Evaluating the spatial distribution of plant function in a prairie-oak savanna

using spectroscopy

by

Paul W. Hacker

A.A., Kwantlen Polytechnic University, 2011

B.Sc., The University of British Columbia, 2015

MGEM, The University of British Columbia, 2018

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The following individuals verify that that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, the dissertation entitled:

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submitted by <u>Paul W. Hacker</u> in partial fulfillment of the requirements for

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Examining Committee:

Nicholas Coops, Professor, Forestry, UBC Supervisor

Etienne Laliberté, Professor, Biological Sciences, Université de Montréal Supervisory Committee Member

Allan Carroll, Professor, Forestry, UBC Supervisory Committee Member

Sean Michaletz, Assistant Professor, Botany, UBC Supervisory Committee Member

Cindy Prescott, Professor, Forestry, UBC University Examiner

Jennifer Williams, Associate Professor, Geography, UBC University Examiner

Abstract

In Canada, prairie-oak ecosystems along the south-east coast of Vancouver Island are both the country's most biodiverse and threatened. Anthropogenic land use change and fire suppression are altering the traditional composition of plant species and structure in these savannas, as well as reducing their total area. In 2001, Environment Canada published a study highlighting the impact of habitat fragmentation, the role of fire and the associated consequences of its removal, as well as the presence and effects of exotic species as fundamental ecosystem characteristics that are currently threatened, central to the recovery of prairie-oak savannas and require further scientific enquiry. Each of these fundamental characteristics required an enhanced understanding of the spatial distribution of plant species, lifeform and function. Due to the potential for high species richness and small individual plant footprints relevant data must be collected at fine spatial resolutions. Remote sensing, specifically drone-based imaging spectroscopy, presents a viable method for gathering information that can be used in analyses evaluating the aforementioned fundamental ecosystem characteristics. My research demonstrates the complexity facing studies examining prairieoak savannas using imaging spectroscopy and provides evidence that plant diversity and function, as well as structural composition, can be spatially evaluated. This work also substantiates the capacity for remote sensing to detect a key exotic species, Cytisus scoparius (L.) Link (Scotch broom) and examine the effects of anthropogenic activities on the primary tree species associated with these ecosystems, *Quercus garryana* Douglas ex. Hook (Garry oak). Combined, this information directly addresses key knowledge gaps and provides land managers with a variety of new methods to monitor prairie-oak savannas.

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Lay Summary

Human activities, including agriculture, road construction and fire suppression affect the environments in which they occur in a variety of ways. One of the most impacted ecosystems in Canada are prairie-oak savannas located on the south-east coast of British Columbia's Vancouver Island. Traditionally, these ecosystems are composed mostly of open meadows with Garry oak trees (*Quercus garryana* Douglas ex. Hook) scattered throughout and experienced low severity fires relatively often. Currently, the encroachment of closed-canopy forests and the introduction of exotic plants threaten the highly diverse plant and animal communities that have inhabited these savannas for thousands of years. I present a method for identifying the invasive Scotch broom (*Cytisus scaparius* (L.) Link) and examine the effects of anthropogenic activities of plant species composition and function across the landscape, as well as address the challenges associated with collecting information from multiple plant species in a single data point.

Preface

The objectives and associated research questions presented in this dissertation were formulated with my supervisory committee. Data were obtained by Etienne Laliberté provided leaf chemical analyses and interpreted results for Chapters 3 and 4. Margaret Kalacska and Juan Pablo Arroyo-Mora collected drone imagery and provided results interpretation for Chapter 4. Partial least squares regression models used in Chapter 6 were collected by Phil Townsend and Zhihui Wang the National Ecological Observatory Network (NEON). Sean Michaletz interpreted results for Chapters 3, 4 and 6, while Allan Carroll interpreted results for Chapter 4. I organized the field work, conducted the primary data analyses and interpretation and preparation of final manuscripts. Information, figures and text presented in this dissertation are derived from the sources presented below:

- Chapter 1/2/3: Hacker, P. W., Coops, N. C., Laliberté, E., & Michaletz, S. T. (2022). Variations in accuracy of leaf functional trait prediction due to spectral mixing. Ecological Indicators 136(February). <u>https://doi.org/10.1016/j.ecolind.2022.108687</u>
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- Chapter 1/2/5: Hacker, P. W., & Coops, N. C. (2022). Using leaf functional traits to remotely detect Cytisus scoparius (Linnaeus) Link in endangered savannas. NeoBiota 71, 149–164. <u>https://doi.org/10.3897/neobiota.71.76573</u>
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List of Abbreviations

- A_{max} Net photosynthetic capacity
- ALS Airborne Laser Scanning
- ASD Analytical Spectral Devices
- ATCOR Atmospheric and Topographic Correction
- AVIRIS Airborne Visible/Infrared Imaging Spectrometer
- BEC Biogeoclimatic Ecosystem Classification
- BC British Columbia
- CABO Canadian Airborne Biodiversity Observatory
- C Carbon
- %C Percent Carbon
- C:N Carbon Nitrogen Ratio
- Car Carotenoid
- CASI Compact Airborne Spectrographic Imager
- CDF Coastal Douglas-fir
- CGOP Cowichan Garry Oak Preserve
- Chl Chlorophyll
- CI Confidence Interval
- CO Colorado

CO² – Carbon dioxide

DBH - Diameter at Breast Height

DSM – Digital Surface Model

DTM – Digital Terrain Model

EMS – Electromagnetic Spectrum

EWT – Equivalent Water Thickness

FT – Functional Trait

GR-DU - Growth- Durability

H - Hypothesis

 $H_2O\text{-}ST-Water-Structure$

hNDVI - hyperspectral Normalized Difference Vegetation Index

HSI - Hyperspectral Imaging system

ITV – Intraspecific Trait Variation

K - Potassium

LES – Leaf Economic Spectrum

LDMC – Leaf Dry Matter Content

LL – Leaf Lifespan

LMA – Leaf Mass per Area

N - Nitrogen

%N – Percent Nitrogen

- N-n High Nitrogen Low Nitrogen
- NaCl Sodium Chloride
- NCC Nature Conservancy of Canada
- NDVI Normalized Difference Vegetation Index
- NEON National Ecological Observatory Network

NIR - Near-infrared

NRMSEP - Normalized Root Mean Squared Error of the Predictor

P – Phosphorus

- PCA Principal Component Analysis
- PC Principal Component
- PES Plant Economic Spectrum
- PLSR Partial Least Squares Regression
- PNEU photosynthetic-N-use efficiency
- R² Coefficient of variation
- RMSEP Root Mean Squared Error of the Predictor
- RPAS Remotely Piloted Aircraft System
- RTM Radiative Transfer Model
- SVC Spectra Vista Corp

SWIR – Short-wave Infrared

- TWI Topographic Wetness Index
- USA United States of America

UV - Ultraviolet

VIS - Visible

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For my family.

Chapter 1: Foundation for research

This chapter establishes the context of this dissertation. The impacts of anthropogenic activities on biodiversity are discussed and research objectives are presented in relation to current scientific knowledge gaps pertaining to Canadian prairie-oak savannas.

1.1 Humans and biodiversity

As the Anthropocene progresses, human activities will continue to modify ecosystems across the planet (Johnson et al., 2017; Pimm et al., 1995; Western, 2001). These changes can lead to the loss of ecological function, threatening the ecological goods and services humans depend on (Chapin III et al., 2000). To monitor the effects of these modifications, plant biodiversity has been proposed as a surrogate indicator of ecosystem health (Pereira et al., 2013). Specifically, plant functional diversity has been identified as a variable that can be used to track biodiversity as it relates directly to the uptake and allocation of resources by individual plants (Jetz et al., 2016). It is essential, then, to monitor the plant functional biodiversity of human-modified ecosystems.

Central to the monitoring of plant functional health is the understanding of leaf properties. The chemical and structural properties of a leaf play fundamental roles in characteristics such as photosynthesis, life span and respiration (Wright et al., 2004). There are a variety of leaf types exhibited by the estimated 298,000 species that span the globe (Butler et al., 2017). This number decreases considerably in the context of remote sensing as only the leaves of species that dominate the canopy are observable. Among dominant plant species there are a variety of leaf types. The cell types discussed below have all been synthesized from Jacquemoud and Ustin's 2019 book *Leaf Optical Properties*, with additional literature cited as necessary.

1.2 Leaf properties

1.2.1 Outer cells

The first leaf component that photons interact with is the epidermis. Usually one-cell thick, the primary functions of the epidermis are mechanical strength and exchange moderation, particularly of water and carbon dioxide (Figure 1). Multiple different cells can be present in the epidermis, each providing a specific function. The outer surface of these cells is coated by a noncellular layer called the cuticle (Figure 1). The cuticle acts in many capacities relating to the regulation of exchanges between the cells in protects and the surrounding medium, as well as in defence against micro-organisms and decomposition. In conjunction with trichomes, which are essentially leaf hairs that extend from the epidermis, the cuticle's thickness and composition can alter the ability of photons to interact with internal leaf structures. Waxes and oils present on cuticle can also influence the interactions of photons with the internal structures. The consequence of this is that the epidermis and associated structures are the initial location of interaction between photons and a leaf.



Figure 1. Leaf cross section depicting various structural components.

1.2.2 Mesophyll

Beneath the epidermis is the mesophyll. All leaf cells that are between the upper and lower epidermis are considered to be in the mesophyll (Figure 1). Parenchyma cells make up the majority of the mesophyll, some of which contain chloroplasts. These 'chlorenchyma' interact with incoming photons in order to generate glucose from photosynthesis. The amount of total chlorophyll in the mesophyll determines leaf's ability to photosynthesize and influences its optical properties. Reflectance, particularly, is affected by the interactions of photons and chlorophyll. This provides opportunities for the estimation of plant characteristics related to stress and growth.

1.2.3 Vascular System

A core function of a leaf is the creation of carbohydrates. These carbohydrates are transferred to other plant organs through the vascular system. This system consists of two tissue types, xylem (transports water and nutrients from roots to leaves) and phloem (transports carbon from leaves to plant), and also transfers water collected by roots to the leaf. The vascular system also contains the vascular cambium, which determines the function of new cells as needed for plant growth. There are anatomical differences between gymnosperms and angiosperms that can lead to variations in the optical properties of, but one of the main optical distinctions relates to the primary pathways of photosynthesis. The bundle sheath cell walls of C4 plants vary to such a degree from those found in C3 plants that the optical properties of the leaves utilizing these systems are different.

1.2.4 Shape and Venation

Leaf shape and venation also play important roles in the expression of leaf optical properties. The distribution and thickness of veins within a leaf can alter the interaction between photons and the organs with the leaves (Figure 1). These characteristics are closely related to the environmental conditions in which the leaf exists. The plasticity of leaf phenotypes under varying environmental,

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including venation and shape, affect biochemical composition as well as the leaves optical properties.

1.2.5 Pigments

Plant colour is related to the distribution of pigments within chloroplasts. When photons interact with these pigments a transfer of energy occurs that enables the light and dark reactions comprising photosynthesis, as well as fluorescence. Chlorophyll *a* and chlorophyll *b* are two photosynthetic pigments that absorb photons primarily in the red and blue visible regions of the electromagnetic spectrum (EMS), as well as at 1660 nm specifically (Kokaly and Skidmore, 2015). Carotenoids are another type of pigment that affects photosynthesis in a leaf. The main functions of carotenoids in relation to leaf optics are to increase the range of light wavelengths that can be absorbed for photosynthesis and to dissipate excess energy. They are also responsible for mitigating damage caused by environmental extremes relating to temperatures or light availability. Carotenoids and chlorophylls both absorb light in the visible section of the EMS, meaning that their presence, distribution and amount greatly influences the optical properties of the leaf.

In the same manner that carotenoids disperse excess energy to deter the degradation of the photosynthetic system, phenolic compounds act as absorbents for high intensity photons in the UV and blue ranges of the EMS. This means that the presence of phenols directly influences the optical properties of the leaves, but a relationship with leaf mass per area (LMA) means that the amount and type of phenolic compounds in a leaf can potentially highlight other leaf characteristics that impact optics.

