq ft; 1860 m^2), with a median surface area of approximately 600 sq ft (56 m^2). The ratio of catchment area to raingarden area (i.e., area loading factor) ranged from 1.6 to 45.8, with a median value of 7.1 for the raingardens sampled. Because the majority of raingardens were located on private property, only limited information was available on the site history; therefore, the installation date was obtained for only 26 of the raingarden sites. Of the sites with known history, raingarden ages ranged from two to five years.

Field Sample Preparation. Collected soil samples were placed on ice in a cooler for transportation to the laboratory. Upon arrival at the laboratory samples were placed in a refrigerator and stored for up to 4 days at 4^{o}C until DNA was extracted. DNA was

extracted from a subsample of the homogenized soil using a MO-BIO PowerSoil™ DNA kit, following the manufacturer's protocol. Extracted DNA and the remaining soil were frozen at -20°C until further use. Soil moisture was determined by drying a subsample overnight in a drying oven at 105°C as per Standard Methods (33).

Batch Biodegradation Tests. Batch tests were conducted to assess the petroleum hydrocarbon biodegradation potential of soil samples from selected raingardens. For these experiments, naphthalene was chosen as the model hydrocarbon because it is the dominant component of coal tar (34) used in some types of pavement sealcoat (14), is a relatively labile and moderately hydrophobic PAH, and its biodegradation has been well studied (e.g., (35)). Eight sites, chosen to represent a range of land use and planting types, were sampled again (6 in November, 2009 and 2 in September 2010) in the manner previously described. The 8 sites chosen for this second round of sampling were expected to contain a wide range of target gene quantities, based on the analysis of the initial 75 samples. Samples were refrigerated at 4°C for up to 20 weeks until used for a batch experiment. The total organic carbon (TOC) content of the samples was measured by the University of Minnesota Soils Research Analytical Laboratory and DNA was extracted as described above. Batch tests were conducted in nephelometric biodegradation flasks (Chemglass) that had been modified to allow both the sidearm and flask top to be sealed with a PTFE-lined septum (Wheaton Scientific) and crimp top (Figure 3.1). The flasks were autoclaved prior to setting up the batch experiments. Twenty-five mL of 2M NaOH (pH=13) was added tothe sidearm and the sidearm was sealed with a septum and crimp top. A moist soil sample (2.5 g) was added to the flask followed by 50.0 mL of pH 7.0 minimal medium containing 10 mg/L of naphthalene as

the sole carbon and energy source. The minimal medium was prepared according to the recipe of Ghoshal et al. (34) except that the concentration of phosphate buffer was doubled to provide additional buffer capacity. Finally, 1,4,5,8-^{14}C radiolabeled naphthalene (Moravek Biochemicals) dissolved in methanol was spiked into the soil slurry via glass syringe at a dose of 300,000 disintegrations per min (DPM) or 1.35×10^{-4} mCi per flask. The flask was immediately sealed with a septa and crimp top. Negative controls were prepared in the same manner except that sodium azide was added to the slurry at a concentration of 50 mM. All flasks and controls were prepared in triplicate. The flasks were incubated in the dark at room temperature (approximately 21ºC) and the soil slurry was mixed using a magnetic stir bar for the duration of each experiment (260 to 430 h). Samples (1.0 mL) were withdrawn periodically from the sidearm and were

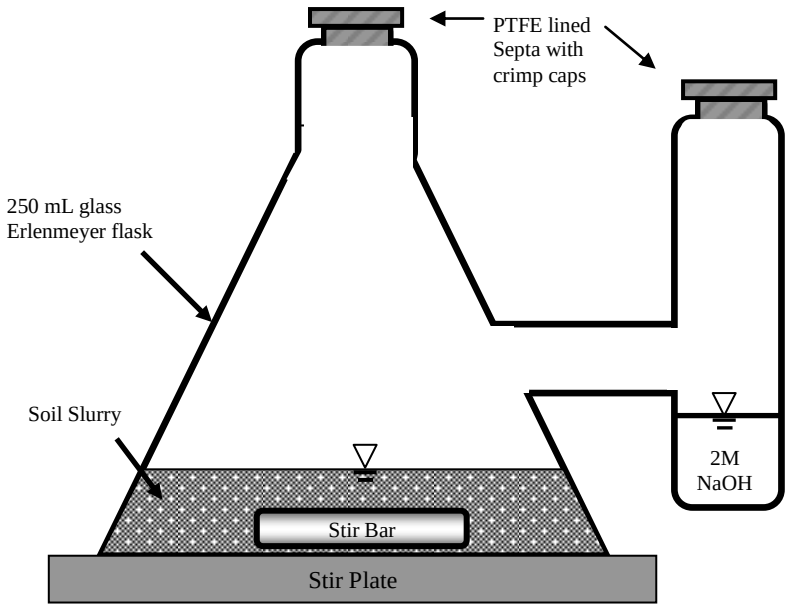

Figure 3.1: Schematic diagram of sidearm flask system used for the batch biodegradation experiments.

air-stripped to remove any volatilized ^{14}C-naphthalene that may have been absorbed by the sidearm solution. A 200µL subsample of the air-stripped solution was injected into 10.5 mL liquid scintillation cocktail (Perkin Elmer) and analyzed on a Packard Tri-Carb 2100TR scintillation counter. The total cumulative $^{14}CO_2$ captured by the NaOH solution as a function of time was computed and then normalized by the total ^{14}C added as naphthalene to obtain the percent naphthalene mineralized. Naphthalene mineralization results are reported as the mean of the triplicate flasks ± standard error.

Quantification of total petroleum hydrocarbon (TPH) concentrations. Petroleum hydrocarbons were extracted from the previously frozen soil samples and analyzed as described in Mohn and Stewart (30). Soil (approximately 3 g dry weight) was thawed then added to 8 g of anhydrous sodium sulfate in a pre-baked (6 h at 550ºC) glass vial. The vial was sealed with a PTFE-lined stopper (Wheaton Scientific) and shaken by hand until the mixture flowed freely. Hexane (8.0 mL, HPLC grade, Fisher Scientific) was added and the mixture was shaken by hand for two min then vortexed for one min. The vials were sonicated in a water bath for 10 min and left overnight on the benchtop. A 2-mL aliquot of supernatant was transferred toan autosampler vial for analysis via gas chromatography with flame ionization detection (GC-FID; HP5890 Series II). The column used was a Restek Rtx-1with a temperature program of 40^{o}C for 2 min, ramped at 30^{o}C /min to 300^{o}C, and held for 2 min. UHP zero-grade nitrogen at a flow rate of 2.4 mL/min was the carrier gas. Inlet and detector temperatures were 280ºC and 300ºC, respectively. The GC-FID was calibrated by making serial dilutions of the Supelco TPH Mix 1 (861424-U, Sigma-Aldrich) in hexane.

The method detection level (33) was 0.50 µg TPH/L solvent (corresponding to approximately 0.13 µg TPH/kg soil). The TPH extraction efficiency was evaluated using laboratory-prepared raingarden soil media (36) comprised of construction sand sieved to a grain size between 0.05 and 0.1 cm (60% by vol.), organic leaf compost (30% by vol.), and top soil (10% by vol.). The raingarden soil media had an organic carbon content of 3.52±0.37%. The TPH Mix 1 in methanol (2.6µg) was added to 3 g of moist soil and mixed end-over-end overnight at room temperature. Then, the soil was extracted using the aforementioned protocol. The mean extraction efficiency (six replicates) was 95.7±15.1%. Reported TPH values were not adjusted for extraction efficiency.

qPCR Analysis. Real time quantitative PCR was used to enumerate functional genes and total 16S rRNA genes from the domain Bacteria in the extracted DNA. The targeted functional genes encode for the naphthalene dioxygenase enzyme (NAH) and phenol monooxygenase enzyme (PHE). PHE was chosen because it is one of the most frequently detected aromatic oxygenases at petroleum hydrocarbon-contaminated sites (26, 37). NAH was chosen because naphthalene comprises the largest component of coal tar (34) and coal tar is often used in pavement sealcoat formulations. The primer sets for the target functional genes were developed by Baldwin et al. (38). The 16S rRNA genes for total Bacteria were enumerated using the primers 341F and 534R (39, 40). All primers were synthesized at the University of Minnesota BioMedical Genomics Center. In addition to the forward and reverse primers (concentration 0.3 µM each), each 25 µL reaction contained 12.5 µL iTaq SYBR Green Supermix w/ ROX (BioRad Laboratories), 1.25 µL bovine serum albumin (Roche Applied Science), 5.5 µL of ultra-pure DNase/RNase free PCR water (Sigma), and 5 µL of 1:10 diluted DNA template. qPCR

was performed in a 96-well plate (Applied BioSystems) using an Eppdendorf AG 22331 real-time thermocycler. The PCR sequence, modified from Baldwin et al. (38), was: 50 ^{o}C for 2 min, 95^{o}C for 10 min, and 40 cycles each consisting of 95^{o}C for 15 seconds and 60^{o}C for 1 min. A final melting curve sequence of 95^{o}C for 15 sec, 60^{o}C for 20 sec, and 95^{o}C for 15 sec was also performed for quality assurance. Plasmids (extracted from *Pseudomonas putida* JS150 [ATCC# 51283] for NAH and *P. putida* CF600 [obtained from Prof. Victoria Shingler, Umeå University, Sweden] for PHE using the QIAprep Spin MiniPrep Kit (Qiagen) following the manufacture's protocol) containing the target gene were used as standards for quantification. Standard curves were linear from $5x10^{2}$ to 10^{9} copies per reaction mixture for NAH, $5x10^{3}$to 10^{9} copies per reaction mixture for PHE, and from $5x10^{5}$ to 10^{10} copies per reaction mixture for Bacteria16S rRNA genes. The detection limits for the NAH, PHE, and the 16S rRNA genes were $4x10^{4}$, $4x10^{5}$, and $4x10^{7}$ copies per g dry soil, respectively. Values below the detection limit were assigned a value of one half the detection limit for data analysis purposes. All samples and standards were run in triplicate. Melting curves were checked for consistency in form and melting temperature. Selected samples were extracted in triplicate to evaluate consistency in the DNA extraction process. Relative error for triplicate DNA extractions was 9.7% for NAH genes (n=16), 1.1% for PHE genes (n=8), and 3.5% for Bacteria 16S rRNA genes (n=5).

Data Analysis. TPH and qPCR data were grouped into categories (i.e. catchment land use type, vegetation type) and the data were tested for normality (and log normality) using the Shapiro–Wilk test. The data were considered to be normally distributed if the null hypothesis of normality could not be rejected at the 95% confidence level.A one-way

analysis of variance (ANOVA) test was used to screen for significant differences between the mean values (or log transformed mean values, if log-normally distributed) of the measured parameter of interest among the different categories. If the ANOVA revealed that at least one mean value was different from the others ($p<0.05$), then subsequent multiple pairwise t-tests were performed. For the multiple pairwise t-tests, a Bonferroni adjustment was employed ($\alpha^{*}=\alpha/k$; where: α=significance level=0.05 and k=number of pairwise comparisons in the analysis) to guarantee that the probability of any false rejection from all comparisons in the analysis was less than 0.05, which is more conservative than setting the probability of false rejection to 0.05 for each separate comparison (41). This method substantially reduces probability of Type I error within the analysis. To corroborate the results obtained from the ANOVA and t-tests, a nonparametric all pairwise comparison Wilcoxon rank sum test was also employed. A p-value of 0.05 was selected for delineating significance for all statistical comparisons in this manuscript.

3.3 RESULTS AND DISCUSSION

Petroleum Hydrocarbon Residuals in Raingarden Soil. Box and whisker plots of the TPH concentration in raingarden soil for the different catchment land uses are shown in Figure 3.2.

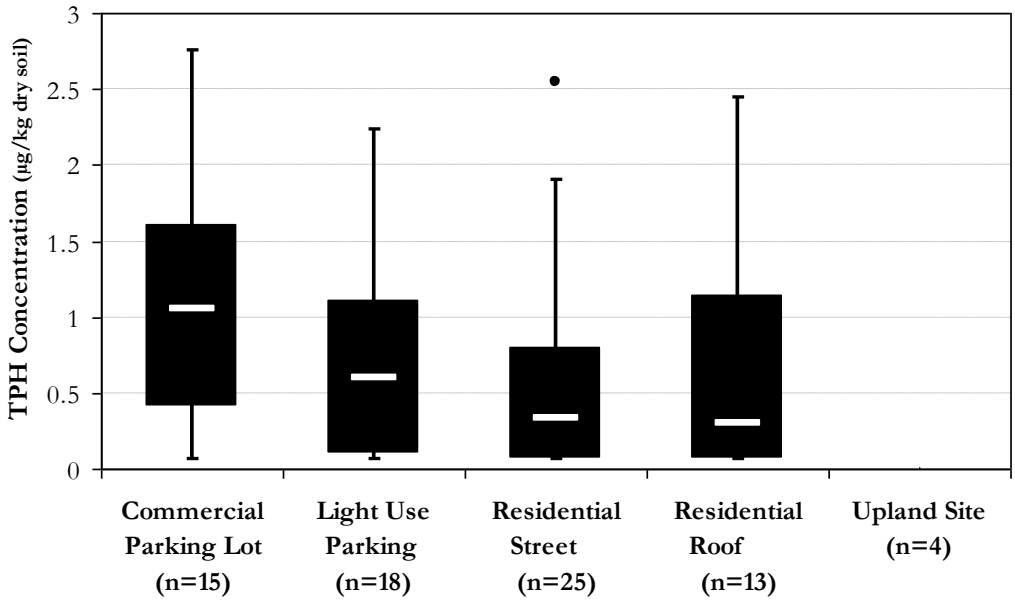

Figure 3.2: Effect of catchment land use on total petroleum hydrocarbon (TPH) residual concentration in raingardens and comparison with upland sites. This plot shows the median (white bar), the upper and lower quadrilles [top and bottom of box, respectively, forming the inter-quartile range (IQR)], and the minimum and maximum values (whiskers). Outliers (points) are values greater than 1.5 IQR beyond the first and third quartile. All of the upland samples were below the detection limit.

None of the upland sites contained TPH residual above the detection limit. In addition, many raingarden soil samples were below the TPH method detection limit (39% of samples) and TPH concentrations in all of the remaining samples (< 3 µg TPH/kg dry soil) were low compared to regulatory limits. For example, the Minnesota Pollution Control Agency (MPCA) requires corrective action when petroleum contamination of surface soil is greater than 10 parts per million (i.e. 10,000 µg TPH/kg; (42)). Thus, even the greatest TPH concentration observed (2.76 µg TPH/kg dry soil) was still several orders of magnitude less than the concentration of concern for human and ecological health. According to the results of the Shapiro-Wilk testing, the log-normal distribution best described data for each catchment land use, and the ANOVA test revealed that the

log-mean raingarden soil TPH concentrations for the different catchment land use types were not significantly different from one another ($p>0.07$). TPH soil concentrations between raingardens containing deeply-rooted vegetation (natives or cultivars; roots depth >15cm) were not different ($p=0.69$) from unplanted raingardens or those planted with turf grass, and there were no significant differences in TPH between any of the vegetation classes ($p>0.28$). In addition, TPH levels did not correlate ($R^2=0.0016$; Figure 3.3) with areal loading factor (catchment area/raingarden area). The lack of an effect of catchment land use and areal loading factor on TPH levels in raingarden soils was surprising, as greater inputs of TPH were expected for commercial parking lots in comparison to the other land uses and for higher areal loading factors. These results suggest that TPH biodegradation or loss is occurring in these raingardens.

For comparison, an expected TPH residual was computed based on measured parameters and conservative assumptions where data were not available. The following equation was used:

Equation 3. 1

$$TPH_{Soil} = \frac{P \times C_{TPH} \times A_{Catchment} \times t \times e \times RC}{A_{Raingarden} \times D_{Soil} \times \rho}$$

where: P is the annual precipitation, C_{TPH} is the concentration of TPH in stormwater (conservatively assumed to be the low value of 0.2 mg/L reported by Wu et al. (7)), $A_{Catchment}$ is the area of the catchment that contributes runoff to the raingarden, *t* is the raingarden age, *e* is the estimated extraction efficiency (95.7%), RC is the runoff coefficient (assumed to be 0.98 for pavement; (43)), $A_{Raingarden}$ is the surface area of the raingarden, D_{Soil} is the depth of soil in which TPH is trapped (assumed to be the top 10 cm (23)), and ρ is the bulk density of the soil (assumed to be 1.3 g/cm^3 (44)). This

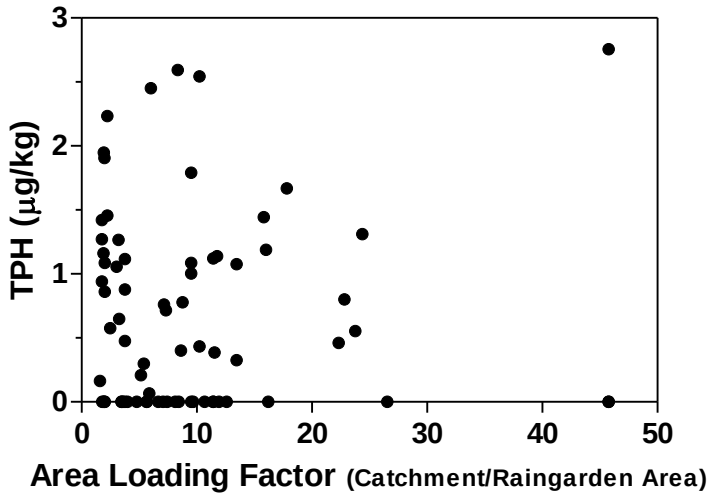

Figure 3.3: Relationship between total petroleum hydrocarbon (TPH) residual measured and raingarden hydraulic loading factor (R^2=0.0008).

calculation assumes that all of the TPH is retained in the raingarden soil via sorption and that there are no loss processes (e.g., biodegradation) occurring. The calculation was performed for the 26 raingardens for which we knew the installation dates, which ranged in age from 2 to 5 years. For those raingardens, the expected soil TPH concentrations were approximately four to five orders of magnitude greater than those of the respective observed TPH concentrations. Although there are limitations to such broad assumptions (e.g., bulk density), the large difference between predicted and observed TPH residuals demonstrates that significant losses of TPH are occurring in raingardens.

Overall, the TPH results show that raingarden soil TPH concentrations are significantly greater ($p \leq 0.002$) than those in upland 'control' soils, but far below levels of concern. In addition, the observed soil TPH concentrations are much less than expected based on a conservative assumption regarding the TPH concentration in stormwater. Furthermore, raingarden soil TPH concentrations did not correlate with areal loading factor or catchment land use type. Taken together, these results suggest that the

raingardens are receiving inputs of petroleum hydrocarbons but that attenuation of petroleum hydrocarbons is occurring in raingarden soils. Thus, biodegradation was investigated as a potential petroleum hydrocarbon loss process in raingardens.

Enumeration of Bacteria and Functional Genes. The quantities of PHE, NAH, and Bacteria 16S rRNA genes were enumerated and normalized per gram dry soil mass. These values are presented as a function of catchment land use in Table 3.1 and as a function of dominant vegetation type in Table 3.2. The statistics provided in Table 3.1 and Table 3.2 are segregated based upon land use type and vegetation, respectively. For all (non-segregated) raingarden samples, PHE copy numbers ranged from 10.6 to 12.9 log copies/g dry soil with a median value of 12.5; NAH copy numbers ranged from non-detect to 12.0 log copies/g dry soil with a median value of 7.9; and Bacteria 16S rRNA copy numbers ranged from 11.5 to 13.6 log copies/g dry soil with a median value of 13.1.

Table 3.1: Effect of catchment land use on gene copy number (log gene copies / g dry soil) in raingarden soils and comparison with soils from upland sites. Values shown are mean ± standard deviation.

Catchment Land Use	n[1]=	Phenol Monooxygenase (PHE)	Naphthalene Dioxygenase (NAH)	Total Eubacteria (16S rRNA)
High Intensity Commercial Parking Lot	15	12.0±0.42	6.8±2.0	12.9±0.36
Light Use Parking Lot	18	11.9±0.71	7.2±1.4	12.9±0.44
Residential Street	25	12.6±0.25	7.7±1.8	13.1±0.39
Residential Roof	13	12.6±0.14	7.9±1.6	13.2±0.22
Upland (non raingarden)	4	11.5±0.67	7.3±1.3	12.6±0.42

1= number of samples in the respective category

Table 3.2: Effect of dominant vegetation regime present in the raingarden on gene copy numbers (log gene copies / g dry soil) in raingarden soils. Values shown are mean ± standard deviation.

Vegetation Type	n[1]=	Phenol Monooxygenase (PHE)	Naphthalene Dioxygenase (NAH)	Total Eubacteria (16S rRNA)
Native Vegetation Plantings	36	12.3±0.51	8.0±2.2	12.3±0.41
Decorative Cultivars	26	12.5±0.22	7.1±1.9	13.2±0.29
Turf Grass	6	11.2±0.40	7.2±1.0	12.6±0.48
Unplanted	7	11.6±0.56	7.0±1.1	12.3±0.30

1= number of samples in the respective category

For the upland sites, the PHE copy numbers ranged from 10.8 to 12.4 log copies/g dry soil with a median value of 11.4; NAH copy numbers ranged from non-detect to 8.2 log copies/g dry soil with a median value of 7.8; and Bacteria 16S rRNA copy numbers ranged from 12 to 12.9 log copies per g dry soil, with a median value of 12.8. Gene copy numbers did not vary significantly with catchment area land use and gene copy numbers in upland sites were not statistically different from those in raingarden sites ($p>0.099$). Additionally, gene densities did not correlate with TPH concentrations or the areal loading factor ($R^2 \leq 0.02$, Figure 3.4 and Figure 3.5). Thus, the two functional genes appear to be ubiquitously distributed throughout the soil environment and were found in raingardens receiving runoff from a variety of catchment land uses and in upland control soils.

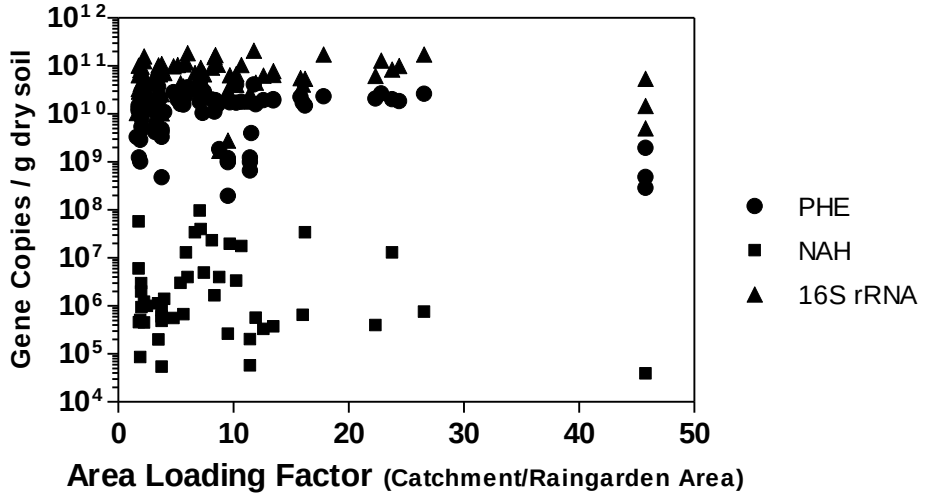

Figure 3.4: Relationship between phenol monooxygenase (PHE), naphthalene dioxygenase (NAH), and 16S rRNA gene quantities and raingarden areal loading factor.

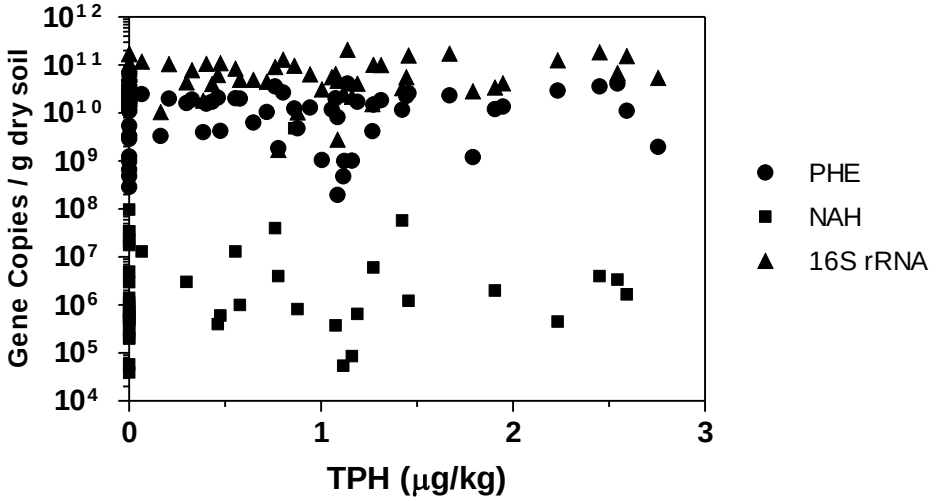

Figure 3.5: Relationship between phenol monooxygenase (PHE), naphthalene dioxygenase (NAH), and 16S rRNA gene quantities and total petroleum hydrocarbon (TPH) residual.

Raingardens that contained deep-rooted (>15cm) vegetation (natives and cultivars categories) had statistically greater densities of both functional genes and total bacteria (p-values: PHE<0.0001, NAH=0.036, Bacteria 16S rRNA=0.0084) when compared as a group to the raingarden sites that contained shallow-rooted or no vegetation (turfgrass

and mulch categories). These results are consistent with reports from the phytoremediation literature in which soil under the immediate influence of plant roots typically contain one to four orders of magnitude greater bacterial density than non-vegetated soils (45-47).

Batch Biodegradation Tests. The seven raingarden samples and one upland sample used in the batch biodegradation tests were all capable of mineralizing naphthalene, but at different rates and to different extents (Figure 3.6 and Figure 3.7).The three-parameter Gompertz curve ($y = ae^{be^{ct}}$) was fitted to the data using a least squares minimization approach and the best-fit curves were used to determine the time to maximum rate of mineralization (inflection point) and the maximum extent of mineralization. Time to maximum rate of mineralization was chosen as the indicator of degradation kinetics whereas maximum mineralization was thought to be more likely determined by bioavailability.

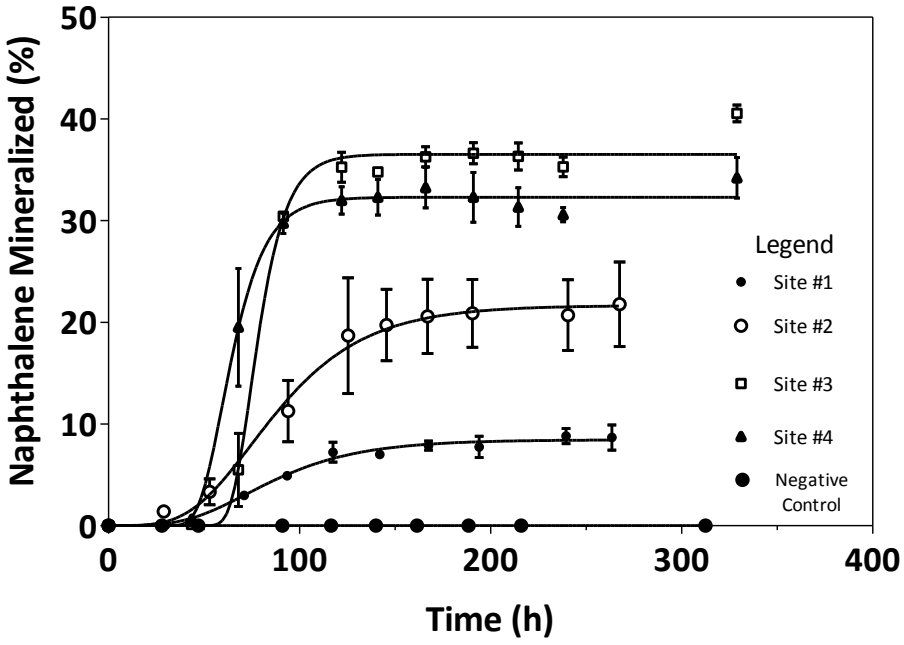

Figure 3.6: Results from the naphthalene biodegradation batch experiments (remaining experiments shown in Figure 3.7 for clarity). Data points represent the mean and error bars represent the standard error of triplicate batch tests. Lines are the least squares regression fit using the Gompertz function.

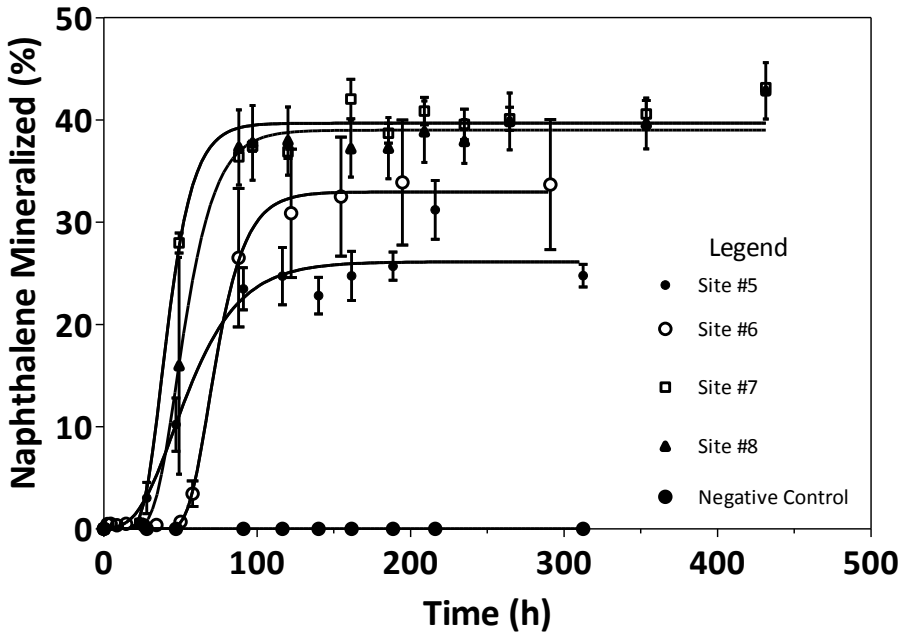

Figure 3.7: Naphthalene mineralization profiles for Sites #5-#8 (continued from Figure 3.6).

