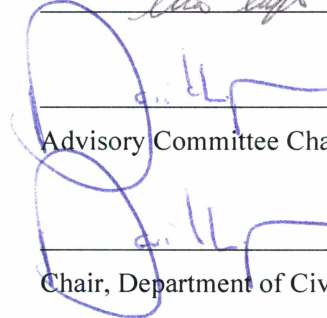
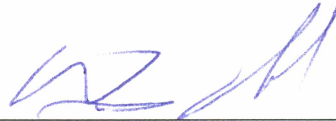


ATTENUATION OF THE HERBICIDE GLYPHOSATE ALONG RAILROAD CORRIDORS
IN ALASKA

By

Nellie B. Ballou

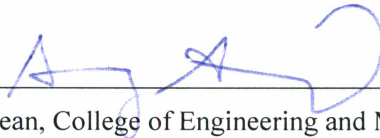
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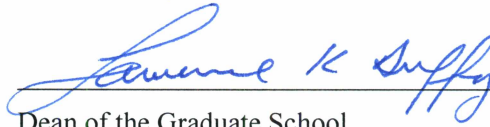
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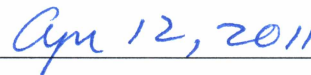
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Dean of the Graduate School



Date

ATTENUATION OF THE HERBICIDE GLYPHOSATE ALONG RAILROAD CORRIDORS
IN ALASKA

A
THESIS

Presented to the Faculty
of the University of Alaska Fairbanks
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

By

Nellie B. Ballou

Fairbanks, Alaska

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Abstract

Following the application of glyphosate in the formulation of AquaMaster[®] at two contrasting sub-arctic zones along the railroad corridor in Alaska, attenuation of the herbicide glyphosate was investigated. Study sites were established in continental and coastal zones. Glyphosate soil attenuation was similar to temperate regions during the growing season but exhibited an extended persistence during the winter months. Although glyphosate microbial degradation likely slowed during winter, both sites showed evidence of slight glyphosate degradation during the winter months. The coastal site attenuated more rapidly than the continental site which is presumably due to increased rainfall relative to the continental site. Glyphosate attenuation at the coastal site was likely driven by dispersion while microbial degradation was responsible for the attenuation of glyphosate at the continental site. Movement to subsurface soils (10-25 cm) at low concentrations was observed at both sites with slightly more transport at the coastal site than the continental site. Glyphosate transport to groundwater along railroad corridors was not conclusive. Vegetation cover reduction was reduced at the continental site but could not be determined at the coastal site.

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1. Introduction

Vegetation management along railroad corridors is a critical and integral part of railroad safety. Heavily vegetated areas of railroad track can pose a safety hazard by obstructing visibility of railroad signs and signals and interfering or restricting railroad employees from performing trackside duties. Vegetation can inhibit drainage through the railbed, which degrades the integrity of the rail system. In accordance with federal safety regulations, all US railroads are required to keep railroad corridors clear of vegetation. In many states, excluding Alaska, herbicide application is permitted and commonly practiced to control vegetation along railroad corridors.

The Alaska Railroad Corporation (ARRC) operates a 650 mile track from the Gulf of Alaska to the Interior of Alaska. In recent years the ARRC has not utilized chemical vegetation control methods but has employed other tactics along railroad corridors including steaming, burning, hand pulling, mechanical cutting and ballast replacement. Although some of the mechanical methods are effective, they are resource intensive. Therefore, the ARRC is pursuing additional strategies to better manage vegetation along railroad corridors including the use of herbicides. Specifically, the ARRC is investigating the use of the herbicide AquaMaster[®], which contains the active ingredient glyphosate.

In 2008 the Alaska Railroad Corporation coordinated with researchers at the University Alaska Fairbanks (UAF) and the USDA Agricultural Research Service (USDA-ARS) to conduct a study in order to better understand the behavior and efficacy of glyphosate in Alaska's unique environment.

1.1 Project Scope

Few studies have investigated the attenuation of glyphosate in sub-arctic regions (Newton et al. 2008). Glyphosate has been the focus of many studies in temperate regions, but verification in sub-arctic regions is needed (Torstensson et al. 2005; Newton et al. 2008). The objectives of this study were to determine the overall attenuation of glyphosate in soil along railroad corridors in Alaska, determine if glyphosate reaches surrounding groundwater, and measure the herbicide

efficacy. Glyphosate and its degradation product, aminomethylphosphonic acid (AMPA) were measured in the soil profile, vadose zone water, and groundwater over time after a typical broadcast spray application. Two contrasting sub-arctic locations, continental and coastal, were chosen as study sites along the railroad corridor. Results will aid State officials in determining if herbicide usage is appropriate in Alaska.

1.2 Methodology Overview

Glyphosate herbicide in the formulation of AquaMaster[®] was applied at a rate of approximately 4.6 kg/ha to a study location near Fairbanks, Alaska, referred to as the continental study site and to a location in Seward, Alaska, referred to as the coastal study site. Soil and water samples were collected over time following the herbicide application and subsequently analyzed for glyphosate and its metabolite AMPA. Liquid chromatography (LC) was used for coastal soil and water analysis and gas chromatography (GC) was used for coastal and continental soil analysis. Due to LC data integrity, focus is given to GC soil analysis. All references to glyphosate in this document refer to the isopropylamine salt form of glyphosate.

2. Glyphosate Background

2.1 Glyphosate Uses

Glyphosate is a non-selective systemic herbicide that was patented by Monsanto in the 1970's under the trade name Roundup[®]. Glyphosate is the active ingredient in over 50 herbicides sold under various trade names since then (U.S. EPA 1993; Monsanto 2005). Glyphosate is generally formulated and sold as the isopropylamine salt and applied via spray in a water mixture. This herbicide is primarily used for agricultural purposes including pastures and food crops, but is also applied in right-of-ways, industrial, and residential settings. Glyphosate is one of the most commonly applied herbicides in the United States. Approximately 10 million tons of glyphosate are applied annually in the U.S. (U.S. EPA 1993).

2.2 Properties of Glyphosate

In pure form, glyphosate is a white crystalline powder and is a weak organic acid. Although glyphosate is soluble in water, it is practically insoluble in most organic solvents (Bleke 1998). The herbicide has a pH of 4.6 to 4.8 as sold in formulation (Monsanto 2005). Glyphosate belongs to a group of herbicides called organophosphorus which contain carbon-phosphorus bonds.

Table 2-1 Selected Glyphosate Properties (IPCS 2010)

Property	Value
Molecular weight	169 g
Melting Point	185 C
Solubility	1.2 g/100mL @ 25 C
Vapor Pressure	7.5×10^{-8} mm Hg
log Kow	-3.5
Henry's constant	1.44×10^{-12} atm-m ³ /mole

The very low vapor pressure (Table 2-1) indicates the compound does not readily volatilize. The glyphosate structure (Figure 2-1) has three functional groups: carboxyl, amino and phosphonate. The glyphosate molecule is connected by a network of hydrogen bonds which easily dissociate (Knuuttila 1985).

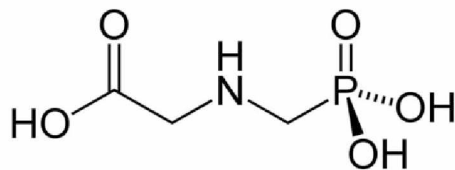


Figure 2-1 Structure of Glyphosate Molecule (IPCS 2010)

2.3 Glyphosate in the Environment

2.3.1 Vegetation Uptake, Translocation and Metabolism

Glyphosate is a systemic, non-selective herbicide used to control many annuals and perennials (Franz et al. 1997). Within four hours after foliar application, glyphosate is translocated throughout the plant via the phloem (Sprankle et al. 1975a). Rapid translocation from the foliage to the roots, rhizomes and apical meristems make glyphosate an effective herbicide on many types of vegetation (Sprankle et al. 1975a). Typically, glyphosate is applied in conjunction with a surfactant which helps penetrate the waxy cuticle of the leaves and prevents the formation of droplets. Glyphosate will kill the above ground plant material as well as the roots. Once the chemical enters the soil it is not absorbed by the plant roots because it binds tightly to the soil particles and therefore is not an effective pre-emergence herbicide (Sprankle et al. 1975b; Sprankle et al. 1975c). Less than one percent of the glyphosate in the soil is absorbed by the roots (Ghassemi 1982). Although glyphosate is not taken up by roots from the soil, it has been shown that foliar applied glyphosate translocated to the roots may exude glyphosate residues from the roots to the soil (Coupland and Caseley 1979; Laitinen et al. 2007).

Physical characteristics of vegetation such as age, leaf area and leaf shape can influence the chemical uptake and translocation of glyphosate (Caseley and Coupland 1985). Environmental factors also play a role in chemical uptake efficiency and translocation including sunlight intensity, temperature, wind speed, humidity, soil moisture and precipitation (Caseley and Coupland 1985). In general, both increased temperatures and sunlight intensity have been found to increase glyphosate uptake and translocation. Wind can cause a decrease in glyphosate plant uptake by causing drift. Higher relative humidity can increase glyphosate plant uptake, especially during the first 24 hours following application (Caseley and Coupland 1985; Sharma and

Singh 2001). Soil moisture is of lesser importance than other environmental factors affecting glyphosate uptake and translocation, however soil moisture could influence the water status of the vegetation as well as the microbial community (Caseley and Coupland 1985). Precipitation influences uptake and herbicide performance by causing wash off if the event closely follows application (Sprankle et al. 1975a; Monsanto 2009). The concentration of glyphosate as well as surfactant concentrations also affect the plant uptake (Caseley and Coupland 1985).

The metabolism of glyphosate in vegetation is less defined than in soil. It is difficult to analyze metabolic process in plants because metabolic process may be impaired with herbicide application. Metabolism of glyphosate by plants requires the glyphosate oxidoreductase enzyme which is responsible for producing AMPA (Duke 1980). A limited number of plant species have been reported to be able to metabolize glyphosate to AMPA (Sandberg et al. 1980; Komossa et al. 1992).

2.3.2 Mode of Action

Glyphosate's mode of action is the inhibition of the 5-enolpyruvylshikimate-3-phosphate synthesis (EPSPS) enzyme (Amrhein et al. 1980; Komossa et al. 1992). The EPSPS enzyme is in the biochemical pathway and is responsible for the formation of the aromatic amino acids tyrosine, tryptophan, and phenylalanine which are essential for the synthesis of proteins linking primary and secondary metabolism in the shikimate pathway (Devine et al. 1993). Glyphosate is the only herbicide designed to block the enzymatic step in the shikimate pathway (Devine et al. 1993). In most species these enzymes are present in the chloroplast (Carlisle and Trevors 1988). Other organisms are not affected by the herbicide in this way because EPSPS is specific to plants (Giesy et al. 2000). Visual symptoms which include wilting and severe discoloration of above-ground parts and deterioration of roots can be noticed within two to seven days after application. Colder temperatures and extensive cloud cover may slow this activity to some degree (Monsanto 2009).

2.3.3 Fate in Soil

2.3.3.1 Sorption

Glyphosate readily binds to soil particles (Sprankle et al. 1975b; Hance 1976; Rueppel et al. 1977; Glass 1987). Similar to inorganic phosphate, glyphosate is bound to soil through phosphonic acid moiety and therefore competes with inorganic phosphate for sorption sites (Sprankle et al. 1975b; Hance 1976). Thus, sorption is dependent upon available unoccupied binding sites (Hance 1976). Desorption of sorbed glyphosate is low (Mamy and Barriuso 2007).

Glyphosate is primarily sorbed to cation saturated surfaces (Sprankle et al. 1975b; Borggaard and Gimsing 2008). Sprankle et al. (1975b) determined that glyphosate sorbs differently to organic matter and clays depending on what cations were present. In organic matter saturated with the following cations, glyphosate sorption was reported $\text{Na}^+ = \text{Mg}^{2+} < \text{Zn}^{2+} < \text{Ca}^{2+} = \text{Mn}^{2+} = \text{Fe}^{3+} = \text{Al}^{3+}$ and in clays saturated with the following cations sorption was reported as $\text{Ca}^{2+} < \text{Mn}^{2+} < \text{Zn}^{2+} < \text{Mg}^{2+} < \text{Fe}^{3+} < \text{Al}^{3+}$. Aluminum oxides and iron oxides are the principle sorption sites for glyphosate sorption (Sprankle et al. 1975b; Hensley et al. 1978). Glyphosate sorption as it relates to soil organic matter has been disputed. Piccolo et al. (1996) suggested to humic substances promoted sorption but Gerritse et al. (1996) determined organic matter reduced sorption. Borggaard and Gimsing (2008) suggested that soil organic matter alone does not necessarily sorb glyphosate, but when organic matter containing metal ions is complexed with glyphosate sorption sites may be blocked. Glyphosate sorption increase slightly with increasing soil pH and increases with increased surface area (McConnell and Hossner 2002; Borggaard and Gimsing 2008).

In summary, glyphosate is tightly bound to soil particles. Soils with low inorganic phosphate capacity, high iron and aluminum cation concentrations, large surface area and high pH tend to exhibit strong binding of glyphosate. However, every soil is unique and absorption of glyphosate difficult to generalize.

2.3.3.2 Movement

Due to the soil binding properties of glyphosate, the overall leaching potential and mobility is low in most soils (Rueppel et al. 1977; Roy et al. 1989). Glyphosate rarely migrates below 15

cm in soil, even at high concentration (Carlisle and Trevors 1988; Roy et al. 1989; Feng and Thompson 1990). A review of glyphosate mobility (Borggaard and Gimsing 2008) determined glyphosate leaching potential was based on a multitude of factors but mainly soil structure and rainfall. In unstructured soils, that have few or no macropores leaching and movement will be limited. In soils with macropores, movement may be possible through preferential flow in certain cases (Kjær et al. 2005; Borggaard and Gimsing 2008). In coarse grained soils with low sorption capacity movement may be possible, especially following a rain event (Strange-Hansen et al. 2004). Generally, in all soil types precipitation appears to be the driving force behind movement of glyphosate (Strange-Hansen et al. 2004; Vereecken 2005; Borggaard and Gimsing 2008). The primary degradation product of glyphosate, AMPA, is considered slightly more mobile (Rueppel et al. 1977). Another potential route for movement of glyphosate is movement along plant roots in sandy soils (Laitinen et al. 2009). Burrowing animals and insects can create secondary porosity in the soil profile creating preferential flow paths for infiltrating waters, contributing to glyphosate movement.

2.3.4 Degradation and Persistence

Microbial degradation is the primary route of degradation in soil (Sprankle et al. 1975b; Rueppel et al. 1977; Franz et al. 1997). Photodegradation and chemical degradation have been examined but do not appear to be major pathways for the degradation of glyphosate (Rueppel et al. 1977). Chemical degradation of glyphosate is extremely slow because it contains a carbon-phosphate bond which is highly resistant to chemical breakdown (Gimsing et al. 2004). Degradation by soil microbial processes can occur in both aerobic and anaerobic conditions, however higher degradation rates are generally seen in aerobic environments (Rueppel et al. 1977). The microbial activity is a co-metabolic process meaning the microorganisms are not using the herbicide as a carbon source; however, microorganisms are able to use it as a phosphorus source (Sprankle et al. 1975b; Dick and Quinn 1995; Franz et al. 1997).

A lag period is typically not associated with the degradation of glyphosate which indicates degradation enzymes are present before application (Sprankle et al. 1975b; Rueppel et al. 1977; Franz et al. 1997). Microbes degrade glyphosate rapidly at first likely due to metabolism of the most bioavailable glyphosate, which is the free, unbound glyphosate. This rapid degradation is

followed by a longer, slower degradation period due to soil bound glyphosate that is less bioavailable (Sprankle et al. 1975b; Hance 1976; Rueppel et al. 1977). Generally, if glyphosate is bound strongly to soil, its degradation will be impeded (Borggaard and Gimsing 2008). Sorption and subsequent microbial degradation is soil specific and is dependent on inorganic phosphorus content, soil cation makeup, pH, and surface area as described in section 2.3.3.1. As with most microbial processes, degradation of glyphosate is more rapid at higher temperatures (Heinonen-Tanski 1989).

There are two microbial degradation pathways for glyphosate (Liu et al. 1991; Dick and Quinn 1995). The most studied pathway involves AMPA as the primary degradation product of glyphosate (Rueppel et al. 1977). The other pathway involves the formation of sarcosine and glycine (Kishore and Jacob 1987; Liu et al. 1991). Complete degradation in both pathways results in CO_2 and NH_4^+ . The two pathways are depicted in Figure 2-2.