1.2.6 Nitrogen

Plant health is limited by the ability of an individual to collect and use nitrogen (Berendse and Aerts, 1987). Within a leaf, nitrogen content is linked with the capacity to photosynthesize (Serrano et al., 2002). In terms of leaf structure, the quantity of nitrogen determines the size of the parenchyma cells and, as a consequence, drives leaf thickness and size(Loomis, 1997). When nitrogen is less available leaves tend to have smaller parenchyma cells and smaller overall size(Morton and Watson, 1948). Any change in leaf size and thickness will affect the manner in which photons interact with the leaf, making nitrogen content a useful trait to measure using optical sensors.

1.2.7 Water

The amount of water in a leaf can range from 50% - 90% of its total mass. Inadequate water supply decreases the ability of the leaf to transpire, which in turns affects its ability to maintain an ideal temperature for metabolic processes and capture CO₂ (Mahan and Upchurch, 1988). Since access to, and efficient use of, water drives many plant functions, leaf water content also affects the underlying processes that govern the reflectance, transmission and absorption of photons (Carter, 1993).

Although important, the aforementioned leaf properties are not the only components related to functional capacity. Cellulose, starch, lignin and other chemicals also contribute to leaf function and add to the complexity of monitoring plant health. However, the limitations imposed on plants by environmental and genetic constraints force individuals to allocate resources into specific traits. The economic nature of this resource allocation offers an opportunity to monitor plant health through the leaf functional trait associations.

1.3 Functional trait associations

1.3.1 Carbon Economics

In leaf economics, carbon is the common currency and is linked to plant energy budgets as these processes govern the rate of carbon assimilation (Michaletz et al., 2015). Generally, successful carbon economic strategies are those that maximize the return on carbon investment over the lifespan of a leaf (Westoby et al., 2002). Michaletz et al., 2016 demonstrated that plants across the planet work to maintain ideal cellular temperatures in pursuit of this maximization and suggest that this can result in the alteration of functional traits.

The fact that plants are limited homeotherms and can thermoregulate has multiple implications (Michaletz et al., 2015). The first, and perhaps largest, is the provision of a mechanistic link between climate and leaf functional traits (Michaletz et al., 2016). In functional ecology, this relationship presents the opportunity to determine how abiotic factors like temperature affect the metabolic processes that drive trait manifestation (Michaletz et al., 2016, 2015). Put simply, it may be possible to determine shifts in the underlying health conditions of a plant based on a relatively observable trait like leaf nitrogen content.

This suggests that remote sensing technologies, such as imaging spectroscopy, could provide information that enables the estimation of plant energetic processes (Michaletz et al., 2016). Such a link between imagery and internal plant processes is necessary to accurately evaluate plant health and presents a multitude of management opportunities. With the capacity for sensors to collect relevant data at the site, landscape and global scale, leaf thermoregulation may play a vital role in monitoring plant health at the global scale (Michaletz et al., 2018).

1.3.2 Leaf economic spectrum

In 2004, Wright et al. published a fundamental paper presenting a multi-species, global leaf economic spectrum (LES) that differentiates between leaf longevity and leaf growth. Their work was based on the findings of Reich et al. (1998), which showed the significant relationship between net photosynthetic capacity, leaf nitrogen and specific leaf area. The underlying principle of the LES is that finite resources are either stored or used immediately in pursuit of a specific purpose (Wright et

al., 2004). The use of a resource for one particular function means that said resource is unavailable for other functions. This limitation requires the individual, in our case a plant, to invest in relation to underlying factors like genetics and environmental conditions. For most living organisms there are three main pursuits: survival, growth and reproduction. Plants must select, then, which pursuit takes precedent. These investments are made throughout the plants lifetime and are based largely on nutrient availability and the environment in which it lives.

Wright et al., 2004 present a concise and informative outline for the LES. Through the creation of a global dataset consisting of over 2,548 species and their associated functional traits, they were able to show that trade-offs exist between six mass-based leaf characteristics (Figure 2). The relationship between leaf lifespan (LL) and leaf mass area (LMA) was found to be particularly interesting as it highlights the need for long-lived leaves to be durable, which relates to investment in defences rather than growth (Figure 2) (Wright et al., 2004). Wright et al., 2004 found other important relationships between LL and both photosynthetic capacity and leaf nitrogen content.



Figure 2. Visualization of the leaf economic spectrum. Leaf mass per area (LMA) and leaf lifespan (LL) are anticorrelated with phosphorous (P), photosynthetic capacity (A), dark respiration (R), and nitrogen content (N). Created using the principal component loadings from the mass-based evaluation presented in Wright et al., 2004.

These trait associations present opportunities to observe the effect that abiotic phenomena, such as climate, have on the plants across multiple sites. First, however, it is necessary to determine if the observed relationships are consistent at a global scale. Wright et al., 2005a conducted such an assessment and found that the LES was transferable at a global scale. They showed that LL-LMA associations were maintained globally and introduced the concept that the ratio of photosynthetic capacity and leaf N, photosynthetic-N-use efficiency (PNUE) can also be considered as part of the LES (Wright et al., 2005a). Compared to LL and LMA, PNUE was negatively correlated, supporting the claim that short-lived leaves with faster growth rates have higher capacity for photosynthesis (Wright et al., 2005a). The leaf trait associations highlighted in the LES provide relevant information for monitoring overall plant health and function, as well as understating leaf investment strategies. These foundational findings prompted further investigation of the linkages between trait manifestation and plant life strategies, such as specific methods of leaf defence and metabolic efficiency.

1.3.3 Leaf defence strategies

The universal relationships of plant functional traits apply directly to the growth differential balance hypothesis: to invest in overall growth or cell specialization (Herms and Mattson, 1992). There are many functions that a cell can specialize in, but one of the most important is defence. This is especially key for plants with long lifespans and is a general driver of the positive correlation of LL and LMA (Wright et al., 2004). Allocation of resources to the creation of defensive leaf compounds, such as phenolics and lignin, means that resources cannot be made in growth, or leaf nutrition.

McManus Chauvin et al., 2018 used the same methodology as Wright et al., 2004 to evaluate 10 traits, including those related to a variety of defensive compounds, and found that there is a primary trade off between a leaf's investment in nutrition and chemical defence, which follows the LES. They also found a secondary spectrum of leaf defences that highlights a gradient of investment in phenolics as well as trade-offs between anti-herbivore compounds like tannins and latex and highlights the complexity of resource allocation that an individual plant faces (Chauvin et al., 2018).

1.3.4 Leaf thermoregulation

Another relevant leaf economic spectrum is that relating to the capacity for a leaf to control its internal temperature. The metabolic efficiency of leaf cells is important for plant health (Enquist et al., 2015; Michaletz et al., 2016). A major component of this is the maintenance of optimal temperatures for enzymes related to photosynthetic and respiratory functions (Michaletz et al., 2016). It was believed that plants were poikilotherms, meaning that the temperature of a plant organ, such as a leaf, was equivalent to and driven by the external temperature (Upchurch and Mahan, 1988). This theory would suggest that a plants ability to function was dependent on the environmental conditions in which it lived (Michaletz et al., 2015).

In the mid-20th century, a variety of studies demonstrated that plant were in fact able to control their internal temperature in order to maintain optimum functioning (Gates, 1964; Mahan and Upchurch, 1988; Upchurch and Mahan, 1988). These findings prompted further study of plant thermoregulation, specifically how the maintenance of internal temperatures in various environmental conditions affected the manifestation of leaf traits. Since plants are limited homeotherms, rather than poikilotherms, it could be possible to determine mechanistic links between functional traits and drivers of environmental temperature, such as climate.

The identification of consistent relationships between plant functional traits and climate would be useful in the management of agricultural and forestry resources. It may also be possible to use variations in plant functional traits as indicators of climate change (Michaletz et al., 2018). To

confirm such relationships, it is necessary to identify the underlying drivers of leaf thermoregulation and quantify its effect on plant energy budgets.

1.3.5 Energy budgets

Plants can manage the temperatures of their internal tissues in a variety of ways, including the alteration of functional traits, the generation of metabolic heat and transpiration cooling (Gates, 1964; Watling et al., 2008). It is hypothesized that the functional characteristics of plant leaves have been selected over time based on their ability to maintain optimal leaf temperatures for photosynthesis (Korner and Diemer, 1987; Michaletz et al., 2015). This follows the leaf economic theory of trade offs between traits associated with growth and defence and suggests that thermal traits follow similar trends. For example, leaves with long functional longevity have a smaller breadth of temperatures at which they can conduct photosynthesis (Michaletz et al., 2016).

The manifestation of one leaf functional trait over another comes down to two things: (1) the need for a specific function and (2) resource availability (Reich, 2014; Wright et al., 2005b). Cumulative manifestations of leaf properties relating to pursuit of specific leaf functional traits constitute a functional strategy. These strategies cost both time and energy, so it is vital for plants to select appropriate strategies for their environment (Freschet et al., 2010). To determine which traits to produce plants employ economic theories similar to those of humans.

1.3.6 Plant economic spectrum

Although leaves provide useful information relating to photosynthetic capacity, defence strategies and overall health, they do not necessarily represent the characteristics of plants as a whole. For example, plant organs such as roots and stems are ignored in these analyses. Freschet et al., 2010 suggested that the summary of functional traits for a plant should include leaves, stems and roots and present findings in support of a plant economic spectrum (PES) in relation to the entire

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plant in subarctic flora. The authors observed a variety of species and determined that significant correlations between the same functional traits existing in the leaves, stem and roots of individual plants (Freschet et al., 2010).

This is evidence that a PES exists, extending the leaf economic spectrum across the entire individual. Such a revelation presents opportunities to expand the study of functional ecology. It also provides the relationships required to estimate the values of one organ from that of another (Reich, 2014). For example, remote sensing technologies that evaluate the functional traits of leaves have the potential to link to the below-ground traits of a plant (Laliberté, 2017). Such a connection could enable airborne imagery to detect soil type and moisture content on a large-scale, which in turn enables the mapping of soil properties and a more complete understanding of relevant environmental conditions (Cavender-Bares et al., 2022).

1.4 Remote sensing of leaf functional traits

1.4.1 Spectroscopy of foliar chemistry

Many technologies collect information about leaf traits. Spectroscopy is a fundamental technology that has proven effective at retrieving relevant functional trait information from plant leaves (Curran et al., 2001; Goetz, 1992). This technique entails the use of a spectroradiometer to observe and record relevant leaf optical properties at many narrow wavelengths across the optical range (300 nm and 2500 nm). Spectroradiometers can be deployed at multiple spatial scales to investigate individual leaves (leaf spectroscopy) or entire landscapes (imaging spectroscopy) (Asner et al., 2008a; Curran, 1989; Schaepman et al., 2009). These sensors have been highly effective in biodiversity science, allowing researchers to assess the content of certain leaf pigments, map species diversity and monitor exotic species (Asner et al., 2018; Butler et al., 2017; Ustin et al., 2009).

This method is also scalable between the leaf and landscape levels, which enables analyses at various functional scales (Figure 3). Spectroscopy also lends itself to prominent validation analyses, such as partial least squares regression, that efficiently link laboratory derived chemical properties of a substance with its spectral signature (Asner and Martin, 2016).



Figure 3. Progression of spectroscopy from (a) collection of leaf spectra to (b) partial least-squares regression before finally being applied at the (c) landscape level using imaging sensors.