The time to the maximum mineralization rate correlated with the total number of Bacteria16S rRNA gene copies in a statistically significant manner (R^2=0.65, p=0.016; Figure 3.8), but not with the number of NAH (R^2<0.07) or PHE (R^2<0.50) gene copies present in a given sample. These results are consistent with those of Baldwin and coworkers (26) who reported that the concentrations of PHE and NAH genes in groundwater did not correlate with BTEX degradation rate at a contaminated field site. These findings suggest that although the measured functional genes are important for degradation, the rate of *mineralization* is likely governed by the abundance of other enzymes. Although some organisms may be able to completely mineralize complex organic pollutants such as naphthalene, individual strains do not typically possess full degradation pathways (46). In addition, a consortium of bacteria is typically more efficient at mineralizing pollutants than a single strain possessing a complete pathway (48). Because the byproducts of naphthalene ring cleavage are relatively labile and can be degraded by a wide variety of organisms (49), greater total quantities of bacteria should facilitate faster rates of mineralization.

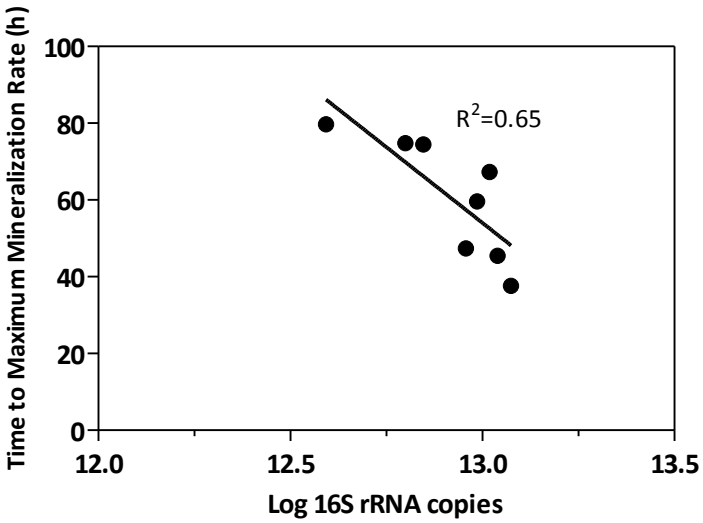

Figure 3.8: Relationship between initial total bacteria (Log 16S rRNA copies) per flask and the time to reach the maximum rate of mineralization (h).

The extent of mineralization ranged from 8 to 39% in the batch tests and can be viewed as a measure of compound bioavailability. These values for extent of mineralization are similar to those obtained by Zhang and Bouwer (50). The incomplete mineralization is not surprising as some of the labeled carbon is incorporated into bacterial biomass. Furthermore, naphthalene is moderately hydrophobic ($\log K_{ow} = 3.3$) and will therefore tend to sorb to organic matter present in soil limiting bioavailability of the compound (51). The extents of mineralization in our experiments did not correlate with the TOC content of the soils in a statistically significant manner ($p=0.08$, Figure 3.9). This may be because a sub-set of TOC (i.e., so called black carbon) could actually be controlling the bioavailability of naphthalene in our system (52, 53). The idea of bioavailabilty limiting biodegradation in soil-containing systems can similarly be used to explain the consistent low residual TPH concentrations observed in the raingarden soil samples discussed above.

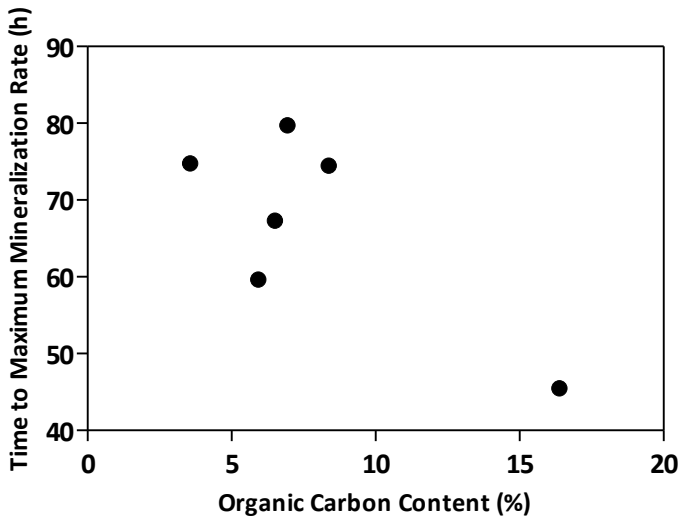

Figure 3.9: Relationship between organic carbon content and time to reach maximum naphthalene mineralization rate in raingarden soil batch biodegradation experiments.

Engineering Implications. Based on studies in the literature showing that petroleum hydrocarbons are removed from stormwater by raingardens (6, 21, 22) and the results of this study demonstrating that the ability to degrade a representative petroleum hydrocarbon was ubiquitous in raingarden soils, raingardens are an effective option for sustainably treating petroleum hydrocarbon-contaminated stormwater. It appears that the ability to biodegrade petroleum hydrocarbons is critical for preventing TPH from accumulating to concentrations of concern in soils from a broad range of raingarden field sites. These results contrast with results from stormwater retention pond sediments, where total PAH levels ranged from 37.8 to 64.5 mg/kg dry weight (29). This finding is important for engineers, planners, and regulators incorporating bioretention into LID site design, as accumulation of toxic compounds in stormwater treatment systems could present a human health risk (18) and a disposal problem (42). An explanation for the relative success of raingardens is that raingardens (when properly draining) maintain aerobic conditions, whereas pond sediments are typically anaerobic (29). Although

anaerobic biodegradation of some petroleum hydrocarbons such as benzene and PAHs is possible, the rate tends to be slow compared to aerobic biodegradation (35, 54-62). Certainly our results are limited as we only investigated the ability of raingarden soils to biodegrade one representative petroleum hydrocarbon, but the ability to mineralize naphthalene suggests that less hydrophobic and more labile petroleum hydrocarbons such as BTEX (benzene, toluene, ethylbenzene, and xylenes) compounds are also likely to be biodegraded. Thus, when the results of this research are compared to data from the literature, it appears that raingardens may be more effective than stormwater ponds at mitigating hydrocarbon pollution.

Results also revealed that NAH, PHE, and total bacterial quantities were statistically greater in the group of raingardens planted with deep-rooted vegetation (natives and cultivars) as opposed to the group planted with shallow-rooted turf grass or containing only mulch, and batch studies linked higher total bacterial quantities to faster naphthalene mineralization rates. Thus, these findings suggest that planting raingardens with deep-rooted vegetation, such as natives and cultivars, not only improves the aesthetic appeal, but may also enhance the stormwater treatment potential of the raingardens. Indeed, Siciliano et al. (63) reported that the degradation of PAHs in vegetated systems was double that in non-vegetated systems, and Aprill and Simms (64) reported that the presence of deeply-rooted native prairie grasses caused a statistically significant increase in the rate of degradation of four PAHs when compared to non-vegetated soils. Biodegradation rates of PAHs and bacterial numbers generally decrease with distance from roots (65-67); thus, greater vegetation density in the raingarden could provide for

increased biodegradation performance. More research into the potential benefits of raingarden vegetation is warranted (6).

3.4 CONCLUSIONS

This investigation was the first to examine petroleum hydrocarbon residuals in raingarden soils and to use molecular methods to assess biodegradation potential in such systems. The key conclusions of the study are:

- Although TPH residual concentrations are statistically greater in raingarden soils than in upland control soils, the raingarden soil residuals are still orders of magnitude below regulatory action levels. Furthermore, raingarden soils contain four to five orders-of-magnitude lower TPH residuals than estimated from a simple loading calculation, suggesting that petroleum hydrocarbons are attenuating rather than accumulating.
- All raingarden soils tested were able to mineralize naphthalene, and the initial rate of mineralization correlated with the number of copies of Bacteria16S rRNA genes present.
- Biodegradation functional genes and Bacteria 16S rRNA genes were significantly greater in raingardens that were planted with deeply-rooted natives or cultivars compared to those with little rooting depth (i.e., turf grass) or no vegetation; thus, greater vegetation density and root permeation in the raingarden could provide for increased biodegradation performance.
- Hydrocarbon residuals in raingardens studied were significantly lower than literature values for stormwater pond sediments. Raingardens may be more effective

than retention ponds for sustainably treating petroleum hydrocarbon- contaminated stormwater.

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Chapter 4: Fate of Naphthalene in Laboratory-Scale Bioretention Cells: Implications for Sustainable Stormwater Management

This chapter was published in *Environmental Science and Technology* and is cited as:

LeFevre, G.H.; Novak, P.J.; Hozalski, R.M. Fate of Naphthalene in Laboratory-Scale Bioretention Cells: Implications for Sustainable Stormwater Management. *Environ. Sci. Technol*. **2012**, 46, 995-1002. DOI: 10.1021/es202266z

Bioretention cells are increasingly popular in low-impact development as a means to sustainably mitigate the environmental problems associated with stormwater runoff. Yet, much remains to be known regarding the removal and ultimate fate of pollutants such as petroleum hydrocarbons in bioretention cells. In this work, laboratory-scale bioretention cells were constructed inside sealed glass columns. The columns were periodically spiked with ^{14}C-naphthalene over a 5-month period and the fate of this representative hydrocarbon and the influence of vegetation on naphthalene fate was studied. Three column setups were used: one planted with a legume (Purple Prairie Clover, *Dalea purpureum*), one with grass (Blue-Joint Grass, *Calamagrostis canadensis*), and one unplanted (*i.e.*, control). Overall naphthalene removal efficiency was 93% for the planted columns and 78% for the control column. Adsorption to soil was the dominant naphthalene removal mechanism (56-73% of added naphthalene), although mineralization (12-18%) and plant uptake (2-23%) were also important. Volatilization was negligible (<0.04%). Significant enrichment of naphthalene-degrading bacteria occurred due to contaminant exposure and plant growth as evidenced by increased biodegradation activity and increased naphthalene dioxygenase gene concentrations in the bioretention media. This research suggests that bioretention is a viable solution for sustainable petroleum hydrocarbon removal from stormwater, and that vegetation can enhance overall performance and stimulate biodegradation.

4.1 INTRODUCTION

Urbanization alters the native hydrologic regime (1, 2) and results in the conveyance of substantial quantities of pollutants to surface waters via stormwater runoff (3, 4). Bioretention cells, often called raingardens, are rapidly gaining popularity as an innovative approach to simultaneously address issues of stormwater quantity through infiltration and stormwater quality through physical, chemical, and biological removal of pollutants (5). Research in the last decade has contributed substantially to our understanding of the ability of bioretention systems to remove pollutants from stormwater (5-7). Nevertheless, much remains to be known regarding the ultimate fate of those pollutants and the effects of bioretention design decisions on pollutant removal and fate. If pollutants pass through these systems, they could contaminate the underlying groundwater, while accumulation of pollutants in bioretention soils could result in a soil contamination problem (8, 9). It is therefore important to determine the ultimate fate of pollutants in such systems.

One class of environmentally relevant stormwater pollutants is petroleum hydrocarbons. The presence of petroleum hydrocarbons in stormwater is well-documented (3, 9) and these compounds are known to have negative human health (10) and ecological impacts (11, 12). Reported concentrations of petroleum hydrocarbons in stormwater runoff vary widely, ranging from 0.2 to 277 mg/L (3, 13-15). There are many potential sources of petroleum hydrocarbon pollution in urban catchments, including motor vehicles, leaky storage tanks, elicit dumping, spills, tire particles, and pavement sealcoats (3, 4, 16, 17). Of these, coal tar-based pavement sealcoats are recognized as a

significant source of carcinogenic polycyclic aromatic hydrocarbons (PAHs) to urban stormwater (16-18); in some urban watersheds, this source accounts for the majority of PAH loading to streams (16).

There are several reports in the peer-reviewed literature regarding the effectiveness of bioretention systems at removing petroleum hydrocarbons from stormwater. For example, removal of oils and grease (introduced as used motor oil at 20 mg/L in synthetic stormwater) in laboratory-scale bioretention columns exceeded 99% (19) with similar results for full-scale bioretention cells (20). In addition, raingardens were able to decrease polycyclic aromatic hydrocarbon (PAH) mean concentrations per storm event by 31 to 99% for an average annual pollutant mass reduction of 87% (21). The results from three separate studies involving stormwater infiltration basins in France, Germany, and Maryland suggest that most of the hydrocarbons removed from the stormwater were retained (*i.e.*, sorbed) in the first few centimeters of soil (15, 21, 22). We are aware of only one report in the peer-reviewed literature concerning an investigation of specific hydrocarbon removal mechanisms in bioretention media, including biodegradation, filtration, and sorption (23). Thus, there is a paucity of information regarding the ultimate fate of petroleum hydrocarbons within bioretention cells and the influence of design parameters, such as vegetation type or density, on pollutant fate. Therefore, we investigated the fate of a ^{14}C-labelled model petroleum hydrocarbon (naphthalene) in laboratory-scale bioretention columns, including both vegetated and non-vegetated (control) columns. We specifically focused on the roles of biodegradation and plant uptake as sustainable pollutant removal mechanisms and examined the influence of vegetation type on these mechanisms.

4.2 MATERIALS AND METHODS

Bioretention Column Apparatus. Three glass columns were assembled with two main compartments (Figure 4.1), a soil zone and an overlying headspace zone. Columns were pressure-tested prior to the experiment to ensure that no leakage occurred at connections or seals. The lower portion of the columns (60 cm x 10 cm ID) was filled with Minnesota Pollution Control Agency (MPCA) "Water Quality Mix A" bioretention soil media (24). The Type A media mix consisted of construction sand (sieved to 1190 μm ≥ grain size ≥ 500 μm), MnDOT (Department of Transportation) Grade 2 leaf compost (Resource Recycling Technologies, Minneapolis, MN), and top soil (Scott's Earthgro®) mixed and homogenized at a 4:2:1 volume ratio, respectively. The bioretention soil media contained 3.25 ± 0.37% total organic carbon. Air flow (100 mL/min) through the upper portion of the column was provided by a vacuum pump, which was connected to the top of the column using Teflon tubing with Swagelok Ultra-Torr fittings. The exit gas flowed through two ORBO activated carbon tubes (20/40 large charcoal, Sigma Aldrich) in series to capture any volatized naphthalene. From the activated carbon traps, the air was bubbled through two carbon dioxide traps (each contained 15 mL of 2 M NaOH) in series using fine bubble diffusers (Supelco) to capture any $^{14}CO_2$ produced via naphthalene mineralization. An activated carbon tube was also affixed to the air input port to prevent any possible contamination from the ambient air in the room; this carbon tube was removed when adding water to the columns.

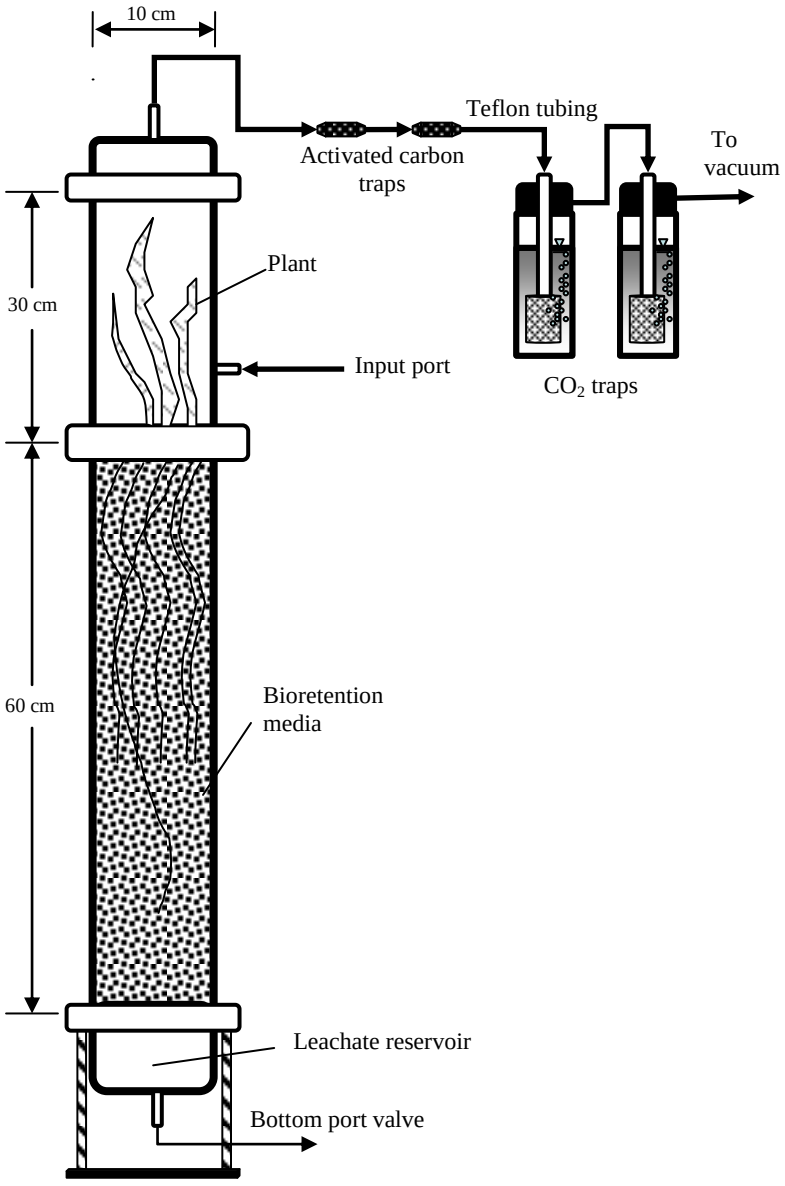

Figure 4.1: Schematic diagram of column bioretention cell.

Bioretention Column Experiments. The three columns were prepared as follows: (1) unplanted (i.e., control), (2) planted with a legume (Purple Prairie Clover, *Dalea purpureum*), and (3) planted with a grass (Blue-Joint Grass, *Calamagrostis canadensis*). These two plant species were used because they are listed in the "Plants for Stormwater

Design Manual" (24, 25), are indigenous to the central Minnesota region, and contain deep root structures. We were unaware of any demonstrated pollution control benefits associated with the use of these plant species in bioretention areas. The plants were purchased as adult plugs (Glacial Ridge Growers, Glenwood, MN). Fluorescent grow lights were located above and to the side of each column and operated on a daily cycle of 18 h on, 6 h off. Columns were planted with the aforementioned species and a fine bark mulch layer was placed on top of the soil media (2 cm thick). Once per week, 250 mL of synthetic stormwater (23, 26) was added to each column to simulate a small storm (¼″ rainfall in a 5:1 catchment:infiltration area ratio). The vegetation was established for six weeks prior to the start of the experiment. Upon initiation of the experiment, 250 mL of synthetic stormwater containing 10 mg/L naphthalene (Fisher Scientific) and 100,000 DPM (or 4.50×10^{-5} mCi) of 1,4,5,8-^{14}C-naphthalene (Moravek Biochemicals Inc., Brea, CA) was introduced into each column via the input port (Figure 4.1). Subsequent identical naphthalene spikes occurred once per month for a total of 4 spikes (Day 0, 31, 72, 107) during the 167-day experiment. Naphthalene was chosen as the model hydrocarbon because it is the dominant component of coal tar used in some types of pavement sealcoat (16, 27) it is a relatively labile and soluble PAH, and its biodegradation has been well-studied (*e.g.*, (28)).

Sampling and analysis were conducted in an effort to account for all of the ^{14}C-naphthalene added to the system. Excess water leached through the column was collected from the leachate reservoir (Figure 4.1), weighed, and a subsample was analyzed for ^{14}C content. The activated carbon traps were sacrificed weekly, hexane-

extracted, and the extracts were analyzed for ^{14}C content. The $^{14}CO_2$ traps were sacrificed weekly or biweekly and 200-µL aliquots were withdrawn for ^{14}C analysis.

At the terminus of the experiment, the columns were disassembled and 50 g samples of the soil were collected from the surface and at 7 cm intervals throughout the entire bed depth. The individual soil samples were separately homogenized, placed in baked glass serum vials with Teflon lined septa, and stored in a refrigerator (4°C) until analysis. The soil samples were analyzed to obtain depth profiles of total ^{14}C, naphthalene, and selected bacterial genes as described below. Plant roots and above ground biomass were carefully separated from the soil and stored in sealed plastic bags in a freezer (-20°C) until analysis. The total mass of dried soil and vegetation in each column was determined for mass balance purposes.

Batch Biodegradation Experiments. Batch biodegradation experiments were conducted on the original soil "Media Mix A" used to pack the columns and on soil samples collected from each column at the surface and at a depth of 35 cm after the 167-day experiment had been completed. The goal of these experiments was to determine the capacity of the microorganisms in the soil samples to mineralize naphthalene, and determine relevant biodegradation kinetic parameters. Details concerning the experimental conditions and procedure for the batch experiments are provided elsewhere in Chapter 3. Briefly, soil was used to inoculate nephelometric biodegradation flasks (Chemglass) containing mineral media spiked with non-labeled and 14C-labeled naphthalene. The flasks were incubated in the dark at room temperature with constant mixing and the evolved 14CO2 was trapped in a NaOH solution contained in the flask sidearm. Aliquots of the NaOH solution were removed periodically and analyzed via

liquid scintillation counting. Each batch experiment was performed in triplicate with a triplicate killed control (50 mM sodium azide).

Analytical Methods. Naphthalene was extracted from the soil and activated carbon using a hexane-sonication method described elsewhere (Chapter 3, ref 29), and analyzed using both liquid scintillation counting and gas chromatography with flame ionization detection (GC-FID; HP5890 Series II). The GC column used was a Restek Rtx-1 with a temperature program of 40^{o}C for 2 min, ramped at 10^{o}C /min to 250^{o}C, and held for 1 min. UHP zero-grade nitrogen at a flow rate of 2.4 mL/min was the carrier gas. Inlet and detector temperatures were 280ºC and 300ºC, respectively. The method detection level (30) was 18 µg/L, corresponding to approximately 60 µg/kg dry soil. Naphthalene extraction efficiency was 100.1% ± 12.5% (n=7); thus, reported values were not corrected for extraction efficiency. Samples of hexane extracts or NaOH used for trapping CO_2 (900 µL or 200 µL, respectively) were added to 10.5 mL liquid scintillation cocktail (Perkin Elmer) and analyzed on a Packard 2100TR liquid scintillation counter (LSC).

Plant biomass was removed from each of the planted columns at the end of the experiment and separated into two fractions: above ground (leaves and stems) and below ground biomass (roots). Small subsamples of each fraction were freeze dried and then analyzed in triplicate for ^{14}C content using an OX600 Biological Oxidizer (RJ Harvey Instruments). The remaining plant biomass was dried in an oven for four days at 100°C and then weighed for mass balance purposes.

Bacterial DNA was extracted from the soil samples using a MO-BIO PowerSoil^{TM} DNA kit, following the manufacturer's instructions. 16S rRNA genes (a proxy for total

bacteria) and two catabolic functional genes (naphthalene dioxygenase, NAH; phenol monooxygenase, PHE) were quantified using real-time quantitative polymerase chain reaction (qPCR) as described in Chapter 3. NAH encodes for enzymes responsible for the initial attack on the rings of PAHs, including naphthalene (31). The PHE genotype encodes for the ring-hydroxylating group of phenol hydroxylase enzymes. These enzymes catalyze the oxidation of the hydroxylated hydrocarbons that result from the biotransformation of petroleum hydrocarbons such as naphthalene (32, 33). PHE is one of the most consistently detected catabolic genes at petroleum-contaminated sites (32, 34-36), and PHE gene expression has correlated with BTEX biodegradation in groundwater (36). Each sample was run in triplicate with negative controls and a standard curve. Melting curves were examined for consistency in form and melting temperature. The detection limits for the NAH, PHE, and the 16S rRNA genes were 4×10^4, 4×10^5, and 4×10^7 copies per g dry soil, respectively. Values below the detection limit (NAH only; noted in Figure 4.7) were excluded from the statistical analyses and are plotted as zero in Figure 4.3.

Data Analysis. Stata 10.1 (StataCorp, College Station, TX) was used to conduct Student t-tests, Wilcoxon-Rank sum tests, and to determine Spearman's rho correlation coefficients. Non-parametric analyses were used to avoid bias introduced by assuming a distribution. All tests were conducted at the 95% confidence level, with Sidak adjustment to reduce Type I error in the correlation matrix. Principle component analysis (PCA) was performed using CANOCO 4.5 (Microcomputer Power, Ithaca, NY). Calculations of the total 14C adsorbed to the soil in the columns were made by integrating (trapezoidal rule) the values obtained from the samples taken every 7 cm.

Mineralization versus time results from the batch biodegradation experiments were modeled using a modified version of the three-parameter Gompertz curve ($f(t) = ae^{-e^{c(t-k)}}$), where: a is the maximum extent of mineralization, k is the time required to reach the inflection point or maximum mineralization rate (henceforth referred to as the lag time), and c is a fitting parameter associated with the mineralization rate. The model was fit to the data by a least squares approach using Microsoft Excel Solver, and error propagation was used to determine the 95% confidence interval for each parameter. The maximum rate of mineralization was determined by taking the derivative of the Gompertz function and setting time equal to k ($f'(k) = -ace^{-1}$).

4.3 RESULTS

Fate of ^{14}C-naphthalene in bioretention cells. A breakdown of the ^{14}C that was leached, volatilized, sorbed to soil, mineralized, and incorporated into plant biomass is shown in Figure 4.2. The total ^{14}C recoveries for the unplanted, grass, and clover columns were 95%, 91%, and 107%, respectively. Overall naphthalene removal efficiencies for the bioretention cells, defined as (added-leached)/added, were 93% for the two planted cells and 78% for the unplanted cell.

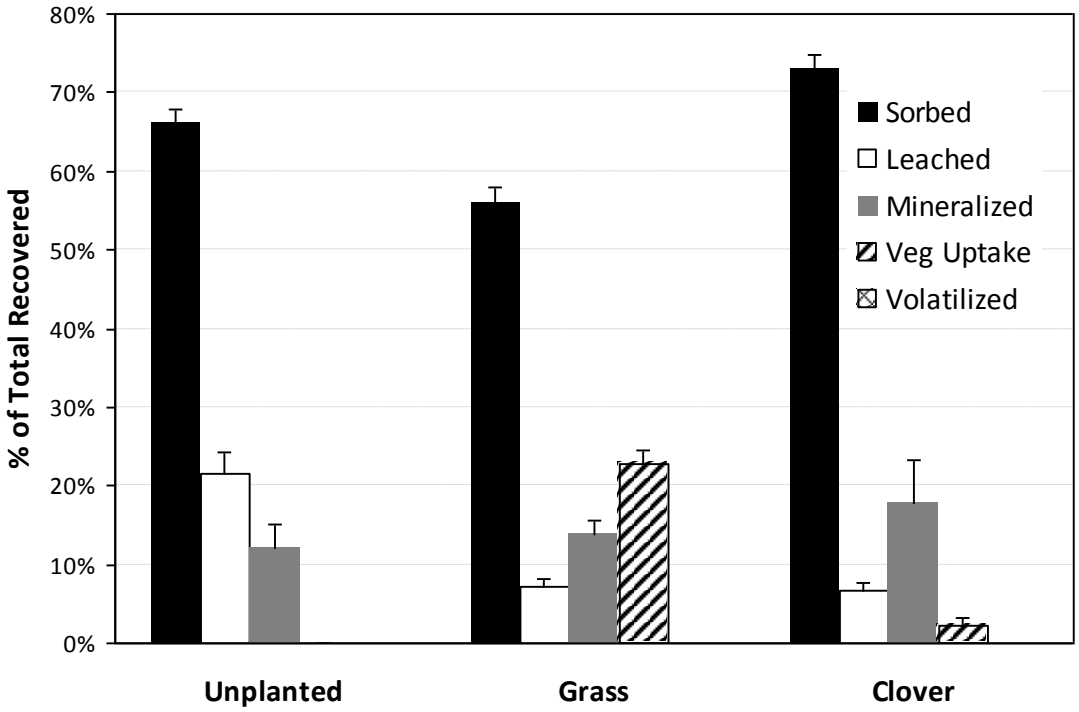

Figure 4.2: Distribution of ^{14}C in the column reactors (volatilization is not visible for any of the columns). The values were normalized to the total ^{14}C recovered (Unplanted: 95%, Grass: 91%, Clover: 107%) and error bars represent relative standard deviation.