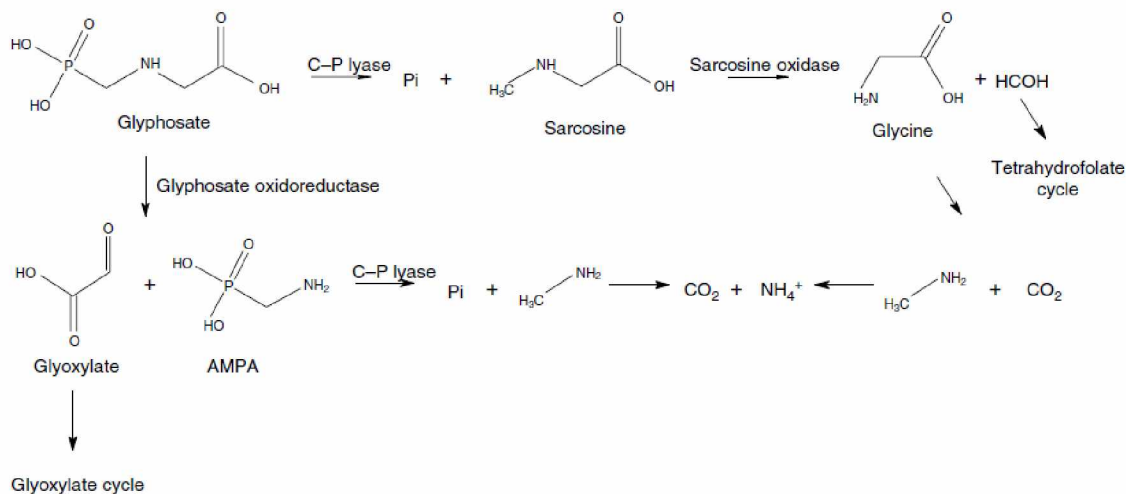


Figure 2-2 Glyphosate Metabolic Pathways (Borggaard and Gimsing 2008)

The initial step in the AMPA pathway is cleaving of the carbon-nitrogen (C-N) bond which produces glyoxolate and AMPA. The carbon-phosphate (C-P) bond in AMPA is then cleaved by C-P lyase to produce inorganic phosphate and methylamine which ultimately forms CO_2 and NH_4^+ (Balthazor and Hallas 1986; Jacob et al. 1988; Liu et al. 1991). The initial step in the sarcosine pathway is cleaving of the C-P bond by C-P lyase. This cleaving then produces

phosphate and sarcosine and is subsequently metabolized to CO_2 and NH_4^+ (Kishore and Jacob 1987; Dick and Quinn 1995). It is not clear which glyphosate degradation pathway is most common, however, AMPA is commonly detected in soil that is treated with glyphosate (Rueppel et al. 1977; Borggaard and Gimsing 2008; Al-Rajab and Schiavon 2010) and sarcosine is not. The lack of sarcosine presence may be due to a quicker degradation via the sarcosine pathway or AMPA's ability to bind to soil which could inhibit its degradation (Moshier and Penner 1978).

The persistence of glyphosate in soil is similar to sorption and degradation in that it is largely dependent on soil type. The half-life of glyphosate can range from days to many months depending on many factors including degradation rates, sorption capacity and soil type. A comprehensive look at several persistence studies by Giesy et al. (2000) reported the average half-life of glyphosate in soil at 47 sites in 13 studies to be 32 days with a range of 1.2 to 197.3 days.

2.3.5 Fate in Aquatic Environments

Glyphosate can enter aquatic systems by direct application, drift, or runoff containing glyphosate bound soil particles (Rueppel et al. 1977; Bronstad and Friestad 1985). Monsanto has developed certain glyphosate herbicides, such as AquaMaster[®], that have been approved for control of aquatic vegetation and therefore detection in water would be expected if there was direct application (Monsanto 2003). Glyphosate exhibits similar behavior in aquatic systems as soil. Similar to soil, the primary mode of degradation in aquatic systems is microbial (Rueppel et al. 1977; Zaranyika 1993). Glyphosate dissipates rapidly from natural waters by direct microbial transformation to AMPA and CO_2 or by adsorption to sediments followed by microbial degradation (Zaranyika 1993). Of these two processes, adsorption to sediment followed by microbial degradation is the primary path of degradation in water (Zaranyika 1993). The degradation rate in aquatic systems depends on the amount of free glyphosate versus the amount of sorbed glyphosate, the microbial density and the sediment or particle content in the system (Zaranyika 1993). The rate of degradation in water is generally slower than soil due to fewer microorganisms present in water (Ghassemi 1982). The half-life of glyphosate in water has been reported between 12 days and 10 weeks (Rueppel et al. 1977).

2.3.6 Atmosphere

Glyphosate has a very low vapor pressure (7.5×10^{-8} mm Hg) and therefore does not volatilize readily. Henry's Gas Constant (Table 2-1) specifies that the chemical has a tendency to partition in water, not air. Soil partitioning values indicate that glyphosate is readily sorbed onto soil particles. Glyphosate may become airborne during application in gusty winds that would allow the chemical to drift. Drift could have particularly damaging impacts if the chemical reached crops not intended for herbicide use (Schuette 1998).

2.3.7 Glyphosate in Cold Climates

As noted previously, glyphosate is a widely used herbicide and has been studied extensively, largely in temperate climates. There have been few studies on herbicides at high latitudes or in cold climates possibly due to population and fewer agricultural opportunities compared with more temperate locations. A study conducted at higher latitudes (Newton et al. 2008) determined dissipation rates did not follow first-order decay rates due to an extended freezing season which slowed microbial activity, therefore, steady state conditions could not be assumed. During non-freezing and summer months glyphosate dissipated at rates consistent with rates reported in temperate regions. However, degradation rates were much slower in winter months. Newton also reported that microbial activity did not completely cease to exist during the winter months, it just slowed. Hence, the extended period in which soils are frozen results in a slightly longer persistence of glyphosate in soil and water at northern latitudes. This finding is consistent with a study undertaken in Norway (Stenrod et al. 2005), which evaluated the effects of freeze-thaw cycles on glyphosate in soils. Stenrod et al. (2005) reported increased microbial activity and subsequent degradation rates during periods of thawing and reduced degradation during periods of freezing.

An earlier study by (Heinonen-Tanski 1989) noted a 10° C decrease reduced the degradation rate of glyphosate to between six and ten percent of the original degradation rate. Puchalski et al. (1999) determined that, in general, degradation of pesticides in soil samples at a constant temperature of -15° C was insignificant, although glyphosate was not tested specifically.

3. Materials and Methods

Two field study sites were established along the Alaska Railroad to study the attenuation of glyphosate along railroad corridors. The two study sites represented contrasting sub-arctic zones in Alaska; a coastal zone in Seward, and a continental zone near Fairbanks. Field studies were conducted during 2008, 2009, and 2010. The overall methodology was similar for both sites; vegetation was measured, herbicide was applied and soil, groundwater, and soil-water samples were taken over time and analyzed for glyphosate and AMPA. Both sites were treated with AquaMaster[®] (glyphosate), which was applied according to the manufacture's label directions.

3.1 Site Descriptions

3.1.1 Continental Study Site Description

The continental study site, established in July 2009, was located southeast of Fairbanks on the Alaska Railroad spur line which connects Fairbanks and Eielson Air Force Base. The region's local climate is characterized by relatively cold, dry winters with warm, dry summers. Mean annual temperature is -3.6° C with a mean temperature of 15.9° C in July during the warmest month and a mean temperature of -8.3° C during the coldest month which is December. Total annual precipitation is 285 mm of rain or snow-water equivalent, including 1288 mm of snow. The summer months typically receive the most precipitation of the seasons with approximately 132 mm (Western Regional Climate Center 2010).

The continental site consisted of a wide variety of vegetation intermixed with patches of bare ground. A photo of the site is included in Appendix A. Vegetation was measured in eight 2 x .5 meter subplots. Percent cover by species was measured in each subplot directly before treatment application. Vegetation cover was approximately 12% prior to application. Vegetation species included Paper Birch (*Betula papyrifera*), White Sweet Clover, (*Melilotus albus*) Balsam Poplar (*Populus balsamifera*) Norwegian Cinquefoil (*Potentilla norvegica*), and several grass species (Appendix A).

The continental study site soil was well graded gravel with sand, GW, according to the Unified Classification System (UCS). The soil pH was 6.0, 6.2, and 6.3 for surface, root and below root depths, respectively. Soil pH was determined using ASTM method D4972-01: Standard Test.

3.1.2 Coastal Study Site Description

The coastal region site, established in August 2008, was located at the Alaska Railroad Yard in Seward, Alaska. This industrial site is the southern terminus of the Alaska Railroad and is utilized for coal loading and storage as well as maintenance and storage of railway equipment. Seward typically has short, cool summers and wet, mild winters. Mean annual temperature in Seward is approximately 4.4° C. Temperatures average -3.7° C in January during the coldest month and reach a mean of 13.4° C in July during the warmest month. The mean annual precipitation is approximately 1.72 m of rain or snow-water equivalent including 2.1 m of snowfall. Autumn months typically receive more precipitation than any other season in Seward (.7 m) (Western Regional Climate Center 2010). Soil temperatures for this study area were not taken.

The coastal site consisted of a mix of vegetated areas and bare ground with low grasses and coarse-grained soil material. A photo of the site is included in Appendix B. Vegetation was measured in eighteen 2 x .5 meter subplots. Percent cover by species was measured in each subplot directly before treatment application. Vegetation cover was approximately 17% before application. Vegetation at the Coastal site included Vetch (*Lathyrus japonicas*), Alaska Willow (*Salix* L.), Common Dandelion (*Taraxacum officinale*), Common Plantain (*Plantago major* L.) and several grass species (Appendix B).

The soil at the Seward site was poorly-graded sand with gravel, SP, according to the UCS. The site had little to no slope. The soil pH was 6.0, 6.6, and 6.2 for surface, root and below root depths, respectively. Soil pH was determined using ASTM method D4972-01: Standard Test.

3.2 Field Experiment

The continental study site was established in July 2009 and consisted of a 61 m (200 ft) long by 4.9 m (16 ft) wide spray zone which included the ground between both railroad tracks, approximately 1.4 m (4.7 ft), as well as the vegetated and soil covered ground adjacent to the west side of the track. See Appendix C for a diagram of the Site.

The coastal study site was established in August 2008 and also consisted of a 61 meter long by 4.9 meter wide zone. The 4.9 meter width included the ground between both railroad tracks,

approximately 1.4 m, and the adjacent vegetated and soil covered ground on the east side of the tracks.

Both sites were treated with AquaMaster[®] (53.8% active ingredient glyphosate) at an application rate of 4.6 kg/ha in a volume of 190 L/ha. The herbicide was applied according to label instructions and mixed with the surfactant AgriDex[®]. At both locations application was via all terrain vehicle (ATV) equipped with spray broadcast nozzles. See Appendix B for a photograph of application equipment. A hand-held GPS was used to monitor the speed of ATV while spraying. Wind conditions were calm during application.

Soil sample collection methodology was generally the same at both sites. The 61 m long spray zone was divided into ten 6.1 m sections. On each sampling event, six of the ten sections were chosen at random to sample. Three soil samples, representing different depths in the soil column, were collected within each of the six selected sections. The three soil depths were surface [S] from 0-5 cm, root [R] from 10-15 cm, and below root [B] from 20-25 cm.

Soil samples were collected using stainless steel trowels. Due to the compaction of the railbed and coarse nature of the material, soil probes were not effective. Each sample was put into HDPE 250 mL bottles marked with the appropriate zone [S, R or B], site location, and date and section number then placed in coolers with ice for transport to UAF laboratory freezers where they were kept frozen until ready for extraction and subsequent analysis. Between each soil layer trowels were cleaned with a coarse brush, de-ionized water and Alconox[®] soap and dried to avoid cross-contamination. Nitrile gloves were worn and replaced after each sample was collected. Soil moisture was determined in the lab using ASTM method D2216-10: Standard Test for Determination of Water Content of Soil and Rock by Mass.

The continental study site was sampled over 12 sampling events. Sampling included 10 events before freeze-up in 2009, one just after spring thaw in 2010 and one before freeze-up in 2010. More than 200 soil samples were collected and analyzed for this site. All continental soil samples were analyzed using GC. A total of nine sampling events occurred at the coastal study site. Seven of the sampling events took place before freeze-up in 2008, one event took place in

the late spring of 2009 after spring thaw, and the last sampling event was before freeze-up in the fall of 2009. Coastal soil samples were analyzed using LC and GC.

Water collection systems were established at each site prior to application. Each site consisted of four water monitoring clusters. Each cluster consisted of a groundwater monitoring well, a .61 m soil lysimeter, and a 1.8 m soil lysimeter. A soil lysimeter is designed to collect soil-water from the vadose zone. The four monitoring clusters were approximately 15.2 m (50 ft) apart and located in the center of the railroad tracks. See Appendix C for a sampling diagram.

Water samples from groundwater wells were pumped into a 250 ml HDPE using a small hand pump and hose. The pump and hose was cleaned with de-ionized water and Alconox[®] soap after each sample. Vadose zone soil-water collection required establishing a vacuum in the lysimeters prior to sampling (generally 18-24 hours). The vacuum created a pressure difference between the surrounding soil-water and the inside of the porous ceramic tip of the lysimeter, resulting in soil-water flowing into the lysimeter. Lysimeter water was collected with a separate pump and hose and was also cleaned in same fashion as the groundwater pump between samples. Water samples were packed in coolers with ice for transport to UAF laboratories where they were kept frozen until analysis.

3.3 Analytical Methods

Liquid chromatography (LC) and gas chromatography (GC) were the chemical analytical methods utilized in this study to determine concentrations glyphosate and AMPA in soil and water samples. All soil samples from the continental and coastal sites were analyzed utilizing GC. Coastal soil and water samples were analyzed using LC. The project initially utilized LC methods for analysis but after complications with sensitivity and instrument availability, analysis was completed using GC. Focus was given to GC soil data due to LC data integrity.

3.3.1 GC Methodology and Analysis

A modified version of the soil extraction and GC analysis method developed by Hu et al. (2008) was used to determine glyphosate and AMPA concentrations in soil. Samples were dried and homogenized using a two mm sieve. Five grams of sample were weighed and the measurement

recorded. The sample was extracted by combining the soil and 30 mL 2M NH₄OH in a flask and stirring for 60-90 minutes. The sample was filtered with a 1.5 µm glass microfiber filter and collected in a 250 mL flask. The NH₄OH extraction was repeated and the filtered sample collected in same flask. The supernatant was transferred to an evaporating tube and evaporated to dryness. All evaporation processes took place using a TURBOVAP[®], RAPIDVAP[®] or REDIVAP[®] depending on instrument availability. The sample was then triple-rinsed with 2.5 mL water: methanol: hydrochloric acid (160:80:10 v/v) and transferred to a centrifuge tube. Then, 20 µL of phosphoric acid was added to each sample and set aside at room temperature for an hour. After centrifugation at 3500 rpm for 15 minutes, the samples were transferred to test vials and again evaporated to dryness. Complete evaporation was followed with a derivatization procedure that consisted of adding 1 mL trifluoroacetic anhydride (TFAA) and 0.5 mL trifluoroethanol (TFE). The sample was capped with a teflon lid and heated to 100° C for one hour. After cooling, the excess reagents were removed via air stream at 40° C.

A methylene chloride and water extraction followed. Ten mL of water was added to the dried sample in the test vial and shaken vigorously then transferred to a separatory funnel. The water addition and shaking step was repeated and poured into the same separatory funnel. Then, 20 mL of methylene chloride was added to the derivitization tube and shaken vigorously before transferring to the separatory funnel. The methylene chloride and water mixture was shaken for approximately two minutes in the separatory funnel and vented occasionally. Water and methylene chloride layers separated and the methylene chloride fraction was collected into an evaporation tube. The methylene chloride extraction was repeated two more times and each time the methylene chloride fraction was collected into the same evaporation tube. Again, the sample was taken to dryness in the evaporation tube. After dryness was achieved, the sample was transferred to a volatile organic analysis (VOA) vial with ten mL methylene chloride. An internal standard of 1,4-dichlorobenzene (1,4-DCB) was added to each sample before injection into the GC.

Samples were analyzed with an Agilent Technologies 7890N Network GC System, 7683 Series Injector, and 5973 Network Mass Selective Detector (GC/MSD). GC method parameters are given in Table 3-1.

Table 3-1 GC Method Parameters

Component	Parameter
Column	30m x 320 μ m, .25 μ m film
Oven	2 min at 50°C, then increase to 150°C at 10°C/min, then increase to 250°C at 100°C/min and hold for 5 min.
Injector	size: 10 μ L volume: 4 μ L
Front Inlet	Initial temp. at 250 °C, pressure 4.52 psi

The GC/MSD produced mass chromatograms that were used to quantify the glyphosate, AMPA and 1,4-DCB concentrations in each sample. The ordinate of the mass chromatogram represented the abundance or intensity of the signal as the compound flows from the GC column into the mass selection detector. The abscissa of the mass chromatogram represented the specific retention time of each compound. 1,4-DCB, AMPA and glyphosate were plotted on the same ordinate and abscissa. Standard solutions of glyphosate, AMPA, and 1,4-DCB were made using known quantities and analyzed on the GC/MSD. From these standards mass chromatograms were created, which were used to determine the specific retention time and several ions specific to each compound. The specific ions were identified by mass to charge ratio (m/z). The specific retention time and ions of each compound were used to quantify concentrations of the field samples.