Curran et al., 1989 used spectroscopy to identify 42 absorption features of vegetation along the visible and near-infrared sections of the electromagnetic spectrum (EMS) (Curran, 1989). These features use the interactions between photons and the molecules within leaves to estimate the content of the organic compound of interest (Table A1)(Curran, 1989). Simply put, the vibrational energy state of the molecules in a leaf will change based on its interaction with energy from the sun. The key chemicals identified by Curran (1989) are highlighted in Table 1. Table 1. Important absorption features identified by Curran (1989). The number of appearances of each chemical, as well as their location along the EMS and functional associations, are outlined. VIS: visible, NIR: near infrared, SWIR: shortwave infrared

Chemical	Appearances	EMS Region(s)	Functional Association
Chlorophyll a	2	VIS	Photosynthesis
Chlorophyll b	2	VIS	Photosynthesis
Nitrogen	7	SWIR	Size of parenchyma cells,
			photosynthesis
Starch	19	NIR, SWIR	Energy storage
Oil	3	NIR	Photon scattering,
			defence, attractant
Lignin	6	SWIR	Cell wall structure
Water	5	NIR, SWIR	Transpiration,
			temperature
Cellulose	11	SWIR	Cell structure
Sugar	7	NIR, SWIR	Energy
Protein	12	NIR, SWIR	Photosynthesis (rubisco)

energy from the visible range of the EMS (400 – 700nm)(Curran, 1989). These transitions occur as the molecules in the chlorophyll are excited to a higher energy level and absorb light (Curran, 1989). This absorption is what creates colour. Some compounds experience stretching and bending of their covalent bonds as they interact with infrared energy (Kumar et al., 2006). This occurs as their springlike structures absorb IR energy and produces variations in the absorption properties of the compound (Kumar et al., 2006).

Chlorophyll a and b, for example, experience electron transitions when they interact with

It is important to discuss the physics that cause certain features to be dominant drivers of absorption. In the visible region of the EMS (400-700nm), absorption is controlled by the transitions of electrons between energy levels (Curran, 1989). In the near infrared (NIR: 700-1300nm) and short-wave infrared (SWIR: 1300-2500nm), absorption is driven by the stretching, bending or deformation of certain molecular bonds (Kumar et al., 2006). In vegetation studies, this difference is highlighted in the transition between the visible and NIR regions, which has been termed the red-edge (690 – 720nm) (Gitelson and Merzlyak, 1998; Kumar et al., 2006). A large increase in reflectance is observed due to a shift in primary photon interaction from leaf pigments in

the visible region to leaf water content and internal leaf structures in the NIR (Kumar et al., 2006). The various influence that leaf properties exert on subsequent leaf optical properties provide valuable information about the internal components of a leaf and can be used to predict functional traits.

1.4.2. Functional trait prediction

In order to validate the trait estimates produced from spectroscopy, the foliage used for the leaf-level spectroscopy undergoes a variety of chemometric tests to determine the chemical composition of the compounds of interest (Asner et al., 2011). Chemical results are commonly associated with spectral reflectance values in two ways: index-based and partial least squares regression. Both methods are prevalent in literature and have been proven successful (Feilhauer et al., 2010; Xue and Su, 2017).

The indices method utilizes individual spectral wavelengths that are associated with certain foliar chemicals (Cornelissen et al., 2003; Curran, 1989). In an index-based analysis the reflectance values of specific wavelengths are used as the input values for predefined equations to estimate the relative amount of chemical abundance, such as carotenoids, chlorophyll, lignin or nitrogen (Blackburn, 1998; Daughtry, 2001; Ferwerda et al., 2005). Although effective, this methodology presents issues related to accuracy as the limited number of spectral bands used lacks statistical sensitivity (Xue and Su, 2017).

Partial least squares regression (PLSR) addresses the issues caused by a limited number of spectral bands by utilizing the entire spectral signature and is commonly used in conjunction with chemometric data (Wold and Sjostrom, 2001). PLSR is particularly adept at averting issues of collinearity, which are implicit in spectroscopy as the reflectance values of adjacent wavelengths can represent similar information (Wang et al., 2016). Recently, PLSR has been used as the predominant

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method of trait extraction when conducting spectranomic analyses for ecological purposes (Chavana-Bryant et al., 2019; Roth et al., 2015). Upon determining associations between leaf spectroscopy and the chemometric results, it is possible to relate the leaf-level spectral data with that acquired through imaging spectroscopy (Feilhauer et al., 2015).

1.4.3 Scaling from land to air

Remote sensing technologies provide information relating to leaf reflectance and plant morphology and are effective for mapping multiple functional traits (Schweiger et al., 2017; Wang et al., 2019). Airborne imaging spectroscopy is not uncommon, but the cost and time associated with collecting the leaf-level validation data is a limiting factor in large studies. Asner et al., 2009 proposed a methodology called 'spectranomics', which combined airborne laser scanning (ALS), airborne imaging spectroscopy and leaf spectroscopy to remotely sense plant functional traits. Spectranomics combines leaf and imaging spectroscopy with leaf chemical analysis, or chemometrics, to evaluate the functional traits of plants. Leaf spectroscopy is competed by scanning leaf samples from the species of interest. The results of this analysis provide reflectance values from 350 nm to 2500 nm for the associated leaf, which can be organized in a two-dimensional plane to provide a spectral signature (Figure 4).


Figure 4. Spectral signatures of thirty Q. garryana (Garry oak) trees. Note that the signatures all have the same general shape, but vary slightly in reflectance value at multiple wavelengths.

The data acquired through imaging spectroscopy is similar to that of leaf spectroscopy as it contains spectral information at multiple wavelengths between 300 nm - 2500 nm, but is presented in a pixelated format similar to the imagery collected form a standard commercial camera (Martin et al., 2008). Each pixel collected using imaging spectroscopy contains reflectance information that can span the same spectral range of the leaf spectroscopy, allowing comparisons between the chemically validated leaf spectroscopy and the spectral information in an image (Butler et al., 2017). Connections between a pixel and the validated leaf-level spectral data facilitate the identification of individual species, species diversity and the distribution of foliar chemistry present within the imagery (Asner et al., 2014, 2012; Yang et al., 2016).

One advantage of spectranomics is that imaging spectroscopy can be completed at a variety of scales. Airborne sensors with a spatial resolution larger than 30 cm² dominate the literature (Asner et al., 2018; Skowronek et al., 2017; Zhao et al., 2018), but recent studies are exploring the use of imaging spectroscopy at finer spatial scales (Schweiger et al., 2018). A larger scale provides good results for the interpretation of forest canopies, but precludes the evaluation of individual leaves or relatively small plant species (Asner et al., 2008a; Serrano et al., 2002). This inherently ignores many non-tree species and creates mixed pixels. The consequent effects of capturing spectral reflectance data from multiple plant species in a single data point has not been explored and could present challenges when attempting to accurately predict leaf functional traits. As a result, this issue is specifically addressed in this thesis. Many spectranomic studies are also conducted in closed canopy ecosystems (Asner et al., 2011; Zhao et al., 2018). This enables spectral analysis of the uppermost foliage across the landscape, but does not allow the evaluation of vertical variation in spectral properties. This research was conducted in the tropics and proved to be both effective and efficient at differentiating the species and functional characteristics of forest canopies (Asner and Martin, 2009). The ability to map leaf functional traits over space and time compliments previous research that evaluated the impact of various abiotic factors on leaf trait distribution and promotes similar studies utilizing remote sensing to determine large-scale leaf economic trends. As such, it is important to understand key global trends already established in leaf functional trait literature.

1.5 Gradients of functional diversity

1.5.1 Elevational Trends

An integral study relating to the diversity of leaf traits in relation to elevation was conducted by Ehleringer (1988). This study examines over 150 vascular plant species along a gradient of elevation and precipitation. The authors found that leaf absorptance remained relatively constant

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except in the driest areas, in which it decreased (Ehleringer, 1988). Leaf angle changed considerably in relation to precipitation, with steeper angles occurring in drier areas (Ehleringer, 1988). Elevation also influenced leaf angle, with the lowest sites having the steepest angles (Ehleringer, 1988). The combination of characteristics observed at the lowest, driest sites suggest that plants are altering functional leaf traits to minimize heating and water loss (Ehleringer, 1988).

Read et al., 2014 observed that some leaf traits vary predictably along elevation gradients. This meta-analysis found that leaf nitrogen (N) content and leaf mass per area (LMA) vary along elevation gradients in relation to mean annual temperature (Read et al., 2014). These variations were found across a multitude of species and existed both inter- and intraspecifically (Read et al., 2014). Asner et al., 2016 found that the spatial variation of foliar N, P and LMA are strongly linked to both elevation and substrate. Other abiotic factors were important in predicting the distribution of specific traits, such as mean annual precipitation and relative elevation in predicting N and LMA, but were insignificant for others (Asner et al., 2016). Many LES relationships, such as the negative correlation of LMA and N, were shown to exist but vary in their strength across elevation and substrate (Asner et al., 2016). This suggests that abiotic conditions play a significant role in determining the variation of functional diversity across landscapes and influences the resource allocation strategies of plants (Asner et al., 2016).

1.5.2 Global Trends

Functional

The relationships of various leaf functional traits transcend biomes and remain consistent across the globe. The methods employed to derive the leaf economic spectrum were used as a springboard for Diaz et al., 2016 to explore the relationships of six plant functional traits considered to be indicators of specific ecological strategies (Díaz et al., 2016). Two important axis were derived.

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The first is related to morphological characteristics of the plant, such as total height, LMA and leaf nitrogen per mass (Díaz et al., 2016). The second dimension follows the LES and reflects the tradeoff between leaf construction and growth (Díaz et al., 2016). These findings support the idea that spectrums relating to plant resource investment strategies span multiple plant organs, rather than just leaves, and are consistent at a global scale (Díaz et al., 2016; Freschet et al., 2010). Evidence of a plant economic spectrum had previously been hypothesized by Freschet et al., 2010, but only considered species sub-arctic flora.

Latitudinal

The theory that plant species diversity varies along a latitudinal gradient was well established in the 20th century (Pianka, 1966). Essentially, this theory outlines the relationship between geography and biodiversity (Usinowicz et al., 2017). In tropical ecosystems, the climate allows for longer growing seasons and supports a larger standing biomass (Wright, 2002). As latitude increases ecosystems experience shorter growing seasons and a more variable climate, limiting growth, reproduction and survival (Usinowicz et al., 2017). Such drastic changes in species diversity along a predictable gradient presents interesting opportunities to study variations in ecosystem processes, such as the primary productivity of forests.

Productivity

As with species diversity, terrestrial net primary productivity varies along latitudinal gradients that can be defined according to their climate (Huxman et al., 2004; Usinowicz et al., 2017). Tropical forests tend to have higher net primary productivity than temperate stands (Usinowicz et al., 2017). The role of precipitation and temperature have been found to be less important than stand properties like age, biomass and growing season length, suggesting that indirect factors drive productivity (Michaletz et al., 2018).

The spatial gradients of plant functional characteristics are of great importance for monitoring plant health and underlying environmental conditions. Elevation and soil substrate have been proven to have significant impact on plant functional traits and demonstrate the need for continued analysis at all spatial scales (Asner et al., 2016; Ehleringer, 1988; Read et al., 2014). A combination of air- and spaceborne imaging spectroscopy will provide the information required to estimate and understand the functional characteristics of earth's vegetation, as well as present timely information from which management decisions can be implemented (Enquist et al., 2015; Vihervaara et al., 2017).

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1.6 Humans and leaf functional traits

Along with identifying the distribution of foliar chemistry and taxonomy, spectranomics presents an opportunity to examine how the spatial distribution of specific anthropogenic landscape modifications interact with biodiversity. Land use change, fire suppression and urban encroachment are common activities that persist worldwide and threaten biodiversity (Gurevitch and Padilla, 2004; MacDougall et al., 2004; Tratalos et al., 2007). These processes can impose shifts in the chemical and physical properties of an ecosystem (Fuchs, 2001). Such changes lead to alterations in the functional traits, and therefore fitness, of individual plants and can unhinge the traditional balance of species richness and abundance (Eldegard et al., 2015; Godbold and Solan, 2009; Stevens et al., 2010). To exploit the full potential of the spectranomic technique, it is important to gather information about a variety of ecosystems.