Sorption of the ^{14}C-naphthalene to soil was the dominant fate mechanism in the columns. The total amount of ^{14}C sorbed to the soil for the unplanted, grass, and clover columns represented 66%, 56%, and 73%, respectively, of the total ^{14}C recovered from each column. The majority of the ^{14}C (>50%) sorbed to the soil was present in the upper portion of the columns (top 15 cm for clover and unplanted, top 30 cm for grass) (Figure 4.3). The concentration of naphthalene in the soil samples with depth, as measured by GC-FID, positively correlated with the ^{14}C concentration in the same samples (Rho=0.42, p=0.0247, n=29). Furthermore, the ratio of $^{14}C/^{12}C$ naphthalene added to the columns in the simulated rain events was not significantly different from that extracted from the soil

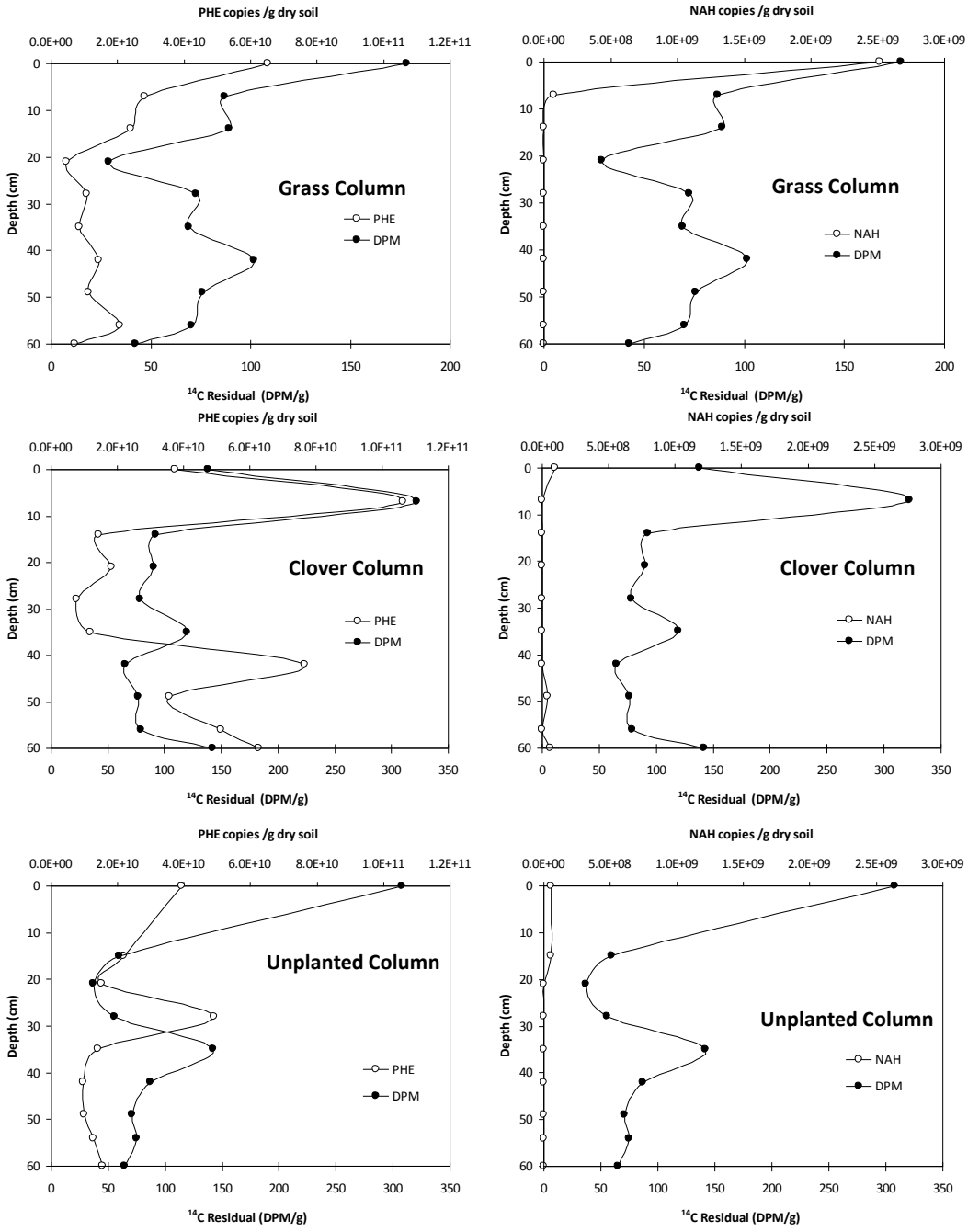

Figure 4.3: Depth profiles of ^{14}C and PHE, NAH, and 16S rRNA genes for the grass, clover, and unplanted columns

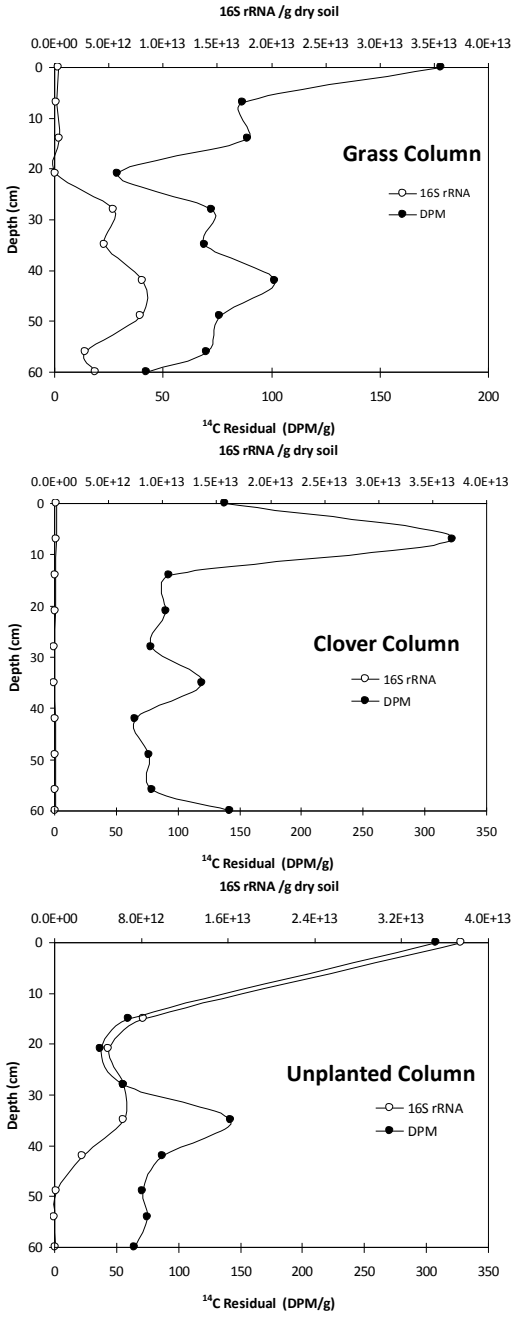

Figure 4.3 (Continued.)

(p=0.61), suggesting that the ^{14}C extracted from the soil was primarily undegraded naphthalene. The presence of naphthalene biodegradation products in the soil samples, however, cannot be entirely ruled out.

Biodegradation of naphthalene, as indicated by $^{14}CO_2$ evolution, was a substantial naphthalene loss mechanism, representing 12-18% of the total ^{14}C recovered from each column. The extent of naphthalene mineralization was not significantly different between the three columns (p>0.5). Vegetative uptake accounted for 23% of the total ^{14}C for the grass column and 2.5% for the clover column. Of the ^{14}C incorporated into plant tissue, 88 and 92% (clover and grass, respectively) was present in the above-ground biomass with the remainder in the roots (Table 4.1). Overall, leaching of ^{14}C was minor for the vegetated columns (7%) but not for the unplanted column (22%). Most of the ^{14}C leached from the control column (91%) was collected immediately following the first naphthalene dose (Figure 4.4). This "first flush" pattern was also observed in the grass and clover columns, but to a lesser extent (39% and 37%, respectively). Thus, following the initial flushing of ^{14}C from the columns, little additional mass (<5% of the $^{14}C_{total}$) was lost via this mechanism. Volatilization of naphthalene was negligible for all of the columns (<0.04% of the total ^{14}C recovered).

Table 4.1: Plant biomass and ^{14}C concentrations in the plant tissue from the vegetated columns

		Mass (g)	DPM/g
Grass	Roots	7.598	1,190
	Stem/leaf	9.774	10,820
Clover	Roots	4.494	350
	Stem/leaf	4.166	2,698

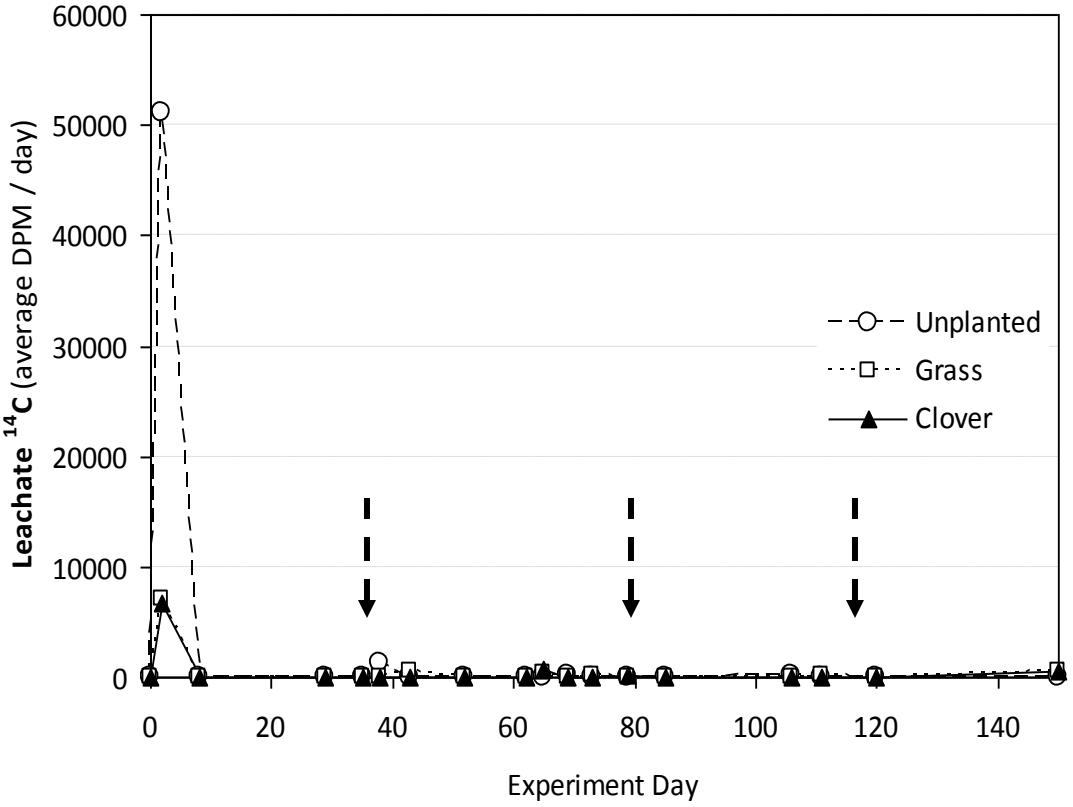

Figure 4.4: Measurement of ^{14}C in the bioretention cell leachate throughout the experiment. The proportion of the ^{14}C measured in the first flush was 91, 39, and 37% of the total ^{14}C leached for the unplanted, grass, and clover column, respectively. The first 14C-naphthalene spike was at day zero and the arrows indicate the timing of subsequent spikes.

Enrichment of naphthalene-degrading bacteria. Not surprisingly, exposure of the bioretention soil media to naphthalene for approximately five months enriched for naphthalene-degrading bacteria. The mineralization curves from the batch experiments are shown in Figure 4.5 and the parameters from the Gompertz model fitting are summarized in Table 4.2. A comparison of the time to reach the maximum rate of mineralization (*i.e.*, lag time) for the batch experiments is presented in Figure 4.6. The lag time for the column soil samples after approximately five months of naphthalene exposure was significantly less than that for the original soil media ($p<0.0001$). Furthermore, the pooled lag times from the surfaces of all of the columns were significantly lower than the pooled lag times from 35-cm depth ($p < 0.0001$), and the lag time for the vegetated columns (pooled, surface and 35-cm depth) was significantly less than that for the unplanted control column ($p < 0.0001$; pooled, surface and 35-cm depth). The maximum mineralization rates, which ranged from 1.12×10^{-3} to 8.73×10^{-3} $mg_{naphthalene}g_{soil}^{-1}\,h^{-1}$, were not significantly different between the aforementioned groups (vegetated vs. non-vegetated columns: $p=0.68$, $n=18$; surface vs. 35 cm depth in columns: $p=0.20$, $n=18$; all columns vs. original soil media; $p=0.06$, $n=21$).

In addition to the reduced lag time, the quantity of NAH genes in the column bioretention soils increased with exposure to naphthalene (Figure 4.7). NAH genes were not detected in the original bioretention soil used to pack the columns, but were detected in the surface soils at low numbers in two of the three columns following establishment of vegetation in the columns prior to naphthalene addition (Figure 4.7). After exposure to naphthalene for 5 months, NAH genes were detected in all of the surface soil samples from the three columns (Figure 4.7, Figure 4.3). Furthermore, the quantities of NAH genes detected

were much higher than those prior to naphthalene exposure (Figure 4.7) and were comparable to surface soil samples collected from field bioretention cells (75 samples from 58 sites) analyzed in Chapter 3. A correlation matrix of the pooled depth samples taken from the columns revealed both PHE and NAH copies/g dry soil correlated with ^{14}C residual (Rho=0.41, p=0.0276, n=29; Rho=0.38, p=0.042, n=29; respectively), further demonstrating that naphthalene in the soil stimulated hydrocarbon-degrading bacterial communities. Depth profiles for each column are included in Figure 4.3.

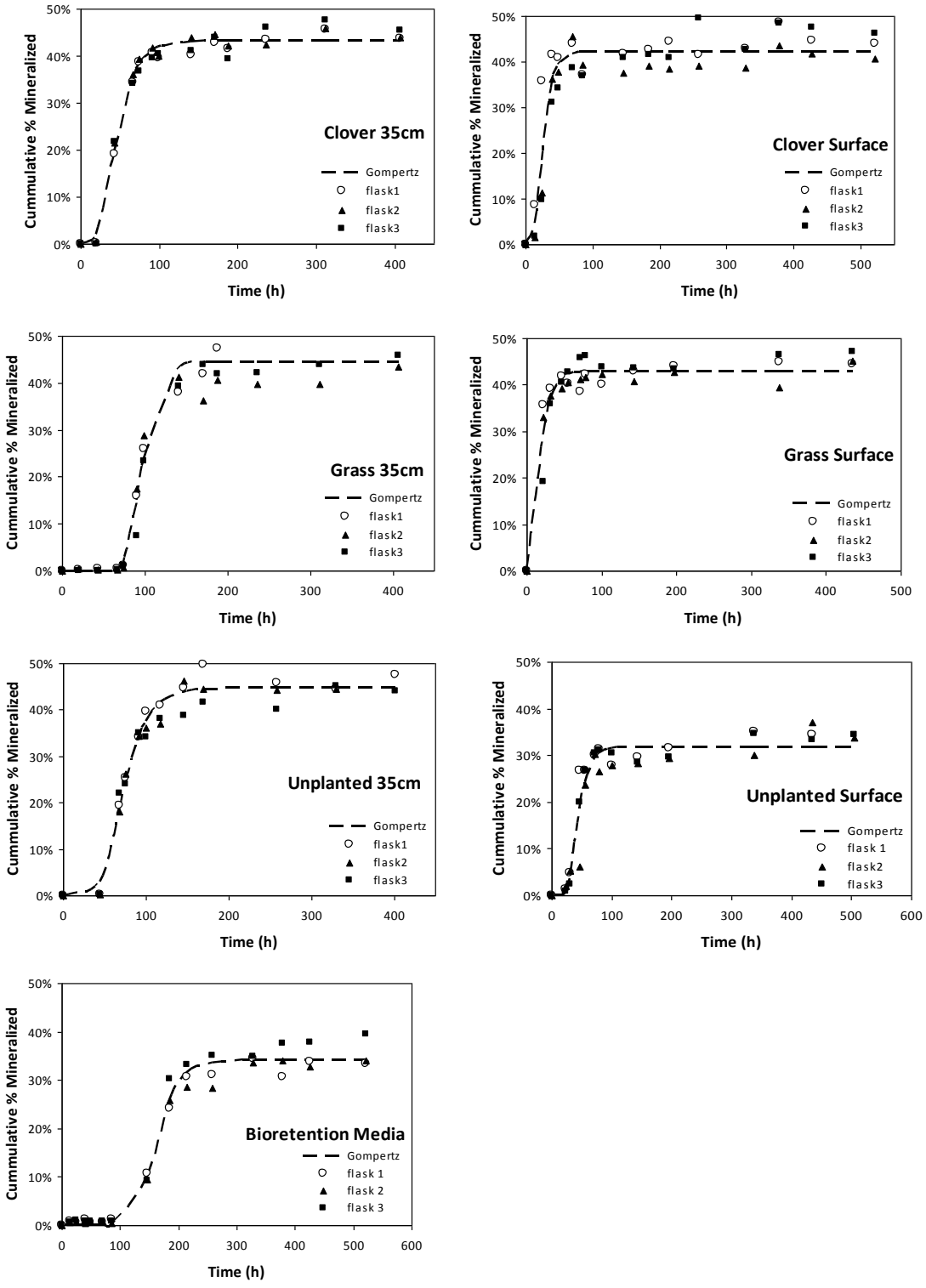

Figure 4.5: Batch biodegradation of naphthalene using soil samples from the column surface and at a depth of 35 cm. Naphthalene biodegradation results for the original bioretention media (used to pack the columns) are also shown.

Table 4.2: Summary of the Gompertz model parameter values (± 95% confidence intervals) obtained from the batch biodegradation experiments.

Sample	Maximum Extent of Mineralization (% of input)	Time to Maximum Mineralization Rate (h)	Maximum Rate of Mineralization ($\mu g_{Naphthalene} g_{soil}^{-1} h^{-1}$)
Clover 35cm	43.2 ± 0.8	38.9 ± 2.2	$2.22 \pm 3.2 \times 10^{-3}$
Grass 35cm	44.6 ± 2.4	92.0 ± 3.6	$2.64 \pm 1.4 \times 10^{-2}$
Unplanted 35cm	44.6 ± 1.5	65.3 ± 3.0	$2.02 \pm 4.1 \times 10^{-3}$
Clover Surface	42.3 ± 1.7	21.9 ± 2.91	$6.86 \pm 2.5 \times 10^{-2}$
Grass Surface	42.9 ± 1.2	13.6 ± 5.1	$8.73 \pm 4.0 \times 10^{-2}$
Unplanted Surface	31.7 ± 1.5	39.6 ± 3.5	$5.57 \pm 1.8 \times 10^{-2}$
Bioretention Soil Media	34.2 ± 1.0	151.4 ± 4.2	$1.12 \pm 2.5 \times 10^{-3}$

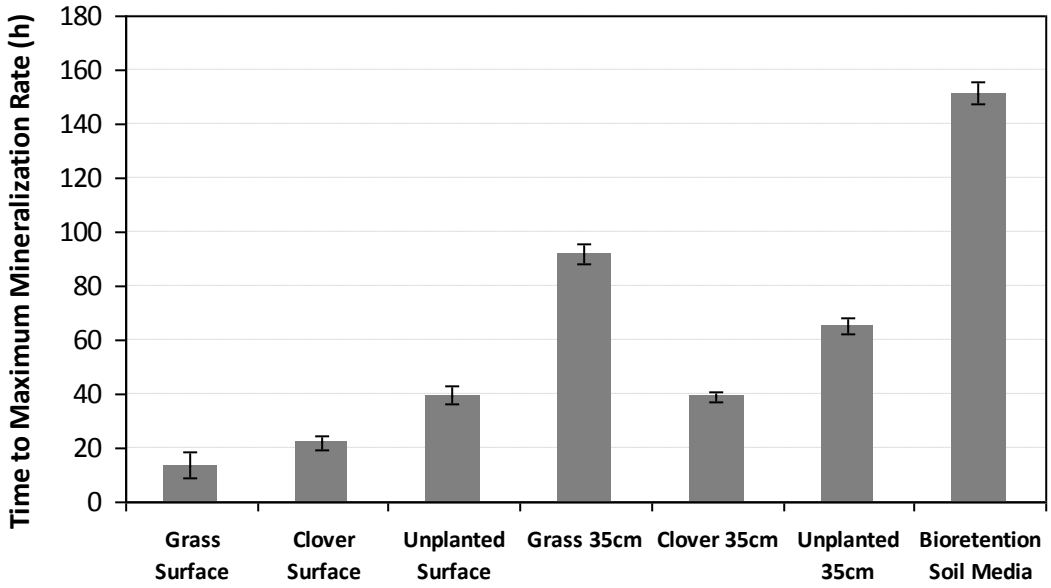

Figure 4.6: Comparison of time to reach maximum rate of mineralization (lag time) in batch tests. Two soils from each column (surface and 35cm depth) and the bioretention soil media prior to any naphthalene exposure are included. Error bars indicate 95% confidence intervals.

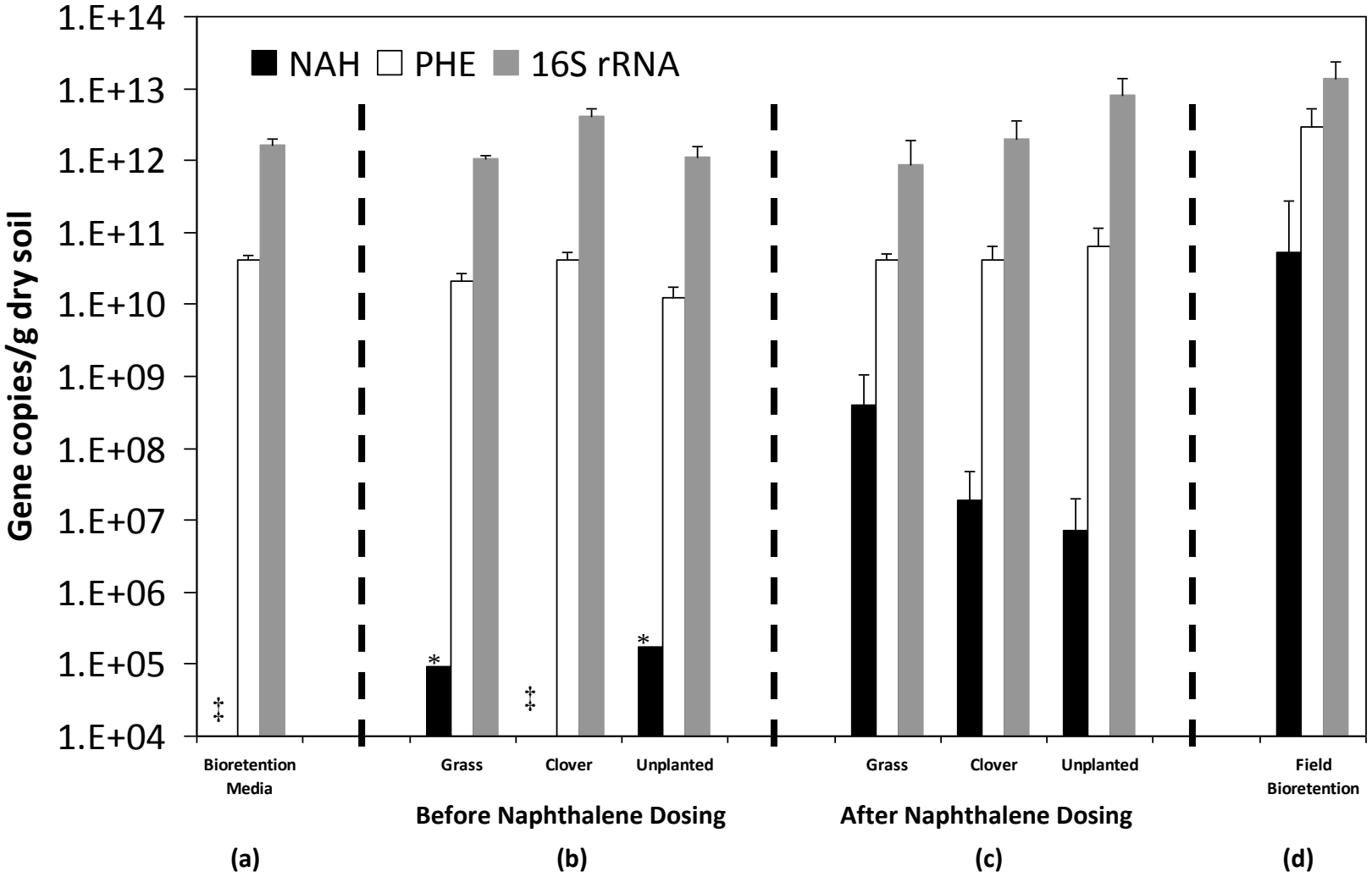
1.E+14
1.E+13
1.E+12
1.E+11
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
Gene copies/g dry soil
NAH
PHE
16S rRNA
‡
*
‡
*
Bioretention Media
Grass
Clover
Unplanted
Grass
Clover
Unplanted
Field Bioretention
Before Naphthalene Dosing
After Naphthalene Dosing
(a)
(b)
(c)
(d)

Figure 4.7: Comparison of the phenol monooxygenase (PHE) and naphthalene dioxygenase (NAH) functional genes and total bacterial 16S rRNA genes for (a) the bioretention soil media used to fill the columns, (b) the three column surface soils after vegetation was established but before naphthalene dosing, (c) the three column surface soils following the naphthalene dosing, and (d) bioretention field samples (n=75). Bars represent the mean of triplicate PCR performed on triplicate DNA extractions. Error bars are bars equal ± one standard deviation. ‡ indicates that all samples were below detection; * indicates only one sample (displayed) was above the detection limit.

Effect of vegetation. The presence and type of vegetation affected the fate of the added naphthalene. As mentioned, vegetative uptake accounted for a significant amount of 14C recovered from the grass column (23%) but considerably less from the clover column (2.5%). The total vegetative biomass was more than two times greater in the grass column than in the clover column and the 14C concentration was approximately three times greater in the grass biomass than in the clover biomass (Table 4.1). The presence of plants altered the composition of the soil at the surface as well. The bioretention soil media prior to placement into the column contained 3.25% organic carbon. After the plants had been established (but prior to naphthalene dosing), the surface soils for the unplanted, grass, and clover columns contained 3.20%, 9.50%, and 15.6% total organic carbon, respectively. Interestingly, the differences in soil organic carbon content did not appear to affect the sorption of naphthalene in the columns (Figure 4.3).

The qPCR results revealed that the clover bioretention column contained a significantly higher ratio of PHE to 16S rRNA genes than the other two bioretention columns ($p<0.0001$). This result was corroborated by correlation matrix analysis of all of the pooled soil sample data in which the PHE to 16S rRNA gene ratio correlated positively with the presence of clover as vegetation cover type ($Rho=0.78$, $p<0.0001$, $n=29$). To further investigate if the clover column was different from the other two columns, principal component analysis (PCA) was conducted on the pooled results from the analyses of the soil samples (Figure 4.8). Input parameters included the vegetation planting type, PHE and 16S rRNA gene copies/g soil, and naphthalene concentration.

The PCA did not include NAH gene concentrations because several samples were below the detection limit (Figure 4.3). The first and second principal components describe 47.3% and 30.4% of the variability, respectively. The results from the soil samples for the clover column grouped separately from those for the unplanted and grass columns, which overlapped.

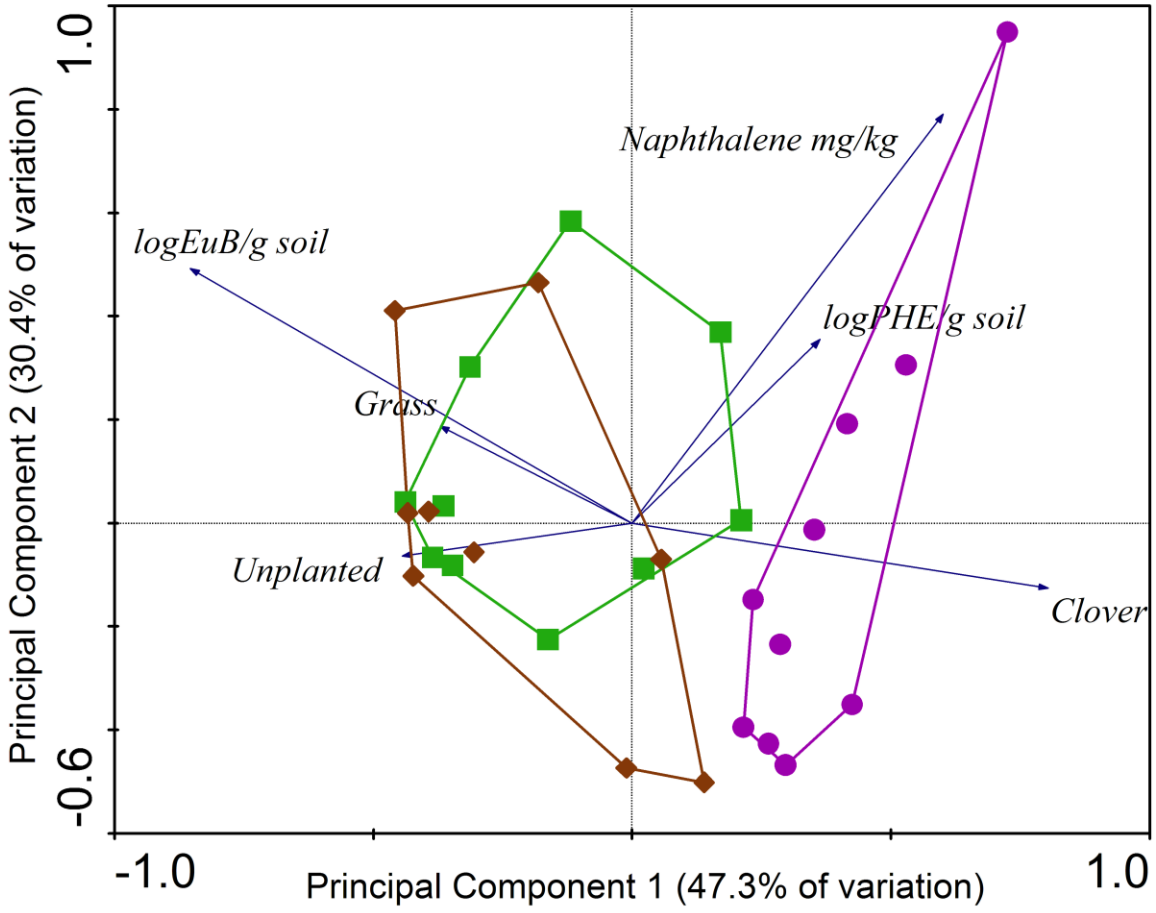

Figure 4.8: Results of a principal component analysis considering cover type, PHE gene concentration (log copies / g soil), total Eubacteria (16S rRNA) gene concentration (log copies / g soil), and naphthalene concentration (mg naphthalene / kg soil). The data are differentiated by vegetation type (clover=purple circles, grass=green squares, unplanted=brown diamonds). The arrows indicate species vectors. The first and second principal components describe 47.3 and 30.4% of the variability, respectively.

4.4 DISCUSSION

Overview of Naphthalene Fate. In our column bioretention cells, sorption to soil accounted for the bulk of the added naphthalene during the course of the experiment, while leaching was minimal after the first flush through the system. Biodegradation (*i.e.*, mineralization) and plant uptake were also significant naphthalene removal mechanisms. The importance of sorption is consistent with field-scale observations (22, 23, 37) and is not surprising as PAHs are relatively hydrophobic compounds. Although our results suggest that the majority of the ^{14}C residual in the soil columns is likely undegraded naphthalene, the presence of naphthalene metabolites cannot be ruled out. In the environment, PAH metabolites are generally more polar and labile than the parent compound; however, some metabolites are toxic to bacteria and may inhibit microbial growth (38). Naphthalene's fairly low air-water partitioning coefficient ($logK_{aw}$= -1.74; (39)) combined with the tendency to sorb to soil organic matter ($logK_{ow}$=3.33; (39)) explains the lack of volatilization. The relatively high naphthalene concentration in the first flush of leachate may be due, at least in part, to slow biodegradation kinetics as the microbial community had insufficient time to adapt (40) to the newly introduced pollutant. Although not relevant in our experiments because the synthetic stormwater was particle free, filtration might also be an important removal mechanism at field bioretention sites as PAHs are often associated with particles (3, 5, 21, 41, 42).

From an environmental protection perspective, it is critical to avoid infiltrating pollutant-laden stormwater to underlying groundwater (8, 43). Similarly, it is also important not to simply transfer pollutants to the bioretention soil, where the pollutants

could potentially accumulate to hazardous levels. Fortunately, sorption is typically reversible in that hydrocarbons initially sorbed to the soil organic matter can later desorb and be biodegraded (23, 40). Indeed, hydrocarbon residual concentrations in soil samples from full-scale bioretention systems (< 3 μg/kg; Chapter 3) were approximately 1000 times lower than naphthalene concentrations in the soil samples from our columns (0.19 - 4.27 mg/kg). Certainly, it is difficult to compare these two sets of results directly because the pollutant loading histories of the field sites are not known. Nevertheless, the much lower levels in the field soil samples suggest that either the loading rates at all of the field sites are extremely low or that sorbed hydrocarbons have been released from the surface soils and subsequently degraded, resorbed at greater depths, or possibly transported out of the bottom of the bioretention cells.

