SPECTRAL FINGERPRINTS PREDICT FUNCTIONAL PHENOTYPES OF A NATIVE SHRUB

by

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DEDICATION

I owe everything to my family—the people that taught me how to appreciate the world by curling up inside with a good book and curling up next to a fire under the stars.

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ABSTRACT

Landscapes are rapidly changing. To understand these changes and how they may influence coexisting herbivores, it is critical that we improve the ways in which we monitor changes in plant species, populations, and functional phenotypic traits over space and time. Near infrared spectroscopy (NIRS) is proving to be a valuable tool when it comes to this goal. NIRS is noninvasive and can provide high-resolution temporal information, including structural and chemical characteristics, on objects that are otherwise expansive, inaccessible, or imperceptible. We used the threatened sagebrush-steppe ecosystem, which spans over 43 million hectares of the Western United States, as a case study to test the accuracy in which NIRS can measure and classify functional phenotypic traits of sagebrush (*Artemisia* spp.) populations. Sagebrush habitats are known to have extreme levels of genetic and chemical heterogeneity and plasticity. Yet, our results showed that NIRS can classify species of sagebrush within a site, populations of sagebrush within a species across sites, and phenology (both seasonally and annually) of sagebrush within a population. These taxonomic, geographic, and phenological phenotypes are functionally important in many ways, including determining species composition and distribution, identifying developmental stages of individual plants, potentially detecting past and present anthropogenic and environmental stressors, and predicting interactions with herbivores. Even so, habitat use by coexisting herbivores is not always explained by these relatively crude phenotypes. Specifically, herbivores make foraging decisions based on specific concentrations of chemical phenotypes that have functional consequences for herbivores. Our research further demonstrated that NIRS can predict concentrations of individual chemical compounds and classes of compounds, in the forms of both nutrients and toxins, in sagebrush plants across species and populations. As such, we further tested if NIRS could directly predict browsing by coexisting sagebrush herbivores, in the form of bite marks on plants. Although NIRS was not able to predict herbivore foraging behavior, it shows promise for predicting foraging behavior indirectly through predicted concentrations of phytochemicals and directly with finer tuned field validation and model calibration. To monitor the threats of climate and anthropogenic disturbances on ecosystems, it is essential we find better ways to quantify the functional phenotypes that mediate interactions among plants, herbivores, and the environment. We show that NIRS can be a powerful tool in achieving this aim.

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LIST OF ABBREVIATIONS

NIRS	Near Infrared Spectroscopy
SVM	Support Vector Machine
PLSR	Partial Least Squares Regression
LOOCV	Leave-One-Out Cross Validation
RMSEC	Root Mean Square Error of Calibration
SEC	Standard Error of Calibration
RMSECV	Root Mean Square Error of Cross-Validation
SECV	Standard Error of Cross-Validation
AUC	Area Under the Curve
DW	Dry Weight
WW	Wet Weight

GENERAL INTRODUCTION

Landscapes are under threat from pervasive changes occurring across all biological scales. The biotic and abiotic features of landscapes are changing in structure, composition, distribution, and function due to increased human disturbance and climate change, creating adverse consequences for ecological communities. Human disturbance has led to degradation and erosion of land cover and habitats (Curtis et al., 2018; Watson et al., 2018; Roopsind et al., 2019), decreased landscape connectivity (Ehrlich and Pringle, 2008; Correa Ayram et al., 2017; Gubili et al., 2017; Carter et al., 2020), diminished biomass and primary production (Allen et al., 2015; Popkin, 2019; Komatsu et al., 2019), impaired fitness and fecundity of plants and wildlife (Carnicer et al., 2011; Requena- Mullor et al., 2019; Komatsu et al., 2019), and lower biodiversity (McRae et al., 2016; Watson et al., 2018; Semper-Pascual et al., 2019). Climate change, often incited by human activities, has exacerbated these changes through increased and intensified natural disasters (Millennium Ecosystem Assessment and World Resources Institute, 2005; Field et al., 2012), warming temperatures (Breshears et al., 2005; Overpeck and Udall, 2010), extensive drought (Breshears et al., 2005; Allen et al., 2010, 2015), and fluctuations in biological and chemical cycles that decrease ecosystem resilience to stress (Millennium Ecosystem Assessment and World Resources Institute, 2005; Field et al., 2012; McRae et al., 2016). These effects compromise the life-sustaining functions of ecosystems by destabilizing their provisional (e.g., food and water), regulatory (e.g., climate and disease control), and supporting (e.g., oxygen production) services (Millennium Ecosystem Assessment and World Resources Institute, 2005). To manage these ecosystem changes, we first need to be able to accurately and efficiently monitor the composition of species.

Monitoring the composition of plant communities is especially important. Human and climatic disturbances change the age class structure of a plant community (e.g., remnant stands versus new growth, recruitment; Klanderud, 2005; Caughlin et al., 2014, 2016; Roopsind et al., 2018). Shifts in microclimates affect the phenology (i.e., timing of emergence and senescence of leaves) of plants across communities (Xu et al., 2018; Andresen et al., 2018; Wang et al., 2018). All of these changing phenotypes, in turn, influence the herbivores that rely on plants for survival. Changes in the structure, composition, and function of plant communities directly impact the distribution, movement, habitat use, and demographic rates of wild and domestic herbivores. For example, plant distribution and size affect the cover and occupancy of herbivore species (Eber and Brandl, 2003; Haynes et al., 2007; Forister and Wilson, 2013; Eby et al., 2014; Anderson et al., 2016). Phenology of plant communities impacts the movement and fitness of migratory species (e.g., migration with the green wave; van der Graaf et al., 2006; Thein et al., 2008), domestic herbivore grazing (Pfister et al., 1988; Frank, 1996), and phenological mismatch (Hogrefe et al., 2017; Xie et al., 2018; Boelman et al., 2019), which has bottom-up cascading trophic effects (Wang et al., 2018). Plant distribution (Shipley et al., 1998; Lanan, 2014), composition (Robinson and Holmes, 1984), size (e.g., out of reach for browsing; Shipley et al., 1998; Peterson et al., 2003; Jager et al., 2009), and quality (e.g., functional chemical traits; Dearing et al., 2005; DeGabriel et al., 2009; Frye et al., 2013) influence habitat use by herbivores. Current methods to monitor suitability of plants for habitat use by herbivores, such as species occurrence or plant structural traits, are time intensive and limited in spatial and temporal scope. Moreover, plant quality is more than just abundance and structure, especially for foraging herbivores. The quality of plants can be measured in nutrients and toxins (i.e., phytochemicals). The concentrations and diversity of these phytochemicals, or functional traits, direct the foraging activity of associated herbivore species (Sorensen et al., 2005b; Shipley et al., 2006; Forbey and Foley, 2009; Nobler et al., 2019). Without stable and nutritious plant communities, the health, fitness, and diversity of associated herbivore species are at risk of decline (Fauchald et al., 2017; Parikh et al., 2017; Schrempp et al., 2019) and even extirpation (Connelly et al., 2000; Larrucea and Brussard, 2008; Knick and Connelly, 2011).

Monitoring changes in the functional diversity of plant communities is essential if we hope to maintain suitable lands for native and domestic herbivores, restore and monitor post-disturbance succession, or gain baseline conditions of a community. Assessing functional diversity requires that we both classify species and quantify the functional traits of those species. However, classification of plants in a community can be challenging due to complex and changing morphology and phenology (Tzionas et al., 2005; Xu et al., 2018), species hybridizations (Rieseberg and Carney, 1998), and habitat restoration practices that include reseeding with non-native taxa (Jones, 2003; Tanner and Gange, 2013). Even when species can be accurately classified, the functional role of plants within a community is dependent on functional traits such as phytochemical properties. As such, the functional diversity of plant communities cannot be fully captured by standard morphometric (e.g., line-point intercepts and quadrats; Pilliod and

Arkle, 2013), genetic (Donaldson and Lindroth, 2007), or chemical (e.g., lab-based assays; Kelsey et al., 1982; DeGabriel et al., 2008) measurements. Moreover, these standard approaches provide only a brief snapshot of information over space and time because they are time-consuming, expensive, and often require convergence among researchers with diverse expertise (e.g., ecology, chemistry, geoscience, conservation, etc.). We need better tools to monitor the coupled diversity and functionality of plants present on a landscape because they are indicators of the health, dynamics, and quality of the habitat. Finding a more efficient means of classifying plants and assessing functional traits of plants at greater spatial and temporal resolutions remains critical.

Alternative methods to effectively classify plant species and monitor functional traits of plants across landscapes are growing in use. These emerging technologies involve the collection of imagery or spectral fingerprints of objects through the use of remote sensing techniques, which do not require direct contact with the objects (Rast and Painter, 2019). This is especially useful in places that are inaccessible (e.g., deep sea exploration; Platt et al., 1988; Klemas and Yan, 2014), imperceptible (e.g., inside geological features; van der Meer et al., 2012; Liang et al., 2014), or expansive (e.g., global snow cover; Bormann et al., 2018). These remote sensing technologies come in a variety of forms, allowing for specificity of the data desired. Platforms for collecting spectral data include, but are not limited to, handheld or ground-based, unmanned aerial systems, manned aerial systems, and satellites (Rast and Painter, 2019). Each platform, in conjunction with one or more sensors, can collect spectral information along different regions and lengths of the electromagnetic spectrum and at different spatial and temporal resolutions. The spectral data can then be linked to geophysical, chemical, or biological information associated with the objects.

Near infrared spectroscopy (NIRS), collected from handheld and airborne systems, is one popular technique for collecting biochemical and phenotypic information in plant communities. The agricultural industry has been using this technology for decades by measuring nitrogen content in domestic feed, both commercially and privately (Abrams et al., 1987; Shenk and Westerhaus, 1994; Corson et al., 1999; Mnisi and Mlambo, 2017; Saha et al., 2018). NIRS has also been used to predict phytochemicals in agriculture in the forms of fruits (Baranska et al., 2004; Sinelli et al., 2008), hops (Garden et al., 2000), and wines (Urbano-Cuadrado et al., 2004; Cozzolino et al., 2008). More recently, NIRS has been used in wild systems to aid wildlife and habitat conservation in tropical rainforests (Asner and Martin, 2009; Asner et al., 2011, 2012; Féret and Asner, 2014), eucalyptus (Foley et al., 1998; Youngentob et al., 2012) and bamboo forests (Wiedower et al., 2009), grasslands (Griggs et al., 1999), savannas (Brunet et al., 2007), arid shrublands (Mitchell et al., 2012b; Olsoy et al., 2016), and sub-arctic taiga (Stolter et al., 2006). However, this methodology is still new and studies are often limited to large spatial scales (e.g., forest canopies; Asner and Martin, 2009; Asner et al., 2011), relatively homogeneous ecological systems (e.g., wheat and poplar in agricultural systems; Maranan and Laborie, 2008; Rincent et al., 2018), or the measurement of specific chemicals or traits (e.g., nitrogen; Abrams et al., 1987; Saha et al., 2018) or greenness (e.g., NDVI; Wylie et al., 2003; Wilson et al., 2017; Hogrefe et al., 2017) in plants. As informative as these broad resolutions or discrete traits are, they do not convey the full range of functional traits of plants. Most plants consist of a myriad of chemicals that are responsible for ecological dynamics including growth, competition, reproduction, and interactions with herbivores. NIRS offers the potential to rapidly and comprehensively assess the full range of functional chemical traits in complex and dynamic systems.

The semiarid sagebrush-steppe serves as a model ecosystem for remotely sensing complex functional traits because of its chemical and phenotypic diversity (Kelsey et al., 1982; McArthur et al., 1988; McArthur and Sanderson, 1999; Rosentreter, 2005; Welch, 2005). Colloquially known as the sagebrush sea, this semiarid ecosystem is distributed across more than 43 million hectares of the Western United States. It provides a patchwork of sagebrush (*Artemisia* spp.) species, subspecies, and hybridizations that support multiple services, including forage and shelter for generalist and specialist herbivores (Welch, 2005), adaptive evolution of plants (Huynh et al., 2015), genetic and chemical diversity (Kelsey et al., 1982; McArthur et al., 1988; McArthur and Sanderson, 1999; Graham et al., 2001; Richardson et al., 2012; Jaeger et al., 2016), and medicinal uses by native people (Kelley et al., 1992). Most importantly, this ecosystem is under severe threat, ecologically and economically, from anthropogenic disturbances, fire, disease, invasive species, and climate change. Historically, up to 50% of its distribution has already been lost (Welch, 2005; Miller et al., 2011). Efficient monitoring of this rapidly changing landscape is critical. Efforts have been made to more effectively monitor the plant communities in the sagebrush-steppe (Wylie et al., 2003; Streutker and Glenn, 2006; Mitchell et al., 2012; Olsoy et al., 2014; Glenn et al., 2016; Olsoy et al., 2016; Pandit et al., 2019), but no efforts have been made to optimize monitoring the functional traits, including taxa, morphology, and nutritional and chemical quality, that are critical for the conservation of associated wildlife.

The purpose of this research is to explore and champion the use of NIRS to assess functional traits in sagebrush-steppe ecosystems. The layout of this thesis serves as a case study to demonstrate how NIRS can be applied in a threatened and chemically complex shrub, sagebrush. Chapter 1 investigates the use of NIRS in classifying taxonomic and phenological phenotypes across sagebrush-steppe habitats. We investigated if NIRS could accurately classify species, populations within a species, and temporal variation within a population, as well as detect herbivory (e.g., browsed versus not browsed plants) by a mammalian herbivore that specializes on sagebrush. Chapter 2 investigates the use of NIRS in predicting concentrations of functional chemical traits in sagebrush that influence foraging by specialist vertebrate herbivores. We investigated if NIRS could accurately predict the concentrations of nutritional and potentially toxic chemicals present in different sagebrush species and populations at increasing chemical resolution (i.e., from classes of compounds to individual compounds) as well as predict the extent of herbivory (e.g., number of bite marks) by a mammalian herbivore that specializes on sagebrush.

Anthropogenic activities and climate change will continue to alter the threatened sagebrush-steppe ecosystem and its interactions with species at all trophic levels. Land managers need tools that allow for effective and continuous monitoring of these changes. Remote sensing, in the form of NIR spectra, is a novel and efficacious way of monitoring these precipitous changes that could be used to detect and manage the repercussions of human activity. Furthermore, NIRS can be used to inform management of functional roles of sagebrush communities to better establish conservation and restoration efforts.

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CHAPTER ONE: USING NIRS TO CLASSIFY TAXONOMIC AND PHENOLOGICAL PHENOTYPES OF PLANTS ACROSS SAGEBRUSH-STEPPE HABITATS

Abstract

Plant communities are composed of complex phenotypes that not only differ among taxonomic groups and habitats but also change over time within a species or habitat. Each phenotype serves an important and measurable function of an individual plant within a community. Phytochemical phenotypes (hereafter, chemotypes) play a particularly important role in plant communities because they serve as a link across all other phenotypes. Chemotypes are genetically determined and can explain resistance to biotic and abiotic stressors, yet can change through interactions with neighboring plants, microbial communities, and herbivores. Near infrared spectroscopy (NIRS) operates by measuring organic bonds and can be used to detect unique chemotypes that characterize plant species, populations, and individual plants. We used the sagebrush-steppe ecosystem as a case study to test the accuracy in which NIRS can measure and classify variation in taxonomic, phenological, and trophic interactions in plants that likely reflect distinct chemotypes. Sagebrush taxa are known to have extreme levels of genetic and chemical heterogeneity and plasticity, yet, our results demonstrated that NIRS can classify species of sagebrush within a population, populations of sagebrush within a species, and phenology (both seasonally and annually) of sagebrush within a population with accuracies ranging between 75-99%. However, accuracy dropped when classifications were spectrally determined at a field site where morpho- and chemotypic variation was extremely high. These results suggest that larger sample sizes or better taxonomic identification in the field may be required for NIRS to classify taxa within phenotypically heterogeneous populations of sagebrush. Results also suggest that NIRS has the potential to detect genetic diversity associated with unique hybrid zones or post-disturbance sites where non-native sources of seed may have been used for restoration. We also used NIRS to classify interactions with herbivores, in the form of browsed versus non-browsed plants. NIRS did not reliably classify browsed state of sagebrush by herbivores. This suggests that subtle, yet important, differences exist between plants browsed and non-browsed by herbivores and suggests a need for more in-depth investigations of the chemotypes mediating these interactions. The taxonomic and phenological phenotypes detected by NIRS are functionally important in determining species composition and distribution, identifying timing of life stages of individual plants, predicting forage quality of plants for herbivores, and determining the functional quality of habitats for translocation or restoration of herbivores dependent on sagebrush. Our research reveals that NIRS can be used to classify and monitor these phenotypes across habitats and could help land managers and researchers detect the health of plant populations over space and time.

Introduction

Plant communities are complex and not only differ among taxonomic groups and habitats but also change over time within a species or habitat. Nested within a species, population, and individual plant there can be numerous smaller communities of traits, i.e., phenotypes. Plant phenotypes are often dependent on genotypes (e.g., genetic makeup, ploidy level, species) and can be represented by quantifiable morphotypes (e.g., size, structure, age) and chemotypes (e.g., chemical composition). Each phenotype serves an important and measurable function in the success of a plant in its habitat. However, chemotypes play particularly important roles. Chemotypes are defined as the chemical makeup or profile of a plant, including both primary and secondary compounds. Chemotypes vary within, among, and across plants in an ecological community. Furthermore, chemotypes are genetically determined (Hayashi et al., 2005; Desjardins, 2008; Karban et al., 2014; Cook et al., 2018), yet can change through interactions with neighboring plants (Shiojiri et al., 2009; Karban et al., 2016b, 2016a; Germino et al., 2019; Zaiats, 2019), soil or leaf microbial communities (Phelan et al., 2012; Rosentreter and Root, 2019; Barnard et al., 2019; Benedek et al., 2019), and herbivores (Shiojiri et al., 2009; Karban et al., 2016b, 2016a). In addition, chemotypes can explain resistance to external biotic (e.g., herbivores) and abiotic (e.g., drought) stressors. In this way, chemotypes serve as an important link among genotypes interacting with the environment and resultant phenotypes. For example, chemotypes influence population genetics and genetic adaptation (Zytynska et al., 2019), plant demographic rates (e.g., population structure, community, distribution; Ehlers and Thompson, 2004), phenology (e.g., green-up; Thoss et al., 2007; Welker et al., 2007; Usano-Alemany et al., 2014), and interactions with herbivores (e.g., foraging behavior and diet selection; Sorensen et al., 2005b; Moore and Foley, 2005; Dearing et al., 2005; DeGabriel et al., 2009; Frye et al., 2013; Ulappa et al., 2014; Bedoya-Pérez et al., 2014; Nobler et al., 2019).

Spectra, in the form of near infrared spectroscopy (NIRS), can detect chemotypic changes in plants (Vance et al., 2016) and potentially even predict habitat use by associated herbivores (Moore et al., 2010). The static and dynamic organic bonds that comprise plants can be both unique to each plant taxa and influenced by climate, soil, size, structure, age class, and herbivory. NIRS can detect these organic bonds. NIRS operates by measuring the reflectance of electromagnetic energy in objects from wavelengths ranging between 350-2500 nm. This electromagnetic range is particularly well-known for reflecting organic bonds (Curran, 1989; Youngentob et al., 2012), establishing NIRS as a great proxy for chemical determination. Because NIRS detects chemotypes, it can classify genotypes (Amar et al., 2009; Gebreselassie et al., 2017), including hybrids (Hicks et al., 2002; Humphreys et al., 2008) and ploidy levels (Rincent et al., 2018), morphotypes (e.g., size, structure, age class; Viana et al., 2009; Hetta et al., 2017; Martínez-Valdivieso et al., 2018), and foraging behavior of herbivores (McIlwee et al., 2001; Siitari et al., 2002; Moore et al., 2010).

The semiarid sagebrush-steppe of the Western United States serves as an ideal system to demonstrate how NIRS can monitor the phenotypic, and more specifically, the chemotypic diversity representing distinct plant genotypes and stages of phenology. The dominant shrub in this system, sagebrush (*Artemisia* spp.), has complex and changing morphotypic and chemotypic diversity (Kelsey et al., 1982; Rosentreter and Kelsey, 1991; Winward, 2004; Rosentreter, 2005; Welch, 2005), which is shown to be genetically determined (Jaeger et al., 2016). Sagebrush is associated with often unique and long-standing ecological and evolutionary relationships with other plants (Casper and Jackson, 1997), soil microbes (Cundell, 1977; Morris and Allen, 1994; Rosentreter and Root, 2019; Condon et al., 2019), insects and reptiles (Winward, 1991; Welch, 2005), pronghorn (*Antilocapra americana*; Hansen et al., 2001; Jacques et al., 2006), mule deer (*Odocoileus hemionus*; Wambolt, 1996), pygmy rabbits (*Brachylagus idahoensis*; Ulappa et al., 2014; Nobler, 2016; Utz et al., 2016), sage sparrows (*Artemisiospiza nevadensis*; Paige and Ritter, 1999), sage thrashers (*Oreoscoptes montanus*; Paige and Ritter, 1999), and sage-grouse (*Centrocercus urophasianus*; Ulappa, 2011; Frye et al., 2013; Fremgen-Tarantino et al., 2020). Some of the associated species are sagebrush obligates, including the threatened vertebrate herbivores, pygmy rabbits and greater sage-grouse, with sagebrush comprising about 50% of their diet in the summer and up to 100% of their diet in the winter (Wallestad and Eng, 1975; Green and Flinders, 1980). As threats from anthropogenic and climatic stressors increase, timely monitoring of stress responses by sagebrush is necessary for successful conservation and restoration of this system. However, monitoring and managing the functional traits of sagebrush that other species depend on remains difficult. For instance, standard techniques for ecological sampling within plant communities include structure (e.g., line-point intercepts) and composition (e.g., quadrats) measurements that do not provide adequate information on functional traits, especially over time. Some research has employed more advanced techniques such as true color (Booth et al., 2005) and multispectral image analysis (Wylie et al., 2003; Glenn et al., 2016), light detection and ranging (LiDAR) surveys (Streutker and Glenn, 2006), terrestrial laser scanning (TLS; Olsoy et al., 2014b, 2014a), NIRS (Mitchell et al., 2012b; Olsoy et al., 2016), and ecosystem demography modeling (Pandit et al., 2019) to assess these functional traits. However, results remain largely focused on individual traits and do not capture the complex interactions of all the functional chemical traits in plants.

Ascertaining a more efficient method for quantifying functional phenotypes remains of significant importance if the goal is to better assess and monitor changing plants and associated herbivores.

We investigated the accuracy of NIRS to classify plants with distinct taxonomic, phenological, and trophic interaction phenotypes within and among sagebrush populations. Specifically, we used NIRS data from sagebrush to ask the following questions (Table 1.1):

i. Can NIRS classify species within a sagebrush site?
ii. Can NIRS classify sagebrush populations within a species?
iii. Can NIRS classify phenology (i.e., years and seasons) within a sagebrush population?
iv. Can NIRS classify herbivore foraging behavior (i.e., browsed versus non-browsed plants) within a sagebrush population?

Methods

Sagebrush Field Sites

Samples were collected at four field sites across four different counties in Idaho, USA (Figure 1.1). These include Magic Reservoir in Blaine County (43° 14’ N, 114° 19’ W, hereafter Magic), Cedar Gulch in Lemhi County (44° 41’ N, 113° 17’ W), Craters of the Moon in Minidoka and Blaine Counties (42° 57’ N, 113° 23’ W, hereafter Craters), and Raft River in Cassia County (42° 9’ N, 113° 24’ W). These field sites were predominantly sagebrush (*Artemisia* spp.) and included variation in phenotypic diversity and environmental conditions (i.e., species, morphology, terrain, elevation, climate, etc.)

and in the samples and data collected and measured (i.e., year, season, species, microhabitat, plant chemicals, etc.; Table 1.1). However, all field sites included NIRS scans of sagebrush plants and were inhabited by one of two threatened herbivores that are heavily reliant upon and selective about the sagebrush consumed, pygmy rabbits (Ulappa, 2011; Utz, 2012; Nobler, 2016) and Greater sage-grouse (Ulappa, 2011; Frye, 2012; Fremgen, 2015).

Phenotype Selection

Phenotypes measured at each field site were those shown to influence foraging and habitat use by herbivore populations and are parameters that land managers can monitor and, at times, manipulate. The taxonomic phenotypes included sagebrush species, which was the broadest classification unit, and geographically distinct populations within a single species. The phenological phenotypes included year of sampling, which represents a different set of leaves and environmental conditions, and season of sampling, which represents different leaf types (i.e., persistent and ephemeral in summer and only persistent in winter; Miller and Shultz, 1987) and environmental conditions within a plant. The trophic interaction phenotype was herbivory (i.e., presence of foraging) within a season and population, which was the narrowest classification unit. Sagebrush species were identified using plant morphology, type of environment (e.g., elevation and soil type and depth; Rosentreter, 2005), and chemotype (e.g., pattern of individual leaf chemicals).

Detection of Herbivory

Sagebrush samples were deemed browsed or non-browsed according to the presence or absence of bite marks by pygmy rabbits on plants within similar distance to an active pygmy rabbit burrow. Bite marks were identified for pygmy rabbits by a clean 45-degree cut in a woody stem and can be differentiated from other lagomorph species by the diameter of the clipping (Crowell et al., 2018) and the lack of leafy material left below the shrub (Figure 1.2). Fresh bite marks were identified by a wetter green or bright brown stem interior, as opposed to a dry dull brown color.

Sample Collection and Analysis

Sagebrush plants were selected for sampling according to browsed state of plant (i.e., browsed versus non-browsed). At the Magic and Cedar Gulch sites, browsed and non-browsed plants by pygmy rabbits were clipped for about 2 g of wet weight (ww) or no more than 25% of the overall biomass of the plant and stored in labeled plastic bags, respectively, on ice. At the Craters and Raft River sites, leaves were collected from plants browsed (n = 3) and non-browsed (n = 3) by sage-grouse as described in Fremgen (2015). Briefly, browsed plants had at least 10 fresh bite marks by sage-grouse on leaves and non-browsed plants had no more than one fresh bite mark by sage-grouse. Plants within a patch were combined to form one composite browsed sample and one composite non-browsed sample, which were stored in labeled plastic bags, respectively, on ice. All samples were stored continuously on ice until transferred to Boise State University. Samples from pygmy rabbit sites (Magic and Cedar Gulch) included both stems and leaves, whereas samples from sage-grouse sites (Craters and Raft River) included only leaves. For NIRS analysis, samples were ground (~2 mm) and dried at 60°C for 48 hours.

NIRS Analysis

The ASD FieldSpec® 4 spectroradiometer was used to measure continuous near infrared wavelength reflectance from 350 nm to 2500 nm in all of the sagebrush samples.

Each ground dried sagebrush sample was placed in a sealed clear plastic bag and spread homogeneously on a black countertop with no countertop surface visible through the biomass. After calibrating and optimizing the ASD FieldSpec® 4 to a pure white reflectance according to standard protocol in the user manual, it was then used to measure the reflectance of each sagebrush sample (Figure A.1). Thirty replicate scans were collected for each sample. The instrument was recalibrated and optimized every 15 samples. The NIRS replicate reflectance scans were exported for each sample, where they were then imported into Camo Analytics Unscrambler® software and checked for outliers using Unscrambler® outlier detection and review of raw values to justify removal. Samples were then averaged to one spectral profile per sample. Each spectrum was converted to absorbance values using a $\log_{10}(1/R)$ transformation, where R is reflectance (Figure A.2). Spectral absorbance values were transformed by taking a 1st gap derivative every 1 nm and then truncated from 450 nm to 2350 nm (Figure A.3).

Statistical Analysis

All phenotype categorical response variables (i.e., species, population, season, herbivory) and the predictor numerical variable (i.e., NIR spectra) were joined for each sagebrush sample and imported into Camo Analytics Unscrambler® chemometric software. Unscrambler® was then used to perform support vector machine (SVM) analyses to classify phenotypes (i.e., response variables) using transformed NIR spectral values (i.e., predictor variables). The SVM type used was C-SVC, with a linear kernel and C value of 1.0 (i.e., large margin). Each model was independently calibrated and validated using leave-one-out cross validation (LOOCV) of 10 segments. The resulting confusion matrix from the support vector machine analysis was used to evaluate the overall accuracy of NIRS to classify phenotypes. Further accuracies were then calculated to differentiate the proportion of phenotypes on the ground that were accurately classified by NIRS (also known as producer's accuracy) versus the proportion of phenotypes classified by NIRS that were actually present on the ground (also known as user's accuracy).

The criteria for inclusion for the phenotypic classifications tested using NIRS were established according to the following:

i. Can NIRS classify species within a sagebrush site?
    a. All species identified at each field site (Table 1.1) were included in the field site analysis. These species included Wyoming big (*A. t. wyomingensis*), three-tip (*A. tripartita*), black (*A. nova*), low (*A. arbuscula*), and "dwarf" sagebrush. Dwarf sagebrush were identified as a generic dwarf sagebrush classification in the field according to relatively smaller morphological size and location of collection (i.e., micro-topographically unique patches) but without clear morphological features of specific dwarf species (*A. nova* and *A. arbuscula*). Each field site analysis also included all other phenotypic data (i.e., consecutive seasons and/or years of collection, microhabitat of collection, browsed state of plant) when available (Table 1.1).

ii. Can NIRS classify sagebrush populations within a species?
    a. Wyoming big sagebrush was used as the species of interest across all field sites because of its consistently large sample size per site (Table 1.1), ubiquitous distribution (Table 1.1; Turi et al., 2014), and functional

relevancy (e.g., selective use in foraging and occupancy by herbivores; Frye, 2012; Utz, 2012; Fremgen, 2015; Nobler, 2016). The analysis also included all other phenotypic data (i.e., consecutive seasons and/or years of collection, microhabitat of collection, browsed state of plant) when available (Table 1.1).

iii. Can NIRS classify phenology (i.e., years and seasons) within a sagebrush population?

    a. Wyoming big sagebrush at the Magic field site was the only population analyzed due to it having the largest collection of repeated temporal measurements (both annually and seasonally) of plants (Table 1.1). This analysis controlled for species, but for the annual temporal analysis season was not controlled due to smaller seasonal sample sizes within one annual collection (i.e., 2012-2013; Table 1.1). All other phenotypic data (i.e., microhabitat of collection, browsed state of plant) were included when available (Table 1.1).

iv. Can NIRS classify herbivore foraging behavior (i.e., browsed versus non-browsed plants) within a sagebrush population?

    a. Wyoming big sagebrush at the Magic field site during the 2012-2013 collection was the only population and year analyzed due to it having the largest sample size and highest homoscedasticity of browsed and non-browsed samples (Table 1.1). This analysis controlled for species but not for season due to its smaller sample size. All other phenotypic data (i.e., microhabitat of collection) was included when available (Table 1.1).

Results

Accuracy of NIRS to Classify Species Within Sagebrush Sites

For the Magic field site, the overall accuracy of the training dataset was 95.90% and the leave-one-out cross validation (LOOCV) dataset of 10 segments was 95.15% (Table 1.2). Wyoming big (*A. t. wyomingensis*) sagebrush (n = 807) was predicted 99% correctly, dwarf sagebrush (n = 112) was 79% correct, and three-tip (*A. tripartita*) sagebrush (n = 8) was 0.0% correct.

For the Cedar Gulch field site, the overall accuracy of the training dataset was 77.01% and the LOOCV validation dataset of 10 segments was 75.92% (Table 1.3). Wyoming big sagebrush (n = 336) was predicted 100% correctly, dwarf sagebrush (n = 100) was 1.0% correct, and black (*A. nova*) sagebrush (n = 25) was 72% correct.

For the Craters field site, the overall accuracy of the training dataset was 94.57% and the LOOCV dataset of 10 segments was 93.48% (Table 1.4). Wyoming big sagebrush (n = 66) was predicted 92% correctly and three-tip sagebrush (n = 26) was 100% correct.

For the Raft River field site, the overall accuracy of the training dataset was 75.19% and the LOOCV dataset of 10 segments was 75.19% (Table 1.5). Wyoming big sagebrush (n = 65) was predicted 0.0% correctly and low (*A. arbuscula*) sagebrush (n = 197) was 100% correct.

Accuracy of NIRS to Classify Sagebrush Populations Within a Species

When using NIRS to classify sagebrush populations within the Wyoming big sagebrush species, the overall accuracy of the training dataset was 96.12% and the LOOCV dataset of 10 segments was 95.99% (Table 1.6). Wyoming big plants from

Magic (n = 1089) were predicted 98% correctly, Cedar Gulch (n = 625) was 100% correct, Craters (n = 94) was 44% correct, and Raft River (n = 263) was 98% correct.

Accuracy of NIRS to Classify Phenology (i.e., Years and Seasons) Within a Sagebrush Population and Species

At the Magic field site, two independent years of sample collection occurred, 2012-2013 and 2014-2015. NIRS classified years of collection within Wyoming big sagebrush at this single site with an overall training accuracy of 91.95% and LOOCV accuracy of 10 segments of 91.95% (Table 1.7). 2012-2013 collections (n = 486) were predicted 89% correctly and 2014-2015 collections (n = 321) were 96% correct. When controlling for season (i.e., winter only) the accuracy to predict year decreased to 77.87% (Table B.1).

At the Magic field site, sagebrush samples were also collected seasonally, during winter 2012 and spring 2013. NIRS classified seasons within individual Wyoming big sagebrush plants at this single site with an overall training accuracy of 99.0% and LOOCV accuracy of 10 segments of 99.0% (Table 1.8). Winter collections (n = 92) were predicted 100% correctly and spring collections (n = 394) were 99% correct.

Accuracy of NIRS to Classify Herbivory (i.e., Browsed versus Non-browsed Plants) Within a Sagebrush Population and Species

At the Magic field site during the 2012-2013 sample collection, Wyoming big sagebrush were sampled according to differing browse states, i.e., browsed versus non-browsed by pygmy rabbits. NIRS classified herbivory of Wyoming big sagebrush at this single site with an overall training accuracy of 60.08% and LOOCV accuracy of 10 segments of 57.20% (Table 1.9). Browsed samples (n = 212) were predicted 24%

correctly and non-browsed samples (n = 274) were 88% correct. When controlling for season (i.e., winter vs. spring) one at a time, every plant was consistently classified as non-browsed except for one (Tables B.2-B.3).

Discussion

Our research has shown that not all sagebrush is created chemically equal and that NIRS can detect some functional inequalities in sagebrush species and populations that may not be obvious from morphological traits. Overall, our results suggest that NIRS has relatively good accuracy in classifying sagebrush species within a site (i.e., geographically distinct habitat), geographically distinct populations within a species, and temporal changes within a geographically distinct population within a single species. However, NIRS has generally poor accuracy at detecting browsing within a species and population. These classification errors are likely related to sample size, poor classification of species in the field, and complex phytochemistry within and among taxa. We offer evidence for each of these potential sources of error in classification via NIRS. Furthermore, we discuss how overcoming these errors will allow NIRS to be a powerful tool in monitoring plant populations, directing restoration efforts and success, and predicting suitable habitats for threatened herbivores in the sagebrush-steppe.

NIRS classification results suggest that larger sample sizes can improve the ability to accurately predict sagebrush species within a site or populations within a species, particularly when chemotypic variation within a species is low but high between species (Au et al., 2020). At the Magic field site, most plants in this population were categorized as Wyoming big sagebrush (*A. t. wyomingensis*), with Wyoming predicted accurately 99% of the time (i.e., producer accuracy). In contrast, “dwarf” species (identified morphotypically in the field as a dwarf sagebrush taxon and, hereby, known simply as dwarf) were predicted accurately 79% of the time and three-tip (*A. tripartita*) was never predicted accurately (Table 1.2). This is likely due to Wyoming big sagebrush having the largest sample size (n = 807), followed by dwarf (n = 112) and then three-tip (n = 8). Misclassifications of three-tip can ultimately be attributed to a very small sample size within this site. In support of this, classification of three-tip at the Craters site was 100% accurate where there was a sample size of 26 plants (Table 1.4). At the Cedar Gulch field site, 100% of the Wyoming big plants (n = 336) were classified correctly, whereas all but one of the dwarf plants (n = 100) were classified as Wyoming (i.e., 1.0% accuracy) and 72% of the black (*A. nova*) sagebrush plants (n = 25) were predicted accurately (Table 1.3). These results indicate that when chemistry is distinct among species (e.g., Wyoming big vs. three-tip; Fremgen, 2015; Fremgen-Tarantino et al., 2020; Figure C.1), large sample sizes may not be required for NIRS to correctly determine species.

When predicting source populations within a species (i.e., Wyoming big sagebrush), the least accurately predicted field site was also the field site with the lowest sample size. Craters (n = 94) was only classified 44% correctly, whereas Magic (n = 1089) was 98% correct, Cedar Gulch (n = 625) was 100% correct, and Raft River (n = 263) was 98% correct (Table 1.6). Large and evenly distributed sample sizes are ideal for ecological analyses. However, in the case of phenotype classification by NIRS, a large sample size may not be required if the phenotypes (i.e., chemotypes) of interest are chemically distinct (Au et al., 2020). This may require a baseline analysis of chemistry at the site, simple trial and error, or a preliminary overestimation of sample size.

