GLYPHOSATE AND AMPA ACCUMULATION

IN *CAMASSIA QUAMASH* BULBS

OF THE COAST SALISH PRAIRIES

by

Claire M. Kerwin

A Thesis
Submitted in partial fulfillment
of the requirements for the degree
Master of Environmental Studies
The Evergreen State College
September 2023

This Thesis for the Master of Environmental Studies Degree

by

Claire M. Kerwin

has been approved for

The Evergreen State College

by

____

Sarah T. Hamman, Ph.D.

Member of Faculty

____

Date

ABSTRACT:

Glyphosate and AMPA Accumulation in *Camassia Quamash* Bulbs of the Coast Salish Prairies

This research investigates the accumulation of glyphosate and its metabolite, aminomethylphophonic acid (AMPA), in *Camassia quamash* bulbs inhabiting Coast Salish Prairies surrounding Olympia, Washington. *Camassia quamash* is a culturally and ecologically significant geophyte endemic to Coast Salish Prairies. As a result of Euro-American attempts to erase Indigenous peoples and lifeways, the reciprocal relationship by which *Camassia quamash*, Coast Salish Prairies, and Indigenous peoples thrive has been eroded. Coast Salish Prairies are presently scarred, dwindling ecosystems, many of which are in active ecological restoration. Herbicides are a key and effective restoration tool in countering the opportunistic, non-native plant species that have displaced native species. Glyphosate-based herbicides are used in ecological restoration efforts and may be accumulating in perennial plants, including the edible *Camassia quamash*. As emerging research shows that consuming glyphosate and AMPA has detrimental health implications, it is necessary to understand if either are accumulating in *Camassia quamash* bulbs. This in vivo study, specific to the ecological, soil, and climatic conditions of prairies surrounding Olympia, WA, found no glyphosate or AMPA in bulbs 4.5 months after 0.96% glyphosate treatments and 17 months after a 2.5% glyphosate treatment. These results should be received with caution and not extrapolated to other regions or species. The soils at treatment sites were “somewhat excessively drained” in Andisols and Inceptisols soil orders. The top 10cm of soil had low pH (4.7-5.4), low clay content (0-6%), and high organic matter content (16.5-31%). Glyphosate-based herbicides were applied in the winter, during the prairies’ rainy season and while *Camassia quamash* was dormant, not directly exposing the plant to the herbicide. I speculate that application during rains, application while *Camassia quamash* was dormant, somewhat excessively drained soil, and soil with low clay content led to glyphosate and AMPA being leached from soil rather than accumulating in *Camassia quamash* bulbs.

Table of Contents:

List of Figures:

List of Tables:

Acknowledgements:

I would like to acknowledge those who supported me on my journey towards a Master of Environmental Studies degree and completing this research. First to my husband, Wayne Dillon, who put his personal aspirations on hold to support us getting closer to the mountain. To my creatures, Theodore and K'wichadi, for providing endless entertainment and recharging me. To the forest that I call home and to the prairies that I waded through for this research. To the Indigenous peoples who tended these ecosystems long before I came to be, since time immemorial. To the camas that I harvested and to the ones that I broke in the process. To my parents, for encouraging and nudging me along the way. To the Chehalis Tribe, for allowing me to harvest camas on their lands and for partially funding this research. To William Thoms, Chehalis, and Dan Penn, Chehalis, for welcoming me, having a laugh, and playing some great games of phone tag. To CNLM and Sanders Freed; WDFW and Josh Cook, Maurice Major, and Bill Kronland; to JBLM, Gina Smith, and Tim Atkinson. To AGQ Labs and B. Jones for stellar communication and answering my every question. To my funding sources, including the Chehalis Tribe, Washington Native Plant Society, and The Evergreen State College Master of Environmental Studies Thesis Fund. To my thesis reader, Dr. Sarah Hamman, for all of the work that she does tending the prairies, the human relationships with them, and the work that I did here. To the Science Support Center staff for the two weeks that I spent in and out of the office. To everyone that I talked incessantly at about camas and herbicides. And to Beyonce, Bad Bunny, and Rosalia for keeping me company and lifting my spirits.

Introduction

Camassia quamash (camas) is a culturally and ecologically significant geophyte endemic to Coast Salish Prairies (Beckwith, 2004). The bulb is traditionally a staple source of carbohydrates for many Coast Salish Tribes and has historically been traded across Tribal Nations (Turner & Kuhnlein, 1983). Since the early 1800s, Euro-American attempts at erasure of Indigenous peoples and lifeways have been detrimental to the reciprocal relationships by which prairies, camas, and humans have thrived since time immemorial (Dunwiddie & Bakker, 2011; Turner & Kuhnlein, 1983).

Coast Salish Prairies are now scarred, dwindling ecosystems, with just 3% of the historic 1.2 million acres remaining. Opportunistic, non-native plant species have taken hold throughout the prairies and are at the forefront of restoration priorities (Dennehy et al., 2011). Herbicides are a key tool in keeping these species at bay and allowing camas and other species integral to prairie habitats to thrive (Dennehy et al., 2011). For over 10 years, glyphosate-based herbicides (GBHs) have been used in tandem with prescribed burns and native plant seeding, an effective protocol based on research by Stanley et al. (2011). GBHs are non-selective herbicides used commonly in ecological restoration (Wagner et al., 2017), but they are known to accumulate in perennial plants in temperate ecosystems (Botten et al., 2021). There is concern amongst Indigenous peoples and restoration practitioners that glyphosate and its metabolite, aminomethylphophonic acid (AMPA), may be accumulating in cultural keystone species like camas (Botten et al., 2021; Garibaldi & Turner, 2004).

While the pathway through which glyphosate kills plants does not exist in animals, it does exist in bacteria and fungi (Rivas-Garcia et al., 2022). Emerging research suggests that chronically consuming low, regulatory doses of glyphosate and metabolites kills bacteria in animal gut microbiomes, causing detrimental systemic effects (van Bruggen et al., 2021). Many of the sites where Indigenous peoples harvest camas are currently or have previously been treated with GBHs and, if glyphosate and AMPA are accumulating in perennial food plants, people and animals consuming the plants may be facing health risks.

To support Indigenous peoples in determining risks of consuming camas bulbs exposed to GBHs and to inform restoration practitioners as to the risk factors of using GBHs in camas prairie habitats, my research seeks to answer the question, "How does the legacy of glyphosate application to a *Camassia quamash* habitat affect the concentrations of glyphosate in *Camassia quamash* bulbs?"

I designed a robust field study that analyzed glyphosate and AMPA concentrations in camas bulbs exposed to a GBH treatment compared to camas bulbs not exposed to a GBH treatment across 6 different sites. I sent camas bulb samples to an off-site lab for glyphosate and AMPA concentration analysis. To account for site specific confounding variables that have been shown to affect glyphosate and AMPA uptake in plants, I sampled soils and tested their pH levels (Gimsing, Borggaard, & Bang, 2004), clay content, and organic matter content (Miles & Moye, 1988). Additionally, I utilized the US Department of Agriculture Natural Resources Conservation Service's Web Soil Survey to determine each site's soil drainage class, soil order, and soil taxonomy. I accessed data from Western Washington's Regional Climate Center to assess weather patterns between GBH treatment and bulb sampling.

I had four criteria when prioritizing sites for this study: (1) the site is culturally significant to Indigenous peoples; (2) the site is currently harvested or could be harvested by Indigenous peoples; (3) the site met the GBH treatment requirements; and (4) the site had a very low risk of being exposed to GBHs through air-drift from neighboring treatment sites, assessed through a GIS analysis.

Of the three GBH exposed sites, two are undergoing ecological restoration with the goal of future traditional harvest, while the other is owned by the US Military and will likely not be harvested from in the near future. Of the three non-exposed sites, two are on Chehalis Tribal lands and one is on non-Tribally owned land but is annually harvested by inter-Tribal gatherers. The 3 exposed sites were treated separately: Site 1 with a GBH treatment 4.5 months prior to testing (0.96% glyphosate treatment); Site 2 with a GBH treatment 5 months prior to testing (0.96% glyphosate treatment); and Site 3 with a GBH treatment 17 months prior to testing (2.5% glyphosate treatment). The 0.96% glyphosate formula is lower than the standard concentration of glyphosate used in restoration ecology, which is more in line with a 1.5-2.5% glyphosate GBH formula (Stanley et al., 2011). All sites had a low or very low risk of being exposed to GBHs by air-drift. Sites were located within a 32 mile radius of Olympia, Washington.

No detectable glyphosate or AMPA was found in camas bulbs at sites exposed or not exposed to glyphosate. These results should be received with caution and are specific to Olympia, WA area Coast Salish Prairie soil characteristics, climate, and ecology, as well as the timing and percent glyphosate used in GBH formulas. Soils at exposed sites were found to be acidic (pH levels 4.7-5.4), with low clay content (0-6%) and high organic matter content (16.5-31%). Web Soil Survey data showed that soils were "somewhat excessively drained", of Andisols or Inceptisols soil orders, and classified as sandy-skeletal taxonomy (NRCS, 2019). GBH exposed sites were treated in late December-January, during months of consistent precipitation and generally above-freezing temperatures (NOAA, 2023). Additionally, *Camassia quamash* was dormant during treatment and the plants were not directly exposed to a GBH.

The results suggest that, under the specific circumstances above, there is no risk of glyphosate or AMPA consumption when consuming *Camassia quamash* bulbs 4.5 months after a 0.96% glyphosate GBH treatment and 17 months after a 2.5% glyphosate GBH treatment. The results are dependent on species, site conditions, climate, and treatment timing. I speculate that the GBH was efficiently leached from the somewhat excessively drained soil during precipitation events, rather than adsorbing to clay and organic matter particles, thus being bioavailable to plants, or being absorbed by *Camassia quamash*. The potential implications of this study, that *Camassia quamash* bulbs are safe to consume regarding this study's glyphosate and AMPA exposure, should not be extrapolated to other circumstances or species.

Chapter 2: Literature Review

Introduction

In this literature review, I will explore the morphology and ecology of *Camassia quamash,* followed by an ethnobotanical summary of camas as a cultural keystone species. I will then examine the ecology, vulnerability, and restoration efforts of the Coast Salish Prairies, where *Camassia quamash* is an integral component of the ecosystem. After establishing grounds for ecological restoration efforts of the prairie habitat, I will investigate the usage of glyphosate and GBHs as a stewardship tool. I will explain the mechanism by which glyphosate functions as an herbicide, then shift to its persistence in soil and accumulation in plants. Finally, I will conclude with regulation of glyphosate, the herbicide and its metabolite's toxicity, and the health implications of their consumption.

2.1 Camas

Camassia quamash, known commonly as camas, is the most widespread and abundant species in the 6-species *Camassia* genus (Chase et al., 2009; Matthews, 2020; Turner & Kuhnlein, 1983). Endemic to North America, the perennial bulbous genus is in the subfamily of Agavoideae, within the Asparagaceae family (Chase et al., 2009). The Coast Salish Prairies are home to a number of subspecies of *Camassia quamash,* as well as the species *Camassia leichtlinii,* or great camas (Beckwith, 2004; Davis, 2018). Research presented here focuses on *Camassia quamash*, the most common and traditionally harvested species on the Coast Salish Prairies (Gould, 1942; Turner & Kuhnlein, 1983).

2.1.A Morphology and Lifecycle

Camas, a geophyte, has an underground perennial bulb that produces a basal whorl of waxy green, linear leaves aboveground (Figure 2-1) (Beckwith, 2004; Davis, 2018). Three to five years after the monocot's germination, a terminal raceme with conspicuous, liliaceous flowers grows from the bulb (Beckwith, 2004; Gould, 1942). The flowers have 6 tepals that range in color from deep violet to light blue to white, with all flowers on a plant typically one shade (Davis, 2018). Each flower has 6 stamens and a three-celled ovary that matures to a tri-locular capsuled fruit containing shiny black seeds (Davis, 2018; Turner & Kuhnlein, 1983). Camas reproduces primarily through seed, but asexual reproduction from offset bulbs has been observed (Thoms, 1989).

Figure 0-1 Camassia Quamash illustration (Kerwin, 2022)

Non-branching, adventitious roots and a contractile root emerge from the basal plate of the bulb (Kawa & De Hertogh, 1992). First developing during the plant's second growing season (Davis, 2018), the contractile root grows and shrinks with moisture, essentially pulling the bulb deeper into the soil and anchoring it at an ideal depth (Kawa & De Hertogh, 1992). As the plant ages, the bulb size increases, as does the number of leaves, number of flowers, and stalk height (Beckwith, 2004).

Maclay (1928), described camas's annual lifecycle in which every organ is replaced, with the possible exception of the basal plate that the bulb's roots and fleshy scales are attached to. At any one-time, mature camas bulbs have multiple years of bulb growth within them, known as the mother bulb, the daughter bulb, and the granddaughter bulb. The mother bulb is the most outer layers of scales, while the granddaughter is the most inner, and each has varying carbohydrate concentrations dependent on their age. In the winter, before leaves emerge, the mother bulb is encased in a tunic: a thin, nearly disintegrated, dark colored tissue that is the remnants of the previous year's growth. The mother bulb's scales are the base of the previous year's leaves and are rich in carbohydrates and nutrients. They encase the daughter bulb, from which leaves extend mid-spring, when prairie soils are saturated and cold. In late spring, the daughter bulb's inner scales erupt into a stem topped by the plant's florescence (Maclay, 1928). A terminal bud develops in the center of the bulb, becoming the granddaughter bulb (Thoms, 1989). Shortly thereafter, in early summer, the flowers rapidly go to seed, the stems and leaves senesce, and the plant becomes dormant (Maclay, 1928). The entire bulb is at its largest just before seeds develop (Davis, 2018). The mother bulb then withers, becoming the new tunic, the daughter bulb sustains the plant through the winter as the new mother bulb, and the granddaughter becomes the daughter (Maclay, 1928; Thoms, 1989). Camas will continue this life cycle for an indefinite period of time (Maclay, 1928) and may sustain dormancy for years (Matthews, 2020).

2.1.A Ecology and Range

Camassia quamash is found in prairies, woodlands, and meadows at elevations less than 10,800 ft from Northern California north to Vancouver Island, and eastward to Northern Utah and Montana (Figure 2-2) (Gould, 1942; Turner & Kuhnlein, 1983). Carbon-dated pollen evidence shows that *Camassia* species have been present in the Pacific Northwest region of the United States for over 70,000 years (Florer, 1972). The plant's range was likely expanded by glaciation and natural dispersal, as well as by Indigenous peoples planting seeds and bulbs as they migrated and traded (Turner & Kuhnlein, 1983).

Tying camas's current habitats together is a temperate wet-dry seasonal cycle (Chance et al., 1977 as cited in Thoms, 1989). This climatic pattern aligns with saturated or near saturated soils in the beginning of the camas growing season, followed by a dry summer as the plant goes dormant (Beckwith, 2004). Camas thrives in open, exposed areas that are seasonally basked in sunlight (Thoms, 1989), and the depth and composition of camas habitat's soils vary, ranging from deep, organic matter rich prairies to rocky outcroppings (Beckwith, 2004; Matthews, 2020). Wherever camas is found, Indigenous peoples have likely historically had a hand in the plant's success, owing to traditional harvesting and tending practices (Turner et al., 2021).

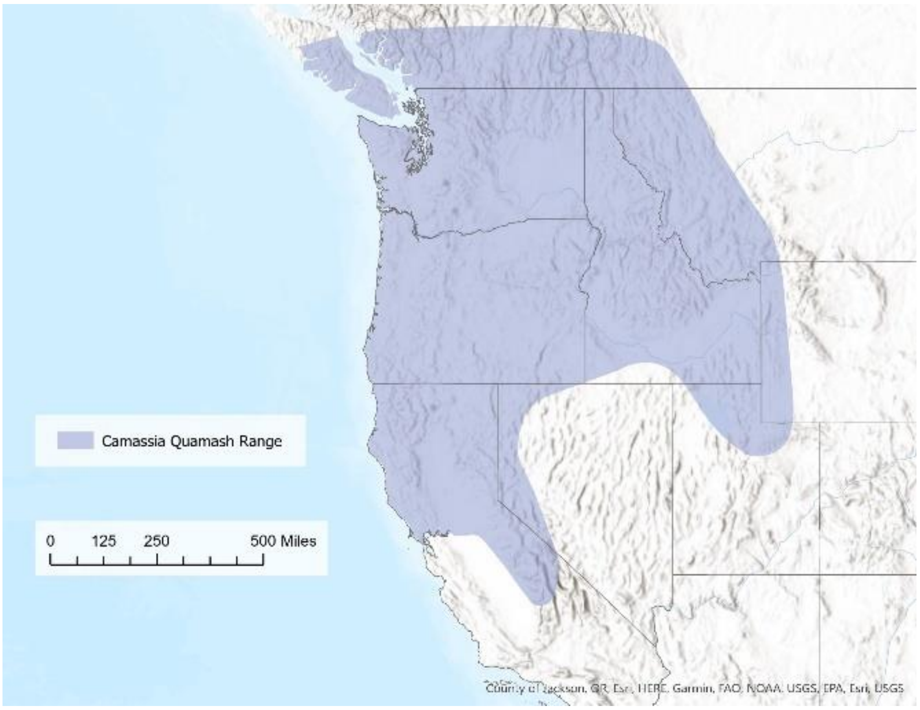

Figure 0-2: Map of Camassia quamash range

<u>2.1.A Ethnobotany</u>

Since time immemorial, Coast Salish peoples have had a symbiotic relationship with camas (Turner, 2014). Indigenous Knowledge unique to tribes and formed over generations informs the harvest, tending, treatment, cooking, and celebration of camas (Turner et al., 2021;

Turner & Kuhnlein, 1983). Once a staple source of carbohydrates to many tribes, the population and health of the plant has suffered without this relationship with Indigenous peoples (Garibaldi & Turner, 2004; Matthews, 2020).

Camas bulbs were once consumed in great quantity and traded across Tribal Nations (Beckwith, 2004; Turner & Kuhnlein, 1983). Their success as a species is intertwined with traditional tending practices of selective harvesting, digging techniques, use of fire, and other horticultural methods (Turner & Kuhnlein, 1983). Digging techniques gently till and aerate soil, promoting soil microbiome health, nutrient cycling, and root growth (Matthews, 2020; Turner & Kuhnlein, 1983). Cyclically burning prairies prevents encroachments of surrounding forests, staves off establishment of unwanted species, and introduces additional nutrients to the ecosystem (Matthews, 2020; Turner & Kuhnlein, 1983). Selective harvesting reduces intraspecies competition and allows spaces for young camas to grow (Anderson, 2005; Maclay, 1928; Thoms, 1989). Additionally, and perhaps most significantly, the Indigenous reciprocal relationship with and reverence for camas habitat cannot be overlooked as a beneficial tending practice (Turner, 2020).

Depending on the Tribal Nation, climate, and species, *Camassia* is traditionally harvested sometime between late spring, when the flowers are beginning to fade, and the fall, when the plant is largely dormant (Turner & Kuhnlein, 1983). Bulbs are at their peak caloric benefit just before the plant goes to seed in the summer (Thoms, 1989). In the Coast Salish Prairie region, harvests typically take place in the late spring, when the flowers and seed heads are identifiable and the soil is still moist enough to dig (W. Thoms, Chehalis Tribe, personal communication, June 6, 2023).

Camas bulbs are largely composed of inulin, a sugar indigestible to humans without substantial cooking (Turner & Kuhnlein, 1983). The Indigenous long, slow cooking process converts the inulin into fructose, a nutritionally available carbohydrate (Konlande & Robson, 1972). Depending on the growing region, cooked camas bulbs can have more protein than beef liver, beans, or potatoes (Scrimsher, 1967), as well as a significant amount of fiber and trace nutrients (Konlande & Robson, 1972).

The loss of access to this traditional food and cultural keystone species has been detrimental to both Indigenous peoples (Blanchet et al., 2021; Garibaldi & Turner, 2004) and to camas ecosystems (Willamette Partnership, 2020). Camas populations and habitats have plummeted with the lack of a reciprocal relationship with humans (Matthews, 2020), land use change (Willamette Partnership, 2020), and systematic decimation of Indigenous Knowledge holders (Boyd, 1990, as cited in Beckwith, 2004).

2.2 Coast Salish Prairies

Coast Salish Prairies spanned 73,000 hectares of Western Washington state prior to Euro-American colonization in the mid 1800s (Chappell et al., 2001). They are home to Indigenous peoples and camas (Willamette Partnership, 2020), as well as a biodiverse plant and fungal community that supports a plethora of wildlife (Fuchs, 2001). The degradation of these prairies can be attributed to removal of Indigenous land tending practices, change in land use, industrial development, climate change, and opportunistic non-native plants (Willamette Partnership, 2020). Recognition of Coast Salish Prairies as cultural landscapes and biodiverse systems has led to an Indigenous and non-Indigenous focus on the ecological restoration of the ecosystems (Dunwiddie & Bakker, 2011).

2.2.A Ecology

Coast Salish Prairies are part of the larger prairie-oak woodland system that spans from Southern British Columbia latitudinally to the Willamette Valley (Figure 2-3) (Dunwiddie & Bakker, 2011), referred to as the Willamette Valley-Puget Trough-Georgia Basin ecoregion (WPG) (Dennehy et al., 2011); Floberg et al.,2004). The ecoregion is further classified into three subregions, with Coast Salish Prairies found in the Puget Trough and Georgia Basin. Compared to their southern neighbor, the Puget Trough and Georgia Basin do not have wetland prairies, though the soil is nearly saturated during the rainy season. Soils here were deposited by glacial outwash, resulting in a coarse texture and a landscape that is flat to rolling. Coast Salish Prairies are more sporadic than those in the Willamette Valley, as they are in smaller valleys surrounded by Douglas fir, western hemlock, and western redcedar dominated forests (Floberg et al., 2004).

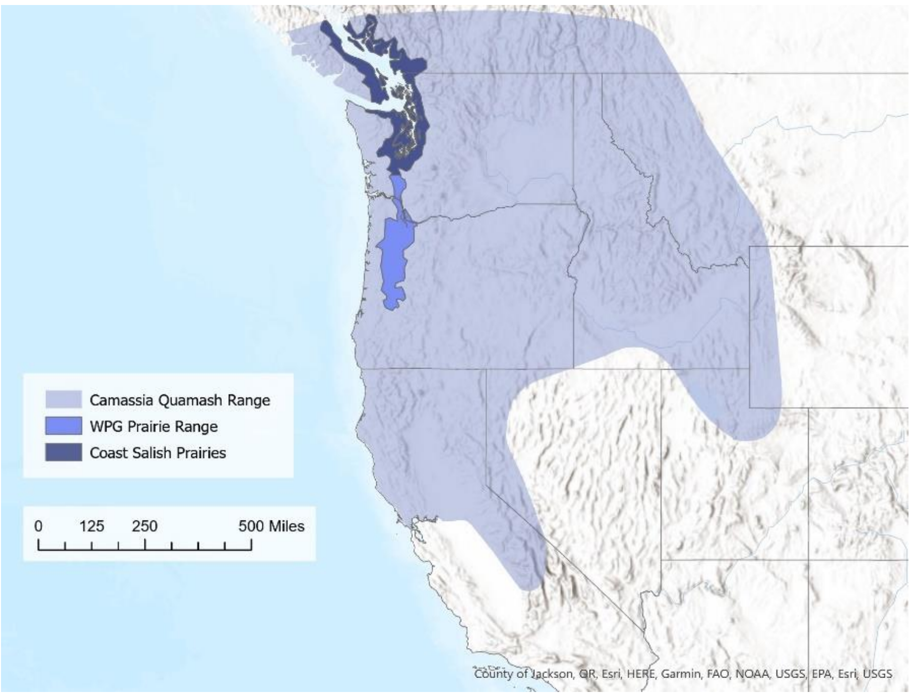

Figure 0-3: Map of Camassia quamash *range, WPG region, and Coast Salish Prairies*

Mere remnants of the WPG prairie habitat remain (Chappell et al., 2001; Dunwiddie & Bakker, 2011). Still, the WPG boasts over 690 plant species, far more than other ecosystems west of the Cascade Mountain Range (Fuchs, 2001). Of the abundance of wildlife that the WPG supports, Floberg et al. (2004) found that at least 526 plants, fungi, mammals, birds, reptiles, and invertebrates are threatened to some extent. Animal species of particular concern are the Taylor's checkerspot butterfly (*Euphydryas editha taylori*), mardon skipper (*Polites mardon*), streaked horned lark (*Eremophila alpestris strigata*), and mazama pocket gopher (*Thomomys mazama*) (Dunwiddie & Bakker, 2011). At least one of these, the mazama pocket gopher, relies on camas bulbs for food and the Taylor's checkerspot butterfly relies on camas's flowers for nectar (WDFW, 2023a).

2.2.B Ecosystem Vulnerability and Restoration

The Coast Salish Prairies are dwindling for several reasons. They have been replaced as the landscape has been converted to agricultural cropland and pasture; growing human population has expanded to occupy prairies; industrial development and traffic has resulted in an influx of pollutants; early conservationist land practices, including cultural fire exclusion, has allowed coniferous forests to overtake prairies; human-caused global warming is resulting in shifting climatic patterns that may be incompatible with the ecosystem; non-native, opportunistic species have filled niches left by human disturbance and subsequently spread to occupy vital native habitat (Willamette Partnership, 2020); and Euro-American attempts at eradication of Indigenous peoples and lifeways have nearly severed the reciprocal relationship by which the prairies have thrived since time immemorial (Garibaldi & Turner, 2004). Opportunistic non-native species are a critical barrier to Coast Salish Prairie health and are at the forefront of ecological restoration priorities (Stanley et al., 2008; Willamette Partnership, 2020).

Non-native plant species have spread with Euro-American and global expansion (Bonnamour et al., 2021). They were planted both purposefully and accidentally, with some being escaped ornamental or agricultural plants (van Kleunen et al., 2018), others being planted to mitigate an environmental hazard (Reeves, 2010), and more being dropped by travelling people and migrating animals (Bonnamour et al., 2021). Currently, many non-native seeds are brought on vehicles passing by and equipment being used on prairies (Dennehy et al., 2011; Willamette Partnership, 2020). Prairies may be particularly susceptible because they are vast, open landscapes exposed to seed rain circulated by neighboring agricultural fields and traffic (Willamette Partnership, 2020). Without globalization and transportation ceasing, new non-native species are expected to continue to take hold (Willamette Partnership, 2020). Opportunistic non-native plants, also known as invasive species, have been particularly successful where native plants have been removed or ecosystems have been weakened and disturbed (Trowbridge et al., 2017).

There are four main techniques that Indigenous and non-Indigenous peoples are using to mitigate the impact of non-native species: mowing, fire, seeding/planting, and herbicides (Trowbridge et al., 2017). The goal of mowing is to cut down invasive plants before they are able to seed and spread (Stanley et al., 2008; Trowbridge et al., 2017). However, the practice has been found to be largely ineffective, especially with non-native grasses, as mowing does not actually thin the grass and native seeds are unable to reach the soil to germinate (Stanley et al., 2008, 2011). Reintroduction of fire to the landscape, whether prescribed or cultural, returns nutrients and carbon to the soil (Pingree & DeLuca, 2017) and strengthens the native plants (Storm & Shebitz, 2006) while not allowing some non-native plants to seed, though many non-natives, particularly grasses, persist (Rook et al., 2011; Trowbridge et al., 2017). Camas is one of the many plants that benefits from reintroduction of fire to its habitat (Storm & Shebitz, 2006). In further efforts to outcompete non-native plants, native seeds are spread and native plants grown in nurseries are transplanted to increase their presence (Krueger et al., 2014). Herbicides are regularly used to eradicate non-native species, but they can have damaging direct and indirect effects on ecosystems (Stanley et al., 2008; Tunnell et al., 2006).