To help clarify the chromatography a selected ion monitoring (SIM) program was developed, which resulted in only the specific ions in glyphosate, AMPA, and 1,4-DCB to be evaluated. After the chromatogram was created for a sample, extracted ion chromatograms were created by specifying an m/z value and time period. The extracted ion chromatograms, displayed a peak that represented the specific ion. The GC detection limit for glyphosate and AMPA was .01 ppm.

The GC was equipped with an auto-sampler which allowed several samples to be run in one batch. Each GC sample batch included lab reagent blanks (LRB), spiked samples, and standards. The LRB contained no glyphosate or AMPA but did contain the internal standard, 1,4-DCB. Spiked samples contained field soil that had small amounts of known concentrations of glyphosate and AMPA added.

The concentrations of glyphosate and AMPA were determined by integrating the area under the peak created by the extracted ion chromatograms that were created for each compound at the specific retention time. Agilent ChemStation software was utilized for this procedure. The ratio of glyphosate abundance to 1,4-DCB abundance and the ratio of AMPA abundance to 1,4-DCB abundance was calculated using the standard solutions. The concentration of glyphosate and AMPA was known in the standard solutions, so a linear relationship was used to determine the glyphosate and AMPA concentrations in the samples using the analyte/internal standard ratio in the samples, the analyte/internal standard, and the glyphosate and AMPA standard solutions.

3.4 LC Methodology and Analysis

Sample preparation for analysis with LC was as follows. Soil samples were removed from the freezer and ten grams were weighed. Exact soil weight measurements were recorded to the nearest tenth. Samples were then mixed with 50 mL di-ionized water and placed on a shaker table for 15 minutes. Samples were then filtered with a 1.5 μ m glass microfiber filter and injected into the LC. Coastal site water samples were filtered with a 1.5 μ m and injected into the LC.

Samples were analyzed by an Agilent1100 Liquid Chromatography/Mass Spectrometry (LC/MS) and a Thermo Scientific Hypercarb 50 mm x 2.1 mm column. LC method parameters and column conditions are listed in Table 3-2 and Table 3-3, respectively.

Table 3-2 LC Method Parameters

Drying Gas Flow Rate (L/min)	8
Drying Gas Temp. (C°)	350
Nebulizer Gas Pressure (psi)	55
Vcap (V)	1000

Table 3-3 LC Column Conditions

Eluent	A: H ₂ O + 0.1% formic acid B: Acetonitrile + 1% formic acid
Gradient	Start : 90% A, 10% B. 2 mins: 90% A, 10% B. 20 mins: 0% A, 100 % B. 25 mins: 90 % A, 10% B.
Flowrate	1 mL/min

4. Results and Discussion

4.1 Continental Site Results and Discussion

4.1.1 Continental Site Glyphosate

A summary of glyphosate soil concentrations analyzed by GC for the continental site is provided in Table 4-1 and complete glyphosate results are listed in Appendix D. All sampling event dates are reported as days after treatment (DAT). Surface soil was sampled prior to application and detected no glyphosate or AMPA. The median glyphosate surface concentration over time indicates a general decreasing trend, as expected (Figure 4-1). Glyphosate surface concentrations were variable in soil. Variability was greatest just after application but decreased by 32 DAT. Vegetation interception was likely the most significant contributing factor to variability. Other factors including sampling methodology and uniformity of application likely had minor contributions to surface glyphosate concentration variability. The continental study site was intermixed with areas of vegetation and areas of bare soil. Vegetation measurements determined the site had approximately 12% vegetation cover which indicated there were areas of vegetation cover, but also areas where the herbicide was likely directly applied to bare soil. Areas where glyphosate application was intercepted by vegetation may result in little to no herbicide contacting soil. Contrastingly, direct application to bare soil would result in maximum glyphosate soil concentrations. Since the soil sampling locations were chosen at random, both vegetated areas and bare soil areas were sampled. It is not possible to distinguish between samples that actually did not receive glyphosate and samples with non-detectable levels. Therefore median concentrations presented are based on all samples, including those without detectable concentrations, which were assigned a value of zero during calculations.

Table 4-1 Continental Site Glyphosate Soil Concentrations. Each sampling event consists of six samples. Three soil depths (surface, root and below root) are represented. Moisture content on a mass basis is also presented. Samples below the detection limit of .01 mg/kg are shown as “nd” and samples not taken are shown as “na”. Concentrations do not account for recovery rates.

Surface						
Sample Date	DAT	Median Conc. (mg/kg)	25% Percentile (mg/kg)	75% Percentile (mg/kg)	Soil Moisture Content (%)	Surface composite Conc. (mg/kg)
7/13/09	0.5	1.42	0.54	3.36	na	na
7/14/09	1	1.37	1.00	1.55	0.78	1.61
7/15/09	2	0.96	0.53	3.25	1.37	0.50
7/16/09	3	1.47	0.60	1.63	6.34	1.07
7/21/09	8	1.05	0.85	1.59	0.14	0.73
7/29/09	16	0.86	0.52	2.37	0.19	0.63
8/3/09	21	1.08	0.62	1.38	0.45	1.27
8/14/09	32	0.44	0.31	0.56	5.53	0.49
8/29/09	47	0.16	0.09	0.21	7.00	0.31
10/2/09	81	0.14	0.13	0.17	7.73	0.22
4/20/10	281	0.22	0.17	0.24	6.90	na
10/6/10	450	0.09	0.05	0.12	5.04	na
Root						
7/14/09	1	0.03	0.03	0.08	2.01	
7/15/09	2	0.14	0.05	0.17	1.40	
7/16/09	3	nd	nd	0.01	1.24	
7/21/09	8	0.18	0.06	0.24	0.91	
7/29/09	16	0.10	0.02	0.19	0.79	
8/3/09	21	0.02	0.01	0.04	1.70	
8/14/09	32	nd	nd	nd	5.32	
8/29/09	47	0.04	0.02	0.07	5.62	
10/2/09	81	nd	nd	nd	5.59	
4/20/10	281	nd	nd	nd	7.28	
10/6/10	450	0.02	0.01	0.03	7.36	
Below Root						
7/14/09	1	0.27	0.24	0.29	1.71	
7/15/09	2	0.52	0.35	0.92	1.83	
7/16/09	3	0.10	0.04	0.27	2.38	
7/21/09	8	0.18	0.05	0.35	1.47	
7/29/09	16	0.01	0.01	0.03	2.65	
8/3/09	21	0.03	0.01	0.03	2.85	
8/14/09	32	0.02	0.01	0.11	4.50	
8/29/09	47	0.07	0.03	0.13	5.26	
10/2/09	81	0.02	0.01	0.04	12.42	
4/20/10	281	0.02	0.01	0.05	5.92	
10/6/10	450	nd	nd	nd	6.86	

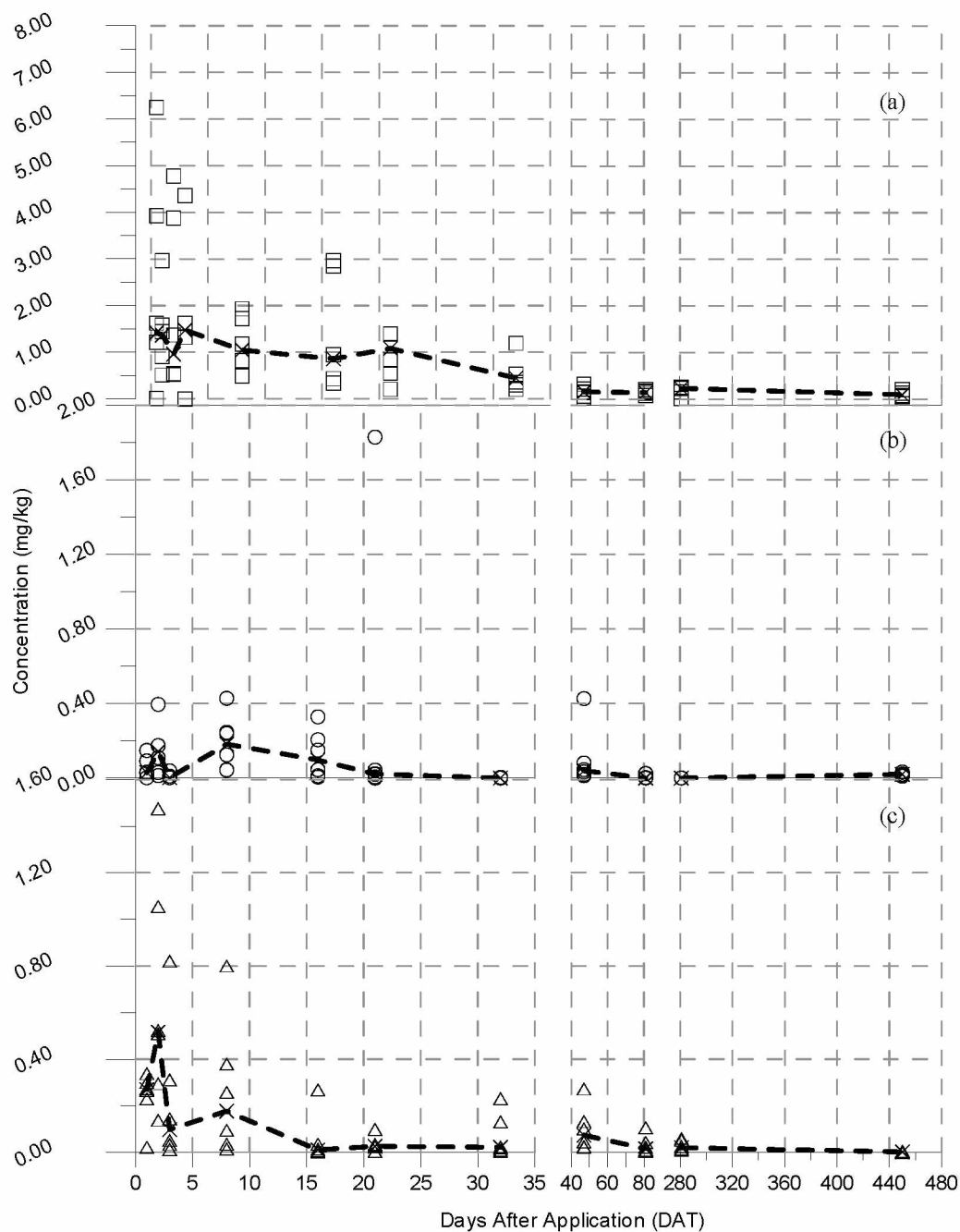


Figure 4-1 Continental Site Median Glyphosate Soil Concentration. Surface (a, 0-5 cm), root zone (b, 10-15 cm), and below root zone (c, 20-25 cm). The trend line in each plot represents the sample concentration median. Herbicide was applied to the site on July 13, 2009. The soil froze approximately 95 days after application and thawed approximately 280 days after application. The first break in the abscissa axis represents a comparably long period between sampling. The second break in the ordinate axis represents the frozen period. Note the concentration scale difference between (a), (b) and (c).

Although efforts were taken to promote a uniform herbicide application, it is challenging to make certain the application was uniform throughout the spray zone owing to the application method. Non-uniform application could contribute to variability in soil concentrations. Due to the coarse nature and heavy compaction of the material in the railroad corridor, using a soil core was not practical, therefore trowels were used. Soil sampling depth (e.g. 0-5 cm, 10-15 cm, 20-25 cm) accuracy was difficult to ensure, which could have also contributed to soil analyte concentration variability. For example, if an individual surface sample consisted of less than a depth interval from 0-5 cm a higher concentration would be expected.

Glyphosate median soil concentrations generally decreased with time, though increases were detected 3, 21, and again at 281 DAT. The highest median surface concentration occurred 3 DAT; however the highest observed individual surface concentration occurred 1 DAT. The increases in median glyphosate surface concentration 3 DAT followed a rain event of 3.81 mm on 2 DAT. Precipitation is shown relative to sampling events and median concentrations in . A rain event within eight hours of application may wash off herbicide from the vegetation and reduce herbicidal effectiveness (Sprankle et al. 1975a; Monsanto 2009). The rain event did not occur within eight hours of application but may have influenced the concentration increase 3 DAT. Glyphosate is rapidly taken up by vegetation in the first four hours following application but uptake continues at a slower rate for more than 48 hours (Sprankle et al. 1975a). During the rain event, a portion of the herbicide residue remaining on the vegetation may have washed off and contacted the soil increasing glyphosate concentration in samples collected 3 DAT.

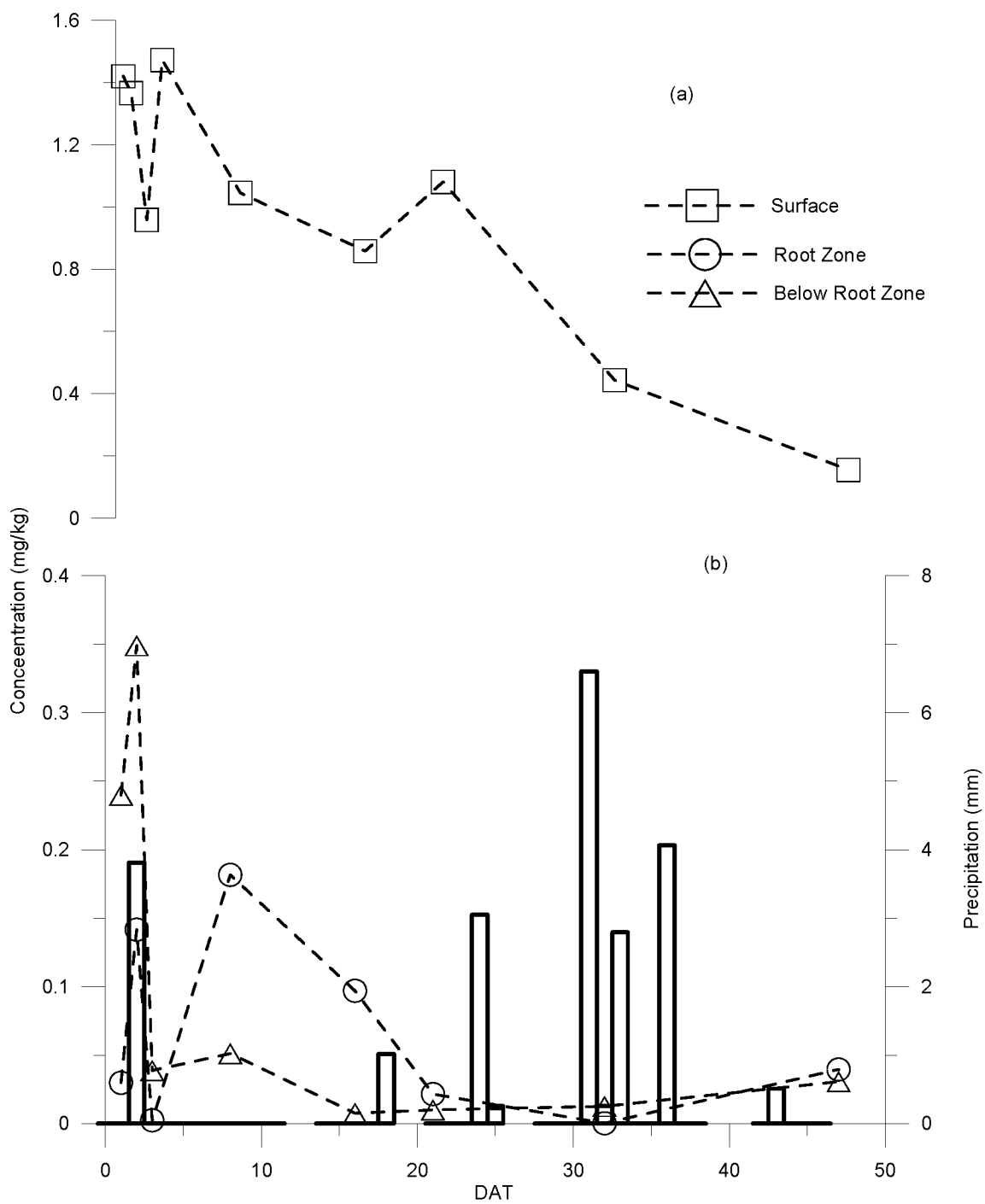


Figure 4-2 Continental Site Precipitation with Median Glyphosate Concentrations. (a) Surface and (b) root and below root concentrations with precipitation for first 47 days of the study.

An additional median surface concentration increase occurred at 21 DAT. There isn't an obvious explanation for this increase; however it could be an artifact of random sampling discussed above. A slight increase was also observed at 281 DAT, just after spring thaw. This increase is not significant but could be due to a release of glyphosate from plant material from fall turnover as hypothesized by Laitinin *et al.* (2007). Laitinin measured an increase in glyphosate in soil eight months after application. The increase was attributed to a release of glyphosate from the vegetation during the freezing and thawing processes.