Even with large sample sizes, misclassified results may be due to possible errors in proper identification of species (or in this case, subspecies) in the field (i.e., user accuracies). At the Cedar Gulch field site, although there was a small sample size of black sagebrush (n = 25), classification was highly accurate (100% user accuracy) because of distinct morphological characteristics that allowed for better identification in the field, such as a greenish color, sticky leaves, and unique black glands on the leaf tissue (Rosentreter, 2005). In contrast, there were 100 dwarf sagebrush plants collected at this site, but only one of the dwarf plants was categorized correctly as the dwarf taxa, which resulted in too few samples for proper user accuracy. Misclassification of dwarf species also occurred at the Magic field site, although not to the same extent (with a 79% producer accuracy and 94% user accuracy). Errors in classifying dwarf taxa could be due to the dwarf plants simply being small or young Wyoming big sagebrush instead of an actual different sagebrush species with a distinct chemotype. Classifying sagebrush species in the field remains a challenging and elusive task for researchers. Sagebrush morphology is highly variable and vulnerable to change. For instance, sagebrush morphology within and among species can vary from soil type and depth (Rosentreter and Kelsey, 1991; Rosentreter, 2005; Barnard et al., 2019), elevation (Rosentreter and Kelsey, 1991; Rosentreter, 2005), climate (Rosentreter, 2005; Germino et al., 2019; Lazarus et al., 2019), herbivory (Kessler et al., 2006; Karban, 2011; Ulappa et al., 2014; Karban et al., 2016a), and even chemical interactions (Karban et al., 2006, 2014; Zaiats, 2019), ploidy level (Richardson et al., 2012) or hybridizations with neighbors (McArthur et al., 1988; McArthur and Sanderson, 1999; Graham et al., 2001; Richardson et al., 2012; Jaeger et al., 2016). Even a strong baseline knowledge of habitat, morphology, and use of chemical traits such as ultraviolet reflectance (Rosentreter, 2005) may not be enough to properly identify sagebrush in the field. We argue that inclusion of existing measures of chemistry, such as ultraviolet reflectance (Stevens and McArthur, 1974), coupled with advances in handheld NIRS can further aid in correct identification of species in the field.

Even when meticulous and deliberate identifications were made in the field, some species were still classified poorly using NIRS, such as the Wyoming big sagebrush (n = 65) at Raft River, with 0.0% producer accuracy (Table 1.5). The user accuracy was 100% (Table 1.5), however this was unreliable due to lack of predicted samples. Notably, the ability for sagebrush to hybridize has been shown between Wyoming big and black species (Garcia et al., 2008), Wyoming big and low species (Winward and McArthur, 1995; Garcia et al., 2008), and Wyoming big and three-tip species (Schlatterer, 1973). We propose that errors in NIRS classifications may indicate hybridization at these sites.

Our results reflect potential errors in field identification of sagebrush species, but they also demonstrate that NIRS could be used as a tool to verify predicted taxonomy or even detect unique environmental or historical conditions or phytochemical diversity. For example, Magic and Cedar Gulch included sagebrush we simply classified as a “dwarf” species based on morphology and phenology. Lower accuracy of dwarf classifications at both of these sites suggests that some, if not most, of the dwarf samples actually differed in taxonomy from dwarf sagebrush species. Alternatively, these plants could be a dwarf species but could also have unique chemistry due to hybridization (McArthur et al., 1988;

Richardson et al., 2012; Jaeger et al., 2016), unique environmental conditions, such as soil type and depth, or history of herbivory, which can cause variation in the reflectance of chemical compounds measured via NIRS.

To illustrate the potential power of NIRS for revealing unique taxonomic, phytochemical, or environmental diversity, we first focus on the Cedar Gulch and Raft River field sites. The dwarf species at Cedar Gulch were consistently classified as Wyoming big sagebrush and, in addition, turned out to be relatively homogeneous in chemistry with the Wyoming big plants at this field site (Olsoy et al., 2020). These results suggest that most of the dwarf sagebrush species at Cedar Gulch are likely small or young Wyoming big sagebrush with different morphology due to environmental conditions or history of herbivory. These results could suggest diverse age classes of Wyoming big sagebrush at Cedar Gulch. Similarly, the seven black sagebrush plants at Cedar Gulch categorized as Wyoming big could be potential hybrids (Garcia et al., 2008) or also have unique chemistry than the other black sagebrush plants due to environmental conditions or history of herbivory. When looking at the Raft River field site, the sagebrush population had extremely high heterogeneous diversity in its morphotypes and chemotypes (Fremgen, 2015; Figure C.1) – the mechanisms of which are still unknown. This diversity could be due to elevational gradients (Lay and Etcheverry, 2004), soil types and depth (Lay and Etcheverry, 2004), hybridizations (McArthur et al., 1988), or unknown history of fire (Morris, 2006) or herbivory (Fremgen, 2015), and, subsequently, attribute to the misclassifications by NIRS. For example, the sagebrush patches at Raft River used by sage-grouse consisted of more unique morphotypes than random patches (unpublished, Forbey personal communication). The Magic and Craters field sites offer similar evidence for identifying unique environmental or historical conditions. When looking at the accuracy of NIRS to classify sagebrush populations within a species (i.e., Wyoming big sagebrush), the population with the least accurate classifications was Craters (44%), where over half of this population was classified as a population from Raft River and to a lesser extent Magic. It is important to note that the user accuracy at Craters was high (98%), indicating an NIRS classification error rather than a field or lab classification error. Craters has very unique terrain and climate (Withey et al., 2014; Fremgen, 2015; Fremgen-Tarantino et al., 2020), as well as a regular and recent fire history (Withey et al., 2014; Fremgen, 2015; Fremgen-Tarantino et al., 2020). These traits may indicate that Craters has undergone unique post-fire succession (Fremgen, 2015; Fremgen-Tarantino et al., 2020) or even was restored with seed collected from foreign, non-local, populations. Ultimately, unexplained results or outliers detected by NIRS classifications could direct researchers to identify species hybrids or novel chemical profiles as well as distinct environmental or historical conditions.

When species (i.e., Wyoming big sagebrush) and populations (i.e., Magic field site) were held constant, NIRS could detect temporal changes between years and between seasons within a year. At the Magic field site, plants collected in 2012-2013 were accurately classified 89% of the time (i.e., producer accuracy) and plants within 2014-2015 were accurately classified 96% of the time (Table 1.7). When species (i.e., Wyoming big sagebrush) and individual plants (i.e., repeat sampling of the same plant from one season to the next) were held constant within the 2012-2013 collection, winter samples were accurately classified 100% of the time and spring samples were accurately classified 99% of the time (Table 1.8). These results suggest that individual sagebrush plants within the same species can be differentiated by season (i.e., phenology) using NIRS. The year and season in which sagebrush is collected or assessed is ecologically important. Annually, age and biotic and abiotic stressors direct the timing of leaf growth, senescence, or survival of plants (Wilt and Miller, 1992; Shiojiri and Karban, 2008; Gull et al., 2019). Seasonally, plant and leaf morphology changes through the emergence and senescence of inflorescence stalks for reproduction (Rosentreter, 2005) and ephemeral leaves for rapid growth and development during spring and summer (Rosentreter, 2005). As these temporal and phenological changes occur, so does the chemistry of the sagebrush (Wilt and Miller, 1992). As a result, NIRS can be used to measure and monitor these chemical changes over time, which can inform the phenological stage and condition of a sagebrush plant or population. If NIR spectra remains relatively constant temporally, a population is likely to be somewhat stable, whereas if NIR spectra is unstable, it could suggest a population is undergoing biotic or abiotic stress. For example, winter sagebrush has only persistent (i.e., chemically stable) leaves, whereas summer sagebrush has both persistent and ephemeral (i.e., chemically variable) leaves. However, individual plants may vary in their investment in ephemeral leaves due to heavy winter herbivory or atypical weather. Moreover, in restored landscapes, variation observed in NIRS within a season, presumed species, and site may suggest variation in the sources of seed used for the restoration, which could create a mismatch not only in investment in ephemeral leaves but also in timing of inflorescence stalks for reproduction. The ability for NIRS to detect variation in vegetative forms within sagebrush could help identify if there are mismatches for flowering times and predict the potential for hybridization among newly seeded and remnant sagebrush plants.

As individual plant traits change over time, spatial variation in these traits develops across populations, which can lead to differential habitat use by herbivores. Plant traits such as protein and toxins can influence the growth, development, metabolism, and energy budgets of herbivores (Sorensen et al., 2005a; Dearing et al., 2005; DeGabriel et al., 2009; Frye et al., 2013; Utz et al., 2016). It is not enough to simply assess if food is available to an herbivore population, the quality of the food must be investigated if the goal is to manage healthy herbivore populations. Furthermore, the quality of plants in a habitat is highly dynamic, making the assessment of forage quality for herbivores challenging. Standard ecological measurements that researchers have developed to describe landscapes that include distribution and abundance of classified species and phenology cannot fully capture the complex dietary traits important to herbivores within a system. Because of this, it can be beneficial to directly measure the traits of plants that influence foraging by herbivores to direct land management decisions for herbivores. Insomuch, we tested how NIRS could classify the browsing of sagebrush plants by a specialist mammalian herbivore, the pygmy rabbit (Figure 1.2).

NIRS was not able to accurately classify herbivory. At the 2012-2013 Magic field site, paired browsed and non-browsed Wyoming big sagebrush samples were collected across both winter and spring seasons. When including both seasons in the analysis, results indicate that NIRS cannot reliably discriminate which plants have been browsed by pygmy rabbits, with the majority of the samples categorized as non-browsed. Browsed samples (n = 212) were classified accurately 24% of the time and non-browsed samples (n = 274) were classified accurately 88% of the time (Table 1.9). When controlling for one season at a time, the accuracy declined, where every sample except for one was predicted as non-browsed for both seasons (Tables B.2-B.3), likely due to smaller sample sizes (i.e., winter (n = 91), spring (n = 394)). The misclassification of browse type could be due to the need for a spatially and temporally larger sample size. Misclassification could also be due to very subtle differences in chemistry between browsed and non-browsed plants, which do not differ in chemical profiles but do differ in concentrations of specific chemicals (Nobler et al., 2019). Herbivores make decisions based on fine-scale mixtures and concentrations, or doses, of the chemicals comprising the plant (Forbey et al., 2013b; Nobler et al., 2019; Patey et al., 2020). Therefore, identifying these mixtures and concentrations may provide a more effective, albeit indirect, way to predict browsing by herbivores than entire chemotypic profiles. We propose that NIRS can be used to predict browsing not through classification, but by predicting concentrations of chemicals in sagebrush plants and populations (see Chapter 2) that direct browsing above specific thresholds (Frye, 2012; Fremgen, 2015; Melody, 2017; Nobler et al., 2019; Figures D.1-D.4).

Overall, when using NIRS to classify phenotypes of sagebrush, results demonstrate that predictions are most reliable when chemistry is consistent within a group (i.e., site, taxa, year, season), but disparate between groups. Given that NIRS is directly measuring chemical bonds in the compounds constituting the sagebrush plants, discriminating plants that differ chemistry is not particularly surprising. However, as the chemistry of these phenotypes become more similar, from species and populations, which are more chemically distinct, to foraged state, which share profiles but have only subtle differences in chemical concentrations, the ability of NIRS to discriminate the phenotypes becomes less reliable.

The capacity to efficiently scale research is becoming increasingly important as our globe continues to change at broader and faster rates. Spectral sensors with increased electromagnetic ranges and resolutions are progressively more available, along with increased spatial and temporal scales from unmanned aerial systems and satellites. Although spectral, spatial, and temporal tradeoffs exist that must be considered when scaling, these different resolutions can lend further insight and capacity into discriminating phenotypes, especially when working in conjunction. For example, our NIRS classification results can be used to inform phenotype predictions made from hyperspectral imagery collected from unmanned aerial systems at our same field sites, which can in turn inform satellite imagery predictions. Integration of similar spectral traits across instruments can minimize the loss of resolution associated with scaling up spatially and temporally (Xiao et al., 2019) and help isolate and potentially remove inherent terrestrial (Dashti et al., 2019) and atmospheric interference (Thompson et al., 2018).

Sagebrush landscapes are rapidly changing and the only way to understand these changes and how they may influence herbivores is to improve the ways in which we monitor changes in species and functional traits over space and time. NIRS is proving to be a valuable tool when it comes to discriminating among taxonomical and phenological phenotypes. These phenotypes are functionally useful in many ways, including delineating species composition and distribution (Bálint et al., 2016), informing size-structured population models (Kirkpatrick, 1988), identifying phenological stages of populations and potential for phenological mismatch with other trophic levels (Richardson et al., 2018), predicting forage quality of plants for herbivores (Fremgen-

Tarantino et al., 2020), detecting past and present anthropogenic and environmental stressors (Withey et al., 2014), and restoring quality habitats for locally adapted herbivores (Oh et al., 2019). It is essential to monitor these functional phenotypes, and we show how NIRS can be a powerful tool to measure them. Misclassifications identified by NIRS, especially in large datasets, might help formulate specific hypotheses related to genetic mixing due to land management practices (Milton et al., 1999). For example, NIRS has the potential to identify post-disturbance sites where diverse non-native sources of seed may have been used for restoration. In this case, we hypothesize that NIR spectra would appear diverse and inconsistent compared to undisturbed remnant populations. We propose this might be the case between our fire-disturbed Craters field site and undisturbed Cedar Gulch field site. Additionally, even more complex (and less predicted) NIRS results could be used to test hypotheses about hybridization. We propose this to be the case at our Raft River field site where multiple phenotype classification analyses failed. Finally, although NIRS cannot reliably classify browsed states of sagebrush by herbivores, it suggests there are subtle yet important differences between browsed and non-browsed plants that herbivores use for diet selection that are likely independent of taxonomic classifications, which call for more in-depth investigations (see Chapter 2). NIRS, especially in combination with standard and emerging monitoring tools, can help to better monitor critical and threatened sagebrush systems through the measurement of functional phenotypes in the plants that comprise them.

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Tables

Table 1.1 Phenotypes associated with data collected at each field site in Idaho, USA.

Field Site	Elevation (m)	Years	Seasons	Sagebrush Species	Microhabitats	Browsed State of Plants	Herbivore Species
Magic Reservoir	1465 - 1480	2014-2015 (n=590)	Winter (n=246), Summer (n=344)	*A. t. wyomingensis* (n=321), Dwarf taxa (n=107)	On-mound (n=226), Off-mound (n=211), Dwarf-patch (n=153)	Browsed (n=30), Non-browsed (n=398)	*Brachylagus idahoensis*
Cedar Gulch	1890 - 1940	2014-2015 (n=625)	Winter (n=387), Summer (n=238)	*A. t. wyomingensis* (n=336), *A. nova* (n=25), Dwarf taxa (n=100)	On-mound (n=199), Off-mound (n=216), Dwarf-patch (n=210)	Browsed (n=43), Non-browsed (n=384)	*Brachylagus idahoensis*
Craters of the Moon	1300 - 1650	2013-2014 (n=94)	Winter (n=94)	*A. t. wyomingensis* (n=66), *A. tripartita* (n=26)	Used-patch (n=58), Random-patch (n=36)	Browsed (n=28), Non-browsed (n=66)	*Centrocercus urophasianus*
Raft River	1380 - 2140	2013-2014 (n=263)	Winter (n=263)	*A. t. wyomingensis* (n=65), *A. arbuscula* (n=197)	Used-patch (n=176), Random-patch (n=87)	Browsed (n=75), Non-browsed (n=188)	*Centrocercus urophasianus*

Table 1.2 Confusion matrix from support vector machine using near infrared spectroscopy to predict distinct species of sagebrush (*Artemisia* spp.) at Magic Reservoir, Idaho, USA.

		Actual					Overall Accuracy (%)	
	Class Name	Wyoming	Dwarf	Three-tip	Total	User Accuracy (%)	Training	Validation
Predicted	Wyoming	801	24	8	833	96.16	95.90	95.15
	Dwarf	6	88	0	94	93.62		
	Three-tip	0	0	0	0	0		
	Total	807	112	8	927			
	Producer Accuracy (%)	99.26	78.57	0				

Table 1.3 **Confusion matrix from support vector machine using near infrared spectroscopy to predict distinct species of sagebrush (*Artemisia* spp.) at Cedar Gulch, Idaho, USA.**

	Actual						Overall Accuracy (%)	
Predicted	Class Name	Wyoming	Dwarf	Black	Total	User Accuracy (%)	Training	Validation
	Wyoming	336	99	7	442	76.02	77.01	75.92
	Dwarf	0	1	0	1	100		
	Black	0	0	18	18	100		
	Total	336	100	25	461			
	Producer Accuracy (%)	100	1	72				

Table 1.4 Confusion matrix from support vector machine using near infrared spectroscopy to predict distinct species of sagebrush (*Artemisia* spp.) at Craters of the Moon, Idaho, USA.

	Actual					Overall Accuracy (%)	
	Class Name	Wyoming	Three-tip	Total	User Accuracy (%)	Training	Validation
Predicted	Wyoming	61	0	61	100	94.57	93.48
	Three-tip	5	26	31	83.87		
	Total	66	26	92			
	Producer Accuracy (%)	92.42	100				

Table 1.5 **Confusion matrix from support vector machine using near infrared spectroscopy to predict distinct species of sagebrush (*Artemisia* spp.) at Raft River, Idaho, USA.**

		Actual				Overall Accuracy (%)	
	Class Name	Wyoming	Low	Total	User Accuracy (%)	Training	Validation
Predicted	Wyoming	0	0	0	100	75.19	75.19
	Low	65	197	262	75.19		
	Total	65	197	262			
	Producer Accuracy (%)	0	100				

Table 1.6 **Confusion matrix from support vector machine using near infrared spectroscopy to predict distinct populations of Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) across four field sites (Figure 1.1) in Idaho, USA.**

	Actual							Overall Accuracy (%)	
	Class Name	Magic Reservoir	Cedar Gulch	Craters of the Moon	Raft River	Total	User Accuracy (%)	Training	Validation
Predicted	Magic Reservoir	1067	0	9	4	1080	98.8	96.12	95.99
	Cedar Gulch	0	625	0	0	625	100		
	Craters of the Moon	0	0	41	1	42	97.62		
	Raft River	22	0	44	258	324	79.63		
	Total	1089	625	94	263	2071			
	Producer Accuracy (%)	97.98	100	43.62	98.1				

Table 1.7 Confusion matrix from support vector machine using near infrared spectroscopy to predict distinct years of collection of Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) at Magic Reservoir, Idaho, USA.

	Actual					Overall Accuracy (%)	
	Class Name	Y2013	Y2015	Total	User Accuracy (%)	Training	Validation
Predicted	Y2013	434	13	447	97.09	91.95	91.95
	Y2015	52	308	360	85.56		
	Total	486	321	807			
	Producer Accuracy (%)	89.3	95.95				

Table 1.8 Confusion matrix from support vector machine using near infrared spectroscopy to predict distinct seasons (phenology) of collection of Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) at Magic Reservoir, Idaho, USA between 2012-2013.

	Actual					Overall Accuracy (%)	
Predicted	Class Name	Winter	Spring	Total	User Accuracy (%)	Training	Validation
	Winter	92	5	97	94.85	99.00	99.00
	Spring	0	389	389	100		
	Total	92	394	486			
	Producer Accuracy (%)	100	98.73				

Table 1.9 Confusion matrix from support vector machine using near infrared spectroscopy to predict foraging behavior (browsed versus non-browsed) by pygmy rabbits (*Brachylagus idahoensis*) on Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) at Magic Reservoir, Idaho, USA between 2012-2013.

	Actual					Overall Accuracy (%)	
	Class Name	Browsed	Non-browsed	Total	User Accuracy (%)	Training	Validation
Predicted	Browsed	50	32	82	60.98	60.08	57.20
	Non-browsed	162	242	404	59.9		
	Total	212	274	486			
	Producer Accuracy (%)	23.58	88.32				

Figures

Figure 1.1 Field sites where individual sagebrush (*Artemisia* spp.) samples were collected that are important to herbivore populations of conservation concern in Idaho, USA. Samples were collected between the years of 2012-2015.

Figure 1.2 Indication of pygmy rabbit (*Brachylagus idahoensis*) browsing on a sagebrush shrub. It can be identified by a clean 45-degree bite mark and can be differentiated from other lagomorph species by the diameter of the clipping (Crowell et al., 2018) and the lack of leafy material left below the shrub. Fresh bite marks were identified by a wetter green or bright brown stem interior, as opposed to a dry dull brown color. Photo provided by Nobler (2016).

CHAPTER TWO: NIRS PREDICTS CONCENTRATIONS OF PLANT CHEMICAL TRAITS IMPORTANT TO SPECIALIST HERBIVORES

Abstract

Interactions between herbivores and plants shape the distribution and density of plant populations, which has cascading effects across other trophic levels. The abundance and composition of the chemicals in plants influences habitat use, demographics, population dynamics, and evolution of herbivores. The combination of these chemical traits (i.e., chemotypes) act as visual, olfactory, and gustatory cues to herbivores. Moreover, chemical traits are dynamic and vary within, among, and across individuals, populations and species of plants. Both the type of chemical (i.e., nutrients and toxins) as well as the concentration affects herbivore foraging decisions. The dose of the chemical determines the pharmacological effect on the herbivore, further informing their foraging decisions. However, identifying and quantifying these chemicals is time intensive, expensive, and requires specialized expertise and instrumentation. Alternatively, near infrared spectroscopy (NIRS) operates by measuring organic bonds and can offer a rapid, inexpensive, and relatively low-tech option to detect and quantify chemical traits in plants. We used the sagebrush-steppe ecosystem as a case study to test the accuracy in which NIRS can predict concentrations of chemicals in plants. Sagebrush habitats are rapidly declining due to biotic and abiotic landscape changes. Moreover, sagebrush is known to have high levels of chemical heterogeneity and plasticity compared to many other plant species and systems. We found that NIRS can accurately predict both nutrient and toxin concentrations in sagebrush. NIRS predicted nitrogen content, a direct indicator of crude protein, with an 80-95% accuracy at all sagebrush field sites except our most phenotypically complex site. Predictions remained reliable when combining field sites to form a global predictive equation. NIRS predicted individual and classes of toxins in sagebrush with a range of accuracies. Monoterpenes were predicted the best, with total monoterpenes predicted 61-79% accurately at all field sites except our most phenotypically complex site. The highest predicted individual monoterpenes ranged in accuracy between 50-71%. Total phenolics were predicted moderately well at all field sites, with accuracies ranging between 42-58%. Total coumarins, a subclass of phenolics, were only predicted well at a single field site, with 77% accuracy. We then tried to bypass predicting chemical concentrations and used NIRS to directly predict foraging behavior by herbivores in the form of number of bites on plants. NIRS was not reliable in predicting browsing, with the best accuracy being 31%. Chemical traits explain how herbivores interact with and use individual plants and populations in their habitats. Finding better ways to monitor and measure these chemical traits is paramount. NIRS can be used to predict chemical concentrations in sagebrush, which can help inform land managers and researchers on ways to detect which plant populations may be the most palatable for herbivores over space and time.

Introduction

Predicting the interactions between plants and herbivores is important. What herbivores eat and where they forage shapes the distribution of plant populations

(Poelman and Kessler, 2016; Anderson et al., 2016), which in turn influences herbivore distribution and habitat use (Viswanathan et al., 2005; von Zeipel et al., 2006; Anderson et al., 2016). One prominent mechanism driving these interactions is the diversity, composition, and concentration of chemicals in plants. These chemical traits, observed as chemotypes, include both primary (e.g., nutrients) and secondary (e.g., defensive toxins) metabolites. Moreover, chemotypes vary within, among, and across plants and can change through interactions with neighboring plants (Casper and Jackson, 1997; McCall and Fordyce, 2010; Keddy and Cahill, 2012), microbial communities (Phelan et al., 2012; Rosentreter and Root, 2019; Barnard et al., 2019; Benedek et al., 2019), and herbivores (Shiojiri et al., 2009; Karban et al., 2016b, 2016a). Specifically, chemical traits explain how herbivores interact with and use individual plants and populations (Sorensen et al., 2005b; Moore and Foley, 2005; Dearing et al., 2005; DeGabriel et al., 2009; Frye et al., 2013; Ulappa et al., 2014; Bedoya-Pérez et al., 2014; Nobler et al., 2019). The abundance and composition of chemical traits in plants act as visual, olfactory, and gustatory cues, alongside physiological feedback loops, to direct habitat use by herbivores (Siitari et al., 2002; Honkavaara et al., 2002; Sorensen et al., 2005a, 2005c; Dearing et al., 2005; DeGabriel et al., 2009; Bedoya-Pérez et al., 2014; McArthur et al., 2019).

The pharmacological effects of chemical traits on herbivores is mediated by the dose of the compound (Sorensen et al., 2006; McLean et al., 2007; Forbey and Foley, 2009; Forbey et al., 2013; Patey et al., 2020). Just as humans differentially react to varying doses of any substance they consume, so do herbivores. Chemicals can be both deleterious or therapeutic depending on the dose consumed and the physiology of the herbivore (Sorensen et al., 2006; Patey et al., 2020). Consequentially, it is these dose-dependent responses (Frye et al., 2013; Fremgen, 2015; Melody, 2017; Nobler et al., 2019) that produce selection thresholds by herbivores. For example, herbivores generally select to forage on plants with higher nutritional and lower toxin content more than available, both within and among taxa (Frye et al., 2013; Ulappa et al., 2014; Fremgen-Tarantino et al., 2020). However, the potential noxious effects of phytochemicals through energetically-costly detoxification, central nervous system depression, and inhibition of nutrient uptake (Sorensen et al., 2005c; Kohl et al., 2015, 2016; Wing and Messmer, 2016), are often not detected until certain concentration thresholds have been reached (Figures D.1-D.4). To better predict and manage plant-herbivore interactions across landscapes, we need more efficient ways to detect chemical traits and their concentrations in plants.

Pioneering technologies are providing quicker and easier ways of measuring nutritional and toxic chemical traits in plants. Specifically, spectra, in the form of near infrared spectroscopy (NIRS), can detect chemical traits in plants, potentially offering an herbivore-eye view of plant traits (Moore et al., 2010). NIRS operates by measuring the reflectance of electromagnetic energy in objects from wavelengths ranging between 350-2500 nm. This electromagnetic range is particularly well-known for reflecting organic bonds (Curran, 1989; Youngentob et al., 2012), establishing NIRS as a great proxy for detecting and quantifying organic chemicals such as nutrients and toxins. Research in the agricultural industry has shown that NIRS can be used to quantify nitrogen (i.e., crude protein; Robbins, 1993) and phytochemical content in domestic feed (Abrams et al., 1987; Shenk and Westerhaus, 1994; Corson et al., 1999; Mnisi and Mlambo, 2017; Saha et al., 2018), fruits (Baranska et al., 2004; Sinelli et al., 2008), and wines (Urbano-

Cuadrado et al., 2004; Cozzolino et al., 2008). NIRS has also begun to be used in wild systems for conservation efforts, including jungles (Foley et al., 1998; Asner and Martin, 2009; Wiedower et al., 2009; Asner et al., 2011, 2012; Youngentob et al., 2012; Féret and Asner, 2014), grasslands (Griggs et al., 1999; Brunet et al., 2007), deserts (Russell et al., 2012; Vaknin and Mishal, 2017), and taiga (Stolter et al., 2006). However, only a small amount of research has shown that NIRS can predict fine-scale chemical changes that mediate interactions with herbivores in complex wild systems (Moore et al., 2010; Mitchell et al., 2012b; Olsoy et al., 2016).

The semiarid sagebrush-steppe of the Western United States serves as the perfect system to demonstrate how NIRS can detect and quantify chemical traits that influence foraging by generalist and specialist herbivore species. This system encompasses complex and changing chemotypic diversity (Kelsey et al., 1982; Rosentreter and Kelsey, 1991; Winward, 2004; Rosentreter, 2005; Welch, 2005), shown to be genetically determined (Jaeger et al., 2016). Additionally, there are specific and unique longstanding ecological and evolutionary (Oh et al., 2019) relationships between sagebrush and associated specialist herbivores. This includes the aroga moth (Winward et al., 1985), certain populations of mule deer (Wambolt, 1996), pronghorn (Hansen et al., 2001; Jacques et al., 2006), sage-grouse (Ulappa, 2011; Frye et al., 2013; Oh et al., 2019; Fremgen-Tarantino et al., 2020), pygmy rabbits (Ulappa et al., 2014; Nobler, 2016; Utz et al., 2016), and more. Subsequently, the threatened vertebrate herbivores, greater sage-grouse (*Centrocercus urophasianus*) and pygmy rabbits (*Brachylagus idahoensis*), rely nearly entirely on sagebrush for food in the winter (Wallestad and Eng, 1975; Green and Flinders, 1980). However, habitat use and diet selection of these specialist herbivores is dependent on concentrations of both protein and toxins (Ulappa, 2011; Frye, 2012; Utz, 2012; Fremgen, 2015; Nobler, 2016). As a threatened ecosystem from anthropogenic and climatic stressors, timely monitoring of the chemical traits that mediate habitat use by threatened herbivores is necessary for successful management of conservation and restoration of the sagebrush steppe. However, the challenges of measuring chemical traits in sagebrush remains daunting, expensive, and time intensive. Ascertaining a more efficient means of quantifying functional chemical traits is of significant importance if the goal is to better assess, monitor, and predict how sagebrush and obligate sagebrush herbivores will respond to changing socioecological systems.

We propose using NIRS to predict functional chemical traits in sagebrush plants. We investigated the accuracy of NIRS to predict concentrations of individual chemicals and classes of chemicals, in the forms of nitrogen (i.e., crude protein) and phytochemicals (i.e., defensive toxins), in sagebrush plants within and among populations across the sagebrush-steppe ecosystem (Table 2.1). In addition, because NIRS can simultaneously detect chemical mixtures and their concentrations, we investigated if NIRS could predict intensity of browsing by herbivores on sagebrush plants. We used NIRS data from sagebrush to ask the following questions:

i. Can NIRS predict concentrations of nitrogen within and among sagebrush species and populations?

ii. Can NIRS predict concentrations of total classes and individual phytochemicals in sagebrush plants?

iii. Can NIRS predict intensity of browsing, in the form of the number of bite marks, by specialist herbivores foraging on sagebrush plants?

Methods

Sample Collection

Sagebrush plants were selected for sampling by finding patches across four field sites in Idaho, USA (Figure 2.1) where herbivores had access to varying diversity of sagebrush species (Table 2.1) but were selective about the specific plants consumed. Pygmy rabbits (*Brachylagus idahoensis*) were the herbivores of interest at the Magic Reservoir, hereby known as Magic, and Cedar Gulch field sites, where they generally selected for higher nutrient and lower toxin content (Ulappa et al., 2014; Nobler, 2016). Greater sage-grouse (*Centrocercus urophasianus*, hereafter sage-grouse) were the herbivores of interest at the Craters of the Moon, hereby known as Craters, and Raft River field sites, where they generally selected for higher nutrient and lower toxin content (Fremgen, 2015; Fremgen-Tarantino et al., 2020). Browsed and non-browsed plants were identified within used foraging patches at each site. At the Magic and Cedar Gulch sites, browsed samples were defined by the presence of pygmy rabbit bite marks (Figure 2.2; see paragraph below) and often seen in conjunction with fresh fecal pellets below and around the plant. Non-browsed samples were identified as the nearest plant to the browsed plant with fresh feces or tracks that were not browsed. Once browsed and non-browsed plants were detected, sprigs from plants were clipped using pruning shears and placed in labeled plastic bags on ice. All plant samples were clipped for about 2 g of wet leaf and stem biomass or no more than 25% of the overall biomass of the plant. At the Craters and Raft River sites, browsed samples were defined by the presence of sage-grouse bite marks (Figure 2.3; see paragraph below) and often seen in conjunction with fresh fecal pellets and tracks below and around the plant. Non-browsed samples were identified as plants nearest to (and within 0.5 m of) the browsed plants with fresh feces or tracks that were not browsed. Once browsed and non-browsed plants were detected, leaves and stems were collected from three browsed and three non-browsed plants within the foraging patch and pooled to form one browsed composite sample and one non-browsed composite sample. Each composite sample contained equal amounts of the three individual plant collections. All samples were stored on ice in the field until return to Boise State University where they were stored at -20°C.

Intensity of Herbivore Browsing

Sagebrush samples that were deemed browsed according to our sample collection protocol were also assessed for intensity of browsing prior to biomass collection. Intensity of browsing was quantified as the number of bite marks on the browsed plant. Bite marks were identified according to methods stated in Chapter 1. Briefly, pygmy rabbit bite marks had a clean 45-degree cut and differed from other lagomorph species by lack of leafy material left below the shrub and the diameter of the clipping (Figure 2.2). Sage-grouse bite marks were identified by a clean bite mark on the leafy material, leaving the sprig stems intact (Figure 2.3). Fresh bites were identified by a wet green or bright brown stem interior for pygmy rabbits and wet green leftover leaf material for sage-grouse.

Sample Preparation

Sagebrush samples were removed from a -20°C freezer and individually ground to a coarse powder (~2 mm) in liquid nitrogen using a ceramic mortar and pestle to minimize loss of leaf volatiles. Samples were ground differently per herbivore species. Whole sprigs (stems and leaves) of samples were ground from the pygmy rabbit sites because the rabbits consume both leaves and stems (Figure 2.2; Nobler, 2016; Crowell et al., 2018). Only leaves of samples were ground from the sage-grouse sites because sage-grouse consume only leaves (Figure 2.3; Fremgen, 2015). For the sage-grouse samples, leaves were separated from the stems using dry ice to freeze the sprigs and then gently tapped until all of the leaves had fallen off. A subset of 100 mg wet weight (ww) of each ground sample was weighed into a 20 mL clear glass headspace vial for gas chromatography analysis to separate monoterpenes based on size and volatility and to determine concentrations of total and individual monoterpenes. A subset of 50 mg ww of each ground sample was weighed into a 1.5 mL microcentrifuge tube for extraction to determine phenolic and coumarin content. All samples were kept on ice throughout the entirety of processing.

Chemical Analysis

Nitrogen

Nitrogen content, measured in the form of detectable nitrogen, was obtained by outsourcing the assay to Dairy One Forage Laboratory (Ithaca, NY, USA) for analysis. At least 1.0 g ww of each ground sample was placed in a labeled paper coin envelope and dried at 60°C for 48 hours. The dry sagebrush samples were shipped to Dairy One Forage Laboratory where total nitrogen values were acquired through the nitrogen combustion method. Final values were reported as total nitrogen (%) dry weight (dw) content.

Monoterpenes

The separation and concentration of individual and total monoterpenes was determined through the use of headspace gas chromatography (Agilent 7694 Headspace Sampler, Agilent, Santa Clara, CA, USA; Agilent 6890N Series Gas Chromatograph, Agilent, Santa Clara, CA, USA). A J&W DB-5 capillary column (30 m x 250 μm x 0.25 μm) was used in which 1.0 mL of headspace gas was injected into for compound separation and analyzed through flame ionization detection. The operating conditions for the headspace autosampler included oven temperature of 100°C, loop temperature of 110°C, transfer line temperature of 120°C, vial equilibrium time of 20 min, pressurization time of 0.20 min, loop fill time of 0.50 min, loop equilibrium time of 0.20 min, and injection time of 0.50 min. The operating conditions for the gas chromatograph included splitless injector temperature of 250°C, flame ionization detector temperature of 300°C, and oven temperature of 40°C for 2 min, then increased 3°C/min to 60°C, 5°C/min to 120°C, 20°C/min to 300°C, and held at 300°C for 7 min. The makeup gas was nitrogen and the carrier gas was helium. The inlet pressure was 80 KPa with a flow rate of 1.0 mL/min. We quantified all individual monoterpenes that were detected prior to the retention time of 24 min. After 24 min no more monoterpene compounds were reliably detected. Using external standards, we were able to identify the compounds 1,8-cineole (CAS # 470-82-6), 3-carene (CAS # 13466-78-9), α-phellandrene (CAS # 99-83-2), α-pinene (CAS # 1686-14-2), β-pinene (CAS # 18172-67-3), borneol (CAS # 464-45-9), camphene (CAS # 79-92-5), camphor (CAS # 76-22-2), p-cymene (CAS # 99-87-6), and terpinolene (CAS # 586-62-9). We also quantified total monoterpene content and the total number of compounds (i.e., peaks) detected per sample. Quantifications were calculated as areas under the curve (AUCs) for each separated chemical, identified by retention time of the peak. For each sample, peaks less than 1% of the total AUC were thrown out. All samples were standardized for their dry weight for final units of AUC/mg dw.