Herbicides are considered a best-practice in WPG prairie restoration and recommended in pamphlets and literature (Stanley et al., 2011; Willamette Partnership, 2020). They are often used in combination with other management techniques, such as after burns or before native seeds are spread (Stanley et al., 2011). Depending on the need, there are a variety of herbicides used in prairie restoration (Stanley et al., 2008; Tunnell et al., 2006). Some, like Clethodim and Fluazifop, are grass-selective (Cascadia Prairie Oak Partnership, 2014) while others are forb-selective (Krueger et al., 2014). Pre-emergent herbicides, like Indaziflam, kill germinating seeds (Terry et al., 2021). A few herbicides kill on contact, where as many are translocated within the plant to inhibit necessary biological processes systemically (Cascadia Prairie Oak Partnership, 2014). The most commonly used herbicides are non-selective and systemic, killing plants that have not built up a tolerance to them (Noland & Carver, 2011; Weidlich et al., 2020). As herbicides are designed to kill plants, they inevitably cause varying degrees of damage, known and unknown, to native species (Olszyk et al., 2017), soil structure and microbiome (Druille et al., 2016), and humans (van Bruggen et al., 2021).

Even with aggressive restoration techniques, it is likely that non-native species will jeopardize the health and resilience of prairie ecosystems for the foreseeable future (Dennehy et al., 2011). Thus, herbicides will most likely continue to be used year after year (Dennehy et al., 2011). The repeated use of herbicides may cause toxins to accumulate in perennials (Botten et al., 2021), many of which are traditional medicines and foods for Indigenous peoples (Deur & Turner, 2005), and consumption of herbicides may have detrimental effects on human health

(Rivas-Garcia et al., 2022). Therefore, the very management practices that are used to restore prairie ecosystems may be threatening the health of those who rely on them (Wood, 2019).

2.3 Glyphosate and AMPA:

Glyphosate is the active chemical compound found in a number of herbicides used in restoration, agriculture, and forestry (Martins-Gomes et al., 2022). Due to its efficiency and relative low toxicity when compared to other herbicides, glyphosate-based herbicides (GBHs) are the most extensively used products in attempts to eradicate non-native plant species worldwide (Rivas-Garcia et al., 2022; Weidlich et al., 2020). Restoration practitioners regularly recommend the use of GBHs in WPG prairies and their use has been considered a best practice (Dennehy et al., 2011; Noland & Carver, 2011).

Heavy usage in agriculture has compelled scientists to extensively study glyphosate and GBHs, with results leading to controversy surrounding their safety (de Castilhos Ghisi et al., 2020). Studies to date are primarily focused on GBH use in agriculture and small amounts of glyphosate and its metabolites are routinely found in the food and water we consume (Kolakowski et al., 2020; Rivas-Garcia et al., 2022). Comparatively, there are few studies that review GBH usage in a restoration setting, and even fewer that focus on how much glyphosate may be consumed when eating traditional foods (Botten et al., 2021).

N-(phophonomethyl)glycine, commonly known as glyphosate, was first developed in 1950, but did not make its herbicidal debut until 1974, when it was approved by the EPA for usage and patented by Monsanto Company (now Bayer) (Ojelade et al., 2022). Monsanto Company began selling glyphosate as the GBH Roundup for use in agriculture (Kolakowski et al., 2020). Other commonly used herbicides at the time were Atrazine and Paraquat, both of which are extremely detrimental to human health and now banned or restricted (Martins-Gomes et al., 2022). Glyphosate, on the other hand, kills plants by inhibiting a biochemical pathway found only in plants, bacteria, and fungi, called the shikimate pathway, which produces amino acids (Duke et al., 2012). It is widely believed to not severely impact humans or other animals (Mesnage & Antoniou, 2020). GBH usage grew exponentially after its introduction in agriculture (Klingelhöfer et al., 2021) and eventually made its way into the ecological restoration field (Weidlich et al., 2020).

Glyphosate is produced as a salt, diluted in water, mixed with other chemicals to support efficient application, then sprayed on or injected into plants (Druille et al., 2016). There are a number of GBHs used in restoration (Noland & Carver, 2011). Some practitioners purchase glyphosate salts and create their own formulas (Druille et al., 2016), while others use pre-formulated products, such as the Bayer's Roundup, Roundup Pro, Vision, and VisionMax (Bayer, 2023b). Exact formulations of pre-formulated GBHs are rarely published, as they are considered proprietary and not required to be made public (Ojelade et al., 2022). They may contain one or multiple adjuvants, including surfactants that promote glyphosate adherence to plant tissues (Czarnota & Thomas, 2013).

GBH application technique is dependent on scale, ecosystem type and target species. For large areas, including forests, aerial spraying from an airplane or helicopter may be used (Wood, 2019). Smaller areas may have GBHs broadcast applied by vehicle with an attached boom sprayer or by personnel with back-pack sprayers. For specific plants and very small areas, spot-spraying with a back-pack sprayer or injecting the herbicide directly into a plant is common (Rana, 2018). Each application method poses a different risk to the surrounding ecosystem (Marrs et al., 1993).

2.3.A Mechanism

Glyphosate interrupts the shikimate pathway (Ojelade et al., 2022), inhibiting the actions of the 5-enolypyruvylshikimate-3-phosphate synthase (EPSPS) enzyme (Martinez et al., 2018). By disrupting ESPS, glyphosate impedes the production of amino acids that are necessary for plant growth and hinders metabolism functions (Martinez et al., 2018). Efficiently translocating the chemical through plants along the photosynthetic route (Preston & Wakelin, 2008), susceptible plants die within days (Gaupp-Berghausen et al., 2015). The shikimate pathway also exists in some fungi and bacteria, in which the EPSPS enzyme performs in a similar capacity (Duke et al., 2012). While glyphosate is not explicitly used to kill fungi or bacteria, it is known to impact fungal and bacterial communities negatively, including the microbiome of soil and animals (van Bruggen et al., 2021).

2.3.B Persistence in Soil

GBHs reach the soil surrounding a target plant by spraying over a plant to achieve full coverage, exuding from the target plant's roots into the rhizosphere (Neumann et al., 2006), decomposing contaminated plant matter (Neumann et al., 2006), drifting through the air from an adjacent treatment site, and/or being transported by water (Rasmussen et al., 2015) (Figure 2-4). Once in the soil, glyphosate itself is either mineralized into its metabolites, immobilized in the soil, or leached into ground or surface water (Ojelade et al., 2022). There is disagreement about the length of time glyphosate persists in soil, as some studies state that glyphosate quickly dissipates from soil and others state that it is highly persistent (EFSA, 2015). Glyphosate's half-life (the amount of time it takes for 50% of applied glyphosate to leave soil) has been found to be between 1 and 300 days (EFSA, 2015) but may persist in soils for at least 12 years (Botten et al., 2021). Glyphosate's persistence in soils is highly dependent on soil composition, climate, and ecosystem dynamics (Eberbach, 1998; EFSA, 2015).

The bulk of glyphosate entering soil may be mineralized within days (EFSA, 2015). When mineralized, 90% of the metabolites are aminomethylphosphonic acid (AMPA), though it may also be metabolized into methylphosphonic acid, sarcosine, glycine, phosphate, CO2, or ammonia (Dick & Quinn, 1995). Bacteria existing in soil prior to GBH application mineralize glyphosate into aminomethylphosphonic acid (AMPA) (Gimsing, Borggaard, & Bang, 2004). This mineralization happens more efficiently in high pH soils with more phosphate, copper, and magnesium and less in soils that are high in iron and aluminum (Gómez Ortiz et al., 2017; Okada et al., 2016). AMPA then degrades into methylamine and phosphate, becoming carbon dioxide and ammonium over time (Ojelade et al., 2022). Research has shown that AMPA has a longer life in soil than glyphosate, with half-life estimates reaching 630 days (EFSA, 2015).

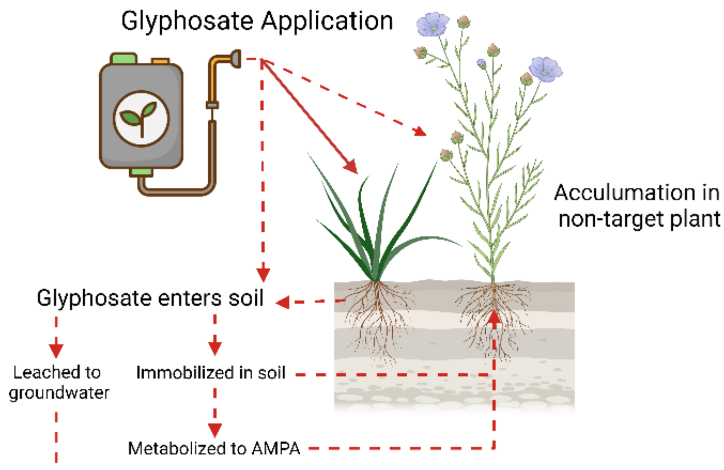

Figure 0-4: Diagram of glyphosate entering and dissipating from an ecosystem

Colder climates inhibit bacterial action and cause mineralization of glyphosate to slow (Botten et al., 2021; Newton et al., 2008). Soils and plants in colder climates accumulate glyphosate for longer and in greater amounts that those in warmer climates. Wood (2018) found more glyphosate in plant roots one year post-application in a northern temperate climate than Thompson (1990) found in a warmer, southern climate after 45 days (Feng & Thompson, 1990; Wood, 2019). Differences in glyphosate and AMPA amounts have even been found in climates that do not differ as drastically as Wood and Thompson's. In a study comparing latitudinally adjacent bio-climatic zones in Canada, Botten et al. (2021) observed that climate had a greater influence on amounts of glyphosate and AMPA in plant tissue than soil type or species of plant.

Glyphosate and AMPA are immobilized when they are adsorbed onto soil particles (Viti et al., 2019). Immobilization is stronger in soils with higher organic matter, clay (Miles & Moye, 1988), aluminum (Gimsing, Borggaard, Jacobsen, et al., 2004), and iron content and a higher cation exchange capacity (Gómez Ortiz et al., 2017). Glyphosate binds to similar geometry as phosphorous, and high amounts of phosphorous in soil can prevent adsorbtion of glyphosate (Laitinen et al., 2009). PH also plays a role, with lower pH promoting immobilization (Gimsing, Borggaard, & Bang, 2004). Stronger immobilization prevents bacteria from mineralizing glyphosate and leads to accumulation of the compound in soils (Newton et al., 1994). Immobilization is not a permanent phenomenon: glyphosate will eventually be degraded and both AMPA and glyphosate will be leached into the water column (Laitinen et al., 2009).

2.3.C Accumulation in Plants

Glyphosate and AMPA both accumulate in plants. Upon application to an ecosystem, plants will absorb glyphosate through leaves, stems, or roots (Neumann et al., 2006). Since AMPA is a result of mineralization within soil, it primarily enters plants through roots, though a plant's microbiome will also mineralize glyphosate into AMPA (Reddy et al., 2004). The effect of the herbicide on the plant depends on amount applied (Botten et al., 2021) and plant resistance (Reddy et al., 2004). Many plants survive exposure to sub-lethal amounts of glyphosate and may store it for years (Botten et al., 2021).

Once glyphosate and AMPA enter a plant, they are translocated through phloem in the same pattern that carbohydrates and sugars from photosynthesis are, concentrating in the most actively growing parts of the plant (Wakelin et al., 2004). Young leaves, shoots, and roots accumulate glyphosate first, followed by older parts of the plant (Wyrill & Burnside, 1976). The compounds then continue to cycle through the plant, are expelled into the rhizosphere as root exudates (Neumann et al., 2006), or are removed as contaminated tissues when the plant senesces (Newton et al., 1994).

Accumulation of glyphosate and AMPA is well studied in an agricultural setting, where annual plants are farmed and removed from the field yearly (Klingelhöfer et al., 2021). Storage of the compounds in perennial plants is less understood (Botten et al., 2021). Glyphosate and AMPA are regularly found in annual plants that have been exposed to the herbicide, even when there are months between application and planting (Kolakowski et al., 2020). It follows that perennial plants uptake glyphosate and AMPA as well and, without plants being removed from the field yearly, the compounds may accumulate in plant tissues and be stored overwinter (Botten et al., 2021; Edge et al., 2021).

While concentrations of glyphosate in soil depends on soil and climate factors, the uptake and accumulation of glyphosate in plants is likely species dependent (Wood, 2019). Neighboring perennial plant species exposed to the same concentration of glyphosate and AMPA will accumulate different amounts of the toxins (Edge et al., 2021; Wood, 2019). Furthermore, separate species accumulate the compounds in different organs and expel them at different rates (Wyrill & Burnside, 1976). The variations may be attributed to root structure (Wyrill & Burnside, 1976), life strategy (Wood, 2019), and permanence of tissues (Botten et al., 2021). In Canadian forest studies, both Wood (2018) and Botten et al. (2021) found that, when compared to woody shrubs, herbaceous perennials had the highest amounts of glyphosate and AMPA one to twelve years post-treatment. Herbaceous perennials have less permanent tissue than woody perennials and must store all carbohydrates and nutrients in their rootstock, rather than throughout the plant (Wood, 2019). Glyphosate has been found in the following year's new shoots, suggesting that it is one of the compounds that can be stored overwinter in herbaceous perennials (Botten et al., 2021).

2.3.D Consumption

Glyphosate and AMPA are regularly found in the modern diet (Kolakowski et al., 2020). Amounts in sampled foods are rarely above maximum residue limits (MRLs) set by the United States Environmental Protection Agency (US EPA), but, with the increase in GBH use and exposure, the US EPA has been steadily raising glyphosate MRLs since the advent of the herbicide (Cuhra et al., 2016). While RoundUp and other Bayer GBHs are known to impact human health, glyphosate is less toxic on its own without the unknown adjuvants in pre-formulated GBHs (Martins-Gomes et al., 2022). With the recognition of adjuvant toxicity, there is disagreement within the scientific community and regulatory agencies on consumption safety of glyphosate and AMPA (Benbrook, 2019). Regulations of glyphosate itself have been called into question by more recent research suggesting that the chronic exposure to low doses of glyphosate and AMPA has negative impacts on human health (Buchenauer et al., 2022; Ojelade et al., 2022).

The US EPA began setting glyphosate's MRLs when the herbicide was approved for agricultural use in 1974 and AMPA was not included in the original MRLs (US EPA, 2022). MRLs have been figured as 100x less than would be needed to have a negative health reaction, or no observed adverse effects limits (NOAELs) (ATSDR, 2020). Many studies of glyphosate consumption use NOAELs as the lowest dose of glyphosate within an experiment, rather than

MRLs, resulting in relatively few studies of glyphosate at low doses (Martins-Gomes et al., 2022; Ojelade et al., 2022).

In the U.S., most foods have a 1.75 ppm (mg/kg/day) MRL, while most EU, Canadian, and Australian agencies list a 0.5 mg/kg/day MRL (BCG Global, 2023; Mesnage et al., 2015). The discrepancy between agencies from different countries is attributed to data weighted in assessments, with the EPA relying primarily on internal data or data submitted by herbicide companies rather than public peer-reviewed research (Klingelhöfer et al., 2021; US EPA, 2023b). MRLs also vary by food and are dependent on how much of an herbicide a plant is exposed to (US EPA, 2023a). For example, glyphosate MRLs in onions, potatoes, and edible bulbs in the U.S. is 0.2 mg/kg/day, while the MRL for soybeans is 20 mg/kg/day because soybeans are exposed to more of the herbicide in the agricultural process (BCG Global, 2023).

Despite regulation, both glyphosate and AMPA are routinely found in human urine, blood, and breastmilk (Mesnage et al., 2015). When consumed through food or water, glyphosate follows two main routes: elimination in urine and feces or metabolization in the intestinal tract to AMPA (Brewester et al., 1991). In the U.S., extensive sampling has produced estimates that 60-95% of the public has glyphosate in their urine (van Bruggen et al., 2021). Glyphosate has even been found in the urine of infants, indicating that it can be transported through breast milk (Trasande et al., 2020). Glyphosate is efficiently dissolved in water, resulting in a low risk of accumulation in tissues and rapid elimination in urine (CCME, 2012). However, studies have found risk of accumulation in kidneys and liver, impacting function of the organs (Gao et al., 2019; Van Eenennaam & Young, 2017).

2.3.E Regulations

The study of GBHs is rife with controversy (McHenry, 2018). Independence of studies has been called into question, with two substantial stakeholders financing the bulk of published research (Klingelhöfer et al., 2021). Monsanto/Bayer, who manufacturers the most widely used GBHs, and the U.S. Department of Agriculture (USDA), who is one of the most widespread users and supporters of the herbicides, are not only the top two publishers and financers of research globally, their work also receives the most citations (Klingelhöfer et al., 2021). Additionally, in 2017, a document release exposed Monsanto/Bayer's ghostwriting of published papers and media, interference with peer review processes, and creation of a website defending their products (McHenry, 2018).

International agencies are in disagreement about potential negative health consequences of the use and consumption of GBHs (Benbrook, 2019). In 2016, both the U.S. EPA and the European Union Environmental Protection Agency (EUPA) reviewed the safety of glyphosate, finding that glyphosate was not carcinogenic at "doses relevant for human risk assessment" (Benbrook, 2019). The following year, the World Health Organization's International Agency for Research on Cancer (IARC) issued a report stating that glyphosate is "probably carcinogenic to humans", citing DNA damage and oxidative stress (Benbrook, 2019). Differences between the findings appear to have resulted from the selection, weighting, and circumstances of evaluated studies (Benbrook, 2019). While the IARC relied on published and peer reviewed materials, the U.S. EPA and EUPA depended on unpublished studies that are not accessible to the public because of their proprietary nature (Benbrook, 2019). Additionally, the IARC weighted studies that used GBH, glyphosate, and AMPA in regulatory consumption, elevated consumption, and application exposure studies, while the U.S. EPA and EUPA weighted data exclusively concerning glyphosate consumption at regulatory levels (Benbrook, 2019).

The IARC's findings are supported by literature suggesting that surfactants and other adjuvants of GBH formulas are carcinogenic because they cause oxidative stress, DNA damage, and are endocrine disruptors at low dosages (Martins-Gomes et al., 2022). Such surfactants, like polyethoxylated tallowamine (POEA), are now illegal in the EU but are still legal and used in the U.S. (Martins-Gomes et al., 2022). Roundup and VisionMax, both owned by Bayer and commonly used in restoration, may be formulated with POEA and other toxic adjuvants (ATSDR, 2020). POEA is less commonly used in restoration of the Coast Salish Prairies, with non-ionic Nu-Film being the primary surfactant applied (Dennehy et al., 2011). With its non-ionic property, Nu-Film is designed to not adsorb to soils and to efficiently dissipate from soils (Miller, 2023). While the surfactant is less studied than others, consumption is not known to have negative health effects (KeyIndustries, 2023).

2.3.F Consumption Health Implications

There are issues with many of the commonly cited studies reporting the toxicity of glyphosate. First, glyphosate is not consistently the only tested substance, as researchers will use pre-formulated GBHs instead of glyphosate in their studies (Peillex & Pelletier, 2020). This makes it difficult to compare studies because formulas of GBHs differ by both brand and country of origin (Ojelade et al., 2022). The practice also provokes confusion because it is not possible to differentiate between toxicity of adjuvants in GBHs and glyphosate toxicity (Defarge et al., 2016; Mesnage et al., 2019). Second, studies finding that glyphosate is toxic tend to use large amounts of the herbicide, often NOAELs for their lowest tested dose, rather than chronically consumed dose estimates (Martins-Gomes et al., 2022). Studies referenced in this review are focused on mammal research using glyphosate and AMPA amounts within 10 mg/kg of U.S. MRLs. Third, it was not until recently that AMPA was included in studies, meaning that glyphosate residue quantities may have been higher than researchers were aware of (van Bruggen et al., 2021).

As GBHs are heavily used across both farmed and natural landscapes, and glyphosate is a now common component of the environment, studies concerning health implications have been abundant (Martins-Gomes et al., 2022). Over decades of study, a near consensus has been reached that glyphosate and AMPA themselves are not carcinogenic (Benbrook, 2019) or endocrine disrupting at low doses (van Bruggen et al., 2021). However, the majority of studies have not analyzed chronic low-dose exposure similar to that which the public experiences. Those that do show correlations between glyphosate and AMPA consumption and various health issues (Buchenauer et al., 2022; Requena-Mullor et al., 2021). There are also a growing number of researchers investigating the impact of glyphosate and AMPA on the human intestinal tract and microbiome (Del Castilo et al., 2022; Qiu et al., 2020).

Glyphosate kills plants through inhibiting the shikimate pathway, a pathway that exists only in plants, fungi, and bacteria, leading regulators of glyphosate to believe that glyphosate's mechanism cannot systemically impact animals (Mesnage & Antoniou, 2020). Since the pathway exists in bacteria, the relatively recent recognition of the importance of the gut microbiome has led to glyphosate's indirect impact on animal health being called into question (van Bruggen et al., 2021). Researchers have found that multiple bacteria present in the human gut microbiome have the shikimate pathway and are impacted by glyphosate (Mesnage & Antoniou, 2020). Moreover, studies have observed immediate and generational microbiome effects with chronic MRL level consumption (Barnett et al., 2023; Buchenauer et al., 2022). While studies have, to date, not been done in vivo with humans, extrapolatory studies have suggested that the human gut microbiome is impacted (Leino et al., 2021; Mesnage & Antoniou, 2020; Puigbò et al., 2022). Additionally, temporal correlations have been drawn between when glyphosate was introduced into the environment and increases in neuropsychiatric conditions known to be associated with microbiome dysbiosis (Vijay & Valdes, 2022).

Chronic dietary exposure to glyphosate has been found to significantly affect the gut microbiome of rats in real-time and generationally (Buchenauer et al., 2022). Buchenauer et al., (2022) performed an experiment on three generations of rats consuming 0.5 mg/kg/day of glyphosate 3 days a week – less than the US glyphosate MRL for many foods. Results showed that the first generation of rats had significantly different microbiome bacteria proportions than the control; the second generation had a suppressed immune system, showed signs of being prone to asthma, had an increased amount of bacteria in the gut, and had fewer offspring; and the third generation again had a different microbiome and suppressed immune system. The microbiome of rats differed significantly by generation and from the controls (Buchenauer et al., 2022).

In the Buchenauer et al. (2022) study, 6 species of bacteria and 1 entire genus was significantly affected by glyphosate consumption. At least two of the species are capable of metabolizing glyphosate into AMPA in in vitro studies (Mesnage & Antoniou, 2020). To explore potential impacts on relevant bacteria species, Mesnage et al. (2020) computationally modelled bacteria in the human gut microbiome with data from the Human Microbiome Project. They found that nearly 70% of the bacteria found in the human gut microbiome has the shikimate pathway and would theoretically be sensitive to glyphosate. However, in many of these, the shikimate pathway is “transcriptionally inactive” (Mesnage & Antoniou, 2020). Leino et al.,

(2021) estimated that 12-26% of bacteria in the active human intestinal tract may be capable of being affected by glyphosate, while others have found this number as high as 54% (Puigbò et al., 2022).

Glyphosate and AMPA consumption may also lead to metabolic dysfunction, a decrease in working memory, and inhibited locomotor activity. A correlation study found a link between glyphosate levels in urine and diabetes diagnosis, particularly in males over the age of 60 (Qi et al., 2023). In vitro, mice who were fed chronic, below U.S. MRL doses of glyphosate had children and grandchildren with signs of “higher fasting blood glucose, inability to efficiently clear glucose, and impaired insulin response”, even though their offspring did not consume glyphosate (Barnett et al., 2023). The same study observed decreases in working memory in mice whose parent consumed glyphosate while pregnant and impaired locomotor function in mice whose grandparent consumed glyphosate while pregnant (Barnett et al., 2023). The researchers hypothesized that the results were due to a dysbiosis of gut bacteria, as dysbiosis can lead to metabolic and behavioral irregularities (Barnett et al., 2023).

2.4 Conclusion:

With confirmed accumulation of glyphosate and AMPA in herbaceous perennials of temperate climates, current literature supports the hypothesis that glyphosate and AMPA could be accumulating in camas bulbs exposed to GBHs in Coast Salish Prairies. Furthermore, research concerning glyphosate, AMPA, and the human gut microbiome exposes risks of consuming MRL doses of glyphosate and AMPA. The potential of glyphosate and AMPA accumulating in *Camassia quamash* bulbs calls for an in vivo analysis to present Indigenous peoples with accurate information about consumption of this cultural keystone species and to provide restoration practitioners with data relevant to land management techniques on Coast Salish Prairies.

Chapter 3: Methods

To answer the question, "How does the legacy of glyphosate application to a *Camassia quamash* habitat affect the concentration of glyphosate and AMPA in *Camassia quamash* bulbs?", I performed an in vivo field study. Bulbs from sites that had been treated with a GBH (treated bulbs) and bulbs from sites that had not been treated with a GBH (control bulbs) were tested for glyphosate and AMPA. To account for potential confounding variables, I also collected soil samples and analyzed them for three factors that have been found to influence the mobility of glyphosate and AMPA in soil: pH levels, clay content, and SOM (Gimsing, Borggaard, Jacobsen, et al., 2004; Laitinen et al., 2009; Shushkova et al., 2009). I utilized the U. S. Department of Agriculture Natural Resources Conservation Service's Web Soil Survey to determine each site's soil drainage class, soil order, and soil taxonomy. I noted weather patterns for the past 2 years, as cold winter temperatures can inhibit the bacterial breakdown of GBHs in soils and precipitation can leach GBHs from soils (Gimsing, Borggaard, Jacobsen, et al., 2004; von Wirén-Lehr et al., 1997). Additionally, I assessed GBH air-drift risk potential from neighboring treatments.

3.1 Site Selection

When selecting sampling sites, my goal was to select sites that have had one broadcast spray GBH treatment within the past 2 years, have a minimal risk of exposure to GBHs by air-drift from neighboring treatments, and are of cultural priority to Indigenous peoples. I determined history of GBH treatment and cultural priority through personal communications with land-managers and a Tribal Nation. I assessed air-drift risk through a GIS analysis.

3.1.A History of Glyphosate Treatment

I communicated with The Confederated Tribes of the Chehalis Reservation (Chehalis Tribe), the Center for Natural Lands Management (CNLM), Washington Department of Fish and Wildlife (WDFW), Ecostudies Institute, Lewis County, Thurston County, Washington Department of Transportation, and Washington Department of Natural Resources (DNR) to locate sites that had been treated with a GBH within the past 2 years.

3.1.B Cultural Priority:

I spoke with the Chehalis Tribe and other land managers to understand site cultural significance. The Chehalis Tribe confirmed sites that are a priority to the Tribe and land managers of non-Tribal sites shared if camas is currently traditionally harvested from the sites they manage. All sites except two, Marion Prairie and Scatter Creek, are currently traditionally harvested.

3.2 Glyphosate Air-drift Exposure Risk GIS Analysis

I performed a GIS air-drift risk analysis in ArcGIS Pro by assessing risk from three types of locations where practitioners can treat with GBHs: commercial farms (Marrs et al., 1993), commercial tree farms (WDNR, 2023b), and roadways (WSDOT, 2023). I was unable to access complete records of GBH use on any of the three types, so I performed an analysis using all commercial farm, commercial tree farm, and roadway sites within a specified area. Noxious weeds may also be treated with GBHs and their locations pose an air-drift risk. Those geolocated and treated by Thurston County (Thurston County Noxious Weeds Department, 2023) and

Washington State Department of Agriculture (WSDA, 2023) were along roadways and therefore would be included in the roadway analysis. I excluded noxious weeds from my analysis.

The distance that glyphosate travels in the air during application is dependent on application technique, application distance from ground, application equipment, and wind speed during application (Kasner et al., 2021; Marrs et al., 1993). Without knowledge of any of these variables at the time of potential neighboring application, I created a worst-case scenario risk scale with 5 levels: (1) Very High, (2) High, (3) Medium, (4) Low, and (5) Very Low. Generally, exposure by air-drift decreases as distance from application increases (Reddy et al., 2010; Yates et al., 1978). For near ground spraying, typical of commercial farm and roadway applications (Strandberg et al., 2021; WDNR, 2023b), the risk scale ranges from 0m to 50m, with 0-5m being "Very High" risk and 40-50m being "Very Low" risk. If a location was outside of the risk area, it was designated "No Risk". For aerial spraying, typical of commercial tree farms greater than 5 acres (WDNR, 2023a), the risk scale goes from 0m to 100m (Reddy et al., 2010; WDNR, 2023b), with 0-10m being "Very High" risk. If a location was outside of the risk area, it was designated "No Risk."