Biodegradation in Bioretention. Mineralization is the most desirable loss mechanism for most organic pollutants. Although biodegradation of petroleum hydrocarbons was presumed to be occurring in bioretention systems (5), up to now only one study has explicitly examined biodegradation in these systems (23). Hong et al. reported 80-95% removal of petroleum hydrocarbons via filtration or sorption in a bench-scale bioretention reactor; subsequently, approximately 90% of the initially sorbed hydrocarbons were biodegraded (23). In our column experiments, only approximately 15% of the added naphthalene was mineralized. The batch experiments suggested that up to 45% of naphthalene could be mineralized to 14CO2, which represents nearly complete naphthalene degradation (with the remaining carbon incorporated into biomass; (44), see also Appendix E. The low fraction mineralized in this study is attributed to the initially

poor naphthalene biodegradation capacity of the soil used to pack the columns. The ability of the soil microorganisms to biodegrade naphthalene improved over time as evidenced by the enhanced biodegradation kinetics (Table 4.2) and increased NAH gene concentrations in the column soils at the end of the experiment. Thus, it is likely that the fraction of naphthalene mineralized would have increase over time had we continued to operate the columns.

Influence of Vegetation. Uptake of ^{14}C into the plant biomass was a significant removal mechanism, particularly for the grass column. Contaminants taken up into vegetation can be metabolized to less toxic substances by enzymes within the plant cell; the so-called "green liver" concept (45-48). Although the form of the ^{14}C in the plant tissue is unknown (*e.g.*, undegraded parent compound, metabolite, or plant biomolecules), most studies indicate that contaminants taken up and sequestered into plant biomass are not available to organisms that may ingest the vegetation (46). Thus, incorporation of stormwater hydrocarbons into plant biomass is likely to be a desirable, benign removal mechanism. Nevertheless, there is some evidence that sequestered pollutants become bioavailable as the plant tissues decay (49). Certainly, more research is needed to investigate the fate and bioavailability of toxic organic compounds taken up by plants (50).

Interestingly, the ^{14}C concentration in the grass tissue was approximately three times greater than that in the clover tissue (Table 4.1). The enhanced incorporation of ^{14}C into the biomass of the grass species could be due to four main factors. First, the roots of the grass were much more fibrous than the clover roots, creating increased surface area for

potential ^{14}C uptake. It is known that naphthalene can readily be taken up by plant roots; indeed, this strategy has been applied at multiple phytoremediation study sites (51-53). Second, differential lipid content between vegetation types may alter hydrophobic contaminant accumulation in plant biomass (50, 54). Differences in vegetative lipid content are likely to be negligible for these plant species, however (55). Third, the grass is likely to have a higher transpiration rate than the clover (56). Naphthalene may be directly transpired through vegetation and evaporated at the plant stomata (52). Naphthalene evaporation was negligible (<0.04% of the influx), however, suggesting that if significant naphthalene transpiration was occurring, the chemical was not released by the stomata into the column headspace. It is also possible that the ^{14}C was taken up in a form other than naphthalene, such as $^{14}CO_2$ or some other naphthalene metabolite, and then incorporated into the plant biomass. Lastly, the higher organic carbon content of the surface soil in the clover column may have decreased the bioavailability of the naphthalene, reducing uptake by the plant roots.

In addition to direct incorporation or transpiration of contaminants, a primary benefit of vegetation in phytoremediation is the stimulation of contaminant-degrading bacteria in the rhizosphere (57-63), which appears to have also occurred in our laboratory-scale bioretention cells. The rhizosphere is an enhanced microhabitat, with microbial biomass concentrations one to four orders of magnitude greater than in soils outside of the root zone (58, 60, 62, 63). Plant roots deliver oxygen (64) and release exudates into the rhizosphere, the latter of which provide a supply of labile carbohydrates and substances that may act as contaminant analogues (65, 66). Our results demonstrated that bacteria in

the soils from the vegetated columns degraded naphthalene with a significantly shorter lag time than the bacteria in the soil from the unplanted control column. These findings are consistent with observations from the literature in which hydrocarbons are degraded more quickly in vegetated than in non-vegetated soils (58-60, 63, 65-73). The interplay between plants and bacteria may explain why the clover columns were differentiated by PCA (Figure 4.8) and had a significantly higher ratio of PHE to 16S rRNA genes than the other columns. Leguminous plants foster a symbiotic relationship between mycorrhizal fungi and rhizosphere bacteria (74-77); which may enhance the quantity and/or activity of contaminant degraders (78). Some plants, including legumes, are also known to release phenols (65, 79, 80), which could act as contaminant analogues to induce biodegradation of xenobiotic chemicals (81) such as naphthalene. Furthermore, PAH degradation can be nitrogen limited (82) and legumes with their associated rhizosphere bacteria fix nitrogen into soils. Because the PHE gene concentration was enhanced by the presence of clover as a cover type and PHE gene concentration is associated with degradation of petroleum hydrocarbons and their metabolites (32, 34-36), we suggest that integrating legumes into bioretention as part of a comprehensive vegetation regime could provide valuable benefits for mitigating hydrocarbon-contaminated stormwater.

ACKNOWLEDGMENT

We thank Pedro Alvarez and Dong Li at Rice University for access to and aid in using the biooxidizer on their campus.

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CHAPTER 5: Root Exudate Impacts on Contaminant Desorption and Biodegradation

5.1 INTRODUCTION

Many persistent organic pollutants are known to degrade more rapidly and completely in root-zone, or rhizosphere, soils (1-15). For example, Aprill and Sims (4) determined that polycyclic aromatic hydrocarbon (PAH) residual was significantly lower in prairie grass-planted pots versus unplanted pots. Similarly, Reilley et al. measured 44% greater PAH degradation in vegetated soils (12). Joner and Leyval (16) and Corgie et al. (17) observed that PAH dissipation in a soil was a function of proximity to plant roots.

There are several proposed hypotheses to explain enhanced contaminant degradation in the rhizosphere. First, plant roots deliver oxygen to soils and may therefore improve conditions for aerobic biodegradation (18). Second, plants release root exudates that may act as contaminant analogues and precondition the microbial community or specifically induce contaminant degradation (1, 3, 19-22). Third, plant root exudates contain large quantities of simple sugars that may stimulate overall bacterial populations (1, 3, 14, 19, 23) or facilitate cometabolic degradation of contaminants (24). There is also evidence, however, that raw or metabolized plant root exudates may supress the biodegradation of target contaminants (25, 26). Finally, it is also possible that raw or metabolized plant root exudates may alter the bioavailability of hydrophobic contaminants in the rhizosphere.

The composition of root exudates or their metabolized derivatives suggests that they may be able to alter pollutant sorption to soil. Exudates contain substances such as amino acids, organic acids, sugars, phenolic compounds, polysaccharides, and humic

compounds (27), some of which are known to increase the desorption of hydrophobic organic compounds from soils (28, 29). For example, Gao et al. (28) reported that artificial root exudates enhanced the desorption of phenanthrene and pyrene from soil. There is also some evidence that root exudates or their metabolized derivatives have surfactant-like properties (22, 29, 30). For example, glycerolipids and glycoproteins can be excreted by plants and these are known to be surface-active compounds (22). Furthermore, soil bacteria that inhabit the rhizosphere are known producers of biosurfactants (22) and there is a significant body of work demonstrating the ability of biosurfactants to increase contaminant desorption (31-38). Because bioavailability of persistent organic contaminants often limits remediation success (9), enhanced desorption in the rhizosphere as a result of the presence of raw or metabolized root exudates could be a critical mechanism for facilitating phytoremediation success.

In this work we examined the effect of both raw and metabolized plant-harvested and artificial root exudates on naphthalene sorption and solubility and the effects of these exudates on naphthalene biodegradation. Naphthalene, a model PAH, was chosen as a model contaminant because it is commonly found in the environment, it is hydrophobic ($\log K_{ow} = 3.3$), and sorbs readily to organic matter in soil. The specific objectives of this research were to: (1) determine if sorption characteristics were altered by raw and metabolized exudates, (2) determine if raw and metabolized exudates acted to induce or supress biodegradation, and (3) evaluate the raw and metabolized exudates for systematic differences in character or behavior that may explain, at least in part, the effect of exudates on contaminant sorption or degradation.

5.2 METHODS

Experimental Design and Apparatus

Experimental Design. To accomplish the stated objectives, the experimental design was divided into several components. First, plants were grown in hydroponic solutions to allow harvest of root exudates, and artificial root exudates (AREs) were mixed using laboratory reagents. These raw exudates were then metabolized in the presence of soil bacteria to create metabolized exudates.

The raw and metabolized exudate solutions were used to determine desorption of naphthalene from an aged soil and naphthalene solubility. The raw and metabolized exudates were assessed for impact on biodegradation kinetics of the target compound, naphthalene.

Additionally, the raw and metabolized exudates were characterized to assess differences between the harvested and AREs, the raw and metabolized exudates, and between plant types.

Production of Root Exudates. Plants that represented three major families of vegetation were chosen for exudate production and harvest: grass (Family: Poaceae; Cord Grass, *Spartinapectinata*), sedge (Family: Cyperaceae; Porcupine Sedge; *Carexhystricina*) and legume (Family: Fabaceae; Purple prairie clover, *Daleapurpurea*]. Grasses are often used for phytoremediation applications (1, 3), sedges are common in moist soil/wetland areas, and legumes are known to have unique root-rhizosphere properties that may enhance hydrocarbon remediation (39). These plants are also

recommended for use in bioretention areas, which may receive organic contaminants such as petroleum hydrocarbons via stormwater (40, 41). All plants were grown by Glacial Ridge Growers (Glenwood, MN).

A hydroponic system (Figure 5.1) was used to harvest root exudates. Replicate plants (3 replicates per species) were maintained in separate hydroponic systems for a total of 9 individual plants. A Teflon tube fitted with a Luer-lock valve for exudate collection was inserted to the exit port of a 1-L vacuum flask filled with filter-sterilized 50%-strength Hoagland's solution (Sigma H2395; (*e.g.*, 2, 42, 43) 4-L flasks were used for the sedges to accommodate the large root mass). The root mass of each plant was cleaned of soil by hand washing with DI water and then suspended in the hydroponic solution either by autoclaved polyurethane foam plugs (grasses or clover) or friction (sedges). Flasks were covered with aluminum foil. A set of florescent grow lights with an illumination regime of 16 h on, 8 h off (42) was used to grow the plants hydroponically for three months, at which point the root exudates were harvested. Periodically, exudate samples were collected for analysis and the balance of the sample volume plus transpiration losses was replaced with 50%-strength Hoagland's solution. Herein, exudates harvested from the hydroponic systems are referred to as 'harvested exudates.'

Solutions of Artificial Root Exudates (AREs) were prepared by mixing solutions described in Joner et al. [(44); recipe in Appendix C], filter-sterilized through a pre-rinsed 0.22 μm nitrocellulose filter (Millipore #SA1J789H5), and stored in a 1-L baked amber flask with autoclaved cap at 4°C in the dark. Diluted AREs were used in all experiments

(10% AREs diluted with 50%-strength Hoagland's solution). This diluted solution is simply referred to as 'AREs' throughout.

Harvested raw exudates and AREs were characterized for dissolved organic carbon (DOC), UV_{254}, specific UV absorbance (SUVA), spectral slope, and florescence (excitation-emission matrices, EEMs; florescence index, FI) as described below.

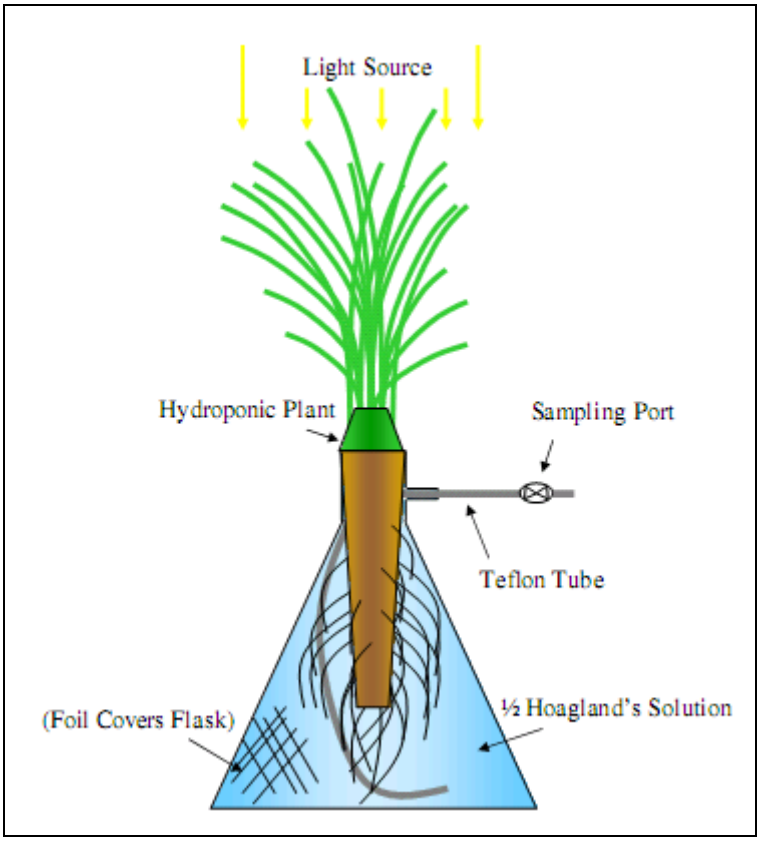


Figure 5.1: Schematic of hydroponic plant apparatus created for the harvest of root exudates.

Production of Metabolized Root Exudates. To produce metabolized exudates or AREs, the raw harvested exudates and AREs were incubated with soil bacteria in batch cultures. Soil bacteria from a bioretention site on the University of Minnesota campus were extracted based on the method of Musovic et al. (45) and frozen for later use. Briefly, soil was collected near the rhizosphere of big bluestem (*Andropogon gerardii*). The soil was sieved to <1190 µm. Soil (1.0 g) was added to 4 mL cell extraction solution (50 mM sodium pyrophosphate and 0.05% Tween 80 (Sigma)), vortexed for 5 min, sonicated for 10 min, then centrifuged three times on a swinging rotor centrifuge at 129 rcf (×*g*) for 5 min. Nycodenz solution (2 mL of 60% v/v, Axis-Shield) was added to a new centrifuge tube and the supernatant from the soil extraction was carefully transferred to the top of the Nycodenz solution. The vial was centrifuged for 30 min at 3226 rcf (× *g*) to further separate the cells from the soil particles. The layers containing cells were collected and washed twice with phosphate buffered saline (PBS). The cells were frozen in 25% glycerol (v/v) at -70°C.

Glycerol stocks of the soil bacteria were thawed and inoculated (10 µL) into R2A broth (46). The cells were grown for 24 h at room temperature (22°C). Cells from this enrichment were washed in PBS (46), measured optically (biomass density=50.5 mg/L; described below), and used to inoculate the harvested plant root exudates and AREs. These solutions were covered with aluminum foil and placed on a shaker table (room temperature, 120 rpm) for 9 days. The metabolized exudates or AREs were then filtered through pre-combusted (550°C) Whatman GF/F filters (Cat # 1825, (47, 48)) and stored

in the dark at 4°C. Metabolized exudates and AREs were characterized for DOC, UV_{254}, SUVA, spectral slope, and fluorescence as described below.

Soil Partitioning. Soil partitioning experiments were used to determined if desorption was enhanced in the presence of raw or metabolized exudates. Soil, described in Chapter 4 as "Water Quality Mix A," (total organic carbon=3.25±0.37%; Chapter 4) was aged in a saturated naphthalene solution for 17 months in the dark at room temperature. Approximately 3 g of the aged soil and 17 mL of solution containing raw or metabolized exudates were added to a carbon-free 20 mL vial. The vial was filled headspace free. The solution consisted of raw or metabolized exudates diluted to 3.0 mg/L DOC with 50%-strength Hoagland's solution and 50 mM NaN_3 was added as a biocide. To assist with mixing, four 3mm diameter glass beads were added to the vial, which was then sealed with a Teflon-lined stopper. The vials were covered with opaque tape and placed on an end-over-end rotator for 18 days, which preliminary kinetics tests revealed to be sufficient time to reach equilibrium (Appendix C). After removal from the rotator, the vials were allowed to settle overnight. The aqueous fraction from each vial was removed, centrifuged in a 20 mL polystyrene tube (Falcon) at 2500 rcf (× g) for 6 minutes, and the supernatant was analyzed for naphthalene via high performance liquid chromatography (HPLC). The solids remaining in the vial were also analyzed for sorbed naphthalene via gas chromatography (GC) as described previously (Chapter 3 and 4). Replicate (3) experiments were conducted on each individual replicate hydroponic plant system (*i.e.*, n=9 for sedge, n=9 for grass, n=9 for clover) for both the raw and metabolized exudates. Nine replicate experiments were also conducted for each of the

following: the raw AREs, the metabolized AREs, 50% Hoagland's solution, and Milli-Q water.

Solubility. The ability of the exudates (raw or metabolized) to alter naphthalene solubility was also investigated. The following were added to vials such that no headspace remained: 0.1 g solid naphthalene (Fisher), 8 mL of a solution containing raw or metabolized exudates (diluted to 3.0 mg/L DOC with 50%-strength Hoagland's solution and 50 mM NaN_3 added as a biocide), and four 3mm diameter baked glass beads. The vials were sealed with Teflon-lined stoppers and placed on an end-over-end rotator for 5 days. Aqueous naphthalene concentration was quantified via HPLC. Again, replicate (3) experiments were conducted on each individual replicate hydroponic plant system (*i.e.*, n=9 for sedge, n=9 for grass, n=9 for clover) for both the raw and metabolized exudates. Six replicate experiments were also conducted for each of the following: the raw AREs, the metabolized AREs, 50% Hoagland's solution, and Milli-Q water.

Biodegradation Kinetics. Batch experiments were performed in stirred vials to determine if raw or metabolized exudates altered biodegradation kinetics. Solution (45 mL) containing the following was added to 160-mL baked vials: 2.5 mg/L naphthalene, 35 mL minimal mineral medium (pH 7.0; described in Chapter 3), and 10 mL of a test treatment. Test treatments consisted of raw or metabolized exudate solution (diluted to 3 mg/L DOC with 50%-strength Hoagland's), minimal medium containing a supressor (3

mg/L glucose), minimal medium containing an inducer (3 mg/L salicylate, (26)), or minimal medium only (negative control). *Pseudomonas putida* JS150 (ATCC #51283) [4.0 µg dry biomass equivalent, determined optically (49)] was added to each vial and vials were sealed with a Teflon-lined stopper and incubated at 33°C on a multipoint stirplate (Variomag Poly 15, 125 rpm). Naphthalene concentration was measured in the aqueous phase via HPLC by removing 1 mL samples every 30 minutes for 10 hours. A no-biomass control was also set-up in a manner identical to the negative control, except that no biomass was added to the vial. The rate of naphthalene loss through time in the no-biomass control was not significantly different from zero ($p=0.4761$; Appendix C).

All experiments were designed such that match-paired statistical analysis was permited to negate any confounding effects of temperature, innoculum growth statge, etc. For the controls, 8 matched-pair tests were conducted. For the exudates, two matched pair experiments were conducted for each plant type and ARE for both the raw and metabolized exudates. Thus, when the data for the plant types were pooled for all plant types and AREs, 8 matched pairs experiments were conducted (e.g., n=8 matched pairs for raw exudates and n=8 matched pairs for metabolized exudates). All biodegradation kinetics experiments used plant replicate 2 for consistency (i.e., sedge 2, grass 2, clover 2). Biodegradation kinetics were determined as described in the Data Analysis section.

Analytical Methods

Quantification of Dissolved Organic Carbon. The samples of exudate solutions were filtered through pre-combusted (550°C oven) Whatman GF/F filters (Cat # 1825)

(47, 48) into carbon-free glass vials. The filtered samples were then analyzed for organic carbon using a GE Sievers 900 Portable TOC Analyzer.

Spectral Measurements. The filtered exudate solutions were analyzed for ultraviolet/visible light absorbance from 200-600 nm using a Shimadzu UV-1601 PC spectrophotometer and a quartz cuvette. SUVA was determined by normalizing the UV_{254} by the DOC concentration (50). The spectral slope value was calculated using a least squares regression fit of the absorbance values over the 300-600 nm range (48) with a reference wavelength of 400 nm using Equation 5. 1 (47):

Equation 5. 1
$$a_\lambda = a_{\lambda o} e^{s(\lambda o - \lambda)}$$

where: a_λ is the measured absorbance at the given wavelength, $a_{\lambda o}$ is the absorbance at the reference wavelength, s is the spectral slope, λ is the wavelength at the given absorbance reading, and λo is the reference wavelength value.

Optical Biomass Density Measurements. Biomass density was determined optically using a Beckman DU 530 UV/Vis spectrophotometer with the following relationship:

Equation 5. 2
$$W = 9929(1 - \sqrt{1 - 0.7347 A_{660}})$$

where W is the dry biomass (mg/L) and A_{660} is the absorbance at 660 nm (49).

Fluorescence Measurements. A Jobin-Yvon Horiba Floromax 3 fluorometer with xenon lamp was used to obtain excitation-emission matrices (EEMs) via the method of

Cory and McKnight (51). EEMs were measured in ratio mode with an excitation range of 240 to 600 nm (at 5 nm intervals) and emission measurements over the range of 320 to 550 nm (at 2 nm intervals) with an integration time of 0.25 seconds. Samples were first filtered through precombusted GF/F filters (47) and then analyzed for UV_{254}; strongly absorbing samples (UV_{254}>0.3 Abs) were diluted by half to avoid inner-filter effects (51). All samples were run at room temperature. Daily lamp checks and checks for cuvette contamination were performed, as well as check of the water-Raman peak for a fresh MilliQ water blank. EEMs are expressed in Raman units (measured value less the nanopure water blank value divided by the water-Raman area). The Fluorescence Index (FI) was calculated from the EEM measurements and is the ratio of the emission intensity at a wavelength of 470 nm to that at 520 nm, at an excitation of 370 nm (48).

Surface Tension. Surface tension of liquids was measured using a Krüss K105T digital tensiometer. Liquid temperatures were measured (18-23°C) and the surface tension values were then corrected to a temperature of 20°C (52). Standard error of estimate for a set of replicate measurements (n=4) was ±0.47%.

HPLC Analysis. Aqueous naphthalene was quantified via high performance liquid chromatography using an Agilent 1200 with UV/Vis detector fitted with a SuplecoAscentis RP-Amide column (Cat# 565324-U; 15cm x 4.6mm, 5µm). The eluent, consisting of 80% HPLC-grade (>99.9%) acetonitrile and 20% ultrapure water, was supplied at a flow rate of 1.0 mL/min. The injection volume was 20 µL. The

naphthalene peak signal was at 218 nm with a reference wavelength of 360 nm. The elution time was 3.8 minutes. The method detection limit (46) for naphthalene was 0.57 µg/L.

Data Analysis

Biodegradation kinetics tests (C/C_0) were modeled using the modified Gompertz equation ($f(t) = ae^{-e^{c(t-k)}}$, described fully in Chapter 4) with least-squares residual fit, allowing determination of the time to reach the maxium degradation rate (h). Kinetic rates (h^{-1}) were determined from the slope of the natural log of C/C_0 plotted against time for data following the time of inflection. These values are reported with standard error and the fit of the respective model (R^2).

A repeated-measures one-way analysis of variance (ANOVA) was conducted to assess systematic differences between the paired kinetics tests. A Tukey-Kramer post-test was used to perform comparisons if ANOVA revealed significant differences ($p<0.05$). Paired Student's t-tests were used to assess matched pairs in data sets. Non-parametric analysis (Wilcoxon rank-sum or matched pairs tests; introduced in Chapter 3) was employed if data were distributed in a non-normal/transformable manner. All statistical analysis was conducted in GraphPad Prism (version 5.1).

5.3 RESULTS

DOC Quantification and SUVA Values. The DOC, UV254, and SUVA for the raw and metabolized exudates are presented in Table 5.1.

Table 5.1: DOC, UV_{254}, and SUVA values for the raw and metabolized exudates.

	Raw Exudates				Metabolized Exudates			
	DOC (mg/L)	Std Dev (mg/L)	UV 254	SUVA ($L\ mg^{-1}\ m^{-1}$)	DOC (mg/L)	Std Dev (mg/L)	UV 254	SUVA ($L\ mg^{-1}\ m^{-1}$)
Sedge 1	5.79	66.6×10^{-3}	0.120	2.07	0.866	20.1×10^{-3}	0.115	13.3
Sedge 2	3.16	15.3×10^{-3}	0.142	4.49	0.782	5.57×10^{-3}	0.157	20.1
Sedge 3	5.38	5.77×10^{-3}	0.140	2.60	0.662	2.52×10^{-3}	0.133	20.1
Grass 1	13.9	436×10^{-3}	0.438	3.15	15.7	200×10^{-3}	0.472	3.01
Grass 2	5.75	56.9×10^{-3}	0.165	2.87	3.49	70.9×10^{-3}	0.255	7.31
Grass 3	3.11	5.77×10^{-3}	0.201	6.46	2.08	5.77×10^{-3}	0.219	10.5
Clover 1	11.0	0.00×10^{-3}	0.221	2.01	24.6*	2060×10^{-3}	0.352	1.43
Clover 2	6.70	1.53×10^{-3}	0.189	2.82	3.46	208×10^{-3}	0.207	5.98
Clover 3	9.95	70.0×10^{-3}	0.178	1.79	2.75	11.5×10^{-3}	0.209	7.60
AREs	35.2	624×10^{-3}	0.222	0.63	18.7	721×10^{-3}	0.556	2.97

*Metabolized Clover 1 was not included in data analysis because of the dramatic and unexpected increase in DOC observed suggesting that primary production or contamination occurred.

The organic carbon levels for the raw exudates varied considerably within each plant type. The AREs (again, diluted to 10% with Hoagland's solution, as described in the methods above) contained substantially higher DOC than any of the harvested plant root exudates. After being metabolized, the DOC of the exudates decreased significantly ($p=0.0117$) and the SUVA and UV_{254} values increased significantly ($p= 0.0098$ and $p=0.0098$, respectively). Because aromaticity is positively correlated with SUVA (50, 53, 54), it is likely that the more aliphatic fractions of the exudates became aromatic compounds following bacterial metabolism. The decrease in DOC upon metabolism was expected (note: the metabolized Clover 1 sample was not included in further data analysis because the dramatic increase suggested that primary production or contamination occurred in this sample).

Spectral Slope. The spectral slope values for raw and metabolized exudate samples are presented in Table 5.2.

Table 5.2: Spectral slopes for the raw exudates and metabolized exudates.

	Raw Exudates	Metabolized Exudates	% change
Sedge 1	0.0014	0.0185	1221%
Sedge 2	0.0016	0.0178	982%
Sedge 3	0.0018	0.0182	931%
Grass 1	0.0112	0.0197	76%
Grass 2	0.0025	0.0157	524%
Grass 3	0.0044	0.0197	344%
Clover 1	0.0127	0.0185*	-
Clover 2	0.0068	0.0202	199%
Clover 3	0.0052	0.0198	278%
AREs	0.0136	0.0137	0.5%

*Not included in data analysis because of the dramatic and unexpected increase in DOC observed, indicating that primary production or contamination likely occurred.

The metabolized exudates had a significantly higher spectral slope than the raw exudates ($p < 0.0001$). The sedge had the greatest increase in spectral slope, followed by the grass, clover, and AREs, respectively. These results agree with the SUVA values for the metabolized exudates. Given that both measurements are related to aromaticity, this was expected (47). Spectral slope is independent of the organic carbon concentration and is an indication of the extent of DOC diagenesis and DOC source (47). Lower slope values indicate a terrestrial DOC source, whereas higher slopes tend to correspond with autochthonous DOC sources (47).The spectral slope data for the raw and metabolized exudates was again consistent with expectations, as metabolized exudates are more likely to have a weathered and autochthonous signature.

Fluorescence Index. The fluorescence index (FI) values for the raw and metabolized exudates are summarized Table 5.3.

Table 5.3: Summary of florescence index (FI) values for the raw and metabolized exudates.

	Raw Exudates	Metabolized Exudates	% change
Sedge 1	1.3686	1.4176	3.6%
Sedge 2	1.4078	1.4047	-0.2%
Sedge 3	1.3735	1.3741	0.0%
Grass 1	1.3787	1.4215	3.1%
Grass 2	1.3923	1.4019	0.7%
Grass 3	1.3901	1.4004	0.7%
Clover 1	1.4586	1.5021*	-
Clover 2	1.4090	1.4100	0.1%
Clover 3	1.3973	1.3886	-0.6%
AREs	2.0383	1.5268	-25.1%

*Not included in data analysis because of the dramatic and unexpected increase in DOC observed, indicating that primary production or contamination likely occurred.

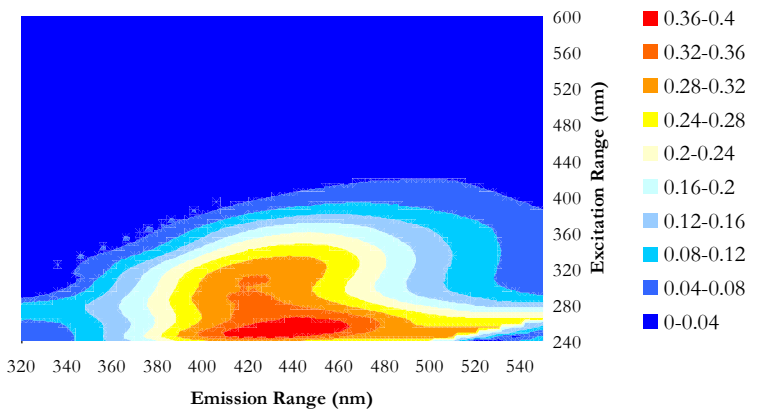
There was no significant systematic difference observed in the FI values of the raw and metabolized exudates ($p=0.4596$). The raw ARE FI value, however, was much larger than that of the raw harvested exudates and also decreased significantly upon metabolism. For natural organic matter in aquatic systems, higher FI values (>1.4) indicate microbiologically-derived carbon sources, whereas low FI values (<1.4) tend to correspond with terrestrially-derived sources (48). All FI values for the harvested exudates were approximately 1.4 (average=1.396, standard deviation= 0.016). The raw AREs, however, were larger than 1.4, thereby differing in character from the raw harvested exudates.