Phenolics

Ground sagebrush material was extracted in HPLC-grade methanol (CAS # 67-56-1) at a tissue-to-solvent ratio of 50 mg/1.0 mL, vortexed for 10 sec, then placed in a sonicating water bath for two separate 3 min sessions. Samples were then centrifuged at 13,000 g for 5 min at room temperature (20ºC) and the resulting supernatant filtered through glass wool to remove particulates. Total phenolic content was then assayed using a modified version of the Folin-Ciocalteu reagent method (Ainsworth and Gillespie, 2007). Gallic acid (CAS # 5995-86-8) diluted in HPLC-grade methanol was used as a standard, ranging from 0 to 5 mM. Each sagebrush sample was diluted at a ratio of 1:2.5 or 1:5 to fit within the standard curve. Each diluted standard and sample (20 µL) was pipetted in triplicate into clear flat-bottomed 96-well plates. Next, 100 µL of 10% Folin-Ciocalteu reagent (MP Biomedicals Inc. # 0219518690) was added to each well, followed by 80 µL of 700 mM (7.5%) sodium carbonate (CAS # 497-19-8), and the reaction was mixed gently. Plates were incubated for two hours at room temperature as color change occurred. Color intensity was then measured on a spectrophotometric BioTek Synergy MX multi-mode plate reader (BioTek, Winooski, VT, USA) set to read absorbance at wavelength 765 nm. The gallic acid standard curve was used to quantify phenolic content for each sagebrush sample in milligrams. All samples were standardized for their dry weight for final units of mg gallic acid equivalents/g dw.

Coumarins

Total coumarin content was measured from each filtered methanol extract (described above) using a colorimetric assay. Scopoletin (CAS # 92-6-15) diluted in HPLC-grade methanol was used as a standard, ranging from 0 to 80 µM. Each sagebrush sample and standard (50 μL) was pipetted in triplicate into a clear flat-bottomed 96-well plate where it was run on a spectrophotometric BioTek Synergy MX multi-mode plate reader (BioTek, Winooski, VT, USA) set to measure fluorescence at an excitation wavelength of 350 nm and emission wavelength of 469 nm (Figure F.7). A standard curve of scopoletin was used to quantify coumarin content for each sample in micromoles. All samples were standardized for their dry weight for final units of µmol scopoletin equivalents/g dw.

NIRS Analysis

The same methods from Chapter 1 were followed. Briefly, an ASD FieldSpec® 4 spectroradiometer was used to measure continuous near infrared wavelength reflectance from 350 to 2500 nm in all of the sagebrush samples. Ground dried samples placed in clear plastic bags were scanned on a black countertop. Standard calibration and optimization methods were applied according to the ASD user manual. Thirty replicate reflectance scans were collected for each sample (Figure A.1) and exported for outlier analysis and spectral averaging. Spectra were converted to absorbance values using a $\log_{10}(1/R)$ transformation, where R is reflectance (Figure A.2) and then derived (1st gap derivative) and truncated to 450-2350 nm (Figure A.3).

Statistical Analysis

All categorical (i.e., field site) and numerical (i.e., chemical concentrations, NIR absorbances) variables were joined for each sagebrush sample and imported into Camo Analytics Unscrambler® chemometric software. Distributions of response variables were checked for normalcy. Unscrambler® was then used to perform partial least squares regressions (PLSR) between NIR spectral values (i.e., predictor variables) and chemistry and herbivory values (i.e., response variables) to produce NIRS-predicted chemistry and herbivory values. Each model was independently calibrated and validated using leave-one-out cross validation (LOOCV) of 20 segments and nonrelevant spectral wavelengths were downweighted (i.e., given lesser weight relative to other wavelengths) to prevent overfitting of the models.

Results

Predicting Nitrogen Concentrations Using NIRS

The results of the partial least squares regression (PLSR) show that NIRS can predict nitrogen concentration in sagebrush plants at all field sites except one (Table 2.2). Magic (n = 585) predicted nitrogen with a coefficient of determination (R^2) of calibration of 0.86 (standard error of calibration (*SEC*) = 0.09; Figure 2.4) and leave-one-out cross validation (LOOCV) R^2 of 0.83 (standard error of cross-validation (*SECV*) = 0.10). Cedar Gulch (n = 619) predicted nitrogen with a calibration R^2 of 0.80 (*SEC* = 0.14; Figure 2.5) and LOOCV R^2 of 0.77 (*SECV* = 0.15). Craters (n = 93) predicted nitrogen with a calibration R^2 of 0.95 (*SEC* = 0.07; Figure 2.6) and LOOCV R^2 of 0.92 (*SECV* = 0.09). Raft River (n = 190) did not predict nitrogen well, with a calibration R^2 of 0.03 (*SEC* = 0.24; Figure 2.7) and was not able to calculate a LOOCV R^2 due to poor calibration results (*SECV* = 0.25). The global model that combined Magic, Cedar Gulch, Craters, and Raft River (n = 1983) sites predicted nitrogen with a calibration R^2 of 0.78 (*SEC* = 0.17) and LOOCV R^2 of 0.77 (*SECV* = 0.18; Figure 2.8). When Raft River was removed from the multi-site analysis, the global model predicted nitrogen with a calibration R^2 of 0.84 (*SEC* = 0.15; Figure 2.9) and LOOCV R^2 of 0.83 (*SECV* = 0.15).

Predicting Concentrations of Total Classes and Individual Phytochemicals With NIRS

The results of the PLSR show that NIRS can be used to predict total monoterpene concentrations in sagebrush plants across different populations (Table 2.3). The best prediction of total monoterpenes was at Magic (n = 569) with a calibration R^2 of 0.79 (*SEC* = 147.75; Figure 2.10) and LOOCV R^2 of 0.77 (*SECV* = 153.97). Cedar Gulch (n = 618) predicted monoterpenes with a calibration R^2 of 0.61 (*SEC* = 102.05; Figure 2.11) and LOOCV R^2 of 0.56 (*SECV* = 108.57). Craters (n = 94) predicted monoterpenes with a calibration R^2 of 0.70 (*SEC* = 126.10; Figure 2.12) and LOOCV R^2 of 0.55 (*SECV* = 156.38). Raft River (n = 262) did not predict monoterpenes well, with a calibration R^2 of 0.02 (*SEC* = 175.41) and was not able to calculate a LOOCV R^2 due to poor calibration results (*SECV* = 183.44).

The results of the PLSR show that NIRS can also be used to predict certain individual monoterpene concentrations in sagebrush plants (Table 2.4). At Magic (n = 586) camphor was best predicted with a calibration R^2 of 0.71 (*SEC* = 99.78; Figure 2.13) and LOOCV R^2 of 0.70 (*SECV* = 102.66). Other individual monoterpenes were predicted moderately well at Magic: camphene with a calibration R^2 of 0.66 (*SEC* = 37.36) and LOOCV R^2 of 0.65 (*SECV* = 38.28), 1,8-cineole with a calibration R^2 of 0.57 (*SEC* = 50.55) and LOOCV R^2 of 0.55 (*SECV* = 51.52), and α-Pinene with a calibration R^2 of 0.52 (*SEC* = 9.48) and LOOCV R^2 of 0.50 (*SECV* = 9.68). Cedar Gulch (n = 621) predicted “unknown 20.1 minutes” the best with a calibration R^2 of 0.54 (*SEC* = 14.40; Figure 2.14) and LOOCV R^2 of 0.52 (*SECV* = 14.69). β-Pinene was also predicted moderately well at Cedar Gulch with a calibration R^2 of 0.51 (*SEC* = 33.56) and LOOCV R^2 of 0.49 (*SECV* = 34.34). Craters (n = 94) predicted α-Pinene the best with a calibration

R^2 of 0.70 (*SEC* = 16.53; Figure 2.15) and LOOCV R^2 of 0.66 (*SECV* = 17.77). Camphene was also predicted well at Craters with a calibration R^2 of 0.69 (*SEC* = 43.63) and LOOCV R^2 of 0.64 (*SECV* = 47.13). Raft River (n = 262) did not predict any individual monoterpene well, with the best being camphene (calibration R^2 of 0.03 (*SEC* = 34.64; Figure 2.16) and no LOOCV R^2 calculated (*SECV* = 36.19)).

The results of the PLSR show that NIRS was able to predict total phenolics concentrations in sagebrush plants moderately well at some sites, but not all (Table 2.5). Magic (n = 523) predicted phenolics the best with a calibration R^2 of 0.58 (*SEC* = 3.23; Figure 2.17) and LOOCV R^2 of 0.52 (*SECV* = 3.47), similar to predictions at Raft River (n = 250; calibration R^2 of 0.58 (*SEC* = 19.53; Figure 2.18) and LOOCV R^2 of 0.53 (*SECV* = 20.76)). This was followed by Craters (n = 87; calibration R^2 of 0.50 (*SEC* = 9.63) and LOOCV R^2 of 0.42 (*SECV* = 10.41)) and Cedar Gulch (n = 601; calibration R^2 of 0.42 (*SEC* = 3.05) and LOOCV R^2 of 0.40 (*SECV* = 3.10)).

The results of the PLSR show that NIRS was able to predict total coumarin concentrations in sagebrush plants moderately well at some sites, but not all (Table 2.6). Magic (n = 546) predicted coumarins with a calibration R^2 of 0.77 (*SEC* = 0.37; Figure 2.19) and LOOCV R^2 of 0.75 (*SECV* = 0.38), but the predictive capacity for coumarins was much lower at the other sites. Coumarins were poorly predicted at Cedar Gulch (n = 576; calibration R^2 of 0.34 (*SEC* = 0.19) and LOOCV R^2 of 0.30 (*SECV* = 0.19)), Craters (n = 94; calibration R^2 of 0.04 (*SEC* = 5.05) and LOOCV R^2 of 0.01 (*SECV* = 5.17)), and Raft River (n = 255; calibration R^2 of 0.01 (*SEC* = 0.33) and no LOOCV R^2 determined due to poor calibration results (*SECV* = 0.33)).

Predicting Intensity of Browsing by Herbivores With NIRS

The results of the PLSR show that NIRS cannot reliably predict intensity of browsing as determined by the number of bite marks by herbivores on sagebrush plants (Table 2.7). The best predictor of browsing intensity was at Magic (n = 30) with a calibration R^2 of 0.31 (SEC = 20.62; Figure 2.20) and LOOCV R^2 of 0.06 ($SECV$ = 25.33). Browsing was not well predicted with NIRS at Cedar Gulch (n = 43; calibration R^2 of 0.09 (SEC = 34.14) and LOOCV R^2 of 0.04 ($SECV$ = 35.78)), Craters (n = 28; calibration R^2 of 0.05 (SEC = 21.57) and no LOOCV R^2 results ($SECV$ = 23.58)), and Raft River (n = 81; calibration R^2 of 0.04 (SEC = 18.06) and LOOCV R^2 of 0.02 ($SECV$ = 18.54)). Combining all field sites (n = 390) did not improve predictions of bite marks (calibration R^2 of 0.09 (SEC = 25.47) and LOOCV R^2 of 0.07 ($SECV$ = 25.82)).

Discussion

Our research shows that NIRS can be used to predict concentrations of some, but not all chemicals, and did not predict extent of herbivory in sagebrush populations. In general, nitrogen was best predicted for sagebrush regardless of site, followed by relatively strong predictions for monoterpenes, moderate predictions for phenolics, relatively low predictions for coumarins and poor predictions for intensity of browsing at all sites. The Magic field site generally had better predictions of all chemical traits than the other sites, whereas Raft River had the worst predictions. This research is one of the first evaluations of NIRS to predict multiple chemical traits, including primary and total and individual secondary metabolites, and herbivore responses across a natural and complex biogeographic gradient. Other work in this field has focused on single chemicals, such as nitrogen in agricultural (Saha et al., 2018) and wild systems (Wiedower et al., 2009) or limited numbers of individual phytochemicals in agricultural (Cozzolino et al., 2008) and wild systems (McIlwee et al., 2001) or a single class of chemicals (Au et al., 2020). Furthermore, all but one of these previous studies (Au et al., 2020) were conducted across smaller biogeographical ranges. Only one (Moore et al., 2010) involved using NIRS to predict herbivore responses, but in this study only one herbivore, compared to two in our study, was observed within a fenced nature reserve, compared to a complex socioecological system in our study. Our results support recent work in another natural system (Au et al., 2020) showing that global NIRS predictive models may be complicated by chemical variation that exists across extensive biogeographical and temporal ranges and gradients. As in other studies (Foley et al., 1998; Stolter et al., 2006; Saha et al., 2018), plant chemicals, such as nitrogen, that have consistent organic bonds performed relatively well within individual sites and in global models regardless of taxonomic and chemical diversity. This was not the case for phytochemicals, which vary not only in concentration but also in composition within a chemical class. We offer potential sources of variation that could explain predictive accuracy of NIRS, including heterogeneity of plant population taxonomy and geography (Table 2.1), range of concentrations of plant chemicals (Figures H.1-H.10), timing of plant leaf emergence and senescence, environmental disturbances, and the way in which samples were collected. We also describe how results could be used to understand plant-herbivore interactions in a changing and threatened ecosystem.

Overall, the Magic site predicted phytochemicals by NIRS better than other sites. Our previous work indicates that Magic has fairly homogeneous taxonomic and chemical diversity (Nobler, 2016; Olsoy et al., 2020), with most of its samples shown chemically and spectrally to be Wyoming big sagebrush (*A. t. wyomingensis*) (see Chapter 1; Table 1.2). Moreover, the lower and smaller range of elevation (Table 2.1), consistent weather patterns (Minshall, 1977; Western Regional Climate Center, 2005), and lack of known recent biotic or abiotic stressors (Minshall, 1977; Buhidar, 2001) suggest a relatively stable environment. The relatively low chemical and environmental variation at Magic likely explained our relatively high NIRS predictions of phytochemicals at this site. In contrast, Cedar Gulch has more heterogeneous diversity across the site than Magic with the presence of another species, black sagebrush (*A. nova*), increased chemical complexity (Olsoy et al., 2020), and a rougher terrain (Western Regional Climate Center, 2005; Olsoy et al., 2020), alongside being at the highest elevation of all of the sites (Table 2.1). The relatively more dynamic environment at Cedar Gulch may explain the lower accuracy of NIRS to predict phytochemicals at this site. However, the extent of dynamic environments is not the only factor influencing NIRS accuracy to predict phytochemicals. Craters had two species with distinct chemotypes (Wyoming big and three-tip (*A. tripartita*) sagebrush) and a regular and recent fire history (Fremgen, 2015; Fremgen-Tarantino et al., 2020). Despite this complexity, predictions at the Craters site yielded the best results for nitrogen (Table 2.2; Figure 2.6) and had relatively high predictions for individual monoterpenes compared to other sites (Tables 2.3-2.4; Figure 2.12; Figure 2.15), even with a small sample size (n = 94). It is also important to note that all of our analyses included all species collected at each field site. Some of the field sites were dominated by a single species, such as Magic and Cedar Gulch (i.e., Wyoming big sagebrush) and Raft River (i.e., low sagebrush). Future analyses should test how controlling for taxa or other classifications might improve predictions (Au et al., 2020). This would reduce sample size but may still provide a cleaner range of variability and stronger predictions.

In addition to environmental conditions, the fashion in which samples were collected may influence NIRS predictions. At Craters, samples were only collected during one season (i.e., winter) instead of being collected across multiple seasons like at Magic and Cedar Gulch. However, a *post hoc* analysis of Magic samples from a single season (i.e., spring only (n = 402)) did not improve NIRS predictions of nitrogen (calibration R^2 of 0.78 and validation R^2 of 0.74) compared to inclusion of all years and seasons (Table 2.2; Figure 2.4). Instead, the fashion in which samples were collected could have influenced NIRS predictions. At Craters, the study design included an herbivore directed collection method (i.e., used versus random sagebrush patches) versus a strategized random design used at Magic and Cedar Gulch. However, Raft River samples also used an herbivore-directed collection method yet failed to accurately predict any chemical traits. We attribute the poor predictive power of NIRS at the Raft River site to highly heterogeneous diversity in regard to taxonomy and chemistry (i.e., high probability of hybridization). Our Raft River results indicated that taxonomic and chemical complexity constrained predictions of chemical traits (Tables 2.2-2.6). Furthermore, the climate (Western Regional Climate Center, 2005), broad elevation gradient (Table 2.1; Lay and Etcheverry, 2004), and geographic size (Lay and Etcheverry, 2004) of Raft River could also influence phytochemical diversity and, thus, reliability of NIRS predictions.

Overall, the capacity for NIRS to predict nitrogen content within and among sagebrush populations and taxonomic groups proved extremely robust. Nitrogen is a direct indicator of crude protein (Robbins, 1993), which is essential to the growth, reproduction, and survival of herbivores (Mattson, 1980). In sagebrush systems, during spring and summer, nitrogen is available in the forms of grasses, forbs, and shrubs that grow across the arid landscape, which are main sources of food for most generalist and specialist sagebrush herbivores (Welch, 2005). During the winter, sagebrush is one of the only reliable sources of nitrogen for sagebrush herbivores, especially specialists (Wallestad and Eng, 1975; Green and Flinders, 1980). As such, increased ability to predict concentrations of nitrogen from NIRS could more rapidly and efficiently locate suitable habitats and determine palatability of forage for both domestic (i.e., browsers and grazers) and wild herbivores. When we combined all of the field sites to determine a global NIRS model to predict nitrogen across geographically disparate populations (Figure 2.1) results were promising (R^2 = 0.78; Table 2.2; Figure 2.8). When removing our poorly predicted field site, Raft River, results improved (R^2 = 0.84; Table 2.2; Figure 2.9). Results were best at sites with lower taxonomic diversity, but more importantly with lower chemical complexity. While a quantitative assessment of this diversity is challenging, the support for this is observed in comparing the means and ranges of chemical diversity among sites (Table G.1; Figures H.1-H.10), chemical (i.e., monoterpene) profiles of taxa among sites (Table 2.1; Figure C.1), and generally simpler chemical makeup of taxa in some sites (Table 2.1; Turi et al., 2014).

Our ability to predict nitrogen in sagebrush compares relatively well to similar studies done in other ecological systems. For example, NIRS predicts nitrogen in eucalyptus differentially foraged on by greater gliders and possums (R^2 = 0.96; McIlwee et al., 2001), bamboo by pandas (R^2 = 0.93-0.97; Wiedower et al., 2009), willows by moose (R^2 = 0.97-0.99; Stolter et al., 2006), seagrass by dugongs (R^2 = 0.99; Lawler et al., 2006), and herbs by gorillas (R^2 = 0.95; Rothman et al., 2009). Finally, our results compare to other NIRS instruments used in specific fields such as agriculture or food science, where nitrogen is assessed for forage quality of domestic livestock (Abrams et al., 1987; Shenk and Westerhaus, 1994; Corson et al., 1999; Mnisi and Mlambo, 2017; Saha et al., 2018). In these fields, NIRS analysis is often outsourced to labs, such as Dairy One Forage Laboratory (Ithaca, NY, USA), where analyses are conducted under highly controlled laboratory conditions, which allows for strong predictive models (e.g., $R^2 > 0.97$). This establishes them as golden standards in the field for NIRS chemical analysis. With this awareness, we sent a subset of our Magic sagebrush samples (n = 489) to Dairy One Forage Laboratory to measure nitrogen content on their advanced FOSS™ instrumentation and developed a calibration equation using their in-house WinISI™ software. Dairy One analysis determined a calibration R^2 of 0.93 (*SEC* = 0.07; Figure E.1) compared to our ASD FieldSpec® 4 NIRS results of calibration R^2 = 0.86 (*SEC* = 0.09). These results demonstrate that our in-house NIRS is a comparable predictor of nitrogen in wild plants and populations.

Just as crucial as nitrogen is to herbivore foraging decisions and health, so are phytochemicals (i.e., toxins). Phytochemicals directly affect the physiological condition and even survival of herbivores through energetically-costly detoxification, CNS depression, and inhibition of nutrient uptake (Sorensen et al., 2005c; Kohl et al., 2015, 2016; Wing and Messmer, 2016). However, it is the dose of the phytochemical that mediates the pharmacological response of the herbivore (Figures D.1-D.4). Unfortunately, measuring these compounds in the lab are difficult, time-intensive, and require specific expertise. Finding more efficient ways to quantify these doses, or concentrations, of phytochemicals in sagebrush would provide an invaluable tool to land managers who want to prioritize conservation and management of quality forage for threatened herbivores. Our research shows that NIRS can be used to predict certain concentrations of classes of phytochemicals and individual phytochemicals in sagebrush populations.

We first assessed total monoterpene concentrations. This is a class of phytochemicals abundantly and diversely present in sagebrush (Kelsey et al., 1982; Turi et al., 2014) and known for acting as CNS depressants (Sorensen et al., 2005c; McLean et al., 2007). Therefore, it is important to know the overall (and additive) concentrations of these compounds. Magic predicted total monoterpenes the best (R^2 = 0.79; Table 2.3; Figure 2.10), likely due to its homogeneous taxonomic and chemical diversity and large sample size. Predictions generally declined with increasing degrees of heterogeneity at each field site: Craters (R^2 = 0.70; Table 2.3; Figure 2.12), Cedar Gulch (R^2 = 0.61; Table 2.3; Figure 2.11), and Raft River (R^2 = 0.02; Table 2.3). Raft River was unable to predict total monoterpene content, similar to nitrogen. This continues to confirm the phenotypic complexity present at Raft River. Other literature using NIRS to predict total monoterpene concentrations in wild systems have shown similar results, however most of these systems have significantly less diversity and abundance of monoterpenes overall. For example, in sagebrush, numbers of individual monoterpenes range from 10 to 120 (Figure H.5) depending on the species, with as many as 8 compounds dominating the overall monoterpene concentration (Figure C.1). In many populations, 3-5 compounds generally dominate the overall concentration, with one compound in particular, camphor (Figure C.1 around minute 21.0; Figure H.6), often predominating. This is in contrast to other studies that have predicted total and subclasses of monoterpenes using NIRS, such as in common sage (*Salvia officinalis*, R^2 = 0.49-0.86; Elementi et al., 2006), which has about 10 monoterpene compounds and is dominated by 2-3 individuals (Elementi et al., 2006), and several citrus fruits (R^2 = 0.92-0.99; Schulz et al., 2002), which have about 10 monoterpene compounds and are all highly dominated (50-99%) by 1 individual chemical (limonene; Schulz et al., 2002; Chidambara Murthy et al., 2012). However, some other studies have accomplished predicting monoterpenes in wild complex plants, including wild lavender (*Lavandula angustifolia*, R^2 = 0.87-0.92; Smigielski et al., 2018), which is known to have more than 40 compounds and can be dominated by as many as 8 individual compounds (Boeckelmann, 2008). This may, though, be due to highly conserved biosynthesis gene pathways and structural characteristics of monoterpenes among lavender species, hybrids, and cultivars (Boeckelmann, 2008), whereas sagebrush is known to have highly complex genotypes, hybrids, and ploidy levels (Richardson et al., 2012; Jaeger et al., 2016).

We next assessed individual monoterpene concentrations. Each monoterpene is known for inducing unique metabolic and physiological responses, from general CNS depression to vasorelaxation (Santos et al., 2011). Every sagebrush population had different top predictors, however, similar monoterpenes appeared in the top models of most populations, including β-Pinene (Figure H.8), camphene (Figure H.7), 1,8-cineole (Figure H.9), and camphor (Figure H.6). This can likely be explained by these monoterpenes being ubiquitous and representing the most abundant monoterpenes in most sagebrush species, particularly those present at our field sites. These specific monoterpenes are not only important to the sagebrush ecosystem but also to the broader natural and anthropogenic world. β-Pinene is present across many plant species, especially pine (*Pinus* spp.), fir (*Abies* spp.), and spruce (*Picea* spp.; Geron et al., 2000), and is used by humans as an essential oil and in products such as turpentine, pesticides, and disinfectants (Duke, 1992). It is also used in traditional medicine to treat inflammation (Chen et al., 2016; Salehi et al., 2019). Camphene is prevalent at lower levels in pines, hemlock (*Tsuga* spp.), fir, birch (*Betula* spp.), and desert shrubs (Geron et al., 2000). It is also used in some essential oils, as well as in fragrances and as a food additive (Duke, 1992). 1,8-cineole, also known as eucalyptol, is present in eucalyptus (*Eucalyptus* spp.), wormwood (*Artemisia absinthium*), rosemary (*Salvia rosmarinus*), and common sage (Duke, 1992). It is used in flavorings, fragrances, cosmetics, insecticides, and repellents, but at low levels due to its higher degree of toxicity (Duke, 1992). It is also known to be a cough suppressant in traditional medicines (Juergens et al., 2003; Chen et al., 2016). Camphor is prevalent in laurel trees (*Laurus* spp.), rosemary, wormwood, common sage, and desert shrubs (Duke, 1992). It is used in fragrances, plastics, pesticides, repellents, and embalming fluids (Duke, 1992). Traditionally, camphor has been used as a mild local analgesic and cough suppressant (Chen et al., 2013, 2016). Camphor is known to be highly toxic in relatively high doses (Duke, 1992). Non-oxygenated monoterpenes, including β-Pinene and camphene, are easily emitted from plants due to their high degrees of volatility (Grosjean et al., 1993; Geron et al., 2000). This can be deleterious to most life forms because these emissions react with atmospheric gases to create ozone (O_3) in the troposphere, a compound that serves as a potent respiratory hazard and pollutant to organic life at high enough concentrations (Grosjean et al., 1993; Geron et al., 2000). Oxygenated monoterpenes, such as 1,8-cineole and camphor, are also found in ambient air but to a lesser extent and are much less reactive (Geron et al., 2000). Measuring and monitoring these compounds in plants and the atmosphere can, therefore, serve as biomarkers of atmospheric chemistry, carbon cycles, and climatic conditions and assist in management of emissions through modified tree planting (Simpson and McPherson, 2011). In regard to sagebrush habitats, these monoterpenes are well-known to have deleterious pharmacological effects on herbivores through CNS, vascular, and metabolic poisoning at high doses (Kohlert et al., 2000; Sorensen and Dearing, 2003; McLean et al., 2007; Bedoya-Pérez et al., 2014; Karban et al., 2016a). In view of this, pygmy rabbits are selective about each of these monoterpenes when given options in captivity to forage on differing doses and mixtures (Nobler, 2016; Nobler et al., 2019). In the wild, pygmy rabbits, selected to avoid 1,8-cineole and β-Pinene (Utz, 2012; Nobler, 2016). Similarly, sage-grouse avoided 1,8-cineole (Frye et al., 2013) and β-Pinene (Fremgen, 2015; Fremgen-Tarantino et al., 2020). To better predict quality habitat and foraging decisions of these threatened herbivores we need more efficient ways to measure these compounds. NIRS has shown relative success in predicting specific individual monoterpenes in sagebrush (R^2 = 0.51-0.71; Table 2.4; Figures 2.13-2.15) depending on the site. Other literature using NIRS to predict individual monoterpene concentrations in domestic and wild systems have shown similar results, including in rosemary (*Rosmarinus officinalis*), basil (*Ocimum basilicum*), pepper (*Piper nigrum*), marjoram (*Origanum majorana*), spearmint (*Mentha spicata*), and ginger

(*Zingiber officinale*, R^2 = 0.95-0.99; Ercioglu et al., 2018), blue (*Eucalyptus globulus*) and shining (*Eucalyptus nitens*) gum (R^2 = 0.55-0.90; Humphreys et al., 2008), common sage (R^2 = 0.43-0.97; Elementi et al., 2006), citrus fruits (R^2 = 0.87-0.99; Steuer et al., 2001; Schulz et al., 2002), and eucalyptus (R^2 = 0.88-0.95; McIlwee et al., 2001). The ability to predict monoterpene concentrations allows for identification and distribution of potentially noxious plants that may be lethal to domestic herbivores. Additionally, our models would allow us to supplement growing research on the potential contribution of volatile organic compound emissions from plants that are negatively influencing our atmosphere.

We also assessed total phenolic concentrations, which are a class of phytochemicals known for antiseptic, endocrine, and nutrient-inhibiting properties (Acamovic and Brooker, 2005; Turi et al., 2014; Marsh et al., 2017). They encompass a diverse group of compounds and are highly prevalent in plant life (Acamovic and Brooker, 2005) and are known for having both noxious and therapeutic effects (Acamovic and Brooker, 2005). While total phenolics was best predicted at Magic (R^2 = 0.58; Table 2.5; Figure 2.17), predictive results were relatively consistent across the sagebrush sites (Table 2.5). This might be due to phenolics containing an extremely broad class of compounds that are relatively conserved in chemical structure (Marsh et al., 2017). That so, NIRS would pick up total phenolic reflectance fairly equivalently across sagebrush taxa and populations. It is important to note that this is the only chemical class where Raft River had a functional prediction above R^2 of 0.04 (R^2 = 0.58; Table 2.5; Figure 2.18). This is expectedly due to the dominant species of sagebrush at that field site, low sagebrush (*A. arbuscula*), which is known for increased types, numbers, and concentrations of phenolics compared to other *Artemisia* species (Figure C.1; Figure H.3; Turi et al., 2014). It might also be attributed to the unique and diverse phenotypes present at this field site (Fremgen, 2015). Even though studies in the sagebrush-steppe have shown that total phenolic content does not always influence herbivore foraging behavior (Frye et al., 2013; Ulappa et al., 2014), preliminary studies show that differing concentrations and mixtures of individual phenolic compounds do influence selection by herbivores (unpublished data; Figure F.1), and some of these individual phenolic compounds can be predicted by NIRS (unpublished data; Figures F.2-F.5). Other literature using NIRS to predict total and individual phenolic concentrations in domestic and wild systems have shown similar and even better results. For example, individual and classes of phenolics were predicted in holm oak (*Quercus ilex*, tocopherol (R^2 = 0.78-98) and total phenolics (R^2 = 0.92); Pintó-Marijuan et al., 2013), willows (*Salix phylicifolia*, total tannins (R^2 = 0.89) and total phenolics (R^2 = 0.98); Stolter et al., 2006), blackberries (*Rubus fructicosus*, total phenolics (R^2 = 0.86); Toledo-Martín et al., 2018), potatoes (*Solanum tuberosum*, total phenolics (R^2 = 0.88); López et al., 2014), and ruminant forage (total tannins (R^2 = 0.89) and total phenolics (R^2 = 0.98); Bomfim, 2013). However, most of these systems have less diversity and abundance of phenolics overall compared to sagebrush. Very few studies have used NIRS to predict complex wild threatened plant-herbivore systems.

Finally, we assessed total coumarin concentrations as a subclass of phenolics that are known for antibacterial, antifungal, and anticoagulant properties (McArthur et al., 1988; Wilt and Miller, 1992; Shultz, 2012). Coumarins show brilliant blue fluorescence under UV-light and have been used to classify sagebrush species (Stevens and McArthur, 1974; McArthur et al., 1988; Rosentreter, 2005) and predict palatability, specifically for the sage-grouse (Figure F.6). Measuring coumarins with a black light is growing as a field technique to classify species (Stevens and McArthur, 1974; Rosentreter, 2005; Jaeger et al., 2016) and palatability (Figure F.6) of sagebrush and can also be quantified colorimetrically in the lab (Figure F.7). Despite the importance of coumarins for taxonomic classification, coumarin content was only reliably predicted at Magic (R^2 = 0.77; Table 2.6; Figure 2.19) likely due to distinct patches of dwarf sagebrush species inhabiting this population (Table 2.1), including low sagebrush (*A. arbuscula*) and possibly early sagebrush (*A. longiloba*), which are known to contain high concentrations of coumarins (Rosentreter, 2005). In contrast, although Raft River was dominated by low sagebrush (Table 2.1), NIRS did not predict coumarins at this site potentially due to the overall lower range of coumarin concentrations at this site (Table G.1; Figure H.2). To our knowledge, there are no studies using NIRS to predict total or individual coumarin concentrations. Increasing our capacity to quantify coumarins would allow for more reliable species identification of sagebrush, currently a challenging task, and identify sites and plants more palatable to certain sagebrush herbivores.

By and large, results of phytochemical predictions suggest that NIR spectra can reliably determine certain classes of phytochemicals and individual phytochemicals in certain species and populations of sagebrush. The variability is most likely explained by the organic properties of the diverse compounds comprising the sagebrush chemotypes in each population and, consequently, how well they reflect light across the NIR spectrum (Curran, 1989; Youngentob et al., 2012). This, in turn, governs the differential spectral profiles seen in sagebrush among field sites (Figure A.4) and the ensuing spectral variables (i.e., weighted beta regression coefficients) that most influence the chemical predictions by NIRS (Figures A.5-A.11). Although nitrogen is consistently predicted at distinct wavelengths in the NIR range (910, 1690, 1980, 2172, 2180, 2300 nm in Youngentob et al., 2012; Figures A.5-A.7), phytochemicals contain organic bonds that emit light across numerous and diverse ranges of the electromagnetic spectrum (e.g., coumarins reflect light strongly in the UV and blue ranges; Stevens and McArthur, 1974; Figure A.11), which might create variable interference with nitrogen bonds. Therefore, observing both the spectral profiles and weighted beta regression coefficients of samples can reveal deviations that potentially inform unexpected outcomes, such as those at Raft River, where reflectance spectra align similarly with other field sites (Figure A.4) but the large variation in phenolics (Figure H.3) may explain why weighted coefficients are distinctive (Figure A.8). Distinct reflectance spectra or weighted coefficients may suggest that sensors with different electromagnetic ranges or higher sensitivity are needed to predict specific phytochemicals. Moreover, the phytochemicals that were predicted with slopes that deviated from a 1:1 reference line suggest that NIRS is underestimating or overestimating concentrations. This could indicate concentrations measured above or below limits of detection in lab equipment. For example, plateauing concentrations of camphor could be due to an overwhelming abundance of camphor in sagebrush (Figure C.1 around minute 21.0) that likely oversaturated detection in our gas chromatograph instrument, leading to an underestimation of actual camphor concentrations. Deviation from the regression line could also be used to identify individual plants within a site or population of sagebrush that are chemically or genetically unique, due to climate or socioecological disturbances, hybridizations, or other disturbances and management practices. Ultimately, this is one of few studies demonstrating the use of NIRS to predict concentrations of a diversity of phytochemicals in a complex wild system.

Quantifying nutrients and phytochemicals in plants is important in explaining herbivore habitat use and informing conservation efforts. Herbivores make foraging decisions based on their immediate circumstances, sensory systems, and feedback loops. Due to this, when working to conserve habitats important to herbivores, it can be beneficial to bypass measuring these arbitrary chemical traits and concentrations and directly measure markers of habitat use by herbivores. In this way, we aimed to predict markers of herbivory, in the form of the number of bite marks on plants by vertebrate herbivores, using NIRS. We did this for two herbivores of conservation concern: pygmy rabbits and sage-grouse (Figures 2.2-2.3). Magic predicted pygmy rabbit bite marks the best, although still poorly (R^2 = 0.31; Table 2.7; Figure 2.20). The other field sites did not predict bite marks (Table 2.7). When combining all of the field sites, bite marks of all herbivores were predicted poorly (R^2 = 0.09; Table 2.7). These results suggest that NIR spectra cannot yet reliably be used to predict herbivory, in the form of bite marks, on individual sagebrush plants. The only sagebrush population with marginal predictive capacity was Magic. This site had one of the smallest sample sizes (n = 30), yet still produced the highest predictive result. This could be due to NIRS actually predicting another parameter that is correlated to bite marks, the simpler phenotypic diversity at this site, or the fact that Magic generally had the widest ranges of chemical values (Table G.1; Figures H.4, H.6-H.10). Other studies, including food intake by koalas, ringtail possums, and greater gliders on eucalyptus (Foley et al., 1999; Moore et al., 2010), demonstrate that NIRS can indirectly predict habitat use in complex wild plant-herbivore systems.

These rather revolutionary outcomes make replicating the results in the sagebrush system promising. Foley et al. (1999) and Moore et al. (2010) differed from our methods by quantifying and predicting foliage intake (g dry matter) in captive koalas relative to specific phytochemicals, and then using predictions of phytochemical concentrations from NIRS to indirectly predict use of trees in the field. This integrated approach inspires the need for similar investigations in the sagebrush-steppe system. Moreover, the more controlled conditions in captivity that are translated to the field may strengthen predictive capacity, as compared to our entirely *in natura* assessment. We also measured bite counts instead of intake due to the inability to quantify intake in wild animals. Other sources of error could be too small of a sample size (Figure 2.7), too small or bimodal of a range of bite mark numbers (Figure 2.20), or the unique dynamics of sagebrush chemistry responding to browsing at different time scales (Karban et al., 2006; Karban, 2011). The chemical composition and abundance of sagebrush changes after browsing by herbivores (Karban, 2011). Browsed plants emit volatile signals that can then be detected by nearby sagebrush plants, which respond accordingly (Karban et al., 2006). These dynamic responses in sagebrush chemistry post-browsing may actually be the traits reflected in the NIR spectra and what is predicted rather than the actual cues that influence browsing (i.e., a delayed time effect may be occurring). Regardless, these early results suggest that NIRS has the potential to indirectly predict herbivore use in sagebrush habitats through predicting phytochemical concentrations and directly predicting herbivore use through more refined methods.