3.2.A Drift Type 1: Commercial Farm Drift

I began by creating the feature class "Regional Polygon", a rectangular area that encompassed all sample sites. I clipped each layer to "Regional Polygon" to enable quicker, more efficient analysis. I used the source 2021 WSDA Agricultural Land Use (WSDA, 2021) to perform the commercial farm drift analysis. I clipped the "2021 WSDA Agricultural Land Use" feature class to "Regional Polygon". I then used the tool 'Select Layer by Attribute' and selected all crops except for commercial tree farms. I used the tool 'Euclidean Distance' on the selected features (Output Cell Size: 5; Distance Method: Planar; Raster Analysis: Maximum of Inputs;

Cell Size Projection Method: Convert units). On the resulting raster, I used the tool 'Reclassify' to reclass it into 5 values: 1 = 0-5m, 2 = 5-10m, 3 = 10-20m, 4 = 20-30m, and 5 = 30-50m. I then relabeled the values to assign risk levels where 1 = Very High, 2 = High, 3 = Medium, 4 = Low, and 5 = Very Low (Figure 3-1).

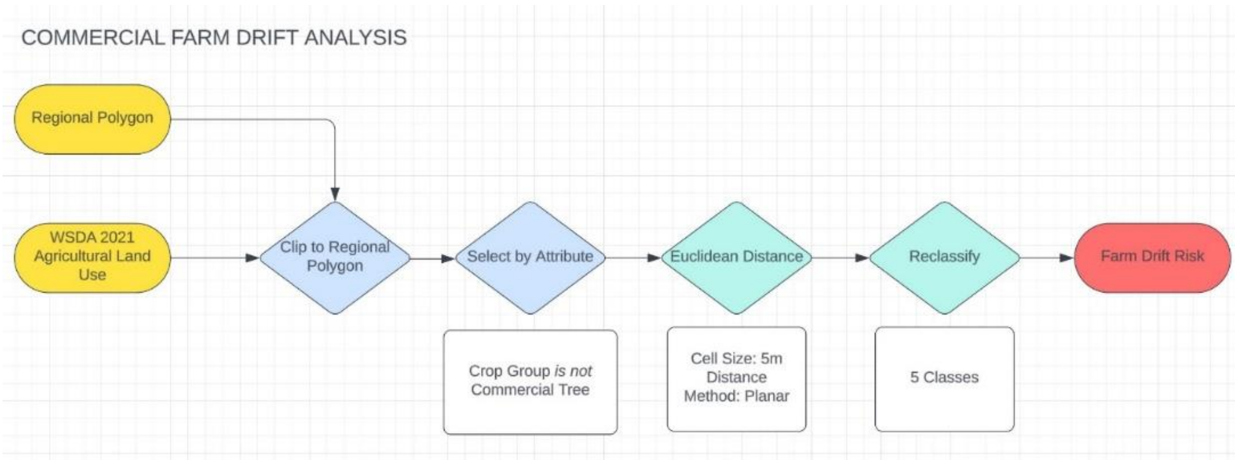

Figure 0-1: GIS workflow Drift Type 1: Commercial Farm Drift Analysis

<u>*3.2.B Drift Type 2- Commercial Tree Farm Drift*</u>

I began by creating the feature class "Regional Polygon", a rectangular area that encompassed all sample sites. I clipped each layer to "Regional Polygon" to enable quicker, more efficient analysis. I used the source 2021 WSDA Agricultural Land Use (WSDA, 2021) to perform the commercial tree farm drift analysis. I clipped the "2021 WSDA Agricultural Land Use" feature class to "Regional Polygon". I then used the tool 'Select Layer by Attribute' and selected features that belonged to Crop Group "tree farm" and were ≥ 5 acres. I used the tool 'Euclidean Distance' on the selected features (Output Cell Size: 5; Distance Method: Planar; Raster Analysis: Maximum of Inputs; Cell Size Projection Method: Convert units). On the resulting raster, I used the tool 'Reclassify' to reclass it into 5 values: 1 = 0-10m, 2 = 10-20m, 3 = 20-30m, 4 = 30-50m, and 5 = 50-100m. I then relabeled the values to assign risk levels where 1 = Very High, 2 = High, 3 = Medium, 4 = Low, and 5 = Very Low. (Figure 3-2)

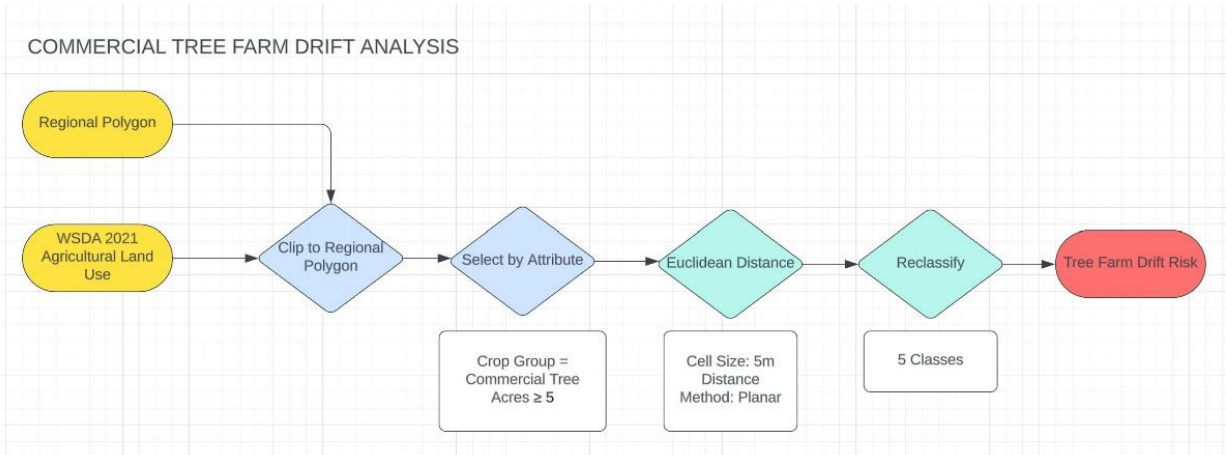

Figure 0-2: GIS workflow Drift Type 2: Commercial Tree Farm Drift Analysis

<u>*3.2.C Drift Type 3: Roadway Drift*</u>

I began by creating the feature class "Regional Polygon", a rectangular area that encompassed all sample sites. I clipped each layer to "Regional Polygon" to enable quicker, more efficient analysis. I sourced transportation data from ESRI US Federal Data (ESRI, 2023) to perform the roadway air-drift risk analysis. The data consisted of 3 relevant feature classes: "Primary Roads", "Secondary Roads" and "Local Roads". I clipped each of the feature classes to "Regional Polygon" separately. I then used the tool 'Euclidean Distance' on each of the feature classes (Output Cell Size: 5; Maximum Distance: 100; Distance Method: Planar; Raster Analysis: Maximum of Inputs; Cell Size Projection Method: Convert units). On the resulting rasters, I used the tool 'Reclassify' to reclass them into 5 values: 1 = 0-5m, 2 = 5-10m, 3 = 10-20m, 4 = 20-30m, and 5 = 30-50m. I then used the tool 'Mosaic to Raster' (Resampling Method = Nearest) to combine all three rasters into one. Finally, I relabeled the values to assign risk levels where 1 = Very High, 2 = High, 3 = Medium, 4 = Low, and 5 = Very Low. (Figure 3-3)

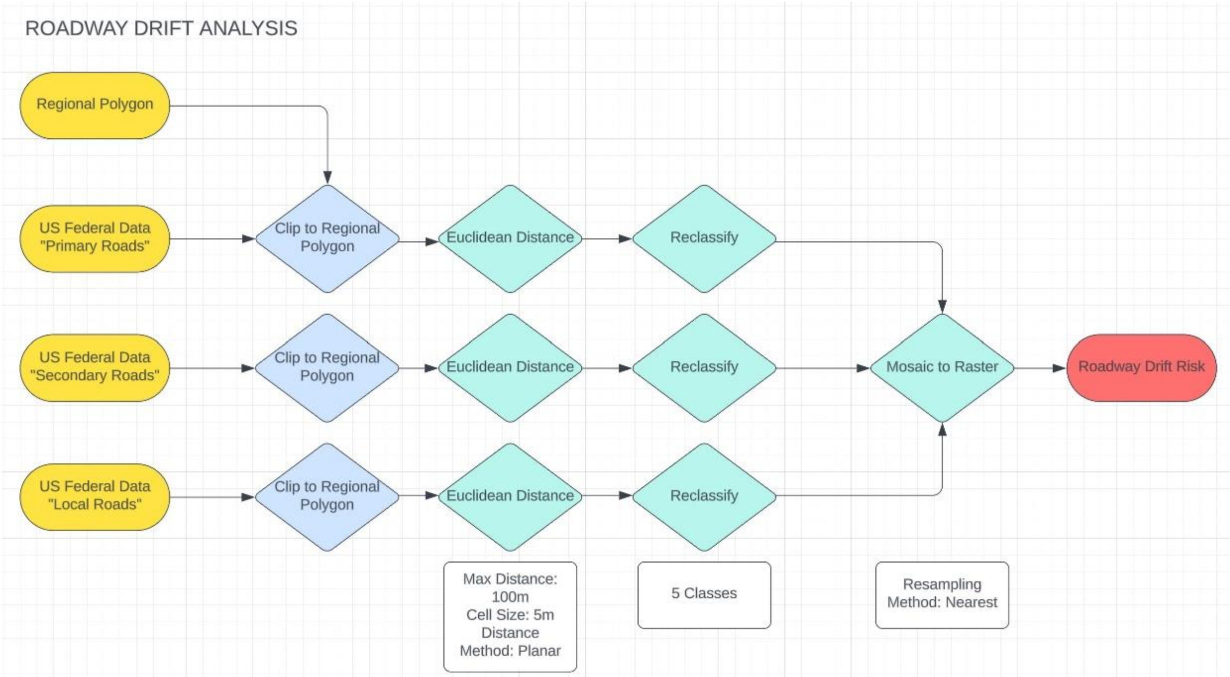

Figure 0-3: GIS workflow Drift Type 3: Roadway Drift Analysis

<u>*3.2.D Data Visualization*</u>

I then visualized the three drift type rasters identically by risk level, resulting in the map that I used to assess site suitability.

3.3 Site Details:

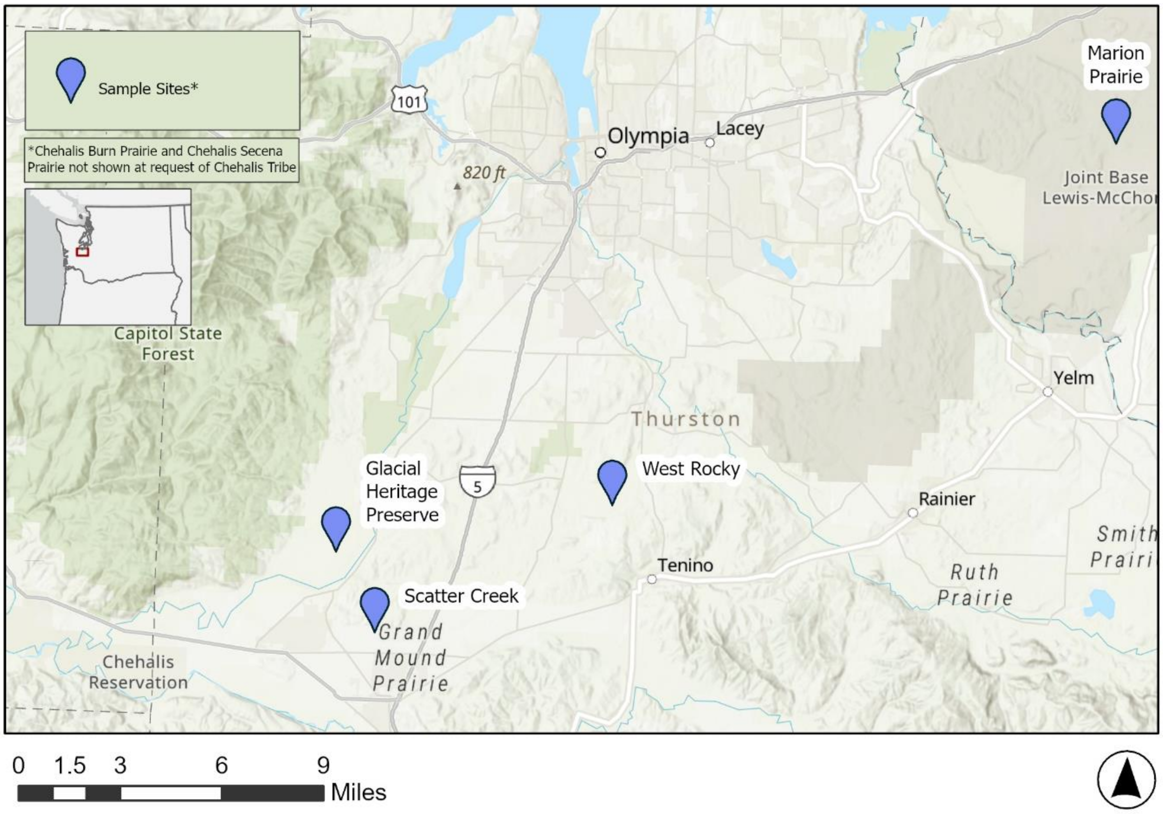

Figure 0-4: Map of all sample sites excluding the Chehalis Tribal sites at request of the Tribe

3.3.A Marion Prairie: Treated

Marion Prairie is managed by the Joint Base Lewis-McChord (JBLM) Sentinel Landscape Partnership as part of the base's artillery range (USFWS, 2023). The camas at Marion Prairie is not currently harvested by Indigenous peoples. The sampled site was treated with a GBH on 12/29/2021 with a formula of 2.5% glyphosate, 0.25% Nufilm. There is no record of GBH application prior to 2017 (T. Atkinson, personal communication, May 31, 2023). I collected samples from Marion Prairie on 6/5/2023, approximately 17 months after GBH treatment.

The sample site sits at an elevation of approximately 330ft (USGS, 2019). According to the USDA Natural Resources Conservation Service's (NRCS's) Web Soil Survey, the area's soil is classified as Everett-Spanaway complex, 3-15% slopes with a drainage class of "somewhat excessively drained". The taxonomy of the soil is sandy-skeletal and the soil type is 5% Histosols and 95% Inceptisols (NRCS, 2019).

3.3.B Scatter Creek Wildlife Area: Treated

Scatter Creek Wildlife Area (Scatter Creek) is managed by WDFW and has been in active restoration for 17 years (WDFW, 2023b). The camas at Scatter Creek is not currently harvested by Indigenous peoples. The sampled site was treated with a GBH on 1/4/2023 with a formula of 2% Roundup Pro (0.96% glyphosate), 0.25% BroncMax, and 0.1% Hi-Lite dye (J. Cook, personal communication, July 17, 2023). There is no prior record of GBH application at the sample site (J. Cook, personal communication, July 17, 2023). I collected samples from Scatter Creek on 6/6/2023, approximately 5 months after GBH treatment.

The sample site sits at an elevation of roughly 185ft (USGS, 2019). According to the USDA NRCS's Web Soil Survey, the area's soil is classified as Spanaway-Nisqually complex, 2-10% slopes with a drainage class of "somewhat excessively drained". The particle size of the soil is sandy-skeletal and the soil type is 60% Andisols and 30% Inceptisols (NRCS, 2019).

The specific location that I sampled from had been control burned the fall prior to sampling (Fall 2022) (J. Cook, personal communication, June 6, 2023). The site was barren with exposed soil and patches of shaded and non-shaded camas.

3.3.C West Rocky: Treated

West Rocky is managed by Washington Department of Fish and Wildlife and has been in active restoration for 17 years (WDFW, 2023c). The camas at West Rocky is currently harvested by Indigenous peoples. The sampled site was treated with a GBH on 1/23/2023 with a formula of 2% Roundup Pro (0.96% glyphosate), 0.25% BroncMax, and 0.1% Hi-Lite dye (J. Cook, personal communication, July 17, 2023). There is no prior record of GBH application at the sample site (J. Cook, personal communication, July 17, 2023). I collected samples from West Rocky on 6/6/2023, approximately 4.5 months after GBH treatment.

The sample site sits at an elevation of roughly 220ft (USGS, 2019). According to the USDA NRCS's Web Soil Survey, the area's soil is classified as Spanaway gravelly sandy loam, 0-3% slopes with a drainage class of “somewhat excessively drained”. The taxonomy of the soil is sandy-skeletal and the soil type is 100% Andisols (NRCS, 2019). The specific location that I sampled had the characteristic mounded landscape of the Coast Salish Prairies, mima mounds (Washburn, 1988).

3.3.D The Prairie at the Mouth of Black River: Control

The Prairie at the Mouth of Black River is a managed by the Chehalis Tribe (W. Thoms, Chehalis Tribe, personal communication, June 6, 2023). In the Chehalis language, the ancestral name for the prairie is sq̓ʷayʼa̓yiłq, translating to “it makes a lake” in English. The literal interpretation of the ancestral name is “The Prairie at the Mouth of Black River” (W. Thoms, Chehalis Tribe, personal communication, August 10, 2023). In charts and tables, The Prairie at the Mouth of Black River may be shortened to PMBR. The camas at the prairie is harvested annually by the Tribe. There is no record of GBH application at the site (W. Thoms, Chehalis Tribe, personal communication, June 6, 2023). I collected samples from PMBR on 6/8/2023.

The sample site sits at an elevation of approximately 95ft (USGS, 2019). According to the USDA NRCS's Web Soil Survey, the area's soil is classified as Daviscreek-Huttula complex, 0-10% slopes with a drainage class of “well drained”. The taxonomy of the soil is medial-skeletal over sandy or sandy-skeletal and the soil type is 75% Andisols and 25% Inceptisols (NRCS, 2019).

3.3.E Glacial Heritage Preserve: Control

Glacial Heritage Preserve (Glacial Heritage) is owned by Thurston County and managed by CNLM. It has been in active restoration since 1995. The camas at Glacial Heritage is harvested annually by Indigenous peoples from multiple Tribal Nations in the region. There is no record of GBH application since 2015 (S. Freed, personal communication, May 31, 2023). I collected samples from Glacial Heritage on 5/31/2023.

The sample site sits at an elevation of approximately 150ft (USGS, 2019). According to the USDA NRCS's Web Soil Survey, the area's soil is classified as Spanaway-Nisqually Complex, 2-10% slopes with a drainage class of "somewhat excessively drained". The taxonomy of the soil is sandy-skeletal and the soil type is 60% Andisols and 30% Inceptisols (NRCS, 2019).

3.3.F Secena Prairie: Control

Secena Prairie is managed by the Chehalis Tribe and the camas at the prairie is harvested annually by the Tribe (W. Thoms, Chehalis Tribe, personal communication, June 6, 2023). There is no written record of GBH application, though there is an oral record that a pesticide was sprayed on the area roughly 20 years ago (W. Thoms, Chehalis Tribe, personal communication, June 6, 2023). I collected samples from Secena Prairie on 6/8/2023.

The sample site sits at an elevation of approximately 105ft (USGS, 2019). According to the USDA NRCS's Web Soil Survey, the area's soil is classified as Grandmound gravelly sandy loam, 0-15% slopes with a drainage class of "somewhat excessively drained". The taxonomy of the soil is sandy-skeletal and the soil type is 10% Andisols and 90% Inceptisols (NRCS, 2019).

Table 0-1: Sample site treatment details, collection dates, and management

Site Treatment and Management

Site	Type	Treatment	Treatment Date	Collection Date	Management
Marion Prairie	Treated	2.5% Glyphosate, 0.25% Nufilm (adjuvant), 1% blue dye	12/29/2021	6/5/2023	Partnership
Scatter Creek	Treated	2% RoundupPro (0.96% glyphosate), 0.25% BroncMax (water conditioning agent), 0.1% Hi-Lite dye	1/4/2023	6/6/2023	WDFW
West Rocky	Treated	2% RoundupPro (0.96% glyphosate), 0.25% BroncMax (water conditioning agent), 0.1% Hi-Lite dye	1/23/2023	6/6/2023	WDFW
The Prairie at the Mouth of the Black River	Control	-	-	6/8/2023	Chehalis Tribe
Glacial Heritage	Control	-	-	5/31/2023	CNLM
Secena Prairie	Control	-	-	6/8/2023	Chehalis Tribe

Table 0-2:Sample site air-drift risk, location, and USDA NRCS's Web Soil Survey results (NRCS, 2019)

Site Location and Charcteristics

Site	Type	Air Drift Risk	Soil Class	Soil Drainage Class	Particle Size	Composition Type	Latitude	Longitude	Elevation
Marion Prairie	Treated	Low	Everett-Spanaway complex, 3-15% slopes	Somewhat excessively drained	Sandy-skeletal	5% Histosols 95% Inceptisols	47.0491132°N	122.5269164°W	330 ft
Glacial Heritage	Treated	None	Spanaway-Nisqually complex, 2-10% slopes	Somewhat excessively drained	Sandy-skeletal	60% Andisols 30% Inceptisols	46.8311434°N	123.0228954°W	185 ft
West Rocky	Treated	None	Spanaway gravelly sandy loam, 0-3% slopes	Somewhat excessively drained	Sandy-skeletal	100% Andisols	46.8888824°N	122.8815013°W	220 ft
The Prairie at the Mouth of the Black River	Control	None	Daviscreek-Huttula complex, 0-10% slopes	Well drained	Medial-skeletal over sandy or Sandy-skeletal	75% Andisols 25% Inceptisols	-	-	95 ft
Glacial Heritage	Control	None	Spanaway-Nisqually complex, 2-10% slopes	Somewhat excessively drained	Sandy-skeletal	60% Andisols 30% Inceptisols	46.8650722°N	123.0486501°W	150 ft
Secena Prairie	Control	Low	Grandmound gravelly sandy loam, 0-15%	Somewhat excessively drained	Sandy-skeletal	10% Andisols 90% Inceptisols	-	-	105 ft

*No location data for Chehalis Tribe sites at request of Tribe

3.4 Experiment Design and Sampling

I designed an in vivo field study in which I selected 6 field sites, 3 treated and 3 control, and I designated five 1m x 1m sample plots at each site. Plots were at least 100ft apart to maintain independence. I collected 5 soil and camas bulb samples from each site (one consolidates sample from each plot), amounting to 30 samples. I sent all bulbs to an off-site lab to be tested for glyphosate and AMPA. To account for confounding variables that may impact glyphosate and AMPA accumulation in camas bulbs, I tested soil samples for pH, clay content, and SOM; recorded relevant soil data from USDA NRCS's Web Soil Survey; and assessed weather events since glyphosate treatment.

I sampled from May 31st through June 8th, just as the camas flower was senescing and seed pods were forming. I aligned collection dates with the timing of the annual traditional gathering on Coast Salish Prairies. Taking a composite sample of 8 camas bulbs, I selected bulbs from a mix of plants with flowering stalks, plants with smaller leaves and no flowering stalks, and plants with larger leaves and no flowering stalks to attempt to sample bulbs of a variety of ages. The bulbs were harvested from 1.25-6.25cm below the soil surface with a traditional digging stick made of elk antler and ocean spray (*Holodiscus discolor*), a welded steel digger, or a hand trowel. I separated the vegetative portion of the plants from the bulbs and dispersed them in the field. I then placed bulbs in labelled plastic bags and stored them in a cooler until they were refrigerated later the same day.

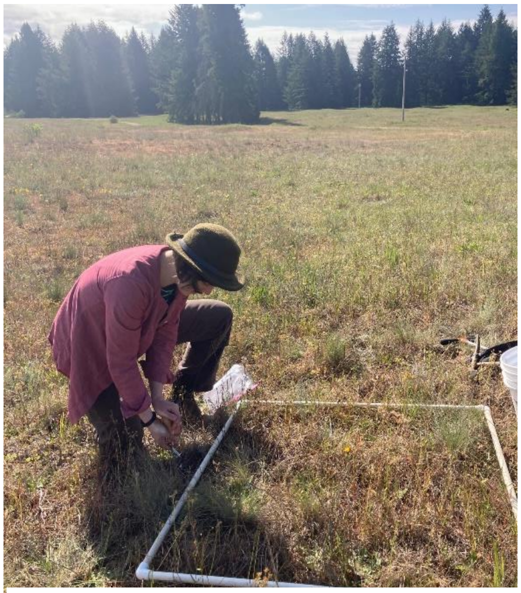
Figure 0-5: Image of soil sampling at Glacial Heritage Preserve

I extracted soil cores on the same day I harvested the bulbs. Core samples were 5cm wide and 10cm deep. These dimensions encompass the depth to which camas bulbs in Coast Salish Prairies typically inhabit the soil. I scraped organic matter away from the top of the soil before I took core samples with a hand trowel. I stored soil cores in labelled plastic bags and placed them in a cooler with ice packs until refrigerated.

Figure 0-6: Image of camas bulb sampling with a traditional digging stick

Figure 0-7: Image of camas bulb sampling within sample plot

Site Specific Sampling Procedures:

Sample sites at Glacial Heritage, Scatter Creek, and Marion Prairie were minimally sloped, at the same elevation, and had similar plot conditions. At Secena Prairie and The Prairie at the Mouth of Black River, the Chehalis Tribe sought to additionally test sampled bulbs for heavy metals and other pesticide residues. At Secena Prairie, I chose sample plots based on areas that might have been exposed to heavy metals, such as prior trash dump locations, resulting in

Plot #1 being more shaded than others and plot #2 having more gravel in the sampled soil. At The Prairie at the Mouth of Black River, I sought to take samples from multiple patches of camas, resulting in differing elevations of plots and the plots being 100-1,000ft apart. The site sampled at West Rocky had mima mounds that were 10ft tall. I chose 3 sample plots at the bases of the mounds and 2 on top of the mounds.

Figure 0-8: Image of bulb samples immediately after extraction at Marion Prairie

3.5 Processing Samples

3.5.A Camas Bulbs

I stored camas bulb samples in a Summit Under Counter Refrigerator for 1 – 7 days before processing at The Evergreen State College Laboratory. While in the refrigerator, a portion of the bulbs froze, becoming more translucent and softer than non-frozen bulbs (Figure 3-11). The manager at AGQ Labs confirmed that that the freeze should not affect the glyphosate and AMPA analysis (B. Jones, AGQ Labs, personal communication, June 3, 2023). Nonetheless, I noted which bulbs showed signs of freeze.

The bulbs were 1-3 cm wide. I cut their stems where the bulb straightened and removed the base, roots, and tunics to mimic traditional consumption (Turner & Kuhnlein, 1983) (Figures 3-9 and 3-10). I then rinsed bulbs with tap water to further remove any soil, air dried them for 24 hours (Figure 3-12), and weighed total sample weight on Radwag analytical scale (AS

82/220.R2). Not all bulbs were intact after processing. Glacial Heritage Plot #1 and The Prairie at the Mouth of Black River Plot #3 both had a camas bulb that was too contaminated with soil to sample, resulting in 7 bulbs being tested for each plot.

I stored bulb samples in labelled plastic bags in the refrigerator for 2 days until they were transported to AGQ Labs via USPS on 6/14/23. They arrived at the lab on 6/16/23 and the lab immediately began processing them. The bulbs were smaller than the lab expected and, as a result, lab technicians hand-ground each sample with dry-ice in preparation for glyphosate and AMPA testing. The lab confirmed that this preparation process would not impact the confidence interval of the results (B. Jones, AGQ Labs, personal communication, June 23, 2023).