Soil samples collected from the root zone (approximately 10-15 cm) and below root zone (approximately 20-25cm) indicated a presence of glyphosate, however at far lower concentrations than surface soils. Results show glyphosate presence in root and below root zones immediately after application 1 DAT. Glyphosate is reported to have a very low leaching potential due to its soil binding properties (Rueppel *et al.* 1977; Roy *et al.* 1989; Bleke 1998) but some researchers report leaching due to rain events that transport soil bound glyphosate in some soils (Edwards *et al.* 1980; Strange-Hansen *et al.* 2004; Borggaard and Gimsing 2008). The site received no precipitation (Figure 4-2) on the day of spray and the soil had low moisture content (Table 4-1).

The lack of rainfall suggests sampling methods may have contributed to the presence of glyphosate in root and below root samples in the few days following application. A discrete sample from both root and below root zones was difficult to obtain because surface soil grains could have fallen into root and below root zone soils and inadvertently been collected during sampling. The influence of surface grains would be more pronounced in the days immediately following application and less pronounced later in the study because of the high surface soil concentrations in the days immediately following application. Yet concentrations are shown consistently (Table 2-1) in the root and below root soils suggesting the presence of glyphosate in the root and below root zones is not solely an artifact of the sampling method. If sampling method was the primary contributor to the observed subsurface glyphosate concentrations, a direct correlation would be measurable between surface and subsurface samples, especially at higher concentrations. However, subsurface concentrations trends only mimic surface concentration trends until 3 DAT suggesting that glyphosate observed in the subsurface soils beyond 3 DAT on in the study may

be due to actual herbicide migration. In total, the glyphosate subsurface concentrations reported 1 DAT to 3 DAT may be higher than actual, but concentrations reported beyond 3 DAT are likely representative of what is taking place in the soil column.

Assuming the concentrations reported longer than 3 DAT are not an artifact of sample contamination, an increase was observed in both the root and below root zones between 3 DAT and 8 DAT. During this time surface soil concentrations decreased slightly and root and below root concentrations increased. This result suggests that glyphosate from surface soils migrated slightly into the root and below root zones, but at far reduced concentrations. The previously mentioned rain event following application may have contributed to transport of glyphosate to the subsurface layers. In combination with the rainfall, movement of glyphosate may have been along plant roots if in sandy soils as suggested by Laitinen et al. (2009). A lag period between a rain event, surface increases, and subsurface increases would be expected and was observed ().

As noted previously, glyphosate has a low leaching potential but if conditions are favorable (precipitation in low sorption capacity, oxide poor, coarse-grained soils) leaching is a possibility (Borggaard and Gimsing 2008). Although determining sorption capacity and soil oxides were not in the scope of this study, Torstensson et al. (2005) reported that railroad embankment material potentially has a relatively high sorption coefficient likely due the abundance of iron commonly found in the railroad embankments. Strange-Hansen et al. (2004) also reported relatively high sorption capacities for some gravels in railway embankments. Embankments containing iron would suggest that sorption could potentially be high, which would limit the mobility of glyphosate.

Median glyphosate concentrations increased in the subsurface soil samples 47 DAT. The increase may again be due to migration of surface glyphosate, but is more likely due to glyphosate exuded from roots into the soil at the subsurface levels. Laitinen (2007) concluded that glyphosate exuded from roots of vegetation which received foliar applied glyphosate could contribute to soil glyphosate concentrations. Throughout the study period 82% of glyphosate detected in the soil was observed in the surface soils. Approximately 6% and 11% were detected in the root and below root zones, respectively.

4.1.2 Continental Site AMPA

Microbial degradation is presumably responsible for the decreasing concentrations in glyphosate as seen in numerous other studies (Sprankle et al. 1975b; Rueppel et al. 1977; Dick and Quinn 1995; Borggaard and Gimsing 2008). Microbial degradation is evidenced by AMPA (Table 4-2, Figure 4-3, and Appendix D). AMPA was observed to a lesser degree in the root and below root zones than in the surface soils throughout the study, but its presence indicates glyphosate that migrated to subsurface level was degraded microbially. Although movement occurred in the subsurface soils, it was limited and at low concentrations. Presence of AMPA is reported to be definitive evidence of biological degradation (Sprankle et al. 1975b; Rueppel et al. 1977; Dick and Quinn 1995; Borggaard and Gimsing 2008).

Table 4-2 Continental Site AMPA Soil Concentrations. Each sampling event consists of six samples. Three soil depths (surface, root and below root) are represented. Moisture content on a mass basis is also presented. Samples below the detection limit of .01 mg/kg are shown as “nd” and samples not taken are shown as “na”. Concentrations do not account for recovery rates.

Surface						
Sample Date	DAT	Median Conc. (mg/kg)	25% Percentile (mg/kg)	75% Percentile (mg/kg)	Soil Moisture Content (%)	Surface composite Conc. (mg/kg)
7/13/09	0.5	nd	nd	nd	na	na
7/14/09	1	0.01	nd	0.01	0.78	0.02
7/15/09	2	0.02	0.01	0.03	1.37	0.01
7/16/09	3	0.01	0.01	0.02	6.34	0.01
7/21/09	8	0.01	0.01	0.01	0.14	0.01
7/29/09	16	0.02	0.01	0.06	0.19	0.01
8/3/09	21	0.01	0.01	0.02	0.45	0.02
8/14/09	32	0.10	0.06	0.13	5.53	0.10
8/29/09	47	0.12	0.06	0.18	7.00	0.06
10/2/09	81	0.07	0.06	0.08	7.73	0.09
4/20/10	281	0.11	0.07	0.14	6.90	na
10/6/10	450	0.21	0.16	0.30	5.04	na
Root						
7/14/09	1	nd	nd	nd	2.01	
7/15/09	2	nd	nd	nd	1.40	
7/16/09	3	nd	nd	nd	1.24	
7/21/09	8	0.01	0.01	0.01	0.91	
7/29/09	16	nd	nd	nd	0.79	
8/3/09	21	nd	nd	nd	1.70	
8/14/09	32	nd	nd	nd	5.32	
8/29/09	47	0.02	0.01	0.06	5.62	
10/2/09	81	nd	nd	nd	5.59	
4/20/10	281	nd	nd	nd	7.28	
10/6/10	450	0.03	0.01	0.05	7.36	
Below Root						
7/14/09	1	0.01	0.01	0.01	1.71	
7/15/09	2	0.01	0.01	0.03	1.83	
7/16/09	3	nd	nd	0.01	2.38	
7/21/09	8	nd	nd	0.01	1.47	
7/29/09	16	nd	nd	nd	2.65	
8/3/09	21	nd	nd	nd	2.85	
8/14/09	32	nd	nd	0.01	4.50	
8/29/09	47	0.02	0.01	0.03	5.26	
10/2/09	81	0.01	nd	0.03	12.42	
4/20/10	281	0.01	0.01	0.02	5.92	
10/6/10	450	nd	nd	0.01	6.86	

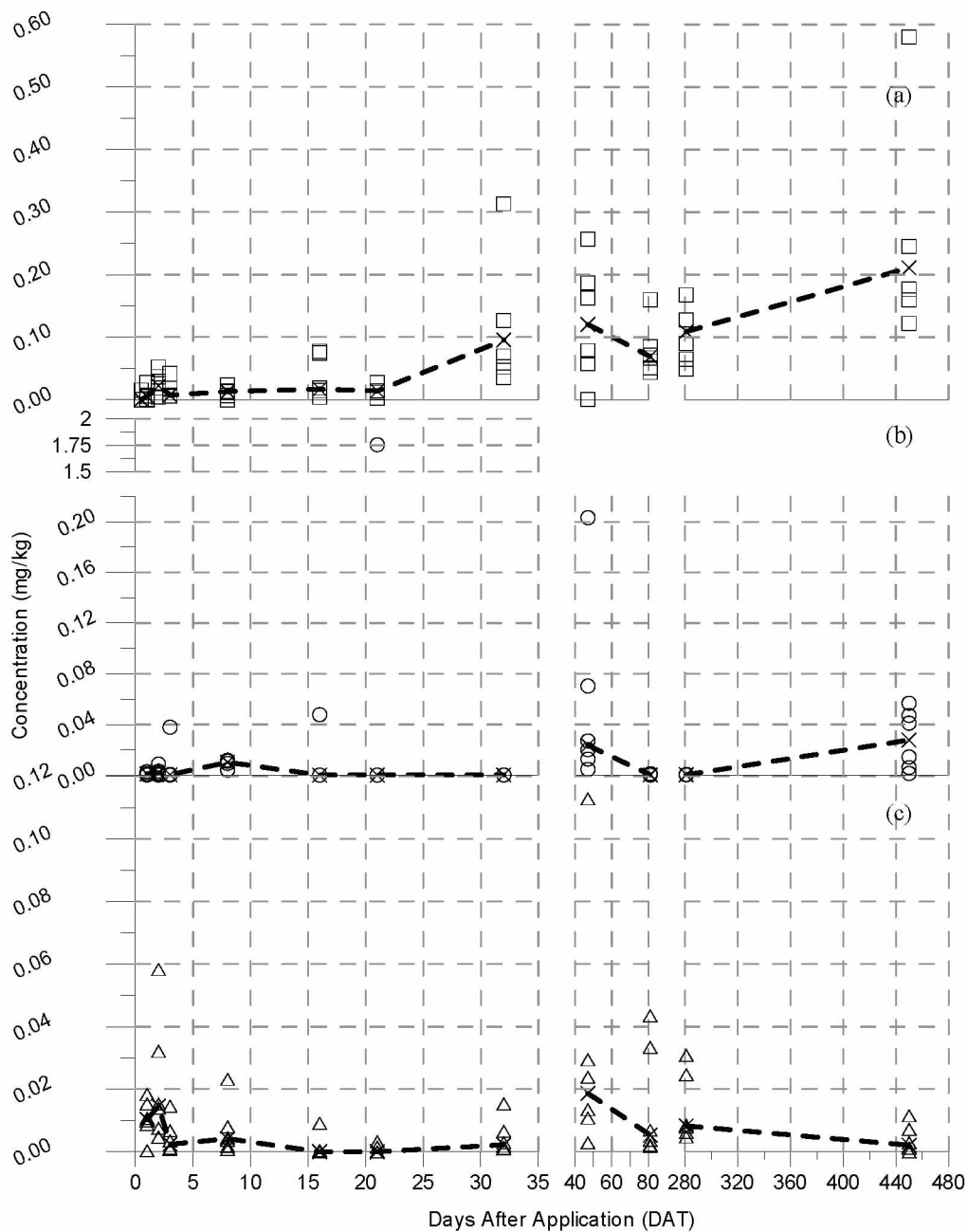


Figure 4-3 Continental Site Median AMPA Concentrations. Surface (a, 0-5 cm), root zone (b, 10-15 cm), and below root zone (c, 20-25 cm). The trend line in each plot represents the sample concentration median. Herbicide was applied to the site on July 13, 2009. The soil froze approximately 94 days after application and thawed approximately 280 days after application. The first break in the abscissa axis represents a comparably long period between sampling. The second break in the ordinate axis represents the frozen period. Note the concentration scale difference between (a), (b) and (c).

The median AMPA concentrations generally trend upward. The highest median AMPA concentration was observed 450 DAT during the last sampling event. Increases were observed between 80 DAT and 281 DAT. Since soil samples were not taken during the winter months it is not possible to determine if there was steady glyphosate degradation during the winter or an increase of microbial activity and degradation at spring thaw. Rike et al. (2008) discusses the possibility of increased microbial activity in soil at spring thaw due to increased movement of nutrients in the soil-water. Thus, it is possible that increased microbial degradation of glyphosate occurred during spring thaw. Investigations by others into glyphosate degradation in frozen soils determined that degradation was not stopped, but slowed when soil temperatures were below freezing (Stenrod et al. 2005; Torstensson et al. 2005; Newton et al. 2008). Because the 281 DAT sample was collected only days after the soil thawed, the AMPA present is most likely a result of slight degradation during the winter. Soil temperatures at three depths were recorded during the first winter of the study period (Figure 4-4).

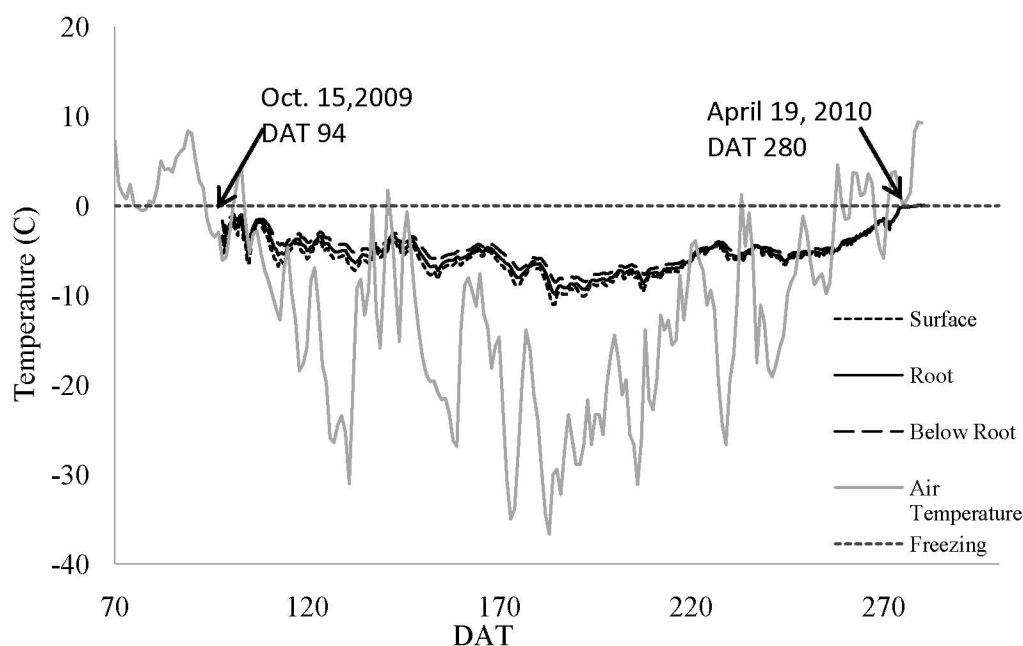


Figure 4-4 Continental Site Winter Soil Temperatures. Temperature recorded at three depths. The beginning and end of frozen soil conditions are shown.

4.1.3 Continental Site Glyphosate Mass

The mass of glyphosate accounted for in the soil was determined by comparing the glyphosate mass in the soil to the total mass of glyphosate applied. An integration of the measured concentrations in the three sample zones was used to determine the amount of mass in the top 25 cm of soil. Two DAT mass calculations show approximately 38% of the mass applied was accounted for, but it could be as high as 110%. This may support the hypothesis that samples taken before 3 DAT may be an overestimation of glyphosate. The overall mass accounted for in the soil generally decreases (Figure 4-5). The mass accounted for in the soil is based on concentrations in the surface, root, and below root zones. Decreases in mass fraction over time also suggest microbial degradation of the herbicide.

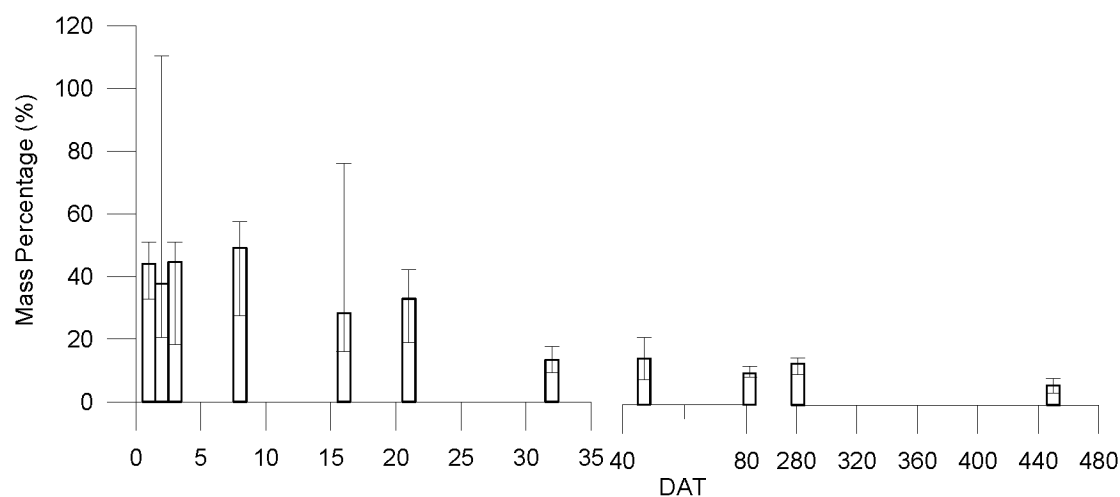


Figure 4-5 Mass of Applied Glyphosate Accounted for in Continental Site Soil Profile. Bars represent the ration of the median value of mass measured in the soil to the total mass applied. Error bars indicate the upper and lower quartile of the measured mass datum set.