Using NIRS to quantify concentrations of chemicals and herbivory in sagebrush populations is beneficial to protecting shrub-steppe ecosystems. Our results demonstrate that NIR spectra can reliably predict certain functional chemical traits, in the forms of nutrient and toxin concentrations, in individual sagebrush plants and populations. Quickly and accurately quantifying chemicals in sagebrush can direct efforts in conserving and restoring forage necessary to domestic and wild herbivore survival. For example, predicting concentrations of chemical constituents in sagebrush in relation to foraging behavior and dose-dependent selection by associated herbivores could help locate sources of plants that are palatable to these herbivores, for conservation, translocation, and reintroduction purposes. Moreover, quantifying chemical concentrations in sagebrush could identify species composition and distribution of restored lands post-disturbance, as well as foster bioprospecting in sagebrush, a practice observed by native peoples for centuries (Kelley et al., 1992). Although NIRS has not yet been shown to reliably predict herbivory—albeit results show room for improvement—threatened herbivores in the sagebrush landscape make foraging decisions based on doses (i.e., concentrations) of chemicals in the sagebrush they are exposed to (Frye et al., 2013; Fremgen, 2015; Nobler et al., 2019). Research also shows the concentration thresholds at which these herbivores are making foraging decisions (Frye et al., 2013; Olsoy et al., 2020; Figures D.1-D.4). Because of this, NIRS provides an efficacious means of quantifying these threshold concentrations across sagebrush habitats and, therefore, indirectly identifying suitable habitats or seed sources that could sustain herbivore populations. In addition, sagebrush populations that have experienced stressors or disturbances, such as drought or fire, might be monitored more effectively using technologies such as NIRS to measure changes in chemicals that are indicators of stress. For example, nitrogen is shown to decrease in leaves and increase in stems in water-stressed sagebrush (Dina and Klikoff, 1973). In view of these advantages, we recommend employing NIRS in monitoring concentrations of phytochemicals in sagebrush with specific focus on those chemicals that respond to human interference (e.g., nitrogen concentrations in plants and habitats after a fire) and elicit responses by wildlife (e.g., induced chemical defenses in plants after browsing).

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Tables

Table 2.1 Phenotypes associated with data collected at each field site in Idaho, USA.

Field Site	Elevation (m)	Years	Seasons	Sagebrush Species	Microhabitats	Browsed State of Plants	Herbivore Species
Magic Reservoir	1465 - 1480	2014-2015 (n=590)	Winter (n=246), Summer (n=344)	*A. t. wyomingensis* (n=321), Dwarf taxa (n=107)	On-mound (n=226), Off-mound (n=211), Dwarf-patch (n=153)	Browsed (n=30), Non-browsed (n=398)	*Brachylagus idahoensis*
Cedar Gulch	1890 - 1940	2014-2015 (n=625)	Winter (n=387), Summer (n=238)	*A. t. wyomingensis* (n=336), *A. nova* (n=25), Dwarf taxa (n=100)	On-mound (n=199), Off-mound (n=216), Dwarf-patch (n=210)	Browsed (n=43), Non-browsed (n=384)	*Brachylagus idahoensis*
Craters of the Moon	1300 - 1650	2013-2014 (n=94)	Winter (n=94)	*A. t. wyomingensis* (n=66), *A. tripartita* (n=26)	Used-patch (n=58), Random-patch (n=36)	Browsed (n=28), Non-browsed (n=66)	*Centrocercus urophasianus*
Raft River	1380 - 2140	2013-2014 (n=263)	Winter (n=263)	*A. t. wyomingensis* (n=65), *A. arbuscula* (n=197)	Used-patch (n=176), Random-patch (n=87)	Browsed (n=75), Non-browsed (n=188)	*Centrocercus urophasianus*

Table 2.2 Partial least squares regression using near infrared spectroscopy to predict nitrogen concentrations (% dw) in individual sagebrush (*Artemisia* spp.) at four individual and combined field sites in Idaho, USA.

	Calibration					Internal cross-validation		
Field Site	N	Components	R^2 [a]	RMSEC [b]	SEC [c]	R^2 [d]	RMSECV [e]	SECV [f]
Magic Reservoir	585	12	0.8583	0.0947	0.0947	0.8273	0.1046	0.1047
Cedar Gulch	619	11	0.802	0.1351	0.1352	0.7667	0.1469	0.147
Craters of the Moon	93	7	0.9487	0.0687	0.0691	0.924	0.0845	0.085
Raft River	190	1	0.0345	0.2417	0.2423	—	0.2533	0.254
Magic Reservoir + Cedar Gulch + Craters of the Moon	1793	11	0.8409	0.1461	0.1462	0.8315	0.1505	0.1506
Magic Reservoir + Cedar Gulch + Craters of the Moon + Raft River	1983	10	0.7839	0.1723	0.1724	0.7712	0.1774	0.1774

[a] Coefficient of determination of calibration

[b] Root mean square error of calibration

[c] Standard error of calibration

[d] Coefficient of determination of cross-validation

[e] Root mean square error of cross-validation

[f] Standard error of cross-validation

Table 2.3 Partial least squares regression using near infrared spectroscopy to predict concentrations of total monoterpenes (AUC/mg dw) in individual sagebrush (*Artemisia* spp.) at four field sites in Idaho, USA.

	Calibration					Internal cross-validation		
Field Site	N	Components	R^{2} [a]	RMSEC [b]	SEC [c]	R^{2} [d]	RMSECV [e]	SECV [f]
Magic Reservoir	569	6	0.7873	147.621	147.7509	0.7703	153.8301	153.965
Cedar Gulch	618	9	0.6121	101.9642	102.0468	0.5632	108.4857	108.573
Craters of the Moon	94	6	0.6971	125.4246	126.0971	0.5548	155.5494	156.3833
Raft River	262	1	0.0155	175.0763	175.4113	—	183.0885	183.4387

[a] Coefficient of determination of calibration

[b] Root mean square error of calibration

[c] Standard error of calibration

[d] Coefficient of determination of cross-validation

[e] Root mean square error of cross-validation

[f] Standard error of cross-validation

Table 2.4 **Partial least squares regression using near infrared spectroscopy to predict concentrations of individual monoterpenes (AUC/mg dw) in individual sagebrush (*Artemisia* spp.) at four field sites in Idaho, USA.**

	Calibration					Internal cross-validation		
Field Site	N	Components	R^2 [a]	RMSEC [b]	SEC [c]	R^2 [d]	RMSECV [e]	SECV [f]
Magic Reservoir								
Camphor	586	6	0.7138	99.6903	99.7755	0.6985	102.5713	102.6587
Camphene	586	6	0.6599	37.3597	37.3916	0.6455	38.2512	38.2838
1,8-cineole	586	6	0.565	50.5066	50.5497	0.5496	51.4785	51.5224
α-Pinene	586	6	0.5221	9.4743	9.4824	0.5046	9.6677	9.6759
Cedar Gulch								
Unknown 20.1 min [g]	621	7	0.5359	14.3845	14.3961	0.5164	14.6824	14.6942
β-Pinene	621	7	0.5126	33.5362	33.5633	0.4915	34.3076	34.3352
Craters of the Moon								
α-Pinene	94	2	0.6998	16.442	16.5302	0.6578	17.6749	17.7695
Camphene	94	2	0.6915	43.395	43.6277	0.6432	46.8764	47.1276
Raft River								

Table 2.5 Partial least squares regression using near infrared spectroscopy to predict concentrations of total phenolics (mg/g dw) in individual sagebrush (*Artemisia* spp.) at four field sites in Idaho, USA.

	Calibration					Internal cross-validation		
Field Site	N	Components	R^{2} [a]	RMSEC [b]	SEC [c]	R^{2} [d]	RMSECV [e]	SECV [f]
Magic Reservoir	523	8	0.5833	3.2318	3.2349	0.5223	3.4664	3.4697
Cedar Gulch	601	4	0.4172	3.0437	3.0462	0.399	3.0987	3.1013
Craters of the Moon	87	2	0.5	9.5784	9.634	0.419	10.3527	10.4125
Raft River	250	6	0.5821	19.491	19.5301	0.528	20.7178	20.7593

[a] Coefficient of determination of calibration

[b] Root mean square error of calibration

[c] Standard error of calibration

[d] Coefficient of determination of cross-validation

[e] Root mean square error of cross-validation

[f] Standard error of cross-validation

Camphene	262	1	0.0312	34.5778	34.644	—	36.1177	36.1865

[a] Coefficient of determination of calibration

[b] Root mean square error of calibration

[c] Standard error of calibration

[d] Coefficient of determination of cross-validation

[e] Root mean square error of cross-validation

[f] Standard error of cross-validation

[g] Monoterpene “Unknown 20.1 min” is an unidentified monoterpene eluted at 20.1 minutes during gas chromatography analysis

Table 2.6 Partial least squares regression using near infrared spectroscopy to predict concentrations of total coumarins (μmol/g dw) in individual sagebrush (*Artemisia* spp.) at four field sites in Idaho, USA.

	Calibration					Internal cross-validation		
Field Site	N	Components	$R^{2\,a}$	RMSEC [b]	SEC [c]	$R^{2\,d}$	RMSECV [e]	SECV [f]
Magic Reservoir	546	6	0.7706	0.3659	0.3662	0.7505	0.3827	0.383
Cedar Gulch	576	4	0.3438	0.1857	0.1858	0.2976	0.1924	0.1926
Craters of the Moon	94	1	0.0381	5.0269	5.0539	0.013	5.1416	5.169
Raft River	255	1	0.0117	0.3279	0.3285	—	0.3321	0.3328

[a] Coefficient of determination of calibration

[b] Root mean square error of calibration

[c] Standard error of calibration

[d] Coefficient of determination of cross-validation

[e] Root mean square error of cross-validation

[f] Standard error of cross-validation

Table 2.7 Partial least squares regression using near infrared spectroscopy to predict intensity of browsing, in the form of numbers of bite marks, by pygmy rabbits (*Brachylagus idahoensis*) [g] or sage-grouse (*Centrocercus urophasianus*) [h] foraging on sagebrush (*Artemisia* spp.) at four field sites in Idaho, USA.

	Calibration					Internal cross-validation		
Field Site	N	Components	R^2 [a]	RMSEC [b]	SEC [c]	R^2 [d]	RMSECV [e]	SECV [f]
Magic Reservoir [g]	30	2	0.3129	20.2781	20.6248	0.0643	24.9304	25.3319
Cedar Gulch [g]	43	1	0.0925	33.7398	34.1391	0.0426	35.3645	35.7827
Craters of the Moon [h]	28	1	0.0534	21.1805	21.5692	—	23.1575	23.5825
Raft River [h]	81	1	0.0435	17.9455	18.0573	0.015	18.4262	18.541
All Sites	390	2	0.0947	25.4421	25.4747	0.0744	25.7828	25.8158

[a] Coefficient of determination of calibration

[b] Root mean square error of calibration

[c] Standard error of calibration

[d] Coefficient of determination of cross-validation

[e] Root mean square error of cross-validation

[f] Standard error of cross-validation

[g] Pygmy rabbits are the predominant specialist herbivores at this site

[h] Greater sage-grouse are the predominant specialist herbivores at this site

Figures

Figure 2.1 Field sites where individual sagebrush (*Artemisia* spp.) samples were collected that are important to herbivore populations of conservation concern in Idaho, USA. Samples were collected between the years of 2012-2015.

Figure 2.2 Indication of pygmy rabbit (*Brachylagus idahoensis*) browsing on a sagebrush shrub. It can be identified by a clean 45-degree bite mark and can be differentiated from other lagomorph species by the diameter of the clipping (Crowell et al., 2018) and the lack of leafy material left below the shrub. Fresh bite marks were identified by a wetter green or bright brown stem interior, as opposed to a dry dull brown color. Photo provided by Nobler (2016).

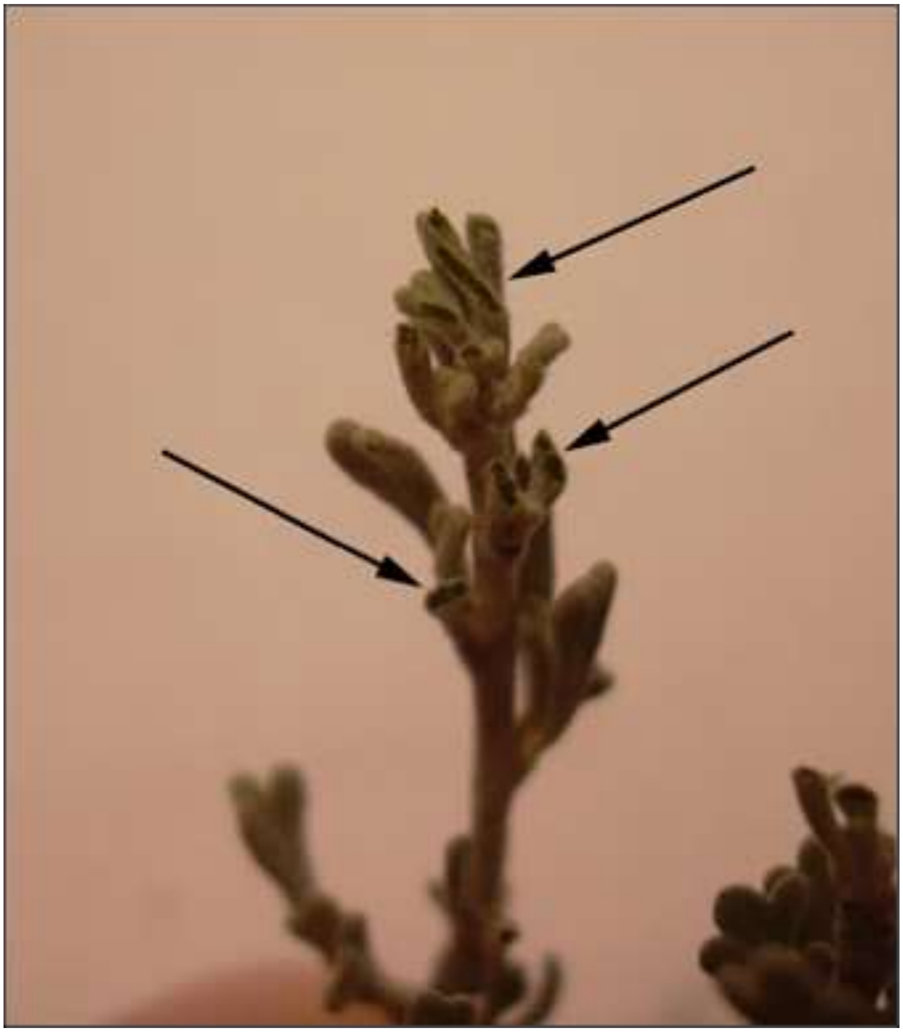

Figure 2.3 Indication of greater sage-grouse (*Centrocercus urophasianus*) browsing on a sagebrush shrub. Bites by sage-grouse can be identified by a clean bite mark on the leafy material, leaving the sprig stems intact. Photo provided by Frye (2012).

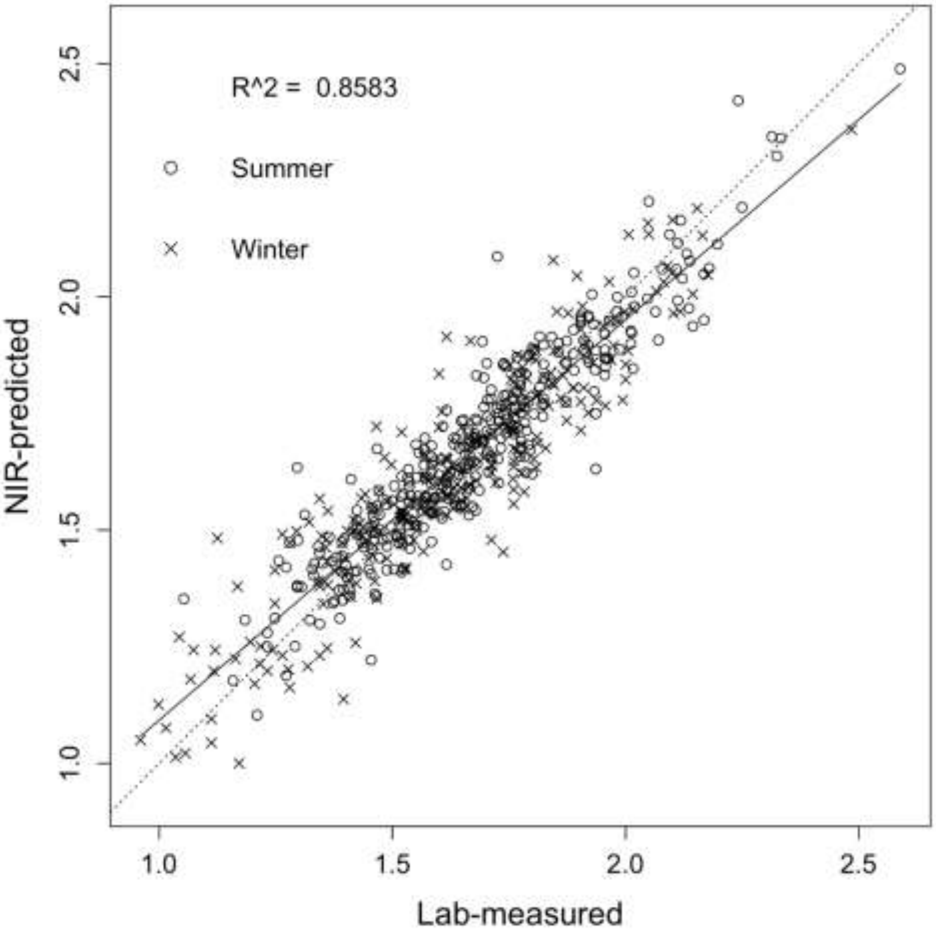

Figure 2.4 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured nitrogen concentrations (% dw) in individual sagebrush (*Artemisia* spp.) at Magic Reservoir, Idaho, USA. The dotted line represents a 1:1 ratio.

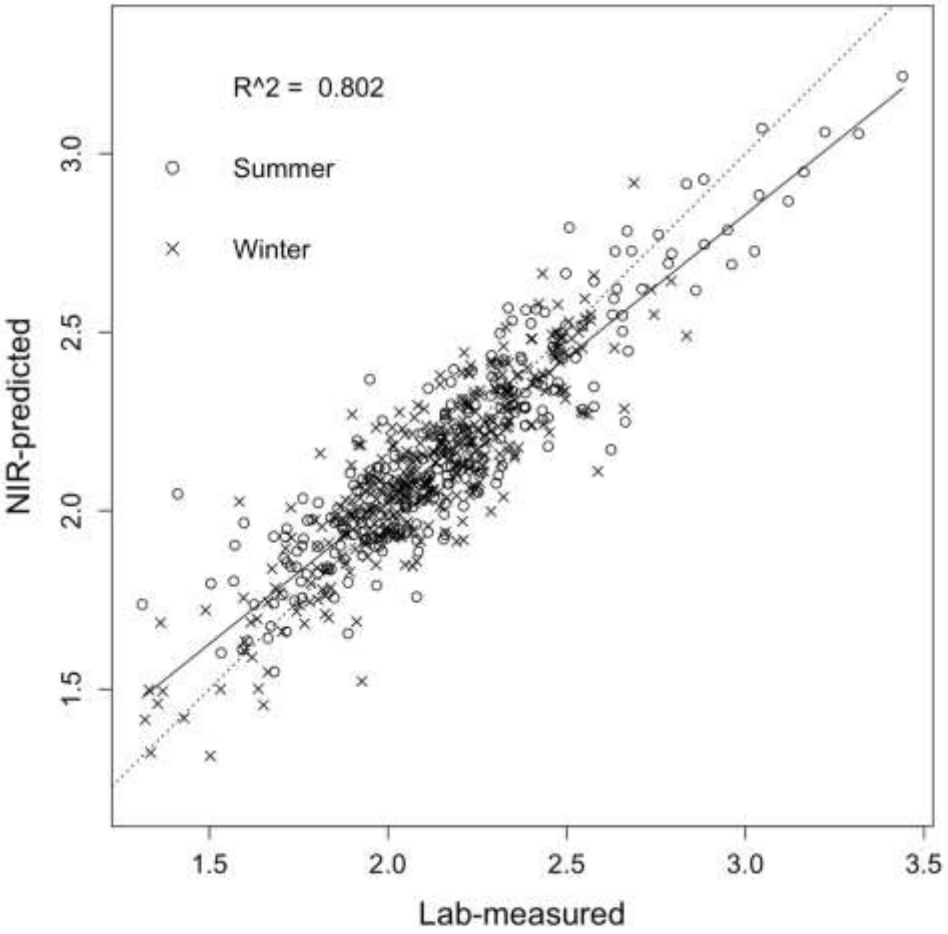

Figure 2.5 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured nitrogen concentrations (% dw) in individual sagebrush (*Artemisia* spp.) at Cedar Gulch, Idaho, USA. The dotted line represents a 1:1 ratio.

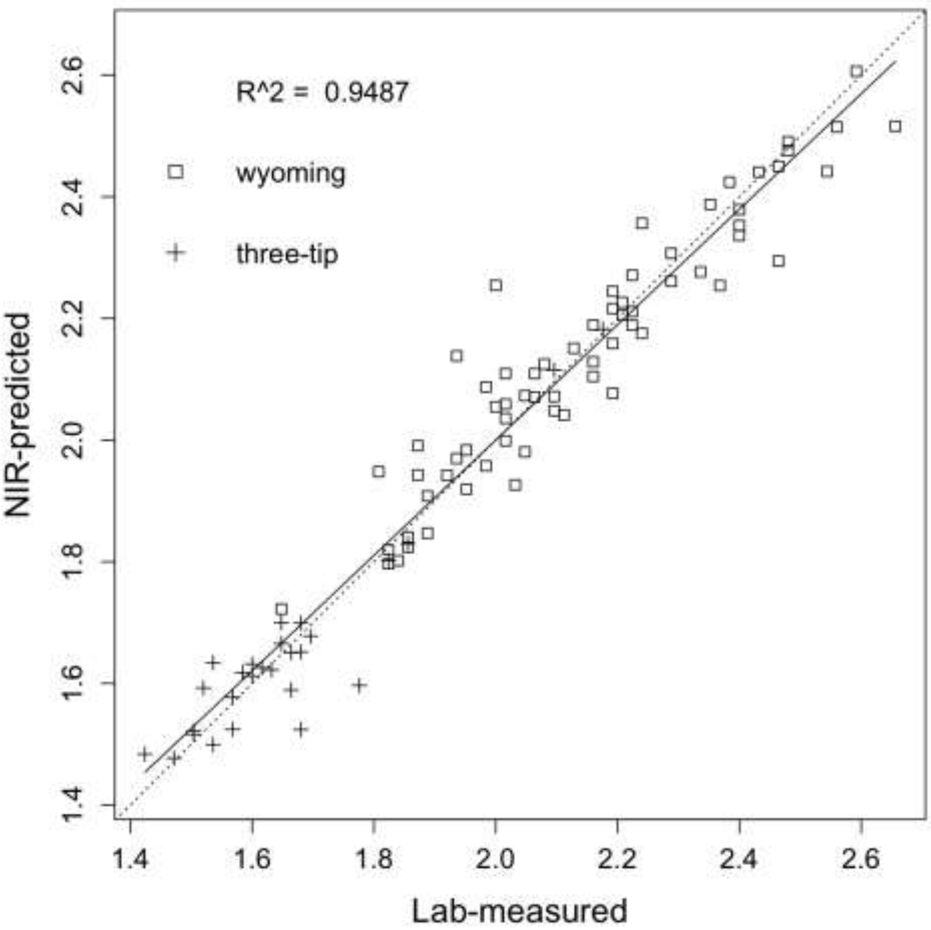

Figure 2.6 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured nitrogen concentrations (% dw) in individual sagebrush (*Artemisia tridentata wyomingensis, A. tripartita*) at Craters of the Moon, Idaho, USA. The dotted line represents a 1:1 ratio.

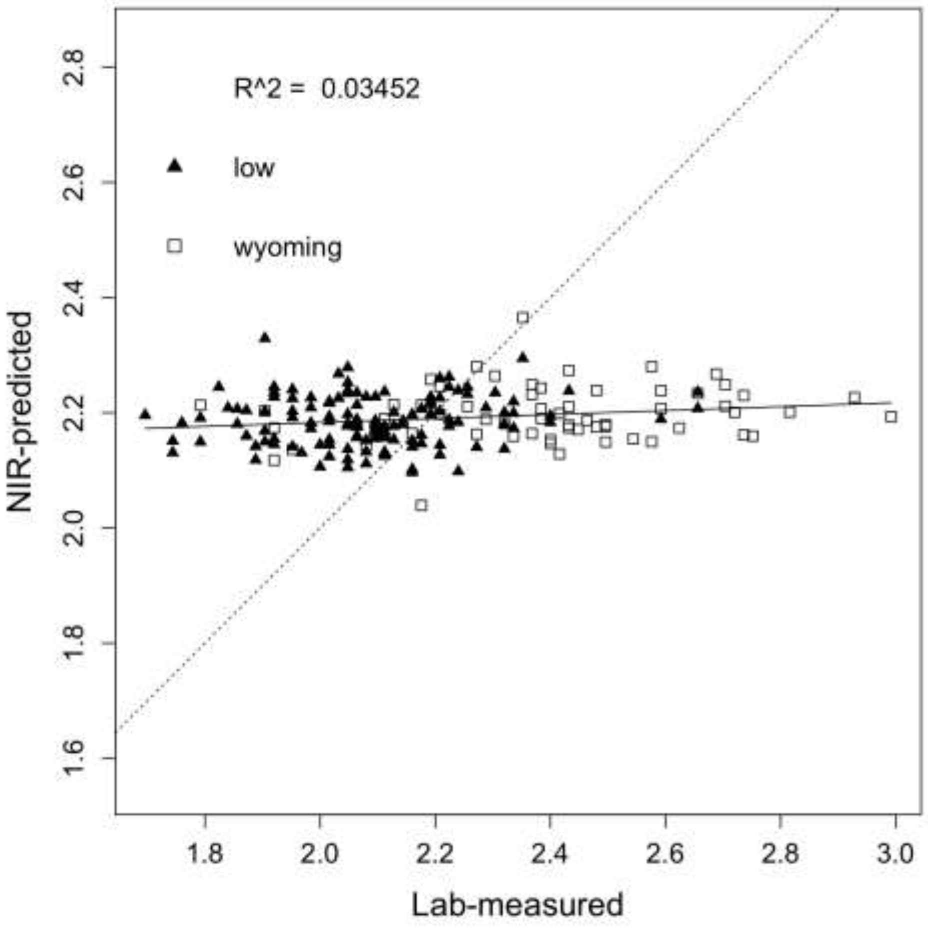

Figure 2.7 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured nitrogen concentrations (% dw) in individual sagebrush (*Artemisia arbuscula, A. tridentata wyomingensis*) at Raft River, Idaho, USA. The dotted line represents a 1:1 ratio.

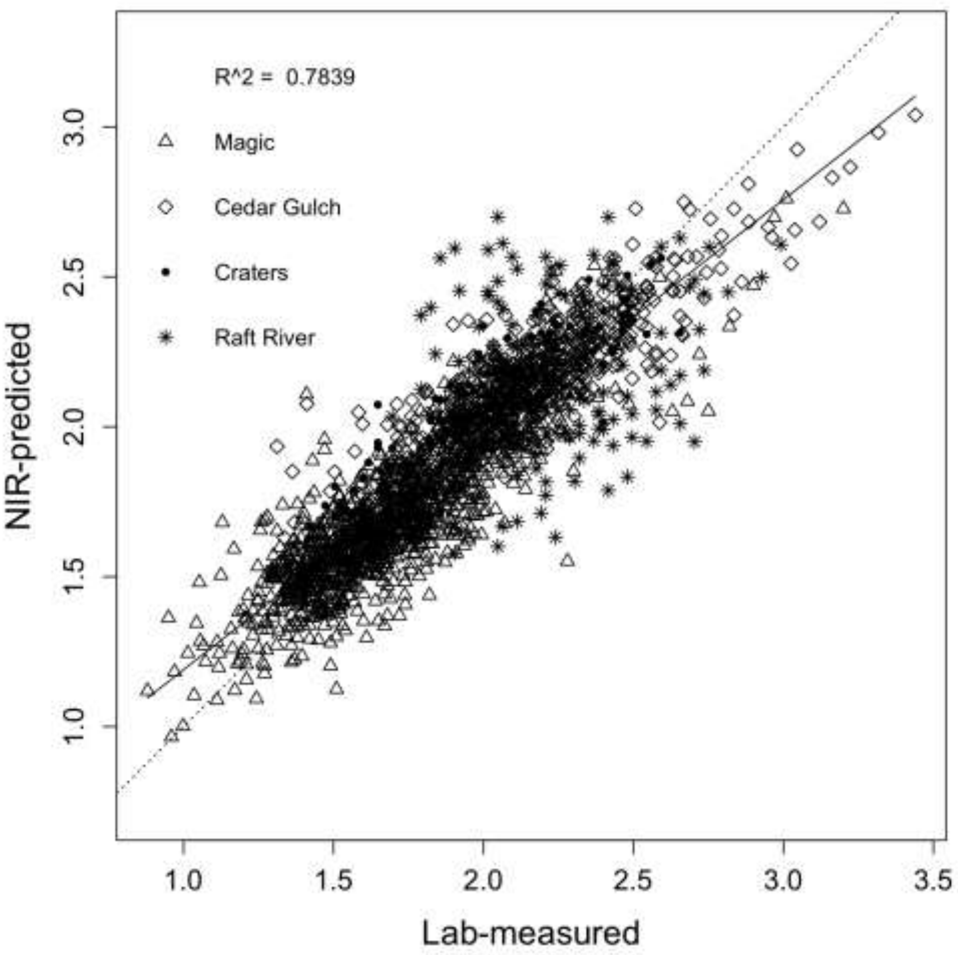

Figure 2.8 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured nitrogen concentrations (% dw) in individual sagebrush (*Artemisia* spp.) at four field sites in Idaho, USA, including Raft River. The dotted line represents a 1:1 ratio.

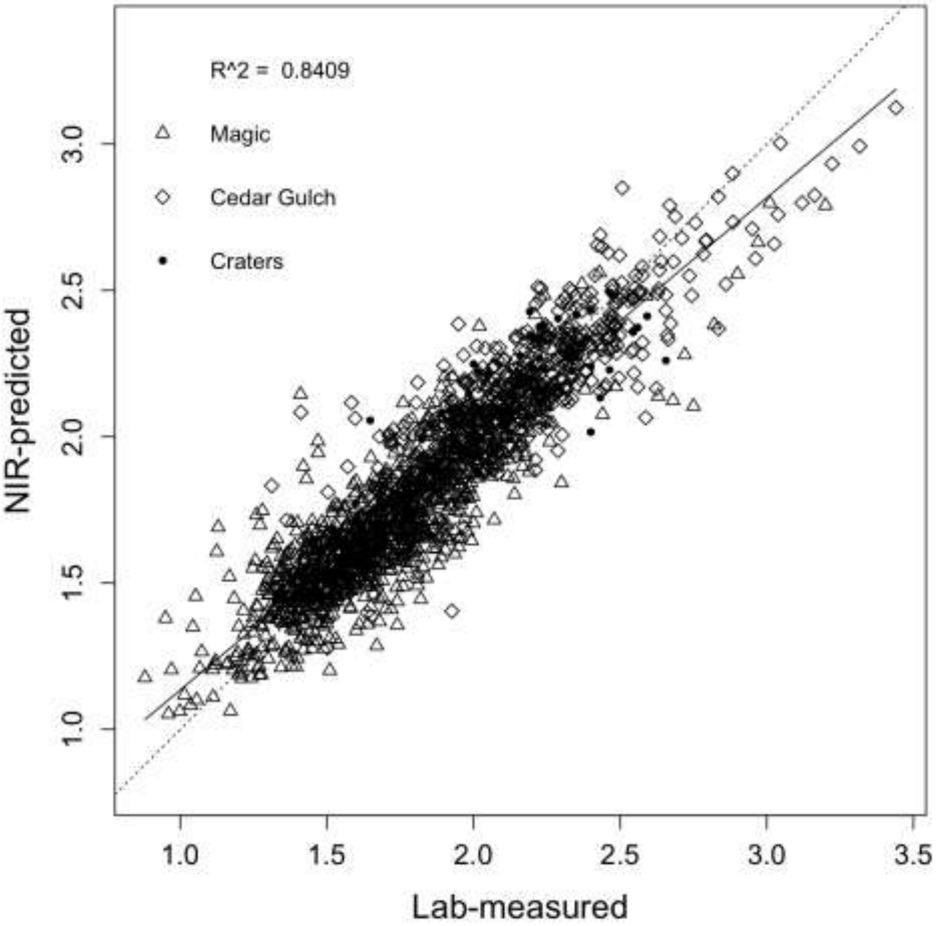

Figure 2.9 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured nitrogen concentrations (% dw) in individual sagebrush (*Artemisia* spp.) at three field sites in Idaho, USA, excluding Raft River. The dotted line represents a 1:1 ratio.

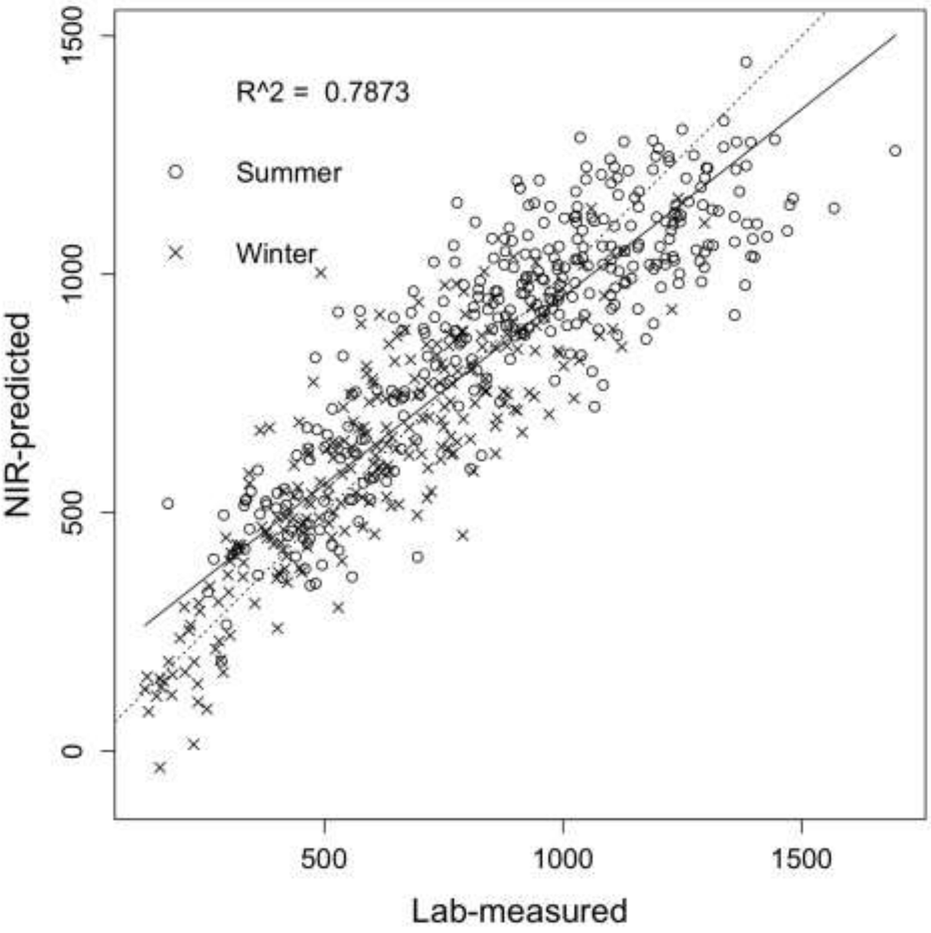

Figure 2.10 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured total monoterpene concentrations (AUC/mg dw) in individual sagebrush (*Artemisia* spp.) at Magic Reservoir, Idaho, USA. The dotted line represents a 1:1 ratio.

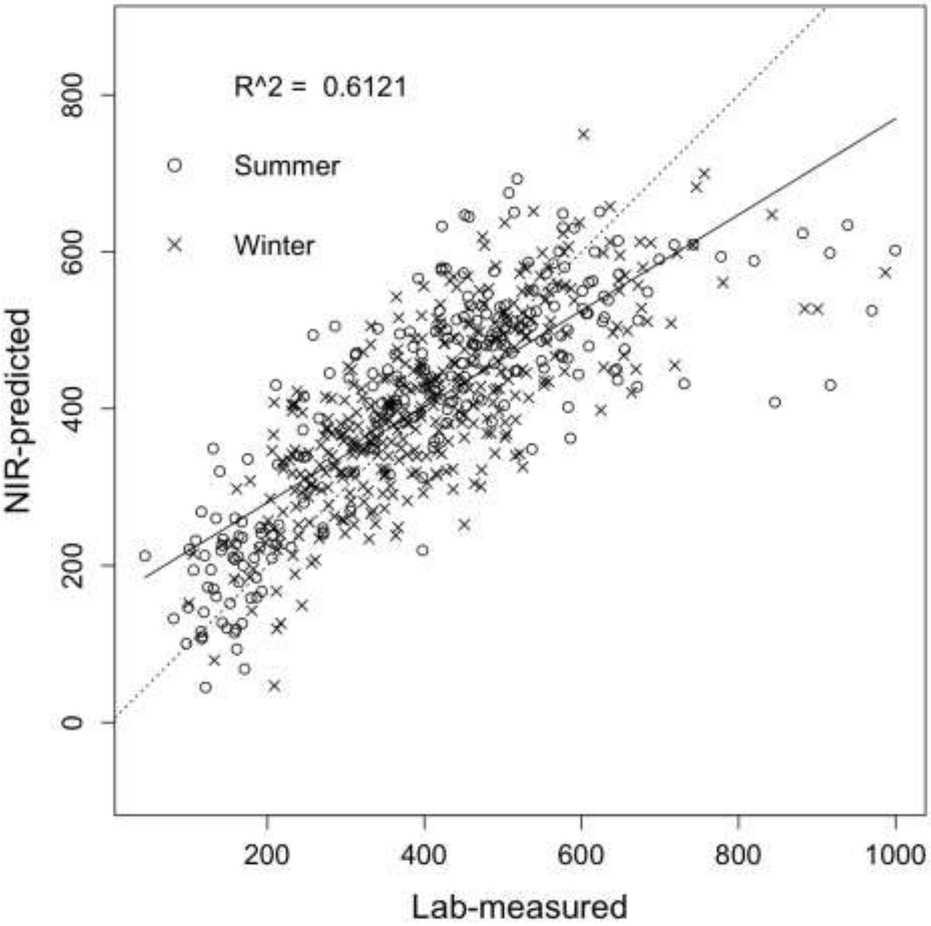

Figure 2.11 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured total monoterpene concentrations (AUC/mg dw) in individual sagebrush (*Artemisia* spp.) at Cedar Gulch, Idaho, USA. The dotted line represents a 1:1 ratio.