Figure 0-9: Camas bulbs pre-processing

Figure 0-10: Camas bulbs post-processing

AGQ Labs, Oxnard, California, is an Accredited Laboratory in Environmental Standard by The International Accreditation Service with ISO 17025, TNI, and GLP certificates (AGQ Labs, 2023). The lab used the Quick Method for the Analysis of Highly Polar Pesticides in Food Involving Extraction with Acidified Methanol and LC- or IC- MS/MS Measurement (Anastassiades et al., 2021), also known as the QuPPe method, with liquid chromatography tandem mass spectrometry to a LOQ of 0.01 mg/kg. Developed in the

European Union, the QuPPe method is the current gold standard for glyphosate and AMPA analysis in food (EURL-SRM, 2023). Uncertainty levels were 24% for glyphosate and 23.8% for AMPA (B. Jones, AGQ Labs, personal communication, July 7, 2023).

Figure 0-12: Image of composite camas bulb bs that became samples air drying after rinsing

<u>*3.5.B Soil Samples*</u>

I stored soil samples in a Summit Under Counter Refrigerator at The Evergreen State College Laboratory for up to 7 days before I processed them. I first removed live organic matter, consisting mostly of grass roots, from samples. To remove large rocks, I then sifted the soil through a 2mm sieve. I spun the roots that did not filter through the sieve between my hands to remove small organic matter and any remaining soil particles. I then ground the sieved soil with a coffee grinder, either a Mr. Coffee 3-speed or a Hamilton Beach 80410, and sifted it through a 2mm sieve a second time. I air-dried the soil for 3-7 days on disposable plates until I analyzed them for pH, clay content, and SOM.

PH Level Analysis

I tested soil pH levels using a 1:2 soil:water ratio method (KBS LTER, 2023). In 50mL beakers, I weighed 15g of air-dried soil on a Radwag analytical scale (AS 82/220.R2). I then added 30mL of deionized water to each beaker and stirred the solution for 1 minute with a glass rod. I lightly covered the mixed solutions and let to sit for 30 minutes. I used a Thermo Scientific Orion Star A111 pH meter fitted with an Oakton Electrode (model 35805-06) and a Thermo Scientific Star ATC Probe (Orion 927007MD) to measure pH levels. The machine was calibrated with 4.0 ± 0.01, 7.0 ± 0.01, and 10.0 ± 0.01 at 20°C buffers prepared by The Evergreen State College Science Support Center. I took 5 pH readings for each sample, thoroughly rinsing the electrode and probe with deionized water and dabbing with non-lint wipes between each reading (Figure 3-13). I averaged the results from each sample and calculated standard deviations and standard errors in Microsoft Excel.

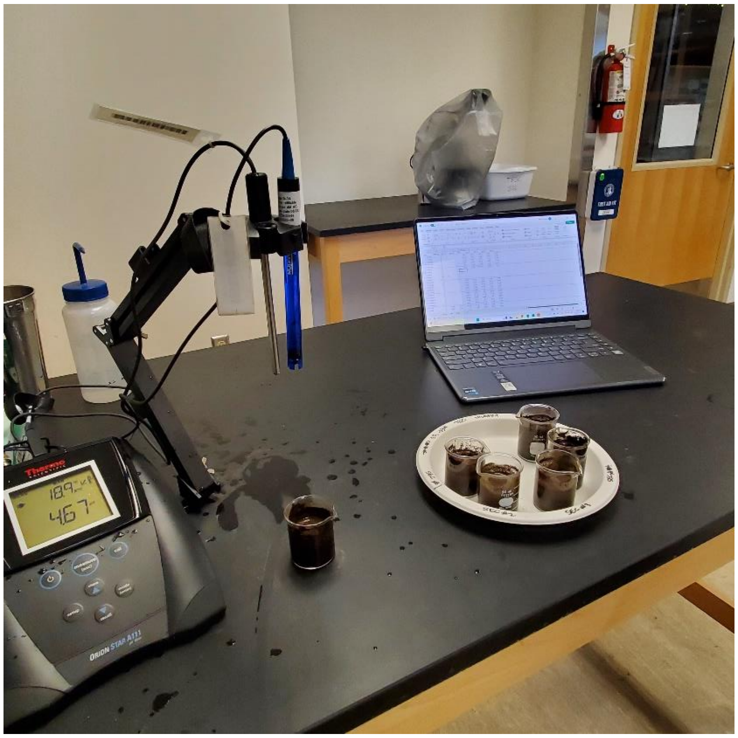

Figure 0-13: Image mid-pH sampling procedure

Clay Content Analysis

I measured percent clay content with the 2 Hour Hydrometer Method, using Oklahoma State University's Soil Texture Protocol (OSU Soil Fertility Lab, 2020). In the method, a 0.5%

Sodium Hexametaphosphate $(NaPO_3)_6$ dispersing solution is combined with a soil and deionized water solution, mixed well, and allowed to sit. Hydrometer and temperature measurements are then taken at intervals to determine particle size.

I performed the clay content analysis in 3 separate sessions, each with 10 samples analyzed at once. I weighed 50 ± 0.1g of air-dried soil on a Radwag analytical scale (AS 82/220.R2) in 250 mL volumetric beakers. I recorded the beaker weight and beaker and soil weight. I then labelled beakers and placed them in a drying oven (Quincy Lab Inc Digital Microprocessor; Model 30E lab oven) at 105°C for at least 4 hours to evaporate remaining moisture in the soil. After 4 hours, I reweighed and recorded the beakers post-oven weight.

I added 25 mL of 0.5% $(NaPO_3)_6$ solution to each beaker and then brought them to a total volume of 200 mL with deionized water. I stirred the solution and soil with a glass rod for 45 seconds, rinsing the glass rod with deionized water between each sample. After 30 minutes, I emptied the beakers into milkshake mixer cups and used a squirt bottle with deionized water to ensure that the entire sample and solution was transferred into the mixer cup. I brought the cups to 355mL and mixed them with a milkshake mixer (Waring Products Drink Mixer, Model 12 DM 19) for 10 minutes on medium speed.

I transferred the mixed solutions into a 1000 mL graduated sedimentation cylinder, using a squirt bottle with deionized water to ensure all the solution was transferred to the cylinder. I then added deionized water to the cylinder to a total volume of 1000 mL. I thoroughly mixed the cylinder's contents with a wooden plunger wrapped in Parafilm, plunging the contents 15 times (Figure 3-15). I prepared blank solutions for each analysis session by adding 25 mL 0.5% $(NaPO_3)_6$ solution to a 1000 mL graduated cylinder, bringing the total volume to 1000 mL with deionized water, and plunging the solution. I recorded Hydrometer (Bel Art hydrometer) and temperature (Celsius thermometer) readings 40 seconds and 2 hours after the plunges (Figure 3-14). I then corrected the Hydrometer readings: for every degree above 20°C, I added 0.36 g L^{-1} to the hydrometer reading. I used Microsoft Excel to compute the following equations to find clay content.

$$\text{Post-oven Weight}_{\text{SOIL}} = \text{Pre-oven Weight}_{\text{SOIL}} - \text{Pre-oven Weight}_{\text{TOTAL}} + \text{Post-oven Weight}_{\text{TOTAL}}$$

$$\text{Clay Content} = \frac{\text{2hr Hydrometer Reading}_{\text{SAMPLE}} - \text{2hr Hydrometer Reading}_{\text{BLANK}}}{\text{Post-oven Weight}_{\text{SOIL}}}$$

Figure 0-14: Image of clay content analysis of Glacial Heritage and Scatter Creek soil samples

Figure 0-15: Example of wooden plunger wrapped in Parafilm for clay content analysis

Clay Content Analysis Discrepancies:

There were discrepancies throughout the clay content analysis. Two samples, The Prairie at the Mouth of Black River Plot #2 and Glacial Heritage Plot #1 did not have an adequate amount of soil to measure the entire 50g of soil. The Prairie at the Mouth of Black River Plot #2 soil analyzed weighed 34.61g and the Glacial Heritage Plot #1 soil analyzed weighed 42.36g before being heated in the lab oven.

Additionally, some sample was lost during plunging of the 1000 mL cylinders. The tools that I used, wooden plungers wrapped in Parafilm, occasionally got soil matter beneath the Parafilm during plunging (Figures 3-16 and 3-17). The matter appeared to mostly be silt and sand. West Rocky #1 was particularly susceptible to this, as the Parafilm broke. Other solutions spilled during plunging. I spilled roughly 1/5 of The Prairie at the Mouth of Black River Plot #1 during plunging and less than 1/10 of The Prairie at the Mouth of Black River Plot #2, West Rocky Plot #4, and Marion Prairie Plot #3 slopped over the edge of the cylinder while plunging.

For the first two analysis sessions in which I analyzed Secena Prairie, The Prairie at the Mouth of Black River, West Rocky, and Glacial Heritage samples, all graduated cylinders with samples were 1000 mL cylinders while the graduated cylinder used for the blank was a 1250 mL cylinder. For the third analysis session in which Marion Prairie and Scatter Creek samples were analyzed, all graduated cylinders were 1000 mL cylinders. During the third analysis, I created an additional blank with the 1250 mL cylinder to compare measurements to the blank in the 1000 mL cylinder. Both measurements were the same when temperature was accounted for and I continued to the calculation portion of my analysis with the assumption that the first two analysis sessions had correct measurements for the blanks.

Figure 0-16: Wooden plunger wrapped in Parafilm pre-plunging

Figure 0-17: Wooden plunger wrapped in Parafilm post-plunging

Soil Organic Matter Content Analysis

I analyzed the organic matter content of soil samples using a Loss-on-Ignition (LOI) protocols from University of California Davis faculty, Missouri State University, and Ozarks Environmental and Water Resources Institute (Missouri State University & OEWRI, 2019; Pasternack, 2023). I measured 5 ± 0.06g of air-dried soil into ceramic crucibles using a Radwag analytical scale (AS 82/220.R2), recording the sample weight and total weight (sample + crucible). I then placed the samples in a drying oven (Quincy Lab Inc Digital Microprocessor: Model 30E Lab Oven) at 105°C for at least 4 hours to remove any moisture and immediately re-weighed the samples upon removal, recording the total weight.

I transferred the samples to a muffle furnace (LindBerg/BlueM Box Furnace: M# BF51894C-1) and heated them at 500°C for 8 hours. After the 8 hours, I allowed the crucibles to cool minimally and then placed them into desiccators to cool completely. Once cooled, I re-weighed the crucibles. The difference between the post-oven sample weight and the post-furnace sample weight equaled the amount organic matter in the soil. I used the following equations in Microsoft Excel to compute the percentage of organic matter in the soil.

$$\text{Post-oven Weight}_{\text{SOIL}} = \text{Pre-oven Weight}_{\text{SOIL}} - (\text{Pre-oven Weight}_{\text{TOTAL}} - \text{Post-oven Weight}_{\text{TOTAL}})$$

$$\text{Post-furnace Weight}_{\text{SOIL}} = \text{Post-oven Weight}_{\text{SOIL}} - (\text{Post-oven Weight}_{\text{TOTAL}} - \text{Post-furnace Weight}_{\text{TOTAL}})$$

$$\text{Percent SOM} = \frac{\text{Post-oven Weight}_{\text{SOIL}} - \text{Post-furnace Weight}_{\text{SOIL}}}{\text{Post-oven Weight}_{\text{SOIL}}} \times 100$$

Soil Organic Matter Content Analysis Discrepancies:

I spilled a small amount of The Prairie at the Mouth of Black River Plot #5 sample when transferring it from the drying oven to the muffle furnace. I was unable to weigh the spilled amount. The sample's calculated SOM was in the range of the other The Prairie at the Mouth of Black River samples' calculated SOMs.

3.6 Weather Data

I obtained temperature and precipitation data collected at the Olympia Airport, elevation 210ft (USGS, 2019), from NOAA's NOWData platform (Figure 3-18) (NOAA, 2023). I downloaded monthly data for each month from October 2021 through early June 2023.

For precipitation, I recorded maximum precipitation in one event, maximum precipitation event date, and total precipitation accumulation (NOAA, 2023). I also recorded any precipitation anomalies, such as days with over 3 inches of accumulation and periods of constant precipitation, and calculated the percentage of days in a month with a trace amount or higher of precipitation.

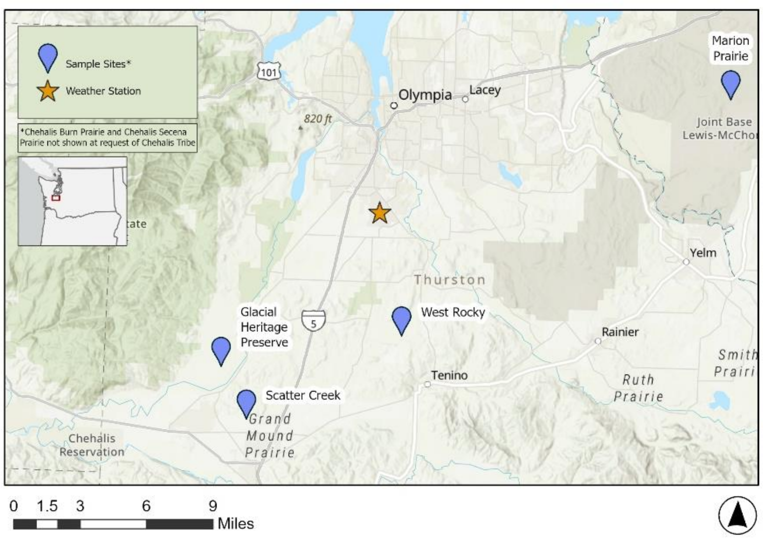

Figure 0-18: Map showing the location of the Olympia Airport weather station and locations of sample sites

For temperature, I downloaded maximum and minimum temperature events, maximum and minimum temperature event dates, average maximum and minimum temperature, and average daily temperature (NOAA, 2023). I also recorded temperature anomalies, such as multiple days in a row with daily averages below freezing and extended periods with low temperatures below freezing, and calculated the percentage of days in a month with average daily temperatures above 41°F.

3.7 Statistical Analysis:

I recorded data in Microsoft Excel and used the program to compute averages, standard deviations, and standard errors as well as to produce tables and charts. I computed average, standard deviation, and standard error of plot results for total camas bulb weight, soil pH level, soil clay content, and SOM to find results per site. I also calculated the averages, standard deviations, and standard errors of the 4 variables for all sites, treated sites, and untreated sites.

To determine if there were statistically significant differences between site variables, I used RStudio to perform an analysis of variance (ANOVA) and post hoc Tukey Honestly Significant Difference (Tukey HSD) analysis on the 30 samples. I observed the histograms of each variable and ran Shapiro Wilks Tests at a 95% confidence interval to assess normality. All variables were close to normally distributed (p-values ≤ 0.55), and I did not transform my data. I then ran ANOVA for each variable, with the sample site as the independent variable.

Using a 90% confidence interval, the ANOVA tests showed significant differences between sites for all 4 variables (p-values ≤ 0.10). I then performed post hoc Tukey HSD analyses on each variable to determine which sites differed in camas bulb weight, soil pH level, soil clay content, and/or SOM.

Chapter 4: Results

Here, I will elaborate on camas and soil specific results, comparison of sample site factors, and glyphosate and AMPA accumulation results.

4.1 Camas Bulb Weight Results

Eight camas bulbs were collected from each sample plot. Bulbs weighed the least at Marion Prairie and the most at The Prairie at the Mouth of Black River (Figure 4-1). On average, camas bulb samples weighed 261.62 ± 20.07g (Table 4-1). Bulbs from sites treated with a GBH averaged 258.33 ± 111.22g and bulbs from untreated sites averaged 264.90 ± 117.34g (Table 4-1; Appendix D). There were significant differences between bulb weights at sites. Marion Prairie and West Rocky ($p = 0.0262$); Marion Prairie and The Prairie at the Mouth of Black River ($p = 0.0057$); West Rocky and Glacial Heritage ($p = 0.0159$); and Glacial Heritage and The Prairie at the Mouth of Black River ($p = 0.0033$) bulb weights were significantly different from each other (Table 4-2).

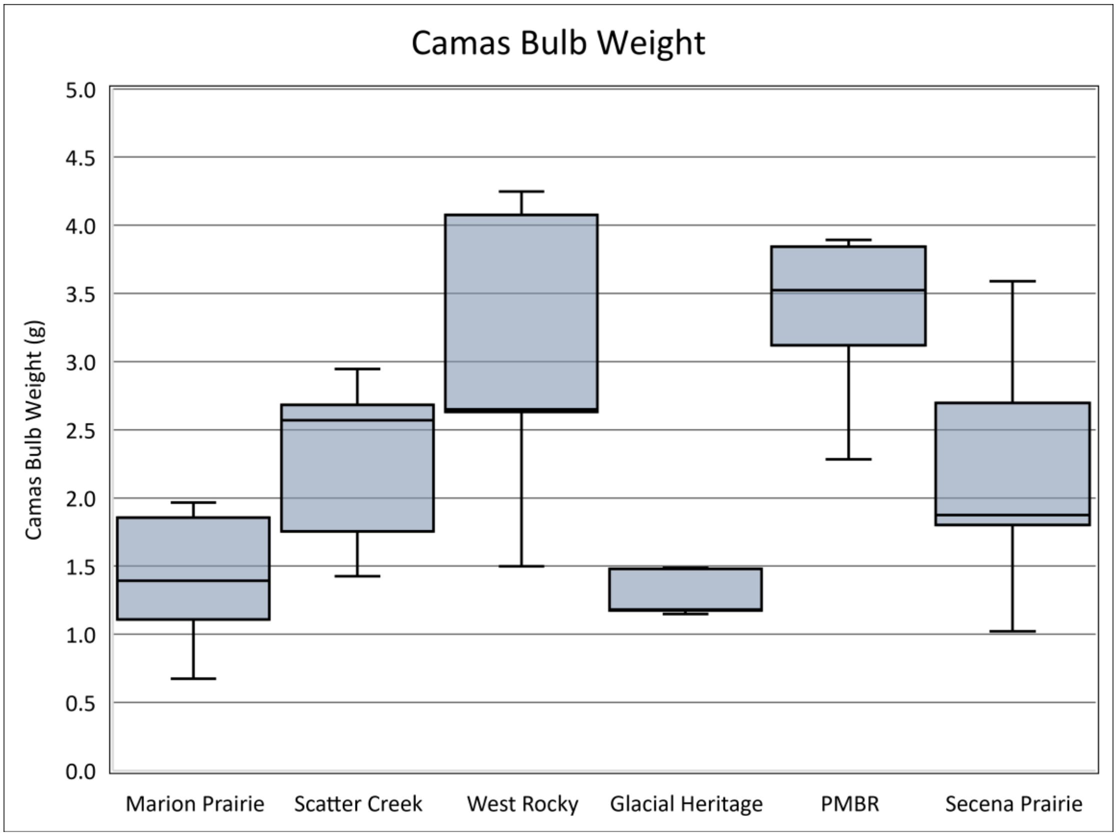

Figure 0-1: Camas bulb mean weight by site

4.2 Soil Results

4.2.A Soil PH Level

All sample plots had acidic pH levels below a pH of 6 (Figure 4-2; Appendix A). The pH averaged 5.13 ± 0.04 across all prairie sites. Average levels were lowest at Scatter Creek and highest at Secena Prairie. Soil pH averaged 5.11 ± 0.06 across treated sites and 5.14 ± 0.05 across untreated sites (Table 4-1). Scatter Creek and Marion Prairie ($p = 0.026$); Scatter Creek and West Rocky ($p = 0.0058$); and Scatter Creek and Secena Prairie ($p = 0.0025$) soil significantly differed in pH levels (Table 4-2).

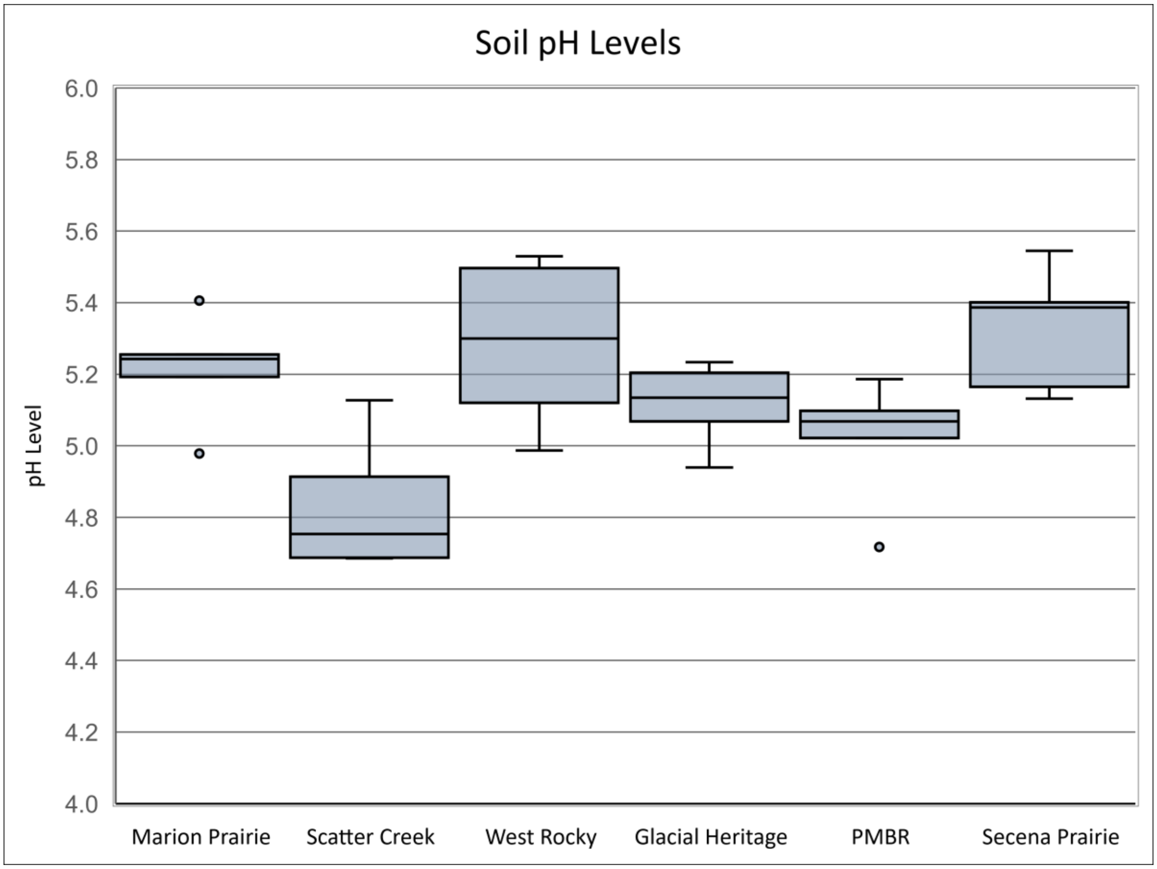

Figure 0-2: PH level and mean by site

<u>*4.2.B Soil Clay Content*</u>

The percentage of clay in the prairie soil measured less than 8% in each sample plot (Figure 4-3; Appendix B). Clay content averaged 2.96 ± 0.44% across all prairie sites (Table 4-1). It ranged from 0% (Scatter Creek plots #1, #3, #4, #5; Marion Prairie plot #2; Glacial Heritage Preserve plots #1 and #3) to 6.94% (Secena Prairie plot #3) (Appendix B). Clay content averaged 2.64 ± 0.69% at treated sites and 3.28 ± 0.48% at untreated sites (Table 4-1). Clay content significantly differed between soils at Scatter Creek and West Rocky ($p = 0.0075$) (Table 4-2).

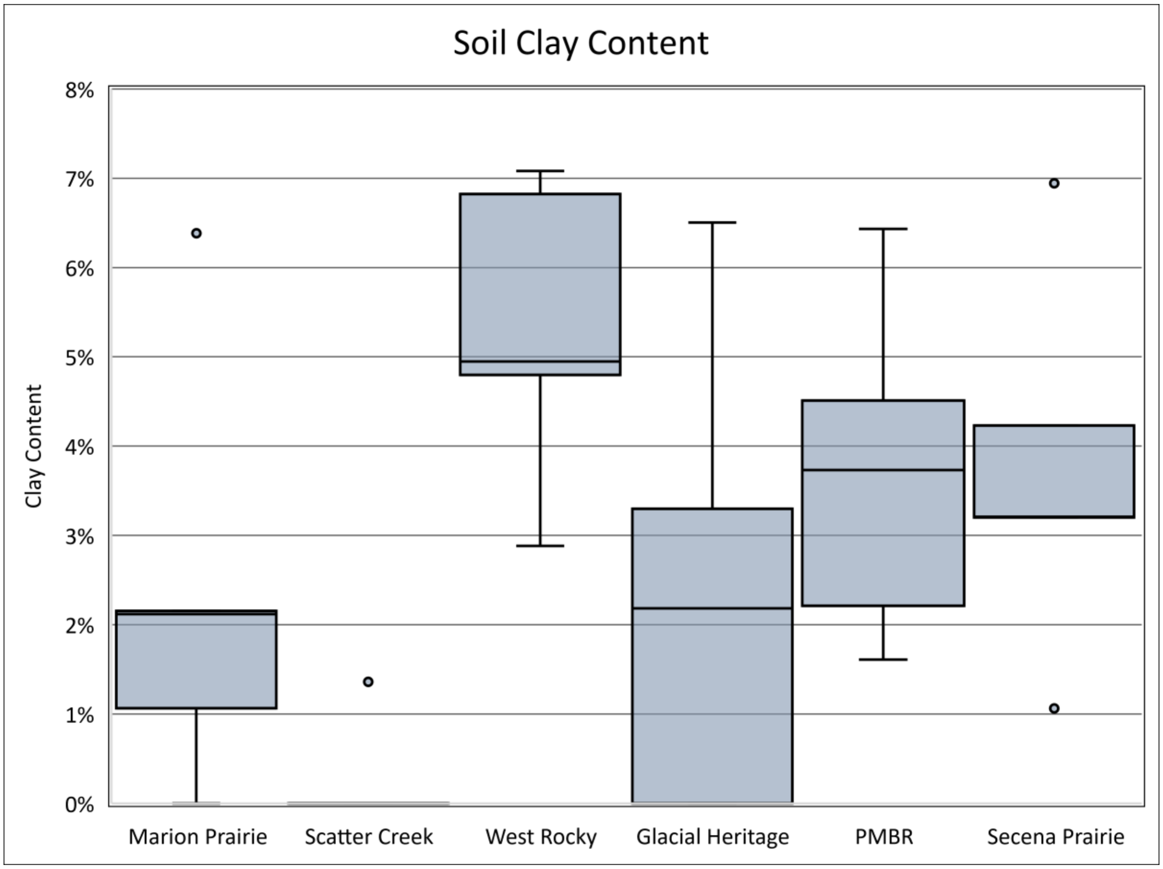

Figure 0-3: Clay content and mean by site

4.2.C Soil Organic Matter Content

Across all sample sites, SOM averaged 27.09 ± 1.42% (Table 4-1). It ranged from 14.98% (Secena Prairie plot #2) to 45.18% (The Prairie at the Mouth of Black River plot #3) across all plots (Appendix C). The Prairie at the Mouth of Black River had the highest average SOM while West Rocky had the lowest (Figure 4-4). SOM averaged 23.64% ± 1.46% at treated sites and 30.55 ± 2.21% at untreated (Table 4-1). SOM was highly variable between sites, with significant differences between Marion Prairie and Scatter Creek ($p = 0.0008$); Marion Prairie and West Rocky ($p = 0.0169$); Marion Prairie and The Prairie at the Mouth of Black River ($p = 0.0377$); Scatter Creek and Glacial Heritage ($p = 0.0513$); Scatter Creek and The Prairie at the Mouth of Black River ($p = 0.0005$); West Rocky and Glacial Heritage ($p = 0.0008$); West Rocky and The Prairie at the Mouth of Black River (p = 0); Glacial Heritage and Secena Prairie (p = 0.0104); and The Prairie at the Mouth of Black River and Secena Prairie (p = 0.0001) (Table 4-2).