Glyphosate degradation has been shown to follow first-order decay kinetics (Weaver et al. 2007), which were modeled in this study to determine half-life. Due to the long winter season and reduced soil temperatures, only data up to 81 DAT, which captures the Alaska growing season, was used for the first-order surface soil decay model to better compare half-life values recorded in temperate climates. A similar analysis was done by Frutiger (2009) for the herbicide 2,4-D in continental sub-arctic conditions.

The half-life for glyphosate at the continental site during the growing season was calculated to be approximately 24 days and the best fit correlation coefficient was .87 (Figure 4-6). The half-life of glyphosate reported in literature has a wide range from a few days to several months, with an average of 32 days (Giesy et al. 2000). Roy et al. (1989) also reported a half-life of glyphosate applied mid summer to be 24 days in sandy soils in a non-subarctic region. The half-life observed during the growing season is not dissimilar to half-lives observed in more temperate climates (Rueppel et al. 1977; Newton et al. 1984; Thompson et al. 1999).

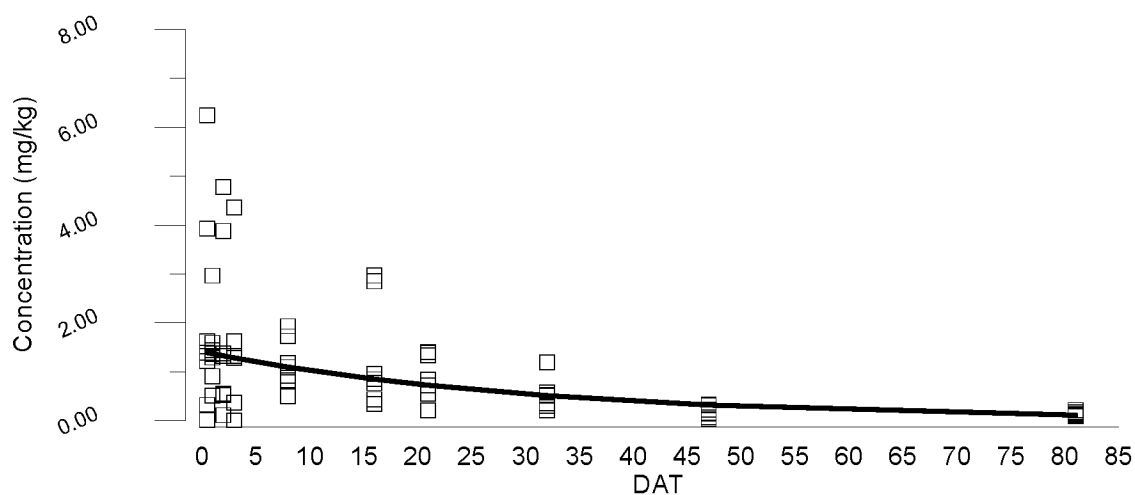


Figure 4-6 First Order Decay Curve for Continental Site. First order decay was based only on the first 81 days of the study.

If the rate constant applied during the growing season was continued, the herbicide would be degraded 90% by approximately 80 DAT. However, this was not observed at the continental site. Glyphosate persisted for more than a year. The extended persistence suggests the rate of degradation decreases during the winter months.

Torstensson *et al.* (2005) conducted a study along Swedish railroads and concluded that glyphosate had a longer persistence in railway embankments than at agricultural or forestry sites potentially due to nutrient poor environments of the ballast material typically used along railroads which may reduce microbial activity and have fewer sorption sites. The Swedish study was conducted at latitudes ranging from 56 ° N to 65.5 ° N, which are similar to the latitudes of this study. Torstensson calculated the half-life of glyphosate in railroad embankments to be

between two and five months. Results from the continental site in this study indicate a faster degradation than reported by Torstensson likely due to soil type at this location. The soil at the continental site was a sandy soil and did not contain much ballast. In Torstensson's study low level glyphosate concentrations were present for more than a year after application. This was also the case at the continental study site. The study conducted by Newton et al. (2008) involving glyphosate dissipation in high latitudes did not attempt to determine a half-life because the herbicide was applied only one month prior to winter freeze-up. Despite more rapid degradation rates than other high latitude sites, the continental glyphosate remained more than a year after application, which indicates a seasonal rate reduction.

Overall, glyphosate attenuated rapidly during the growing season at the continental site. A half-life of 24 days was calculated during the growing season. An extended period of frozen soil conditions during winter months likely caused a reduction in degradation and an extended persistence of the herbicide at the continental site. Glyphosate persisted in the soils at low levels for more than a year. Glyphosate attenuation was driven by microbial degradation in the soil. In the soils of the continental site, glyphosate movement to subsurface soils was limited, but presence of glyphosate was detected in subsurface soils at low concentrations.

4.2 Coastal Site Results and Discussion

Soil and water samples analyzed using LC is discussed in Section 4.2.2. Due to previously mentioned analytical instrument issues results for the coastal site focus on GC analysis.

4.2.1 Coastal Site Glyphosate

Coastal study site glyphosate soil concentrations analyzed by GC are summarized in Table 4-3 Figure 4-7, Appendix E. Approximately 190 soil samples were collected and analyzed with GC at this site. Surface soil was sampled prior to application and detected no glyphosate or AMPA. The median surface concentration decreased rapidly after application. Results show a large amount of variability in soil samples as indicated by the range of concentrations. The variability is greatest in the first days following application but is reduced by 15 DAT. Similar to the continental study, factors including vegetation interception, sampling methodology and uniformity of herbicide application may have contributed to the variability observed in soil

concentrations. Vegetation cover was approximately 17% at the coastal site. Vegetation cover at this location was dense in some areas and sparse in others resulting in different amounts of herbicide contacting the soil during application. The differences in vegetation cover most likely explain some of the variability in concentrations between sample locations measured for each sampling event. As in the continental study, it is not possible to distinguish between samples that actually did not receive glyphosate and samples with non-detectable levels. Therefore median concentrations presented are based on all samples, including those without detectable concentrations, which were assigned a value of zero during calculations.

Surface median glyphosate concentrations decline rapidly over time, though a noticeable increase was observed 1 DAT and less significant increases occurred 15 DAT and again 421 DAT. The highest median surface concentration occurred on the second sampling event 1 DAT. The highest observed surface concentration also occurred 1 DAT.

Table 4-3 Coastal Site Glyphosate Soil Concentrations. Each sampling event consists of six samples. Three soil depths (surface, root and below root) are represented. Moisture content on a mass basis is also presented. Samples below the detection limit of .01 mg/kg are shown as “nd”. Concentrations do not account for recovery rates.

Surface						
Sample Date	DAT	Median Conc. (mg/kg)	25th Percentile (mg/kg)	75th Percentile (mg/kg)	Soil Moisture Content (%)	Surface composite Conc. (mg/kg)
8/4/08	0.5	1.02	0.14	1.90	1.48	3.29
8/5/08	1	1.81	0.98	2.46	9.19	2.51
8/8/08	4	0.14	0.10	0.29	1.30	1.29
8/13/08	9	0.41	0.22	1.68	6.86	1.82
8/19/08	15	0.01	nd	0.07	2.25	0.03
8/26/08	22	0.06	0.02	0.07	8.13	0.19
10/22/08	79	nd	nd	nd	4.27	nd
5/18/09	287	nd	nd	0.01	na	0.22
9/29/09	421	0.01	0.01	0.03	3.67	0.01
Root						
8/4/08	0.5	0.01	nd	0.03	3.97	
8/5/08	1	0.03	0.02	0.06	4.52	
8/8/08	4	0.08	0.03	0.15	2.77	
8/13/08	9	0.51	0.14	1.01	8.32	
8/19/08	15	0.02	0.01	0.06	3.07	
8/26/08	22	0.68	0.16	1.03	12.48	
10/22/08	79	nd	nd	0.02	7.31	
5/18/09	287	0.08	0.05	0.16	2.06	
9/29/09	421	nd	nd	nd	5.06	
Below Root						
8/4/08	0.5	0.02	0.01	0.04	4.59	
8/5/08	1	nd	nd	0.09	3.96	
8/8/08	4	nd	nd	0.01	3.03	
8/13/08	9	0.14	0.11	0.31	5.14	
8/19/08	15	0.08	0.05	0.13	3.08	
8/26/08	22	0.21	0.19	0.33	5.13	
10/22/08	79	nd	nd	0.01	5.33	
5/18/09	287	0.05	0.03	0.09	10.63	
9/29/09	421	nd	nd	nd	5.66	

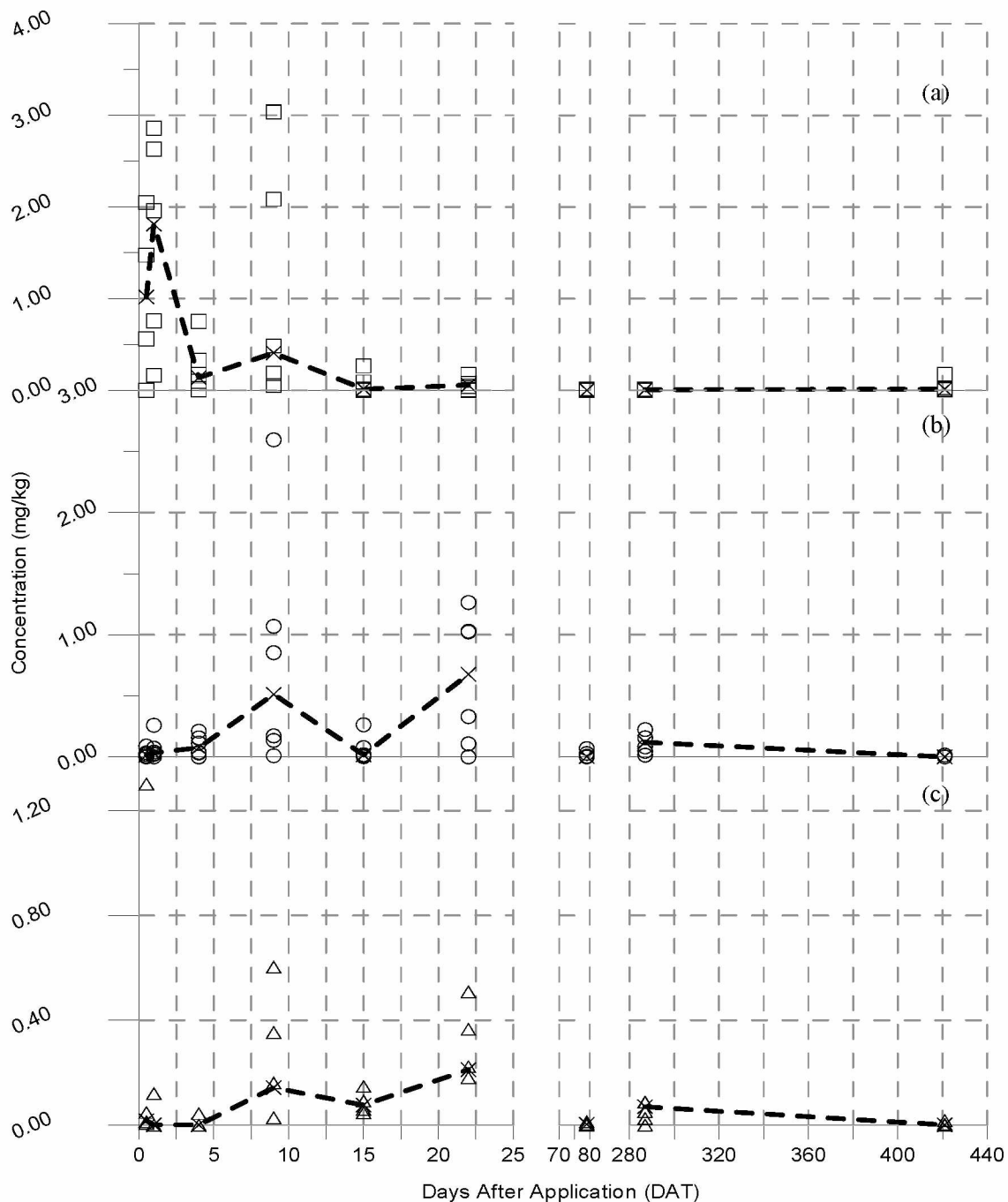


Figure 4-7 Coastal Site Median Glyphosate Concentrations. Surface (a, 0-5 cm), root zone (b, 10-15 cm), and below root zone (c, 20-25 cm). The trend line in each plot represents the sample concentration median. Herbicide was applied to the site on August 4, 2008. The first break in the ordinate axis represents a comparably long period between sampling. The second break in the ordinate axis represents the frozen period. Note the concentration scale difference between (a), (b) and (c).

The increase in median surface concentration between .5 DAT and 1 DAT may be a result of additional herbicide contacting the soil that was washed off the vegetation by a rain event occurring shortly after application. Although substantial reduction in herbicide effectiveness was not noted (Section 4.4), it is probable the rain event caused glyphosate wash off from the vegetation the day of application causing a glyphosate concentration increase 1 DAT. Precipitation events likely resulted in the dispersion of glyphosate from surface soils in the days immediately following application. Precipitation events relative to median glyphosate concentrations are shown in Figure 4-8.

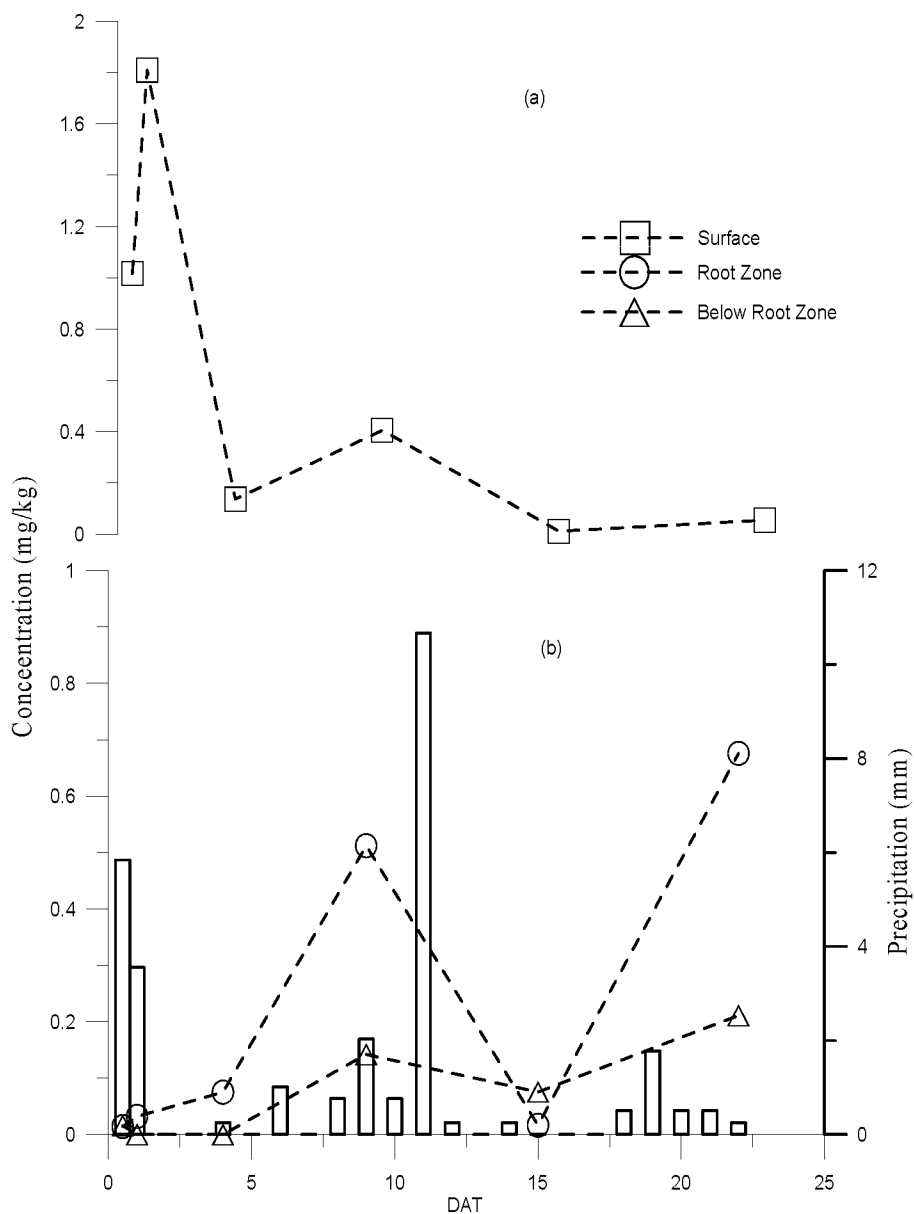


Figure 4-8 Coastal Site Precipitation with Median Glyphosate Concentrations. Data for first 25 days of study period with (a) surface and (b) root, and below root glyphosate concentrations.