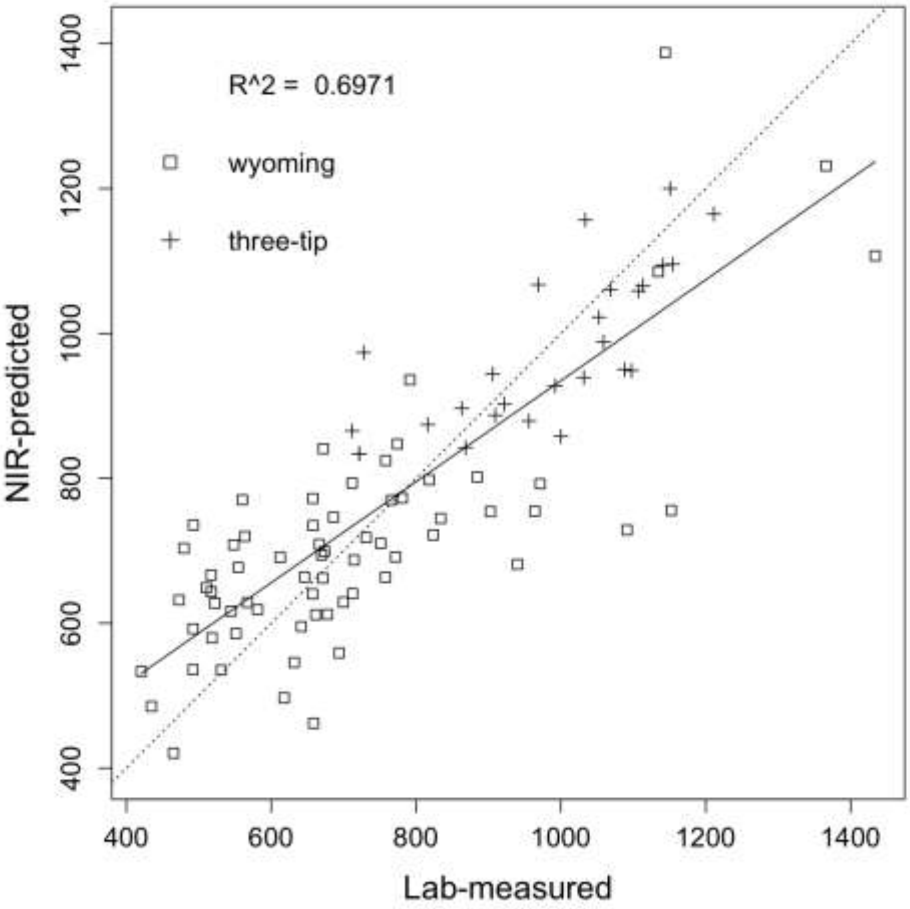

Figure 2.12 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured total monoterpene concentrations (AUC/mg dw) in individual sagebrush (*Artemisia tridentata wyomingensis, A. tripartita*) at Craters of the Moon, Idaho, USA. The dotted line represents a 1:1 ratio.

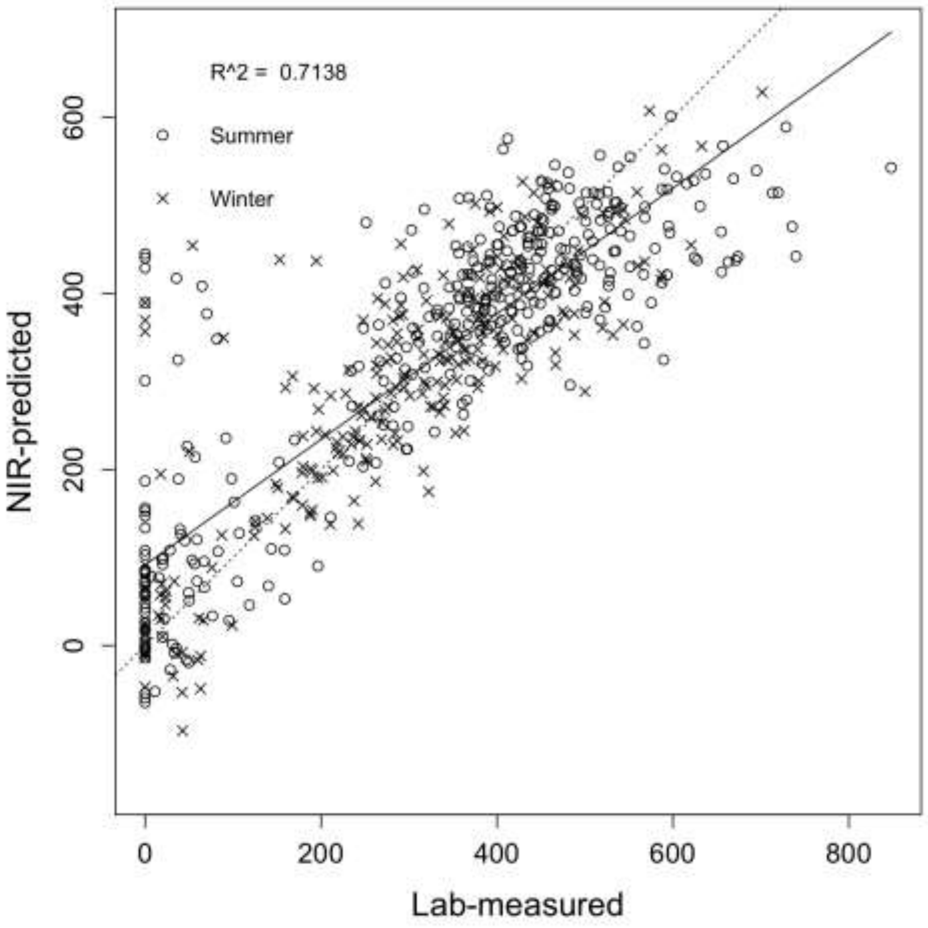

Figure 2.13 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured individual monoterpene concentrations (AUC/mg dw), with camphor shown as the best predicted model, in individual sagebrush (*Artemisia* spp.) at Magic Reservoir, Idaho, USA. The dotted line represents a 1:1 ratio.

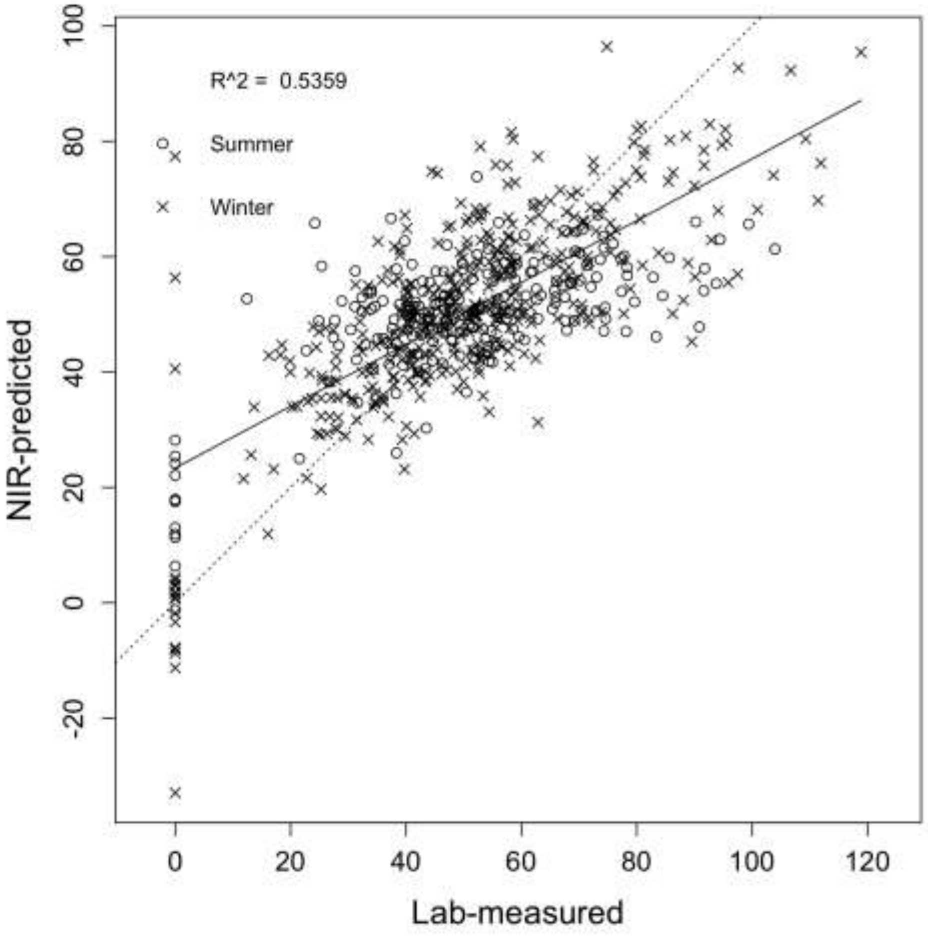

Figure 2.14 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured individual monoterpene concentrations (AUC/mg dw), with unidentified monoterpene "20.1 min" shown as the best predicted model, in individual sagebrush (*Artemisia* spp.) at Cedar Gulch, Idaho, USA. The dotted line represents a 1:1 ratio.

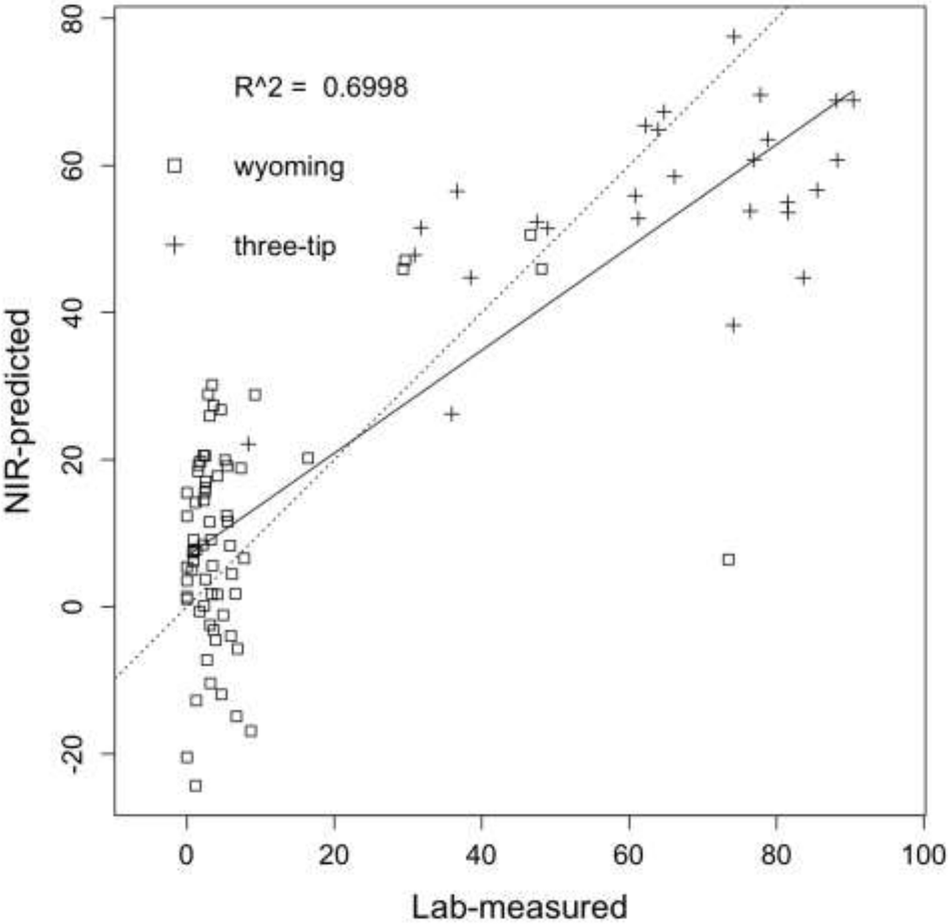

Figure 2.15 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured individual monoterpene concentrations (AUC/mg dw), with α-Pinene shown as the best predicted model, in individual sagebrush (*Artemisia tridentata wyomingensis, A. tripartita*) at Craters of the Moon, Idaho, USA. The dotted line represents a 1:1 ratio.

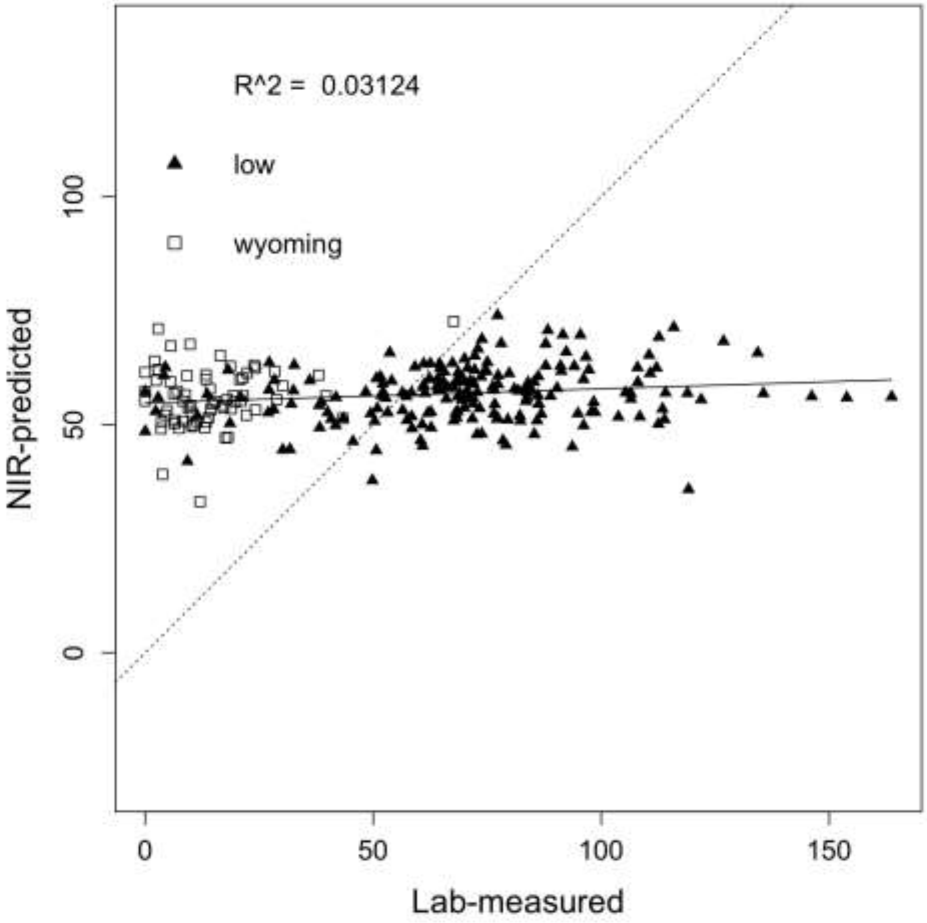

Figure 2.16 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured individual monoterpene concentrations (AUC/mg dw), with camphene shown as the best predicted model, in individual sagebrush (*Artemisia arbuscula, A. tridentata wyomingensis*) at Raft River, Idaho, USA. The dotted line represents a 1:1 ratio.

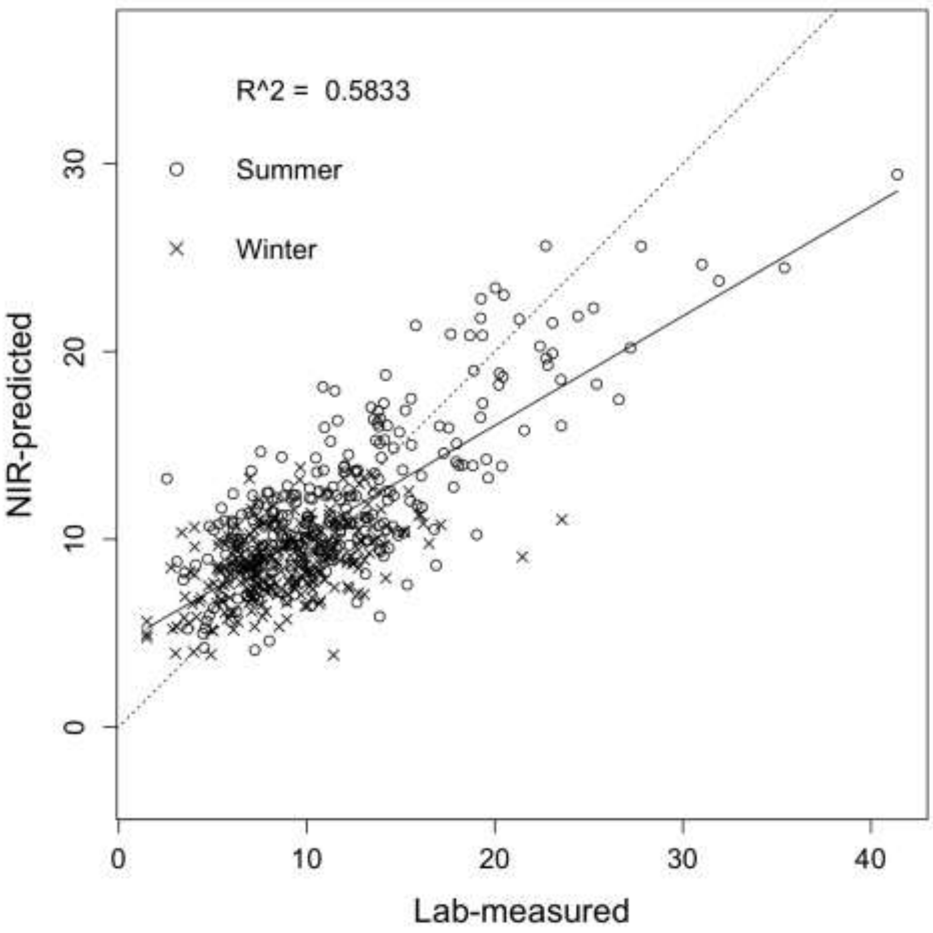

Figure 2.17 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured total phenolic concentrations (mg/g dw) in individual sagebrush (*Artemisia* spp.) at Magic Reservoir, Idaho, USA. The dotted line represents a 1:1 ratio.

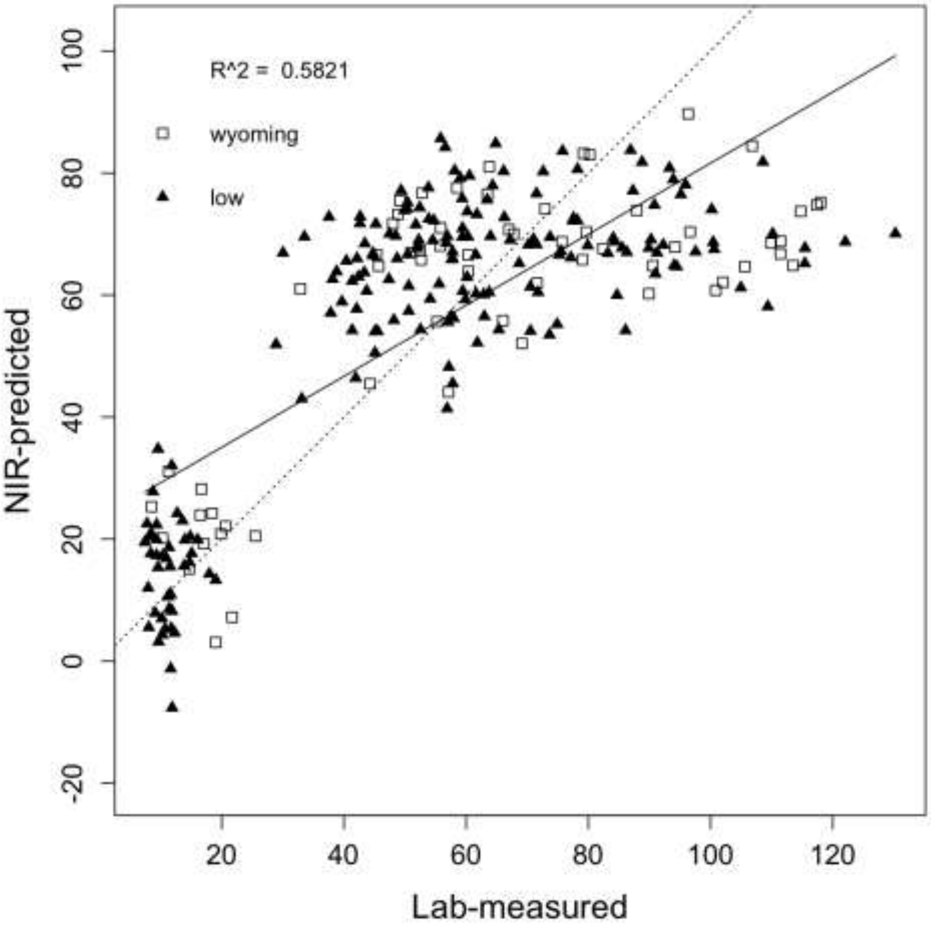

Figure 2.18 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured total phenolic concentrations (mg/g dw) in individual sagebrush (*Artemisia tridentata wyomingensis, A. arbuscula*) at Raft River, Idaho, USA. The dotted line represents a 1:1 ratio.

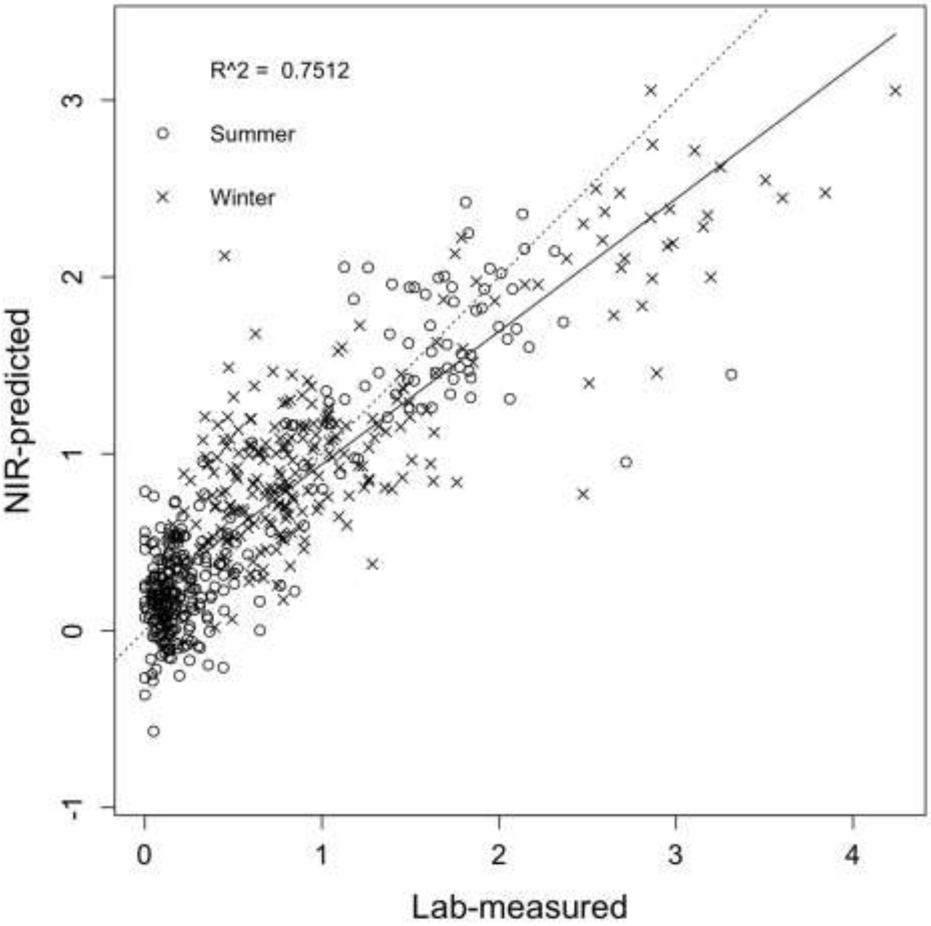

Figure 2.19 Partial least squares regression using near infrared spectroscopy (NIRS) to predict lab-measured total coumarin concentrations (µmol/g dw) in individual sagebrush (*Artemisia* spp.) at Magic Reservoir, Idaho, USA. The dotted line represents a 1:1 ratio.

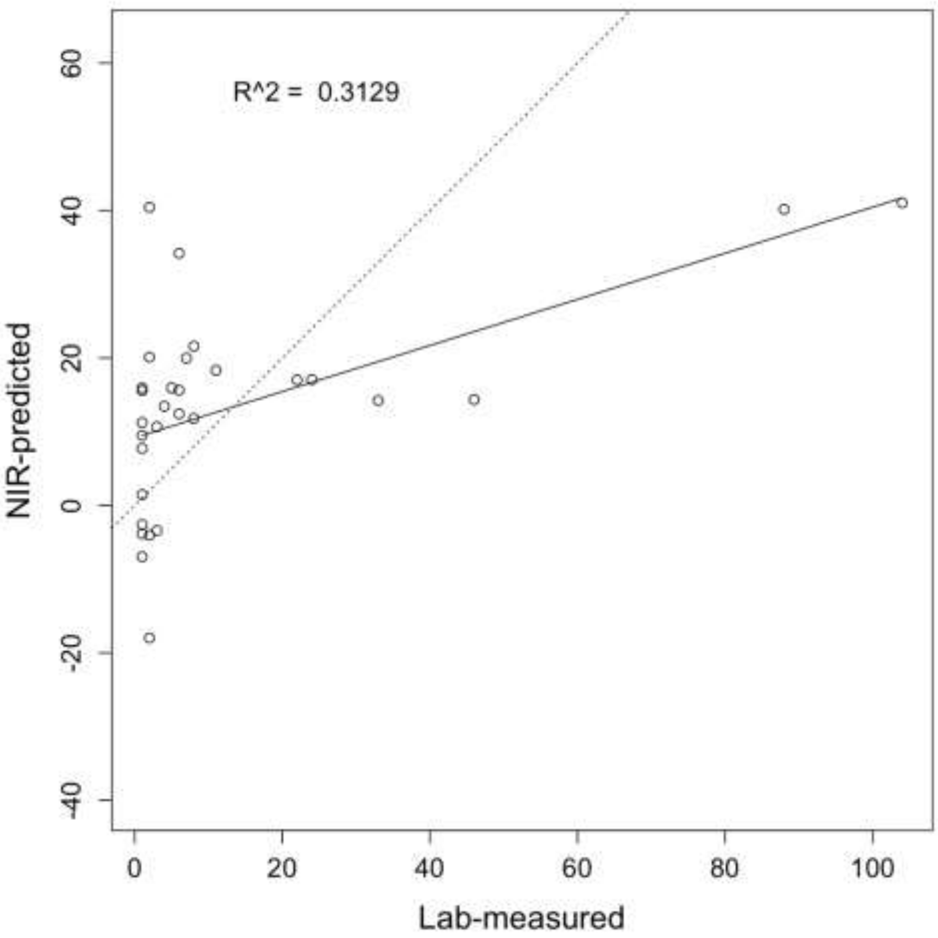

Figure 2.20 Partial least squares regression using near infrared spectroscopy (NIRS) to predict the intensity of browsing, in the form of bite mark counts by pygmy rabbits (*Brachylagus idahoensis*), on individual sagebrush (*Artemisia* spp.) at Magic Reservoir, Idaho, USA. The dotted line represents a 1:1 ratio.

GENERAL CONCLUSION

As climate change and anthropogenic disturbances continue to alter landscapes across our globe (Millennium Ecosystem Assessment and World Resources Institute, 2005), it is imperative that we find improved ways of measuring and monitoring these drastic changes. New technological advances are needed to rapidly detect these changes. One such technology is that of near infrared spectroscopy (NIRS), a form of remote sensing that provides unique spectral fingerprints of objects. Moreover, it does not require immediate contact with the objects, permitting comprehensive looks into expansive (e.g., global snow cover; Bormann et al., 2018), inaccessible (e.g., deep sea exploration; Platt et al., 1988; Klemas and Yan, 2014), and imperceptible places (e.g., inside geological features; van der Meer et al., 2012; Liang et al., 2014). NIRS has demonstrated to be a profound and robust tool in measuring a myriad of environmental parameters (Asner and Martin, 2008; Viana et al., 2009; Hogrefe et al., 2017), especially those that define the functional traits of plants across landscapes (Moore et al., 2010; Olsoy et al., 2016; Vance et al., 2016). Moreover, because NIRS can be done non-destructively, it can be repeated on the same plant to monitor changes in functional traits.

NIRS is already being used in numerous domestic and wild systems to measure, monitor, and restore habitats important to humans and wildlife. On a global scale, satellites equipped with NIR sensors are traveling around the planet on a regular basis and providing nitrogen, greenness (i.e., NDVI), and other biogeochemical and productivity measurements and imagery from jungles (Eckert, 2012; Bi et al., 2015), forests (Martin et al., 2008; Magney et al., 2019), grasslands (Kennedy, 1989; Zhou et al., 2019), deserts (St-Louis et al., 2009; Glenn et al., 2016), the arctic (Winther et al., 1999; Raynolds et al., 2008), and the atmosphere (Thompson et al., 2018, 2019). Temporal assessments have also been conducted using global NIRS data, such as 50 years' worth of terrestrial carbon cycling (Xiao et al., 2019) that informs large-scale long-term ecosystem changes. Regionally and locally, manned and unmanned aerial vehicles are doing the same at finer resolutions, including measuring spatiotemporally changing nitrogen content (Asner and Martin, 2009), nitrous oxide emissions (Soper et al., 2018), and biodiversity (Asner and Martin, 2009; Féret and Asner, 2014) in rainforests, monitoring health of horticultural crops (Griffel et al., 2018; Tu et al., 2018), mapping forage quality for domestic and wild herbivores in savannas (Skidmore et al., 2010), and detecting invasive weed encroachment (Lass et al., 2005). At population scales, low flying unmanned and terrestrial NIR sensors are being used in the field to monitor finer-scale habitat changes across parallel parameters, including species diversity (Mitchell et al., 2012a), chemical diversity (Aasen et al., 2018), and forage quality for wild herbivores (Olsoy et al., 2020).

Additionally, handheld NIR instruments in the lab are being used on samples collected across these spatiotemporal gradients to acquire high-resolution measurements in the associated systems (Au et al., 2020). This is seen through moisture content in leaf litter important to decomposition and biogeochemical cycles (Kim et al., 2017), root growth and production in regard to changing temperatures and carbon supplies (Wang et al., 2018), soil properties and organic matter determinant of soil quality for agricultural and biological systems (Romsonthi et al., 2018; Ludwig et al., 2018), and nutrient and toxin loads in forage important to wild herbivores (Vance et al., 2016). However, as important and informative as all of these NIRS applications are, they do not always reflect the entire picture by limiting species involved or focusing on model organisms, trading off between geographic or temporal scale and resolution, or simply missing the actual ecologically functional parameters that represent interacting species within communities across landscapes. Few studies have used NIRS to measure functional traits in habitats, such as changing phenotypes in relation to use by associated organisms. These measurements are not only functionally important to the dynamics and health of a habitat but represent the combination of all of the parameters, such as biogeochemical cycling and biodiversity, measured in the studies listed above. Assessing functional traits allows for the measurement of myopic or arbitrary parameters to be bypassed and can directly measure the phenotypes relative to our changing planet and interacting organisms. And understanding these interactions is particularly pressing in complex socioecological systems. The sagebrush-steppe ecosystem is one of those systems in peril due to a rapidly changing world and is in need of more efficient ways to measure and monitor functional traits that define species interactions.

The sagebrush-steppe ecosystem, spread across 43 million hectares of the Western United States, remains a highly threatened biome, with over 50% of its lands lost historically (Welch, 2005). The chemically, structurally, and functionally diverse phenotypes across this ecosystem house a multitude of vertebrate and invertebrate herbivores (Welch, 2005), including those with rapidly declining and threatened populations, such as pygmy rabbits (*Brachylagus idahoensis*; Ulappa et al., 2014; Utz et al., 2016; Nobler et al., 2019) and greater sage-grouse (*Centrocercus urophasianus;*

Ulappa, 2011; Frye et al., 2013; Fremgen-Tarantino et al., 2020). These two species rely on sagebrush wholly for occupancy and reproduction and for about 50% of their diet in summer and up to 100% in winter (Wallestad and Eng, 1975; Green and Flinders, 1980). However, they select specific sagebrush species for nesting (Severson et al., 2017; Zabihi et al., 2019) and feeding, and even select within species for specific concentrations of phytochemicals (Frye et al., 2013; Ulappa et al., 2014; Utz et al., 2016; Nobler et al., 2019; Fremgen-Tarantino et al., 2020). Therefore, procuring better ways to measure and monitor these complex and diverse phenotypes, specifically chemotypes, remains paramount to successful management of these threatened herbivores.

Our research reveals the proficiency of NIRS to classify and predict diverse, functional, and critical phenotypes across the sagebrush-steppe. Results indicate that NIRS can accurately classify sagebrush taxonomic and phenological phenotypes. As the chemical similarity of sagebrush phenotypes increased from species and geographically distinct populations within a species to subtle foraging decisions by herbivores between individual plants within a population, discrimination among phenotypes became less clear until, at times, indistinguishable. One exciting outcome is that identification of misclassifications from NIRS may indicate hybridizations, histories of disturbance, or unique climatic conditions at a site that offers important insight when monitoring habitats. Additionally, our results demonstrate that NIRS can effectively predict concentrations of nutrients and phytochemicals, in the forms of both classes and individual compounds, in sagebrush. Detecting and quantifying these chemical mixtures and concentrations can determine sagebrush patches and populations suitable to herbivore species known to be selective about the chemical types and doses they consume

(Sorensen et al., 2006; Nobler et al., 2019; Patey et al., 2020). However, when assessing the capacity for NIRS to predict herbivory, in the form of categorical browsing (i.e., yes or no) or intensity of browsing (i.e., bite marks), NIRS models were not reliable. This can likely be attributed to sample size (Au et al., 2020), range of measured values (Au et al., 2020), or spatiotemporal dynamics of sagebrush chemistry in relation to browsing (Karban et al., 2016b). Despite this limitation, NIRS predictions can benefit land managers and researchers in measuring and monitoring sagebrush habitats important to both humans and wildlife by providing baseline assessments of habitat conditions and quality, taxonomic and chemical compositions, distributions, and abundances, and past or present biotic and abiotic stressors. NIRS can also direct land managers and researchers to plants and populations palatable to herbivores, with distinctive and noteworthy phenotypic traits, or high chemical, structural, and potential genetic diversity (Welch, 2005; Richardson et al., 2012; Turi et al., 2014; Jaeger et al., 2016). Moreover, NIRS can be used to help restore disturbed habitats by identifying unique phenotypic traits in remnant stands versus new growth and reseeding habitats with these traits.

Finally, although known to be precise and reliable, remote sensing is not without its flaws. Sources of error exist at each stage of the process that must be taken into consideration when analyzing and interpreting results. Sources of error can stem from sample and spectral collections, data storage and manipulation, and data processing and analysis. To ensure results are grounded in reality, it is important to establish confidence in the reliability of the data at each step by assessing proper instrument calibration and reproducibility, visualizing spectral scans and frequency distributions of variables, and performing outlier analyses. For example, we tested each of these areas as potential explanations for the lack of predictive accuracy at Raft River. We determined sample collection was not an issue because samples were collected similarly by the same researcher to those at Craters, which produced highly effective predictions. We verified NIRS instrument calibration and reproducibility by comparing spectral scans and transformations with other field sites, and inspections of means and variation were consistent with other populations (Figure A.4). Tests of frequency distributions and means of chemical variables, alongside outlier analyses, also resulted in similar outcomes between Raft River and other field sites (Table G.1; Figures H.1-H.2, H.4-H.10), except for total phenolics (Figure H.3), which was the only chemical that NIRS was able to predict relatively well at Raft River. The only other dissimilarity between Raft River and the other field sites was observed upon inspection of the spectral variables that most influence NIRS predictions (Figure A.8). Further investigations of these dissimilarities may help to explain some of our unreliable or inaccurate predictions at Raft River. However, if similar error tests corroborate reliability of the data, surprising or inexplicable analysis outcomes may be due to biotic or abiotic conditions—such as insect galls, microbes, fungi, or moisture—masking phytochemical detection in plant material. Therefore, these confounding factors must also be taken into account when interpreting and explaining remote sensing outcomes.