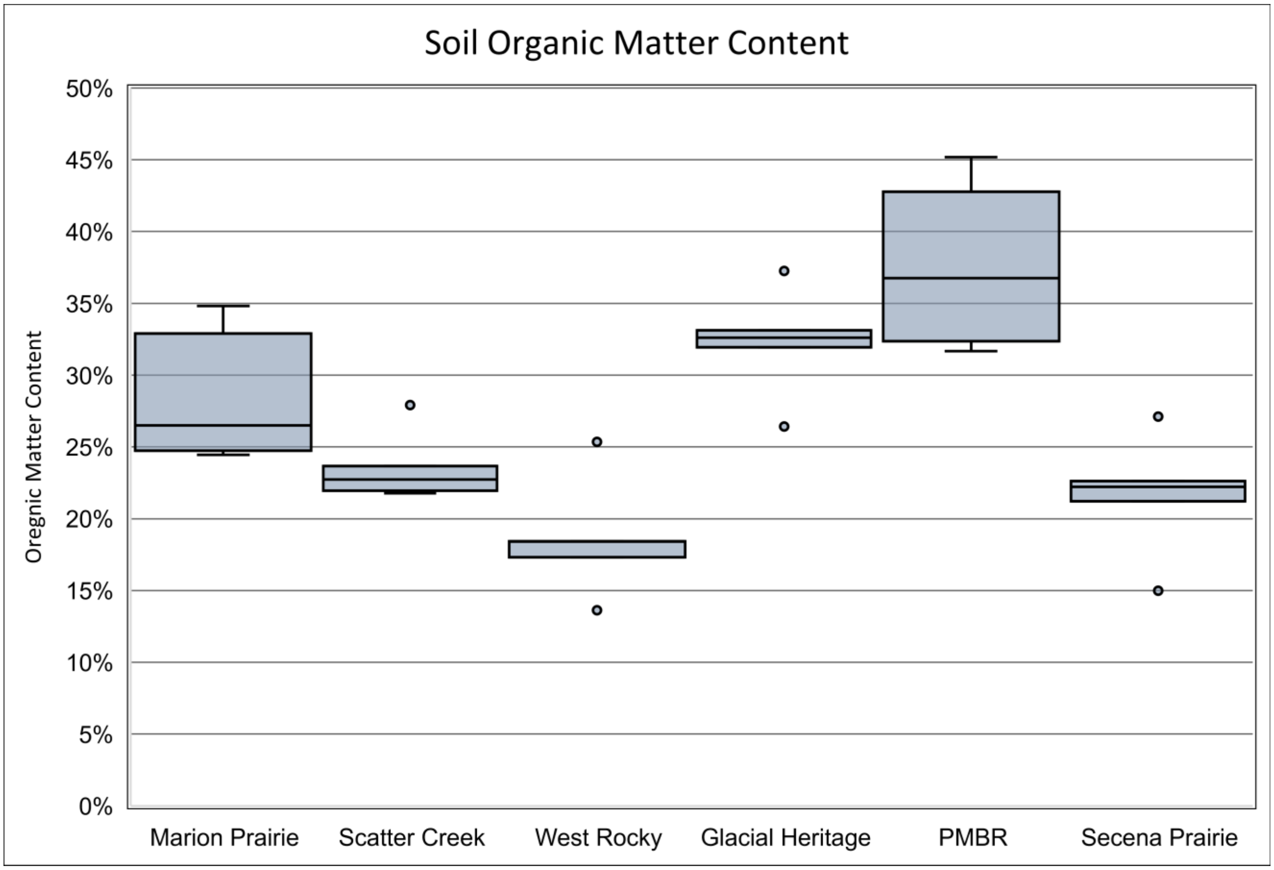

Figure 0-4: SOM and mean by site

Table 0-1: Camas bulb weight and soil characteristic results for all sites

Site Characteristics Results

Type	Site	Camas Bulb Weight (g)	Soil pH	Soil Clay Content (%)	Soil Organic Matter (%)
Treated	Marion Prairie	1.40 ± 0.24	5.22 ± 0.07	2.34 ± 1.08	28.68 ± 2.17
	Scatter Creek	2.28 ± 0.30	4.83 ± 0.08	0.27 ± 0.27	23.61 ± 1.13
	West Rocky	3.02 ± 0.98	5.29 ± 0.11	5.31 ± 0.76	18.62 ± 1.9
	Average	2.23 ± 0.98	5.11 ± 0.06	2.64 ± 0.69	23.64 ± 1.46
Control	The Prairie at the Mouth of the Black River	3.33 ± 0.03	5.02 ± 0.08	3.7 ± 0.86	37.74 ± 2.72
	Glacial Heritage	1.30 ± 0.08	5.12 ± 0.05	2.4 ± 1.21	32.27 ± 1.73
	Secena Prairie	2.10 ± 0.44	5.33 ± 0.08	3.73 ± 0.95	21.63 ± 1.95
	Average	2.28 ± 1.04	5.15 ± 0.05	3.28 ± 0.48	30.55 ± 2.21
All Sites	Average	2.25 ± 0.19	5.13 ± 0.04	2.96 ± 0.44	27.09 ± 1.42

Table 0-2: Statistically significant differences between site camas weight and soil characteristic results

Sample Site Differences

Site 1	Site 2	Camas Bulb Weight p-value	Soil pH p-value	Soil Clay Content p-value	Soil Organic Matter Content p-value
Marion*	Scatter Creek*	0.4667	**0.0260**	0.5976	**0.0008**
Marion*	West Rocky*	**0.0262**	0.9868	0.2299	**0.0169**
Scatter Creek*	West Rocky*	0.6342	**0.0058**	**0.0075**	0.5007
Marion*	Glacial Heritage	1.0000	0.9415	1.0000	0.7950
Marion*	PMBR	**0.0057**	0.5214	0.8941	**0.0377**
Marion*	Secena	0.5660	0.9156	0.8848	0.1615
Scatter Creek*	Glacial Heritage	0.3463	0.1687	0.5725	**0.0513**
Scatter Creek*	PMBR	0.2714	0.5757	0.1190	**0.0005**
Scatter Creek*	Secena	1.0000	**0.0025**	0.1135	0.9798
West Rocky*	Glacial Heritage	**0.0159**	0.6412	0.2461	**0.0008**
West Rocky*	PMBR	0.9855	0.2039	0.8070	**0.0000**
West Rocky*	Secena	0.5332	0.9992	0.8191	0.8886
Glacial Heritage	PMBR	**0.0033**	0.9581	0.9086	0.4005
Glacial Heritage	Secena	0.4354	0.4272	0.9000	**0.0104**
PMBR	Secena	0.2061	0.1053	1.0000	**0.0001**

*Treated sites

Bold: Adjusted p-values indicating signficant difference at 90% confidence ($p \leq 0.1$) interval

4.2.D Web Soil Survey Findings

The soil characteristic results found in this study were comparable with the USDA's NRCS's Web Soil Survey (Table 4-3) and other soil analyses in the prairies (Reynolds et al., 2015). For most sites, pH results were slightly lower than the Web Soil Survey's findings but, with typical pH analysis error levels being ± 0.5 (Cole-Parmer, 2021), my results were within the range of error for all sites. Not all soil series listed in the Web Soil Survey had clay content data, namely Spanaway and Spanaway-Nisqually complex (Scatter Creek, West Rocky, and Glacial Heritage sites). For sites that did have Web Soil Survey clay content data (Marion Prairie, PMBR, and Secena Prairie), my results aligned with the survey's findings. SOM was not listed in the Web Soil Survey, but the high SOM of my results agrees with other prairie studies analyzing the top 10cm of soil (Lowther, 2022; Reynolds et al., 2015).

Table 0-3: Soil characteristic results and Web Soil Survey data

Site Characteristic and Results

Site	Type	Soil Class	Soil Drainage Class	Particle Size	Composition Type	Typical pH	Typical Clay Content (%)	pH Result	Clay Content Result (%)	SOM Result (%)
Marion Prairie	Treated	Everett-Spanaway complex	Somewhat excessively drained	Sandy-skeletal	5% Histosols 95% Inceptisols	Everett: 5.3-5.4 Spanaway: 5.4	Everett: 2-10% Spanaway: --	5.22 ± 0.07	2.34 ± 1.08	28.68 ± 2.17
Scatter Creek	Treated	Spanaway-Nisqually complex	Somewhat excessively drained	Sandy-skeletal	60% Andisols 30% Inceptisols	Spanaway: 5.4 Nisqually: 5.6	Spanaway: -- Nisqually: --	4.83 ± 0.08	0.27 ± 0.27	23.61 ± 1.13
West Rocky	Treated	Spanaway gravelly sandy loam	Somewhat excessively drained	Sandy-skeletal	100% Andisols	5.4	----	5.29 ± 0.11	5.31 ± 0.76	18.62 ± 1.9
The Prairie at the Mouth of the Black River	Control	Daviscreek-Huttula complex	Well drained	Medial-skeletal over sandy, Sandy-skeletal, Loamy-skeletal	75% Andisols 25% Inceptisols	Daviscreek: 5.3 Huttula: 5.3	Daviscreek: 10-20% Huttula: 5-18%	5.02 ± 0.08	3.7 ± 0.86	37.74 ± 2.72
Glacial Heritage Preserve	Control	Spanaway-Nisqually complex	Somewhat excessively drained	Sandy-skeletal	60% Andisols 30% Inceptisols	Spanaway: 5.4 Nisqually: 5.6	Spanaway: -- Nisqually: --	5.12 ± 0.05	2.4 ± 1.21	32.27 ± 1.73
Secena Prairie	Control	Grandmound gravelly sandy loam	Somewhat excessively drained	Sandy-skeletal	10% Andisols 90% Inceptisols	5.3	≤ 15%	5.33 ± 0.08	3.73 ± 0.95	21.63 ± 1.95

4.3 Weather Results

There were below freezing temperature events at Olympia Airport throughout December 2021 – April 2022 and October 2022 – April 2023 with occasional daily averages below freezing in February 2022, November 2022, December 2022, and February 2023 (Appendix E). A temperature of less than 41°F correlates to Coast Salish Prairie soil temperatures that are too low for microbial activity (Reynolds et al., 2015). Temperatures were consistently above 41°F March-October, 2022, and April-June 2023. While there were months in which less than 40% of days were about 41°F, average monthly temperatures did not dip below 35°F (Figure 4-5).

October 2021 – February 2022, June 2022, and November – December 2022 had precipitation events with greater than 1” accumulation (Appendix F). In 17 months between treatment and sampling at Marion Prairie (12/29/2021 – 6/5/2021), 69.1 inches of precipitation accumulated; in 5 months between treatment and sampling at Scatter Creek (1/4/2023 – 6/6/2023), 17.5 inches of precipitation accumulated; and in the 4.5 months between treatment and sampling at West Rocky (1/23/2023 – 6/6/2023) 13.99 inches of precipitation accumulated (Figure 4-6; Appendix F). Additionally, 2 months after Marion Prairie treatment, there was a two day precipitation event resulting in 4.45 inches of accumulation and daily precipitation for 16 days during and after Scatter Creek treatment (Appendix F).

Weather events that may have influenced leaching potential at treated sites are as follows. Marion Prairie was treated on 12/29/2021 and there was a large volume precipitation event recorded at the Olympia Airport 8 days later, on 1/6/2022, where 4 inches of rain fell (NOAA, 2023). In the time from GBH application to bulb harvest at Marion Prairie, 69.1 inches of precipitation was recorded at the airport’s weather station (NOAA, 2023). Scatter Creek, treated on 1/4/2023, was treated amid a period of constant precipitation. From 1/4/2023 – 1/18/2023, 3 inches of precipitation was recorded, varying from 0.01 to 0.51 inches per day (NOAA, 2023).

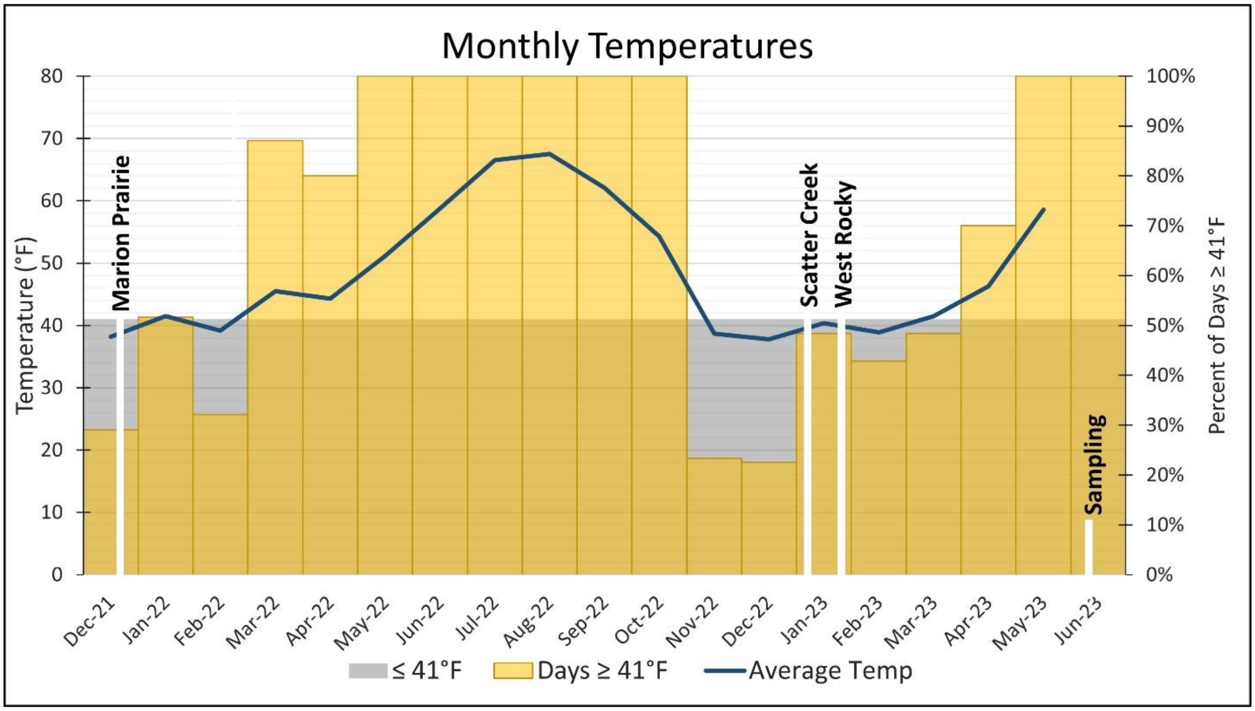

Figure 0-5: Chart showing temperature statistics at the Olympia Airport from Dec-2021 through June-2023

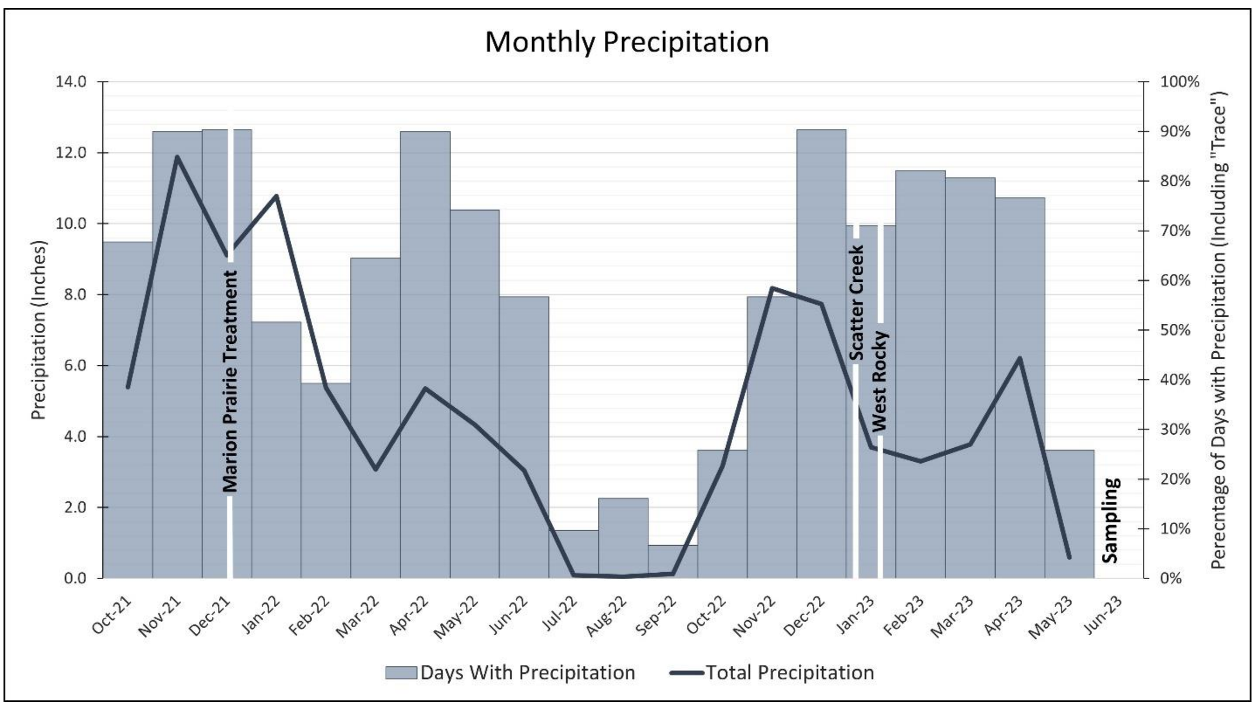

Figure 0-6: Chart showing precipitation statistics at the Olympia Airport from Oct-2021 through June-2023

4.4 Glyphosate Air-Drift Risk Analysis Results

With the exception of Marion Prairie, all sites had “no risk” of exposure to glyphosate by air-drift. Samples at Marion Prairie were located within 50-100 feet of a road and had a “low risk” of glyphosate exposure by air-drift. (Figures 4-7 – 4-11)

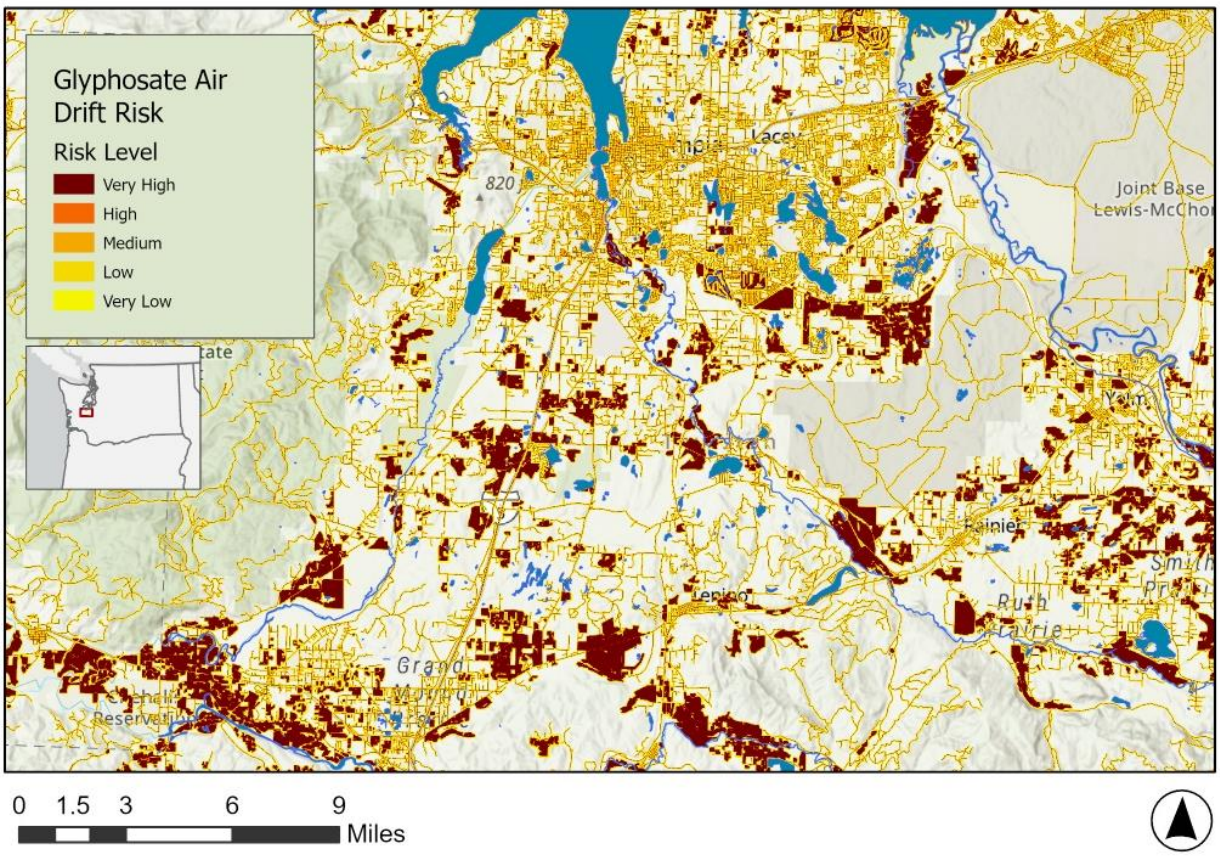

Figure 0-7: Map of glyphosate air-drift risk results

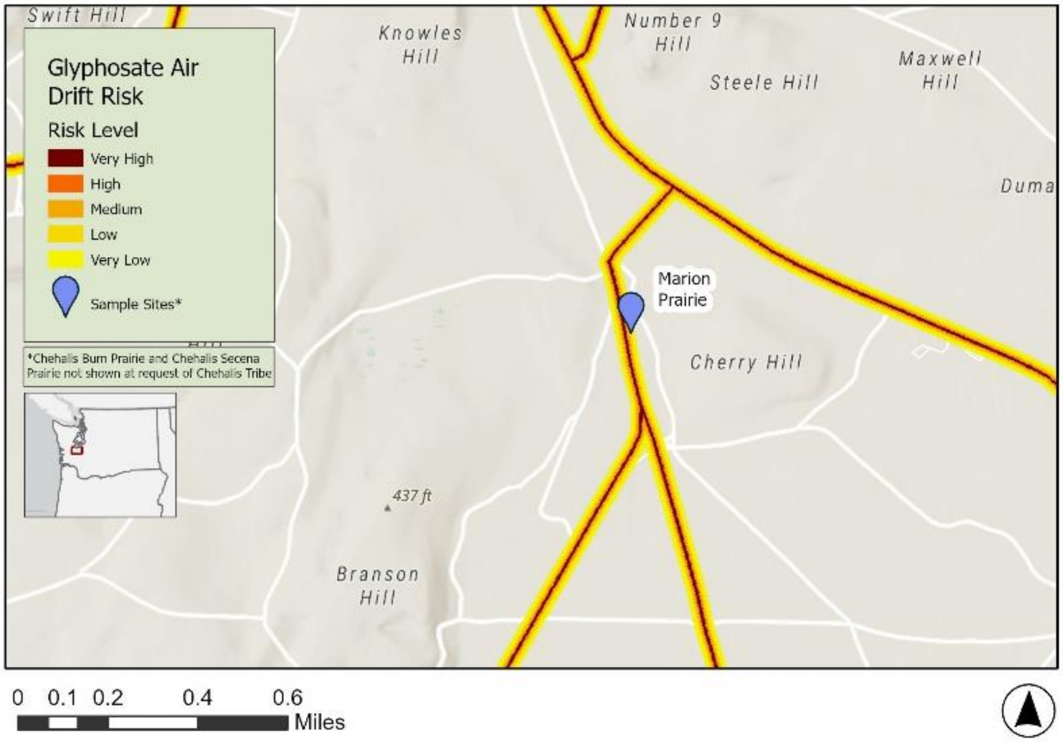

Figure 0-8: Map of glyphosate air-drift risk at Marion Prairie

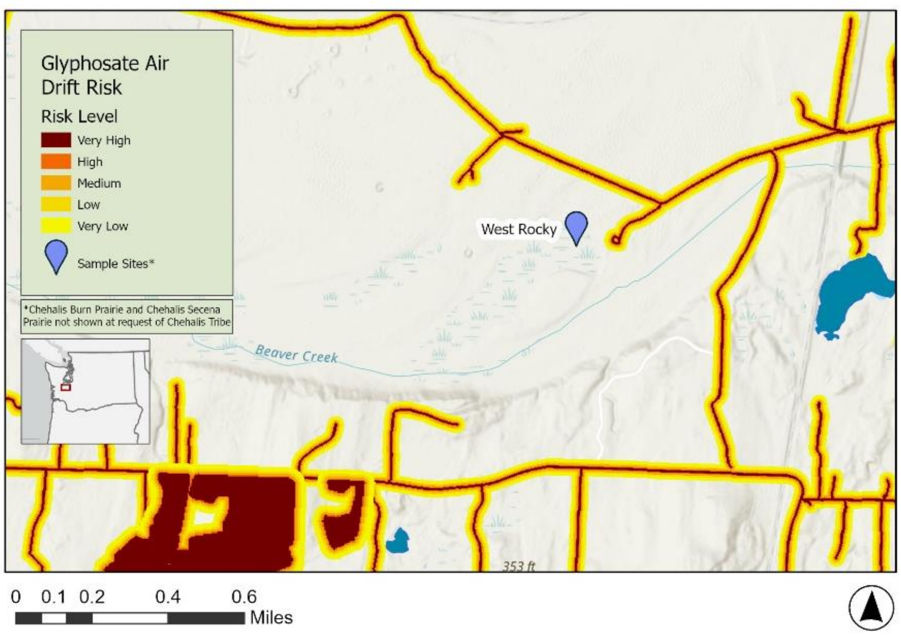

Figure 0-9: Map of glyphosate air-drift risk at West Rocky

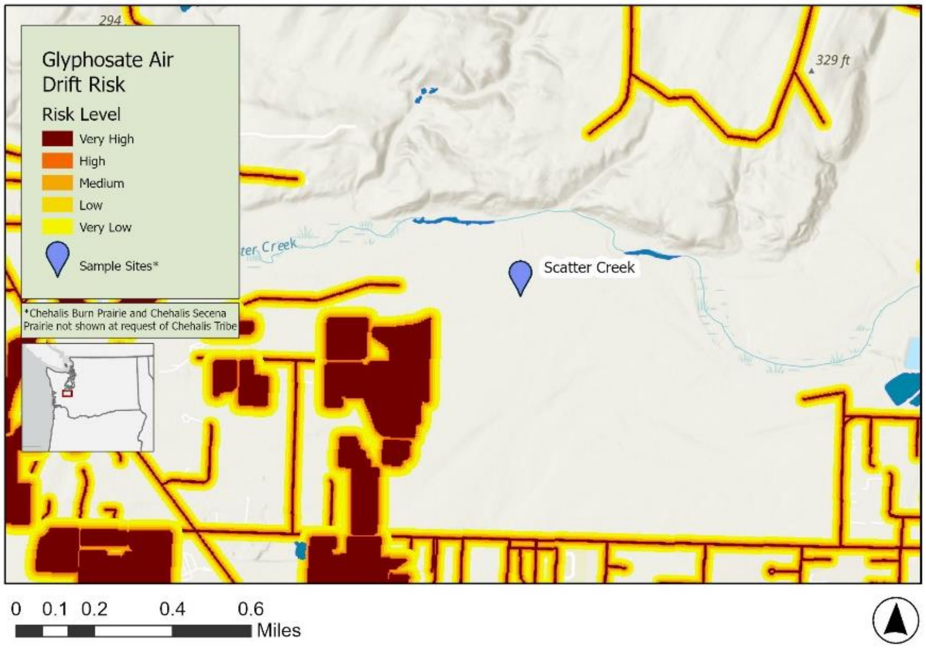

Figure 0-10: Map of glyphosate air-drift risk at Scatter Creek

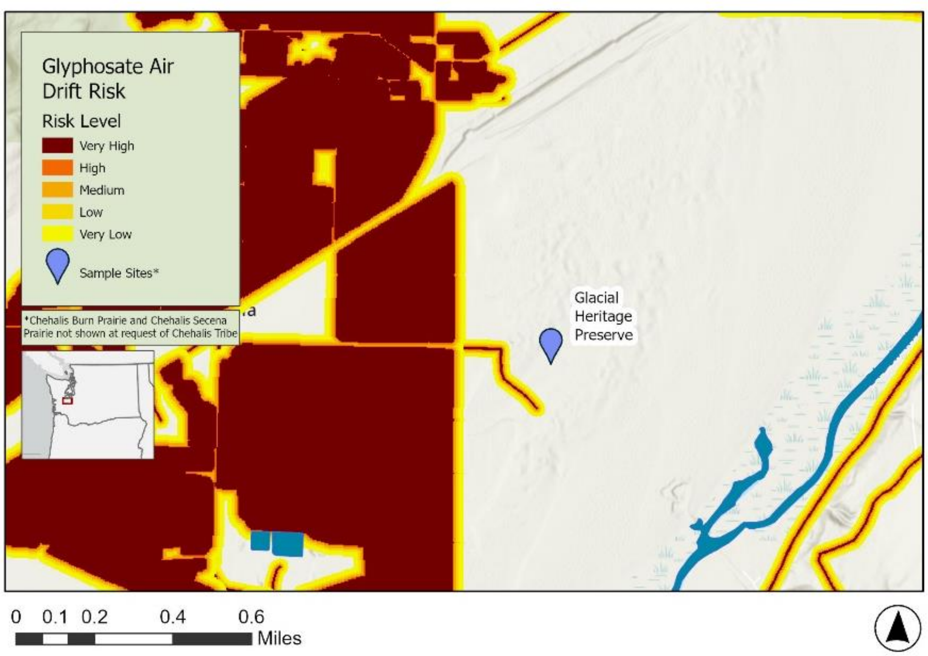

Figure 0-11: Map of glyphosate air-drift risk at Glacial Heritage

4.5 Glyphosate and AMPA Results

AGQ Labs did not find detectable glyphosate in camas bulb samples. The lab's limit of quantification (LOQ) for glyphosate was 0.01mg/kg and their uncertainty level was 24%. Therefore, <0.01 ± 0.0024 mg/kg of glyphosate was found in each sample (Appendix D). AGQ Labs did not find detectable AMPA in bulb samples. The lab's LOQ for AMPA was 0.01 and their uncertainty level was 23.8%. Therefore, <0.01 ± 0.00238 mg/kg of AMPA was found in each sample (Appendix D).