Excitation-Emission Matrices. Excitation-emission matrices (EEMs) were used to elucidate differences and alterations in the character of dissolved organic carbon (47, 55). Contour plots of the raw and metabolized exudate EEMs are shown in Table 5.4. Note

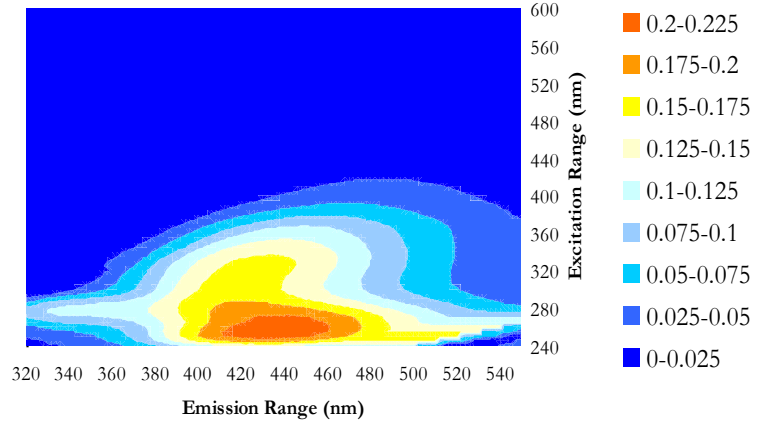
that by convention, all plots have an equal number of contour intervals to accent variations in shape (51); therefore, the scales for each plot vary.

Changes in EEM shape can indicate alterations in the character or source of organic carbon (47, 48, 51, 56). For example, Baker (57) used EEMs to examine the impact of wastewater on receiving streams. A shift of intensity of the EEM can be indicative of the carbon source. Maximum intensity in the EEM humic region (emission range: approximately 450 nm) is indicative of vegetative sources, whereas an intensity shift to the amino-acid region (emission range: approximately 350 nm) indicates a microbiologically-derived organic carbon source. Although these EEMs are presented for qualitative comparisons only, some of the samples (note samples Grass 2, Grass 3, AREs) show a distinct shift from the humic region to the microbiologically-derived region following metabolism. This is again consistent with the observations made regarding spectral slopes, SUVA values, and more generally, DOC concentrations.

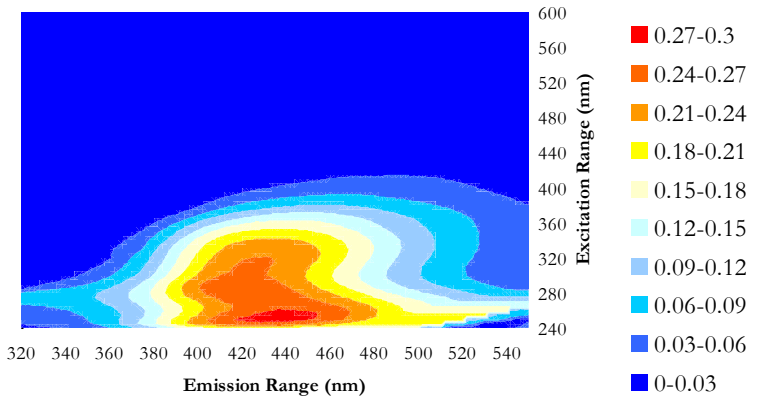
Table 5.4: Excitation-Emission Matrices (EEMs) for the raw and metabolized exudates for sedge (1,2,3), grass (1,2,3), clover (1,2,3), and AREas.



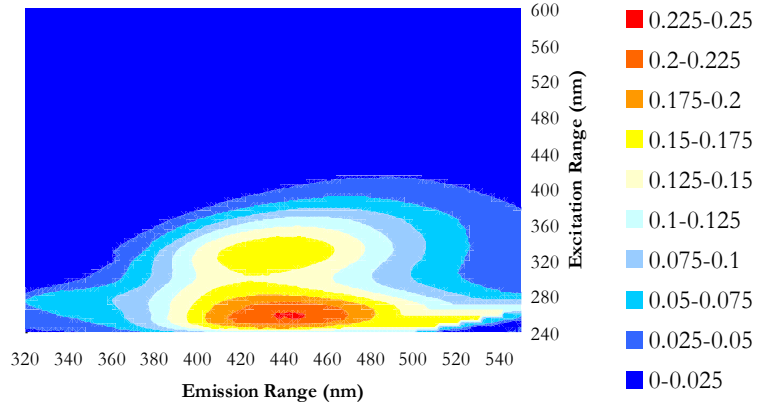
Sedge 1 Raw Exudate



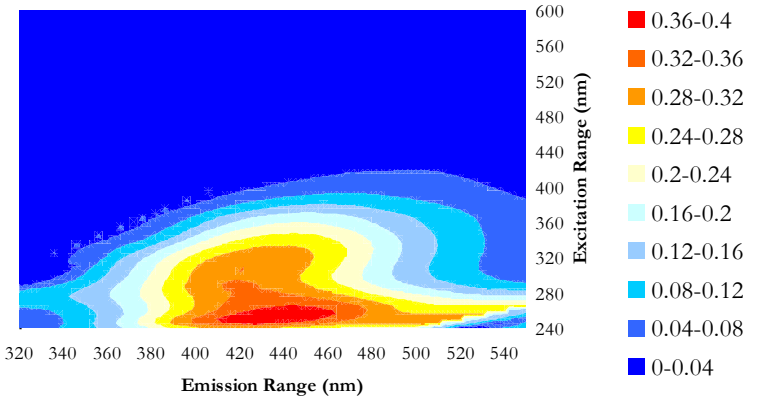
Sedge 1 Metabolized Exudate



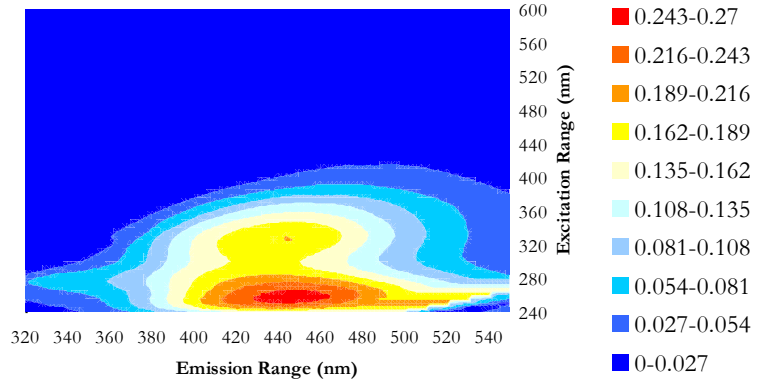
Sedge 2 Raw Exudate



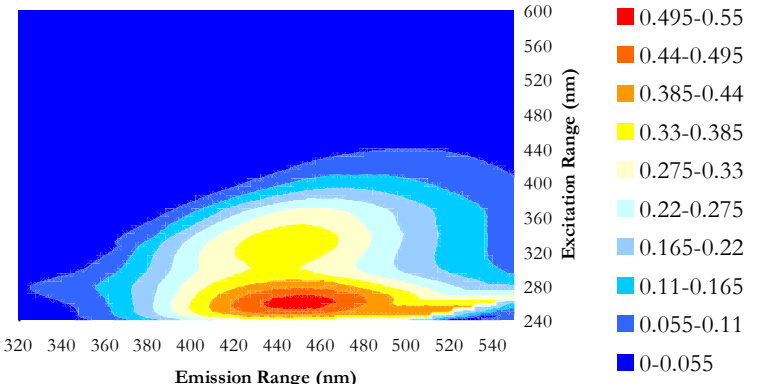
Sedge 2 Metabolized Exudate



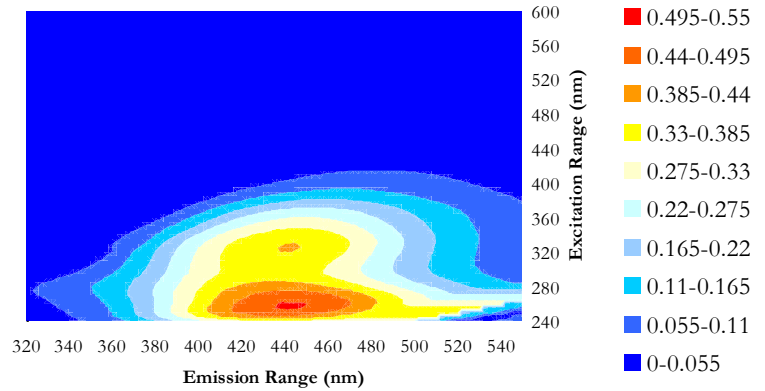
Sedge 3 Raw Exudate



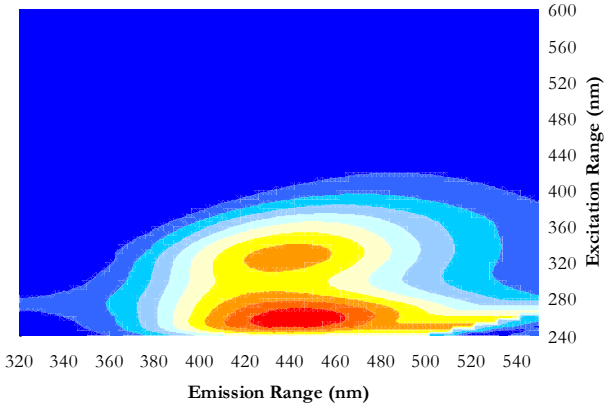
Sedge 3 Metabolized Exudate



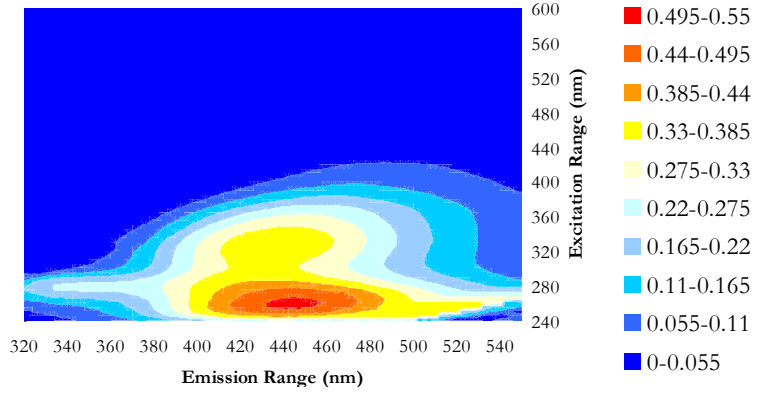
Grass 1 Raw Exudate



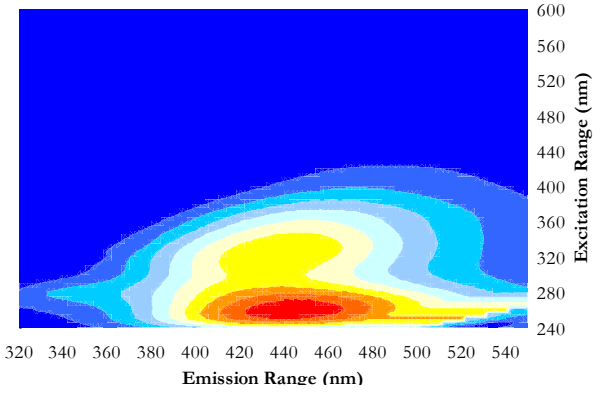
Grass 1 Metabolized Exudate



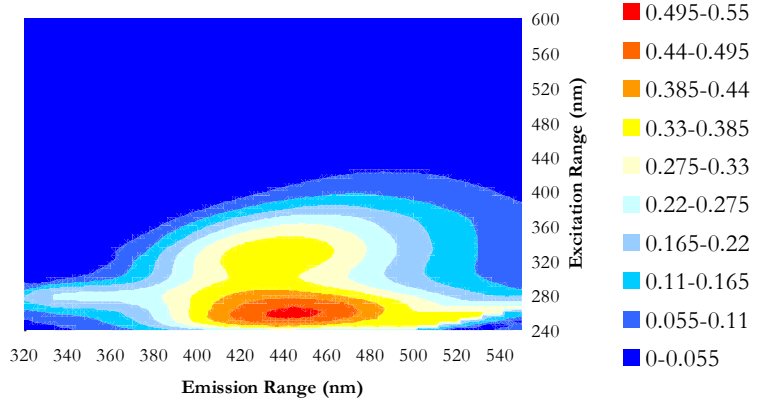
Grass 2 Raw Exudate



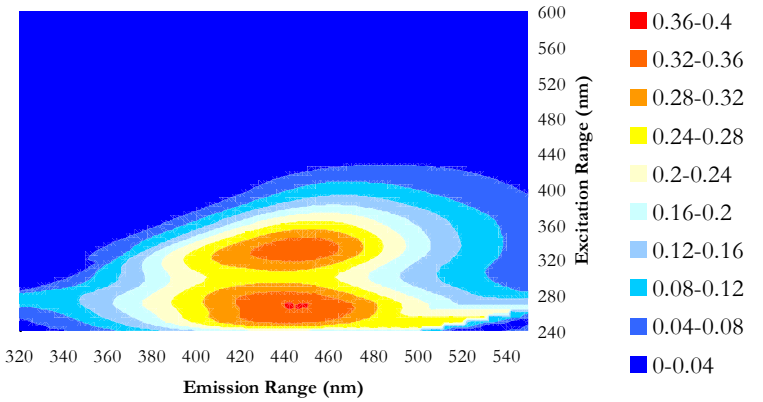
Grass 2 Metabolized Exudate



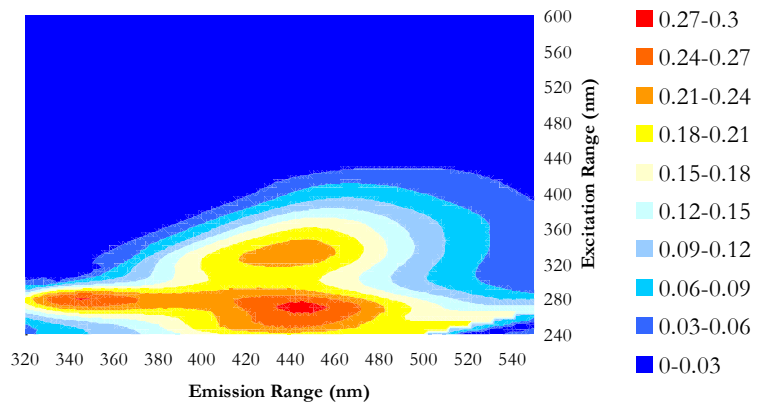
Grass 3 Raw Exudate



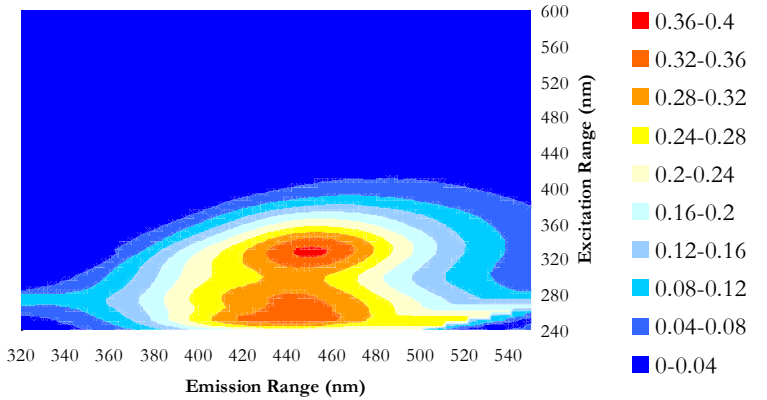
Grass 3 Metabolized Exudate



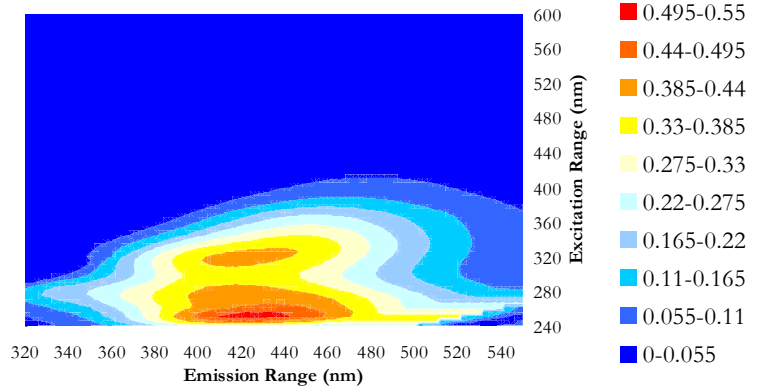
Clover 1 Raw Exudate



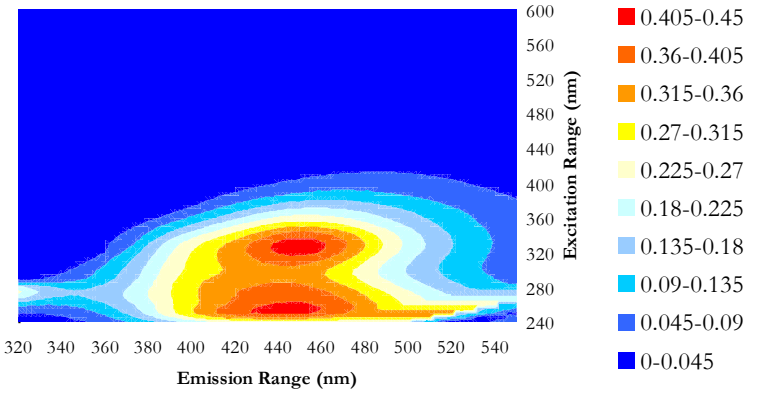
Clover 1* Metabolized Exudate (*sample likely contaminated, shown only for reference**)**



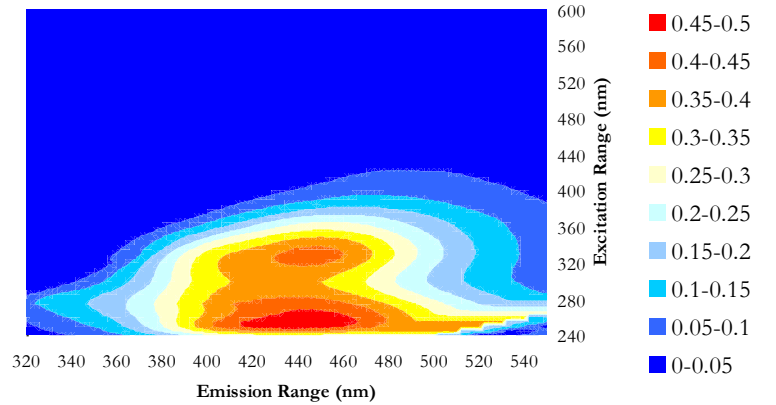
Clover 2 Raw Exudate



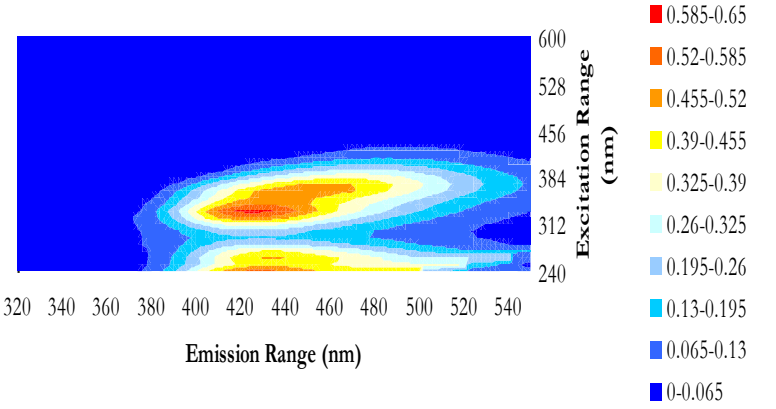
Clover 2 Metabolized Exudate



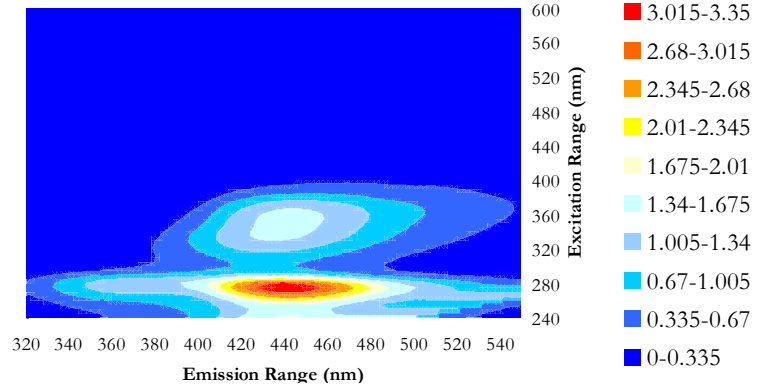
Clover 3 Raw Exudate



Clover 3 Metabolized Exudate



1/10 ARE Raw Exudate



1/10 ARE Metabolized Exudate

Surface Tension. Surface tension measurements for the raw and metabolized exudates are summarized in Table 5.5 with the surface tension value for water and 50%-strength Hoagland's solution provided as reference.

Table 5.5: Surface tension values (mN/m) for the raw and metabolized exudates and water/Hoagland's solution reference values.

	Raw Exudates	Metabolized Exudates	% change
Sedge 1	73.2	73.3	0.2%
Sedge 2	65.6	72.6	10.6%
Sedge 3	58.0	68.5	18.0%
Grass 1	66.2	61.0	-7.8%
Grass 2	61.4	72.4	17.9%
Grass 3	57.0	72.8	27.6%
Clover 1	57.1	66.5*	-
Clover 2	71.5	68.2	-4.7%
Clover 3	65.8	68.5	4.1%
1/10 AREs	71.0	64.5	-9.1%
Milli-Q†	71.4	N/A	N/A
50%-strength Hoagland's	72.4	N/A	N/A

*Not included in data analysis because of the dramatic and unexpected increase in DOC observed, indicating that primary production or contamination likely occurred.

†The literature value for pure water surface tension at 20° C is 72.8 mN/m (52).

The raw harvested exudates appear to have a lower surface tension than the metabolized harvested exudates ($p=0.0408$). When the raw and metabolized AREs were included in the analysis, however, there was no difference between the surface tension values before and after metabolism at the 95% confidence interval ($p=0.0881$). Surface tension values lower than that of pure water (or in this case Milli-Q or 50%-strength Hoagland's solution) indicates the presence of surface-active compounds (31).

Soil-Water Partitioning. Soil-water partioning coefficient (K_d) values for naphthalene-aged soil are summarized in Figure 5.2 and 5.3. In Figure 5.2, K_d values are separated based on the plant type (or AREs) and in Figure 5.4 the K_d values are pooled into the general categories of raw and metabolized exudates.

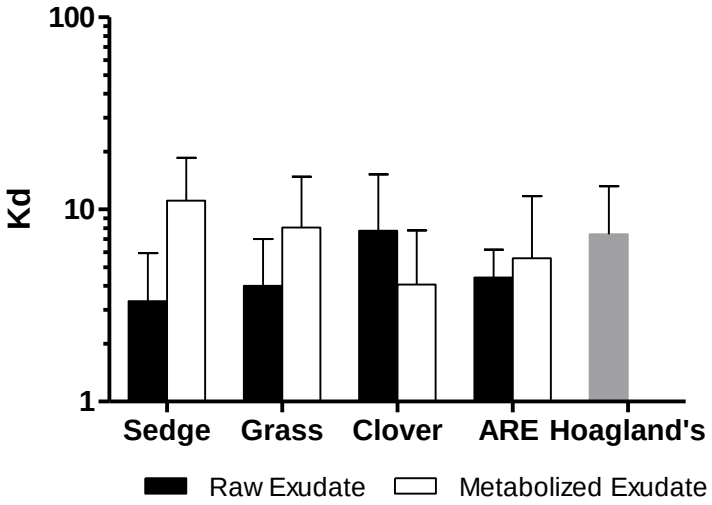


Figure 5.2: Effect of exudate source (i.e., plant type) and bacterial metabolism on the mean naphthalene soil-water partitioning constant (K_d) and comparison with the mean value for a reference solution (50%-strength Hoagland's solution). Error bars indicate the 95% confidence interval (note log-scale axis).

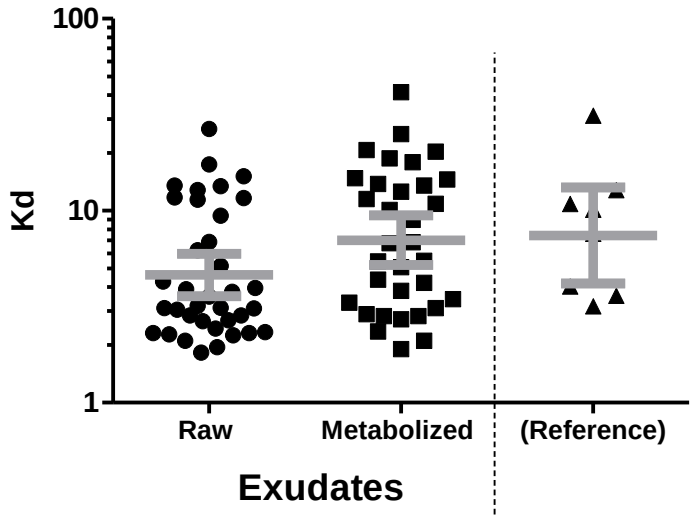


Figure 5.3: Summary of naphthalene K_d values for solutions of raw and metabolized exudates and comparision with values for a reference solution (50%-strength Hoagland's solution). The bars indicate the geometric mean ±95% confidence intervals (note log-scale on y-axis).

When the values were pooled, the soil-water partitioning coefficient was significantly lower for the raw exudates than for the metabolized exudates (Wilcoxon rank sum test, p=0.0292; the non-parametric tests was used because at least one of the data sets or its log transformation was significantly non-Gaussian using the Kruskal-Wallis test, p=0.001). As a result of the smaller sample size for the 50%-strength Hoagland's reference solution experiments (reference, n=9; raw exudates, n=36; metabolized exudates, n=33), the raw exudates did not show a significant difference from the reference at the 95% confidence interval (p=0.0672). The metabolized exudates showed no difference from the reference (p=0.8301). No significant differences existed between the plant types (p=0.7841).

Solubility Alteration. Neither the raw nor metabolized exudates nor 50%-strength Hoagland's solution impacted bulk naphthalene aqueous solubility (Figure 5.4, p=0.9253). Plant type did not affect solubility (p=0.8726).

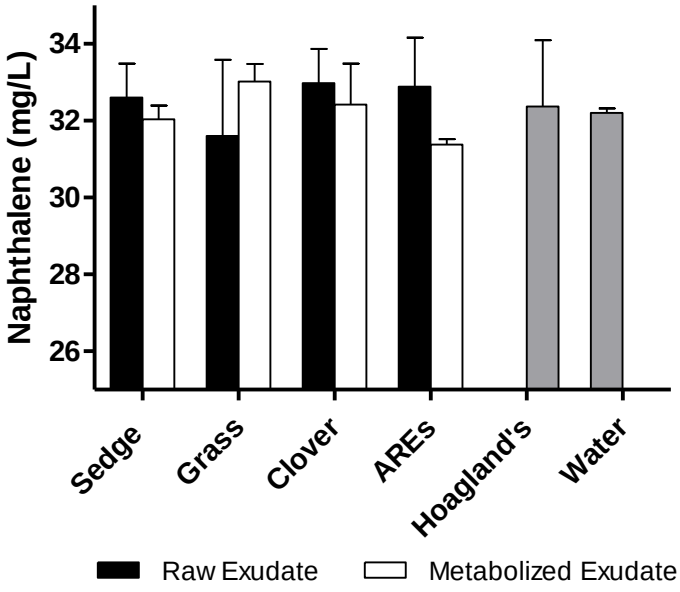


Figure 5.4: Solubility of aqueous naphthalene in solutions of raw and metabolized exudates and comparison with reference solutions. Error bars indicate 95% confidence intervals.

Exudate Effects on Biodegradation. The biodegradation experiments were analyzed with respect to the time required to reach the maximum rate of degradation (h) and for the rates of biodegradation (h^{-1}) (Table 5.6). An example graph of naphthalene biodegradation in the presence of raw AREs and metabolized AREs is shown in Figure 5. 5. The positive and negative controls behaved as expected, with the time to reach the maximum rate of degradation being greatest in the experiments to which the supressor was added, followed by the negative (no addition) control, followed by the experiments

to which the inducer was added (Table 5.7). The supressor (glucose) had a significantly greater time to the maximum degradation rate than the inducer (salicylate) ($p=0.0156$), although neither was significantly different from the negative control (no treatment; $n=8$ pairs, $p>0.07$). Interestingly, naphthalene biodegradation showed evidence of supression in the presence of the raw exudates, with the pooled samples requiring a significantly greater time to reach the maximum degradation rate when compared to the metabolized exudates ($p=0.0495$). The rates of naphthalene biodegradation were not significantly different between the controls ($p=0.1495$), nor were they different between the raw and metabolized exudates ($p=0.262$). There was no significant naphthalene loss in the abiotic controls (Table 5.7).

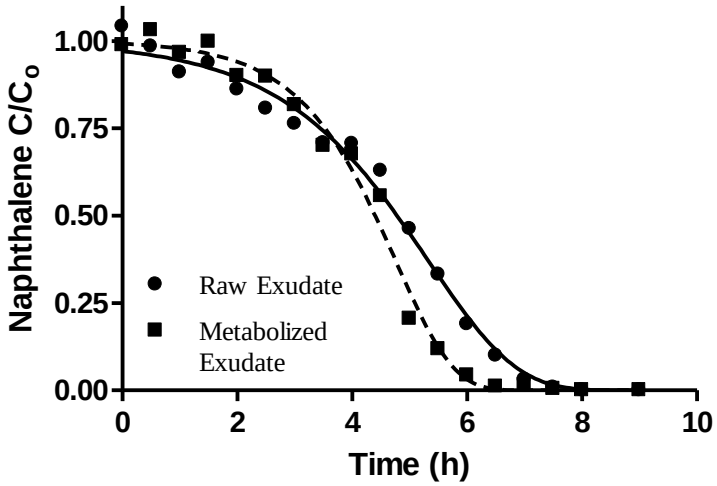


Figure 5. 5: Example graph (AREs shown) of naphthalene biodegradation kinetics for the raw and metabolized exudates.

Table 5.6: Parameters for the paired biokinetics tests using raw and metabolized exudates from Sedge 2, Grass 2, Clover 2, and AREs. The values are the time to reach maximum degradation rate (h) and the rate of naphthalene biodegradation (h^{-1}). Experimental Pair 1 and Pair 2 were conducted separately on different days to allow for subsequent paired statistical analysis; paired data were later pooled between raw and metabolized exudates for analysis (n=8 matched pairs).