Our aim was to explore and champion a pioneering technology, NIRS, in monitoring plant and animal populations in a changing landscape, and our research demonstrates the capacity for which it can be done. Moreover, it is not enough to just monitor these changes, we need to understand the mechanisms by which the changes are occurring, and NIRS can measure some of the functional mechanisms underlying changing plant communities. NIRS has been shown to determine functional phenotypes, as directed by chemical composition, in an extremely complex and dynamic wild system—individual sagebrush plants and populations—important to both wildlife (Welch, 2005) and humans (Kelley et al., 1992). NIRS remains a powerful, reliable, and expeditious technology in discriminating, describing, and predicting components of sagebrush crucial to the effective management of sagebrush-steppe landscapes. Furthermore, integrating NIRS outcomes with traditional (e.g., morphology), advanced (e.g., metabolomics and genomics), and combinatorial methods will further illuminate and help monitor the novel chemical interactions responsible for the morphology, physiology, and demographics of plant and animal communities. If we hope to effectively manage the changing trajectories of our natural lands facing rampant climate change and anthropogenic disturbance, we must find and employ new tools that better allow us to monitor these changes. This is already being done in other systems where NIRS is being used to measure changing terrestrial carbon cycles (Xiao et al., 2019), soil properties and organic matter (Romsonthi et al., 2018; Ludwig et al., 2018), root growth and production (Wang et al., 2018), horticultural crop health (Griffel et al., 2018; Tu et al., 2018), and nitrous oxide emissions (Soper et al., 2018). The synthesis of these studies and our results provides powerful promise of the functional and invaluable use of NIRS in monitoring global landscape change.

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APPENDIX A

Near Infrared Spectra, Transformations, and Regression Coefficients Within and Among Field Sites

Near Infrared Spectra, Transformations, and Regression Coefficients Within and Among Field Sites

Near infrared spectroscopy (NIRS) uses electromagnetic energy from organic and inorganic bonds in objects to measure reflectance values, which result in unique spectral profiles for objects (Rast and Painter, 2019). The NIR electromagnetic spectrum ranges from 350 to 2500 nm, which is particularly suited for detecting organic bonds (Curran, 1989; Youngentob et al., 2012). This substantiates NIRS as a reliable proxy for chemical determination in natural objects, such as plants and shrubs (Vance et al., 2016). NIRS has been shown, through the detection of chemotypes, to classify genotypes (Amar et al., 2009; Gebreselassie et al., 2017), morphotypes (Viana et al., 2009; Hetta et al., 2017; Martínez-Valdivieso et al., 2018), and foraging behavior of herbivores (McIlwee et al., 2001; Siitari et al., 2002; Moore et al., 2010). NIRS can also quantify concentrations of chemicals in natural objects. Research has demonstrated NIRS measuring nitrogen and phytochemical content in domestic feed (Abrams et al., 1987; Mnisi and Mlambo, 2017; Saha et al., 2018), fruits (Baranska et al., 2004; Sinelli et al., 2008), wines (Urbano-Cuadrado et al., 2004; Cozzolino et al., 2008), and wild systems, including jungles (Foley et al., 1998; Wiedower et al., 2009; Féret and Asner, 2014), grasslands (Griggs et al., 1999; Brunet et al., 2007), deserts (Russell et al., 2012; Vaknin and Mishal, 2017), and taiga (Stolter et al., 2006). Our research used NIRS to classify chemotypes of sagebrush in different species and sites in Idaho, along with quantifying concentrations of chemicals within sagebrush.

To develop the models for NIR spectra to classify and predict these chemotypes, we incorporated standard spectral and mathematical transformations to the spectra. Raw spectral profiles, collected from an ASD FieldSpec® 4 spectroradiometer, were measured in reflectance units (Figure A.1). These values were then converted to absorbance units via a log transformation, $\log_{10}(1/R)$, where R was reflectance (Figure A.2). Absorbance spectra then underwent a standard first gap derivative transformation (Figure A.3). Resulting spectra were truncated to 450-2350nm to remove noise from the NIR sensor. Classification and predictive models on sagebrush chemotypes were then performed using these transformed spectra. Differences in spectral profiles can be seen among sagebrush field sites (Figure A.4). Variables that most contributed to explaining the models can also be seen for each chemical class across some of the field sites (Figures A.5-A.11).

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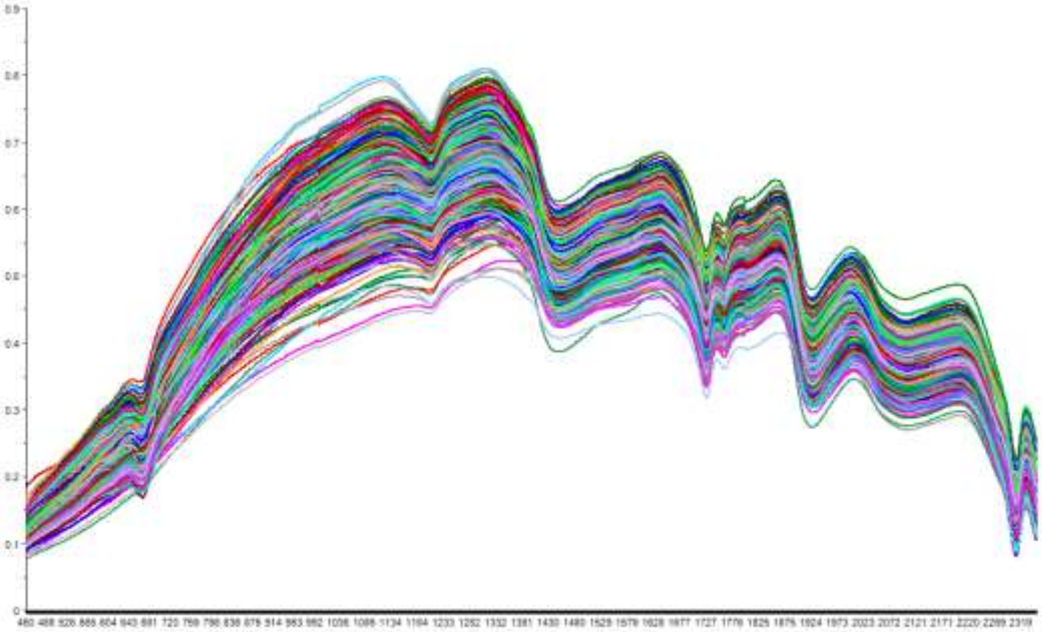

Figure A.1 Example of near infrared reflectance values of sagebrush samples collected using an ASD FieldSpec® 4 spectroradiometer and visualized on Camo Analytics Unscrambler®. The X-axis is the electromagnetic spectrum from 450-2350 (nm) and the Y-axis is the proportion of reflectance out of 1.0, where 1.0 is 100% reflectance.

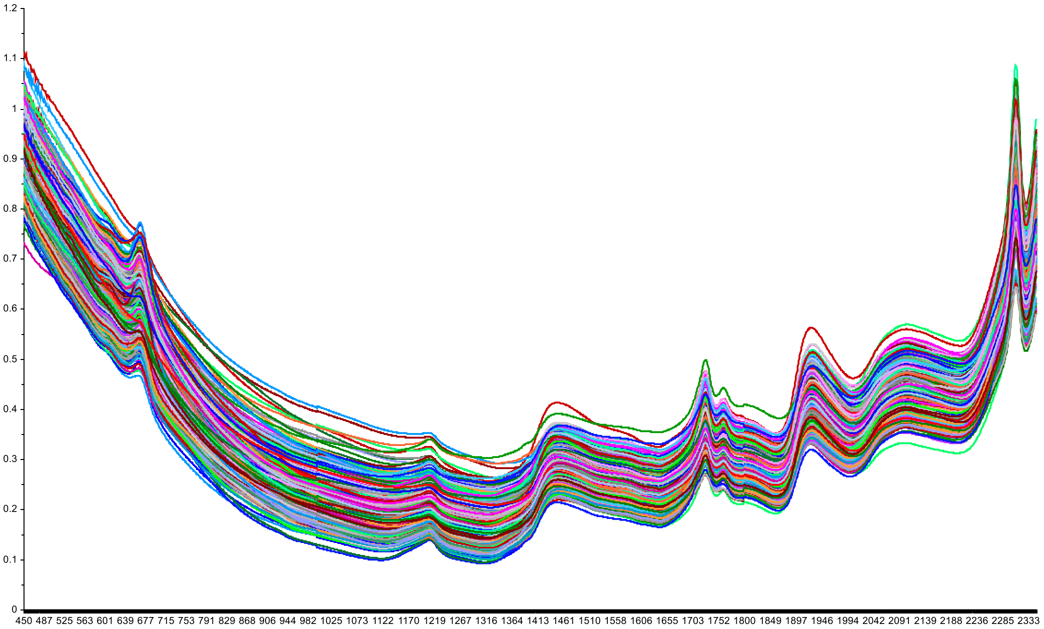

Figure A.2 Example of near infrared absorbance values ($\log_{10}(1/R)$ transformation of reflectance (R)) of sagebrush samples collected using an ASD FieldSpec® 4 spectroradiometer and visualized on Camo Analytics Unscrambler®. The X-axis is the electromagnetic spectrum from 450-2350 (nm) and the Y-axis is absorbance units ($-\log_{10}R$).

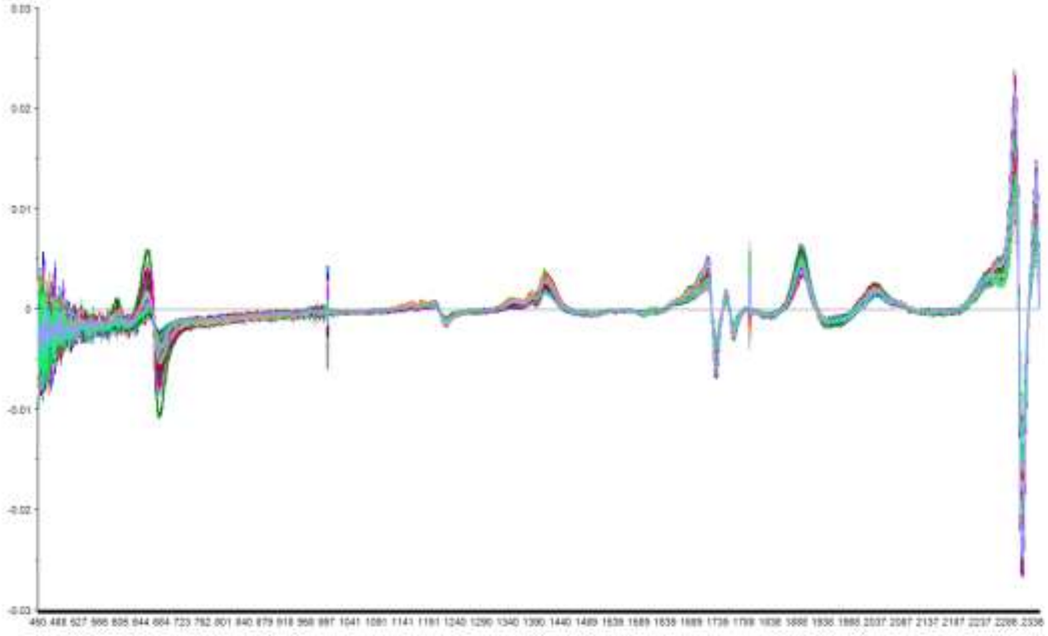

Figure A.3 Example of standard first derivative reflectance calculated on near infrared absorbance values of sagebrush samples collected using an ASD FieldSpec® 4 spectroradiometer and visualized on Camo Analytics Unscrambler®. The X-axis is the electromagnetic spectrum from 450-2350 (nm) and the Y-axis is the first derivative of absorbance units (-log$_{10}$*R*).

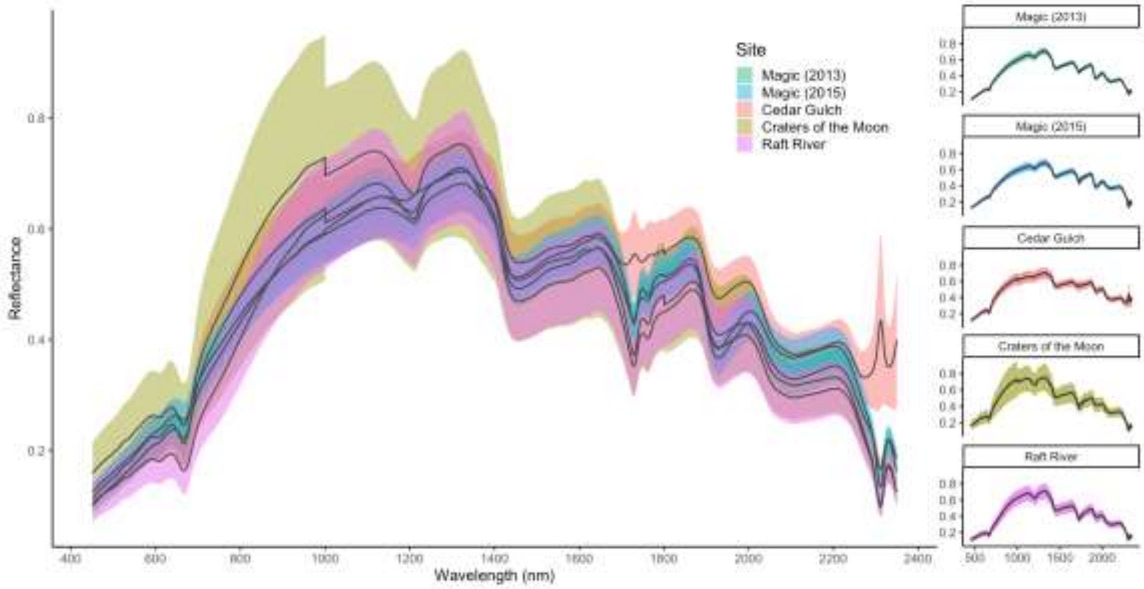

Figure A.4 **Example of variation in near infrared reflectance values of sagebrush samples across four field sites and two years in Idaho, USA, collected using an ASD FieldSpec® 4 spectroradiometer. Solid black lines represent means and color ribbons represent confidence intervals. The year listed in parentheses after “Magic” represents year of sample collection.**

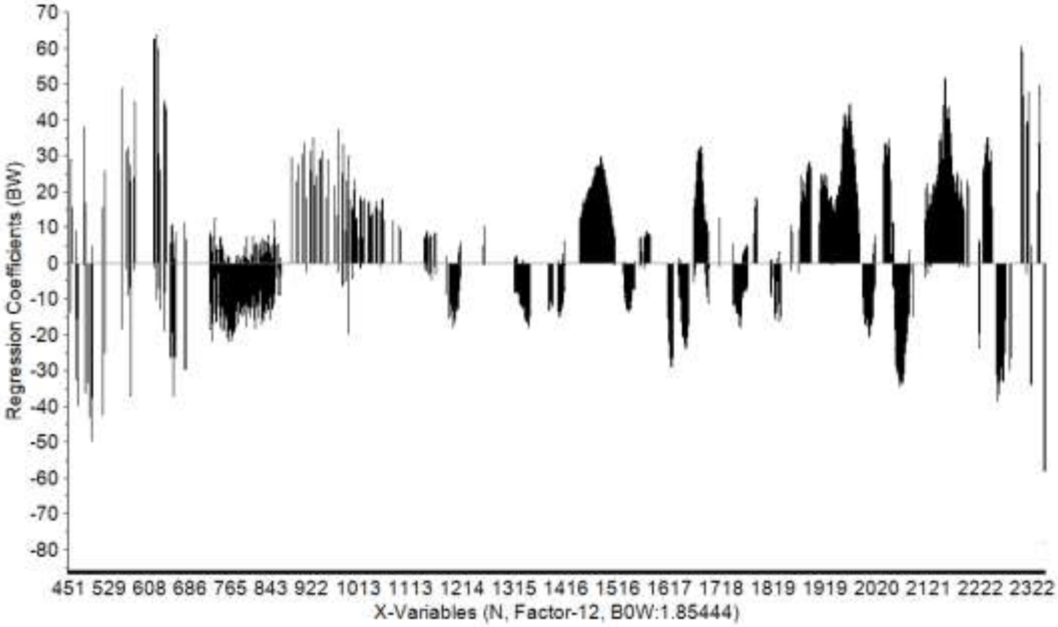

Figure A.5 Relative influence of spectral variables from the partial least squares regression (PLSR) analysis predicting nitrogen at Magic Reservoir, Idaho, USA during 2014-2015. The x-axis is the electromagnetic spectrum (nm) and the y-axis is the relative influence of the weighted beta regression coefficients. The higher the black bar, the greater the influence that spectral variable has in explaining nitrogen in the model. Bars above the zero line represent positive predictive relationships and bars below the zero line represent negative predictive relationships.

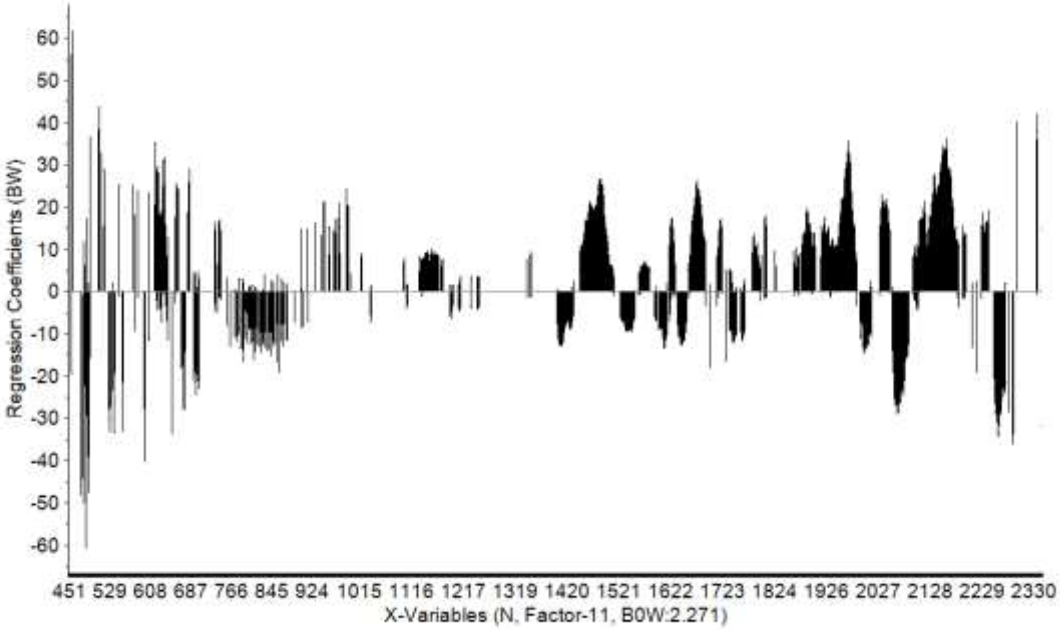

Figure A.6 Relative influence of spectral variables from the partial least squares regression (PLSR) analysis predicting nitrogen at Cedar Gulch, Idaho, USA. The x-axis is the electromagnetic spectrum (nm) and the y-axis is the relative influence of the weighted beta regression coefficients. The higher the black bar, the greater the influence that spectral variable has in explaining nitrogen in the model. Bars above the zero line represent positive predictive relationships and bars below the zero line represent negative predictive relationships.

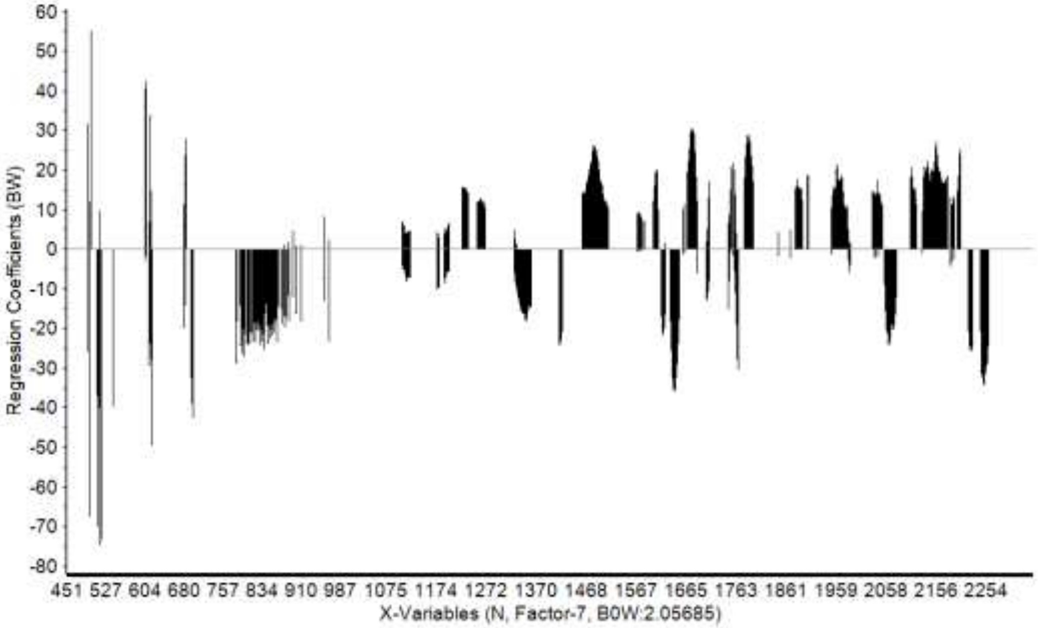

Figure A.7 Relative influence of spectral variables from the partial least squares regression (PLSR) analysis predicting nitrogen at Craters of the Moon, Idaho, USA. The x-axis is the electromagnetic spectrum (nm) and the y-axis is the relative influence of the weighted beta regression coefficients. The higher the black bar, the greater the influence that spectral variable has in explaining nitrogen in the model. Bars above the zero line represent positive predictive relationships and bars below the zero line represent negative predictive relationships.

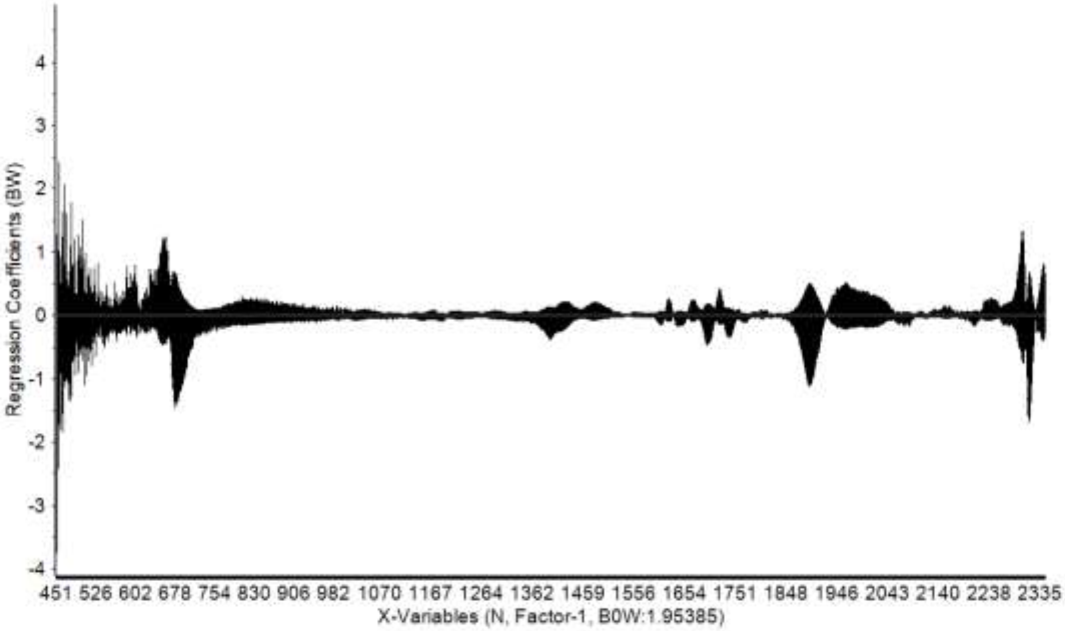

Figure A.8 Relative influence of spectral variables from the partial least squares regression (PLSR) analysis predicting nitrogen at Raft River, Idaho, USA. The x-axis is the electromagnetic spectrum (nm) and the y-axis is the relative influence of the weighted beta regression coefficients. The higher the black bar, the greater the influence that spectral variable has in explaining nitrogen in the model. Bars above the zero line represent positive predictive relationships and bars below the zero line represent negative predictive relationships.

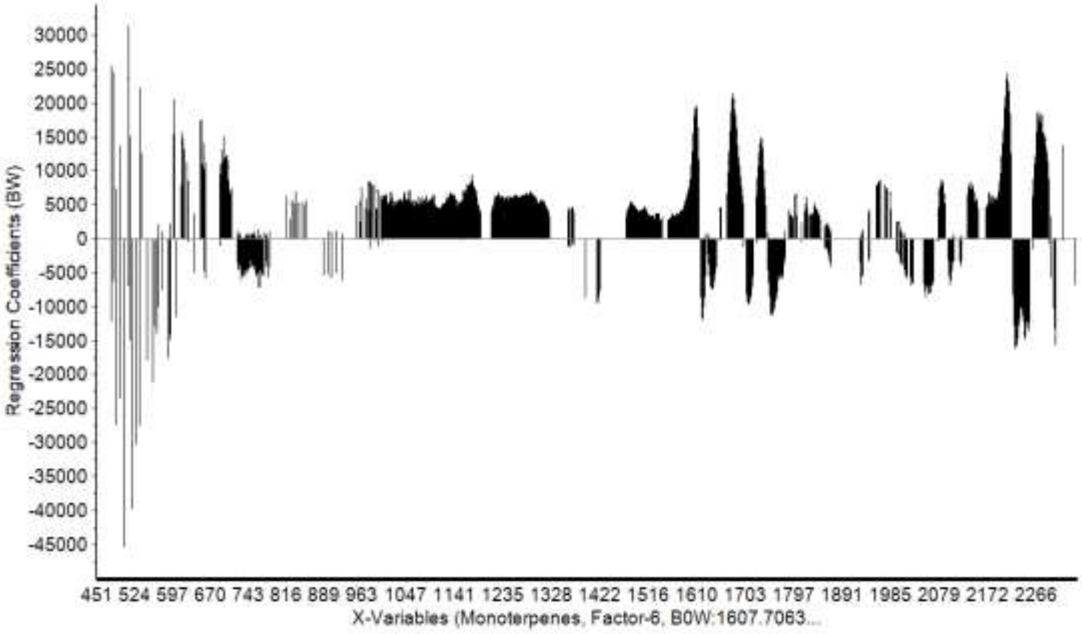

Figure A.9 Relative influence of spectral variables from the partial least squares regression (PLSR) analysis predicting total monoterpenes at Magic Reservoir, Idaho, USA during 2014-2015. The x-axis is the electromagnetic spectrum (nm) and the y-axis is the relative influence of the weighted beta regression coefficients. The higher the black bar, the greater the influence that spectral variable has in explaining nitrogen in the model. Bars above the zero line represent positive predictive relationships and bars below the zero line represent negative predictive relationships.

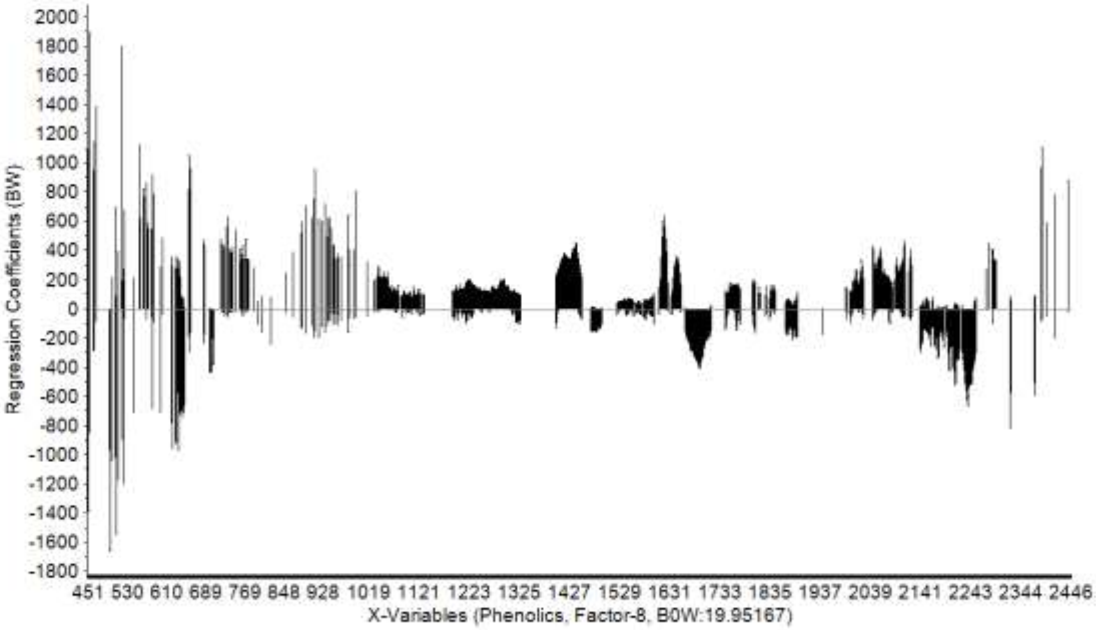

Figure A.10 Relative influence of spectral variables from the partial least squares regression (PLSR) analysis predicting total phenolics at Magic Reservoir, Idaho, USA during 2014-2015. The x-axis is the electromagnetic spectrum (nm) and the y-axis is the relative influence of the weighted beta regression coefficients. The higher the black bar, the greater the influence that spectral variable has in explaining nitrogen in the model. Bars above the zero line represent positive predictive relationships and bars below the zero line represent negative predictive relationships.

Figure A.11 Relative influence of spectral variables from the partial least squares regression (PLSR) analysis predicting total coumarins at Magic Reservoir, Idaho, USA during 2014-2015. The x-axis is the electromagnetic spectrum (nm) and the y-axis is the relative influence of the weighted beta regression coefficients. The higher the black bar, the greater the influence that spectral variable has in explaining nitrogen in the model. Bars above the zero line represent positive predictive relationships and bars below the zero line represent negative predictive relationships.

APPENDIX B

Additional Phenotype Classifications Using NIRS

Additional Phenotype Classifications Using NIRS

Near infrared spectroscopy (NIRS) was tested for the accuracy by which it could classify phenotypes (i.e., chemotypes) of sagebrush species and field sites across years and seasons, as well as categorical representation of herbivore browsing (i.e., browsed vs. non-browsed) at one field site in Idaho, USA, by pygmy rabbits (*Brachylagus idahoensis*). Results in Chapter 1 suggest a general reliability of NIRS to classify sagebrush phenotypes at these scales of increasing similarity, however relevant and more thoroughly controlled analyses were conducted that were not discussed in Chapter 1 and are presented here. The first analysis included the classification of phenology (both years and seasons) within sagebrush populations and species. Measuring phenology is important because it represents the timing of emergence and senescence of sagebrush leaves, which affects the habitat use and survival of associated herbivores. At our Magic Reservoir field site, when controlling for species (i.e., Wyoming big sagebrush, *A. t. tridentata*), years of sample collection were classified accurately 91.95% of the time (Table 1.7). However, samples included both winter and spring/summer collections, so when controlling for season (i.e., winter only) and species, results decreased to 77.87% (Table B.1). This is likely attributed to sample size. At our Magic Reservoir field site, we also assessed the accuracy by which NIRS could classify herbivore browsing, which is a highly relevant indicator of herbivore habitat use and quality of habitat. When controlling for species (i.e., Wyoming big sagebrush), browsing was classified accurately 60.08% of the time (Table 1.9). However, samples included both winter and spring collections, so when controlling for season (i.e., winter vs. spring) one at a time, every plant was consistently classified as non-browsed except for one (Tables B.2-B.3). Results indicate that being more inclusive, which also increased the samples size, provided better results.

Table B.1 Confusion matrix from support vector machine using near infrared spectroscopy to predict distinct years of collection of Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) at Magic Reservoir, Idaho, USA during winter.

	Actual					Overall Accuracy (%)	
	Class Name	Y2013	Y2015	Total	User Accuracy (%)	Training	Validation
Predicted	Y2013	92	52	144	63.89	77.87	77.87
	Y2015	0	91	91	100		
	Total	92	143	235			
	Producer Accuracy (%)	100	63.64				

Table B.2 Confusion matrix from support vector machine using near infrared spectroscopy to predict foraging behavior of pygmy rabbits (*Brachylagus idahoensis*) on Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) in Magic Reservoir, Idaho, USA between 2012-2013 during winter.

	Actual					Overall Accuracy (%)	
	Class Name	Browsed	Non-browsed	Total	User Accuracy (%)	Training	Validation
Predicted	Browsed	0	0	0	100	52.75	48.35
	Non-browsed	43	48	91	52.75		
	Total	43	48	91			
	Producer Accuracy (%)	0	100				

Table B.3 Confusion matrix from support vector machine using near infrared spectroscopy to predict foraging behavior of pygmy rabbits (*Brachylagus idahoensis*) on Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) in Magic Reservoir, Idaho, USA between 2012-2013 during spring.

		Actual				Overall Accuracy (%)	
	Class Name	Browsed	Non-browsed	Total	User Accuracy (%)	Training	Validation
Predicted	Browsed	0	1	1	0	56.85	57.11
	Non-browsed	169	224	393	57		
	Total	169	225	394			
	Producer Accuracy (%)	0	99.56				

APPENDIX C

Representative Chromatograms for Sagebrush Taxa and Hybridizations

Representative Chromatograms for Sagebrush Taxa and Hybridizations

The sagebrush field sites that were investigated for how well NIRS could classify and predict chemotypes in sagebrush had varying degrees of taxonomic and chemical diversity. Chromatograms from gas chromatography serve as an excellent visualization for this diversity. Gas chromatography operates by separating volatile compounds in samples and quantifying their relative concentrations. Sagebrush is known to have a high abundance and diversity of volatile compounds known as monoterpenes (Turi et al., 2014), which are often identifiers of speciation and herbivore use (Frye et al., 2013; Turi et al., 2014; Olsoy et al., 2020). We used the output chromatograms from this chemical analysis technique to demonstrate chemical variation within and among sagebrush species found in varying compositions at each field site. Each peak in the chromatogram represents a different compound and the size of the peak, also known as the area under the curve (AUC), represents the relative concentration. Each sagebrush sample was quantified accordingly and standardized for by its dry weight (dw). The chemical (i.e., monoterpene) profiles for Wyoming big (*A. t. tridentata*), three-tip (*A. tripartita*), black (*A. nov*a), and low (*A. arbuscula*) sagebrush are displayed in Figure C.1 from bottom to top, respectively. Our results indicate that NIRS could serve as an identifier of taxonomically and chemically complex sagebrush plants and sites in Idaho.

References

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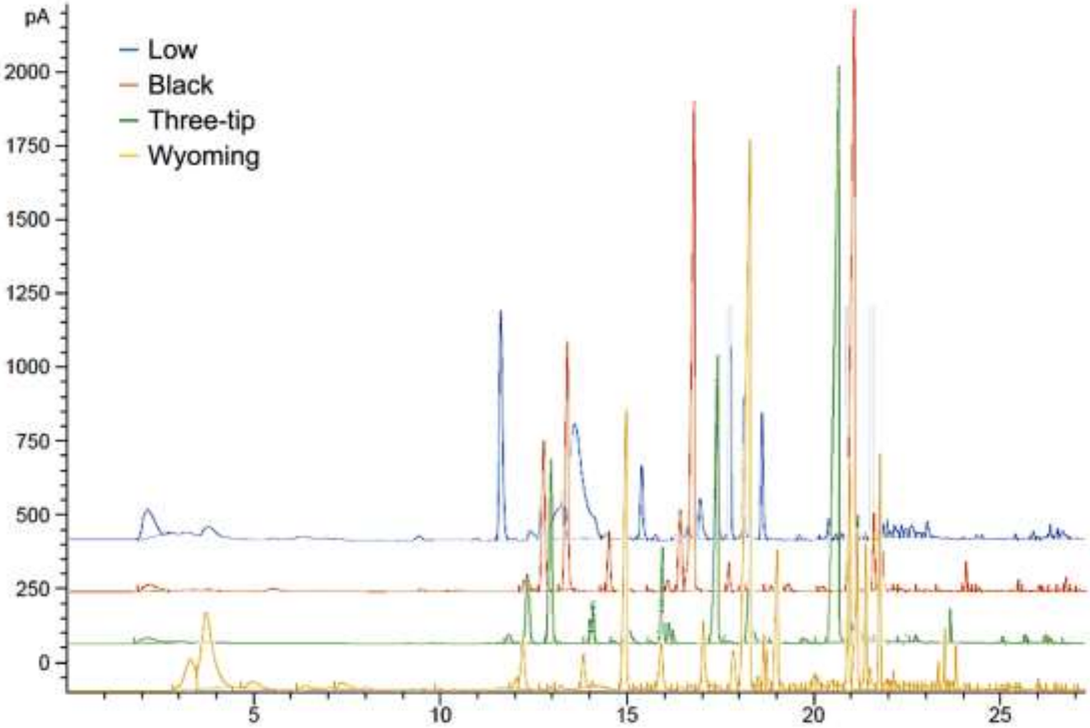

Figure C.1 Representative monoterpene profiles from gas chromatography for Wyoming big (*Artemisia tridentata wyomingensis*; Magic Reservoir, Cedar Gulch, Craters of the Moon, Raft River), three-tip (*Artemisia tripartita*; Magic Reservoir, Craters of the Moon), black (*Artemisia nova*; Cedar Gulch), and low (*Artemisia arbuscula*; Raft River) sagebrush (Table 1.1), from bottom to top, respectively, from Idaho, USA. X-axis is retention time (min) and Y-axis is the relative abundance of the compounds (pA). These chemical profiles demonstrate the chemical diversity among sagebrush taxa.