Chapter 5: Discussion

Introduction

I hypothesized that both glyphosate and AMPA would be found in camas bulbs. Though previous research supports my hypothesis (Botten et al., 2021; Edge et al., 2021; Wood, 2019), I did not find detectable concentrations of either compound in the bulbs. Even with significant differences between site soil characteristics and camas bulb size, glyphosate and AMPA test outcomes were consistent across sites. This study's results are specific to Coast Salish Prairie ecosystems, as their soils, climate, and weather patterns differ from other areas in which *Camassia quamash* grows (Beckwith, 2004; Floberg et al., 2004). Furthermore, uptake of glyphosate and AMPA varies between plants (Botten et al., 2021; Wood, 2019), and the lack of concentrations in camas should not be extrapolated to other plants.

In this section, I will address differences in site GBH application and formulas as well as disparities in laboratory analysis between comparative studies. I will then discuss how soil factors, climate and weather patterns, and the specific biology of camas may have impacted results. Finally, I will lay out the implications of the study and further research areas to consider.

5.1 GBH Formula and Application

Sites were treated with two different GBH formulas: one that was 2.5% glyphosate with 0.25% NuFilm as a surfactant (Marion Prairie) and two that were 0.96% glyphosate (2% Roundup Pro) with 0.25% BroncMax as a water conditioning agent (Scatter Creek and West Rocky). Marion Prairie's treatment (2.5% glyphosate) is more in line with typical restoration practitioner use of 1.5-3% glyphosate (Newton et al., 2008; Stanley et al., 2011). Scatter Creek and West Rocky's lower concentration treatments were 2.6x less than Marion Prairie's.

Accordingly, it cannot be inferred that a 2.5% glyphosate treatment would result in undetectable glyphosate and AMPA in camas bulbs after 4.5 months, as in West Rocky's bulbs.

Other research analyzing glyphosate and AMPA in temperate perennial plants is sparse, but those that have studied this used a higher glyphosate concentration than Marion Prairie: 4.3-4.5% (Botten et al., 2021; Edge et al., 2021; Newton et al., 1994; Wood, 2019). Additionally, the studies used aerial spray application (Botten et al., 2021; Edge et al., 2021; Newton et al., 1994; Wood, 2019) rather than the on-the-ground broadcast spray application in this study. Researchers that aerial sprayed GBHs at 4.3-4.5% glyphosate found glyphosate and AMPA in perennial plant foliage up to 12 years post-treatment (Botten et al., 2021). Most measured glyphosate and AMPA concentrations over one year and all found glyphosate and/or AMPA in herbaceous and shrub perennials at the end of their research period (Edge et al., 2021; Newton et al., 1994, 2008; Wood, 2019). The amount of glyphosate used in the studies, roughly 1.7x the glyphosate used at Marion Prairie and 4.5x the glyphosate used at Scatter Creek and West Rocky, along with the application technique, may explain the discrepancy between the findings.

Surfactants, a component of most GBH formulas, increase the effectiveness of the herbicide by enhancing its ability to stick to plants and be absorbed (Dennehy et al., 2011). In this case, 0.25% NuFilm was used as a surfactant at Marion Prairie and an unknown surfactant was used at Scatter Creek and West Rocky. The GBH used at the latter two sites was Roundup Pro, made by Bayer, and the exact formula of the GBH is proprietary (Bayer, 2023a). Without knowledge of surfactants used, it is not known how they impacted the results. However, the surfactant used may not be an issue. A study in a western Oregon forest found that one aerial spray of simply glyphosate and water with no surfactant resulted in 0.162 mg/kg of glyphosate in sword fern leaves 120 days and 346 days post-treatment (Newton et al., 1994). The authors also found that the soil contained glyphosate at 120 days (0.15 mg/kg), 180 days (0.15 mg/kg), and 346 days (0.08 mg/kg) after treatment (Newton et al., 1994). The results suggest that, even without a surfactant, glyphosate can remain in herbaceous tissues and available to plant root uptake a year post-application.

5.2 Glyphosate and AMPA Analysis

Glyphosate and AMPA testing procedures differed between the study presented here and previous studies. AGQ Labs, the lab that analyzed camas bulb samples for this research, used LC MS/MS and the QuPPe method (AGQ Labs USA, 2023), the current gold standard for glyphosate and AMPA detection used by the FDA and other regulatory agencies (USDA & AMS, 2022). With an LOQ of 0.01 mg/kg, AGQ Labs had the lowest LOQ of all studies that analyzed perennial plants but not the lowest minimum detection limit (Botten et al., 2021; Edge et al., 2021; Feng & Thompson, 1990; Newton et al., 1994, 2008; Wood, 2019). However, the minimum detection limit was not noted in the findings of all studies. Of the three studies in which researchers analyzed roots of herbaceous perennials, two used different laboratory detection methods than I did. Botten et al. (2021) detected glyphosate and AMPA in roots with high performance liquid chromatography mass spectrometry (HPLC-MS), as did Wood (2019) with high performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICPMS). Considering the differences in laboratory analysis methods and the high quality of the method used in this research, glyphosate and AMPA detectability should not have been affected, and these results can be compared to other researchers' findings.

5.3 Soil Characteristics.

A statistical analysis yielded results showing significant differences between site soil conditions (Table 4-2), especially amongst treated sites. Marion Prairie had higher SOM content than West Rocky and Scatter Creek; Scatter Creek had lower pH levels than Marion Prairie and West Rocky; and West Rocky had higher clay content than Scatter Creek. The site differences between Scatter Creek and West Rocky have the potential to be most consequential because the two were treated at similar times with the same GBH formula not be of consequence, as low pH (Laitinen et al., 2009; Miles & Moye, 1988) and weather patterns likely have a greater impact on glyphosate and AMPA mobility than clay content.

Glyphosate is immobilized in soils with lower pH, higher clay content, and higher SOM (Gimsing, Borggaard, & Bang, 2004; Laitinen et al., 2009; Ojelade et al., 2022; Shushkova et al., 2009), and my lab analysis results identified that the prairie soils have low pH, low clay content, and high SOM. With two of three immobilization factors being met, there is potential for both glyphosate and AMPA to be bioavailable to camas (Neumann et al., 2006; Viti et al., 2019). A one-year study in a Canadian forest with loamy sand/sandy loam soils low in clay content (0%) with pH levels from 4.7-7 found detectable glyphosate and AMPA ($\geq$ 0.02 mg/kg) in all 4 perennial plant species roots tested 1-year post-treatment (Wood, 2019). However, in this study, it is possible that the low pH and high SOM lead to such high sorption of glyphosate and AMPA to soil particles that the compounds were unavailable for plant uptake (Miles & Moye, 1988).

Not all soil factors that influence the mobility, adsorption, and leaching of glyphosate and AMPA were tested for in this study. Phosphorous and glyphosate compete when adsorbing to soil particles and high amounts of organic and inorganic phosphorous in soil may increase the mobility of glyphosate (Gómez Ortiz et al., 2017; Miles & Moye, 1988). Cation exchange capacity, iron, aluminum ions, and mineral content can also play important roles in glyphosate mobility and plant uptake (Gómez Ortiz et al., 2017; Okada et al., 2016). Thus, there may be more soil characteristics influencing availability of glyphosate and AMPA in soil than studied here.

5.4 Climate and Weather

The Coast Salish Prairie climate, with its mild, rainy winter, may intensify the leaching of glyphosate and AMPA into the soil water column (Laitinen et al., 2009; Rasmussen et al., 2015). This may drastically decrease the amount of glyphosate in soil and, the less glyphosate in soil, the less likely that plants with absorb it through their roots (Neumann et al., 2006). Another way that glyphosate dissipates from soil is through mineralization into AMPA and other metabolites by bacteria (Rivas-Garcia et al., 2022). Bacteria are most active in soils with a non-saturated moisture level during mild air temperature events (Bento et al., 2016; Reynolds et al., 2015). Coast Salish Prairie summers may be too warm and dry for bacteria to actively mineralize glyphosate (Bento et al., 2016; Newton et al., 1994), but, with the well-drained soils of the prairies rarely reaching saturation levels during rainy periods, it is possible that the mineralization of glyphosate into AMPA is quite rapid in winter months (Bento et al., 2016; Reynolds et al., 2015).

There is a high potential for the leaching of glyphosate at sampled sites. Leaching is more apt to occur in well drained soils and all treated sites are classified as "somewhat excessively drained" by the USDA's NRCS's Web Soil Survey (NRCS, 2019). Furthermore, in sandy soils, modelling shows the extent of glyphosate leaching depends on volume of rainfall rather than intensity (Rasmussen et al., 2015). With the sites being treated during the consistently rainy winter months of the prairies, it is possible than the high volume of precipitation led to the efficient leaching of glyphosate and AMPA. Even if glyphosate and AMPA were leached deeper into the soil rather than completely out of the site, camas of the Coast Salish Prairies may not be able to access the compounds, as the plants generally inhabit the top 10cm of soil (Beckwith, 2004; Personal observation).

Temperature may also have influenced dissipation from soil, as mineralization of glyphosate into AMPA and other metabolites may correlate to soil respiration rates driven by temperature (Gimsing, Borggaard, Jacobsen, et al., 2004; Newton et al., 2008; von Wirén-Lehr et al., 1997). When glyphosate is mineralized, it is no longer available to plants, but its metabolites may be. A study in a Coast Salish Prairie found that respiration was lowest when soil temperatures dipped below 50°F in the wet winter, corresponding with average monthly air temperatures below 41°F (Reynolds et al., 2015). Respiration was also low when soil temperatures were above 80°F in the dry summer, corresponding with average monthly air temperatures of 63°F and higher. If glyphosate mineralization does correlate to soil respiration rates (Bento et al., 2016), then extrapolating on Reynolds et al. (2015), there were 7-9 peak months where glyphosate could efficiently be mineralized at Marion Prairie and 2-3 peak mineralization months at Scatter Creek and West Rocky.

However, the Olympia Airport's weather station takes data on location and site specific temperature and precipitation will vary from what the weather station recorded (WRCC, 2023). As the crow flies, Marion Prairie is 26.8 km (16.7 miles) away from the airport, Scatter Creek is 17.9km (11.2 miles) away, and West Rocky is 9km (5.6 miles) away (Figure 3-18). While the Olympia Airport weather station's data indicate general precipitation and temperature conditions for the surrounding area, specific site data can vary widely (WRCC, 2023). Therefore, accurately predicting leaching and mineralization potential is reliant on approximate weather data.

5.5 Camas Biology

A plant's life strategy has a significant impact on glyphosate and AMPA's presence in its tissues, with herbaceous perennials both accumulating more of the compounds and retaining them longer than similarly treated shrubs (Botten et al., 2021; Wood, 2019). Botten et al. (2021) and Wood (2019) hypothesized that herbaceous plants' concentrated storing of carbohydrates and nutrients in roots for much of the year results in elevated levels of AMPA and glyphosate in the plants, particularly in their roots. Though camas is also an herbaceous perennial, its life strategy is different than other plants that have been studied. With a bulb rather than a branching root structure, camas stores large amounts of carbohydrates in a concentrated area that is relatively small compared to its above ground structure (Beckwith, 2004; Maclay, 1928). Although surprising because of camas's significant carbohydrate storage capabilities, the difference in life strategy may impact the extent to which camas retains glyphosate and AMPA.

In studies that have found persistent glyphosate and AMPA in herbaceous perennials, foliage was directly exposed to a GBH (Botten et al., 2021; Edge et al., 2021; Wood, 2019). The timing of GBH treatment in this study may have limited camas's exposure to the herbicide. Sites were treated with a GBH in the winter while camas was dormant, and with no above ground vegetative structure at the time, camas was not directly exposed to the herbicide. Glyphosate and AMPA are most efficiently translocated through plants in the same manner that sugars are during photosynthesis, from leaves to root (Preston & Wakelin, 2008; Wyrill & Burnside, 1976). The lack of direct exposure to the GBH may have played a role in the camas findings in this study.

Off-target and dormant plants can uptake glyphosate and AMPA from soil contaminated by treated plant root exudates and dropped foliage (Neumann et al., 2006; Viti et al., 2019), but camas does not appear to have done so in this case.

It is also possible that camas is resistant to glyphosate, as some plants quickly metabolize glyphosate into AMPA and others isolate it in specific tissues (Duke, 2011; Preston & Wakelin, 2008; Wakelin et al., 2004). Plants that are resistant still tend to have a detectable amount of AMPA in their tissues and a minimal observable adverse reaction to the GBH (Duke, 2011; Mueller et al., 2003). Neither of these characteristics were seen in this study, as AMPA was not detected in any camas bulbs and bulb weight was not significantly different between treated and untreated sites (Table 4-2). Resistance to glyphosate can differ between species in the same plant family (Tahmasebi et al., 2018) and the potential of *Camassia quamash* being resistant should not be inferred to sister species.

5.6 Implications

Based on the results of this study, *Camassia quamash* of the Coast Salish Prairies are free from detectable glyphosate and AMPA 4.5 months after one GBH treatment of ≤ 0.96% glyphosate and 17 months after one GBH treatment of ≤ 2.5% glyphosate when the GBH is applied during winter months and the camas plant is not directly sprayed. The implications are specific to soils low in pH, very low in clay content, and high in SOM that are "somewhat excessively drained" and primarily Andisols or Inceptisols. Results are also climate-dependent, with the study area's warm, dry summers and cool, wet winters potentially influencing glyphosate and AMPA leaching and glyphosate mineralization. Furthermore, results should not be extrapolated beyond *Camassia quamash* to sister species or other plants.

5.7 Areas of Further Research

The implications of this study are encouraging for the prospect of traditional consumption of *Camassia quamash* bulbs from Coast Salish Prairies undergoing restoration. However, the findings are limited in scope and further research is needed, particularly in areas concerning GBH formula and application. Due to the specific soil conditions and climate of the study sites, research beyond Coast Salish Prairies is necessary to understand safety of camas consumption in other regions. Furthermore, land managers commonly use herbicides other than glyphosate that have not yet been studied and may be accumulating in camas bulbs.

Two of the study sites were treated with Roundup Pro to a concentration of 0.96% glyphosate. This is less than the typical amount used in restoration, which aligns more with the 2.5% glyphosate application at Marion Prairie (Stanley et al., 2011). Camas at the Roundup Pro treated sites (Scatter Creek and West Rocky) were tested 4.5 months or more after application, while Marion Prairie was tested 17 months after application. Though glyphosate and AMPA were not found in camas at any of the sites, it is important to understand the concentration of glyphosate used before assuming that a site’s camas is not contaminated. Additionally, since Roundup Pro’s formula is not publicly available, further research is needed to understand if there are differences between glyphosate and AMPA from Roundup Pro application vs. glyphosate and Nufilm application.

GBH application technique and repeat treatments are other areas of research to be explored. In this study, each site was treated with a single broadcast application, but spot spraying is not uncommon (S. Freed, personal communication, May 31, 2023). Spot spraying could lead to elevated levels of glyphosate in soils if more of a formula is applied to a concentrated area than would be applied during broadcast application. Similarly, if GBH treatments are repeated in an area, glyphosate and AMPA could build up in soil and be bioavailable to plants.

The “somewhat excessively drained” soils of the Coast Salish Prairies coupled with a climate of consistent winter rains and GBH application during the rainy season may have led to the efficient leaching of the herbicide and its metabolites from the soil. It is possible that this trifecta is essential to the lack of detectable glyphosate and AMPA in sampled camas bulbs. Therefore, research in other regions where camas grows is necessary. The soil of the Coast Salish Prairies are uniquely low in pH and clay content, especially compared to other camas habitats (Beckwith, 2004; Floberg et al., 2004; NRCS, 2019), and Andisols and Inceptisols soil orders may have contributed to findings as well.

Winters with consistent rains and mild temperatures are unique to the camas prairies on the west side of the Cascade Range (Floberg et al., 2004) and, with the timing of GBH application, glyphosate and AMPA may have been quickly leached out of the camas’s reach by the rain. Thus, camas will need to be tested in habitats with different soil and climate characteristics to establish if camas can absorb glyphosate and AMPA through its roots. Timing of GBH application will also need to be studied to assess how leaching, mineralization, and camas foliage exposure impacts accumulation. Furthermore, if leaching is significant, local groundwater, ponds, and streams should be tested for glyphosate, AMPA, and any adjuvants used.

Because glyphosate is a non-selective herbicide (Martins-Gomes et al., 2022), it may not be a land manager’s best choice for the restoration work needed (Stanley et al., 2008; Tunnell et al., 2006). Selective, pre-emergent, post-emergent, forb-specific, and grass-specific herbicides are being used as well, sometimes more frequently than GBHs (personal communication: Sanders, JBLM). Imazypyr, triclopyr, clethodim, indaziflam, and diquat dirpomide are five such herbicides that need further research.

Camas is also exposed to other toxins outside of herbicides. An un-published component of this study found lead in camas bulbs at both of the Chehalis Tribe sites. At Secena Prairie, two camas plot samples contained lead (0.044 ± 0.004 mg/kg and 0.036 ± 0.003 mg/kg) and at The Prairie at the Mouth of Black River, one sample contained lead (0.016 ± 0.001 mg/kg). Samples that tested positive for lead at Secena Prairie were taken from areas that had been exposed to trash dumping, while the samples at The Prairie at the Mouth of Black River may have been exposed to lead bullets from hunting or shooting practice. The lead results call for further research to establish safe traditional harvest areas, especially within hunting areas, and to weigh ecological restoration priorities for a site.

Finally, *Camassia quamash* is one of many culturally significant species that are being exposed to herbicides and other toxins (Archuleta et al., 2020; Botten et al., 2021; Wood, 2019). It is necessary to test these and other potentially hazardous compounds in other species of cultural significance to support Tribes and Indigenous peoples, providing knowledge so that they can make informed decisions when harvesting and consuming first foods.

Chapter 6: Conclusion

The results presented in this research indicate that there is no risk of ingesting glyphosate or AMPA when consuming *Camassia quamash* in Coast Salish Prairies surrounding Olympia, WA, 4.5 months following a 0.96% glyphosate treatment and 17 months following at 2.5% glyphosate treatment. The implications are that, when considering human health and *Camassia quamash* consumption, restoration practitioners may continue to treat sites with GBHs in the tested amounts under specific soil, treatment timing, and weather conditions. When the conditions are met, Indigenous peoples can gather and consume bulbs without risk of glyphosate or AMPA health repercussions. However, a condition of these results is that bulbs were tested after a singular GBH treatment. Multiple treatments occurring within the timespan sampled in this study may result in accumulation of glyphosate and AMPA in bulbs.

To my knowledge, this study is the first to research glyphosate and AMPA concentrations in *Camassia quamash* bulbs and the first to research glyphosate and AMPA accumulation in a Coast Salish Prairie perennial plant. It provides baseline information for continued research. *Camassia quamash* has a range that spans 850,000 square miles and countless individual prairies (Gould, 1942). Further research is essential to understanding if *Camassia quamash* is at risk of glyphosate and AMPA accumulation under other climatic, soil, and treatment conditions. As these results cannot be extrapolated to other *Camassia* species, other species such as *Camassia leichtlinii* (great camas), must be analyzed. Additionally, to establish safety protocols for consuming exposed plants, testing of other edible and medicinal perennial plants on Coast Salish Prairies is necessary. Suggested species are *Lomatium sp.* (biscuitroot), *Balsamorhiza sp.* (balsamroots), *Fritillaria sp.* (checker lily), *Quercus sp.* (oaks), *salix sp.* (willows), *Allium sp.* (wild onions), *Trifolium wormskioldii* (springbank clover), and *Fragaria sp.* (wild strawberries).

Correlations between amounts of glyphosate and AMPA in soil and accumulation in perennial plants have not thus far been made. Considering the potential that glyphosate and AMPA leached from well-drained soils leading to a lack of glyphosate and AMPA in *Camassia quamash* bulbs, testing soils for glyphosate and AMPA could provide further knowledge on whether glyphosate and AMPA remain in soil at all. If not, it is possible that plants that are dormant during application, with no foliage exposed, are at a low risk of glyphosate and AMPA accumulation.

To deduce if other herbicides that are now frequently used on Coast Salish Prairies are accumulating in perennial plants, more sampling and analysis of different herbicides is needed. Health impacts of herbicides with active ingredients triclopyr, clethodim, indaziflam, and diquat dibromide are less studied than GBHs, but implications of consumption may come to light. There is also a legacy of using now banned or restricted herbicides on prairies, such as 2,4-D, which was banned locally for its detrimental human and ecological health impacts. As camas plants live for an indefinite period of time (Maclay, 1928), there is a potential that plants were exposed to and accumulated the compound.

The baseline information presented in this study adds to the minimal literature regarding herbicide accumulation in culturally significant edible and medicinal plants of temperate, dry-land ecosystems (Botten et al., 2021; Edge et al., 2021; Wood, 2019). It is an area of study worth continuing so that the reciprocal relationship between Indigenous peoples, ecosystems, and plants may continue to thrive and all three may benefit. The use of herbicides as a component of land tending and restoration practices, coupled with Traditional Ecological Knowledge, Western Ecological Knowledge, and modern technology are all necessary to sustain ecosystems in this era of globalization, where no end to opportunistic, non-native species is in sight (Willamette Partnership, 2020).

Bibliography

AGQ Labs. (2023). AGQ Labs Accreditations [AGQ Labs]. *AGQ Labs USA*. https://www.agqlabs.us.com/accreditations/

AGQ Labs USA. (2023). *Glyphosate testing in food and water*. AGQ Labs USA. https://www.agqlabs.us.com/glyphosate-testing/

Anastassiades, M., Mack, D., Wildgrube, C., Barth, A., Sigalov, I., Görlich, S., Dörk, D., Cerchia, G., Wachtler, A.-K., Kolberg, D. I., Eichhorn, E., Marks, H., Benkenstein, A., & Zechmann, S. (2021). *Quick Method for the Analysis of Highly Polar Pesticides in Food Involving Extraction with Acidified Methanol and LC- or IC-MS/MS Measurement—I. Food of Plant Orgin (QuPPe-PO-Method) Version 12*. https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN

Anderson, M. K. (2005). *Tending the Wild: Native American Knowledge and the Management of California's Natural Resources*. University of California Press.

Archuleta, G., Rempel, C., & Rodriguez, N. (2020, July 7). *Wapato on the Slough*. ArcGIS StoryMaps. https://storymaps.arcgis.com/stories/6f39855ee1694d1aa72252838fba4ef1

ATSDR. (2020). *Toxicological Profile for Glyphosate*. US Department of Health and Human Services. https://www.atsdr.cdc.gov/toxprofiles/tp214.pdf

Barnett, J. A., Josephson, J. K., Bandy, M. L., Haskey, N., Gibon, J., Chiang, H., & Gibson, D. L. (2023). A55 YOU ARE WHAT YOUR GREAT, GREAT GRANDPARENTS PARENTS ATE: PRE-NATAL GLYPHOSATE EXPOSURE INDUCES DYSBIOSIS, METABOLIC DYSFUNCTION AND BEHAVIOURAL ABNORMALITIES THREE GENERATIONS AFTER EXPOSURE. *Journal of the Canadian Association of Gastroenterology*, *6*(Supplement_1), 30–31. https://doi.org/10.1093/jcag/gwac036.055

Bayer. (2023a). *RoundUp Pro Label*. Bayer. https://natseed.com/pdf/Roundup%20Pro%20Label.pdf

Bayer. (2023b). *Products from A to Z*. Bayer Global. https://www.bayer.com/en/products/products-from-A-to-Z

BCG Global. (2023). *FoodChain ID: Pesticide MRLs*. FoodChain ID Regulatory Units by BCG Global. https://bcglobal.bryantchristie.com/db#pesticides/query

Beckwith, B. R. (2004). *The queen root of this clime: Ethnoecological investigations of blue camas (Camassia leichtlinii (Baker) Wats., C. quamash (Pursh) Greene ; Liliaceae) and its landscapes on southern Vancouver Island, British Columbia*. https://dspace.library.uvic.ca/handle/1828/632

Benbrook, C. M. (2019). How did the US EPA and IARC reach diametrically opposed conclusions on the genotoxicity of glyphosate-based herbicides? *Environmental Sciences Europe*, *31*(1), 2. https://doi.org/10.1186/s12302-018-0184-7

Bento, C. P. M., Yang, X., Gort, G., Xue, S., van Dam, R., Zomer, P., Mol, H. G. J., Ritsema, C. J., & Geissen, V. (2016). Persistence of glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness. *Science of The Total Environment*, *572*, 301–311. https://doi.org/10.1016/j.scitotenv.2016.07.215

Blanchet, R., Batal, M., Johnson-Down, L., Johnson, S., Louie, C., Terbasket, E., Terbasket, P., Wright, H., Willows, N., & Okanagan Nation Salmon Reintroduction Initiatives. (2021). An Indigenous food sovereignty initiative is positively associated with well-being and cultural connectedness in a survey of Syilx Okanagan adults in British Columbia, Canada. *BMC Public Health*, *21*(1), 1405. https://doi.org/10.1186/s12889-021-11229-2

Bonnamour, A., Gippet, J. M. W., & Bertelsmeier, C. (2021). Insect and plant invasions follow two waves of globalisation. *Ecology Letters*, *24*(11), 2418–2426. https://doi.org/10.1111/ele.13863

Botten, N., Wood, L. J., & Werner, J. R. (2021). Glyphosate remains in forest plant tissues for a decade or more. *Forest Ecology and Management*, *493*, 119259. https://doi.org/10.1016/j.foreco.2021.119259

Boyd, R. T. (1990). Demographic History, 1774-1874. In *Handbook of North American Indians: Vol. 7: Northwest Coast* (pp. 135–148). Smithsonian Institute.