1.7 Canadian prairie-oak savannas

Traditionally, prairie-oak savanna ecosystems contained the highest level of biodiversity in BC, with a range spanning the south-east coast of Vancouver Island and the Gulf Islands (Bjorkman and Vellend, 2010). The range of these ecosystems stretches south into the states of Washington and Oregon and have played pivotal roles in the placement of European settlements. Also named Garry oak ecosystems, these spaces were created and maintained by First Nations through prescribed burning and thoughtful species selection . For centuries prior to European colonization these partially wild and partially managed ecosystems provided essential overwintering food through the bulbs of *Camassia* sp., amenable living quarters and designated meeting spaces for West Coast communities (Dunwiddie and Bakker, 2011; Fuchs, 2001). Ultimately, however, these favourable characteristics guided Sir James Douglas' decision to settle in the harbour of Camosack, now Victoria, rather than relatively larger Esquimalt, kindling European immigration (Acker, 2012). The settlement of almost 16 km² of prairie-oak savanna surrounding the harbour made land use change

easy and fostered the spread of urbanization and agriculture into similar landscapes (Acker, 2012). Specifically, settlers were attracted to the fertile and relatively deep soils associated with some portions of prairie-oak savannas. At the turn of the 21st century, the relatively small land area of woodland-grassland savannas (< 4%) contained over 75% of the area's human population (Floberg et al., 2004).

Currently, prairie oak ecosystems are threatened by multiple anthropogenic landscape modifications and continue to experience losses in both biodiversity and area (Bjorkman and Vellend, 2010; MacDougall et al., 2004). Prairie-oak ecosystems are considered endangered in Canada as the percent of near-natural habitat is less than 5% of it's original footprint (Bjorkman and Vellend, 2010; Dunwiddie and Bakker, 2011; MacDougall et al., 2004). Abiotic threats stem mainly from the complete suppression of fire, which has enabled woody plants to establish unabated (Fuchs et al., 2000). Biotic threats include invasive plant species, such as *C. scoparius*, herbivory and the encroachment of Coastal Douglas-fir forests (Fuchs, 2001).

In 2001, Environment Canada published a formal recovery strategy for these ecosystems. Their report highlighted that the health status of prairie-oak savannas are linked with the plant species composition, structure underlying plant function (Fuchs, 2001). Three fundamental environmental features were identified by Fuchs et al. (2001) as being under threat from contemporary anthropogenic activities:

- 1. Spatial integrity, in terms of the consequences of habitat fragmentation;
- 2. The role of fire as a natural disturbance agent; and
- 3. Biotic integrity, in terms of the presence and effects of exotic species.

Fuchs et al (2004), and subsequently Dunwiddie and Bakker (2011), also state that minimal research has been conducted in British Columbia related to the quantitative impact of these issues.

Although 20 years have passed since the publication of Environment Canada's recovery strategy, prairie-oak savannas continue to be relatively underrepresented in the literature, especially in relation to the effects of anthropogenic activities on plant habitat, function and invasive species.

1.8 Objectives

The goal of this research is to address knowledge gaps related to the estimation and scaling of leaf functional traits in a heterogenous ecosystem. Specifically, I will explore the consequences of scaling trait prediction from a single leaf to an airborne sensor. I will also explore the capacity for imaging spectroscopy to evaluate the influence that anthropogenic activities have on the three fundamental environmental features of prairie-oak ecosystems (spatial integrity, fire suppression and biotic integrity) considered to be significantly at risk by Fuchs et al. (2001). These objectives will be achieved by:

- Evaluating the accuracy of remotely sensed leaf functional trait predictions
- Mapping leaf functional traits and assessing the impact of anthropogenic activities, including land use change and the subsequent habitat fragmentation, as well fire suppression, on their spatial distribution
- Using leaf functional traits to identify invasive plant species

1.9 Research Questions

The aforementioned objectives will be addressed through the following research questions:

- How are the predictions of plant functional traits affected by the inclusion of reflectance information from multiple species in a single reflectance spectrum?
- 2. How do anthropogenic activities influence the spatial distribution of interspecific plant functional traits?
- 3. How are the functional traits of a single plant species affected by anthropogenic activities?
- 4. What is the capacity for remotely sensed plant functional traits to distinguish invasive plant species?

1.9 Dissertation Overview

Chapter 2 provides an overview of the site at which data for this research was collected and discusses the methods used for data acquisition. **Chapter 3** is the first research chapter of this dissertation and explores how the prediction of functional traits are impacted by spectral mixing. **Chapter 4** maps plant functional traits across the landscape and evaluates the influence of environmental and anthropogenic influences on their spatial distribution. **Chapter 5** assesses the capacity of plant functional traits to distinguish an invasive shrub, *Cytisus scoparius* (L.) Link, in prairie-oak savannas. **Chapter 6** investigates the impact of anthropogenic activities on the intra-specific variation of leaf functional traits in the dominant tree species in prairie-oak savannas, *Quercus garryana* var *garryana* Douglas ex Hook. **Chapter 7** provides conclusions relative to the scientific objectives pursued in this dissertation, highlighting the innovations and limitations of this research.

Chapter 2: Site Overview and Data Curation

2.1 Study site

The field work relating to this research was conducted at the Cowichan Garry Oak Preserve (CGOP; Lat: 48°48'29.85"N, Long: 123°37'54.34"W) in Duncan, British Columbia (BC), Canada between May 4 – 19, 2019 (Figure 5). Managed by the Nature Conservancy of Canada (NCC), this site contains a variety of ecosystems, including prairie-oak savannas. Known commonly as Garry oak savannas, these mixed grassland-woodlands harbour a large variety of plant, vertebrate and insect species. Another component of the site is a recently converted agricultural pasture that was traditionally managed to grow hay. This portion of the CGOP likely experienced a variety of agricultural activities including fertilization and irrigation. Due to the variety of traditional land management practices (prairie-oak savanna, agriculture) it is also likely that the site contains subzones that vary in soil depth.

Anthropogenic activities such a fire suppression and land use change pose an immediate threat to the traditional structure of these ecosystems (Fuchs, 2001). Roads abut the site from the south, east and west, while *Q. garryana* var. *garryana*, a native tree species, has been able to increase its canopy cover due to the removal of burning. The presence of roads and increased canopy cover impact a variety of ecological factors, including soil wetness, availability of photosynthetic radiation and microclimate (Caldwell et al., 1986; Forman and Alexander, 1998). Considering the nature of this ecosystem as a hybrid of woodland and grassland plant species, as well as the direct impact of fire suppression on the presence of trees, it is logical to evaluate subsites based on their relative tree canopy cover.



Figure 5. Study area composite depicting (a) the range extent of Quercus garryana, (b) the Cowichan Garry Oak Preserve (CGOP) in Duncan, BC, Canada and (c) a section of the meadow in bloom during May 2019. The range of Q. garryana includes both Canadian and North American areas (Little, Elbert L., 1971).

Plant communities have been evaluated and richness within 1 m² vegetation plots can range from 4 – 26 species. Many of the plant species have small spatial footprints and can represent between 1 – 19% of vegetation plot cover. This capacity for high species richness and low individual cover means that the accurate prediction of leaf functional traits is unrealistic and that relative trait values should be used when comparing spatial relations (Hacker et al., 2022). The CGOP exists within the Coastal Douglas-fir moist maritime Biogeoclimatic Ecosystem Classification (BEC) zone (Fuchs, 2001; Hamann and Wang, 2006). The mean annual temperature at the CGOP is 9.5 °C with approximately 1200 mm of annual precipitation.

2.2 Data Overview

The data used to conduct the research presented in this dissertation was collected over two field programs. The first occurred from July 22 - 28, 2018 and collected information relative to Chapter 5. A second field campaign took place from May 4 - 19, 2019 and collected data relative to Chapters 3, 4 and 6. A total of 23 plant species were sampled during the aforementioned field work (Table 2). The specific methodologies employed for data collection are detailed in the remainder of Chapter 2 and reiterated in Chapters 3, 4, 5 and 6 when required.

Table 2. Species list of the 23-plant species and the number of individuals collected (parentheses) at the CGOP in May 2019.

Berberis aquifolium Pursh (10)	Lomatium utriculatum (Nuttall ex Torrey & A. Gray) J.J. Coulter & Rose (10)	
Bromus sitchensis var. carinatus (Hooker & Arnott) R.E. Brainerd & Otting (10)	Oemleria cerasiformis (Torrey & A. Gray ex Hooker & Arnott) J.W. Landon (10)	
Bromus sterilis Linnaeus (6)	Plectritis congesta (Lindley) de Candolle (10)	
Camassia leichtlinii (Baker) S. Watson (10)	Poa pratensis Linnaeus (10)	
Camassia quamash (Pursh) Greene (10)	Polystichum munitum (Kaulfuss) C. Presl (7)	
Claytonia perfoliata Donn ex Willdenow (10)	<i>Quercus garryana</i> Douglas ex Hooker (10)	
Crataegus monogyna Jacquin (10)	Rosa nutkana C. Presl (10)	
Dactylis glomerata Linnaeus (10)	Sanicula crassicaulis Poeppig ex de Candolle (10)	
Festuca idahoensis Elmer (6)	Sericocarpus rigidus Lindley (3)	
Holodiscus discolor (Pursh) Maximowicz (10)	Symphoricarpos albus Poeppig ex de Candolle (10)	
Lathyrus sphaericus Retzius (6)	Vicia sativa Linnaeus (10)	

2.3 Leaf Spectroscopy

2.3.1 July 22 – 29, 2018

Sun-lit leaf samples were collected from each of the 40 *Q. garryana* individual using 18m long pruners. All leaf samples were stored on ice for no longer than 1 hour. Samples stored in the cooler underwent spectroscopy, scanning and weighing no more than 8 hours after collection, after which they were flash frozen until drying. An Analytical Spectral Devices (ASD) FieldSpec3 portable

spectroradiometer and ASD Integrating Sphere, which is a closed illumination system (Analytical Spectral Devices Inc., Boulder, Colorado (CO), USA) was used to measure the reflectance spectra of each sampled leaf. White and dark references were taken before each sample was scanned to normalize the recorded radiance, which enables the calculation of reflectance (Analytical Spectral Devices, 2010). Each full scan comprised the average of 20-sub scans. Three leaves per tree underwent full scans an average of 20 times to provide a mean of approximately 400 scans per leaf sample. Reflectance was measured from 350–2500 nm and the values of each spectral band were the inputs for the PLSR that was used to estimate plant functional trait values.

2.3.2 May 4 - 19, 2019

Between May 4 – 19, 2019 the leaves of 23 plant species were collected using metal pruners and latex gloves to avoid interaction of skin oils with leaf surfaces (Table 2). Only healthy, nondamaged leaves were sampled for all individuals. All leaves were considered to be fully sunlit, fully expanded and mature. Individuals were sampled randomly within species and across site. Leaves were stored in sealed plastics immediately after collection. Prior to sealing the bags, the collector breathed into the bag to increase CO^2 and water vapour concentration to minimize leaf water loss. Sealed bags were stored in a cool box surrounded by (no direct contact) with ice and spectroscopy was conducted within 8 hours of collection. An Spectra Vista Corp (SVC) HR-1024i spectroradiometer and integrating sphere were used to measure leaf spectral reflectance and transmittance between 350 nm – 2500 nm (Laliberté and Soffer, 2018). I sampled six leaves from each of 201 individuals belonging to 23 plant species. All spectra were vector normalized prior to modelling to normalize brightness and emphasize differences in spectral shape and only bands 400 – 2400 nm were used in analyses. A Savitzky-Golay filter was used to reduce noise, ambient light or slight variations in sensor conditions (Gautam et al., 2015).

2.4 Leaf Chemistry

A total of 14 leaf functional traits were measured during this study (Table 3). These traits represent a subset of plant functional traits and were selected based on their proven correlation with other key traits such as phenolics, flavonoids and anthocyanins (Díaz et al., 2016; Reich, 2014; Wright et al., 2004). Three pigments, chlorophyll a (chl a; mg/g), chlorophyll b (chl b; mg/g) and carotenoids (Car; mg/g), were selected based on their association with plant growth (Curran et al., 2001; Girard et al., 2019). The percentage of carbon (%C) and nitrogen (%N), and their ratio (C:N) were included as these chemicals are related to optimal plant growth, with %N also being identified as an important trait in the leaf economics spectrum (Ayotte et al., 2019; Wright et al., 2004; Zheng, 2009). Two traits associated with leaf mass, leaf mass per area (LMA; g/m^2) and leaf dry matter content (LDMC; mg/g) were selected based on their relationship to plant ecological strategies, primary production and the leaf economics spectrum (Reich et al., 1997; Smart et al., 2017; Westoby et al., 2002; Wright et al., 2004). Equivalent water thickness (EWT; cm) was included due to associations with plant ecological strategy (Féret et al., 2019). Structural leaf components cellulose (%), hemicellulose (%), lignin (%), solubles (%) and recalcitrants (%) were also evaluated based on their relationship with nutrient cycling (Ayotte and Laliberté, 2019; Nagler et al., 2000; Van Cleemput et al., 2018).