Overall glyphosate soil concentrations decreased with increasing depth (Table 4-3). Small concentrations of glyphosate were measured in the subsurface 1 DAT. The previously mentioned rain event following application may have contributed to transport of glyphosate to the subsurface layers.

The highest median glyphosate concentration in both the root and below root zones occurred 22 DAT. The site received approximately 29 mm between application and 22 DAT. This amount of rain likely caused dispersion in the surface samples and transport of glyphosate from the surface layer to subsurface layers 22 DAT. The soil moisture increased as well 22 DAT.

Slight increases were observed in the root and below root zones but not surface soils on 278 DAT. Because the 278 DAT sampling event took place after spring thaw (Figure 4-10), plant residues released during fall and winter freezing and thawing process potentially could have migrated to subsurface soils. Another explanation for the increase could be a portion of glyphosate exuded from roots into the subsurface soils as suggested by Laitinen et al. (2007).

4.2.2 Coastal Site AMPA

Median AMPA soil concentrations are presented in Table 4-4, Figure 4-9 and Appendix E. AMPA was present in relatively high concentrations in the surface samples immediately following application. It is possible that due to the long daylight hours and moisture content, microbial degradation took place very quickly. However, it should be noted that samples were taken during the summer of 2008 but not analyzed using GC until the summer of 2010 due to complications with analysis methods. Soil samples were kept frozen (except for a freezer malfunction in which samples thawed for one day) between sample collection and analysis. Although there are indications that pesticides don't degrade under constant freezing temperatures (Puchalski et al. 1999) some studies have noted minor degradation in soils under freezing temperatures (Stenrod et al. 2005; Newton et al. 2008). Due to the lengthy time between sample collection and GC analysis, minor degradation may have taken place. Lag time may explain the presence of AMPA in the days immediately following application. Median surface AMPA concentrations mimic glyphosate concentrations, although at far lower concentrations, for the first 15 DAT.

Table 4-4 Coastal Site AMPA Median Concentrations. Each sampling event consists of six samples. Three soil depths (surface, root and below root) are represented. Moisture content on a mass basis is also presented. Samples below the detection limit of .01 mg/kg are shown as “nd”. Concentrations do not account for recovery rates.

Surface						
Sample Date	DAT	Median Conc. (mg/kg)	25th Percentile (mg/kg)	75th Percentile (mg/kg)	Soil Moisture Content (%)	Surface composite Conc. (mg/kg)
8/4/08	0.5	0.03	nd	0.05	1.48	0.02
8/5/08	1	0.04	0.02	0.06	9.19	0.04
8/8/08	4	nd	nd	nd	1.30	0.04
8/13/08	9	0.01	0.01	0.10	6.86	0.09
8/19/08	15	nd	nd	0.01	2.25	nd
8/26/08	22	nd	nd	nd	8.13	0.01
10/22/08	79	nd	nd	nd	4.27	nd
5/18/09	287	0.01	nd	0.01	NA	0.05
9/29/09	421	nd	nd	nd	3.67	nd
Root						
8/4/08	0.5	nd	nd	nd	3.97	
8/5/08	1	nd	nd	0.01	4.52	
8/8/08	4	nd	nd	0.01	2.77	
8/13/08	9	0.02	0.01	0.04	8.32	
8/19/08	15	nd	nd	nd	3.07	
8/26/08	22	0.02	nd	0.05	12.48	
10/22/08	79	nd	nd	nd	7.31	
5/18/09	287	0.02	0.01	0.02	2.06	
9/29/09	421	nd	nd	nd	5.06	
Below Root						
8/4/08	0.5	nd	nd	0.01	4.59	
8/5/08	1	nd	nd	nd	3.96	
8/8/08	4	nd	nd	nd	3.03	
8/13/08	9	0.01	0.01	0.01	5.14	
8/19/08	15	0.01	0.01	0.03	3.08	
8/26/08	22	0.01	0.01	0.01	5.13	
10/22/08	79	nd	nd	0.01	5.33	
5/18/09	287	0.01	nd	0.01	10.63	
9/29/09	421	nd	nd	nd	5.66	

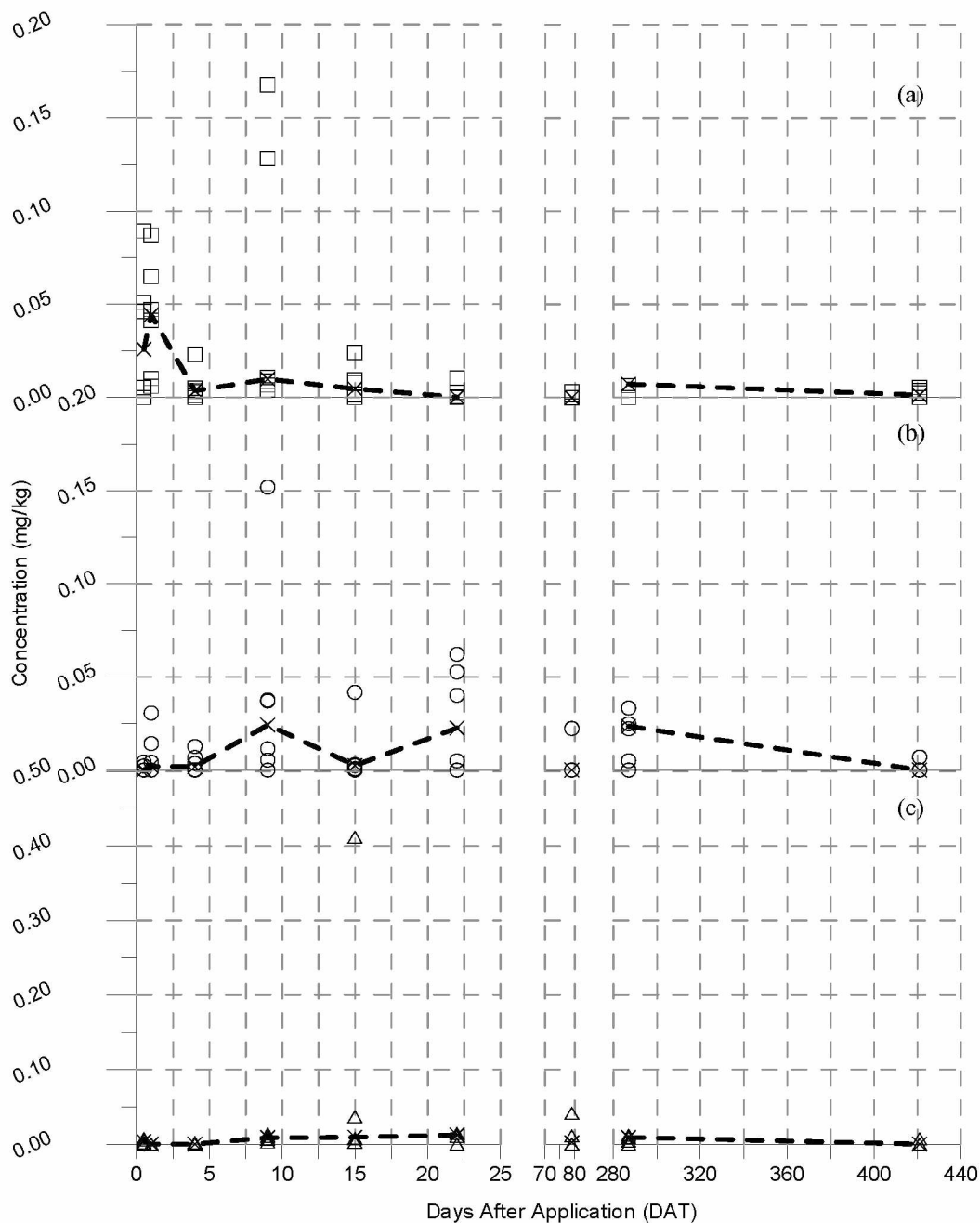


Figure 4-9 Coastal Site Median AMPA Soil Concentrations. Surface (a, 0-5 cm), root zone (b, 10-15 cm), and below root zone (c, 20-25 cm). The trend lines in each plot represent the sample concentration median. Herbicide was applied to the site on August 4, 2008. The first break in the ordinate axis represents a comparably long period between sampling. The second break in the abscissa axis represents the frozen period. Note the concentration scale difference between (a), (b) and (c).

Generally, median AMPA concentrations did not increase as glyphosate decreased in surface samples which suggest processes other than microbial degradation were influencing the reduction of glyphosate. However, notable AMPA concentrations were observed in the root zone 22 DAT and again 287 DAT suggesting microbial degradation in the subsurface and indicating degradation takes place during the winter months. Microbial degradation is likely especially if the soils are not held at freezing temperatures for substantial amounts of time. Soil temperature data was not recorded during the study period at the coastal site, however ambient air temperature is displayed in Figure 4-10. The air temperature fluctuated throughout the winter and was not consistently below freezing for the entire winter. Fluctuating air temperatures data suggests that the soil may also have fluctuated above and below freezing conditions throughout winter. These findings are consistent with Stenrod *et al.* (2005) who also measured degradation of glyphosate to continue throughout freeze thaw cycles.

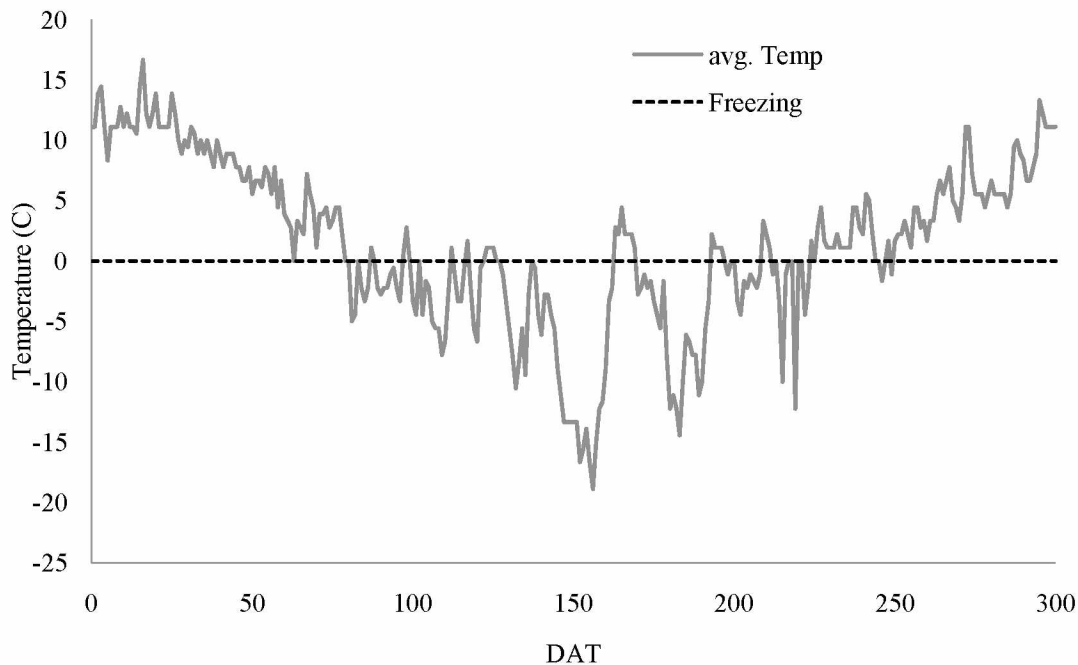


Figure 4-10 Coastal Site Winter Air Temperatures

A mass fraction was calculated in the same manner as the continental site. The mass measured in the soil was compared to the total mass applied. On 79 and 421 DAT less than 1% of the mass was accounted for which is too insignificant to appear on Figure 4-11. The trend is either linear or exponential decay which suggest microbial degradation was not the primary driver in glyphosate reduction.

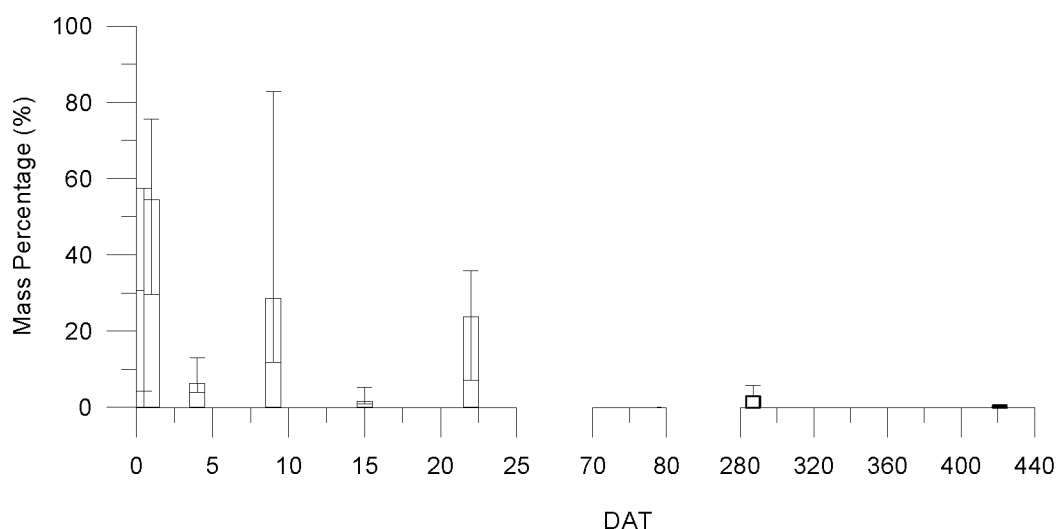


Figure 4-11 Mass of Applied Glyphosate Accounted for in Coastal Site Soil Profile. Bars represent the ratio of the median value of mass measured in the soil to the total mass applied. Error bars indicate the upper and lower quartile of the measured mass datum set.

Glyphosate degradation does not always follow first order decay models as noted by Feng and Thompson (1990) who reported on the results of a persistence study in Canadian watersheds. At the coastal site soil glyphosate concentrations decrease rapidly over time, but poor regression coefficients excluded the use of first order decay models for this study site during the entire growing season. If only the first 22 days of the study are used, a half-life of 4.7 days can be calculated. Compared to several studies done elsewhere, glyphosate at the coastal study site dissipated more rapidly (Giesy et al. 2000). However, glyphosate dissipation in the weeks following application is not out of the range of reported half-life (Giesy et al. 2000).

Glyphosate persisted in the surface soils at the coastal study site for approximately 79 days but was detected in the subsurface soils until 287 DAT. This extended persistence is likely due to winter conditions and reduced degradation rates. The coastal site results are similar to the

findings of Torstensson *et al.* (2005) who reported the detection of glyphosate one year after application at a northern latitude location. This suggests that glyphosate can persist longer in areas where degradation rates can be reduced by winter conditions.

Overall, the coastal site exhibited rapid attenuation of glyphosate after application. Attenuation at the coastal site was likely driven by dispersion from rain events. Migration to the subsurface soils was observed and is also likely due to rain events. Although degradation may not be the primary driver for glyphosate reduction, AMPA was observed in all soil zones indicating that glyphosate that does migrate is degraded microbially. Winter conditions and freeze thaw cycles likely contributed to an extended persistence of the herbicide compared to more temperate climates.

4.2.3 LC Soil and Water Results and Discussion

Liquid Chromatography (LC) analysis of soil samples at the coastal study site differed greatly from the GC analysis of the same site. LC analysis results overall indicated lower concentrations than GC results. GC analysis detected glyphosate presence in more samples than LC analysis. LC results are shown in Table 4-5.

Table 4-5 LC Coastal Site Glyphosate Soil Concentrations. Each sampling event consists of six samples. Three soil depths (surface, root and below root) are represented. Moisture content on a mass basis is also presented. Samples below the detection limit of .001 mg/kg are shown as “nd” and samples not taken are shown as “na”. Concentrations do not account for recovery rates.