Brewester, D. W., Warren, J., & Hopkjns, W. E. I. (1991). Metabolism of Glyphosate in Sprague-Dawley Rats: Tissue Distribution, Identification, and Quantitation of Glyphosate-Derived Materials following a Single Oral Dose1. *Toxicological Sciences*, *17*(1), 43–51. https://doi.org/10.1093/toxsci/17.1.43

Buchenauer, L., Junge, K. M., Haange, S.-B., Simon, J. C., von Bergen, M., Hoh, A.-L., Aust, G., Zenclussen, A. C., Stangl, G. I., & Polte, T. (2022). Glyphosate differentially affects the allergic immune response across generations in mice. *Science of The Total Environment*, *850*, 157973. https://doi.org/10.1016/j.scitotenv.2022.157973

Cascadia Prairie Oak Partnership. (2014). *Velvet Grass Control*. Cascadia Prairie Oak Partnership. https://cascadiaprairieoak.org/documents/velvet-grass-control

CCME. (2012). Canadian Water Quality Guidelines for the Protection of Aquatic Life—Glyphosate. *Canadian Council of Ministers of the Environment: Canadian Environmental Quality Guidelines*. https://ccme.ca/en/res/glyphosate-en-canadian-water-quality-guidelines-for-the-protection-of-aquatic-life.pdf

Chance, D., Choquette, W., Peterson, W. R., & Rice, D. G. (1977). Prehistoric Land Use in Southern British Columbia, Northeastern Washington, and the Panhandle of Idaho. *Archaeological Reconnaissance of Timber Sales and Land Exchanges in Idaho Panhandle National Forests 1975-1976, by Keo E. Boreson*.

Chappell, C., Mohn Gee, M. S., Stephens, B., Crawford, R., & Farone, S. (2001). *Distribution and Decline of Native Grasslands and Oak WOodlands in the Puget Lowland and Willamette Valley Ecoregions, Washington* (Washington Natural Heritage Program, pp. 124–139). Washington Department of Natural Resources. https://www.dnr.wa.gov/publications/amp_nh_decline_grass_oak.pdf

Chase, M. W., Reveal, J. L., & Fay, M. F. (2009). A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. *Botanical Journal of the Linnean Society*, *161*(2), 132–136. https://doi.org/10.1111/j.1095-8339.2009.00999.x

Cole-Parmer. (2021, July 21). *Testing the pH of Soil Samples*. Cole-Parmer: An Antylia Scientific Company. https://www.coleparmer.com/tech-article/soil-samples-ph-testing

Cuhra, M., Bøhn, T., & Cuhra, P. (2016). Glyphosate: Too Much of a Good Thing? *Frontiers in Environmental Science*, *4*. https://www.frontiersin.org/articles/10.3389/fenvs.2016.00028

Czarnota, M., & Thomas, P. A. (2013, January 22). *Using Surfactants, Wetting Agents, and Adjuvants in the Greenhouse*. University of Georgia Extension. https://extension.uga.edu/publications/detail.html?number=B1319&title=using-surfactants-wetting-agents-and-adjuvants-in-the-greenhouse

Davis, M. (2018). *Determining the Influence of Nutrition and Temperature on the Growth and Development of Camassia spp.* [Masters Thesis, Oregon State University]. https://ir.library.oregonstate.edu/concern/graduate_thesis_or_dissertations/db78tj303

de Castilhos Ghisi, N., Zuanazzi, N. R., Fabrin, T. M. C., & Oliveira, E. C. (2020). Glyphosate and its toxicology: A scientometric review. *The Science of the Total Environment*, *733*, 139359. https://doi.org/10.1016/j.scitotenv.2020.139359

Defarge, N., Takács, E., Lozano, V. L., Mesnage, R., Spiroux de Vendômois, J., Séralini, G.-E., & Székács, A. (2016). Co-Formulants in Glyphosate-Based Herbicides Disrupt Aromatase Activity in Human Cells below Toxic Levels. *International Journal of Environmental Research and Public Health*, *13*(3), E264. https://doi.org/10.3390/ijerph13030264

Del Castilo, I., Neumann, A. S., Lemos, F. S., De Bastiani, M. A., Oliveira, F. L., Zimmer, E. R., Rêgo, A. M., Hardoim, C. C. P., Antunes, L. C. M., Lara, F. A., Figueiredo, C. P., & Clarke, J. R. (2022). Lifelong Exposure to a Low-Dose of the Glyphosate-Based Herbicide RoundUp® Causes Intestinal Damage, Gut Dysbiosis, and Behavioral Changes in Mice. *International Journal of Molecular Sciences*, *23*(10), 5583. https://doi.org/10.3390/ijms23105583

Dennehy, C., Alverson, E. R., Anderson, H. E., Clements, D. R., Gilbert, R., & Kaye, T. N. (2011). Management Strategies for Invasive Plants in Pacific Northwest Prairies, Savannas, and Oak Woodlands. *Northwest Science*, *85*(2), 329–351. https://doi.org/10.3955/046.085.0219

Deur, D., & Turner, N. J. (2005). *Keeping it Living: Traditions of Plant Use and Cultivation on the Northwest Coast of North America*. University of Washington Press.

Dick, R. E., & Quinn, J. P. (1995). Glyphosate-degrading isolates from environmental samples: Occurrence and pathways of degradation. *Applied Microbiology and Biotechnology*, *43*(3), 545–550. https://doi.org/10.1007/BF00218464

Druille, M., García-Parisi, P. A., Golluscio, R. A., Cavagnaro, F. P., & Omacini, M. (2016). Repeated annual glyphosate applications may impair beneficial soil microorganisms in temperate grassland. *Agriculture, Ecosystems & Environment*, *230*, 184–190. https://doi.org/10.1016/j.agee.2016.06.011

Duke, S. O. (2011). Glyphosate Degradation in Glyphosate-Resistant and -Susceptible Crops and Weeds. *Journal of Agricultural and Food Chemistry*, *59*(11), 5835–5841. https://doi.org/10.1021/jf102704x

Duke, S. O., Lydon, J., Koskinen, W. C., Moorman, T. B., Chaney, R. L., & Hammerschmidt, R. (2012). Glyphosate Effects on Plant Mineral Nutrition, Crop Rhizosphere Microbiota, and Plant Disease in Glyphosate-Resistant Crops. *Journal of Agricultural and Food Chemistry*, *60*(42), 10375–10397. https://doi.org/10.1021/jf302436u

Dunwiddie, P. W., & Bakker, J. D. (2011). The Future of Restoration and Management of Prairie-Oak Ecosystems in the Pacific Northwest. *Northwest Science*, *85*(2), 83–92. https://doi.org/10.3955/046.085.0201

Eberbach, P. (1998). Applying non-steady-state compartmental analysis to investigate the simultaneous degradation of soluble and sorbed glyphosate (N-(phosphonomethyl)glycine) in four soils. *Pesticide Science*, *52*(3), 229–240. https://doi.org/10.1002/(SICI)1096-9063(199803)52:3<229::AID-PS684>3.0.CO;2-O

Edge, C. B., Brown, M. I., Heartz, S., Thompson, D., Ritter, L., & Ramadoss, M. (2021). The Persistence of Glyphosate in Vegetation One Year after Application. *Forests*, *12*(5), Article 5. https://doi.org/10.3390/f12050601

EFSA. (2015). Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. *European Food Safety Authority Journal*, *13*(11), 4302. https://doi.org/10.2903/j.efsa.2015.4302

ESRI. (2023). *Transportation_v1 (FeatureServer)* [dataset]. https://services2.arcgis.com/FiaPA4ga0iQKduv3/arcgis/rest/services/Transportation_v1/FeatureServer

EURL-SRM. (2023, March 17). *QuPPe: About the Method*. European Reference Laboratorities for Residues of Pesticides. https://www.quppe.eu/method.asp

Feng, J. C., & Thompson, D. G. (1990). Fate of glyphosate in a Canadian forest watershed. 2. Persistence in foliage and soils. *Journal of Agricultural and Food Chemistry*, *38*(4), 1118–1125. https://doi.org/10.1021/jf00094a046

Floberg, J., Goering, M., Wilhere, G., Macdonald, C., Chappell, C., Rumsey, C., Ferdaña, Z., Holt, A., Skidmore, P., Horsman, T., Alverson, E., Tanner, C., Bryer, M., Iachetti, P., Harcombe, A., McDonald, B., Cook, T., Summers, M., & Rolph, D. (2004). *Willamette Valley-PugetTrough-Georgia Basin ecoregional assessment*.

Florer, L. E. (1972). Quaternary paleoecology and stratigraphy of the sea cliffs, western Olympic Peninsula, Washington. *Quaternary Research*, *2*(2), 202–216. https://doi.org/10.1016/0033-5894(72)90039-7

Fuchs, M. A. (2001). *Towards a Recovery Strategy for Garry Oak and Associated Ecosystems in Canada: Ecological Assessment and Literature Review* (Technical Report Technical

Report GBEI/EC-00-030; Environment Canada). Canada Wildlife Service, Pacific and Yukon Region.

Gao, H., Chen, J., Ding, F., Chou, X., Zhang, X., Wan, Y., Hu, J., & Wu, Q. (2019). Activation of the N-methyl-D-aspartate receptor is involved in glyphosate-induced renal proximal tubule cell apoptosis. *Journal of Applied Toxicology*, *39*(8), 1096–1107. https://doi.org/10.1002/jat.3795

Garibaldi, A., & Turner, N. (2004). Cultural Keystone Species: Implications for Ecological Conservation and Restoration. *Ecology and Society*, *9*(3). https://doi.org/10.5751/ES-00669-090301

Gaupp-Berghausen, M., Hofer, M., Rewald, B., & Zaller, J. G. (2015). Glyphosate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations. *Scientific Reports*, *5*(1), Article 1. https://doi.org/10.1038/srep12886

Gimsing, A. L., Borggaard, O. K., & Bang, M. (2004). Influence of soil composition on adsorption of glyphosate and phosphate by contrasting Danish surface soils. *European Journal of Soil Science*, *55*(1), 183–191. https://doi.org/10.1046/j.1365-2389.2003.00585.x

Gimsing, A. L., Borggaard, O. K., Jacobsen, O. S., Aamand, J., & Sørensen, J. (2004). Chemical and microbiological soil characteristics controlling glyphosate mineralisation in Danish surface soils. *Applied Soil Ecology*, *27*(3), 233–242. https://doi.org/10.1016/j.apsoil.2004.05.007

Gómez Ortiz, A. M., Okada, E., Bedmar, F., & Costa, J. L. (2017). Sorption and desorption of glyphosate in Mollisols and Ultisols soils of Argentina: Glyphosate sorption and desorption in soils of Argentina. *Environmental Toxicology and Chemistry*, *36*(10), 2587–2592. https://doi.org/10.1002/etc.3851

Gould, F. W. (1942). A Systematic Treatment of the Genus Camassia Lindl. *The American Midland Naturalist*, *28*(3), 712–742. https://doi.org/10.2307/2420901

Kasner, E. J., Prado, J. B., Yost, M. G., & Fenske, R. A. (2021). Examining the role of wind in human illness due to pesticide drift in Washington state, 2000–2015. *Environmental Health*, *20*(1), 26. https://doi.org/10.1186/s12940-021-00693-3

Kawa, L., & De Hertogh, A. A. (1992). Root Physiology of Ornamental Flowering Bulbs. In *Horticultural Reviews* (pp. 57–88). John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470650523.ch2

KBS LTER. (2023). *Protocol: Soil pH*. Kellogg Biological Station: Long-Term Ecological Research. https://lter.kbs.msu.edu/protocols/39

KeyIndustries. (2023). *Nu-Film-17 (KEYI) SDS.pdf*. KeyIndustries. https://horticentre.co.nz/wp-content/uploads/Safety%20Datasheets/Nu-Film-17%20(KEYI)%20SDS.pdf

Klingelhöfer, D., Braun, M., Brüggmann, D., & Groneberg, D. A. (2021). Glyphosate: How do ongoing controversies, market characteristics, and funding influence the global research landscape? *Science of The Total Environment*, *765*, 144271. https://doi.org/10.1016/j.scitotenv.2020.144271

Kolakowski, B. M., Miller, L., Murray, A., Leclair, A., Bietlot, H., & van de Riet, J. M. (2020). Analysis of Glyphosate Residues in Foods from the Canadian Retail Markets between 2015 and 2017. *Journal of Agricultural and Food Chemistry*, *68*(18), 5201–5211. https://doi.org/10.1021/acs.jafc.9b07819

Konlande, J. E., & Robson, J. R. K. (1972). The nutritive value of cooked camas as consumed by Flathead Indians. *Ecology of Food and Nutrition*, *1*(3), 193–195. https://doi.org/10.1080/03670244.1972.9990289

Krueger, J., Bois, S., Kaye, T., Steek, D., & Taylor, T. (2014). *Practical Guidelines for Wetland Prairie Restoration in the Willamette Valley, Oregon*. Institue for Applied Ecology, Oregon State University.

Laitinen, P., Rämö, S., Nikunen, U., Jauhiainen, L., Siimes, K., & Turtola, E. (2009). Glyphosate and phosphorus leaching and residues in boreal sandy soil. *Plant and Soil*, *323*, 267–283. https://doi.org/10.1007/s11104-009-9935-y

Leino, L., Tall, T., Helander, M., Saloniemi, I., Saikkonen, K., Ruuskanen, S., & Puigbò, P. (2021). Classification of the glyphosate target enzyme (5-enolpyruvylshikimate-3-phosphate synthase) for assessing sensitivity of organisms to the herbicide. *Journal of Hazardous Materials, 408*, 124556. https://doi.org/10.1016/j.jhazmat.2020.124556

Lowther, E. (2022). *Understanding lək̓ʷəŋən soils: The foundation of environmental stewardship in coastal anthropogenic prairies* [Thesis]. https://dspace.library.uvic.ca/handle/1828/14078

Maclay, A. (1928). *Studies of the life history of Camassia quamash (Pursh) Greene*. Washington State University.

Marrs, R. H., Frost, A. J., Plant, R. A., & Lunnis, P. (1993). Determination of buffer zones to protect seedlings of non-target plants from the effects of glyphosate spray drift. *Agriculture, Ecosystems & Environment*, *45*(3), 283–293. https://doi.org/10.1016/0167-8809(93)90077-3

Martinez, D. A., Loening, U. E., & Graham, M. C. (2018). Impacts of glyphosate-based herbicides on disease resistance and health of crops: A review. *Environmental Sciences Europe*, *30*(1), 2. https://doi.org/10.1186/s12302-018-0131-7

Martins-Gomes, C., Silva, T. L., Andreani, T., & Silva, A. M. (2022). Glyphosate vs. Glyphosate-Based Herbicides Exposure: A Review on Their Toxicity. *Journal of Xenobiotics*, *12*(1), Article 1. https://doi.org/10.3390/jox12010003

Matthews, K. (2020). *Restoration Strategies for Propagation of Camassia Quamash on the Weippe Prairie* [University of Idaho]. https://www.proquest.com/openview/96c9c64a0a3b31dcb3e6ab3eaeb63834/1?pq-origsite=gscholar&cbl=44156

McHenry, L. (2018). The Monsanto Papers: Poisoning the scientific well. *International Journal of Risk & Safety in Medicine*, *29*, 1–13. https://doi.org/10.3233/JRS-180028

Mesnage, R., & Antoniou, M. N. (2020). Computational modelling provides insight into the effects of glyphosate on the shikimate pathway in the human gut microbiome. *Current Research in Toxicology*, *1*, 25–33. https://doi.org/10.1016/j.crtox.2020.04.001

Mesnage, R., Benbrook, C., & Antoniou, M. N. (2019). Insight into the confusion over surfactant co-formulants in glyphosate-based herbicides. *Food and Chemical Toxicology*, *128*, 137–145. https://doi.org/10.1016/j.fct.2019.03.053

Mesnage, R., Defarge, N., Spiroux de Vendômois, J., & Séralini, G. E. (2015). Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. *Food and Chemical Toxicology*, *84*, 133–153. https://doi.org/10.1016/j.fct.2015.08.012

Miles, C. J., & Moye, H. A. (1988). Extraction of glyphosate herbicide from soil and clay minerals and determination of residues in soils. *Journal of Agricultural and Food Chemistry*, *36*(3), 486–491. https://doi.org/10.1021/jf00081a020

Miller. (2023). *Nu-Film® P*. Miller: A Huber Company. https://www.millerchemical.com/products/adjuvants/nu-film-p/

Missouri State University, & OEWRI. (2019). *Standard Operating Procedure for: Organic Matter in Sediment, Loss on Ignition Method (2030R02 Org Matter LOI.doc)*. Missouri State University and Ozarks Environmental and Water Resources Institute. https://oewri.missouristate.edu/_Files/SOP_OrganicMatter_LOI_2019.pdf

Mueller, T. C., Massey, J. H., Hayes, R. M., Main, C. L., & Stewart, C. N. (2003). Shikimate Accumulates in Both Glyphosate-Sensitive and Glyphosate-Resistant Horseweed (Conyza canadensis L. Cronq.). *Journal of Agricultural and Food Chemistry*, *51*(3), 680–684. https://doi.org/10.1021/jf026006k

Neumann, G., Kohls, S., Richter, E., Stock, K., Souza, O., Yamada, T., & Römheld, V. (2006). Relevance of glyphosate transfer to non-target plants via the rhizosphere. *Journal of Plant Diseases and Protection*, *20*.

Newton, M., Cole, E. C., & Tinsley, I. J. (2008). Dissipation of four forest-use herbicides at high latitudes. *Environmental Science and Pollution Research International*, *15*(7), 573–583. https://doi.org/10.1007/s11356-008-0039-7

Newton, M., Horner, L. M., Cowell, J. E., White, D. E., & Cole, E. C. (1994). Dissipation of Glyphosate and Aminomethylphosphonic Acid in North American Forests. *Journal of Agricultural and Food Chemistry*, *42*(8), 1795–1802. https://doi.org/10.1021/jf00044a043

NOAA. (2023). *NOWData—NOAA Online Weather Data*. National Weather Service; NOAA's National Weather Service. https://www.weather.gov/wrh/Climate?wfo=sew

Noland, S., & Carver, L. (2011). *Prairie Landowner Guide for Western Washington*. Cascadia Prairie Oak Partnership. https://cascadiaprairieoak.org/wp-content/uploads/2013/08/Prairie-Landowner-Guide-Western-WA1.pdf

NRCS. (2019). *Web Soil Survey—Home* [dataset]. https://websoilsurvey.nrcs.usda.gov/app/

Ojelade, B. S., Durowoju, O. S., Adesoye, P. O., Gibb, S. W., & Ekosse, G.-I. (2022). Review of Glyphosate-Based Herbicide and Aminomethylphosphonic Acid (AMPA): Environmental and Health Impacts. *Applied Sciences*, *12*(8789). https://doi.org/10.3390/app12178789

Okada, E., Costa, J. L., & Bedmar, F. (2016). Adsorption and mobility of glyphosate in different soils under no-till and conventional tillage. *Geoderma*, *263*, 78–85. https://doi.org/10.1016/j.geoderma.2015.09.009

Olszyk, D., Pfleeger, T., Shiroyama, T., Blakeley-Smith, M., Lee, E. H., & Plocher, M. (2017). Plant reproduction is altered by simulated herbicide drift to constructed plant communities. *Environmental Toxicology and Chemistry*, *36*(10), 2799–2813. https://doi.org/10.1002/etc.3839

OSU Soil Fertility Lab. (2020). *Texture Protocol—OSU Soil Fertility Lab*. Oklahoma State University. https://soilfertility.osu.edu/sites/soilf/files/imce/Protocols/Texture%20Protocol%20-%20OSU%20Soil%20Fertility%20Lab%20%28July%202020%29.pdf

Pasternack, G. (2023). *Loss-On-Ignition Protocol: Pasternack*. University of California Davis. https://pasternack.ucdavis.edu/research/methods/loss-ignition-protocol

Peillex, C., & Pelletier, M. (2020). The impact and toxicity of glyphosate and glyphosate-based herbicides on health and immunity. *Journal of Immunotoxicology*, *17*(1), 163–174. https://doi.org/10.1080/1547691X.2020.1804492

Pingree, M. R. A., & DeLuca, T. H. (2017). Function of Wildfire-Deposited Pyrogenic Carbon in Terrestrial Ecosystems. *Frontiers in Environmental Science*, *5*. https://www.frontiersin.org/articles/10.3389/fenvs.2017.00053

Preston, C., & Wakelin, A. M. (2008). Resistance to glyphosate from altered herbicide translocation patterns. *Pest Management Science*, *64*(4), 372–376. https://doi.org/10.1002/ps.1489

Puigbò, P., Leino, L. I., Rainio, M. J., Saikkonen, K., Saloniemi, I., & Helander, M. (2022). Does Glyphosate Affect the Human Microbiota? *Life*, *12*(5), 707. https://doi.org/10.3390/life12050707

Qi, X., Huang, Q., Chen, X., Qiu, L., Wang, S., Ouyang, K., & Chen, Y. (2023). *Associations between urine glyphosate and diabetes mellitus in the U.S. general adult: A cross-sectional study from NHANES program* [Preprint]. In Review. https://doi.org/10.21203/rs.3.rs-3195831/v1

Qiu, S., Fu, H., Zhou, R., Yang, Z., Bai, G., & Shi, B. (2020). Toxic effects of glyphosate on intestinal morphology, antioxidant capacity and barrier function in weaned piglets. *Ecotoxicology and Environmental Safety*, *187*, 109846. https://doi.org/10.1016/j.ecoenv.2019.109846

Rana, S. (2018). *Lecture 14. Herbicide application techniques and equipments* [Academic]. Department of Agronomy, Palampur 176062, HP, India. https://doi.org/10.13140/RG.2.2.22602.11200

Rasmussen, S. B., Abrahamsen, P., Nielsen, M. H., Holm, P. E., & Hansen, S. (2015). Effects of Single Rainfall Events on Leaching of Glyphosate and Bentazone on Two Different Soil Types, using the DAISY Model. *Vadose Zone Journal*, *14*(11), vzj2014.11.0164. https://doi.org/10.2136/vzj2014.11.0164

Reddy, K. N., Ding, W., Zablotowicz, R. M., Thomson, S. J., Huang, Y., & Krutz, L. J. (2010). Biological responses to glyphosate drift from aerial application in non-glyphosate-resistant corn. *Pest Management Science*, *66*(10), 1148–1154. https://doi.org/10.1002/ps.1996

Reddy, K. N., Rimando, A. M., & Duke, S. O. (2004). Aminomethylphosphonic Acid, a Metabolite of Glyphosate, Causes Injury in Glyphosate-Treated, Glyphosate-Resistant Soybean. *Journal of Agricultural and Food Chemistry*, *52*(16), 5139–5143. https://doi.org/10.1021/jf049605v

Reeves. (2010). *Exotic Species: Scotch Broom*. National Park Service. https://www.nps.gov/articles/scotch-broom.htm

Requena-Mullor, M., Navarro-Mena, A., Wei, R., López-Guarnido, O., Lozano-Paniagua, D., & Alarcon-Rodriguez, R. (2021). Evaluation of Gonadal Alterations in a Population Environmentally Exposed to a Mixture of Endocrine Active Pesticides. *International Journal of Environmental Research and Public Health*, *18*(5), Article 5. https://doi.org/10.3390/ijerph18052355

Reynolds, L. L., Johnson, B. R., Pfeifer-Meister, L., & Bridgham, S. D. (2015). Soil respiration response to climate change in Pacific Northwest prairies is mediated by a regional Mediterranean climate gradient. *Global Change Biology*, *21*(1), 487–500. https://doi.org/10.1111/gcb.12732

Rivas-Garcia, T., Espinosa-Calderón, A., Hernández-Vázquez, B., & Schwentesius-Rindermann, R. (2022). Overview of Environmental and Health Effects Related to Glyphosate Usage. *Sustainability*, *14*(11), Article 11. https://doi.org/10.3390/su14116868

Rook, E. J., Fischer, D. G., Seyferth, R. D., Kirsch, J. L., LeRoy, C. J., & Hamman, S. (2011). Responses of Prairie Vegetation to Fire, Herbicide, and Invasive Species Legacy. *Northwest Science*, *85*(2), 288–302. https://doi.org/10.3955/046.085.0216

Scrimsher, L. S. (1967). *Native Foods Used by the Nez Perce Indians of Idaho Masters Thesis*. University of Idaho, Moscow ID.

Shushkova, T. V., Vasilieva, G. K., Ermakova, I. T., & Leontievsky, A. A. (2009). Sorption and microbial degradation of glyphosate in soil suspensions. *Applied Biochemistry and Microbiology*. http://dx.doi.org/10.1134/S0003683809060040

Stanley, A. G., Dunwiddie, P. W., & Kaye, T. N. (2011). Restoring Invaded Pacific Northwest Prairies: Management Recommendations from a Region-Wide Experiment. *Northwest Science*, *85*(2), 233–246. https://doi.org/10.3955/046.085.0212

Stanley, A. G., Kaye, T. N., & Dunwiddie, P. W. (2008). Regional strategies for restoring invaded prairies: Observations from a multisite, collaborative research project. *Native Plants Journal*, *9*(3), 247–254. https://doi.org/10.2979/NPJ.2008.9.3.247

Storm, L., & Shebitz, D. (2006). Evaluating the Purpose, Extent, and Ecological Restoration Applications of Indigenous Burning Practices in Southwestern Washington. *Ecological Restoration*, *24*(4), 256–268.

Strandberg, B., Sørensen, P. B., Bruus, M., Bossi, R., Dupont, Y. L., Link, M., & Damgaard, C. F. (2021). Effects of glyphosate spray-drift on plant flowering. *Environmental Pollution*, *280*, 116953. https://doi.org/10.1016/j.envpol.2021.116953

Tahmasebi, B. K., Alebrahim, M. T., Roldán-Gómez, R. A., Silveira, H. M. da, Carvalho, L. B. de, Alcántara-de la Cruz, R., & De Prado, R. (2018). Effectiveness of alternative herbicides on three Conyza species from Europe with and without glyphosate resistance. *Crop Protection*, *112*, 350–355. https://doi.org/10.1016/j.cropro.2018.06.021

Terry, T. J., Madsen, M. D., Gill, R. A., Anderson, V. J., & St. Clair, S. B. (2021). Herbicide effects on the establishment of a native bunchgrass in annual grass invaded areas: Indaziflam versus imazapic. *Ecological Solutions and Evidence*, *2*(1), e12049. https://doi.org/10.1002/2688-8319.12049

Thoms, A. (1989). *The Northern Roots of Hunter-Gatherer Intensification: Camas and the Pacific Northwest*.

Thurston County Noxious Weeds Department. (2023). *Thurston Noxious Weed Sites* [dataset]. https://gisdata-thurston.opendata.arcgis.com/datasets/thurston::thurston-noxious-weed-sites/about

Trasande, L., Aldana, S. I., Trachtman, H., Kannan, K., Morrison, D., Christakis, D. A., Whitlock, K., Messito, M. J., Gross, R. S., Karthikraj, R., & Sathyanarayana, S. (2020). Glyphosate exposures and kidney injury biomarkers in infants and young children. *Environmental Pollution*, *256*, 113334. https://doi.org/10.1016/j.envpol.2019.113334

Trowbridge, C. C., Stanley, A., Kaye, T. N., Dunwiddie, P. W., & Williams, J. L. (2017). Long-term effects of prairie restoration on plant community structure and native population dynamics: Long-term effects of prairie restoration. *Restoration Ecology*, *25*(4), 559–568. https://doi.org/10.1111/rec.12468

Tunnell, S. J., Stubbendieck, J., Palazzolo, S., & Masters, R. A. (2006). Forb Response to Herbicides in a Degraded Tallgrass Prairie. *Natural Areas Journal*, *26*(1), 72–77. https://doi.org/10.3375/0885-8608(2006)26[72:FRTHIA]2.0.CO;2

Turner, N. J. (2014). *Ancient Pathways, Ancestral Knowledge: Ethnobotany and Ecological Wisdom of Indigenous Peoples of Northwest North America* (Vol. 2). McGill-Queen's University Press.