Trait	Mean	Standard Deviation	Trait	Mean	Standard Deviation
Chlorophyll <i>a</i> (mg/g)	10.4	4.81	LDMC (mg/g)	219.0	87.2
Chlorophyll $b (mg/g)$	3.53	1.81	EWT (cm)	0.013	0.0056
Carotenoids (mg/g)	2.24	0.86	Cellulose (%)	14.7	6.23
N (%)	3.05	0.88	Hemicellulose (%)	16.2	8.35
C (%)	45.6	1.56	Solubles (%)	62.3	13.1
C:N	16.3	5.31	Lignin (%)	6.57	5.04
LMA (g/m^2)	40.7	17.6	Recalcitrants (%)	0.22	0.16

Table 3. Measured values of 14 traits for 201 samples at the CGOP

2.5 Imaging Spectroscopy

The imagery utilized in this dissertation was collected using a Mjolnir VS-620 (HySpex NEO, Skedsmokorset, Norway) hyperspectral imaging system (HSI) deployed using an remotely piloted aircraft system (RPAS) consisting of an XL gimbal (Gremsy, Ho Chi Minh City, Vietnam) mounted on an octocopter over three consecutive days from May 15 – 17, 2019 (Arroyo-Mora et al., 2021). Three unique subsites covering a total of 11,900 m² and experiencing varying levels of anthropogenic influence (distance to roads, canopy cover, etc.) were surveyed using this HSI-RPAS. Flight altitude and speed remained consistent across all flights at 60 m above ground level and 1.0 m/s, respectively (Arroyo-Mora et al., 2021).

Image pre-processing was conducted following the workflow presented in Arroyo-Mora et al. 2021 in order to compensate for variations in illumination conditions. A total of 331 spectral bands are usable for images collected on May 16 and 17, while imagery collected on May 15 had 333 usable bands. The spatial resolution of imagery collected on May 16 and 17 is 3.25 cm², while the image collected on May 15 has a spatial resolution of 3.0 cm². A greenness mask was applied by calculating the hyperspectral normalized difference vegetation index (hNDVI) using ranges of spectral bands surrounding those traditionally used in NDVI in order to identify pixels containing vegetation (Equation 1). Pixels with hNDVI values less than 0.2 were excluded from further analyses. Metrics derived from the CGOP DSM enabled the identification of pixels higher than 2 m above the ground, which were subsequently removed.

 $hNDVI = \frac{avg(890:910) - avg(675:995)}{avg(890:910) + avg(675:995)}$

Equation 1.

2.6 Vegetation Plots

A total of 30 plots were evaluated, each 3 x 3 m and consisting of 9, 1 x 1 m subplots. A total of 61 plant species were observed with a minimum and maximum number of species per subplot of 4 and 26, respectively (Table 4). *Vicia sativa* was the most common species, occurring in all vegetation plots with cover ranging from 1% - 19%. *Poa pratensis* showed the largest single subplot coverage (85%) and was present in 23 plots. Four different lifeforms were observed in the vegetation plots: forb, shrub, graminoid and tree.

Table 4. Summary statistics of the vegetation surveys conducted at the Cowichan Garry Oak Preserve in May 2019.

Total Subplots	Total	Max	Min	Lowest Cover	Highest
$(1m^2)$	Species	Species/Plot	Species/Plot	(%)	Cover (%)
270	61	26	4	1	85

2.7 Airborne Laser Scanning and Roads

Road vector information and airborne laser scanning (ALS) data were acquired from the BC Data Catalogue maintained by the BC Ministry of Forest, Lands and Natural Resource Operations and Rural Development and is freely available online (www.catalogue.data.gov.bc.ca). The road dataset was last updated April, 2017, while the ALS data of the CGOP was acquired by the Municipality of North Cowichan on 29 May 2017. The ALS data were collected using a Riegl Q1560 dual-channel ALS system at a nominal flight height of 1700 m above ground level and has a density of 15 returns/m².

Chapter 3: Effects of spectral mixing on leaf functional trait prediction¹

3.1 Background and Motivation

In this chapter I will address the theoretical knowledge gap relating to effects spectral mixing on leaf functional trait prediction. Spectral mixing occurs when reflectance information from multiple plant species in a single data point is recorded. This complication arises as an issue of scaling from leaf-level to the canopy-level analyses in which an airborne sensor, in my case an imaging spectrometer deployed on a drone, is used to capture optical reflectance information across a landscape, rather than a single leaf. The issue of spectral mixing is particularly challenging are heterogenous ecosystems, such as prairie-oak savannas, which are often rich in plant diversity with high variations of interspecific adjacencies (House et al., 2003).

The plants occurring in these ecosystems are small, increasing the likelihood that pixels generated from airborne or spaceborne imaging spectroscopy contain multiple species (Van Cleemput et al., 2018). This leads to concerns about the capacity of imaging spectroscopy to accurately predict leaf functional traits from models generated using the spectral information of species that may not be providing a large portion of the measured reflectance. Variations in trait prediction due to spectral mixing may lead to a misestimation of the number of species present in a pixel or in the trait value itself, compromising the effectiveness of functional trait estimation and any associated evaluations of biodiversity or ecosystem health. It is of vital importance, then, to understand the complications presented by spectral mixing on the accuracy of leaf trait predictions if we are to properly evaluate their spatial distribution and associated ecosystem health metrics.

¹ Content presented in this chapter has been adapted from:

Hacker, P. W., Coops, N. C., Laliberté, E., & Michaletz, S. T. (2022). Variations in accuracy of leaf functional trait prediction due to spectral mixing. 136(February). https://doi.org/10.1016/j.ecolind.2022.108687

Using a framework developed to recreate the effects of spectral mixing in a prairie-oak ecosystem, this research will explore three research questions:

- 1. How does spectral mixing affect the prediction error of functional trait estimation?
- 2. Are leaf functional traits equally affected by spectral mixing?
- 3. Does spectral mixing with different plant lifeforms affect trait prediction?

3.2 Materials and Methods

3.2.1 Data Overview and PLSR modelling

The data used for this research was collected during the May 2019 field campaign. Leaf spectroscopy and chemical results from 23-plant species (Table 2) were used as input spectra for the body of this analysis, as well as to generate leaf functional trait PLSR models. PLSR was used to create site-wide, species-inclusive models consisting of 201 samples from 23 plant species for the 14 functional traits of interest (Haaland and Thomas, 1988; Serbin et al., 2019) (Table A2). Data were split into training (70%) and validation sets (30%). The ideal number of components was determined using one-sigma heuristic cross-validation (Mevik and Wehrens, 2015). Model accuracy was evaluated using the coefficient of determination (R²), root mean squared error of the predictor (RMSEP) and normalized RMSEP (NRMSEP) (Wehrens, 2007). Bias was not included as RMSEP are bias-corrected. The threshold for good and adequate R² is considered to be 0.65 and 0.50, respectively. Good and adequate thresholds for NRMSEP are considered to be 20% and 30%, respectively. Coefficients from each of these models were applied to each band of the pure and mixed spectral signatures to generate trait predictions.

3.2.2 Theory

To evaluate the effects of spectral mixing on the prediction of leaf functional traits among species I developed a simple scaling framework (Coops et al., 2003). First, I generated speciesinclusive PLSR models for 14 functional traits. Of the 14 models evaluated, it is necessary to select only those with acceptable R² and NRMSEP. For this study, only 8 traits were accepted (R²: 0.54 – 0.85, NRMSEP: 16-31%) (Table A2). Second, I selected a target species which was relatively ubiquitous throughout the study area and used the accepted PLSR models to predict baseline trait values to which to compare future trait prediction values . Multiple fractionated spectra were then created for each species by completing per-band weighted averages of each species pure spectral reflectance signature, or endmember (Figure 6). Next, I generated mixed spectral signatures by combining fractionated leaf spectra of various species (Figure 6). I then applied the accepted PLSR models to these mixed spectra to predict each functional trait and evaluate the effects of spectral mixing based on our predefined hypotheses.



Figure 6. Flow diagram depicting the generation of mixed spectral signatures. This example demonstrates how a 1) theoretical pixel containing two species contributing 75% (Species A) and 25% (Species B) of the spectral reflectance, respectively, is mixed. The 2) endmembers, or pure spectral reflectance signatures, of Species A and B are selected from a candidate database and 3) undergo per-band weighting according their respective spectral contributions. These fractionated spectra are 4) combined linearly to generate a 2-species mixed spectral signature.

I chose V. sativa as the baseline species as it was present in 100% of vegetation plots and

had subplot coverage ranging from 1-15%. This nitrogen-fixing legume also demonstrates capacity

to entangle and climb with other plant species, causing the overlapping of leaves. Mixed spectra were generated by linearly mixing the fractionated spectra of two or more species across all bands using set percentage combinations on the assumption that any portion of a leaf surface contributing to reflectance is equal across all wavelengths (Figure A1).I allow co-occurrence of species at 3 mixing levels: 2-, 3- and 4-species. 2-species mixes consist of the target species and one other species (22 mixes per target species) with target species presence decreasing by 10% from 100% to 0%. A total of 110 3-species mixes were generated by combining the spectra of two non-target species with the target species. To ensure that all lifeforms were mixed equally all non-target species were split into 2 groups before mixing.

Spectral mixes containing four species were generated using four separate species lists. The first list enabled each species to be mixed with each species from a different lifeform. The remaining three lists enabled the target species to be mixed with 3 species of the same lifeform. Combined, this 4-species mixing strategy created 409 unique mixed spectral signatures that captured the effects of mixing within and across lifeforms. A total of 541 unique mixed spectra were created across all mixing levels. Upon completion, this framework was used to examine our three research hypotheses.

3.2.3 Hypotheses

H1: Spectral mixing increases the prediction error of functional trait estimation

Comparison between the predicted trait value for the target species endmember, or baseline trait value, and the predicted mixed spectra trait values were conducted for 8 functional traits using 95% confidence intervals (CI) from the baseline. The confidence interval was calculated as two standard errors from the mean. Observed mean trait values beyond the 95% CI are significantly different.

H2: Traits are equally affected by spectral mixing

Variations in the average percent difference of a predicted trait value from the predicted trait value of the target species endmember, or average percent change, were calculated for each trait by summing the percent variation of each 50% mix and dividing by the number of species (n = 23). Average percent change is relevant for determining if the addition of 2, 3, or 4 other species within a spectral signature causes an observable change in the predicted trait value from the predicted value of the pure target species spectra. Variation from the baseline would suggest that the spectral mixing does alter the capacity to estimate a trait and can highlight which traits are affected the most by mixing. Cumulative percent was calculated by summing the absolute value of percent change in trait value of each 50% mix. Cumulative percent change is relevant for two reasons. First, it presents the effect that each additional species has on the prediction of a specific trait. Second, it highlights which traits are more affected than others at each specific mixing level (2, 3 or 4 species). The average and cumulative percent changes of each trait at 50% target presence were calculated for 2-, 3- and 4-species mixes.

H3: Spectral mixing with different plant lifeforms does not affect trait prediction

The evaluation of significant trait variation between lifeforms was conducted by grouping measured trait values by lifeform. I tested each lifeform group for normality using the Shapiro-Wilk test and found many traits exhibited a non-parametric distribution. Significance of trait variation between lifeforms was therefore tested using the Kruskal-Wallis one-way analysis of variance. If the Kruskal-Wallis test was significant a pairwise Wilcox test was used to determine which specific lifeforms were different.

3.2.4 Software

Modelling, data manipulation and plotting were conducted using the R computing language 4.0.0 (R Core Team, 2021), the 'pls' (Liland et al., 2021) and 'tidyverse' (Wickham et al., 2019) packages . The mathematical functions used to conduct vector normalization and generate fractionated spectra were provided by the 'spectrolab' package (Meireles and Schweiger, 2021).