		Raw Exudate				Metabolized Exudate			
		Sedge	**Grass**	**Clover**	**ARE**	**Sedge**	**Grass**	**Clover**	**ARE**
Exp. Pair 1	**Time to maximum degradation rate (h)**	5.30	5.41	5.05	5.11	5.21	5.32	4.29	4.11
	std. err.	0.24	0.16	0.23	0.17	0.19	0.29	0.66	0.19
	Fit (R^2)	0.98	0.99	0.97	0.98	0.99	0.98	0.97	0.99
	Degradation Rate (h^{-1})	0.87	0.97	1.32	0.78	0.82	1.18	0.91	1.54
	std. err.	0.18	0.08	0.12	0.62	0.17	0.14	0.02	0.60
	Fit (R^2)	0.92	0.99	0.99	0.61	0.92	0.97	0.99	0.87
Exp. Pair 2	**Time to maximum degradation rate (h)**	4.83	5.48	4.88	5.34	5.12	5.06	4.73	4.77
	std. err.	0.08	0.16	0.07	0.09	0.12	0.11	0.11	0.07
	Fit (R^2)	0.99	0.96	0.99	0.99	0.97	0.98	0.99	0.99
	Degradation Rate (h^{-1})	0.86	0.96	1.12	1.21	0.85	1.45	1.06	1.52
	std. err.	0.15	0.09	0.02	0.05	0.05	0.08	0.03	0.20
	Fit (R^2)	0.87	0.98	0.99	0.99	0.98	0.99	0.99	0.93

Table 5.7: Biokinetic parameters for the controls (baseline=no treatment, inducer=salicylate, supressor=glucose) for the paired tests conducted in Chapter 5. The values are the time to reach maximum degradation rate (h) and the rate of naphthalene biodegradation (h^{-1}) with the model fits.

		Gompertz Fit of Degradation Kinetics (C/C_0) through time			**Determination of Degradation Rate** ($\ln[C/C_0]$ through time)		
		Time to maximum degradation rate (h)		Model fit	**Degradation Rate (h^{-1})**		Model fit
	Experimental Pairing	Value	std. err.	R^2	Value	std. err.	R^2
Baseline (no treatment)	1	4.90	0.10	0.99	0.85	0.13	0.93
	2	4.92	0.09	0.98	1.17	0.05	0.99
	3	5.32	0.12	0.98	1.12	0.28	0.84
	4	5.43	0.07	0.99	1.63	0.52	0.83
	5	5.20	0.12	0.99	1.04	0.14	0.98
	6	5.62	0.11	0.98	1.47	0.09	0.99
	7	5.46	0.12	0.98	1.286	0.08	0.98
	8	4.70	0.11	0.99	1.40	0.11	0.97
Inducer (salicylate)	1	4.66	0.18	0.98	0.88	0.20	0.86
	2	4.72	0.07	0.99	1.26	0.11	0.96
	3	5.10	0.08	0.98	2.86	0.37	0.98
	4	5.07	0.12	0.98	1.56	0.50	0.83
	5	5.07	0.09	0.98	1.45	0.29	0.89
	6	4.77	0.07	0.99	1.33	0.12	0.97
	7	5.08	0.09	0.99	1.25	0.06	0.99
	8	4.99	0.12	0.99	1.34	0.14	0.96
Supressor (glucose)	1	5.19	0.14	0.99	0.89	0.12	0.95
	2	4.95	0.07	0.99	1.72	0.31	0.88
	3	7.43	0.13	0.95	0.44	0.17	0.76
	4	5.15	0.09	0.98	1.41	1.17	0.59
	5	7.22	0.14	0.96	0.42	0.13	0.78
	6	4.99	0.17	0.99	1.64	0.28	0.90
	7	5.25	0.15	0.99	1.86	0.29	0.93
	8	4.97	0.37	0.96	1.67	0.29	0.89

5.4 DISCUSSION

The raw exudates were able to increase the desorption of naphthalene when compared to the metabolized exudates (Figure 5.4). Additionally, the raw exudates appeared to have surface active properties, as evidenced by the reduction in surface tension measured in the harvested raw exudate samples (Table 5.4). In our study it appeared that the surface active compounds were subsequently biodegraded, as the metabolized exudates had a significantly greater K_d value and a higher surface tension. There is evidence in the literature of root exudates that contain surface-active compounds, such as glycerolipids and glycoproteins (22), or enhance contaminant bioavailability via other mechanisms, such as organic acid competition for adsorption binding sites (29, 30). Interestingly, although compounds in the raw exudates resulted in a significant decrease in K_d, there was no concomitant increase in naphthalene solubility in the raw exudate samples. This behavior is consistent with the presence of surface-active compounds at concentrations below the critical micelle concentration (CMC), where desorption of a sorbed hydrophobic compound is enhanced through the reduction of surface tension without affecting the aqueous solubility of the same compound (31). Indeed, the observed desorption effects may be magnified for high molecular weight PAHs or PCBs because the effects of surface-altering compounds tend to be more pronounced for larger and more nonpolar molecules (58, 59). Roughly 10-40% of the assimilated vegetative carbon may be deposited into the rhizosphere in the field (1, 3, 9, 60), generating approximately 10-100 mg soluble exudates g^{-1} assimilated root material (60); thus, the cumulative influence of plant exudates at the field scale is expected to be substantial. The findings of

this work are therefore important because they suggest that enhanced desorption of hydrophobic organic contaminants by plant root exudates could ultimately decrease contaminant residuals in planted soils by increasing contaminant bioavailability,.

The biokinetic experiments demonstrated that the raw exudates also supressed naphthalene biodegradation compared to the metabolized exudates. It is well established that plants and their associated microbial communities interact symbiotically, affecting contaminant removal (9-11, 18, 20, 22, 23, 27, 60-68), but little work has focused on how exudates may interfere with the actual biodegradation of a target contaminant. In the rhizosphere, raw plant exudates may provide a desirable carbon and energy source that competes with contaminant degradation through a variety of mechanisms, including repression at the enzyme activity or transcription level. This is consistent with the observations of Kamath et al. (26) who showed that root-derived substances could repress the expression of the naphthalene dioxygenase gene while simultaneously promoting greater total contaminant degradation rate by increasing overall microbial activity. Exudates can serve as carbon and energy sources for bacteria to non-specifically increase microbial populations, which tends to enhance the degradation of target contaminants and their metabolites (Chapter 3; refs 1, 5, 9, 11, 16, 22, 69). Nevertheless, the addition of exudates to enhance degradation may not be consistant in all cases, as these compounds can clearly also supress some specific degradation pathways.

An unexpected finding of this research was that AREs differ markedly from the harvested plant root exudates that we examined in terms of their organic carbon character. This difference was observed with multiple quantitative (DOC, SUVA,

spectral slope, FI) and qualitative (EEMs) characterization approaches. For the experiments conducted, no differences were observed between the AREs and the plant root exudates (raw and metabolized) regarding their impact on naphthalene sorption, solubility, or biodegradation. Nevertheless, because the structure and character (*e.g.*, surface tension) of organic carbon is known to affect both biological and abiotic processes (47, 51, 56, 57), it is possible that the significant differences in organic carbon character between AREs and plant root exudates could result in very different behavior in other situations. AREs used in this study were created using a literature recipe (44) and have been used by others to simulate exudates in the rhizosphere (*e.g.*, 28, 70). Our work was conducted at environmentally relevant concentrations testing multiple plant types, constrasting Gao et al. (28) which tested AREs but not harvested root exudates for PAH desorption. Therefore, based on our characterization work, caution is recommended when extrapolating results obtained with AREs to what might be expected with plant root exudates.

In conclusion, this work demonstrates that root exudates can enhance desorption of hydrophobic contaminants from soil. This abiotic mechanism may contribute to the rhizosphere effect observed in phytoremediation research, thereby making contaminants more bioavailable. Nevertheless, this research also found that harvested root exudates and artificial root exudates are significantly different in organic carbon character, and some of these differences may impact contaminant sorption characteristics. Furthermore, it appears that the surface active components of the root exudates are readily degraded and that metabolized exudates do not significantly improve desorption. The degradation

of root exudates to metabolites can cause suppression of the target contaminant in a batch system, but addition of labile carbon is unlikely to have a net negative effect in field applications. Thus, greater quantities of plants with deep root structures would likely improve contaminant remediation at contaminated legacy sites and minimize accumulation of residual in bioretention areas.

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Chapter 6: Conclusions and Recommendations

Four main conclusions and recommendations are offered from the results of this work:

First, hydrocarbons in stormwater runoff enter bioretention areas but do not accumulate to high levels as a result of biodegradation and other loss mechanisms. Risks of soil contamination and groundwater pollution from hydrocarbon residuals appear to be minimal. Nevertheless, it is recommended that caution be used when siting bioretention practices in karst geology, which can act as an open conduit for conveying pollutants to underlying groundwater.

Second, bioretention is successful at removing hydrocarbons from stormwater infiltrate. Conceptually, hydrocarbon fate can be envisioned as a two-step removal process: instantaneous sorption followed by desorption and subsequent biodegradation. Hydrocarbon removal in the field is spatially and temporally variable; therefore, additional research into as the effects of toxic micropollutants, including pesticides, or of road-salt washout on biodegradation capacity is warranted.

Based on the results of this work, it appears that bioretention is superior to the current technology, stormwater ponds, at capturing and degrading hydrocarbons. In the literature, stormwater ponds have been found to accumulate PAHs. The success of bioretention is due to the aerobic soil zone, which allows for faster biodegradation than in the typically anoxic sediment layers of ponds. Nevertheless, this work only examined naphthalene, a relatively labile PAH, and some high molecular weight and more

recalcitrant PAHs are known to exist in stormwater. Additional research is needed to determine the long-term fate of high molecular weight, pyrogenic PAHs in bioretention areas.

Finally, vegetation improves hydrocarbon removal performance in bioretention; this finding is consistent with the phytoremediation literature and is applicable to other best management practices for managing non-point source pollutants. Vegetation influences hydrocarbon removal through multiple means, including direct uptake, enhanced desorption of contaminants, rhizodeposition of exudates, and stimulation of hydrocarbon degrading bacteria. Further research into the pollution control benefits of vegetation and the impact of plant type is needed due to the complexities of the rhizosphere ecosystem.

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Appendix A: Supporting Information for Chapter 3

Table A.1: Information on the field sites sampled in Chapter 3.

Sample Number	Site Number	Raingarden area (sq ft)	Drainage area (sq ft)	Year	Location-	City	Drain type	Vegetation
1	1	680	2560	2006	Random Park	Mounds View	Light-use parking lot	Mulch / unplanted
2	1	680	2560	2006	Random Park	Mounds View	Light-use parking lot	Mulch / unplanted
3	1	680	2560	2006	Random Park	Mounds View	Light-use parking lot	Mulch / unplanted
4	2	750	1420	2006	Random Park	Mounds View	Light-use parking lot	Turf grass
5	2	750	1420	2006	Random Park	Mounds View	Light-use parking lot	Turf grass
6	Upland 1	N/A	N/A	N/A	Random Park	Mounds View	Upland	Turf grass
7	Upland 2	N/A	N/A	N/A	Random Park	Mounds View	Upland	Turf grass
8	3	375	670	2006	Random Park	Mounds View	Light-use parking lot	Turf grass
9	4	2460	28140	2006	Bel Air Elementary	New Brighton	Commercial parking lot	Mulch / unplanted
10	4	2460	28140	2006	Bel Air Elementary	New Brighton	Commercial parking lot	Mulch / unplanted
11	4	2460	28140	2006	Bel Air Elementary	New Brighton	Commercial parking lot	Mulch / unplanted
12	Upland 3	N/A	N/A	N/A	Midtown EcoYard	Minneapolis	Upland	Native plantings
13	5	1248	11880	2005	Midtown EcoYard	Minneapolis	Light-use parking lot	Turf grass
14	5	1248	11880	2005	Midtown EcoYard	Minneapolis	Light-use parking lot	Native plantings

Sample Number	Site Number	Raingarden area (sq ft)	Drainage area (sq ft)	Year	Location-	City	Drain type	Vegetation
15	5	1248	11880	2005	Midtown EcoYard	Minneapolis	Light-use parking lot	Native plantings
16	5	1248	11880	2005	Midtown EcoYard	Minneapolis	Light-use parking lot	Native plantings
17	6	440	4500	Unknown	Asbury & Frankson	Saint Paul	Residential street	Native plantings
18	6	440	4500	Unknown	Asbury & Frankson	Saint Paul	Residential street	Native plantings
19	7	1520	5250	Unknown	Asbury & Midway	Saint Paul	Residential street	Native plantings
20	8	1991	11250	Unknown	McKineley & Frankson	Saint Paul	Residential street	Native plantings
21	9	1991	31500	Unknown	Albert & Frankson	Saint Paul	Residential street	Native plantings
22	10	2200	49125	Unknown	Hamline & Midway	Saint Paul	Residential street	Mulch / unplanted
23	11	1054	2625	Unknown	McKineley & Arlington	Saint Paul	Residential street	Native plantings
24	12	504	3750	Unknown	McKineley & Pascal	Saint Paul	Residential street	Native plantings
25	13	428	3750	Unknown	McKineley & Pascal	Saint Paul	Residential street	Native plantings
26	14	592	1168	Unknown	McKineley & Pascal N	Saint Paul	Residential street	Native plantings
27	15	367	2627	Unknown	McKineley & Pascal N	Saint Paul	Residential street	Native plantings
28	16	298	594	Unknown	W 49th St	Minneapolis	Residential roof	Native plantings
29	17	112	391	Unknown	W 49th St	Minneapolis	Residential roof	Decorative cultivars
30	18	108	648	Unknown	W 49th St.	Minneapolis	Residential roof	Decorative cultivars
31	19	122	216	Unknown	Beard Ave S	Minneapolis	Residential roof	Native plantings

Sample Number	Site Number	Raingarden area (sq ft)	Drainage area (sq ft)	Year	Location-	City	Drain type	Vegetation
32	19	122	216	Unknown	Beard Ave S	Minneapolis	Residential roof	Native plantings
33	20	48	600	Unknown	York Ave S	Minneapolis	Residential roof	Native plantings
34	21	115	621	Unknown	Xerxes Ave S	Minneapolis	Residential roof	Native plantings
35	22	24	384	Unknown	Thomas Ave S	Minneapolis	Residential roof	Native plantings
36	23	40	477	Unknown	Russell Ave S	Minneapolis	Residential roof	Decorative cultivars
37	Upland 4	N/A	N/A	N/A	Midtown EcoYard	Minneapolis	Upland	Native plantings
38	24	881	5850	Unknown	U of M vet clinic	Saint Paul	Light-use parking lot	Native plantings
39	25	425	3000	Unknown	U of M vet clinic	Saint Paul	Residential roof	Decorative cultivars
40	26	959	7800	Unknown	Gortner &Commonwealth	Saint Paul	Residential street	Native plantings
41	27	884	5200	Unknown	Gortner Ave	Saint Paul	Residential street	Native plantings
42	28	912	1610	2003	Rushmore & Southwind	Burnsville	Residential street	Decorative cultivars
43	29	408	3944	2003	Rushmore & Southwind	Burnsville	Residential street	Decorative cultivars
44	30	133	2154	2003	Rushmore Dr.	Burnsville	Residential street	Decorative cultivars
45	31	240	2566	2003	Rushmore Dr.	Burnsville	Residential street	Decorative cultivars
46	32	336	1903	2003	Rushmore Dr.	Burnsville	Residential street	Decorative cultivars
47	33	198	1669	2003	Rushmore Dr.	Burnsville	Residential street	Decorative cultivars
48	34	180	4777.5	2003	Rushmore Dr.	Burnsville	Residential street	Decorative cultivars

Sample Number	Site Number	Raingarden area (sq ft)	Drainage area (sq ft)	Year	Location-	City	Drain type	Vegetation
49	35	168	2262	2003	Rushmore Dr.	Burnsville	Residential street	Decorative cultivars
50	36	136	2424	2003	Rushmore Dr.	Burnsville	Residential street	Decorative cultivars
51	37	234	5706	2003	Rushmore Dr.	Burnsville	Residential street	Decorative cultivars
52	38	240	861	2003	Rushmore Dr.	Burnsville	Residential street	Decorative cultivars
53	39	146	700	2003	Rushmore Dr.	Burnsville	Residential street	Decorative cultivars
54	40	126	2876	2003	Rushmore Dr.	Burnsville	Residential street	Decorative cultivars
55	41	14	160	Unknown	Washburn Ave	Minneapolis	Residential roof	Native plantings
56	42	14	323	Unknown	Washburn Ave	Minneapolis	Residential roof	Native plantings
57	43	82	420	Unknown	Beard Ave	Minneapolis	Residential roof	Decorative cultivars
58	44	17999	57750	Unknown	Mall of America	Bloomington	Commercial parking lot	Decorative cultivars
59	45	17999	34524	Unknown	Mall of America	Bloomington	Commercial parking lot	Decorative cultivars
60	46	4080	29900	Unknown	Holyoke Ave	Lakeville	Parking lot	Decorative cultivars
61	47	4080	35200	Unknown	Holyoke Ave	Lakeville	Parking lot	Decorative cultivars
62	48	1071	12350	Unknown	Wentworth Ave E	West St. Paul	Parking lot	Decorative cultivars
63	49	918	12350	Unknown	Wentworth Ave E	West St. Paul	Parking lot	Decorative cultivars
64	50	20631	46200	Unknown	Thompson Park	West St. Paul	Parking lot	Decorative cultivars
65	50	20631	46200	Unknown	Thompson Park	West St. Paul	Parking lot	Decorative cultivars

Sample Number	Site Number	Raingarden area (sq ft)	Drainage area (sq ft)	Year	Location-	City	Drain type	Vegetation
66	51	598	1820	2003	Park & Ride Lot	Cottage Grove	Commercial parking lot	Native plantings
67	52	398	1500	2003	Park & Ride Lot	Cottage Grove	Commercial parking lot	Native plantings
68	53	459	1500	2003	Park & Ride Lot	Cottage Grove	Commercial parking lot	Native plantings
69	54	75	625	2003	Park & Ride Lot	Cottage Grove	Commercial parking lot	Native plantings
70	55	105	625	2004	UMN Arboretum	Chaska	Commercial parking lot	Native plantings
71	56	3750	7500	2004	UMN Arboretum	Chaska	Commercial parking lot	Native plantings
72	56	4000	8000	2004	UMN Arboretum	Chaska	Commercial parking lot	Native plantings
73	57	9375	15000	2004	UMN Arboretum	Chaska	Commercial parking lot	Native plantings
74	58	900	3575	2004	UMN Arboretum	Chaska	Commercial parking lot	Native plantings
75	56	3750	7500	2004	UMN Arboretum	Chaska	Commercial parking lot	Native plantings

Table A.2: Values for field samples extracted in Chapter 3.

Sample No.	PHE copies/g dry	NAH copies/g dry	16S rRNA copies/g dry	TPH Concentration (µg TPH/kg dry soil)	Soil Moisture Content
1	4.83E+08	5.44E+04	2.62E+10	1.115	0.381
2	4.25E+09	6.04E+05	1.10E+11	0.476	0.418
3	3.36E+09	4.88E+05	9.09E+10	0.000	0.517
4	2.92E+09	5.10E+05	8.28E+10	0.000	0.671
5	1.03E+09	8.64E+04	2.22E+10	1.160	0.352
6	1.24E+09	4.63E+05	2.96E+10	0.000	0.320
7	1.25E+09	2.03E+05	2.71E+10	0.000	0.412
8	4.88E+08	3.97E+04	1.48E+10	0.000	0.194
9	6.70E+08	5.78E+04	1.95E+10	0.000	0.105
10	1.99E+09	0.00E+00	5.42E+10	2.755	0.481
11	1.00E+09	0.00E+00	2.46E+10	1.121	0.270
12	2.90E+08	0.00E+00	5.08E+09	0.000	0.039
13	1.99E+08	0.00E+00	2.82E+09	1.085	0.029
14	1.22E+09	0.00E+00	2.87E+10	1.790	0.371
15	1.06E+09	0.00E+00	3.13E+10	1.004	0.440
16	9.88E+08	2.66E+05	2.45E+10	0.000	0.348
17	4.11E+10	3.37E+06	6.90E+10	2.544	0.301
18	1.73E+10	0.00E+00	4.05E+10	0.433	0.321
19	3.69E+10	1.13E+06	9.38E+10	0.000	0.516
20	1.57E+10	6.71E+05	3.75E+10	0.000	0.187
21	2.29E+10	0.00E+00	5.63E+10	1.444	0.209
22	2.12E+10	3.67E+05	6.22E+10	0.462	0.212
23	2.00E+10	1.32E+06	4.95E+10	0.577	0.207
24	2.94E+10	5.10E+06	6.81E+10	0.000	0.223
25	1.87E+09	4.32E+06	1.72E+09	0.778	0.218
26	1.21E+10	1.71E+06	3.46E+10	1.906	0.196
27	3.60E+10	4.39E+07	9.15E+10	0.760	0.282
28	1.97E+10	2.86E+06	7.09E+10	0.000	0.256
29	2.28E+10	1.53E+05	1.04E+11	0.000	0.298
30	3.61E+10	3.79E+06	1.85E+11	2.450	0.330
31	1.31E+10	0.00E+00	6.42E+10	0.942	0.207
32	1.51E+10	6.08E+06	1.02E+11	1.272	0.088
33	1.96E+10	3.34E+05	6.39E+10	0.000	0.091
34	1.63E+10	3.04E+06	4.38E+10	0.300	0.141
35	1.73E+10	6.53E+05	4.14E+10	1.190	0.132
36	1.59E+10	5.71E+05	4.56E+10	0.000	0.148
37	1.21E+10	7.75E+05	4.59E+10	0.000	0.189
38	3.24E+10	3.46E+07	7.16E+10	0.000	0.243
39	1.80E+10	9.81E+07	6.47E+10	0.000	0.238
40	2.00E+10	2.33E+07	9.35E+10	0.000	0.107
41	2.50E+10	1.30E+07	1.20E+11	0.066	0.172
42	1.18E+10	5.79E+07	3.33E+10	1.422	0.155

Sample No.	PHE copies/g dry	NAH copies/g dry	16S rRNA copies/g dry	TPH Concentration (µg TPH/kg dry soil)	Soil Moisture Content
43	1.76E+10	1.98E+07	6.48E+10	0.000	0.184
44	1.50E+10	3.43E+07	5.48E+10	0.000	0.151
45	1.80E+10	1.78E+07	1.06E+11	0.000	0.141
46	2.15E+10	0.00E+00	1.08E+11	0.000	0.136
47	1.94E+10	0.00E+00	1.69E+11	0.000	0.220
48	2.67E+10	7.64E+05	1.74E+11	0.000	0.178
49	1.93E+10	0.00E+00	7.88E+10	0.327	0.136
50	2.33E+10	0.00E+00	1.76E+11	1.670	0.175
51	1.87E+10	0.00E+00	1.00E+11	1.312	0.233
52	2.10E+10	0.00E+00	6.75E+10	0.000	0.165
53	2.85E+10	5.63E+05	9.96E+10	0.000	0.138
54	2.68E+10	0.00E+00	1.31E+11	0.802	0.149
55	4.14E+10	0.00E+00	2.10E+11	1.138	0.138
56	2.04E+10	1.32E+07	8.53E+10	0.555	0.156
57	2.00E+10	0.00E+00	1.05E+11	0.208	0.178
58	4.19E+09	0.00E+00	1.56E+10	1.267	0.098
59	1.38E+10	0.00E+00	4.22E+10	1.948	0.122
60	1.05E+10	0.00E+00	4.53E+10	0.717	0.188
61	1.57E+10	0.00E+00	1.07E+11	0.402	0.197
62	4.04E+09	0.00E+00	1.84E+10	0.386	0.050
63	2.05E+10	3.79E+05	6.57E+10	1.075	0.120
64	2.61E+10	1.22E+06	1.59E+11	1.456	0.215
65	2.94E+10	4.55E+05	1.26E+11	2.233	0.245
66	1.20E+10	0.00E+00	5.63E+10	1.056	0.078
67	4.84E+09	0.00E+00	1.04E+10	0.878	0.041
68	6.33E+09	0.00E+00	4.98E+10	0.648	0.049
69	1.13E+10	1.66E+06	1.55E+11	2.593	0.108
70	8.10E+09	9.68E+08	4.92E+10	2.343	0.115
71	1.24E+10	4.84E+09	9.71E+10	0.860	0.141
72	8.25E+09	0.00E+00	4.72E+10	1.086	0.128
73	3.35E+09	0.00E+00	1.05E+10	0.164	0.090
74	1.11E+10	1.41E+06	7.16E+10	0.000	0.156
75	5.45E+09	9.42E+05	1.98E+10	0.000	0.124

Table A.3: P-values for comparing the total petroleum hydrocarbon concentration between the different land use types.

TPH p-values	Commercial parking lot	Light-use parking lot	Residential street	Residential roof	Upland
Commercial parking lot					
Light-use parking lot	0.180				
Residential street	0.070	0.540			
Residential roof	0.130	0.715	0.877		
Upland	<.0001	<.0001	<.0001	0.002	

TPH Spike Recovery Test Data

1) Weigh out ~ 3g masses of soil in 6 baked vials (550 C for 6 h) with Teflon lined stoppers

Sample	Mass Soil (g)
1	3.0001
2	3.0033
3	3.0008
4	3.0032
5	3.0064
6	3.0213

2) Create a dilution of Supelco TPH Mix 1 (861424-U, Sigma-Aldrich). The original solution is purchased at a concentration of 2000 µg/mL (vol=1.0 mL). A master stock solution was created by adding the purchased TPH 1.0 mL standard to 27.6287g methanol (gravimetrically determined; density=0.791 g/mL, volume = 34.9288 mL). This created a master stock concentration of 55.6656 mg/L.

3) Create stock "A" for spiking into the soils. A volume of 50.0 µL of the master stock was added to 1.093 mL of analytical grade hexane (mass of vial empty: 2.4790g, mass vial full 3.1949g; mass of hexane=0.7159g; density of hexane=0.6548 g/mL; volume of hexane=1.093 mL). This created a stock "A" solution of 2434.40 µg/L TPH.

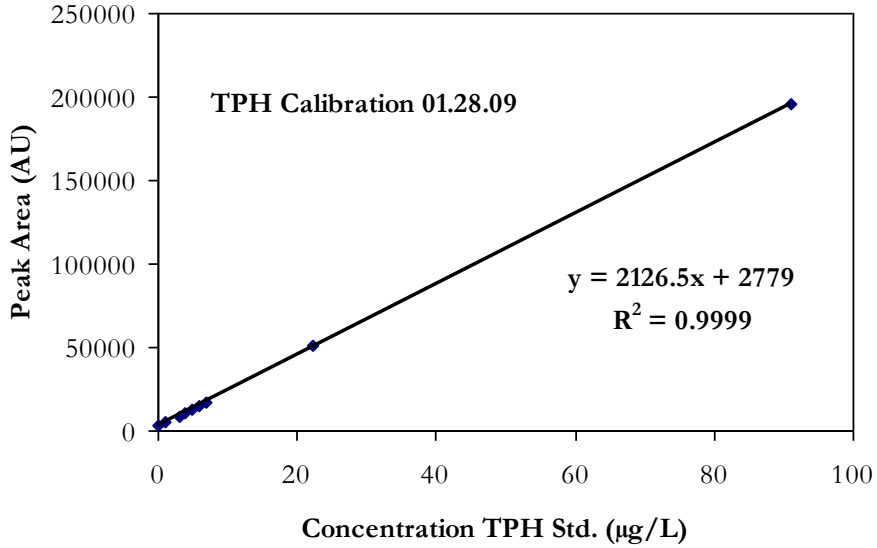
Empty Vial Mass (g)	Full w/ hexane (g)	Mass hexane (g)	vol Hex (mL)	vol Master stock (µL)	Conc (µg/L)
2.4790	3.1949	0.7159	1.0933	50.0	2434.40

4) 150 µL of stock "A" was added to each of the soil samples and allowed to equilibrate (via shaking vigorously and end-over-end rotator overnight).

5) The extraction followed the procedure described in the manuscript (add 8g anhydrous sodium sulfate to remove soil moisture, add 8.0 mL of hexane, sonicate for 10 minutes, place on end-over-end rotator overnight). Let set on benchtop for one hour the next day to allow contents to settle.

6) The septa were pierced via a gas-tight syringe and transferred to autosampler vials. The samples were run on the GC-FID, and the areas were peak areas were determined. The peak areas and the calibration curve used for the spike recovery test are below. The concentration in the extract recovered was determined using these values.

Sample	Area units
1	86004
2	117597
3	87273
4	91817
5	94426
6	72972



7) Below is a summary of the TPH spike recovery test, including the spike and recovered concentration (µg TPH/g soil).

Sample	Mass Soil (g)	Start conc (µg TPH/gsoil)	Final Conc (µg TPH/g soil)	Percent Recovery	GC measure. (µg/L)	Area units
1	3.0001	0.1217	0.1044	85.74%	39.1371	86004
2	3.0033	0.1216	0.1438	118.29%	53.9939	117597
3	3.0008	0.1217	0.1059	87.05%	39.7338	87273
4	3.0032	0.1216	0.1115	91.73%	41.8707	91817
5	3.0064	0.1215	0.1147	94.42%	43.0976	94426
6	3.0213	0.1209	0.0874	72.32%	33.0087	72972

A summary statistic is listed below regarding the percent recovery:

Mean Recovery	**95.70%**
Standard Deviation	**15.1%**

Appendix B: Supporting Information for Chapter 4

Table B.1: Numerical supporting data for columns shown in Figures 4.2 and 4.3.

Sample Column	Sample depth (cm)	Moisture Content	16S rRNA gene copies / g dry soil	PHE gene copies / g dry soil	NAH gene copies / g soil	^{14}C DPM / g dry soil	Conc. Naph. mg / kg
Clover	0	0.486	1.54E+11	3.76E+10	9.97E+07	138.22	1.01
	7	0.687	2.15E+11	1.06E+11	0.00E+00	322.62	4.27
	14	0.180	4.73E+10	1.45E+10	0.00E+00	92.73	0.54
	21	0.177	7.80E+10	1.82E+10	0.00E+00	90.91	0.98
	28	0.180	3.21E+10	7.67E+09	0.00E+00	78.65	0.56
	35	0.172	1.98E+10	1.20E+10	0.00E+00	119.60	1.03
	42	0.189	1.09E+11	7.67E+10	0.00E+00	65.45	1.47
	49	0.219	1.37E+11	3.60E+10	3.92E+07	76.58	2.10
	56	0.193	9.27E+10	5.13E+10	0.00E+00	79.17	2.55
	60	0.216	7.69E+10	6.28E+10	6.07E+07	142.55	0.39
Grass	0	0.536	3.67E+11	6.54E+10	2.52E+09	178.48	2.13
	7	0.159	2.15E+11	2.83E+10	7.38E+07	87.00	0.91
	14	0.148	4.83E+11	2.40E+10	0.00E+00	89.24	0.27
	21	0.173	7.54E+10	4.63E+09	0.00E+00	29.03	1.63
	28	0.165	5.44E+12	1.07E+10	0.00E+00	72.62	2.18
	35	0.157	4.60E+12	8.56E+09	0.00E+00	69.35	0.52
	42	0.203	8.13E+12	1.45E+10	0.00E+00	101.66	1.16
	49	0.199	7.94E+12	1.14E+10	0.00E+00	76.00	0.37
	56	0.227	2.82E+12	2.06E+10	0.00E+00	70.42	0.23
	60	0.224	3.78E+12	7.31E+09	0.00E+00	42.53	0.27
Unplanted	0	0.611	3.75E+13	3.93E+10	5.61E+07	307.87	1.16
	15	0.179	8.23E+12	2.19E+10	5.64E+07	59.90	0.41
	21	0.221	5.00E+12	1.52E+10	0.00E+00	37.16	0.20
	28	0.233	6.40E+12	4.91E+10	0.00E+00	55.66	0.43
	35	0.200	6.35E+12	1.42E+10	0.00E+00	142.30	1.91
	42	0.227	2.55E+12	9.69E+09	0.00E+00	86.97	0.56
	49	0.239	1.97E+11	1.00E+10	0.00E+00	71.33	1.23
	54	0.258	2.57E+10	1.27E+10	0.00E+00	75.33	0.44
	60	0.253	8.12E+10	1.56E+10	0.00E+00	65.05	0.19

Table B.2: Numerical data for Figure 4.4.