Turner, N. J. (2020). From "taking" to "tending": Learning about Indigenous land and resource management on the Pacific Northwest Coast of North America. *ICES Journal of Marine Science*, *77*(7–8), 2472–2482. https://doi.org/10.1093/icesjms/fsaa095

Turner, N. J., Geralda Armstrong, C., & Lepofsky, D. (2021). Adopting a Root: Documenting Ecological and Cultural Signatures of Plant Translocations in Northwestern North America. *American Anthropologist*, *123*(4), 879–897. https://doi.org/10.1111/aman.13658

Turner, N. J., & Kuhnlein, H. V. (1983). Camas (Camassia spp.) and riceroot (Fritillaria spp.): Two liliaceous "root" foods of the Northwest Coast Indians. *Ecology of Food and Nutrition*, *13*(4), 199–219. https://doi.org/10.1080/03670244.1983.9990754

US EPA. (2022, September 23). *Glyphosate* [Overviews and Factsheets]. EPA: United States Environmental Protection Agency. https://www.epa.gov/ingredients-used-pesticide-products/glyphosate

US EPA. (2023a, May 10). *Assessing Human Health Risk from Pesticides* [Collections and Lists]. EPA: United States Environmental Protection Agency. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/assessing-human-health-risk-pesticides

US EPA. (2023b, May 11). *Setting Tolerances for Pesticide Residues in Foods* [Overviews and Factsheets]. EPA: United States Environmental Protection Agency. https://www.epa.gov/pesticide-tolerances/setting-tolerances-pesticide-residues-foods

USDA, & AMS. (2022). *Pesticide Data Program: Annual Summary, Calendar Year 2021*. https://www.ams.usda.gov/sites/default/files/media/2021PDPAnnualSummary.pdf

USFSWS. (2023). *The Joint Base Lewis-McChord Sentinel Landscape | U.S. Fish & Wildlife Service* [U.S. Fish & Wildlife Service]. FWS.Gov. https://www.fws.gov/project/joint-base-lewis-mcchord-sentinel-landscape

USGS. (2019). *The National Map—Data Delivery | U.S. Geological Survey*. United States Geological Survey. https://www.usgs.gov/the-national-map-data-delivery

van Bruggen, A. H. C., Finckh, M. R., He, M., Ritsema, C. J., Harkes, P., Knuth, D., & Geissen, V. (2021). Indirect Effects of the Herbicide Glyphosate on Plant, Animal and Human Health Through its Effects on Microbial Communities. *Frontiers in Environmental Science*, *9*. https://www.frontiersin.org/articles/10.3389/fenvs.2021.763917

Van Eenennaam, A. L., & Young, A. E. (2017). Detection of dietary DNA, protein, and glyphosate in meat, milk, and eggs. *Journal of Animal Science*, *95*(7), 3247–3269. https://doi.org/10.2527/jas.2016.1346

van Kleunen, M., Essl, F., Pergl, J., Brundu, G., Carboni, M., Dullinger, S., Early, R., González-Moreno, P., Groom, Q. J., Hulme, P. E., Kueffer, C., Kühn, I., Máguas, C., Maurel, N., Novoa, A., Parepa, M., Pyšek, P., Seebens, H., Tanner, R., … Dehnen-Schmutz, K. (2018). The changing role of ornamental horticulture in alien plant invasions. *Biological Reviews of the Cambridge Philosophical Society*, *93*(3), 1421–1437. https://doi.org/10.1111/brv.12402

Vijay, A., & Valdes, A. M. (2022). Role of the gut microbiome in chronic diseases: A narrative review. *European Journal of Clinical Nutrition*, *76*(4), Article 4. https://doi.org/10.1038/s41430-021-00991-6

Viti, M. L., Alves, P. a. T., Mendes, K. F., Pimpinato, R. F., Guimarães, A. C. D., & Tornisielo, V. L. (2019). Translocation and Root Exudation of Glyphosate by *Urochloa brizantha* and its Transport on Sugarcane and Citrus Seedlings. *Planta Daninha*, *37*, e019183334. https://doi.org/10.1590/S0100-83582019370100030

von Wirén-Lehr, S., Komoßa, D., Gläßgen, W. E., Sandermann Jr, H., & Scheunert, I. (1997). Mineralization of [14C] glyphosate and its plant-associated residues in arable soils originating from different farming systems. *Pesticide Science*, *51*(4), 436–442. https://doi.org/10.1002/(SICI)1096-9063(199712)51:4<436::AID-PS630>3.0.CO;2-7

Wagner, V., Antunes, P. M., Irvine, M., & Nelson, C. R. (2017). Herbicide usage for invasive non-native plant management in wildland areas of North America. *Journal of Applied Ecology*, *54*(1), 198–204. https://doi.org/10.1111/1365-2664.12711

Wakelin, A. M., Lorraine-Colwill, D. F., & Preston, C. (2004). Glyphosate resistance in four different populations of Lolium rigidum is associated with reduced translocation of glyphosate to meristematic zones. *Weed Research*, *44*(6), 453–459. https://doi.org/10.1111/j.1365-3180.2004.00421.x

Washburn, A. L. (1988). *Mima Mounds: An Evaluation of Proposed Origins with Special Reference to the Puget Lowland*. https://www.dnr.wa.gov/Publications/ger_ri29_mima_mounds.pdf

WDFW. (2023a). *Mazama Pocket Gopher (Thomomys mazama)*. Washington Department of Fish and Wildlife. https://wdfw.wa.gov/species-habitats/species/thomomys-mazama

WDFW. (2023b). *Scatter Creek Wildlife Area Unit*. Washington Department of Fish and Wildlife. https://wdfw.wa.gov/places-to-go/wildlife-areas/scatter-creek-wildlife-area-unit

WDFW. (2023c). *West Rocky Prairie Wildlife Area Unit*. Washington Department of Fish and Wildlife. https://wdfw.wa.gov/places-to-go/wildlife-areas/west-rocky-prairie-wildlife-area-unit

WDNR. (2023a). *Applying and Managing Pesticides | WA - DNR*. Washington State Department of Natural Resources. https://www.dnr.wa.gov/applying-and-managing-pesticides

WDNR. (2023b). *Forest Pesticide Applications | WA - DNR*. Washington State Department of Natural Resources. https://www.dnr.wa.gov/forest-chemical-applications

Weidlich, E. W. A., Flórido, F. G., Sorrini, T. B., & Brancalion, P. H. S. (2020). Controlling invasive plant species in ecological restoration: A global review. *Journal of Applied Ecology*, *57*(9), 1806–1817. https://doi.org/10.1111/1365-2664.13656

Willamette Partnership. (2020). *Willamette Valley Oak and Prairie Cooperative Strategic Action Plan*. Willamette Partnership.

Wood, L. J. (2019). The presence of glyphosate in forest plants with different life strategies one year after application. *Canadian Journal of Forest Research*, *49*(6), 586–594. https://doi.org/10.1139/cjfr-2018-0331

WRCC. (2023). *Climate of Washington*. Western Regional Climate Center. https://wrcc.dri.edu

WSDA. (2021). *Agricultural Land Use | Washington State Department of Agriculture* [dataset]. https://agr.wa.gov/departments/land-and-water/natural-resources/agricultural-land-use

WSDA. (2023). *Washington State Noxious Weed Data Viewer* [ArcGIS]. https://www.arcgis.com/apps/webappviewer/index.html?id=cec83bd1b9fc4d7681afd219a9197654

WSDOT. (2023). *Using herbicides | WSDOT*. Washington State Department of Transportation. https://wsdot.wa.gov/construction-planning/protecting-environment/maintaining-vegetation-along-our-highways/using-herbicides

Wyrill, J. B., & Burnside, O. C. (1976). Absorption, Translocation, and Metabolism of 2,4-D and Glyphosate in Common Milkweed and Hemp Dogbane. *Weed Science*, *24*(6), 557–566. https://doi.org/10.1017/S0043174500062949

Yates, W. E., Akesson, N. B., & Bayer, D. E. (1978). Drift of Glyphosate Sprays Applied with Aerial and Ground Equipment. *Weed Science*, *26*(6), 597–604. https://doi.org/10.1017/S0043174500064638

Appendix:

Appendix A: Complete pH lab results

PH Lab Results

Site	Plot	Run 1	Run 2	Run 3	Run 4	Run 5	Average	Standard Deviation	Standard Error
Marion Prairie	1	5.43	5.37	5.36	5.43	5.43	5.40	0.0358	0.0160
	2	5	5.04	5.02	4.95	4.95	4.99	0.0409	0.0183
	3	5.2	5.18	5.19	5.19	5.2	5.19	0.0084	0.0037
	4	5.27	5.28	5.25	5.23	5.28	5.26	0.0217	0.0097
	5	5.23	5.31	5.22	5.29	5.21	5.25	0.0449	0.0201
Scatter Creek	1	4.85	4.92	4.9	4.93	4.92	4.90	0.0321	0.0144
	2	5.14	5.13	5.12	5.13	5.13	5.13	0.0071	0.0032
	3	4.69	4.69	4.68	4.7	4.68	4.69	0.0084	0.0037
	4	4.71	4.71	4.7	4.67	4.68	4.69	0.0182	0.0081
	5	4.71	4.74	4.75	4.74	4.78	4.74	0.0251	0.0112
West Rocky	1	5.59	5.52	5.5	5.49	5.48	5.52	0.0439	0.0196
	2	5.05	5.05	4.99	4.97	4.98	5.01	0.0390	0.0174
	3	5.32	5.34	5.29	5.27	5.33	5.31	0.0292	0.0130
	4	5.55	5.56	5.54	5.51	5.53	5.54	0.0192	0.0086
	5	5.11	5.14	5.13	5.12	5.11	5.12	0.0130	0.0058
Glacial Heritage	1	5.05	5.11	5.08	5.06	5.06	5.07	0.0239	0.0107
	2	5.22	5.19	5.17	5.08	5.13	5.16	0.0545	0.0244
	3	4.93	4.94	4.93	4.95	4.94	4.94	0.0084	0.0037
	4	5.19	5.17	5.22	5.25	5.25	5.22	0.0358	0.0160
	5	5.21	5.22	5.22	5.2	5.19	5.21	0.0130	0.0058
The Prairie at the Mouth of Black River	1	5.07	5.09	5.11	5.09	5.1	5.09	0.0148	0.0066
	2	4.72	4.72	4.71	4.72	4.72	4.72	0.0045	0.0020
	3	4.99	5.03	5.02	5.02	5.03	5.02	0.0164	0.0073
	4	5.23	5.2	5.18	5.18	5.19	5.20	0.0207	0.0093
	5	5.05	5.06	5.06	5.07	5.08	5.06	0.0114	0.0051
Secena Prairie	1	5.57	5.55	5.55	5.55	5.53	5.55	0.0141	0.0063
	2	5.19	5.17	5.19	5.14	5.16	5.17	0.0212	0.0095
	3	5.4	5.43	5.37	5.4	5.38	5.40	0.0230	0.0103
	4	5.37	5.39	5.42	5.39	5.4	5.39	0.0182	0.0081
	5	5.1	5.13	5.15	5.1	5.15	5.13	0.0251	0.0112

Appendix B: Complete clay content lab results

Clay Content Lab Results

Site	Plot	Total Pre-oven Weight (g)	Sample Pre-oven Weight (g)	Total Post-oven Weight (g)	Sample Post-oven Weight (g)	Session	40s Hydro-meter(g/mL)	40s Temp (°C)	2hr Hydro-meter (g/mL)	2hr Temp (°C)	Corrected 40s Hydrometer (g/mL)	Corrected 2hr Hydrometer (g/mL)	Clay Fraction of Sample	Clay Content (%)	Notes
Marion Prairie	1	160.0862	50.0109	157.2134	47.1381	3	15	25	0	23	16.8	1.08	2.1214	2.12%	
	2	145.7565	50.0765	142.2853	46.6053	3	14.5	25	-1	23	16.3	0.08	0.0000	0.00%	
	3	161.9432	49.9718	158.9763	47.0049	3	14	25	2	23	15.8	3.08	6.3823	6.38%	Small amount of solution lost during plunging
	4	154.3914	49.9978	150.7665	46.3729	3	10.5	25	0	23	12.3	1.08	2.1564	2.16%	
	5	148.8519	49.9953	145.8251	46.9685	3	15.5	24.75	-0.5	23	17.21	0.58	1.0645	1.06%	
Scatter Creek	1	170.2873	49.977	167.4093	47.099	2	12	25	-0.5	23	13.8	0.58	-1.8259	-1.83%	
	2	161.201	50.0094	158.1361	46.9445	2	10	25	1	23	11.8	2.08	1.3633	1.36%	
	3	153.4505	50.0995	150.3253	46.9743	2	10	25	-1	23	11.8	0.08	-2.8952	-2.90%	
	4	164.0209	50.0267	161.2162	47.222	2	10.5	25	-1	23	12.3	0.08	-2.8800	-2.88%	
	5	148.5315	50.0605	145.5283	47.0573	2	10	25	-1	23	11.8	0.08	-0.0289	-0.03%	
West Rocky	1	161.3821	50.1851	158.8822	47.6852	3	10	25	1	24	11.8	2.44	4.9491	4.95%	Parafilm on plunger broke more than others
	2	153.2844	49.9572	151.2622	47.935	3	11	25	2	23.75	12.8	3.35	6.8217	6.82%	
	3	148.4375	50.0245	145.8611	47.4481	3	12	25	2	24	13.8	3.44	7.0814	7.08%	
	4	170.2204	49.9841	167.5653	47.329	3	11	25	1	23.75	12.8	2.35	4.7962	4.80%	Small amount of solution lost during plunging
	5	145.7224	50.0778	142.7898	47.1452	3	10.5	25	0	24	12.3	1.44	2.8847	2.88%	
The Prairie at	1	149.0675	50.0592	144.401	45.3927	1	13	24.75	3.1	23.5	14.71	4.36	6.4328	6.43%	Lost ~1/5 of solution during plunging
	2	139.1346	34.6098	135.5895	31.0647	1	10.5	24.75	0.5	24	12.21	1.94	1.6095	1.61%	Small amount of solution lost during plunging
	3	170.4315	50.0258	164.7591	44.3534	1	15	25	2	24	16.8	3.44	4.5092	4.51%	
	4	164.1805	50.0192	159.1661	45.0048	1	15.5	24.5	1.5	24.5	17.12	3.12	3.7329	3.73%	
	5	148.5951	50.0232	143.7823	45.2104	1	16	25	1	24	17.8	2.44	2.2119	2.21%	
Glacial Heritage	1	156.4041	42.3623	152.6628	38.621	2	17	25	-0.5	24	18.8	0.94	-1.2946	-1.29%	
	2	148.873	49.9902	145.0041	46.1213	2	23.5	25	3	24	25.3	4.44	6.5046	6.50%	
	3	160.0518	50.008	156.2179	46.1741	2	22.5	25	0	24	24.3	1.44	0.0000	0.00%	
	4	154.5027	50.0979	149.8877	45.4829	2	22.5	25	1.5	24	24.3	2.94	3.2979	3.30%	
	5	162.0712	50.0673	157.8056	45.8017	2	20	25	1	24	21.8	2.44	2.1833	2.18%	
Secena Prairie	1	162.09	50.0133	158.8928	46.8161	1	12	24.75	1.5	24	13.71	2.94	3.2040	3.20%	
	2	153.5181	50.0146	150.7592	47.2557	1	12	25	2	24	13.8	3.44	4.2323	4.23%	
	3	160.1778	50.074	157.1946	47.0908	1	10.5	24	3	24.75	11.94	4.71	6.9440	6.94%	2nd hydro reading taken above 1.5" of OM
	4	161.3153	50.0076	158.0644	46.7567	1	11	25	1.5	24	12.8	2.94	3.2081	3.21%	
	5	145.8494	50.117	142.7181	46.9857	1	11	25.5	0.5	24	12.98	1.94	1.0642	1.06%	
Session 1 Blank						1	0	24	0	24	1.44	1.44			
Session 2 Blank						2	0	25	0	24	1.8	1.44			
Session 3 Blank						3	-1	25.5	-1	23	0.98	0.08			

Appendix D: Complete soil organic matter content lab results

Soil Organic Matter Lab Results

Site	Plot	Soil Pre-oven Weight (g)	Total Pre-oven Weight (g)	Total Post-oven Weight (g)	Soil Post-oven Weight (g)	Total Post-furnance Weight (g)	Soil Post-furnace Weight (g)	Loss-on-Ignition (g)	Soil Organic Matter (%)	Notes
Marion Prairie	1	5.0225	41.5617	41.2561	4.7169	40.1029	3.5637	1.1532	24.45%	
	2	5.006	44.9958	44.6244	4.6346	43.0992	3.1094	1.5252	32.91%	
	3	5.0179	46.4474	46.1243	4.6948	44.8802	3.4507	1.2441	26.50%	
	4	4.9981	37.181	36.8111	4.6282	35.1993	3.0164	1.6118	34.83%	
	5	5.0185	40.8323	40.5273	4.7135	39.3613	3.5475	1.166	24.74%	
Scatter Creek	1	5.02	43.2871	43.1552	4.8881	42.0442	3.7771	1.111	22.73%	
	2	4.99	44.9775	44.8554	4.8679	43.7031	3.7156	1.1523	23.67%	
	3	5.02	41.2331	41.0452	4.8321	39.696	3.4829	1.3492	27.92%	
	4	5	49.3422	49.2163	4.8741	48.1464	3.8042	1.0699	21.95%	
	5	5.01	42.3312	42.1772	4.856	41.1198	3.7986	1.0574	21.78%	
West Rocky	1	5.0269	43.2907	43.0531	4.7893	42.224	3.9602	0.8291	17.31%	
	2	5.0216	40.3733	40.1737	4.822	39.5166	4.1649	0.6571	13.63%	
	3	5.0172	41.2343	40.971	4.7539	40.0945	3.8774	0.8765	18.44%	
	4	4.99141	49.3291	49.0628	4.72511	48.1933	3.85561	0.8695	18.40%	
	5	5.0231	42.341	42.0465	4.7286	40.8482	3.5303	1.1983	25.34%	
Glacial Heritage	1	4.9894	42.3115	41.869	4.5469	40.1745	2.8524	1.6945	37.27%	
	2	5.0035	44.8024	44.4415	4.6426	43.2149	3.416	1.2266	26.42%	
	3	5.0174	44.6865	44.319	4.6499	42.7787	3.1096	1.5403	33.13%	
	4	5.0462	45.1468	44.7042	4.6036	43.2028	3.1022	1.5014	32.61%	
	5	5.0179	46.4376	46.0167	4.597	44.5488	3.1291	1.4679	31.93%	
The Prairie at the Mouth of Black River	1	5.06	45.6368	45.49	4.9132	43.9004	3.3236	1.5896	32.35%	
	2	5.002	47.7632	47.3938	4.6326	45.4127	2.6515	1.9811	42.76%	
	3	4.95	40.7675	40.5814	4.7639	38.4289	2.6114	2.1525	45.18%	
	4	4.996	46.8912	46.7606	4.8654	45.2196	3.3244	1.541	31.67%	
	5	5	41.9051	41.7579	4.8528	39.9746	3.0695	1.7833	36.75%	Spilled small amount of sample
Secena Prairie	1	5.02	41.8347	41.7771	4.9624	40.4317	3.617	1.3454	27.11%	
	2	5.011	40.3633	40.2888	4.9365	39.5493	4.197	0.7395	14.98%	
	3	5.01	46.4406	46.3373	4.9067	45.2962	3.8656	1.0411	21.22%	
	4	5.06	37.2441	37.1443	4.9602	36.0222	3.8381	1.1221	22.62%	
	5	5.0007	40.8156	40.7263	4.9114	39.6346	3.8197	1.0917	22.23%	

Appendix F: Complete results

Complete Site Results

Site	Plot	Type	Average Camas Bulb Weight (g)	Glyphosate (mg/kg)	AMPA (mg/kg)	Soil pH Level	Soil Clay Content	Soil Organic Matter
Marion Prairie	1	Treated	1.97	<0.01 ± 0.0024	<0.01 ± 0.00238	5.41	2.12%	24.45%
	2		1.39	<0.01 ± 0.0024	<0.01 ± 0.00238	4.98	0.00%	32.91%
	3		0.67	<0.01 ± 0.0024	<0.01 ± 0.00238	5.19	6.38%	26.50%
	4		1.86	<0.01 ± 0.0024	<0.01 ± 0.00238	5.26	2.16%	34.83%
	5		1.11	<0.01 ± 0.0024	<0.01 ± 0.00238	5.24	1.06%	24.74%
	Total		7.00					
	Average		1.40			5.22	2.34%	28.68%
	St Error		0.24			0.07	1.08%	2.17%
	St Deviation		0.53			0.15	2.43%	4.84%
Scatter Creek	1	Treated	1.76	<0.01 ± 0.0024	<0.01 ± 0.00238	4.91	0.00%	22.73%
	2		2.68	<0.01 ± 0.0024	<0.01 ± 0.00238	5.13	1.36%	23.67%
	3		1.43	<0.01 ± 0.0024	<0.01 ± 0.00238	4.69	0.00%	27.92%
	4		2.57	<0.01 ± 0.0024	<0.01 ± 0.00238	4.69	0.00%	21.95%
	5		2.95	<0.01 ± 0.0024	<0.01 ± 0.00238	4.75	0.00%	21.78%
	Total		11.38					
	Average		2.28			4.83	0.27%	23.61%
	St Error		0.29			0.08	0.27%	1.13%
	St Deviation		0.65			0.19	0.61%	2.52%
West Rocky	1	Treated	2.63	<0.01 ± 0.0024	<0.01 ± 0.00238	5.50	4.95%	17.31%
	2		4.25	<0.01 ± 0.0024	<0.01 ± 0.00238	4.99	6.82%	13.63%
	3		2.65	<0.01 ± 0.0024	<0.01 ± 0.00238	5.30	7.08%	18.44%
	4		4.08	<0.01 ± 0.0024	<0.01 ± 0.00238	5.53	4.80%	18.40%
	5		1.50	<0.01 ± 0.0024	<0.01 ± 0.00238	5.12	2.88%	25.34%
	Total		15.10					
	Average		3.02			5.29	5.31%	18.62%
	St Error		0.51			0.11	0.76%	1.90%
	SD		1.14			0.23	1.71%	4.24%
Glacial Heritage	1	Control	1.18	<0.01 ± 0.0024	<0.01 ± 0.00238	5.07	0.00%	37.27%
	2		1.49	<0.01 ± 0.0024	<0.01 ± 0.00238	5.13	6.50%	26.42%
	3		1.18	<0.01 ± 0.0024	<0.01 ± 0.00238	4.94	0.00%	33.13%
	4		1.15	<0.01 ± 0.0024	<0.01 ± 0.00238	5.23	3.30%	32.61%
	5		1.48	<0.01 ± 0.0024	<0.01 ± 0.00238	5.20	2.18%	31.93%
	Total		6.47					
	Average		1.29			5.12	2.40%	32.27%
	St Error		0.08			0.05	1.21%	1.73%
	St Deviation		0.17			0.12	2.70%	3.88%
The Prairie at the Mouth of Black River	1	Control	3.12	<0.01 ± 0.0024	<0.01 ± 0.00238	5.10	6.43%	32.35%
	2		3.84	<0.01 ± 0.0024	<0.01 ± 0.00238	4.72	1.61%	42.76%
	3		3.52	<0.01 ± 0.0024	<0.01 ± 0.00238	5.02	4.51%	45.18%
	4		3.89	<0.01 ± 0.0024	<0.01 ± 0.00238	5.19	3.73%	31.67%
	5		2.28	<0.01 ± 0.0024	<0.01 ± 0.00238	5.07	2.21%	36.75%
	Total		16.66					
	Average		3.33			5.02	3.70%	37.74%
	St Error		0.30			0.08	0.86%	2.72%
	St Deviation		0.66			0.18	1.92%	6.07%
Secena Prairie	1	Control	1.87	<0.01 ± 0.0024	<0.01 ± 0.00238	5.55	3.20%	27.11%
	2		1.80	<0.01 ± 0.0024	<0.01 ± 0.00238	5.17	4.23%	14.98%
	3		1.02	<0.01 ± 0.0024	<0.01 ± 0.00238	5.39	6.94%	21.22%
	4		3.59	<0.01 ± 0.0024	<0.01 ± 0.00238	5.40	3.21%	22.62%
	5		2.70	<0.01 ± 0.0024	<0.01 ± 0.00238	5.13	1.06%	22.23%
	Total		10.99					
	Average		2.20			5.33	3.73%	21.63%
	St Error		0.44			0.08	0.95%	1.95%
	St Deviation		0.98			0.17	2.14%	4.35%
All Sites	Average		2.25			5.13	2.96%	27.09%
	St Error		1.03			0.04	0.44%	1.42%
	St Deviation		0.19			0.24	2.43%	7.78%
Treated Sites	Average		2.61			5.11	2.64%	23.64%
	St Deviation		4.39			0.25	2.68%	5.67%
	St Error		1.13			0.06	0.69%	1.46%
Control Sites	Average		2.27			5.15	3.28%	30.55%
	St Deviation		4.01			0.18	1.86%	8.56%
	St Error		1.04			0.05	0.48%	2.21%

Appendix G: Monthly temperature data

Monthly Temperature Data

Month - Year	Site Treated	Treatment Date	Minimum Temp (°F)	Average Minimum Temp (°F)	Average Temp (°F)	Anomilies
Dec-21	Marion Prairie	12/29/2021	7	34	38	
Jan-22			7	34	42	
Feb-22			14	30	39	2/23/22 - 2/24/22: Daily average temperatures below fre
Mar-22			21	37	46	
Apr-22			25	34	44	
May-22			32	42	51	
Jun-22			40	48	59	
Jul-22			44	52	67	
Aug-22			44	52	68	
Sep-22			37	48	62	
Oct-22			31	42	54	
			17	29	39	11/8 - 11/20: Minimum temperatures below freezing (17
Dec-22			22	33	38	12/21-12/23: Daily average temperatures below freezing
Jan-23	Scatter Creek West Rocky	1/4/2023 1/23/2023	15	34	40	
Feb-23			16	31	39	2/23-2/25: Daily average temperatures below freezing
Mar-23			22	31	42	
Apr-23			25	37	46	
May-23			36	45	59	
Jun-23			37			

*Months with below freezing temperatures highlighted

Appendix I: Monthly precipitation data

Monthly Precipitation Data

Month - Year	Site Treated	Treatment Date	Site Drainage	Max Precipitation (inches)	Total Precipitation (inches)	Days With Precipitation	Anomilies
Oct-21				1.23	5.39	68%	
Nov-21				1.78	11.88	90%	
Dec-21	Marion Prairie	12/29/2021	somewhat excessively	1.75	9.11	90%	
Jan-22				3.99	10.78	52%	
Feb-22				3.12	5.37	39%	2/27-2/28: 4.4[illegible] inches of rain tota
Mar-22				0.71	3.07	65%	
Apr-22				0.85	5.35	90%	
May-22				0.92	4.33	74%	
Jun-22				1.16	3.04	57%	
Jul-22				0.06	0.09	10%	
Aug-22				0.03	0.05	16%	
Sep-22				0.08	0.13	7%	
Oct-22				0.84	3.17	26%	
Nov-22				3.33	8.18	57%	
Dec-22				1.60	7.73	90%	
Jan-23	Scatter Creek West Rocky	1/4/2023 1/23/2023	somewhat excessively	0.51	3.7	71%	1/2-1/18: Constant rain with daily accumulations 0.01-0.51 inches
Feb-23				0.76	3.30	82%	
Mar-23				0.49	3.78	81%	
Apr-23				0.70	6.21	77%	
May-23				0.26	0.59	26%	

*Months with precipitation events greater than 1 inch highlighted

- Precipitation since Marion Prairie treatment: 69.1 inches
- Precipitation since Scatter Creek treatment: 17.5 inches
- Precipitation since West Rocky Treatment: 13.99 inches