APPENDIX D

Dose-Dependent Selection Thresholds of Plant Compounds by Herbivores

Dose-Dependent Selection Thresholds of Plant Compounds by Herbivores

Herbivores respond differentially to chemicals just as humans do. And the pharmacological effect of the chemical is mediated by the dose the herbivore consumes (Sorensen et al., 2006; Forbey and Foley, 2009; Patey et al., 2020). The chemicals that herbivores consume from plants can be both noxious and therapeutic to their health (Sorensen et al., 2006; Patey et al., 2020). Due to this, herbivores are selective not only about the types of chemicals they consume but also the dose (Sorensen et al., 2006; Forbey and Foley, 2009; Patey et al., 2020). These selective responses produce dose-dependent thresholds at which herbivores make foraging decisions (Frye et al., 2013; Melody, 2017; Nobler et al., 2019). For example, greater sage-grouse (*Centrocercus urophasianus*) positively select to forage on sagebrush with increasing nutritional content (Figure D.1), however they do not avoid sagebrush with a particular toxin, 1,8-cineole, until it reaches a certain threshold (Figure D.2). The same can be seen in pygmy rabbits (*Brachylagus idahoensis*) in regard to the tradeoff between nutrient and toxin (e.g., monoterpene) content (Olsoy, 2019; Figure D.3). Understanding these dose-dependent selection thresholds and tradeoffs coupled with using NIRS to rapidly predict those doses in sagebrush (Chapter 2) will better allow us to manage plant-herbivore interactions across landscapes.

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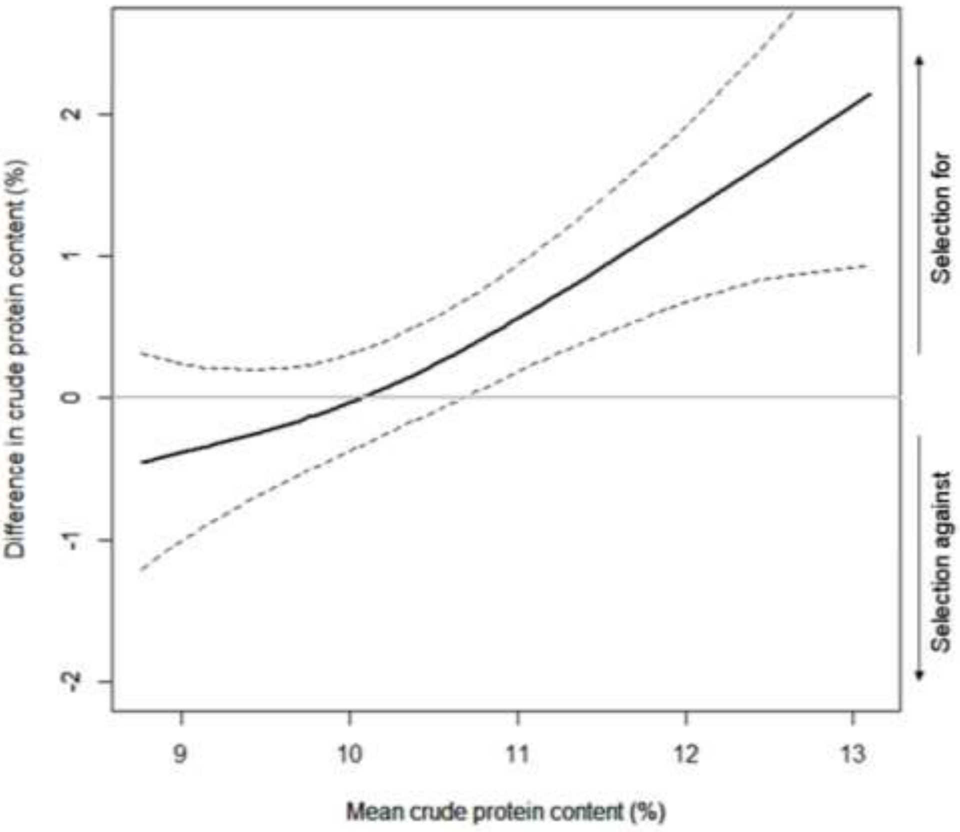

Figure D.1 The dose-dependent selection threshold of crude protein (% dw) by greater sage-grouse (*Centrocercus urophasianus*) in Southern Idaho, USA (taken from Frye (2012)). The difference in crude protein content is calculated using paired browsed and non-browsed sagebrush (*Artemisia* spp.) samples as a function of mean crude protein content within foraging patches. The fitted black line and 95% confidence intervals were derived from generalized additive models. The zero line represents crude protein concentrations at which no selection by sage-grouse is occurring. Values above the zero line indicate higher crude protein content in plants browsed by sage-grouse than those not browsed (i.e., selection for crude protein) and values below the zero line indicate higher content in non-browsed plants (i.e., selection against crude protein).

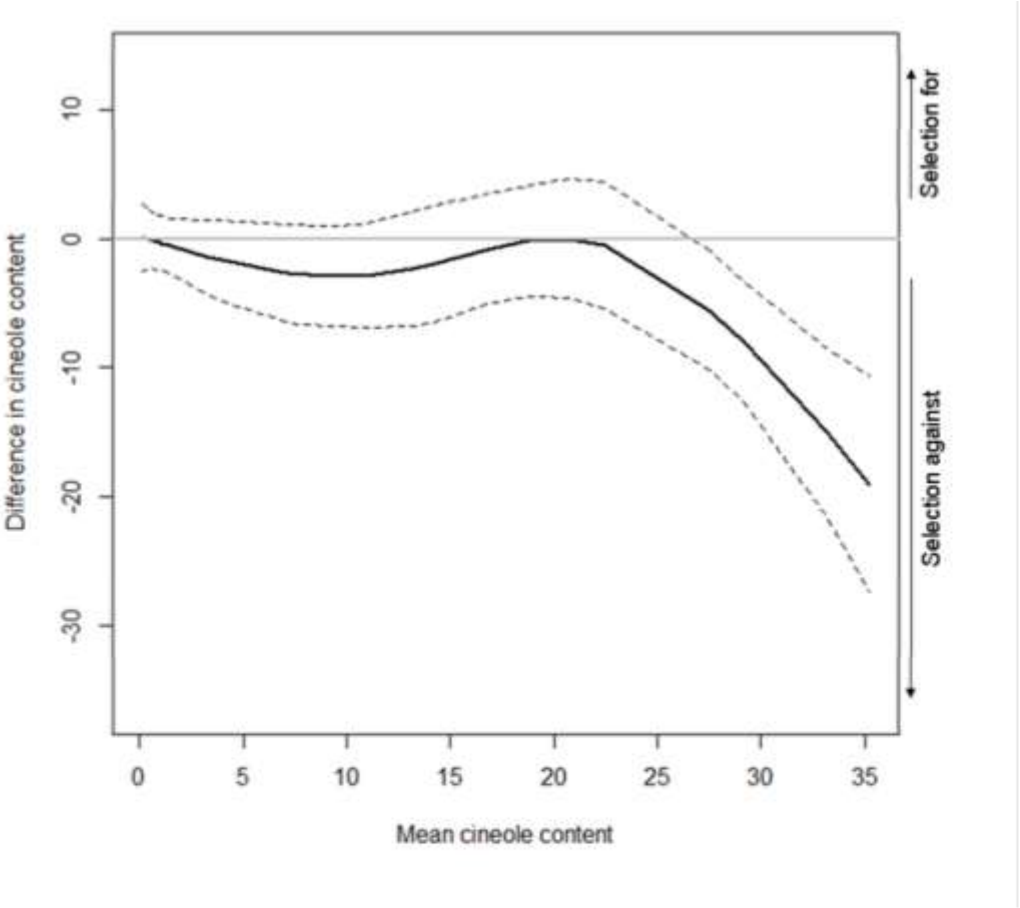

Figure D.2 The dose-dependent selection threshold of the phytochemical, 1,8-cineole (AUC/100 µg dw), by greater sage-grouse (*Centrocercus urophasianus*) in Southern Idaho, USA (taken from Frye (2012)). The difference in cineole content is calculated using paired browsed and non-browsed sagebrush (*Artemisia* spp.) samples as a function of mean cineole content within foraging patches. The fitted black line and 95% confidence intervals were derived from generalized additive models. The zero line represents cineole concentrations at which no selection by sage-grouse is occurring. Values above the zero line indicate higher cineole content in plants browsed by sage-grouse than those not browsed (i.e., selection for cineole) and values below the zero line indicate higher content in non-browsed plants (i.e., selection against cineole).

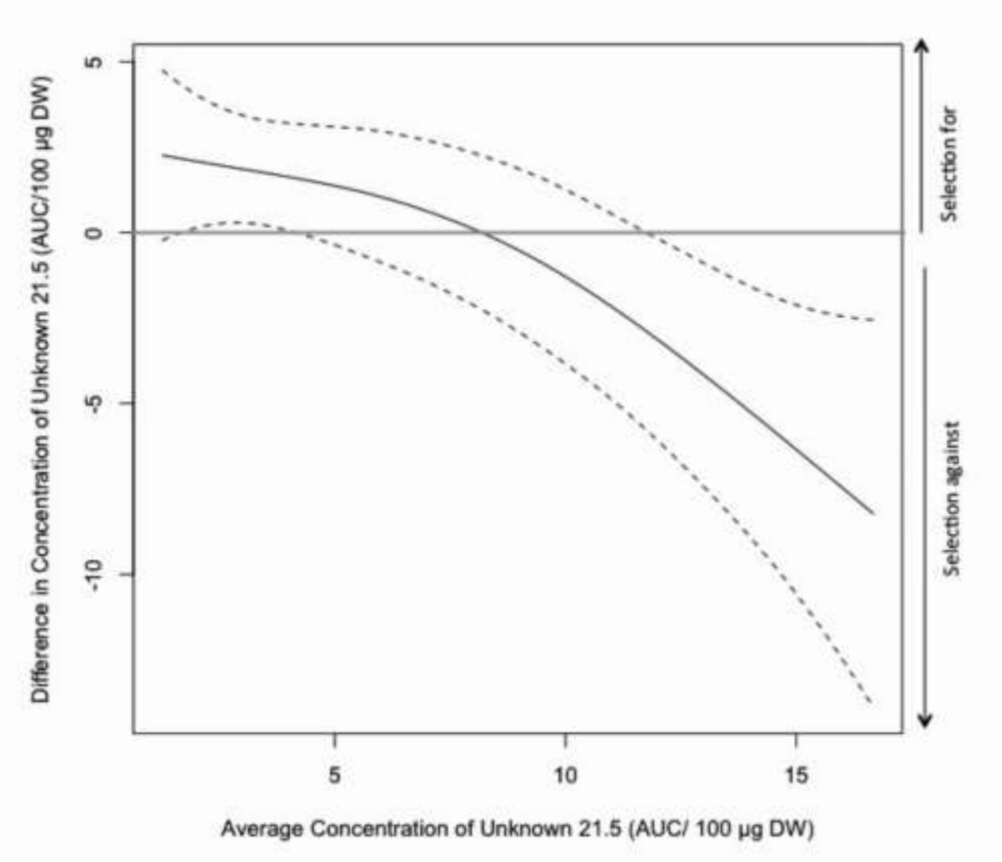

Figure D.3 The dose-dependent selection threshold of the phytochemical, Unknown 21.5 min (AUC/100 µg dw), by greater sage-grouse (*Centrocercus urophasianus*) in Southern Idaho, USA (taken from Fremgen (2015)). The difference in Unknown 21.5 content is calculated using paired browsed and non-browsed sagebrush (*Artemisia* spp.) samples as a function of mean Unknown 21.5 content within foraging patches. The fitted black line and 95% confidence intervals were derived from generalized additive models. The zero line represents Unknown 21.5 concentrations at which no selection by sage-grouse is occurring. Values above the zero line indicate higher Unknown 21.5 content in plants browsed by sage-grouse than those not browsed (i.e., selection for Unknown 21.5) and values below the zero line indicate higher content in non-browsed plants (i.e., selection against Unknown 21.5).

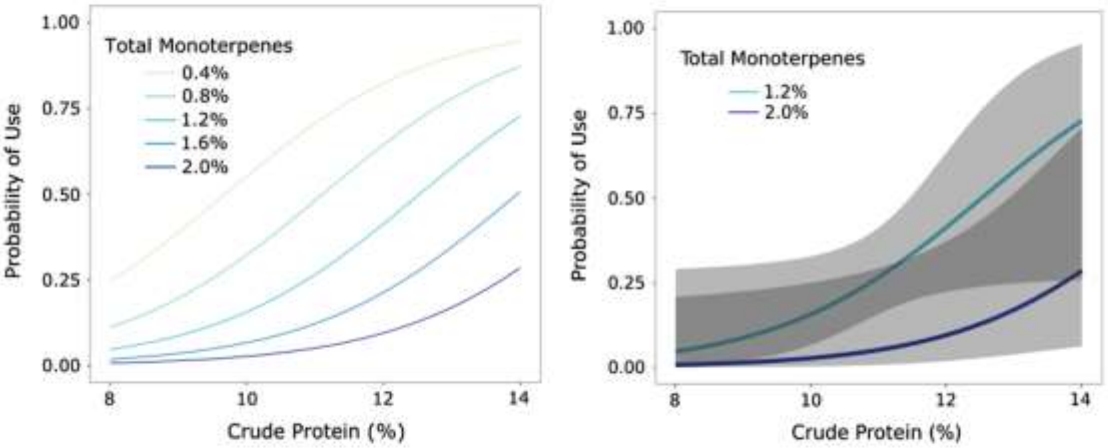

Figure D.4 The dose-dependent selection threshold of phytochemicals in the form of increasing concentrations of total monoterpenes (AUC/mg dw) against increasing concentrations of crude protein (% dw) by pygmy rabbits (*Brachylagus idahoensis*) in Magic Reservoir, Idaho, USA (Olsoy, 2019). Probability of pygmy rabbit use increases with higher crude protein content but lower total monoterpene content in sagebrush (*Artemisia* spp.).

APPENDIX E

External Near Infrared Spectroscopy Analysis

External Near Infrared Spectroscopy Analysis

In agriculture and food sciences, near infrared spectroscopy (NIRS) analyses are often outsourced to professional labs, such as Dairy One Forage Laboratory in Ithaca, NY, USA. NIRS analyses are then conducted under highly controlled laboratory conditions using advanced instrumentation, such as FOSS™ NIRS instruments. These conditions are conducive to developing strong predictive models (e.g., $R^2 > 0.97$), establishing Dairy One as a golden standard in the field for NIRS chemical analysis. To compare our in-house NIRS nitrogen predictions to this golden standard, we sent all of our Magic Reservoir sagebrush samples to Dairy One to measure nitrogen content on their equipment and receive a calibration equation from their internal WinISI™ software. Their analysis produced an R^2 of 0.93 ($SEC = 0.07$; Figure E.1), as compared to our ASD FieldSpec® 4 NIRS results of $R^2 = 0.86$ ($SEC = 0.09$). This demonstrates comparable predictions of nitrogen in wild plant systems using our in-house equipment and protocol.

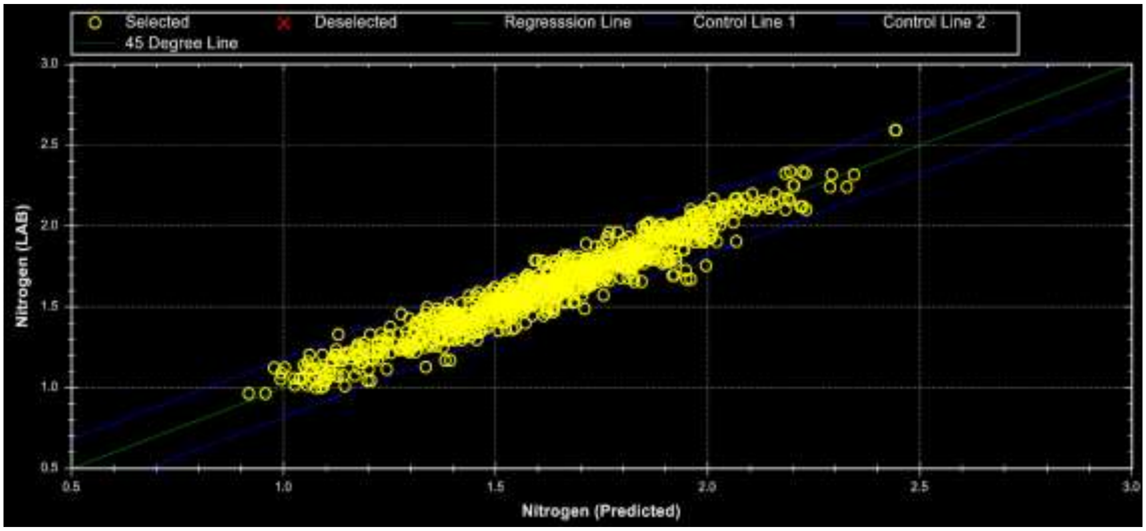

Figure E.1 Nitrogen predicted values and calibration equation of individual sagebrush samples scanned at Dairy One Forage Laboratories (Ithaca, NY, USA). Samples were collected from Magic Reservoir, Idaho, USA in 2014-2015 and prepped according to methods listed in Chapter 2, then shipped to Dairy One and scanned on their FOSS NIRS™ System II instrument. Resulting spectra were log transformed and the 2nd derivative was taken, and calibration equations (n = 489, R^2 = 0.9283, *SEC* = 0.0728) were developed using the FOSS WinISI™ 4 chemometric calibration software.

APPENDIX F

Measuring Individual Phenolics and Coumarin Content for Herbivore Palatability

Measuring Individual Phenolics and Coumarin Content for Herbivore Palatability

Phenolics are a class of phytochemicals known for antiseptic, endocrine, and nutrient-inhibiting properties (Acamovic and Brooker, 2005; Turi et al., 2014; Marsh et al., 2017). They comprise a large and diverse group of compounds that are highly prevalent in plant life (Figure F.1; Acamovic and Brooker, 2005). They can produce both noxious and therapeutic effects in consumers (Acamovic and Brooker, 2005). Plant-herbivore research across many systems has shown that total and individual phenolic content in plants influences herbivore foraging decisions. However, we have yet to show that total phenolic content significantly influences foraging by specialist herbivores, pygmy rabbits (*Brachylagus idahoensis*) and greater sage-grouse (*Centrocercus urophasianus*), in sagebrush systems. This could be due to the diverse ranges and mixtures of phenolics found in sagebrush (Turi et al., 2014) that could be counteracting each other and, therefore, not evoking physiological responses in herbivores after consumption. However, our research has shown that some individual phenolics do in fact elicit responses by these herbivores (Figure F.1). Because of this, we tried to use NIRS to predict individual phenolics in sagebrush at one field site where we had assayed these individual compounds, Craters of the Moon, Idaho, USA, and produced promising results (R^2 = 0.53-0.82; Figures F.2-F.5).

Coumarins are another class of phytochemicals nested within the phenolics class that are known for antibacterial, antifungal, and anticoagulant properties (McArthur et al., 1988; Wilt and Miller, 1992; Shultz, 2012). They tend to be highly polar (Figure F.1) and fluoresce a bright blue color under UV-light. This fluorescent nature has been exploited to aid in sagebrush classification (Stevens and McArthur, 1974; McArthur et al., 1988;

Rosentreter, 2005), as well as predict sagebrush palatability for the greater sage-grouse (Figure F.6) and other herbivore species using field (Figure F.6) and lab-based (Figure F.7) colorimetric tests. Although both phenolics and coumarins are yet to be predicted in sagebrush with great reliability using NIRS, they are compounds that are important to measure given their effects on herbivores, as well as species identification. This indicates that further research and fine-tuning of methods need to be done to better predict total and individual phenolics and coumarins in sagebrush systems.

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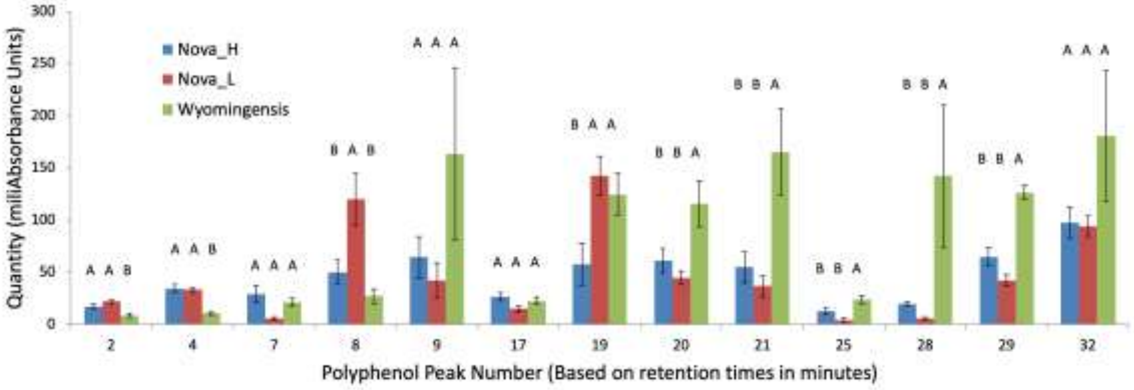

Figure F.1 Concentrations (mAU) of 13 individual phenolics of interest (not correlated with other peaks and showing initial quantitative differences between browsed types or species) detected in high (H) and low (L) browsed black (*Artemisia nova*, Nova) and Wyoming big (*Artemisia tridentata wyomingensis*, Wyoming) sagebrush by greater sage-grouse (*Centrocercus urophasianus*) from Idaho, USA. Letters represent significant differences among means using a Tukeys HSD test with A having higher concentrations than B (unpublished data). Peaks at lower retention times represent more polar phenolics that include coumarins (Figures F.5-F.6) and peaks with higher retention times represent less polar phenolics.

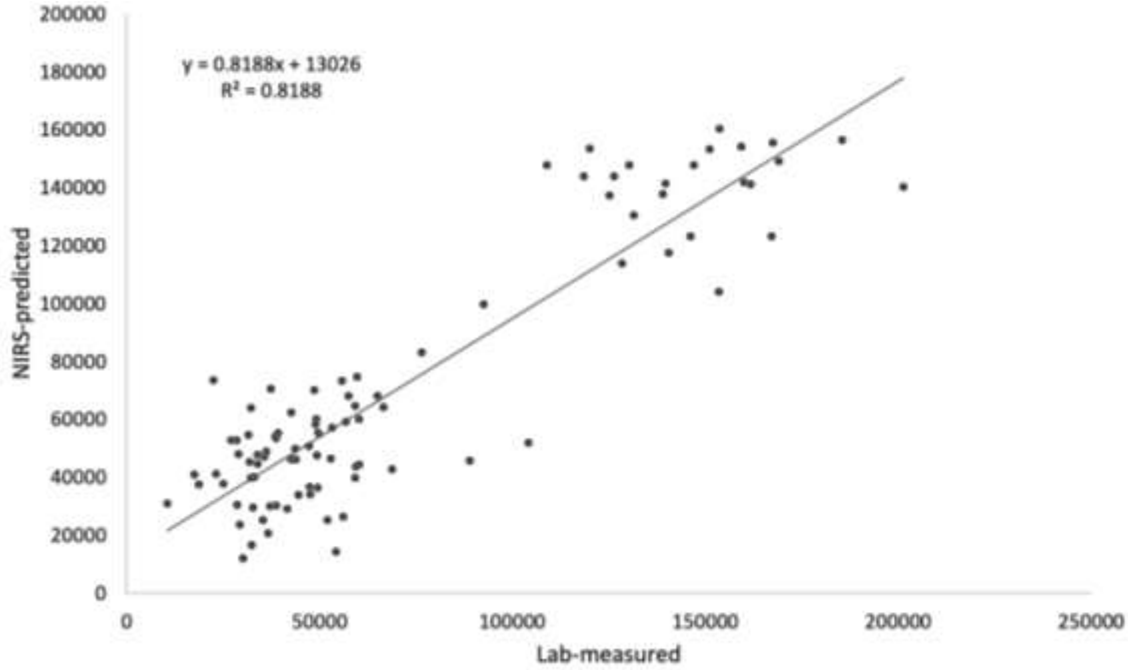

Figure F.2 Partial least squares regression using near infrared spectroscopy to predict individual phenolic, unknown "C", concentrations (mAU) in individual sagebrush (*Artemisia* spp.) at Craters of the Moon, Idaho, USA. Unknown "C" eluted around 11 min (of 45 total min) using high performance liquid chromatography and was one of the most polar compounds.

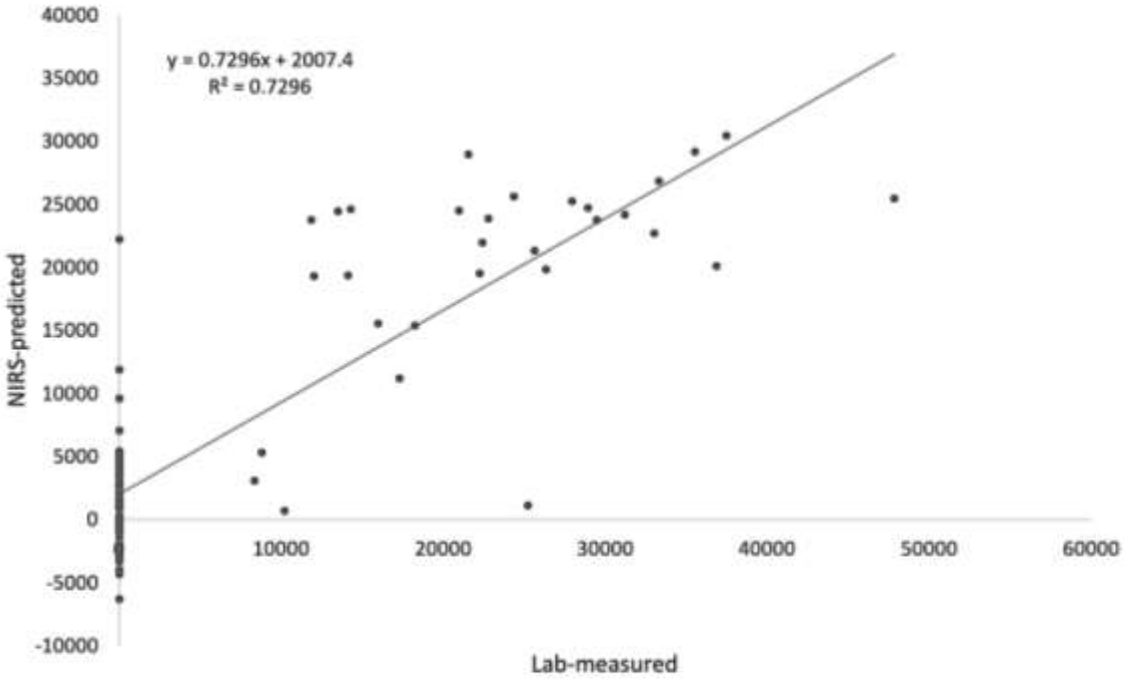

Figure F.3 Partial least squares regression using near infrared spectroscopy to predict individual phenolic, unknown "A", concentrations (mAU) in individual sagebrush (*Artemisia* spp.) at Craters of the Moon, Idaho, USA. Unknown "A" eluted around 8 min (of 45 total min) using high performance liquid chromatography and was one of the most polar compounds.

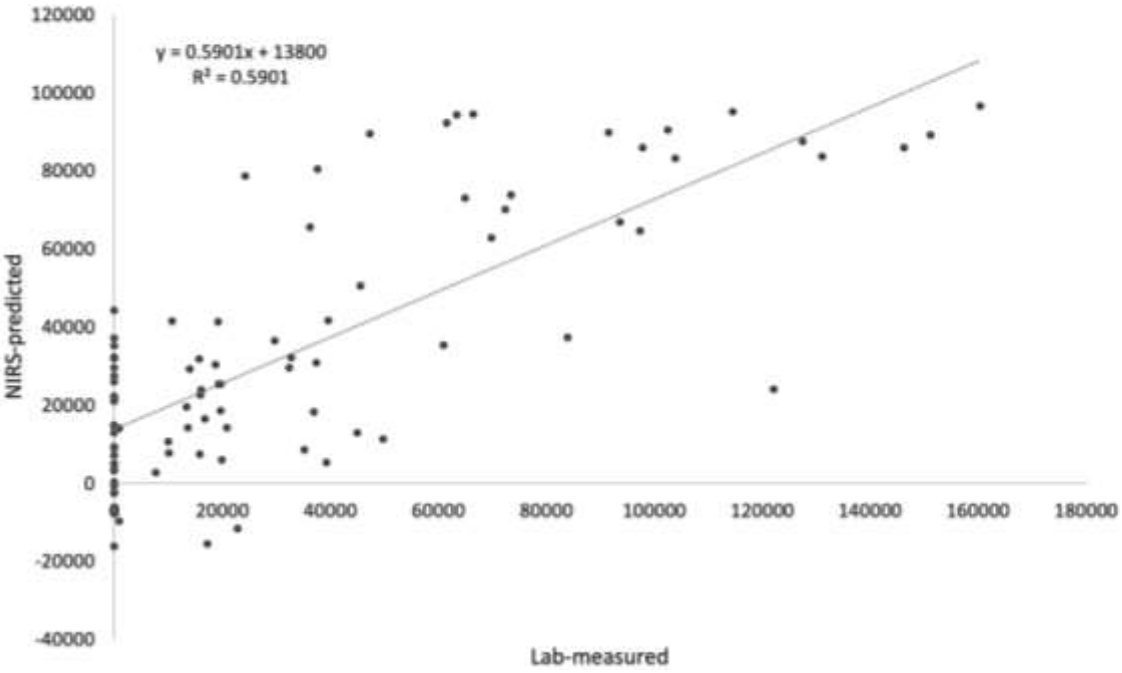

Figure F.4 **Partial least squares regression using near infrared spectroscopy to predict individual phenolic, unknown "K", concentrations (mAU) in individual sagebrush (*Artemisia* spp.) at Craters of the Moon, Idaho, USA. Unknown "K" eluted around 22 min (of 45 total min) using high performance liquid chromatography and was one of the intermediate polar compounds.**

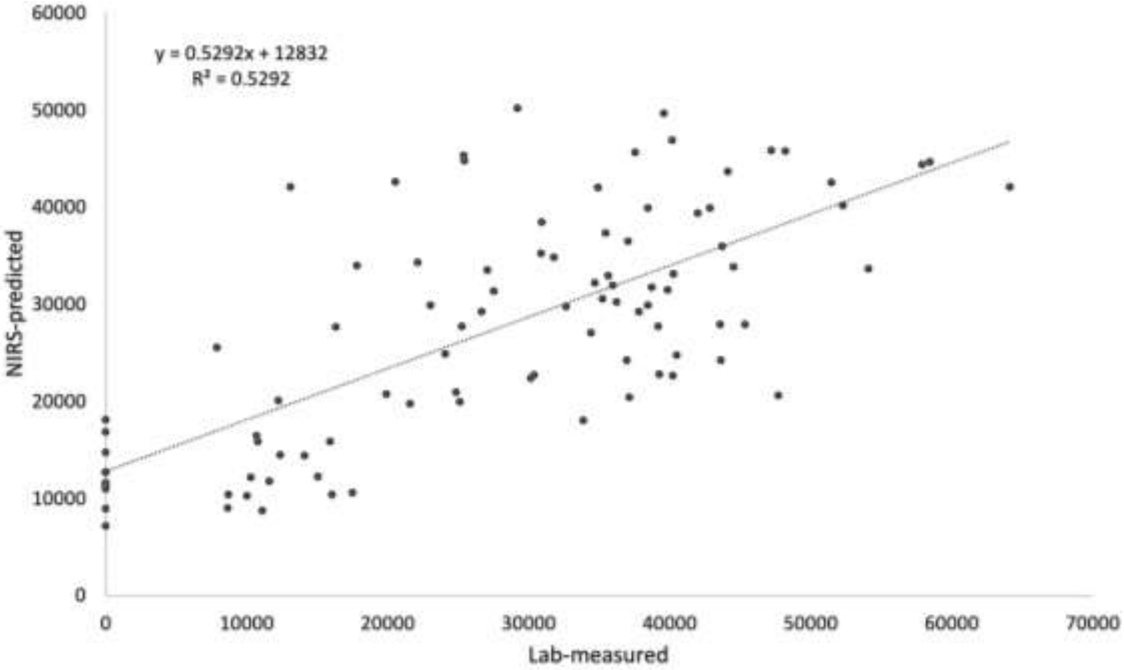

Figure F.5 Partial least squares regression using near infrared spectroscopy to predict individual phenolic, unknown "H", concentrations (mAU) in individual sagebrush (*Artemisia* spp.) at Craters of the Moon, Idaho, USA. Unknown "H" eluted around 17 min (of 45 total min) using high performance liquid chromatography and was one of the intermediate polar compounds.

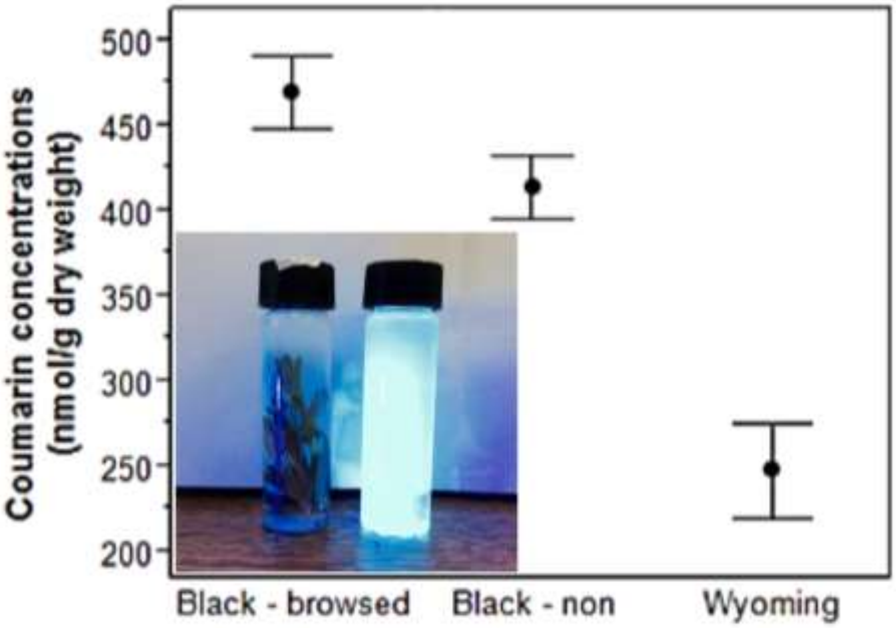

Figure F.6 Coumarin concentrations (nmol scopoletin equivalents/g dw) in relation to browsing by specialist sagebrush herbivore, greater sage-grouse (*Centrocercus urophasianus*), in Idaho, USA. Black sagebrush (*Artemisia nova*) is selectively foraged on more than available by greater sage-grouse (Frye et al., 2013).

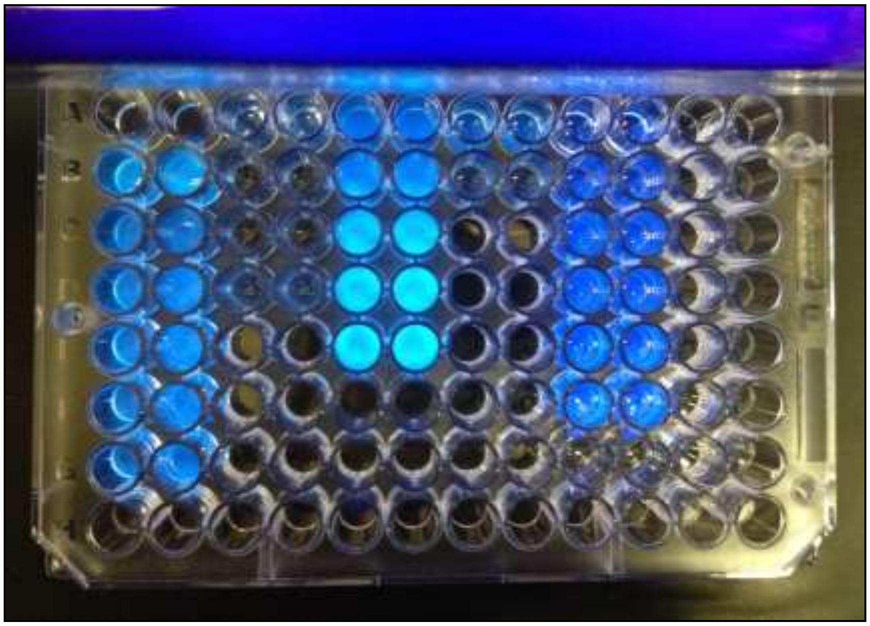

Figure F.7 Representative fluorescence analysis of coumarin concentrations (nmol scopoletin equivalents/g dry weight) on a spectrophotometric plate reader to assess sagebrush palatability for specialist herbivores according to in-house lab protocol.