3.3 Results

3.3.1 Measured trait values and PLSR modelling

All but two of the 14 measured leaf functional traits varied significantly between graminoid, forb, shrub and tree lifeforms (Figure A2). Carotenoid values for graminoid and tree species and LMA values between graminoids and shrubs were the only trait-lifeform relationships that did not significantly differ from each other. The accuracy of the trait prediction using the single species leaf spectra models varied, with R² ranging from 0.10 to 0.85. Due to their low accuracy, carotenoid content (R² = 0.36), carbon % (R² = 0.48), solubles % (R² = 0.41), hemicellulose % (R-2=0.36), lignin % (R² = 0.46) and recalcitrant % (R² = 0.28) were not included in further analyses. The remaining 8 traits demonstrated acceptable predictive accuracies (R² = 0.54 – 0.85), with LDMC (R² = 0.67), LMA (R² = 0.69) and EWT (R² = 0.85) producing the highest R² (Table A2). The 8 accepted traits demonstrated NRMSEP values between 16% and 33%. Model accuracies of the 8 accepted traits are within our thresholds and consistent with other scientific literature evaluating plant functional traits in grasslands using leaf spectroscopy and PLSR (Wang et al., 2019). The number of components used in a single model varies from 4-8.

3.3.2 Effects of spectral mixing on functional trait prediction

The predicted means and 95% confidence intervals of the eight accepted traits derived from spectra with 100% target species (*V. sativa*) presence are presented in Table 5. The values displayed

under "Percent of Deviation" in Table 5 represent the lowest percentage of target species for which the predicted functional trait values would be considered accurate (within the 95% CI). Any mix containing a lower percentage of the target species resulted in a significantly different trait prediction and precluded target detection using that specific trait.

As target species presence decreased, the predicted values of each trait deviated from the baseline mean (Table 5). When target species presence dropped below 80% the predicted values for both C:N and EWT crossed beyond the 95% CI for 2-species mixes, indicating that the effects of spectral mixing were significant when the target species comprised less than 80% of the mixed spectral signature (Table 5). Nitrogen % demonstrated significant changes in prediction accuracy at 70% when two species were mixed together, while LMA and LDMC remained accurately predicted until target species presence dropped below 30% (Table 5). Predicted values of chl *a* remained accurate until only 20% of the 2-species mix was composed of *V. sativa* (Table 5). Chlorophyll *b* and cellulose were accurately predicted as long as target species presence remained 20% or higher (Table 5).

When the number of species present in a spectral mix increased, the accuracy of trait prediction was affected more heavily for certain traits than others. The effects of spectral mixing on the predicted values of EWT, C:N, cellulose and chl *b* were realized at the same percent of target species presence, regardless of 2-, 3-, or 4-species being included (Table 5). The accuracy of %N predictions were compromised when target species was below 70% for 2- and 3-speices mixes, while 4-species mixing effects occurred when *V. sativa* dropped below 80% (Table 5). Chlorophyll *a* was significantly affected by spectral mixing at 20% target species presence for 2- and 4- species presence, but 10% for 3-species mixing. Spectral mixing affected LMA and LDMC inconsistently as the number of species included in the mix increased (Table 5). LMA was significantly affected when target species dropped below 30% for 2-species mixes and 40% for 4-species mixes, yet prediction accuracy of 3-species mixes was only affected below 10% (Table 5). Even when mixing significantly affected LMA, predicted vales were only slightly outside the 95% confidence interval when target species presence was 20% or higher (Figure 7). Visualizations similar to Figure 7 for the other seven functional traits evaluated in this research are available in the appendices (Figure A3a – g). LDMC demonstrated prediction inaccuracies below 30% and 50% for 2- and 3-sepcies mixes, respectively, while 4-species mixes were unaffected by mixing (Table 5).

Table 5. Mean predicted trait value for the target species endmember, or baseline trait value, and lowest percent target species at which the predicted trait value remains within 95% confidence interval (CI) for each accepted trait. Percent of target species when mean trait invariant refers to the percent of target species (V. sativa) in the mix when the mean predicted trait remains within the 95% CI. All mixes containing target species presence lower than the displayed values result in significantly different trait predictions. "Within 95% CI" denotes that the predicted trait values for this trait at a given mixing level is not significantly affected by spectral mixing when target species presence is less than 20%.

		Percent of target species when mean trait invariant			
Trait	Baseline mean (95% CI)	2-species mix	3-species mix	4- species mix	
Chlorophyll a	11.69 (10.48, 12.90)	20%	10%	20%	
Chlorophyll b	3.84 (3.41, 4.27)	Within 95% CI	Within 95% CI	Within 95% CI	
%N	3.60 (3.40, 3.79)	70%	70%	80%	
C:N	12.79 (11.77, 13.82)	80%	80%	80%	
LMA	36.91 (33.9, 39.90)	30%	10%	40%	
LDMC	235.86 (225.11, 246.61)	30%	50% (226.37)	Within 95% CI	
EWT	0.010 (0.0096, 0.011)	80%	80%	80%	
Cellulose	15.00 (14.27, 15.74)	Within 95% CI	Within 95% CI	20%	



Figure 7. Changes in the predicted leaf mass per area (LMA g/m^2) for four levels of percent target species presence (20, 40, 60 and 80%). 2- (n = 22), 3- (n = 110) and 4-species (n = 409) mixes refer to the total number of species included in the mix. The horizontal red line represented the predicted trait value for the target species endmember. Horizontal black lines within each box indicate the median predicted trait value for the value and the whiskers of each box represent the largest value equal to or within 1.5 times the interquantile range. Mean trait values outside the 95% confidence interval (horizontal dotted lines) are significantly affected by spectral mixing.

Further comparison of species mixing with 50% target species presence at 2-, 3- and 4-

species mixing levels indicated that the effects of spectral mixing vary. The average percent changes of the predicted trait values for each accepted trait demonstrated that some traits are more affected when additional species contribute to the spectral profile analysed (Figure 8a). For example, the average predicted values of C:N and EWT decrease substantially due to mixing, while LMA and cellulose decrease slightly, but remain relatively unaffected (Figure 8a). The predicted values of %N, chl *a* and chl *b* increase slightly due to mixing, while LDMC is relatively unchanged (Figure 8a).

Cumulative percent changes were greatest in C:N and increased by a factor of four for each additional species (Figure 8b). Cellulose and LDMC demonstrated the lowest cumulative change due

to an increase in the number of species, with cumulative change from 4-species mixes being at least half of all other traits (Figure 8b). Like C:N, the remaining five traits demonstrated a 4-fold increase in cumulative percent change for each additional species, however their maximum cumulative change value was 4000 (Figure 8b).



Figure 8. The (a) average and (b) cumulative percent change of trait prediction for eight traits using a mixed spectra with 50% of the target species (Vicia sativa). A value of 0 (black line) is equivalent to the predicted trait value using the target species endmember.

Unique lifeforms reacted in different ways when combined with the target species at the 2species mixing level, and these effects varied by trait. Median LMA values for tree mixes at all ratios are above 95% CI (Figure 9a). Median LMA values for all non-tree species remain within 95% CI until target species presence drops below 30% (Figure 9a). Shrub and graminoid median LMA values exceed 95% CI when target species is equal to 20% (Figure 9a).

The predicted value of %N, for example, increased with target species presence (Figure 9b). This relationship was observed in all four lifeforms, with the only exception to this trend being the shrub *C. scoparius*. Predicted %N of tree and graminoid lifeforms are significantly affected by spectral mixing when target species presence is lower than 80% in a 2-species mix (Figure 9b). Predicted %N of forb and shrub lifeforms are significantly affected by spectral mixing when target species presence is lower than 60% (Figure 9b).



🖶 Forb 🗎 Graminoid 🗮 Shrub 🗰 Tree

Figure 9. Predicted trait values of (a) nitrogen percent (%N) and (b) leaf mass per area ((LMA g.m-2) of graminoid (light grey), forb (grey), shrubs (dark grey) and trees (black) mixtures for four levels of target species (Vicia sativa) presence (20, 40, 60, and 80 %). The red line represents the predicted trait value for the target species endmember (%N = 3.56%; LMA = 36.91 g/m-2) and dotted lines indicate (95% CI from the baseline trait mean (%N = 3.40%, 3.79%; LMA = 33.92 g/m-2, 39.90 g/m-2). Horizontal black lines within each box indicate the median predicted trait value and the whiskers of each box represent the largest value equal to or within 1.5 times the inter-quantile range.

3.4 Discussion

This research determined that the prediction accuracy of leaf functional traits using PLSR and leaf spectra changes as the number of species present in a pixel increases, suggesting that spectral mixing should be taken into consideration when mapping prairie-oak plant species or leaf traits at spatial scales larger than the footprint of the smallest species. It is also the case that some traits are more affected by spectral mixing than others and that the selection of a specific trait for analyses should take this into account. The introduction of different lifeforms also alters the capacity of PLSR models to predict leaf functional traits, which presents both opportunities and complications when evaluating leaf functional traits in prairie-oak ecosystems.

3.4.1 PLSR Modelling

This study suggests that five leaf functional traits (EWT, C:N, %N, LDMC and LMA) are well predicted using leaf spectroscopy and PLSR modelling, while three others (cellulose, chl *a* and chl *a*) are relatively accurate, meaning eight traits were deemed to have acceptable prediction accuracies. The capacity for PLSR to accurately model these eight accepted traits is likely due to their strong association with absorption features in the EMS and the large amount of spectral information captured by the sensor (350 nm – 2500 nm). This latter justification enables the accurate prediction of traits that have influence in multiple regions of the EMS, rather than one. Carotenoids, hemicellulose, lignin, solubles and recalcitrants were deemed to have unacceptably low predictive accuracies and were not included in further analyses. Each accepted trait was considered for site-wide analyses following methods similar to other studies that employed spectroscopy to evaluate leaf functional traits or diversity (Asner and Martin, 2009; Van Cleemput et al., 2018). However, evaluation of spectral mixing on the prediction of these traits shows that some models are more affected than others by the inclusion of multiple species within a single pixel. For example, predicted

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LMA does not vary significantly from the pure prediction value until the presence of the target species was below 30%, while EWT is significantly changed when target presence is lower than 80%. These effects suggest that models with relatively high prediction accuracy can still be subject to the effects of mixing, and that mixing must be taken into consideration when deciding which trait(s) to analyse.

3.4.2 Spectral mixing increases the prediction error of functional trait estimation

Although the presence of more than one species within a single pixel has the capacity to affect trait prediction, the inclusion of additional species does not have a large effect on the capacity to predict functional traits. Overall, the resilience in predictive accuracy varies depending on the trait. This suggests that the trait being evaluated and percent of the target species present are key drivers of changes in predictive capacity, highlighting findings from other studies that suggest the selection of pixel size, and in turn flight altitude and speed, is of great importance during study design (Melville et al., 2018; Wang et al., 2018).

3.4.3 Specific traits vary due to spectral mixing more than others

Examination of how the eight accepted traits are predicted as the number of species observed increases suggests that some traits are affected uniquely. The average percent change in predicted values does not vary due to increased mixing for each of the eight traits evaluated, suggesting that the effects of mixing with one additional species are offset by the effects of a different additional species. This is interesting as it implies that each trait is relatively well suited for prediction despite mixing, which is in fact untrue.

Despite the average change of predicted trait values remaining relatively constant, the absolute cumulative change of predicted values does increase as the level of mixing increases for all traits, with some predicted values quadrupling. This proves that even traits which are accurately

predicted, such as LMA, chl *a* and EWT, may experience large variations in predicted values depending on both how many and which species contribute to reflectance. This is consistent with previous literature suggesting that practical management applications utilizing imaging spectroscopy should select a pixel size smaller than that of the smallest expected plant footprint in ecosystems with high likelihood of spectral mixing, regardless of the trait being analyzed (Feilhauer et al., 2017; Gholizadeh et al., 2019).