Surface					
Sample Date	DAT	Median Conc. (mg/kg)	25th Percentile (mg/kg)	75th Percentile (mg/kg)	Soil Moisture Content (%)
8/4/08	0.5	0.004	nd	4.751	1.48
8/5/08	1	0.372	nd	0.778	9.19
8/8/08	4	nd	nd	0.002	1.30
8/13/08	9	nd	nd	0.020	6.86
8/19/08	15	nd	nd	nd	2.25
8/26/08	22	nd	nd	nd	8.13
10/22/08	79	nd	nd	0.031	4.27
5/19/09	288	nd	nd	nd	na
9/29/09	421	nd	nd	nd	3.67
Root					
8/4/08	0.5	nd	nd	nd	3.97
8/5/08	1	nd	nd	0.060	4.52
8/8/08	4	nd	nd	0.006	2.77
8/13/08	9	nd	nd	0.001	8.32
8/19/08	15	nd	nd	0.002	3.07
8/26/08	22	nd	nd	0.005	12.48
10/22/08	79	nd	nd	0.064	7.31
5/19/09	288	nd	nd	nd	2.06
9/29/09	421	nd	nd	nd	5.06
Below Root					
8/4/08	0.5	nd	nd	3.846	4.59
8/5/08	1	nd	nd	0.006	3.96
8/8/08	4	nd	nd	nd	3.03
8/13/08	9	nd	nd	0.102	5.14
8/19/08	15	nd	nd	0.055	3.08
8/26/08	22	nd	nd	0.000	5.13
10/22/08	79	0.011	nd	0.055	5.33
5/19/09	288	nd	nd	nd	10.63
9/29/09	421	nd	nd	nd	5.66

Median soil concentration results indicate a presence of glyphosate only .5 DAT and 1 DAT. In the subsurface layers, glyphosate median concentrations indicate no presence in the root layer and one presence in the below root layer on 79 DAT. In addition, LC detection of AMPA was also very limited. Only one sampling event, 4 DAT, indicated a detectable AMPA concentration in the six samples analyzed for that sampling event. These results are inconsistent with other studies (Rueppel et al. 1977; Feng and Thompson 1990; Laitinen 2009). Likely the most substantial reason for differences in concentration between LC and GC results was the different extraction techniques used. The LC extraction method was solely a water extraction whereas the GC extraction method was much more aggressive and involved multiple extractions.

Groundwater and vadose zone water samples taken from the lysimeters and groundwater wells were not analyzed by GC in time for this report but were analyzed using LC. Glyphosate LC water results are presented in Table 4-6. Median glyphosate concentrations in the soil lysimeters indicate a presence of glyphosate on 1 DAT and 8 DAT in the .61 m lysimeter and on 15 DAT in the 1.8 m lysimeter. Median AMPA concentrations (not shown) in water samples indicated a presence in groundwater at 80 DAT, and in the 1.8 m lysimeter 16 DAT. The median AMPA concentration in the .61 m lysimeter was non-detectable for all sample days. The AMPA results are an indication that glyphosate present in the soil-water and groundwater was degraded microbially.

Table 4-6 LC Coastal Site Glyphosate Water Concentrations. A-D represents the different monitoring clusters. Non-detectable levels are represented as “nd” and samples that were not taken are represented as “na”. Concentrations do not account for recovery rates. The LC detection limit was .001mg/L.

.61 m Lysimeters								
Date	DAT	A	B	C	D	Median Conc (mg/L)	25th Percentile (mg/L)	75th Percentile (mg/L)
8/5/2008	1	0.009	0.014	0.008	na	0.009	0.009	0.011
8/8/2008	3	0.008	nd	nd	na	nd	nd	0.004
8/13/2008	8	0.012	0.029	0.037	na	0.029	0.021	0.033
8/20/2008	15	nd	nd	nd	na	nd	nd	nd
8/27/2008	22	0.009	nd	nd	na	nd	nd	0.004
10/23/2008	79	nd	nd	nd	na	nd	nd	nd
5/19/2009	287	nd	nd	nd	nd	nd	nd	nd
9/29/2009	420	nd	nd	nd	nd	nd	nd	nd
1.8 m Lysimeters								
8/5/2008	1	0.013	nd	nd	na	nd	nd	0.007
8/8/2008	3	0.006	nd	nd	na	nd	nd	0.003
8/13/2008	8	nd	0.004	nd	na	nd	nd	0.002
8/20/2008	15	nd	0.008	0.004	na	0.004	0.002	0.006
8/27/2008	22	nd	0.061	nd	na	nd	nd	0.031
10/23/2008	79	nd	nd	nd	na	nd	nd	nd
5/19/2009	287	nd	nd	nd	nd	nd	nd	nd
9/29/2009	420	nd	nd	nd	nd	nd	nd	nd
Groundwater Wells								
8/5/2008	1	nd	nd	0.011	0.004	0.002	nd	0.006
8/8/2008	3	nd	0.007	nd	0.011	0.003	nd	0.008
8/13/2008	8	nd	nd	0.017	na	nd	nd	0.008
8/20/2008	15	nd	nd	0.014	nd	nd	nd	0.004
8/27/2008	22	nd	na	na	nd	nd	nd	nd
10/23/2008	79	0.005	na	nd	na	0.002	0.001	0.003
5/19/2009	287	nd	nd	nd	nd	nd	nd	nd
9/29/2009	420	nd	nd	nd	nd	nd	nd	nd

LC water results indicate a presence of glyphosate in groundwater monitoring wells on the day of application and the day immediately following application. The groundwater monitoring wells are approximately 2.6 m below the surface. It is unlikely that glyphosate could migrate through the soil column to a distance of 2.6 m only a few hours after application. Preferential transport of glyphosate via the well annulus combined with the rain event the day of application is a possible explanation for the observations .5 DAT and 1 DAT.

All of the concentrations observed in the groundwater wells and soil lysimeters are extremely low. Considering the issues with the LC at time of analysis, additional analyses should be conducted before conclusions are made.

4.3 Comparison of GC Coastal Results and Continental Results

Results from the coastal and continental study sites differed greatly, especially in the weeks following application. The continental site exhibited first order decay during the growing season and an increase in AMPA with subsequent reduction in glyphosate in surface soils. The coastal study site did not exhibit first order decay throughout the growing season and was reduced to lower concentrations more quickly than the continental study site. Attenuation at the continental site appears to be due to other attenuation mechanisms. The attenuation of glyphosate at the coastal site may be driven by dispersion likely caused from rain events. The coastal study site received 353 mm of rain during the first 60 days following application while the continental study site only received 28 mm. The herbicide was likely diluted and dispersed more quickly due to the rainfall at the coastal study site.

Figure 4-12 records the differences in microbial degradation between the two sites. Mass of AMPA measured in the soil horizon was compared to mass of glyphosate measured in the soil horizon. The continental site has a distinct increase in the ratio of AMPA to glyphosate over time. By the end of the study period, more AMPA was measured in the soil at the continental site than glyphosate. This large ratio of AMPA to glyphosate is a clear indication that microbial degradation is taking place and is a primary factor in the attenuation of glyphosate at the continental site. At the coastal site AMPA to glyphosate ratios are very minor in comparison to the continental site and do not steadily increase over time. The relatively small amount of AMPA

indicates that microbial degradation was not a primary factor in attenuation of glyphosate from the coastal site. It is possible that the glyphosate was dispersed prior to degradation and therefore AMPA was not observed in larger quantities. The only sampling event at the coastal site that exhibited a significant AMPA to glyphosate ratio was on 279 DAT which was after spring thaw. A high ratio of AMPA to glyphosate following spring thaw strengthens the hypothesis that degradation occurred at the coastal location during winter months.

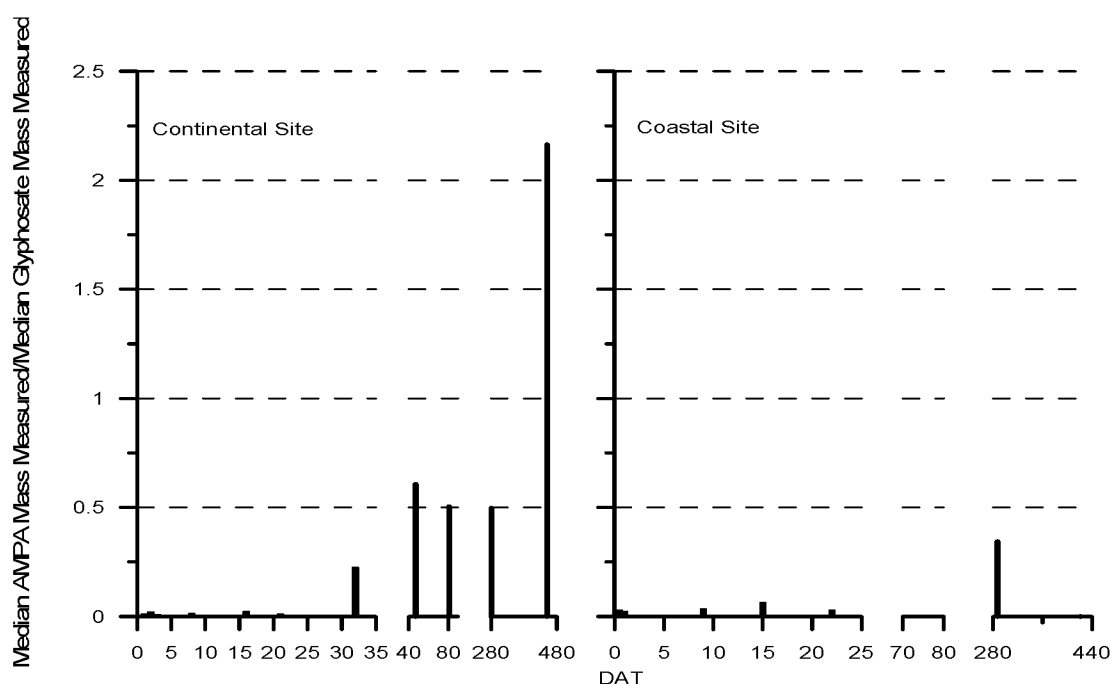


Figure 4-12 Median AMPA Mass vs. Median Glyphosate Mass for Continental and Coastal Sites.

The differences in soil type likely played a role in glyphosate movement. Coarser grained soil at the coastal site compared to the sandy soil at the continental study site may have resulted in more water infiltrating at the coastal site and caused increased dispersion into the subsurface soils. A maximum median concentration of .68 mg/kg was observed in the root zone of the coastal site compared with .18 mg/kg glyphosate at the continental site.

At both sites glyphosate was observed for more than a year. Glyphosate persisted longer in the surface soils at the continental site compared to the coastal site. This is likely due to the winter temperature differences between the two locations. The continuous freezing conditions at the

continental site likely resulted in the extended persistence compared to fluctuating temperatures during the winter at the continental site. Degradation was likely more reduced at the continental site during winter than the coastal site during winter.

4.4 Vegetation Efficacy Results

Vegetation measurements were taken at eight separate plots at the continental study site. AquaMaster[®] was the only herbicide applied at the eight continental plots. Percent cover by species was measured in each of the eight plots on three occasions; pre-application 0 DAT (July 13, 2009), 47 DAT (August 29, 2009), and 430 DAT (September 16, 2010). Average percent cover is presented in Table 4-7. The continental site was dominated by shrub species. Between 0 DAT and 47 DAT all vegetative groups were reduced. Forb species were reduced by 85% and non-native forbs species reduced by 64%. Grasses and shrubs were reduced by 65% and 72%, respectively. Overall cover was reduced by 72%. The total number of species present declined from 16 species 0 DAT to 13 species 47 DAT.

Table 4-7 Continental Site Vegetation Average Percent Cover

Vegetation Group	DAT 0	DAT 47	DAT 230
Invasive Forbes	1.75%	0.64%	8.54%
shrubs	4.26%	1.18%	1.01%
forbs	2.59%	0.39%	2.75%
grass	3.25%	1.13%	6.63%
TOTAL	11.85%	3.33%	18.93%
# species	16	13	15

Based on the percent cover reduction between pre-application and 47 DAT, the herbicide treatment was effective in reducing vegetation cover at the continental site, especially in shrub species. A notable visible difference was observed between the application areas and the non-application areas as shown in Appendix A.

Vegetation measurements recorded at 430 DAT documents the recovery of the vegetation. Native forbs, non-native forbs, and grasses increased in cover but shrubs were still reduced by 72% compared to the pre-application measurement and 14 % when compared to the 47 DAT measurement. Vegetation cover reduction was not expected 430 DAT in non-perennial species because glyphosate is a post-emergence herbicide with no soil activity. The herbicide only kills

or causes injury to actively growing vegetation at the time of application. Once in the soil, there are no herbicidal effects. The vegetation measured 430 DAT likely was not affected by the glyphosate application which occurred more than one year prior.

Although there is no herbicidal activity to vegetation emerging after application, glyphosate may prevent or reduce some plant species from going to seed which would reduce vegetation in subsequent growing seasons. This may explain the continued reduction in shrubs observed as well as the reduction in total number of species present at this site. Shrub reduction observed 430 DAT may also be the result of competition against faster growing forbs and grasses.

Overall, these results indicate that glyphosate adequately controlled vegetation in the railroad corridor for the growing season in which it was applied. The glyphosate application did not have lasting effects on non-perennial vegetation reduction beyond one growing season and would need to be re-applied annually to effectively manage vegetation along railroad corridors.

Due to limited permitted area, vegetation plots at the coastal study site contained more than one herbicide. Each of the 20 plots was treated with AquaMaster[®] (glyphosate) and Oust[®] Extra (sulfometuron methyl + metsulfuron methyl). Oust[®] is effective as a pre and post-emergence herbicide. Vegetation injury is visible within four to six weeks after application. Because the vegetation measurements were not taken until the following year and due to the combination of the herbicides applied at the coastal study site, glyphosate-specific effectiveness cannot be determined.

5. Conclusions

Glyphosate attenuated rapidly from two sub-arctic soils along the Alaska railroad corridor during the growing season, similar to the attenuation of glyphosate in temperate regions. Unlike temperate regions, glyphosate persisted for an extended period in sub-arctic regions in Alaska during the winter months likely due to a reduced degradation rate. Summer applications resulted in significant glyphosate reduction during the growing season which led to low level concentrations at the start of winter. Glyphosate attenuation at the continental site was dominated by microbial degradation while attenuation at the coastal site was likely driven by dispersion and dilution from rain events. In the two study sites, glyphosate had the potential to migrate to the subsurface soils (15-25 cm below the surface), but at much reduced concentrations compared to surface soil concentrations. Movement was more likely at the coastal site; however glyphosate that migrated to the subsurface soils was degraded microbially to AMPA.

Due to herbicide application, vegetation cover at the continental site was reduced 72% 47 DAT. Glyphosate application was most successful in reducing shrub species. As expected, the herbicidal activity lasted one season. Although shrubs continued to be reduced, overall vegetation cover recovered in the following growing season. Glyphosate efficacy of the coastal site could not be determined because more than one herbicide was applied.

Although glyphosate was detected in groundwater wells and vadose zone water at the coastal study site at small concentrations, water results from this study are not conclusive.

5.1 Future Research Needs

Soil type and environmental conditions which drive the behavior of glyphosate and its persistence can vary greatly throughout the region and railroad corridor as noted in differences measured between the continental and coastal study site in this report. Large differences in glyphosate behavior between soils points out the need for continued studies in different soil types including railroad ballast which is underlain in large sections of the of the railroad corridor. Neither of the sites in this study was underlain with ballast typically placed throughout the corridor. Future research should involve application to ballast.

Future research of this topic could benefit from a method of computing mass balance and determining how much herbicide is taken up by vegetation. This research and other sub-arctic research warrant the investigation into the mechanisms of herbicide behavior during winter freeze-up and spring thaw. Vegetation residues should be analyzed to track seasonal changes and to assist in mass balance calculations.

Application timing is important to the attenuation of glyphosate, especially in regions where freezing soil conditions are typical. Persistence and movement in soil could be altered if glyphosate was applied in the fall when there is less time to attenuate before winter freeze-up. Application timing should be investigated in future assessments.

Future research needs also include the completion of groundwater and vadose zone water analyses. Additional monitoring of waters outside the application zone to determine movement in subsurface waters could also be helpful in understanding of the movement of the herbicide glyphosate in Alaska.

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Appendix A-Continental Site Information

Table A-1 Coastal Site Species List. Species list provided by the USDA Subarctic Agricultural Research unit.

Common Name	Scientific Name
Common yarrow	<i>Achillea millefolium</i>
Siberian yarrow	<i>Achillea sibirica</i>
Alder	<i>Alnus sp.</i>
Alpine milkvetch	<i>Astragalus alpinus</i>
Birch	<i>Betula papyrifera</i>
Narrowleaf hawksbeard	<i>Crepis tectorum</i>
Pink	<i>Dianthus sp.</i>
Fireweed	<i>Epilobium angustifolium</i>
Grass	<i>Grass</i>
Eskimo potato	<i>Hedysarum alpinum</i>
Foxtail barley	<i>Hordeum jubatum</i>
Toadflax	<i>Linaria vulgaris</i>
White sweet clover	<i>Melilotus albus</i>
Moehringia	<i>Moehringia lateriflora</i>
Locoweed	<i>Oxytropis sp.</i>
Alpine groundsel	<i>Packera pauciflora</i>
Spruce	<i>Picea sp.</i>
Balsam poplar	<i>Populus balsamifera</i>
Quaking aspen	<i>Populus tremuloides</i>
Staghorn cinquefoil	<i>Potentilla bimundorum</i>
Norwegian cinquefoil	<i>Potentilla norvegica</i>
Nagoonberry	<i>Rubus arcticus</i>
Raspberry	<i>Rubus idaeus</i>
Willow	<i>Salix L.</i>
Dandelion	<i>Taraxacum officinale</i>



Figure A-1 Continental Study Site Vegetation Day of Application Photograph. Herbicide application via ATV. Site was treated AquaMaster[®] (glyphosate).