APPENDIX G

Summary Statistics of Chemical and Herbivory Values

Summary Statistics of Chemical and Herbivory Values

Not all sagebrush is created equal. The following table (Table G.1) displays the summary statistics for chemical and herbivory values measured at four sagebrush field sites in Idaho, USA. The list is organized by constituent and demonstrates the variability of chemical compositions and abundances and herbivore use among sagebrush species and sites.

Table G.1 Summary statistics of chemical and herbivory values at four field sites in Idaho, USA.

Field Site	Values	N	Min	Max	Mean	SD
Magic Reservoir (2012-2013)	Nitrogen [a]	499	0.88	3.2	1.68	0.31
Magic Reservoir (2014-2015)	Nitrogen [a]	587	0.96	2.59	1.65	0.25
Cedar Gulch	Nitrogen [a]	619	1.31	3.44	2.14	0.3
Craters of the Moon	Nitrogen [a]	94	1.42	2.66	2	0.3
Raft River	Nitrogen [a]	192	1.7	2.99	2.19	0.25
Magic Reservoir (2012-2013)	Coumarins [b]	425	0.07	3.18	1.05	0.49
Magic Reservoir (2014-2015)	Coumarins [b]	551	0	6.36	0.78	0.84
Cedar Gulch	Coumarins [b]	576	0	1.44	0.39	0.23
Craters of the Moon	Coumarins [b]	94	0.08	25.67	3.4	5.15
Raft River	Coumarins [b]	255	0	2.78	0.46	0.33
Magic Reservoir (2012-2013)	Phenolics [c]	428	0.95	40.99	11.95	4.44
Magic Reservoir (2014-2015)	Phenolics [c]	531	1.5	52.12	10.95	6.08
Cedar Gulch	Phenolics [c]	602	1.73	29.07	10.91	3.99
Craters of the Moon	Phenolics [c]	90	0.37	118.88	16.67	22.03

Field Site	Values	N	Min	Max	Mean	SD
Raft River	Phenolics [c]	253	7.42	130.24	55.52	30.35
Magic Reservoir (2012-2013)	Monoterpenes [d]	499	432.31	6207.39	2212.98	910.69
Magic Reservoir (2014-2015)	Monoterpenes [d]	578	109.16	2060.05	787.65	332.54
Cedar Gulch	Monoterpenes [d]	625	44.05	1952.12	410.2	177.67
Craters of the Moon	Monoterpenes [d]	94	420.58	1433.86	784.68	229.12
Raft River	Monoterpenes [d]	262	160.29	1318.81	563.89	176.78
Magic Reservoir (2012-2013)	α-Pinene [e]	499	0	282.35	77.73	45.58
Magic Reservoir (2014-2015)	α-Pinene [e]	590	0	70.25	19.67	13.77
Cedar Gulch	α-Pinene [e]	625	0	150.4	2.01	10.92
Craters of the Moon	α-Pinene [e]	94	0	90.41	22.91	30.17
Raft River	α-Pinene [e]	262	0	155.27	41.44	31.99
Magic Reservoir (2012-2013)	Camphene [f]	499	0	1400.5	420.67	205.49
Magic Reservoir (2014-2015)	Camphene [f]	590	0	349.5	100.84	65.1
Cedar Gulch	Camphene [f]	625	0	87.25	5.79	7.82
Craters of the Moon	Camphene [f]	94	1.99	295.4	73.42	78.55

Field Site	Values	N	Min	Max	Mean	SD
Raft River	Camphene [f]	262	0	163.69	56.54	35.2
Magic Reservoir (2012-2013)	β-Pinene [g]	499	0	352.38	63.07	38.07
Magic Reservoir (2014-2015)	β-Pinene [g]	590	0	127.02	19.56	14.15
Cedar Gulch	β-Pinene [g]	625	1.14	644.68	101.9	52.85
Craters of the Moon	β-Pinene [g]	94	0.47	177.49	39.72	38.79
Raft River	β-Pinene [g]	262	0	164.8	28.8	26.97
Magic Reservoir (2012-2013)	p_cymene [h]	499	0	293.88	28.09	22.14
Magic Reservoir (2014-2015)	p_cymene [h]	590	0	112.71	4	10.76
Cedar Gulch	p_cymene [h]	625	0	45.98	0.72	4.53
Craters of the Moon	p_cymene [h]	94	0	57.18	13.46	14.92
Raft River	p_cymene [h]	262	0	53.69	17.54	13.26
Magic Reservoir (2012-2013)	1,8-Cineole [i]	499	0	1152.76	279.86	177.66
Magic Reservoir (2014-2015)	1,8-Cineole [i]	590	0	396.96	95.68	76.77
Cedar Gulch	1,8-Cineole [i]	625	0	180.18	12.17	18.67
Craters of the Moon	1,8-Cineole [i]	94	0	246.19	31.66	40.4

Field Site	Values	N	Min	Max	Mean	SD
Raft River	1,8-Cineole [i]	262	0	207.3	42.62	34.1
Magic Reservoir (2012-2013)	Unknown 20.1 min [j]	—	—	—	—	—
Magic Reservoir (2014-2015)	Unknown 20.1 min [j]	—	—	—	—	—
Cedar Gulch	Unknown 20.1 min [j]	625	0	140.27	50.3	21.56
Craters of the Moon	Unknown 20.1 min [j]	—	—	—	—	—
Raft River	Unknown 20.1 min [j]	—	—	—	—	—
Magic Reservoir (2012-2013)	Camphor [k]	499	0	3018.87	1075.24	442.15
Magic Reservoir (2014-2015)	Camphor [k]	590	0	881.7	321.07	188.95
Cedar Gulch	Camphor [k]	625	0	341.47	37.5	28.2
Craters of the Moon	Camphor [k]	94	0	212.5	51.88	51.17
Raft River	Camphor [k]	262	0	392.1	148.36	82.08
Magic Reservoir (2012-2013)	Borneol [l]	499	0	84.93	26.75	12.93
Magic Reservoir (2014-2015)	Borneol [l]	590	0	313.57	31.56	42.37
Cedar Gulch	Borneol [l]	625	0	69.62	5.83	8.86
Craters of the Moon	Borneol [l]	—	—	—	—	—

Field Site	Values	N	Min	Max	Mean	SD
Raft River	Borneol [l]	—	—	—	—	—
Magic Reservoir (2012-2013)	No. Monoterpenes [m]	—	—	—	—	—
Magic Reservoir (2014-2015)	No. Monoterpenes [m]	578	11	71	32.61	7.01
Cedar Gulch	No. Monoterpenes [m]	625	20	116	47.27	14.94
Craters of the Moon	No. Monoterpenes [m]	94	23	62	45.1	11.96
Raft River	No. Monoterpenes [m]	262	19	66	35.5	12.39
Magic Reservoir (2012-2013) [q]	Bite Marks [n]	487	0	172	6.6	18.96
Magic Reservoir (2014-2015) [q]	Bite Marks [n]	428	0	104	0.94	7.33
Cedar Gulch [q]	Bite Marks [n]	427	0	197	2.2	13.03
Craters of the Moon [r]	Bite Marks [n]	94	0	122.6	12.22	22.33
Raft River [r]	Bite Marks [n]	171	0	113.33	11.15	17.3
Magic Reservoir (2012-2013) [q]	Bites x Plant Height [o]	67	0	5.29	0.21	0.69
Magic Reservoir (2014-2015) [q]	Bites x Plant Height [o]	—	—	—	—	—
Cedar Gulch [q]	Bites x Plant Height [o]	—	—	—	—	—
Craters of the Moon [r]	Bites x Plant Height [o]	94	0	2.63	0.38	0.67

Field Site	Values	N	Min	Max	Mean	SD
Raft River [r]	Bites x Plant Height [o]	171	0	5	0.7	1
Magic Reservoir (2012-2013) [q]	Fecal Pellets [p]	487	0	250	13.24	30.34
Magic Reservoir (2014-2015) [q]	Fecal Pellets [p]	428	0	168	1.16	9.34
Cedar Gulch [q]	Fecal Pellets [p]	427	0	966	20.53	84.61
Craters of the Moon [r]	Fecal Pellets [p]	57	0	2.67	0.25	0.54
Raft River [r]	Fecal Pellets [p]	171	0	7	0.18	0.71

[a] Units are in % dry wt	Nitrogen
[b] Units are in µmol/g dry wt	Coumarins
[c] Units are in mg/g dry wt	Phenolics
[d] Units are in AUC/g dry wt	Monoterpenes
[e] Units are in AUC/g dry wt	α-Pinene
[f] Units are in AUC/g dry wt	Camphene
[g] Units are in AUC/g dry wt	β-Pinene
[h] Units are in AUC/g dry wt	p_cymene
[i] Units are in AUC/g dry wt	1,8-Cineole

Field Site	Values	N	Min	Max	Mean	SD
[j] Units are in AUC/g dry wt	Unknown 20.1 min					
[k] Units are in AUC/g dry wt	Camphor					
[l] Units are in AUC/g dry wt	Borneol					
[m] Values are counts	No. Monoterpenes					
[n] Values are counts	Bite Marks					
[o] Plant height is in cm	Bites x Plant Height					
[p] Values are counts	Fecal Pellets					
[q] Pygmy rabbit is predominant specialist herbivore at this site						
[r] Greater sage-grouse is predominant specialist herbivore at this site						

APPENDIX H

Box Plots of Chemical Values by Field Site

Box Plots of Chemical Values by Field Site

Variation in concentrations of phytochemicals comprising sagebrush differ among four field sites in Idaho, USA. The figures demonstrate spatiotemporal variation among sagebrush populations that may influence capacity for NIRS classifications and predictions.

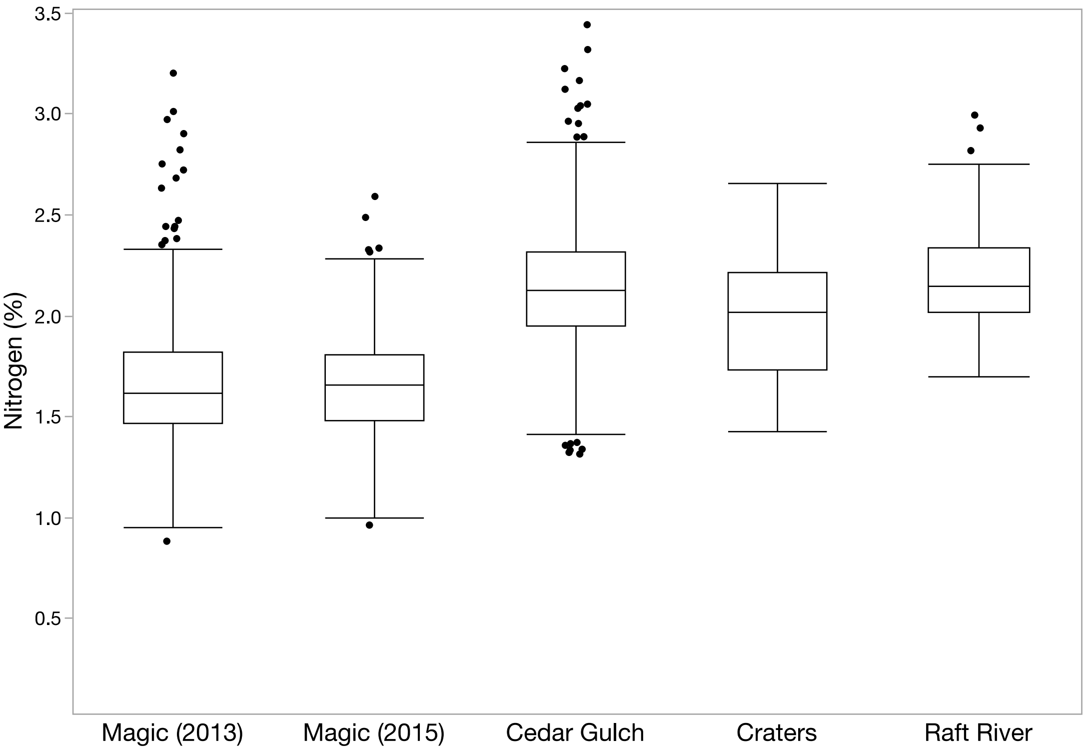

Figure H.1 **Variation in nitrogen content (% dw) in sagebrush plants among four field sites and two years in Idaho, USA. The year listed in parentheses after "Magic" represents year of sample collection.**

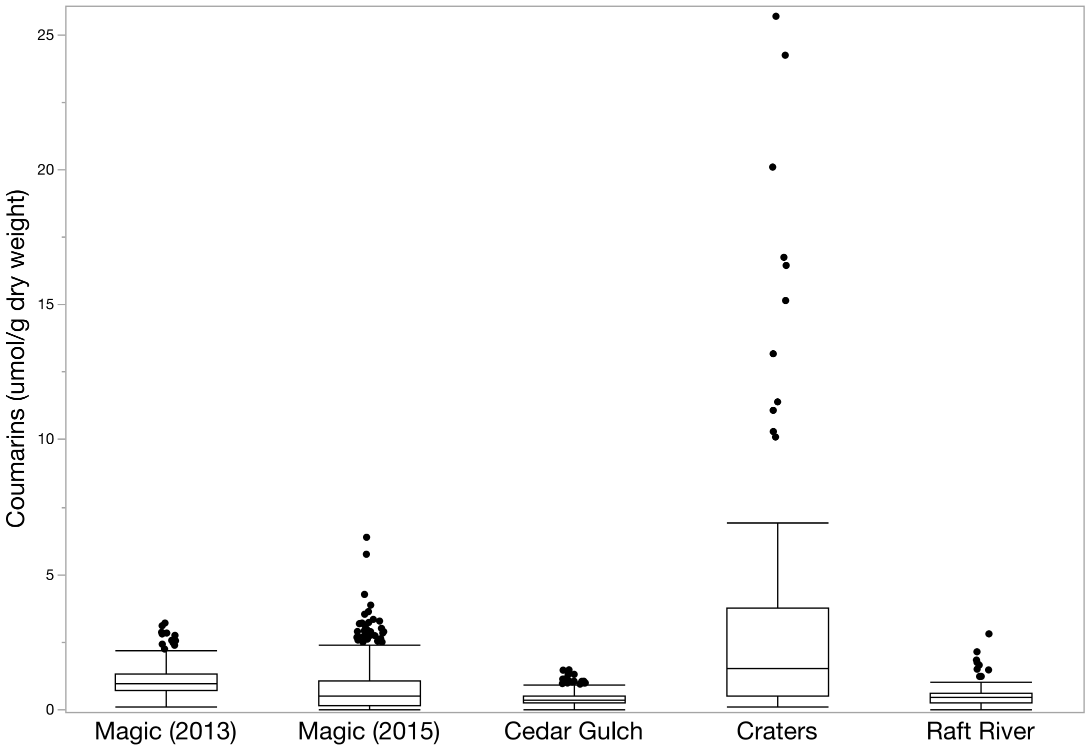

Figure H.2 Variation in coumarin content (µmol/g dw) in sagebrush plants among four field sites and two years in Idaho, USA. The year listed in parentheses after “Magic” represents year of sample collection.

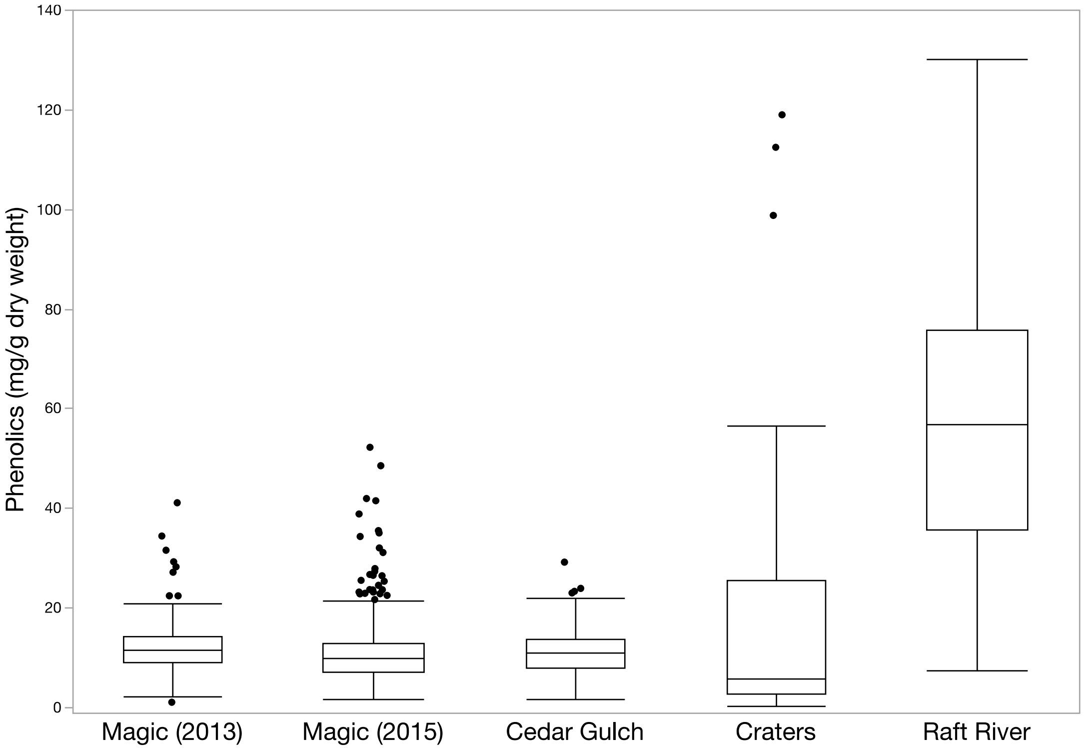

Figure H.3 Variation in phenolic content (mg/g dw) in sagebrush plants among four field sites and two years in Idaho, USA. The year listed in parentheses after "Magic" represents year of sample collection.

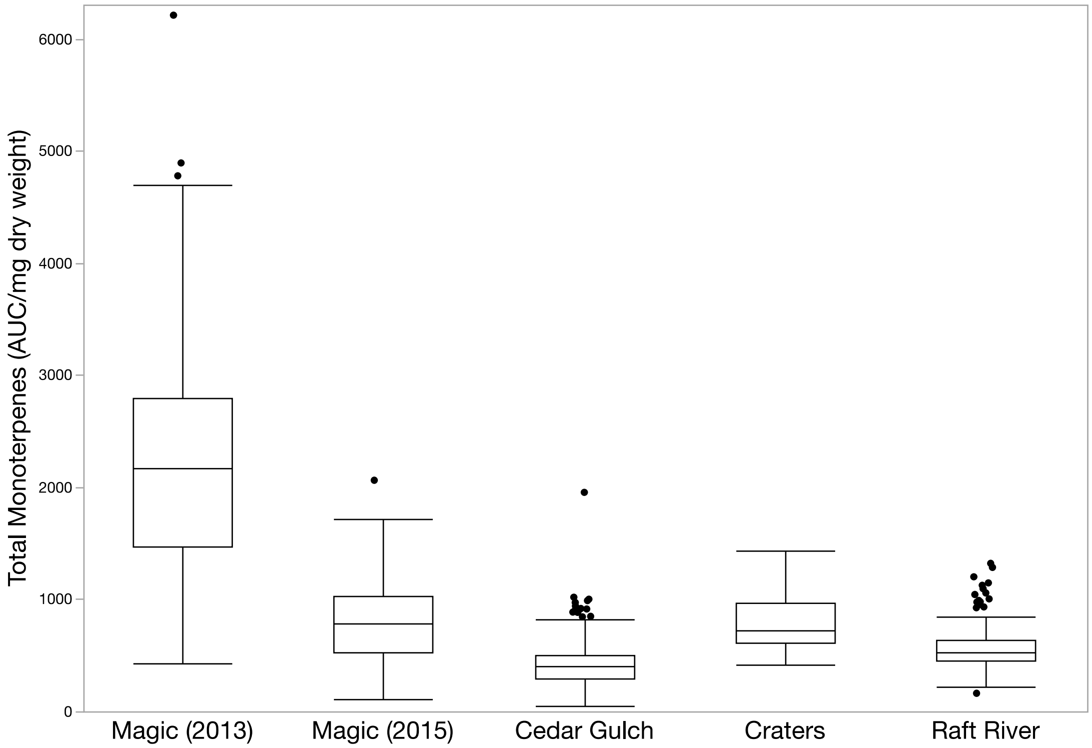

Figure H.4 Variation in total monoterpene content (AUC/mg dw) in sagebrush plants among four field sites and two years in Idaho, USA. The year listed in parentheses after "Magic" represents year of sample collection.

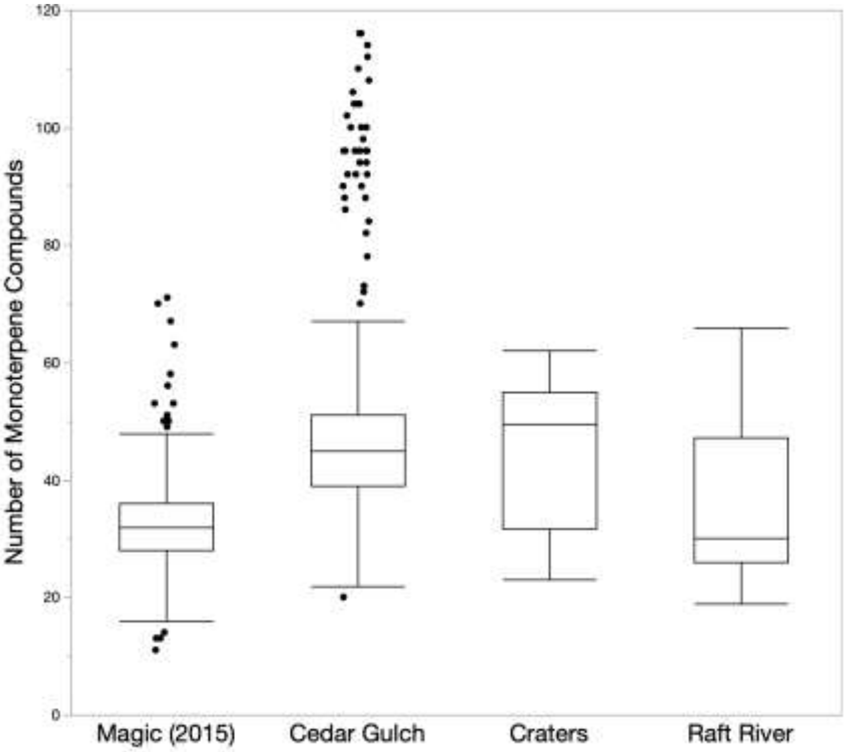

Figure H.5 Variation in number of monoterpene compounds found in sagebrush plants among four field sites in Idaho, USA. The year listed in parentheses after "Magic" represents year of sample collection.

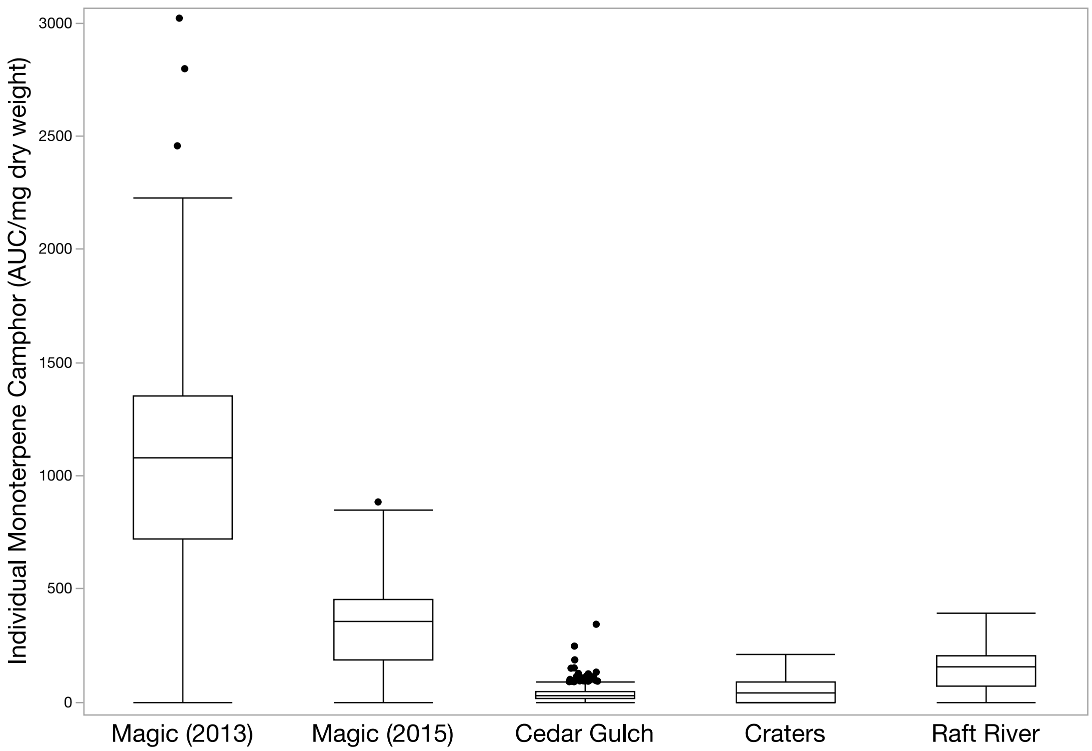

Figure H.6 Variation in individual monoterpene, camphor, content (AUC/mg dw) in sagebrush plants among four field sites and two years in Idaho, USA. The year listed in parentheses after "Magic" represents year of sample collection.

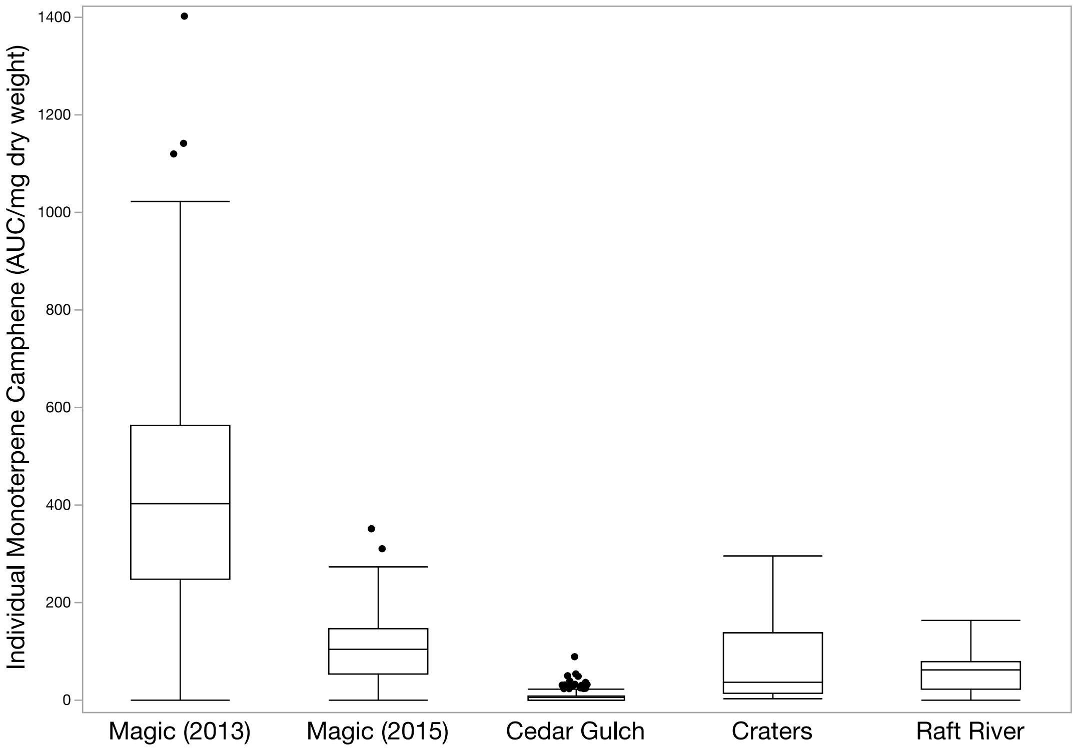

Figure H.7 Variation in individual monoterpene, camphene, content (AUC/mg dw) in sagebrush plants among four field sites and two years in Idaho, USA. The year listed in parentheses after "Magic" represents year of sample collection.

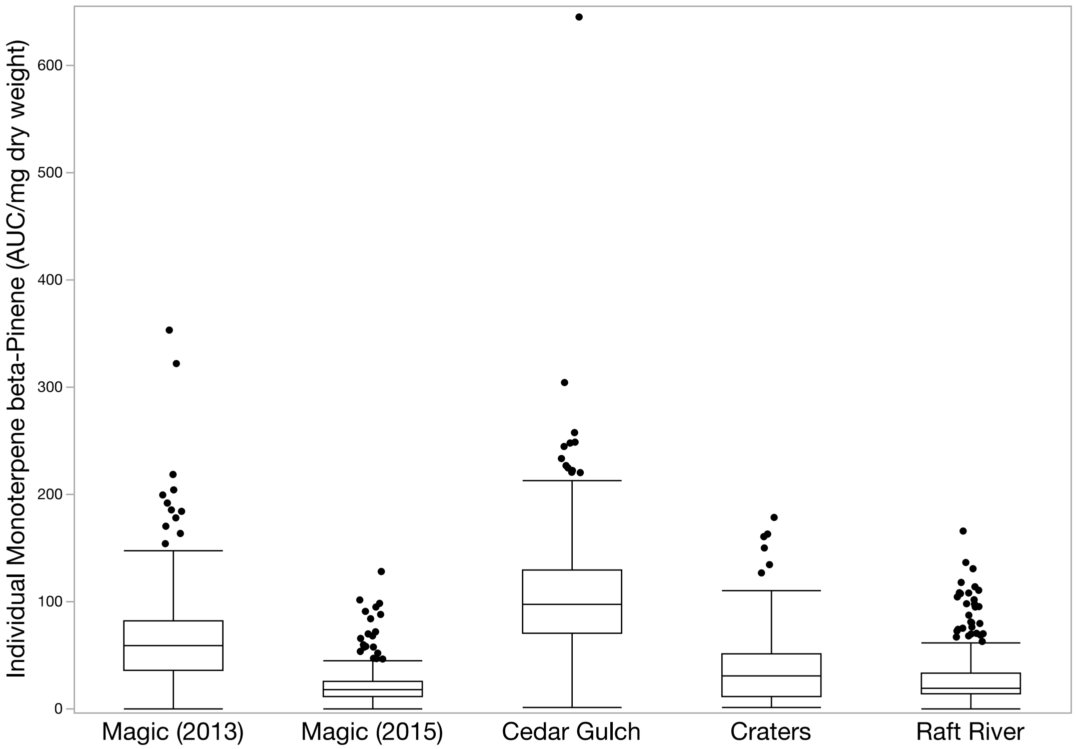

Figure H.8 Variation in individual monoterpene, β-pinene, content (AUC/mg dw) in sagebrush plants among four field sites and two years in Idaho, USA. The year listed in parentheses after "Magic" represents year of sample collection.

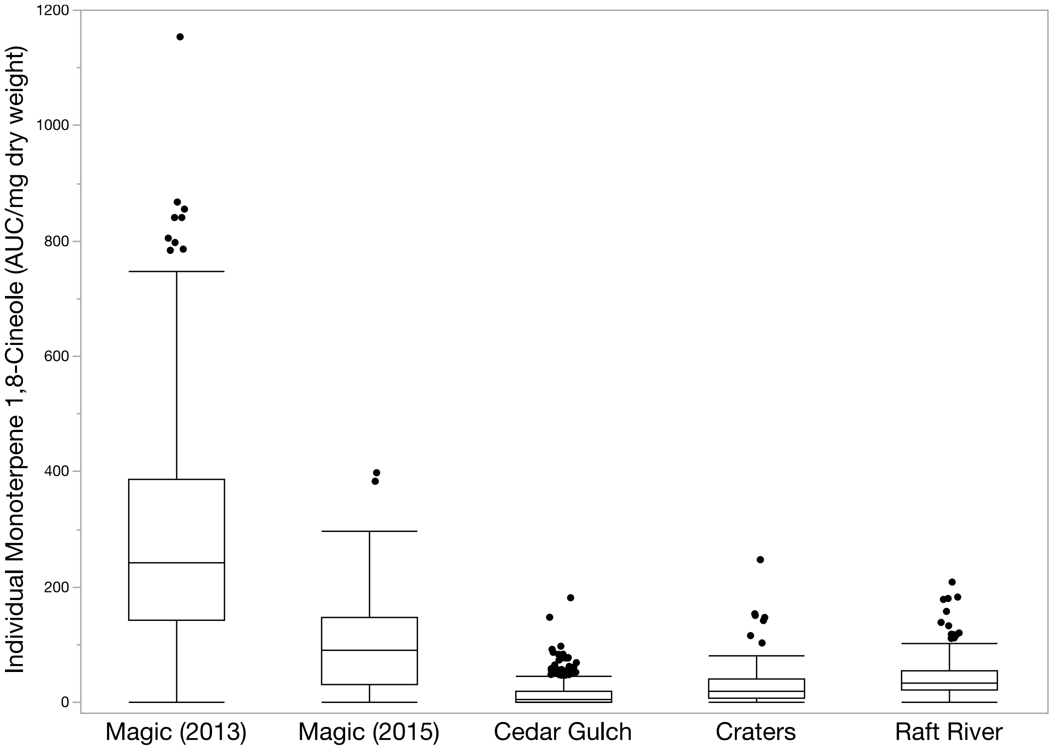

Figure H.9 Variation in individual monoterpene, 1,8-cineole, content (AUC/mg dw) in sagebrush plants among four field sites and two years in Idaho, USA. The year listed in parentheses after “Magic” represents year of sample collection.

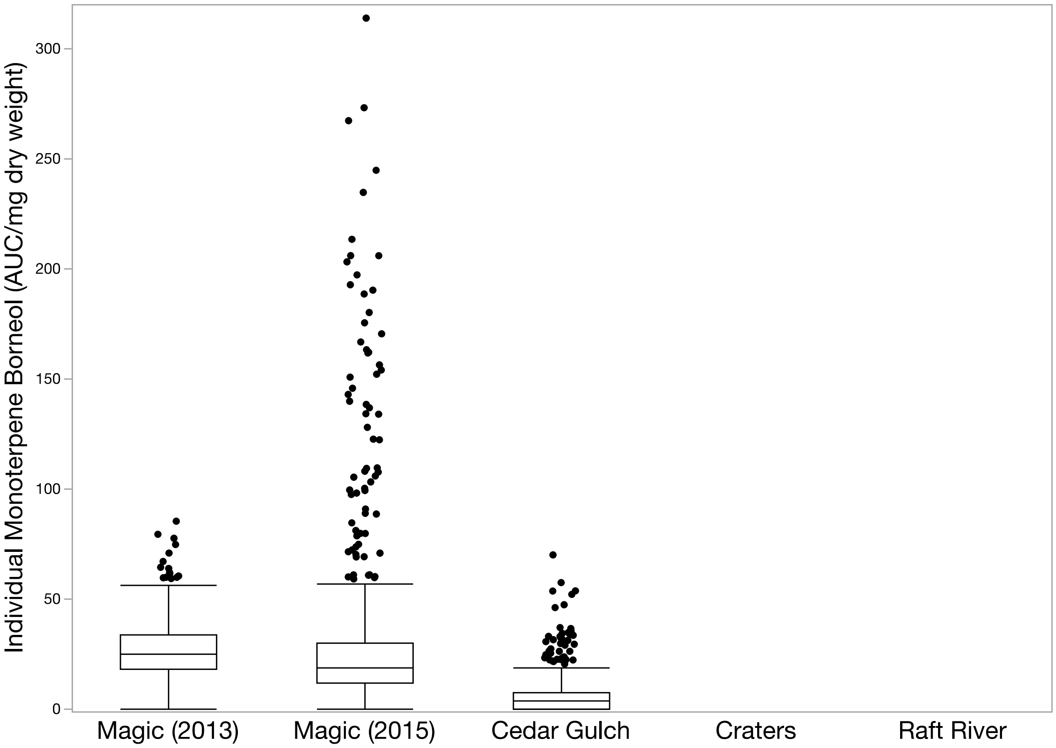

Figure H.10 Variation in individual monoterpene, borneol, content (AUC/mg dw) in sagebrush plants among four field sites and two years in Idaho, USA. The year listed in parentheses after "Magic" represents year of sample collection.