3.4.4 Certain lifeforms alter predicted trait values more than others

Spectral mixing of different lifeforms affects the accuracy of functional trait prediction in a variety of ways. For example, mixes that included graminoid species tended to have a lower predicted %N than the three other lifeforms. Such knowledge could make trait selection more straightforward for grassland managers attempting to evaluate changes in diversity or community composition over time. This analyses of the spectral mixing on predicted %N also isolated a species with significantly higher %N values than all other species when only 20% present. *C. scaparius* is a highly invasive shrub introduced to North America from Europe (Brandes et al., 2019). This species is a nitrogen-fixing legume and has the capacity to alter the soil chemistry and biodiversity of its environment, presenting a threat to traditional ecosystem processes in many North American prairie-oak ecosystems (Carter et al., 2018; Shaben and Myers, 2010). The fact that the analyses of a single functional trait, %N, could potentially provide coverage estimates for graminoids and isolate an invasive shrub promote its selection as the focal trait for future analyses, and is consistent with other literature highlighting the capacity of foliar N concentration to identify nitrogen-fixing invaders (Asner et al., 2008b).

Another interesting management opportunity comes in the isolation of *Q. garryana*, the dominant tree species found in Garry oak savannas, when evaluating the effects of its inclusion in

the prediction of LMA using a mixed spectra. Compared to all other species, *Q. garryan* has much higher measured and predicted LMA values, even when mixed. This could be due to a variety of factors, including the evolution of a defence mechanism against local predators and parasites (Fuchs et al., 2000) or to improve the capacity for leaves withstand changes in the thermal environment (higher LMA leads to slower responses to extreme heat, enabling the avoidance of lethal temperatures during fire or drought) (Michaletz et al., 2016).

The capability of predicted leaf LMA to identify *Q. garryana* individuals presents an opportunity to distinguish grassland boundaries, highlight the location of young seedlings and enable more accurate analyses of the distribution of trees across the landscape, which are all important considerations when managing structural diversity in an ecosystem with relatively low (Larue et al., 2019). Interestingly, the identification of any species exhibiting a significantly different predicted trait value, especially when only 20% of said species is present within a pixel, suggests that pixel size may not need to be equivalent to the smallest expected footprint and that the acquisition of imaging spectroscopy may remain accurate at larger spatial resolutions, depending on which species and trait are being evaluated.

The overarching issue of spectral mixing is on the capacity for airborne sensors to accurately predict functional traits. In prairie-oak savannas it is possible to have as many as 26 unique species present in a single 1 m², meaning that imagery with spatial resolution smaller than this would be required to accurately detect a single species or generate an accurate map of functional traits. Currently, there are a variety of airborne sensors that can capture imagery at sub-meter spatial resolution.

Airborne sensors mounted aboard aircraft, e.g., Compact Airborne Spectrographic Imager (CASI) and Airborne Visible/Infrared Imaging Spectrometer (AVIRIS), could provide accurate functional trait mapping if a single plant species covered more than 60 cm² of the landscape, but would likely be more useful in forested ecosystems, rather than prairie-oak savannas. Sensors deployed on drone platforms, however, are much more likely to collect imagery with the requisite spatial and spectral resolutions to capture reflectance values from a single plant species. Specifically, the ease at which drone pilots can manipulate altitude and flight speed to alter the spatial resolution of the images they capture presents an opportunity more suitable for prairie-oak savannas.

3.5 Conclusion

The framework presented in the paper provides a general method from which to consider the effects of spectral mixing on leaf functional trait prediction in any biome. In prairie-oak ecosystems, such as those found on North America's west coast, this framework demonstrated that the inclusion of leaf optical reflectance from more than one plant species in a spectral signature spanning 400 – 2400 nm can lead to changes in the predictive capacity of subsequent models and that these changes vary by trait. LMA demonstrates the greatest capacity to withstand spectral mixing, suggesting it is a useful trait for mapping functional trait variation in prairie-oak ecosystems where the probability of mixing is high. Nitrogen % presents an opportunity to isolate a key invader, *C. scaparius*, and has the potential to quantify the presence of graminoids within a pixel, but is less accurately predicted by a general site model when spectral mixing is high. The mixing of unique lifeforms also affects the prediction of individual functional traits differently and highlights the capacity for spectroscopic data to capture underlying differences in ecological strategies between some lifeforms. These differences provide opportunities to streamline components of data acquisition, including trait selection and flight parameters.

In this chapter, I've provided evidence that spectral mixing can have a variety of effects on the accuracy of leaf functional trait prediction. This knowledge addresses the knowledge gap relating

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to the impact of spectral mixing and provides information relevant for researchers using imaging spectroscopy to estimate functional traits in heterogenous ecosystems similar to prairie-oak savannas. In the following chapters, I shift from the exploration of a theoretical knowledge gap to analyses that use predicted leaf functional traits to address the fundamental ecosystem characteristics of prairie-oak ecosystem that Environment Canada have deemed to be at risk (Fuchs, 2001).
Chapter 4: Distribution of leaf economic strategies across a prairie-oak ecosystem

4.1 Background and Motivation

The research in this chapter addresses two knowledge gaps highlighted by Fuchs et al. 2001 regarding the impact of habitat fragmentation and fire suppression on prairie-oak ecosystems. Specifically, I explore the impact of anthropogenic activities on the spatial distribution of leaf functional traits. A variety of studies have demonstrated that plant nutrient investment strategies are coupled with leaf functional traits and can be evaluated remotely by observing leaf reflectance across many narrow wavelengths (Asner and Martin, 2009). The combination of leaf and imaging spectroscopy enable the accurate prediction of functional traits and has proven to be accurate across multiple biomes (Asner et al., 2016; Wang et al., 2020). At the site-level, however, it is important to evaluate the influence of specific environmental factors on leaf functional traits and the underlying leaf economics as climatic variation is negligible.

Site-level analyses of high-value ecosystems can provide vital information for land managers in relation to external pressures, including anthropogenic land use change and fire suppression (Wyborn and Evans, 2021). In Canada, prairie-oak savanna ecosystems on the south-east coast of Vancouver Island are endangered, covering less than 5% of their historic area (Fuchs, 2001). These ecosystems are highly biodiverse and provide habitat for some of red-listed plant, bird and insect species, as well as represent refuge for a variety of native bird and insect populations (Bjorkman and Vellend, 2010; Fuchs, 2001; House et al., 2003). Recent advances in imaging spectrometers and RPAS provide an opportunity to quantitatively evaluate the distribution of FTs in across a landscape using imagery with high spatial and spectral resolution (Arroyo-Mora et al., 2021).

In this study, I will explore the spatial relationship of leaf economics and anthropogenic activities in a prairie-oak ecosystem using leaf and imaging spectroscopy. First, I use PLSR to generate predictive models for 14 leaf FTs spanning 23 species and four plant lifeforms. Second, I determine correlations between FTs that can be accurately predicted using PLSR. Third, I apply our PLSR models to reflectance data collected using imaging spectroscopy to assign relative FT values to each pixel. Principal component analysis will determine if these spatially explicit FT layers present similar correlation to leaf economic strategies identified at the leaf level, as well as in the literature. Finally, I will explore the association of leaf economic, or functional, strategies with both anthropogenic and ecological variables. Three research questions are addressed to determine the significance of these influences on leaf FT distribution and leaf economics:

- 1. Can leaf economic strategies be identified from FTs using leaf spectroscopy?
- Do leaf economic strategies scale between leaf level measurements and predicted FTs using RPAS imaging spectroscopy?
- 3. Are leaf economic strategies spatially autocorrelated (clustered) across a prairie-oak savanna with anthropogenic activities?
- 4. Are leaf economic strategies spatially associated with anthropogenic activities?

4.2 Materials and Methods

4.2.1 Data Overview and surface modelling

The data used for this research was collected during the May 2019 field campaign. Leaf spectroscopy and chemical results from 23 plant species were used as input spectra for the body of this analysis, as well as to generate 14 leaf functional trait PLSR models. Specifics regarding leaf and chemical data curation from the May 2019 campaign are outlined in Sections 2.3 and 2.4 respectively, while the details of the PLSR models are presented in Section 3.2.1. The imaging spectroscopy data used for this research was also collected during the May 2019 field campaign, details of which have already been presented in Section 2.5.

A point cloud collected using ALS by the Municipality of North Cowichan on May 29, 2017 was used to generate surface models relevant for calculating canopy cover and creating digital terrain (DTM) and surface (DSM) models. The DTM was created using a Kriging method with 10-k nearest neighbours at a 50 cm² resolution and used as input to calculate a site-wide topographic wetness index (TWI). The DSM was generated using a pit-free algorithm at a resolution of 10 cm² and provide information relevant to the light regime and distance from trees of each pixel. The DSM also enabled the determination of subsite canopy cover, and the identification of Low, Medium and High cover subsites) by comparing the number of pixels above 2 m in height with the total number of pixels (Table A3).

4.2.3 Leaf trait and economic mapping

Spatially explicit trait maps were generated by applying the FT PLSR models to each pixel in the RPAS imaging spectroscopy data collected in May 2019. A final outlier mask was applied to remove the highest and lowest 2.5% of each trait value and all traits layers were normalized. Leaf economic strategies present in the imagery were evaluated by conducting principal component

analysis (PCA) on the eight accepted trait layers, which enabled a comparison of leaf economic strategies present in the chemical data with those in the imagery. PCA also allowed the mapping of leaf economic strategies and the evaluation of spatial autocorrelation using Global Moran's I (Moran, 1950).

4.2.4 Association with anthropogenic activities

Three anthropogenic variables were identified as having direct impact on the landscape due to their influence on fire suppression and land cover change. The distance of each pixel to the nearest road was calculated using open-source spatial data accessed via the BC Data Catalogue (www.catalogue.data.gov.bc.ca). A total of 10 strata with equal widths were calculated for each subsite before the random sampling of 30 pixels/ strata was conducted. Distance to the nearest tree crown was determined using pixels in the DSM that were greater than or equal to 2 m. A total of 9 strata of 1 m width were created before 30 pixels/strata were sampled randomly.

The annual light regime of each pixel in an image was estimated by determining the hourly shadow value for each pixel between 7 am and 7 pm on the 15^{th} day of each month for the entire 2019 growing season (May 1 – October 17). A total of 78 rasters were summed to determine the number of hours each pixel spent in the shade. Pixels experiencing 0 – 26 hours of shade were considered to be sunlit, while pixels shaded for over 56 hours were considered shaded. A summed shadow value between 26 and 52 representing partially shaded pixels. A topographic wetness index (TWI) was generated for the entire site using the slope and flow accumulation products derived from the DTM. TWI products for each subsite were generated and classified as wet, damp or dry.

4.2.5 Software

Image pre-processing, specifically manual feature removal, was conducted using ENVI 5.6 (Exelis Visual Information Solutions, Boulder, Colorado) and Atmospheric and Topographic Correction (ATCOR) (Arroyo-Mora et al., 2021), while image analyses were conducted in R using the 'terra' and 'sf' packages (Core et al., 2021; Hijmans, 2022; Pebesma, 2018). Statistical analyses, including sampling, were conducted in R using 'terra' and 'sgsR'(Goodbody and Coops, 2021). Data manipulation relating to model derivation and plotting utilized the R packages 'tidyerverse', 'ggplot2', 'ggfortify', 'ggpubr' and 'ggpmisc' (Aphalo, 2021; Horikoshi and Tang, 2018; Kassambara, 2020; Wickham et al., 2021, 2019). Shadow masks spanning the 2019 growing season were created in R using the 'rayshader' package (Morgan-Wall, 2021). The topographic wetness index was created in R using the 'Whitebox' and 'rgdal' packages (Bivand et al., 2021; Lindsay, 2016).

4.3 Results

4.3.1 Trait Modelling and Prediction

Of the 14 leaf FTs modelled, only 8 were considered acceptable for use in further analyses (Table A2). The accuracy of the accepted trait models varied, with R^2 and NRMSEP values ranging from 0.54 – 0.85 and 16 – 33%, respectively. EWT demonstrated the highest R^2 (0.85), while LMA (0.69) and LDMC (0.67) were also considered good. Overall, the accuracies of our accepted models fall are within the range of other PLSR models conducted on grassland plant species presented in the literature (Wang et al., 2019).