Bottom Port Effluent ^{14}C (Ave DPM/day)			
Experiment Day	**Unplanted**	**Grass**	**Clover**
0	0	0	0
2	51081	7138	6756
8	0	0	0
29	21	0	56
35	0	0	38
38	1350	0	0
43	0	564	0
52	0	0	0
62	0	0	0
65	0	371	693
69	224	0	7
73	0	121	0
79	0	42	243
85	0	0	0
106	121	0	0
111	0	151	0
120	0	0	0
150	42	551	589

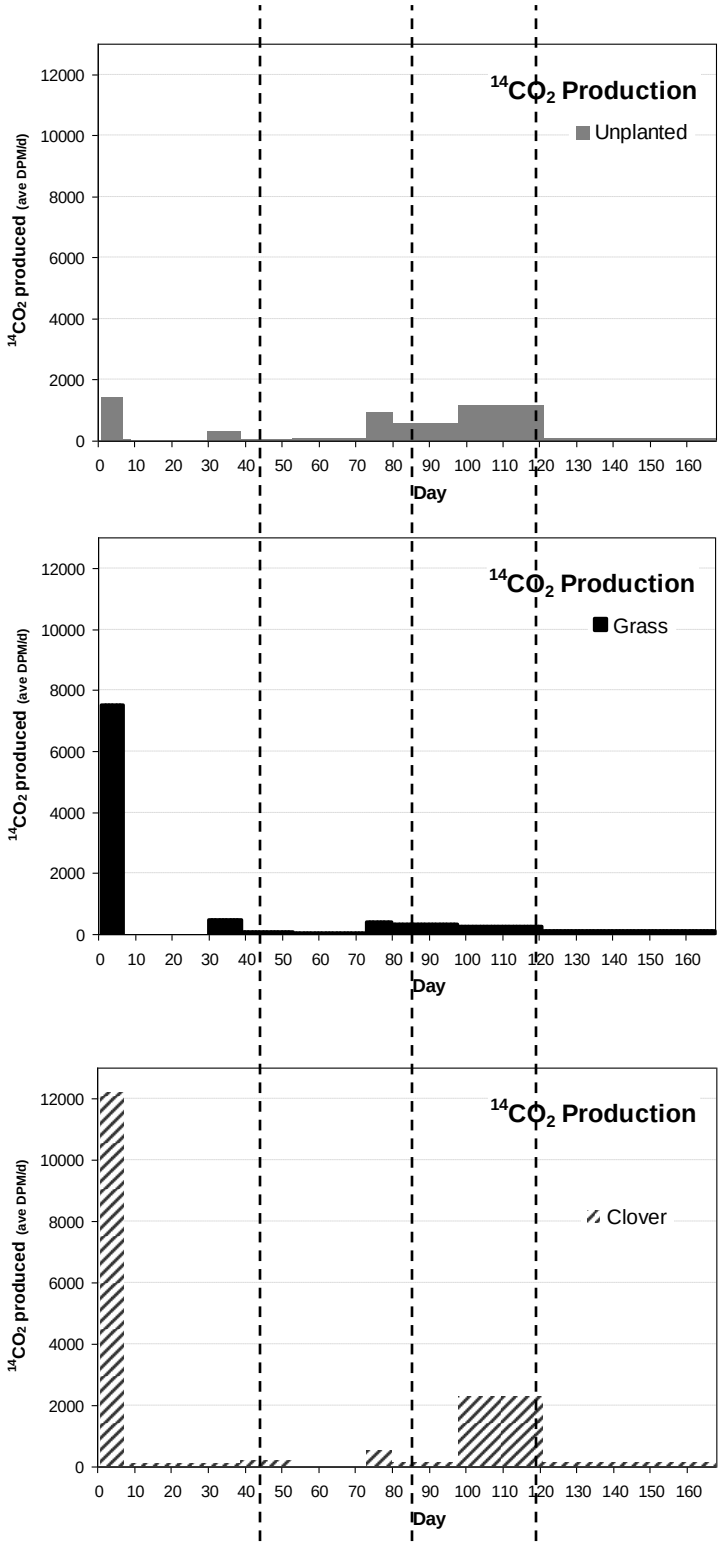


Figure B.1: $^{14}CO_2$ production for the three columns through time. The bars represent the average DPM measured over the given time interval. The first spike was at day zero, and subsequent spikes are indicated with the dashed line.

Table B.3: Measurements of ^{14}C captured in NaOH traps (numerical of Figure B.1).

^{14}C DPM measured in NaOH traps			
Day	**Unplanted**	**Grass**	**Clover**
0	0	0	0
6	8660	44944	72968
8	52	19	0
29	0	19	368
38	2810	4197	632
52	284	848	1014
72	1437	753	75
79	6453	2726	2970
97	10115	5572	2040
120	26814	5517	13524
167	3877	5448	4692

Table B. 4: Measurements of ^{14}C captured in ORBO tubes (numerical supporting data to Figure 4.2).

^{14}C DPM measured in ORBO tubes			
Day	**Unplanted**	**Grass**	**Clover**
1	62	0	6
3	0	0	15
6	0	0	0
8	0	0	0
15	0	0	0
24	0	0	0
29	58	0	0
35	58	0	7
38	0	0	0
52	0	0	0
62	0	25	0
72	50	48	0
79	113	37	0
82	0	2	7
97	0	19	80
99	0	24	56
120	0	11	16
167	4	5	0

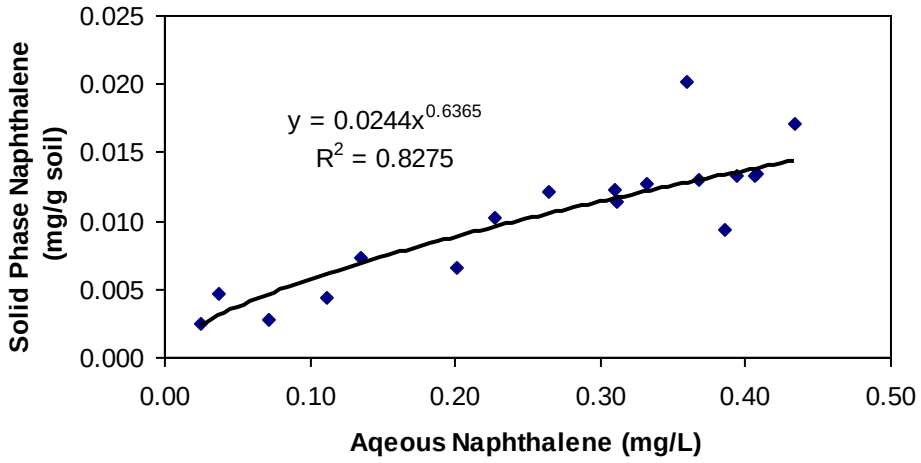


Figure B.2: Soprtion equilibrium isotherm for soil used to pack columns described in Chapter 4.

Figure B.3: Photo of column set apparatus described in Chapter 4.

Figure B. 4: Photo of biodegradation batch flask described in Chapters 3 and 4.

Figure B. 5: Photo of biological oxidizer at Rice University described in Chapter 4.

Appendix C: Supporting Information for Chapter 5

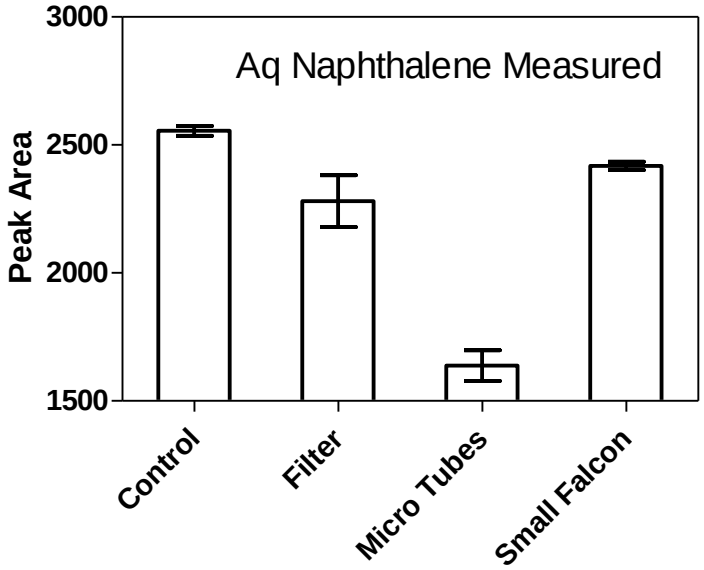


Figure C.1: Comparison of naphthalene recovery between various methods of solids separation from aqueous solution prior to HPLC analysis. Control was the naphthalene solution with no separation method, Filtered was through a baked GF/F filter, Micro Tubes were centrifugation with polypropylene microcenterfuge tubes, and "small Falcon" was centrifugation with 15 mL polystyrene centrifuge tubes. The latter was chosen as the solids separation method due to the recovery and consistancy. Error bars are ±95% confidence interval.

Table C.1: pH measurements of the exudates and biosurfactants. Most hydroponic nutrient solutions have an initial pH between 5.0 and 6.0 (1); the ½ strength Hoagland's is within this initial range.

	pH	
	Exudate	**Biosurfactant**
Sedge 1	4.35	5.69
Sedge 2	3.76	4.18
Sedge 3	4.08	5.09
Grass 1	6.77	3.87
Grass 2	4.28	5.93
Grass 3	4.92	2.73
Clover 1	5.89	5.20
Clover 2	4.28	5.11
Clover 3	3.97	4.64
1/10 AREs	3.41	3.92
1/2 Hoagland's	5.4	

Figure C.2: Photos of hydroponic plant apparatus. Upper (left to right): Porcupine Sedge (*Carex hystricina*), Cord Grass (*Spartina pectinata*); Lower (left to right): Purple prairie clover (*Dalea purpurea*), view of hydroponic root growth.

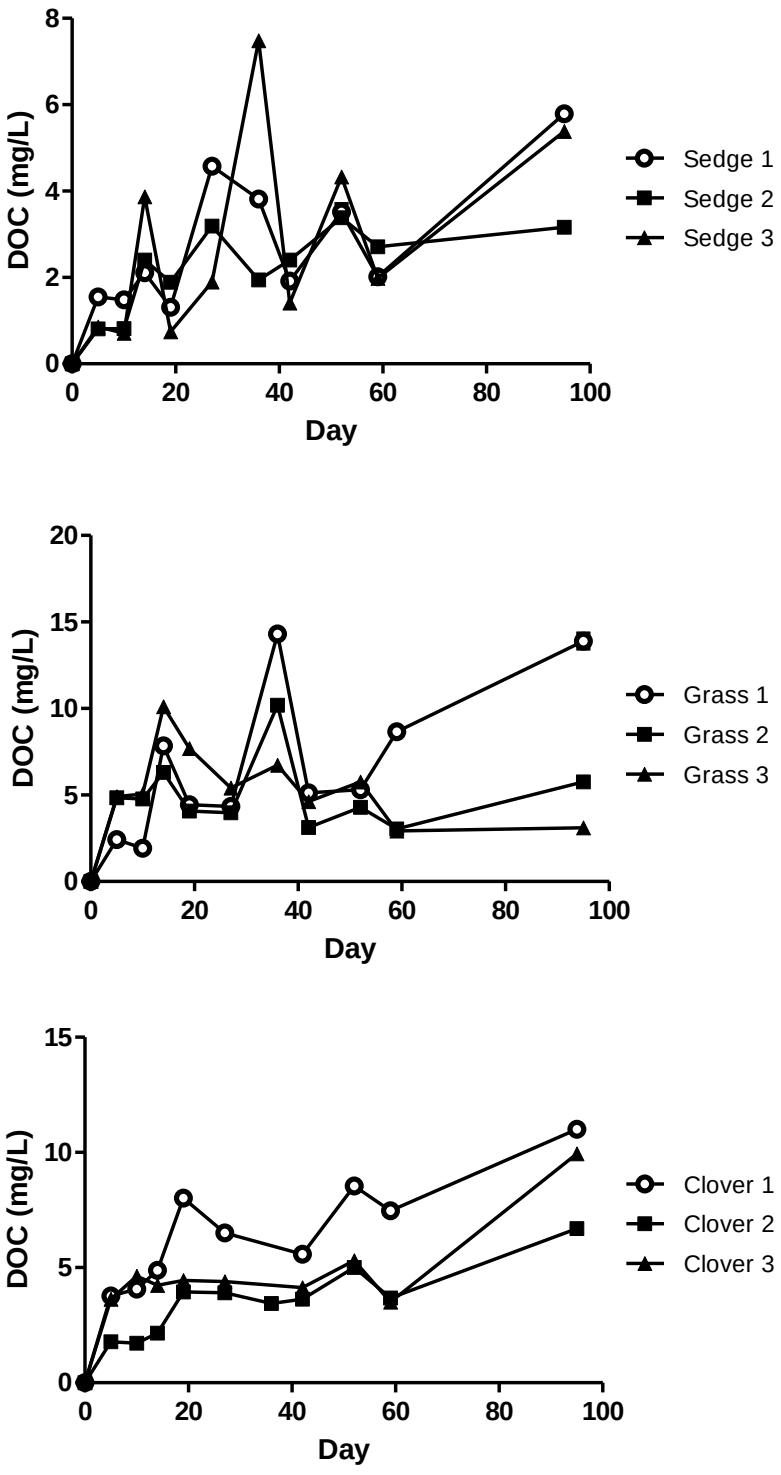


Figure C.3: Dissolved organic carbon (mg/L) measured in the hydroponic flasks as a function of time for the sedge (top), grass (center), and clover (lower). Error bars represent the standard deviation of triplicate measurements of the DOC measurement for the time point.

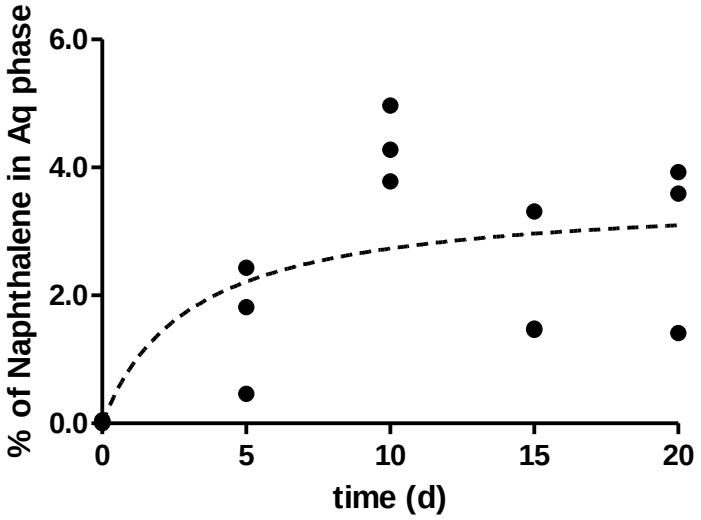


Figure C.4: Preliminary kinetics examined the time to reach equilibrium for the desorption of aged naphthalene from soil.

Table C.2: Artificial Root Exudates (from Joner et al. (2) and Brandt et al. (3)). Analytical grade reagents of >99% purity were used.

Compound	Concentration (mM)	CAS #
D-fructose	50	57-48-7
D-glucose	50	50-99-7
Sucrose	50	57-50-1
Succinic acid	25	110-15-6
L-malic acid	25	97-67-6
L-arginine	12.5	74-79-3
Serine	12.5	312-84-5
L-cysteine	12.5	52-90-4

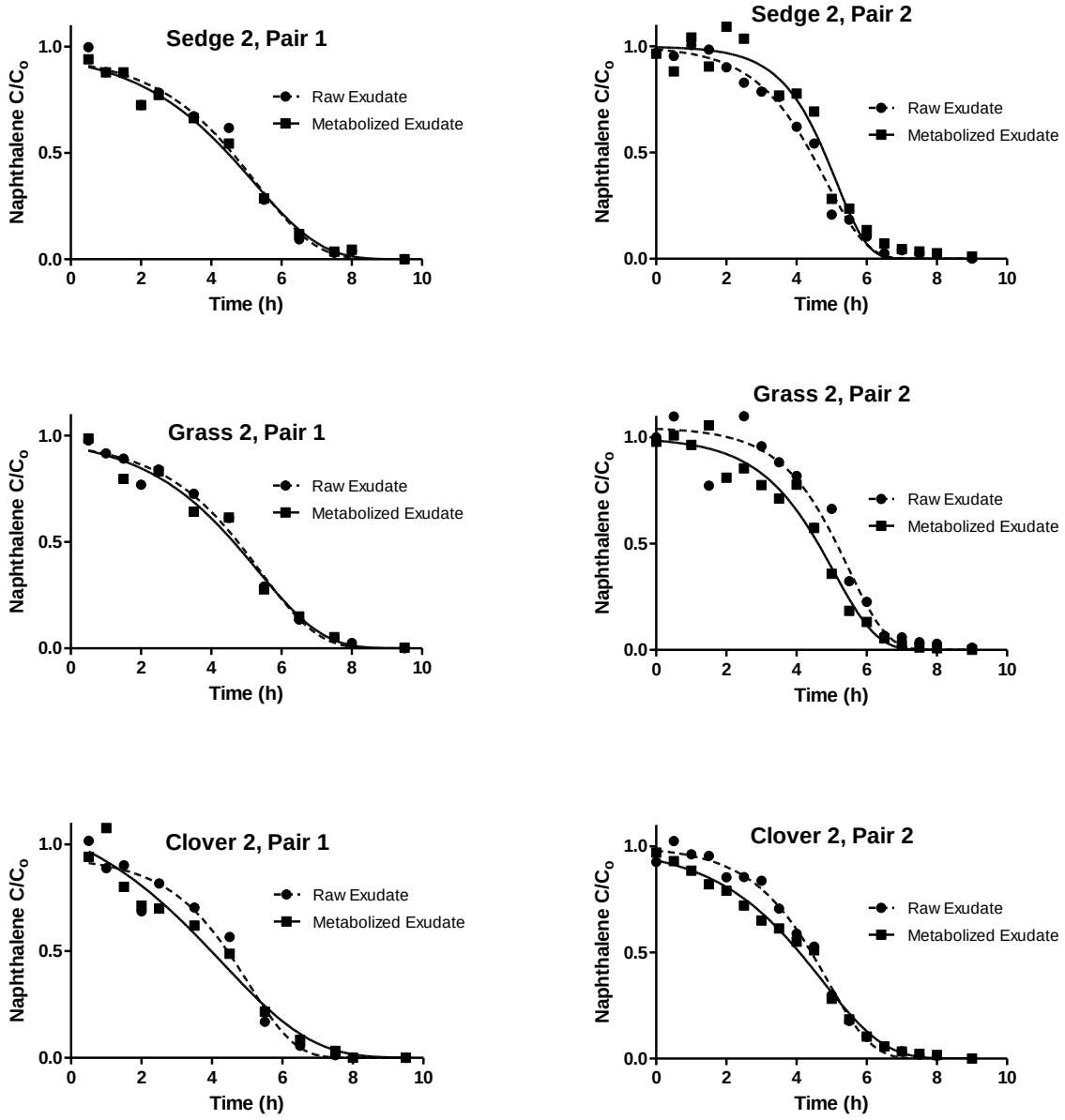


Figure C.5: Biokinetic test results for the paired raw and metabolized exudates for sedge 2, grass 2, and clover 2 (AREs are shown in the chapter).

Table C. 3: Quality control data for the abiotic control used in the biokinetic tets described in Chapter 5.

Abiotic Control (n=3)	Measurements per replicate	**Slope**	Std. err.	R^2	**Slope non-zero?**	**y-intercept**	std. err.
	9	-0.00583	0.008052	0.0205	No (p=0.4761)	1.056	0.03834

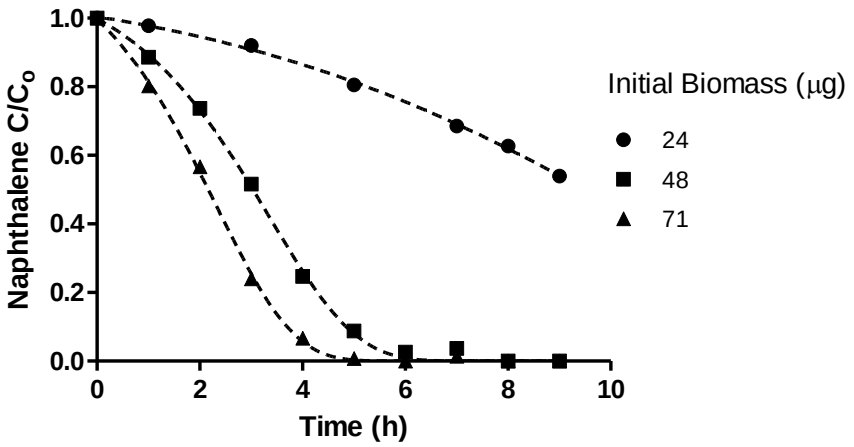


Figure C.6: Effect of varying initial P. pudita JS 150 initial biomass (determined optically) on degradation rate (results of a preliminary experiment to design biokinetics tests).

References

1. Jones, J.B. Hydroponics: A practical guide for the soilless grower. CRC Press: New York, **2005**.

2. Joner, E.J.; Corgié, S.C.; Amellal, N.; Leyval, C. Nutritional constraints to degradation of polycyclic aromatic hydrocarbons in a simulated rhizosphere. *Soil Biol. Biochem.* **2002,** *34,* 859-864.

3. Brandt, K.K.; Sjøholm, O.R.; Krogh, K.A.; Halling-Sørensen, B.; Nybroe, O. Increased pollution-induced bacterial community tolerance to sulfadiazine in soil hotspots amended with artificial root exudates. *Environ. Sci. Technol.* **2009,** *43,* 2963-2968.

Appendix D: Derivation of Modified Gompertz Function with Error Propagation

This section provides a derivation of the modified version of the Gompertz function, used in Chapter 4. Determination of error propagation for the modified equation is also included.

The Gompertz function is expressed as f(t). The first derivative is the rate of mineralization, and setting the second derivative equal to zero determines the time at which the maximum rate of mineralization occurs (inflection point; in this case we choose "k" to represent that time).

$$f(t) = ae^{be^{ct}};b,c<0$$

$$f'(t) = abce^{be^{ct}}e^{ct}$$

$$p = be^{ct}$$

$$dp = be^{ct}c$$

$$f(p) = ae^{p}$$

$$f'(p) = ae^{p}dp$$

$$f'(t) = abce^{be^{ct}}e^{ct}$$

$$f''(t) = abce^{be^{ct}}bce^{ct}e^{ct} + abce^{be^{ct}}ce^{ct}$$

$$f''(t) = abce^{be^{ct}}ce^{ct}[be^{ct}+1] = 0$$

$$0 = be^{ce^{ct}} + 1$$

$$be^{ct} = -1$$

$$e^{ct} = \frac{-1}{b}$$

$$\ln(e^{ct}) = \ln(\tfrac{-1}{b})$$

$$t_{f''(t)=0} = \tfrac{1}{c}\ln(\tfrac{-1}{b}) = -\frac{1}{c}\ln(-b)$$

Let “k” equal the time at which the second derivative equals zero (the time of inflection):

$$k = t_{f''(t)=0} = \frac{-1}{c}\ln(-b)$$

$$b = -e^{-ck}$$

$$\therefore\ f(t) = ae^{be^{ct}} = ae^{(-e^{-ck})e^{ct}}$$

$$f(t) = ae^{[(-e^{-k})e^{t}]^{c}} = ae^{[-e^{(t-k)}]^{c}}$$

$$f(t) = ae^{-e^{c(t-k)}}$$

Now the least squares minimization process can be used (MS Excel Solver was used) to solve for the variables in terms of more useful terms which correspond to physical points on our graph. “a” is the max growth achieved, and “**k**” is the **time at which the maximum mineralization rate occurs**.

To determine the **maximum rate of mineralization,** find the value of the first derivative at the time at which the max mineralization occurs (find the value of the derivative at the inflection point):

$$f'(t) = abce^{be^{ct}}e^{ct}$$

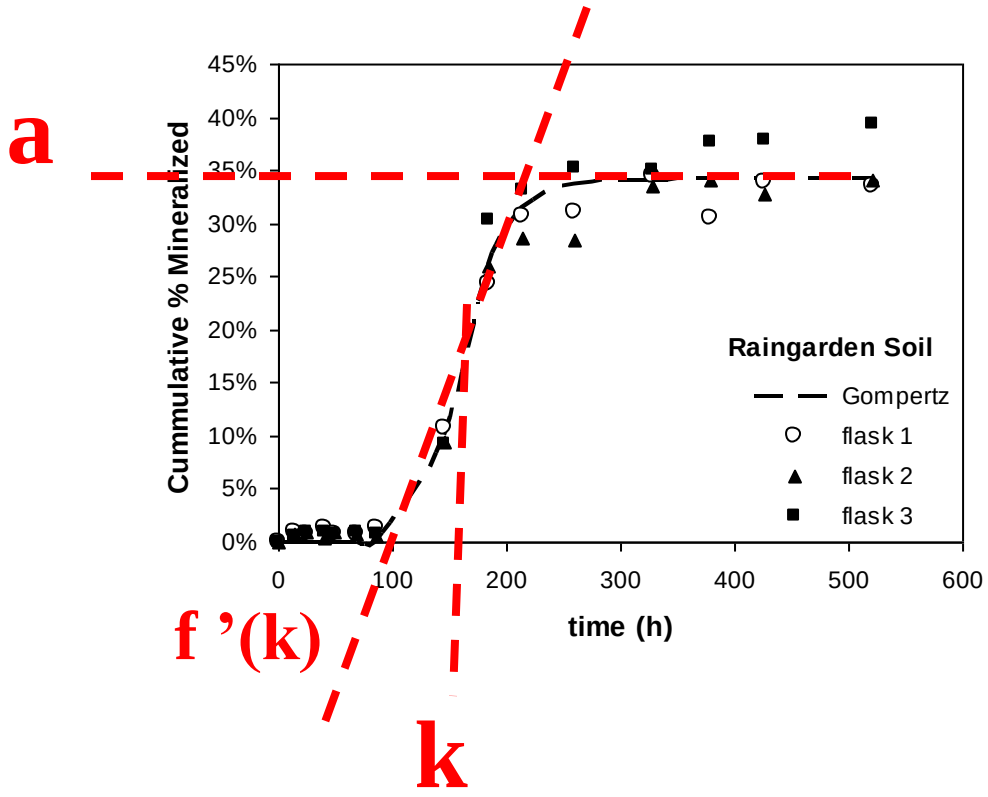
$$f'(k)_{f''(t)=0} = abce^{be^{ck}}e^{ck}$$

$$b_{f''(t)=0} = -e^{-ck}$$

$$f'(k) = a(-e^{-ck})ce^{(-e^{-ck})e^{ck}}e^{ck}$$

$$f'(k) = -ace^{-1}$$

This latter form of the equation allows for substantially easier error propagation analysis than the original form of the first derivative. This figure provides a conceptual value for the variables determined above. "a" is the maximum extent of mineralization, "k" is the time required to reach the maximum rate of mineralization, and f'(k) is the value of the maximum rate of mineralization.



In order to accurately determine the confidence intervals for the rates of mineralization, error propagation must be conducted to ensure that uncertainty represented in various measurements is accurately represented. Below is a derivation of the error propagation for the slope equation determined above:

Error propagation for the slope (rate equation):

$$f'(k) = -ace^{-1}$$

$$f = wA; \quad \sigma_f^2 = w^2\sigma_A^2$$

$$f = AB; \quad \left(\frac{\sigma_f}{f}\right)^2 = \left(\frac{\sigma_A}{A}\right)^2 + \left(\frac{\sigma_B}{B}\right)^2$$

$$SE = \frac{\sigma}{\sqrt{n}}; \quad \sigma^2 = SE^2 \cdot n$$

$$Let\ a = A; c = B; -e^{-1} = w$$

$$\sigma_{f_1}^2 = (a \cdot c)^2 \left(\frac{SE_a^2 \cdot n_a}{a^2} + \frac{SE_c^2 \cdot n_c}{c^2}\right)$$

$$\sigma_{f_2}^2 = (-e^{-1})^2 \cdot \sigma_{f_1}^2$$

$$\sigma_{f'(k)}^2 = (-e^{-1})^2 \cdot (a \cdot c)^2 \left(\frac{SE_a^2 \cdot n_a}{a^2} + \frac{SE_c^2 \cdot n_c}{c^2}\right)$$

Appendix E: Batch Reactor Biological Stoichiometry

Herein, we attempt to use first principle thermodynamics and biological stoichiometry to estimate the proportion of the naphthalene in the batch reactors evolves to carbon dioxide and biomass. Begin with the "R" Equation (1):

Equation E. 1 $$R = f_e R_a + f_s R_c - R_d$$

where f_e is the fraction of energy to catabolism (cell maintenance), f_s is the fraction of energy to anabolism (cell growth / synthesis), R_a is the half reaction for the electron acceptor, R_c is the half reaction for cell synthesis, and R_d is the half reaction for the electron donor.

Assume ammonium as the nitrogen source from NH_4Cl in media (pH in the reactors was neutral 7.0). Assume bicarbonate present. Adequate oxygen (enough to completely convert all naphthalene to carbon dioxide) was available based upon reactor headspace analysis (GC-TCD). Assume cell biomass is represented by $C_5H_7O_2N$ and naphthalene is limiting and the reaction goes to completion.

The value for f_s and f_e must be determined.