Figure A-2 Continental Site Vegetation Photograph DAT 47.

Appendix B-Coastal Site Information

Table B-1 Coastal Site Species List. Species list provided by the USDA Subarctic Agricultural Research unit.

Common Name	Scientific Name
Common yarrow	<i>Achillea millefolium</i>
Alder	<i>Alnus sp.</i>
Tilesius wormwood	<i>Artemisia tilesii</i>
Aster sp.	<i>Aster L.</i>
Dogwood	<i>Cornus canadensis</i>
Narrowleaf hawksbeard	<i>Crepis tectorum</i>
Strawberry	<i>Fragaria virginiana</i>
Grass	<i>Grass</i>
Beach pea	<i>Lathyrus japonicus</i>
Beach pea 2	<i>Lathyrus L.</i>
Toadflax	<i>Linaria vulgaris</i>
White spruce	<i>Picea glauca</i>
Common plantain	<i>Plantago major</i>
Snow cinquefoil	<i>Potentilla nivea</i>
Norwegian cinquefoil	<i>Potentilla norvegica</i>
Sheep sorel	<i>Rumex acetosella</i>
Willow	<i>Salix L.</i>
Dandelion	<i>Taraxacum officinale</i>



Figure B-1 Coastal Site Day of Application Photograph. Site was treated with AquaMaster[®] (glyphosate) and Oust[®] Extra (sulfometuron methyl + metsulfuron methyl).

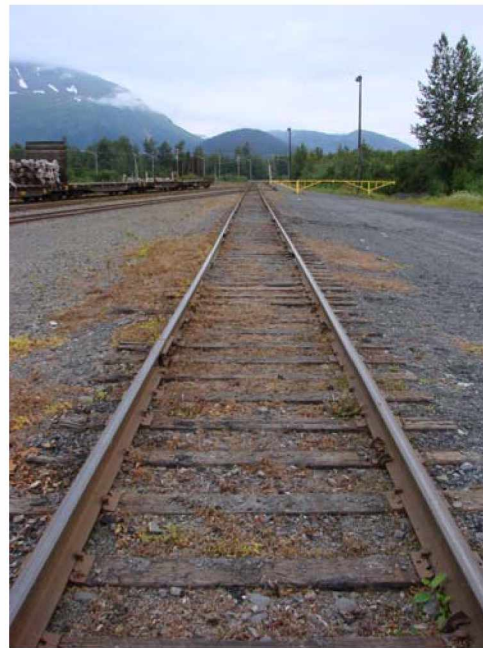


Figure B-2 Coastal Site Photograph 24 DAT. Vegetation treatment of AquaMaster[®] + Oust[®].

Appendix C-Sampling Diagram

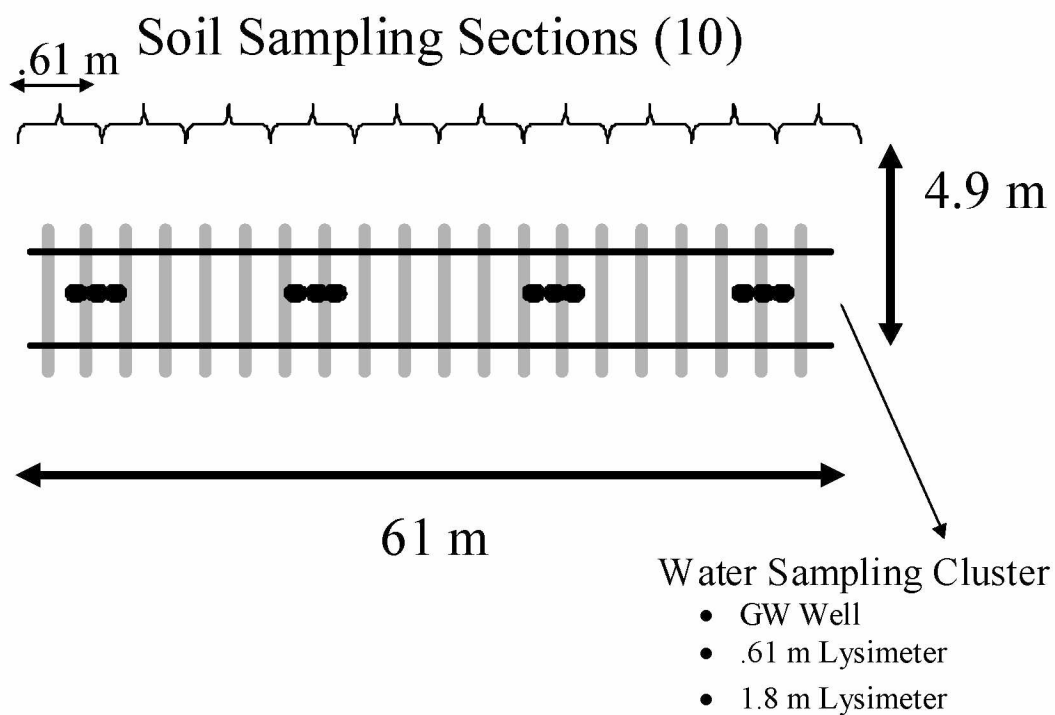


Figure C-1 Site Sampling Diagram. The herbicide spray zone was approximately 61 m long, parallel to the tracks. The spray zone included the area between the tracks. The 61 m section was divided into ten, 6.1 m sections. On each sampling event, six of the ten sections were chosen at random to sample. Three samples were taken at different depths (surface, root and below root) at each of the selected six sections. At each site there were four water sampling clusters consisting of a groundwater well, a .61 m lysimeter, and 1.8 m lysimeter.

Appendix D-Continental Site Soil Results

Table D-1 Complete Glyphosate GC Data Set from Continental Site. nd=non-detect, na=not collected
sc=surface composite.

Date	DAT	Section							sc
		1	2	3	4	5	6		
Surface (mg/kg)									
7/13/2009	0.5	1.62	0.01	1.22	3.93	6.25	0.32	na	
7/14/2009	1	0.51	1.44	2.97	0.91	1.59	1.29	1.61	
7/15/2009	2	1.37	4.78	0.52	3.88	0.54	0.11	0.50	
7/16/2009	3	4.36	1.62	nd	1.63	1.32	0.36	1.07	
7/21/2009	8	1.72	1.93	0.82	1.17	0.49	0.92	0.73	
7/29/2009	16	0.95	2.85	2.97	0.43	0.34	0.77	0.63	
8/3/2009	21	1.40	0.21	0.55	1.39	0.83	1.33	1.27	
8/14/2009	32	0.53	0.30	1.19	0.21	0.36	0.57	0.49	
8/29/2009	47	0.03	0.07	0.16	0.22	0.33	0.15	0.31	
10/2/2009	81	0.17	0.21	0.13	0.13	0.16	0.08	0.22	
4/20/2010	281	0.01	0.23	0.35	0.15	0.22	0.25	na	
10/6/2010	450	0.04	0.20	0.11	0.05	0.12	0.07	na	
Root (mg/kg)									
7/13/2009	0.5	na	na	na	na	na	na		
7/14/2009	1	0.03	0.03	0.15	0.03	0.09	0.09	nd	
7/15/2009	2	0.03	0.40	0.11	0.17	0.17	0.01		
7/16/2009	3	nd	nd	0.04	nd	0.01	nd		
7/21/2009	8	0.25	0.12	0.04	0.04	0.24	0.43		
7/29/2009	16	0.01	0.33	0.04	0.15	0.01	0.21		
8/3/2009	21	0.01	0.02	1.83	nd	0.04	0.02		
8/14/2009	32	nd	nd	nd	nd	nd	nd		
8/29/2009	47	0.08	0.03	0.43	0.01	0.02	0.04		
10/2/2009	81	nd	nd	nd	nd	0.02	nd		
4/20/2010	281	nd	nd	nd	nd	nd	nd		
10/6/2010	450	0.02	0.01	0.03	0.01	0.03	0.02		
Below Root (mg/kg)									
7/13/2009	0.5	na	na	na	na	na	na		
7/14/2009	1	0.27	0.34	0.30	0.23	0.27	0.02		
7/15/2009	2	0.52	0.30	0.51	1.47	1.06	0.14		
7/16/2009	3	0.82	0.05	0.31	0.15	0.01	0.03		
7/21/2009	8	0.04	0.80	0.02	0.10	0.38	0.26		
7/29/2009	16	0.01	nd	0.01	0.04	0.01	0.27		
8/3/2009	21	0.03	0.02	0.01	0.01	0.10	0.03		
8/14/2009	32	0.13	0.01	0.02	0.02	0.23	0.01		
8/29/2009	47	0.10	0.02	0.05	0.27	0.03	0.13		
10/2/2009	81	0.02	0.04	nd	0.01	0.11	0.01		
4/20/2010	281	0.01	0.01	0.06	0.03	0.05	0.01		
10/6/2010	450	nd	0.01	nd	nd	nd	nd		

Table D-2 Complete AMPA GC Data Set from Continental Site. nd=non-detect, na=not collected
sc=surface composite.

Date	DAT	Section						
		1	2	3	4	5	6	sc
Surface (mg/kg)								
7/13/2009	0.5	nd	nd	nd	nd	0.02	nd	na
7/14/2009	1	0.01	nd	nd	0.01	0.03	0.01	0.02
7/15/2009	2	nd	0.04	0.03	0.02	0.05	nd	0.01
7/16/2009	3	0.04	0.01	0.00	0.02	0.01	0.01	0.01
7/21/2009	8	0.01	0.02	0.01	0.01	nd	0.01	0.01
7/29/2009	16	0.02	0.08	0.07	0.01	nd	0.02	0.01
8/3/2009	21	0.01	nd	0.01	0.03	0.01	0.03	0.02
8/14/2009	32	0.13	0.05	0.31	0.04	0.07	0.12	0.10
8/29/2009	47	nd	0.08	0.19	0.26	0.16	0.06	0.06
10/2/2009	81	0.04	0.16	0.07	0.07	0.08	0.05	0.09
4/20/2010	281	0.05	0.09	0.14	0.06	0.17	0.13	na
10/6/2010	450	0.12	0.58	0.32	0.16	0.25	0.18	na
Root (mg/kg)								
7/13/2009	0.5	na	na	na	na	na	na	
7/14/2009	1	nd	nd	nd	nd	nd	nd	
7/15/2009	2	nd	0.01	nd	nd	nd	nd	
7/16/2009	3	nd	nd	nd	nd	nd	nd	
7/21/2009	8	nd	0.01	0.01	nd	0.01	0.01	
7/29/2009	16	nd	nd	nd	0.05	nd	nd	
8/3/2009	21	nd	nd	1.75	nd	nd	nd	
8/14/2009	32	nd	nd	nd	nd	nd	nd	
8/29/2009	47	0.02	0.07	0.20	0.03	nd	0.01	
10/2/2009	81	nd	nd	nd	nd	nd	nd	
4/20/2010	281	nd	nd	nd	nd	nd	nd	
10/6/2010	450	0.06	0.01	nd	0.01	0.04	0.05	
Below Root (mg/kg)								
7/13/2009	0.5	na	na	na	na	na	na	
7/14/2009	1	0.01	0.01	0.02	0.02	0.01	nd	
7/15/2009	2	0.01	0.01	0.02	0.03	0.06	nd	
7/16/2009	3	0.01	nd	0.01	nd	nd	nd	
7/21/2009	8	nd	0.02	nd	nd	0.01	nd	
7/29/2009	16	nd	nd	nd	0.01	nd	nd	
8/3/2009	21	nd	nd	nd	nd	nd	nd	
8/14/2009	32	0.01	nd	nd	nd	0.02	nd	
8/29/2009	47	0.02	0.01	0.01	0.11	nd	0.03	
10/2/2009	81	0.01	0.03	nd	nd	0.04	nd	
4/20/2010	281	0.01	nd	0.03	0.01	0.02	0.01	
10/6/2010	450	0.01	0.01	nd	nd	nd	nd	

Appendix E-Coastal Site Soil Results

Table E-1 Complete Glyphosate GC Data Set from Coastal Site. nd=non-detect, na=not collected sc=surface composite

Date	DAT	Section						
		1	2	3	4	5	6	sc
Surface (mg/kg)								
8/4/2008	0.5	nd	2.05	0.56	1.47	nd	2.72	3.29
8/5/2008	1	0.16	0.76	2.63	2.86	1.96	1.66	2.51
8/8/2008	4	0.17	nd	0.33	0.10	0.75	0.10	1.29
8/13/2008	9	0.48	2.08	3.04	0.18	0.05	0.33	1.82
8/19/2008	15	nd	0.09	0.01	nd	0.27	0.01	0.03
8/26/2008	22	nd	0.04	0.08	0.01	0.18	0.07	0.19
10/22/2008	79	nd	nd	nd	nd	0.02	nd	nd
5/18/2009	287	nd	nd	0.05	nd	0.01	na	0.22
9/29/2009	421	0.17	nd	0.01	0.01	0.03	na	0.01
Root (mg/kg)								
8/4/2008	0.5	nd	0.02	nd	0.09	0.01	0.03	
8/5/2008	1	0.02	0.07	0.26	0.04	nd	0.02	
8/8/2008	4	0.03	nd	0.16	0.11	0.21	0.04	
8/13/2008	9	1.07	2.59	0.17	0.85	0.01	0.13	
8/19/2008	15	nd	0.01	0.02	0.26	0.01	0.08	
8/26/2008	22	0.11	1.03	1.26	nd	1.02	0.33	
10/22/2008	79	0.01	nd	nd	0.07	nd	0.03	
5/18/2009	287	0.01	0.08	0.22	0.05	0.16	na	
9/29/2009	421	0.01	nd	nd	nd	nd	na	
Below Root (mg/kg)								
8/4/2008	0.5	0.01	0.05	nd	0.02	1.30	0.01	
8/5/2008	1	nd	nd	0.17	nd	nd	0.12	
8/8/2008	4	nd	nd	0.01	nd	nd	0.04	
8/13/2008	9	0.35	0.60	0.12	0.11	0.03	0.16	
8/19/2008	15	0.15	0.09	nd	0.40	0.05	0.06	
8/26/2008	22	0.22	0.51	0.20	0.10	0.18	0.37	
10/22/2008	79	0.01	nd	nd	0.01	nd	nd	
5/18/2009	287	0.03	0.09	0.09	0.05	nd	na	
9/29/2009	421	0.02	nd	nd	nd	nd	na	

Table E-2 Complete AMPA GC Data Set from Coastal Site. nd=non-detect, na=not collected, sc=surface composite.

Date	DAT	Section						
		1	2	3	4	5	6	sc
Surface (mg/kg)								
8/4/2008	0.5	nd	0.05	0.01	0.05	nd	0.09	0.02
8/5/2008	1	0.01	0.01	0.09	0.07	0.05	0.04	0.04
8/8/2008	4	nd	nd	nd	nd	0.02	nd	0.04
8/13/2008	9	0.01	0.13	0.17	0.01	nd	0.01	0.09
8/19/2008	15	nd	0.01	nd	nd	0.02	0.01	nd
8/26/2008	22	nd	nd	nd	nd	0.01	nd	0.01
10/22/2008	79	nd	nd	nd	nd	nd	nd	nd
5/18/2009	287	nd	nd	0.01	0.17	0.01	na	0.05
9/29/2009	421	0.01	nd	nd	nd	nd	na	nd
Root (mg/kg)								
8/4/2008	0.5	nd	nd	nd	nd	nd	nd	
8/5/2008	1	nd	0.03	0.01	nd	nd	nd	
8/8/2008	4	nd	nd	nd	0.01	0.01	nd	
8/13/2008	9	0.04	0.15	0.01	0.04	nd	0.01	
8/19/2008	15	nd	nd	nd	0.04	nd	nd	
8/26/2008	22	nd	0.06	0.05	nd	0.04	nd	
10/22/2008	79	nd	nd	nd	0.02	nd	nd	
5/18/2009	287	nd	0.02	0.02	0.01	0.03	na	
9/29/2009	421	0.01	nd	nd	nd	nd	na	
Below Root (mg/kg)								
8/4/2008	0.5	0.01	0.01	nd	nd	0.01	nd	
8/5/2008	1	nd	nd	nd	nd	nd	nd	
8/8/2008	4	nd	nd	nd	nd	nd	nd	
8/13/2008	9	0.01	0.03	0.01	0.01	nd	0.01	
8/19/2008	15	0.04	0.01	nd	0.41	0.01	0.01	
8/26/2008	22	nd	0.03	0.01	0.01	0.01	0.01	
10/22/2008	79	nd	nd	nd	0.04	nd	0.01	
5/18/2009	287	nd	0.01	0.01	0.01	nd	na	
9/29/2009	421	0.01	nd	nd	nd	nd	na	