Equation E.2 $$f_s = \frac{1}{1+A}$$ **Equation E.3** $$f_e = 1 - f_s = \frac{A}{1+A}$$

Equation E.4 $$A = -\frac{\frac{\Delta G_p}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon}}{\varepsilon \Delta G_r}$$

where A is the equivalents of donor used for energy production per equivalent of cells formed, ΔG_p is the energy required to convert the carbon source to pyruvate, ΔG_{pc} is the energy required to convert the pyruvate to cellular carbon, ε is the energy transfer efficiency (typically 0.55-0.7; usually assumed to be 0.6), ΔG_r is the free energy released per equivalent of donor oxidized for energy generation, n (either 1 or -1) corrects for energy transfer efficiency sign by accounting for the fact that some electron donors are negative (energy is obtained via conversion to pyruvate) and some are positive (energy required to convert to pyruvate).

To determine each of the above components:

Equation E.5 $$\Delta G_p = 35.09 - \Delta G_c^{0'}$$

where 35.09 kJ/e^- eq is the free energy for the half reaction for pyruvate, and $\Delta G_c^{0'}$ is the free energy of the half reaction for the carbon source. This value for naphthalene at 25°C was found in the literature (McFarland and Sims, 1991; (2)) to be equal to 6.74 kcal or 28.26 kJ/e^- eq. Therefore, ΔG_p=35.09-28.26=6.83 kJ/ e^- eq.

The above value could also be estimated by using the free energies of formation for the components of the half reaction:

Equation E.6 $$\Delta G^0 = \Sigma m \Delta G^0_{products} - \Sigma m \Delta G^0_{react}$$

where m is the number of moles of each species. For the half reaction, look up the energies of formation for each species in a chemistry table (the values given below are in kcal/mol at 25°C):

Equation E.7 $$\tfrac{5}{24} CO_2 + H^+ + e^- \rightarrow \tfrac{1}{48} C_{10}H_8 + \tfrac{5}{12} H_2O$$

$$\Delta G^0 = [\tfrac{1}{48}(53.6) + \tfrac{5}{12}(-56.7)] - [\tfrac{5}{24}(-94.22) + 1(-9.67)]$$
$$= 6.8 kcal / e^- eq = 28.5 kJ / e^- eq$$

This estimate is quite close to the value provided by McFarland and Sims (1991; (2)). Thus, we have shown two different ways to determine $\Delta G_c^{0'}$ and consequently ΔG_p.

For ammonium nitrogen (used in this case), the value for ΔG_{pc} is 18.8 kJ/e^- eq.

The value for ΔG_p is the difference between the free energies of the electron acceptor and donor:

Equation E.8 $$\Delta G_r = \Delta G_a^{0'} - \Delta G_d^{0'}$$

For an aerobic reaction, $\Delta G_a^{0'}$ is -78.72 kJ/e^- eq. We know from above that the value of the half reaction for the electron donor is 28.26 kJ/e^- eq. Therefore,

$$\Delta G_r = \Delta G_a^{0'} - \Delta G_d^{0'} = -78.72 - 28.26 = -106.98 kJ / e^- eq$$

Now all components of the equation to determine A are available (Equation E.4):

$$A = -\frac{\frac{\Delta G_p}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon}}{\varepsilon \Delta G_r} = -\frac{\frac{6.83}{0.6^1} + \frac{18.8}{0.6}}{0.6(-106.98)} = 0.665$$

Now all components of the equation to determine f_s and f_e are available (Equation E.2 and Equation E. 3):

$$f_s = \frac{1}{1+A} = \frac{1}{1+0.665} = 0.6006 \approx 0.6 \Rightarrow \therefore f_s = 0.6; f_e = 0.4$$

Recall the R equation from the beginning (Equation E. 1):

$$R = f_e R_a + f_s R_c - R_d$$

Write out each of the half reactions, taking into account the fractions appropriated to cell growth and maintenance determined above.

$$R_a : \frac{2}{5}\left(\tfrac{1}{4} O_2 + H^+ + e^- \rightarrow \tfrac{1}{2} H_2O\right)$$

$$R_c : \frac{3}{5}\left(\tfrac{1}{5} CO_2 + \tfrac{1}{20} NH_4^+ + \tfrac{1}{20} HCO_3^- + H^+ + e^- \rightarrow \tfrac{1}{20} C_5H_7O_2N + \tfrac{9}{20} H_2O\right)$$

$$-R_d : \tfrac{1}{48} C_{10}H_8 + \tfrac{5}{12} H_2O \rightarrow \tfrac{5}{24} CO_2 + H^+ + e^-$$

Therefore, the entire balanced reaction for naphthalene biodegradation and cell formation can be written as:

Equation E.9

$$R : C_{10}H_8 + \tfrac{24}{5} O_2 + \tfrac{36}{25} NH_4^+ + \tfrac{36}{25} HCO_3^- \rightarrow \tfrac{36}{25} C_5H_7O_2N + \tfrac{106}{25} CO_2 + \tfrac{64}{25} H_2O$$

Therefore, (106/25)/10 units of the original naphthalene carbon results in CO_2 and (36/25*5)/10 of the original naphthalene carbon forms biomass. Thus, we would expect a ***maximum mineralization extent of approximately 42.4%***. Although there are certainly several inherent assumptions herein, this provides a reasonable theoretical basis for the observed results.

The table below provides values for the maximum extent to mineralization observed in the batch reactors from Chapter 4. Most of the reactors appear to have reached the theoretical maximum level as calculated above. Those that are slightly over may be due to secondary scavenging of ^{14}C-biomass.

Table E.1: Maximum values of mineralization in column batch reactors.

Sample	Maximum Total Mineralization (%)
	Regression value ± 95% CI
Clover 35cm	43.2 ± 0.82
Grass 35cm	44.6 ± 2.4
Unplanted 35cm	44.6 ± 1.5
Clover Surface	42.3 ± 1.7
Grass Surface	42.9 ± 1.2
Unplanted Surface	31.7 ± 1.5
Raingarden Soil Media	34.2 ± 1.0

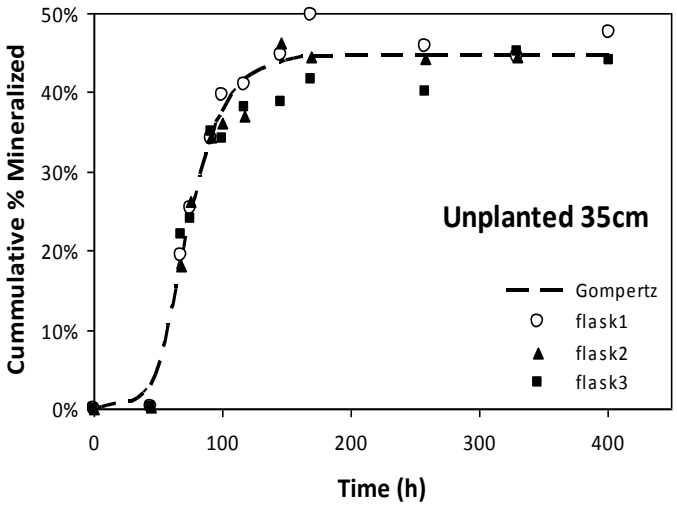


Figure E.1: Example naphthalene batch mineralization curve.

References

1. Rittmann, B. and McCarty, P. Environmental Biotechnology: Principles and Applications. McGraw Hill: Madison, WI, **2001**.

2. McFarland, M.J. and Sims, R.C. Thermodynamic framework for evaluating PAH degradation in the subsurface. *Ground Water* **1991,** *29,* 885-896.

Appendix F: Mass balance Model of Batch and Column Reactors

Below is a mass-balance approach to creating a model for the batch and column reactors used in Chapter 4.

I. Model of Batch Reactors

The following section creates a mass balance model for the naphthalene in batch reactors. The control volume in this setup has been drawn to around the aqueous phase, i.e., *C* is the concentration of naphthalene in the aqueous phase. Thus, sorption to soil, volatilization, and biodegradation would be considered loss mechanisms from the control volume. (L=length, m=mass, t=time for fundamental units.)

First, set up a mass balance on the reactor. Assume that there is no mass flux in or out of the sealed vessel. This reduces mass flux to losses due to reaction.

Equation F.1 $$\frac{dm}{dt} = \dot{m}_{in} - \dot{m}_{out} \pm \dot{m}_{rxn} \Rightarrow \frac{dm}{dt} = -\dot{m}_{rxn} \Rightarrow \frac{dm}{dt} = V\frac{dC}{dt} = -Vk_{loss}C$$

The loss rate constant is the sum of the loss rates for the factors that affect the contaminant in the given environment. In the case of the batch tests, the loss mechanisms of concern are biodegradation, sorption, and volatilization. The development of these respective rate parameters are shown below.

Equation F.2 $$\frac{dC}{dt} = -k_{loss}C = -(k_{sorb} + k_{bio} + k_{vol})C$$

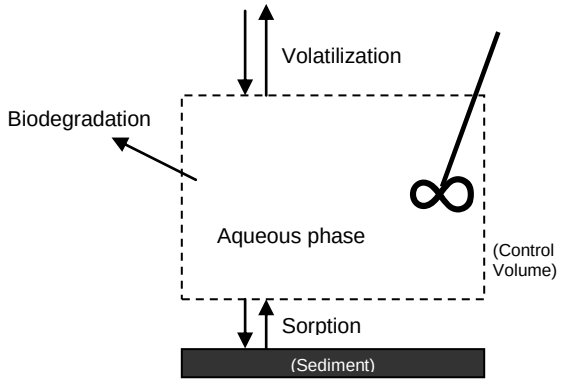


Figure F.1: Control volume schematic for batch reactor mass balance.

A. Development of Biodegradation Kinetic parameter:

Monod Growth Rate Model:

Equation F.3 $$\frac{dX}{dt} = \mu_{max} \frac{S}{K_s + S} X$$

where μ_{max} is the maximum specific growth-rate coefficient (1/t), X is biomass (m/L^3), K_s is the half saturation constant (m/L^3), and S is substrate concentration (m/L^3). The Monod model is used to describe microbial growth. For this batch study, we assume that S is equivalent to C from the assumptions that only the aqueous phase of the contaminant is bioavailable and that the contaminant acts as the sole substrate for microbial growth.

The parameters within this equation could be established experimentally for the batch tests. S, the susbstrate concentration, could be measured directly (i.e., GC or HPLC) and the biomass concentration could be measured through time (i.e. using optical density, heterotrophic plate counts, qPCR). To determine μ_{max} and K_s, a separate experiment could be conducted to measure specific growth rate as a function of susbtrate concentration (Figure F.2).

Logistic Growth Rate Model:

Equation F.4 $$\frac{dX}{dt} = \mu_{\max}\left(1 - \frac{X}{K}\right)X$$

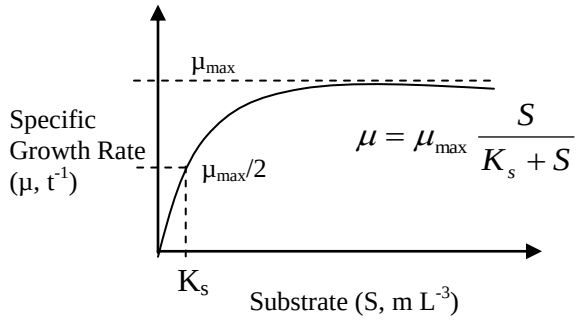


Figure F.2: Monod model, illustrating relationship between specific growth rate and substrate concentration.

where K is the carrying capacity (m/L^3) or maximum sustainable biomass population. This model is useful to account for non-substrate limitations to growth (e.g. space). The K value can be determined for the batch tests by examining the value at which S-shaped growth curve plateaus, and growth ceases (X=K).

Respiration Rate:

Equation F.5 $$\frac{dX}{dt} = -k_d X$$

where k_d is the respiration rate (1/t) or decay coefficient. The decay coefficient could be measured by taking the slope of the log of the population as a function of time during the death phase.

Yield Coefficient:

Equation F.6 $$Y = \frac{\Delta X}{\Delta S} \rightarrow \frac{dS}{dt} = -\frac{1}{Y}\frac{dX}{dt}$$

Yield can be determined by measuring the change in biomass per time divided by the change in substrate per time.

Descriptions of approaches to measuring parameters in the batch experiments have been included above; however, it is also useful to compare typical values. For example, Table F.1 contains typical values from biological activated sludge treatment. Values from activated sludge processes will likely be larger than those encountered for naphthalene, which is more recalcitrant. Table F.2 provides kinetic values for that were determined for naphthalene in a batch reactor.

Table F.1: Ranges and typical values for selected biokinetic coefficient for activated sludge treatment (1).

Coefficient	Range of Values	Typical Value
μ_{max}	0.1-0.5 hr^{-1}	0.12 hr^{-1}
K_s	25-100 mg BOD_5/L	60 mg BOD^5/L
Y	0.4-0.8 mg VSS/mg BOD_5	0.6 mg VSS/mg BOD_5
k_d	0.002-0.003 hr^{-1}	0.0025 hr^{-1}

Table F.2: Experimentally obtained biokinetic rate constants for naphthalene NAPL in batch reactors (2, 3).

Coefficient	Value
μ_{max}	0.067 hr^{-1}
K_s	3.4 mg/L
Y	0.25
k_d	0.0047 hr^{-1}

Equations 3-5 can be combined to describe batch growth characteristics.

Batch Growth:

Equation F.7 $$\frac{dX}{dt} = \left[\mu_{\max} \left(1 - \frac{X}{K} \right) \left(\frac{S}{K_s + S} \right) - k_d \right] X$$

Combining Equations 6 and 7 can determine the substrate utilization rate for batch growth. Manipulation of this equation can determine a biodegradation rate parameter for use in Equation 2.

Substrate Utilization Rate:

Equation Set F. 8

$$\frac{dS}{dt} = -\frac{1}{Y} \left[\mu_{\max} \left(1 - \frac{X}{K} \right) \left(\frac{S}{K_s + S} \right) - k_d \right] X$$

$$\frac{dS}{dt} = -\frac{X}{Y} \left[\mu_{\max} \left(1 - \frac{X}{K} \right) \left(\frac{1}{K_s + S} \right) + \frac{k_d}{S} \right] S$$

$$\therefore \Rightarrow k_{bio} = -\frac{X}{Y} \left[\mu_{\max} \left(1 - \frac{X}{K} \right) \left(\frac{1}{K_s + C} \right) + \frac{k_d}{C} \right] \qquad [t^{-1}]$$

B. Development of sorption kinetic parameter

Determination of a sorption rate constant begins with a mass flux. Then assume that the concentration of naphthalene at the solid-boundary interface is at local equilibrium and can be determined from the soil-water equilibrium partitioning coefficient. Thus, this equation will account for sorption to and desorption from the sediment to the aqueous phase. The sorption rate constant is then determined from the equation.

Equation Set F.9

$$J = \frac{dm}{dt}\left(\frac{1}{A}\right) = k_f (C - C_s) \Rightarrow \frac{dC}{dt} = k_f \left(\frac{A}{V}\right)(C - C_s)$$

$$K_d = \frac{C_s}{C} \Rightarrow C_s = K_d C$$

$$\frac{dC}{dt} = k_f \left(\frac{A}{V}\right)(C - K_d C)$$

$$\frac{dC}{dt} = k_f \left(\frac{A}{V}\right)(1 - K_d)C$$

$$\therefore \Rightarrow k_{sorb} = k_f \left(\frac{A}{V}\right)(1 - K_d) \qquad [t^{-1}]$$

$$K_d = f_{oc} K_{OC}$$

$$K_{OC} = f(K_{OW}) \rightarrow \log K_{oc} = 0.98 \log K_{ow} - 0.32$$

where k_f is the fluid phase mass transfer coefficient (L/t), surface area available for mass transfer (L^2), C is the concentration in the bulk liquid, C_s is the concentration of solute at the solid interface, K_d is the soil-water equilibrium distribution coefficient, K_{OC} is the organic carbon partitioning coefficient, and K_{OW} is the octanol-water equilibrium partitioning coefficient. K_{oc}/K_{ow} regression coefficients are specific to PAHs, and can be used to estimate values (this correlation was taken from Schwarzenbach et al. (4)).

Measurement of the organic carbon content of the soil and knowledge of the K_{ow} (naphthalene $\log K_{ow}$=3.33) can provide a reasonable estimate of the soil-water equilibrium distribution coefficient. K_d could also be measured using performing an equilibrium partitioning experiment (measure aqueous phase via HPLC, solvent extract from the solid phase and run on the GC). The specific surface area of fine grained soils can be measured using approaches including the ethylene glycol monoethyl ether (EGME) method (5), and the volume of the liquid is known. A value for k_f can be estimated from the Sherwood number (see Crittenden et al. (6) for correlations).

The organic carbon partitioning coefficient can altered by the presence of cosolvents in solution. The change in the K_{oc} in the presence of cosolvents can be approximated by:

Equation F.10 $$K_{OC_{Solvent,Water}} = K_{OC} \cdot 10^{-\sigma^c \cdot fv}$$

where σ^c is the cosolvancy power, and f_v is the volume fraction of cosolvent (4).

C. Development of volatilization kinetic parameter:

The volatilization mass balance begins in the manner of Equation Set 9, but now the Henry's Law constant or air-water equilibrium partitioning coefficient is used for substitution (7).

Equation F.11

$$\frac{dC}{dt} = K_L \frac{A}{V}(C_{SAT} - C) = K_L \frac{A}{V}\left(\frac{p_g}{H} - C\right) = K_L \frac{A}{V}\left(\frac{p_g}{HC} - 1\right)C$$

$$\Rightarrow \therefore k_{vol} = K_L \frac{A}{V}\left(\frac{p_g}{HC} - 1\right) \qquad [t^{-1}]$$

where K_L is the overall mass transfer coefficient (L/t) derived for expression of the gas transfer in terms of a liquid phase concentration, A is the interfacial surface area (L^2), V is the volume of liquid (L^3), C_{SAT} is the saturation solubility of the chemical, p_g is the partial pressure of the gas in the headspace, H is the Henry's Law constant or air-water equilibrium partitioning coefficient. $H=p_g/C_{SAT}$.

The Henry's Law constant can be taken from the literature (Naphthalene –logH=1.72; (4)). The surface area of the air-water boundary could be measured, as well as the partial pressure of naphthalene in the headspace (puncture septa; run headspace sample on a GC). The K_L is perhaps the most troublesome parameter in that it would be incredibly difficult to directly determine for the batch or column systems. In theory, one could measure change in concentration of naphthalene in solution and in the headspace through time in an abiotic reactor and deduce a K_L value. There also exist various methods for estimating K_L for special situations, such as wind blowing over a lake or an air stripping tower. Additionally, a literature value can be employed; an estimated K_L value for naphthalene at 20 °C is 9.6 cm/h (8).

D. Model of Batch Mass Balance

The rate constants above for sorption, biodegradation, and volatilization were substituted into Equation 2 to create a complete model for kinetics of naphthalene concentration in the batch reactor.

Equation F.2

$$\frac{dC}{dt} = -k_{loss}C = -(k_{sorb} + k_{bio} + k_{vol})C$$

Equation F.12

$$\frac{dC}{dt} = -\left[(k_f \frac{A}{V}(1-K_d) + \frac{X}{Y}\left[\mu_{\max}\left(1-\frac{X}{K}\right)\left(\frac{1}{K_s + C}\right) - \frac{k_d}{C}\right] + K_L \frac{A}{V}\left(\frac{p_g}{HC} - 1\right)\right] C$$

II. Modeling the Column Reactor

The column is not a simple batch experiment as in Part I, nor should it be modeled as a CMFR because it should not assumed to be completely mixed. Therefore, use of a plug flow model would be more appropriate. Therefore, a one dimensional advection, dispersion, and loss model is proposed. Dispersion results from the random motion of particles (diffusion) and random eddies (turbulent dispersion) (9). A 1-D model is assumed to represent the column system adequately because the column walls restrict lateral fluid movement to sufficiently conclude that the slug input acts a "plug" moving through the column pipe rather than a three dimensional expanding wetting bulb. First, we begin with the change in mass flux on given volume (Figure F.3). (J=flux density $[mL^{-2}t^{-1}]$.)

Equation F.13 $$\frac{d(VC)}{dt} = V\frac{dC}{dt} = AJ_x(0) - AJ_x(\Delta x) \qquad [m/t]$$

where the cross sectional area $A = \Delta y \Delta z$.

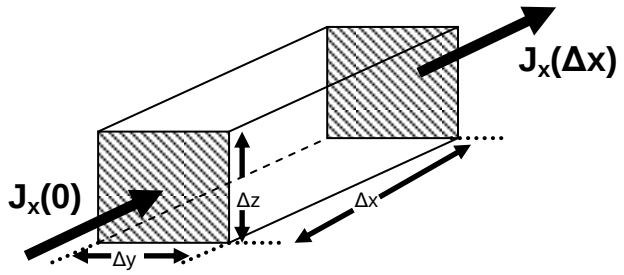


Figure F.3: Conceptual model of test volume and mass flux along the x-axis.

Dividing by the area and Δx yields:

Equation F.14 $$\frac{dC}{dt} = \frac{J_x(0) - J_x(\Delta x)}{\Delta x} \; [m\ L^{-3}\ t^{-1}]$$

Taking the limit of the right side as Δx approaches infinity yields the derivative:

Equation F.15 $$\lim_{\Delta x \to 0} = \frac{J_x(0) - J_x(\Delta x)}{\Delta x} = -\frac{dJ_x}{dx}$$

combined with Equation 14 yields:

Equation F.16 $$\left(\frac{\partial C}{\partial t}\right)_{transport} = -\frac{\partial J_x}{\partial x} \qquad [\text{m L}^{-3}\ \text{t}^{-1}]$$

The total mass flux due to transport in the system is the sum of advective and dispersive forces.

Equation F.17 $$J_{transport} = J_{advection} + J_{disp} \qquad [\text{m L}^{-2}\ \text{t}^{-1}]$$

Dispersion, both molecular diffusion and turbulent dispersion, can be modeled by Fick's Law; thus, total dispersion will be modeled using lumped dispersion coefficient. Advection is the movement of the plug.

Equation F.18 $$J_{disp} = -E\frac{\partial C}{\partial x} \qquad [\text{m L}^{-2}\ \text{t}^{-1}]$$

Equation F.19 $$J_{advection} = u_x C \qquad [\text{m L}^{-2}\ \text{t}^{-1}]$$

Equation F.20 $$u_x = \frac{Q}{A}; Q = \frac{V}{\tau} \rightarrow u_x = \frac{V}{\tau A} \qquad [\text{L/t}]$$

where E is the effective hydrodynamic dispersion coefficient through the media (accounting for molecular diffusion and turbulent dispersion) (L^2/t), u_x is the mean linear velocity of fluid flow (L/t), Q is the mean flow rate through the column (L^3/t), A is the cross sectional area (L^2), and τ is the hydraulic retention time (t). Despite the fact that this model is approximating a plug flow system, Equation 20 can still be a valid means of determining mean velocity of the liquid through the column. The hydraulic retention time, volume, and cross sectional area can be easily measured. The dispersion coefficient could be estimated by conducting a conservative tracer study through the column to determine the exit age distribution. The centroid of the tracer curve (t bar) can be used to

determine the Peclet number, which in turn can be used to determine the dispersion coefficient (6), E (see Section III):

Equation F.21

$$\bar{t} = \left[1 + \frac{2}{Pe}\right]\tau$$

Thus, combining Equations 17-20:

Equation F.22

$$J_{transport} = J_{advection} + J_{disp} = \frac{V}{\tau A}C - E\frac{\partial C}{\partial x} \qquad [\text{m L}^{-2}\ \text{t}^{-1}]$$

Combining Equation 22 and 16 yields:

Equation F.23

$$\left(\frac{\partial C}{\partial t}\right)_{transport} = -\frac{\partial J_x}{\partial x} = E\frac{\partial^2 C}{\partial x^2} - \frac{V}{\tau A}\frac{\partial C}{\partial x} \qquad [\text{m L}^{-3}\ \text{t}^{-1}]$$

The total rate of concentration change is due to transport of the plug (advection and dispersion) established in Equation 23 and losses due to reaction.

Equation F.24

$$\left(\frac{\partial C}{\partial t}\right)_{Total} = \left(\frac{\partial C}{\partial t}\right)_{transport} + \left(\frac{\partial C}{\partial t}\right)_{reaction} = \left(E\frac{\partial^2 C}{\partial x^2} - \frac{V}{\tau A}\frac{\partial C}{\partial x}\right) - k_{reaction}C$$

The reaction constant for compound loss was established in the Part I Batch Reactor Model. For the column model, the loss mechanisms and rate constants will be assumed to be the same, with the addition of a term for vegetative uptake:

Equation F.25

$$k_{reaction} = k_{bio} + k_{sorption} + k_{vol} + k_{plant}$$

The sorption, biodegradation, and volatilization rate constants are assumed to be the same as the Batch Model:

$$k_{bio} = \frac{X}{Y}\left[\mu_{\max}\left(1 - \frac{X}{K}\right)\left(\frac{1}{K_s + C}\right) - \frac{k_d}{C}\right] \qquad k_{sorb} = k_f\frac{A}{V}(1 - K_d)$$

$$k_{vol} = K_L\frac{A}{V}\left(\frac{p_g}{HC} - 1\right)$$

In the column, there must also be a rate coefficient for vegetative uptake. The rate of plant uptake is a function of several factors. T_{scf} is the transpiration stream concentration factor, or the dimensionless ratio between the concentration of the contaminant taken up into the xylem of the plant to the concentration of the contaminant in the bulk solution (10). The rate of water uptake by the plant (L^3t^{-1}) is important, and would vary between plant species and under different growing conditions; however, it is often assumed constant for given conditions rather than time variable. The T_{scf} value can be estimated from the K_{ow} of the contaminant. For example, the following relationship is from Dettenmaier et al. (11):

Equation F.26 $$T_{scf} = \frac{11}{11 + 2.6^{\log K_{OW}}}$$

The change in concentration per time of the contaminant in the bulk solution is expressed as:

Equation F.27 $$\left(\frac{dC}{dt}\right)_{uptake,plant} = -\left(\frac{T_{scf} q}{V}\right) C \qquad [\text{m L}^{-3}\ \text{t}^{-1}]$$

Therefore,

Equation F.28 $$\therefore \Rightarrow k_{plant} = \left(\frac{T_{scf} q}{V}\right) \qquad [\text{t}^{-1}]$$

The water uptake rate of the individual plants could be measured separately for each species using hydroponic experiments, and also compared to other plant species from the agricultural literature (e.g., alfalfa (12)).

Thus, a complete 1-D advection, diffusion, and degradation plug flow model of the column system could be constructed as:

Equation F.29

$$\frac{\partial C}{\partial t} = \left(E\frac{\partial^2 C}{\partial x^2} - \frac{V}{\tau A}C\right) - \left[(k_f \frac{A}{V}(1-K_d) + \frac{X}{Y}\left[\mu_{\max}\left(1-\frac{X}{K}\right)\left(\frac{1}{K_s + C}\right) - \frac{k_d}{C}\right] + K_L \frac{A}{V}\left(\frac{p_g}{HC} - 1\right) + \left(\frac{T_{scf} q}{V}\right)\right] C$$

III. Dimensionless Numbers

The Peclet number (P_e) is a dimensionless value measuring the ratio of the rate of advective transport to the rate of dispersive/diffusive transport. The Damkohler number (D_a) is the ratio of rate of consumption by decay to the rate of advective transport.

Equation F.30 $$P_e = \frac{Lu}{E}$$ **Equation F.31** $$D_a = \frac{kL}{u}$$

where L is the critical length. Very small Peclet numbers ($P_e << 1$) indicate that diffusion/dispersion is much faster than advection; likewise, larger values ($>>1$) indicate that advection dominates. When P_e values are high (>10), the system approaches plug flow characteristics, and when values are low (<0.1), the system is better modeled by a CMFR. These dimensionless values could aid in the characterization of the system and indicate relative importance to loss and transport mechanisms.

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Appendix G: Photos from Field Sites Sampled

Samples 1-11 Random Park Mounds View, MN (no photos available)

Samples 12-16, 37 Midtown Eco Yard, Minneapolis, MN

Sample 17 and 18:

Sample 19:

Sample 20 and 21:

Sample 20 and 21:

Sample 31 and 32:

Sample 33:

Sample 35:

Sample 36:

Sample 38:

Sample 39:

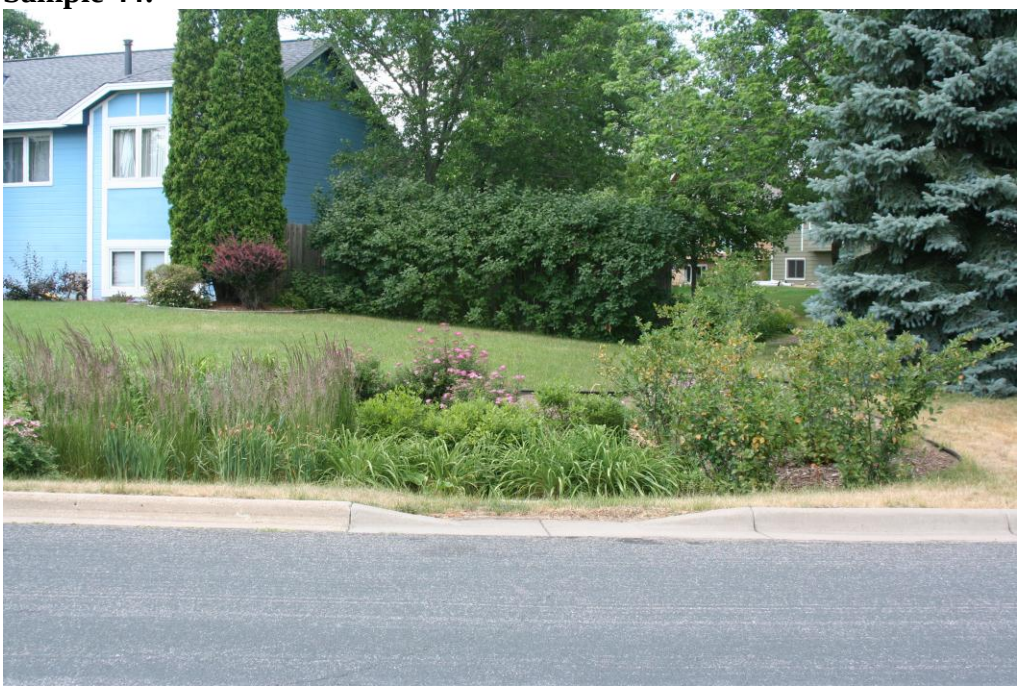
Sample 40:

Sample 41:

Sample 42:

Sample 43:

Sample 44:



Sample 45:

Sample 46:

Sample 47:

Sample 48:

Sample 49:

Sample 50:

Sample 51:

Sample 52:

Sample 53:

Sample 54:

Sample 58:

Sample 59:

Sample 60 and 61:

Sample 62 and 63:

Sample 64:

Sample 65:

Samples 66, 67, 68, 69:

Samples 70-75: No Photos available.