4.3.2 Site-level PCA

The first three components generated from a site-wide PCA of the predicted FTs explained 91.71% of trait variance (Table 6, Figure A4). The loading values of PC1 represent a trade-off between high leaf growth (Chl *a* and *b*) and overall leaf functioning (LMA), which follows the global 'fast-slow' growth strategy highlighted by the leaf economic spectrum (Table 6, Figure A4a). A trade off between leaf water content (EWT) and leaf structure (Cellulose) is identified by the loading values of PC2, while PC3 represents the strategy to invest in leaves with high %N and all other traits (Table 6, Figure A4b). PC1 therefore represents an axis of resource allocation along a leaf growth – defence (GR-DU) economic spectrum, while PC2 highlights investment in either leaf water content or structural defences (H₂0-ST). PC3 is indicative of high levels of investment in leaf %N (N-n).

4.3.3 Subsite PCA

The first three PCs of each subsite explained at least 93.78% of trait variation and followed the same trade-offs highlighted at the site level (Table 7, Tables A4 – 6, Figure A5). As cover increased, the association of all traits along GR-DU and H₂0-ST spectrums remained relatively constant (Table 7). The amount of variation explained along GR-DU at the low cover subsite was less than that of the Medium and High subsites (Table 7). Explained variance of H₂0-ST increased an average of 7.31% with cover, while N-n decreased by 5.75% (Table 7). The N-n spectrum became less important as cover increased.

4.3.4 Principal Component Prediction

The predicted values of PCs 1 - 3 for all pixels across the three subsites were mapped, enabling analysis of various spatial relationships. Each of PC1 – 3 are spatially autocorrelated (Table A7). PC1 (GR-DU) and PC2 (H₂0-ST) become more spatially autocorrelated as cover increases, while PC3 (N-n) becomes less spatially autocorrelated as cover increases (Table A7). The capacity to map principal components enables the evaluation of spatial relationships between leaf economics

and anthropogenic activities (Figure 10).

Site	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Proportion of Variance (%)	55.91	24.40	11.4	4.58	2.07	1.03	0.56	0.05
Chl a	-0.45	0.17	-0.08	0.08	-0.04	0.3	0.3	0.76
Chl b	-0.4	0.24	-0.27	0.3	0.65	-0.42	0.02	-0.16
C:N	-0.36	-0.4	-0.23	0.07	0.05	0.37	-0.71	-0.02
%N	-0.18	0.3	0.86	-0.04	0.22	0.08	-0.3	0.02
EWT	0.34	0.43	-0.22	-0.02	0.36	0.69	0.04	-0.18
LDMC	-0.37	-0.33	0.08	-0.64	0.25	0.17	0.4	-0.28
LMA	0.43	-0.2	-0.05	-0.39	0.49	-0.22	-0.22	0.54
Cellulose	0.19	-0.57	0.28	0.58	0.3	0.2	0.31	0.02

Table 6. Loading and proportion of variance values for each principal component derived from analyses of eight functional trait layers at the CGOP.

Table 7. Explained variance of the first three principal components at low, medium and high cover subsites.

Proportion of Variation (%)	PC1	PC2	PC3
Low	61.36	19.34	13.08
Medium	64.59	25.79	8.06
High	64.06	33.95	1.59



Figure 10. Values of (a) principal component 1, (b) 2 and (c) 3 in a 10 m2 subsection of the CGOP. Each component represents a spectrum along which plants invest resources in pursuit of a specific leaf economic strategy.

4.3.5 Relation to anthropogenic activities

There was significant shift from a growth economic strategy to one of leaf durability as distance to the nearest tree increased at Low and Medium cover subsites (Table 8, Figure A6). Shifts along H₂0-ST were significant as the distance from the nearest tree increased at all cover levels. At the Low cover subsite, plants grew leaves favouring structural defence over water content as the distance from trees increased, while the opposite economic strategies occurred at Medium and High cover subsites. Leaf %N increased and decreased in relation to distance from the nearest tree at Low and Medium cover subsites, respectively. The manifestation of leaves with high %N at High cover sites was not associated with anthropogenic or environmental variables.

The distance of a pixel to the nearest road influenced values of each economic strategy in various ways across all cover levels (Table 8, Figure A7). At the High cover subsite fast-growth was favoured as distance from the nearest road increased. PC2 values decreased with distance to the nearest road at all cover levels. The values of PC3 were not influenced by distance to the nearest road at the Low cover site, but increased with distance when cover was Medium. The High cover subsite demonstrated a decrease in PC3 values with increased distance from roads.

Variations in light regime over the course of the growing season influenced PCs 1 -3 in different ways across cover levels (Table 9, Figure A8). PC1 was unaffected by the light regime at Low and High cover subsites, but had a positive correlation at Medium cover. The relationship of PC2 values and light was negative when cover was Low, but positive at the Medium and High cover subsites. PC3 values increased with increasing light at Low and High cover subsites, while the decreased in Medium cover. At all subsites PC1 and PC3 were not influenced by topographic wetness (Table 9, Figure A9). PC2 values did not change in relation to TWI at Low and High cover subsites. The PC2 values at the Medium cover demonstrated a positive relationship with wetness.

	Low		Medium		High	
Trees	R	p-value	R	p-value	R	p-value
PC1	0.22	<0.001*	0.23	<0.001*	0.082	0.18
PC2	-0.13	0.04*	0.27	<0.001*	0.31	< 0.001*
PC3	0.18	0.004*	-0.44	4.90E-13*	0.031	0.62
	Lov	W	Medium		High	
Roads	R	p-value	R	p-value	R	p-value
PC1	-0.089	0.12	-0.084	0.15	0.13	0.023*
PC2	-0.49	< 0.001*	-0.17	0.0038 *	-0.39	< 0.001*
PC3	-0.0028	0.96	0.37	< 0.001*	-0.22	< 0.001*

Table 8. Correlation and significance values derived using Pearson's r for PCs 1 - 3 in relation to their distance from the nearest tree (top) and road (bottom) at each subsite.

 Table 9. Relationships and significance values derived by fitting an analysis of variance model for PCs 1

 - 3 in relation to the local light regime (top) and topographic wetness (bottom) at each subsite.

	Low		Med	ium	High	
Light	F	p-value	F	p-value	F	p-value
PC1	0.22	0.64	6.88	0.01*	0.63	0.43
PC2	4.59	0.04*	38.91	< 0.001*	26.4	< 0.001*
PC3	5.65	0.02*	8.11	0.01*	11.71	< 0.001*
	Lo	W	Medium		High	
TWI	F	p-value	F	p-value	F	p-value
PC1	0.27	0.6	0.01	0.92	0.13	0.72
PC2	1.55	0.22	4.86	0.03*	0.13	0.72
PC3	0.03	0.85	0.05	0.82	0.07	0.79

4.4 Discussion

In leaf economics, carbon is the common currency and is linked to plant energy budgets as these processes govern the rate of carbon assimilation (Michaletz et al., 2015). These energy budgets are guided by a variety of environmental and biological variables that ultimately lead to the manifestation of leaf FTs that will optimize a plant's carbon economic balance. The selection of a specific leaf FT over another represents a trade-off that can be characterized as a leaf functional strategy and generalized based on the correlations inherent in the trade-off (Reich et al., 1998; Wright et al., 2004). Evaluation of these functional strategies in space can provide information relating to the underlying health of an ecosystem and any differences that occur across a landscape. In the case of this research, we have shown that anthropogenic activities that change the composition of prairie-oak ecosystems can significantly impact the spatial distribution of leaf functional strategies.

At the site-level, the trade-off between rapid leaf growth and leaf durability explained the largest amount of trait variation, which follows the growth – longevity LES presented by Wright et al 2004 and could be due to a variety of factors including access to water, key nutrients or solar radiation. The second most variation was explained by the relationship between EWT and leaf structure, highlighting a trade-off between low and high leaf construction costs, respectively, (Féret et al., 2019; Wright et al., 2004). This H₂0-ST spectrum is likely driven by a combination of a plants access to resources and the presence of herbivorous insect species (Fuchs et al., 2000). The third principal component isolated leaf %N from all other traits and, although it explained only 11.40 % of the total variation, suggests that access to nitrogen may drive trait manifestation across the site. Combined, the first three components suggest that plants at this site grow leaves that are either fast-growing or long-lived, depending on a variety of environmental factors.

The economic trends represented at the site-level scale across all three subsites, although the relative influence of PCs 1 - 3 vary with canopy cover (Table 7). At all cover levels, the GR-DU spectrum explains between 61 - 65 % of trait variation and highlights the scalability of this leaf economic trade-off. Investment in leaf water content or leaf structure explains an increasing amount of variation with increased cover (Low = 19.34 %, High = 33.95 %). This could be associated with differences in soil water or temperature due to the influence of tree cover on microclimate (De Frenne et al., 2019). Trait variation explained by the N-n spectrum has an inverse relationship with canopy cover, decreasing in importance as tree cover increases. This could highlight an allocation of resources in leaf productivity and growth in sunlit areas despite the likely higher N content in soil closer to the tree canopy (Jackson et al., 1990; Vetaas, 1992).

Spatial autocorrelation of all economic spectra occurred at all cover levels, suggesting that leaf economic strategies are pursued in relation to environmental conditions. GR-DU and H₂0-ST become more autocorrelated as cover increases, suggesting that the presence of trees is a key driver of leaf resource allocation. The N-n spectrum becomes less spatially autocorrelated with increasing canopy cover, supporting the notion that increased leaf litter or enhanced nutrient cycling associated with overstory trees reduces the limitation of N (Jackson et al., 1990). Overall, the spatial autocorrelation of leaf economic strategies and their relationships with tree cover support the concept that they are driven by environmental factors, anthropogenic or otherwise.

Distance to the nearest tree was a significant driver of at least one economic strategy at all cover levels. A positive correlation exists between high growth strategies and the distance to trees at Low and Medium cover subsites and suggests that as tree cover increases, plants grow leaves with higher LMA at the cost of chlorophyll, perhaps due to decreasing access to solar radiation. The lack of a significant correlation in relation GR-DU at High cover could be due to a reduction in variation of soil nutrients, light regime or microclimate. Increasing distance from trees also led to a shift in investment strategies along H₂0-ST at Medium and High cover subsites as leaves further from tree cover elect to have relatively high water content. This is indicative of fast-growing leaves and could be related to an increase in available solar radiation, increased soil water increased soil N from leaf litter or a combination of these three (Féret et al., 2019; Jackson et al., 1990). The inconsistent relationship with N-n and the distance to trees at the Low and Medium cover subsites combine with the lack of any correlation at the High cover subsite to suggest that, although potentially limiting across the site, leaf strategies related to %N are not directly driven by a plant's distance to the nearest tree.

Adjacent roadways and their specific distance from a plant significantly influenced GR-DU, H₂0-ST and N-n in at least one subsite. Leaf economic strategies related to GR-DU were impacted at the High cover site, while insignificant at Low and Medium cover. At High cover, an increase in the distance from a road resulted in greater investment in leaf structural at the cost of growth. This could be due differences in nutrient availability associated with roadside inputs, but seems more likely to be correlated with the amount leaf litter input and therefore associated with tree canopy cover (Forman and Alexander, 1998; Jackson et al., 1990). At all cover levels plants elected to grow leaves favouring structure over water content as the distance from a road increased, suggesting that the increased soil water content associated with roadway runoff influence leaf economics (Forman and Alexander, 1998; Trombulak and Frissell, 2000). The growth of leaves with relatively high %N was not affected by the distance from the nearest road at low cover, perhaps indicating again that leaf %N is not directly driven by the presence of roads. Plants growing in the Medium and High cover subsites demonstrated positive and negative relationships with leaf %N, suggesting that although roads drive increases in some soil nutrients, such as salt, they are not driving leaf economic strategies relating to growth or cell structure (Amrheln et al., 1992). Instead, these results support the idea that the increased access to water may be driving the growth of water rich-leaves.

V regime impacted the GR-DU spectrum at the Medium cover subsite, but not Low or High. Such inconsistencies suggest that light regime is not a key driver of the growth-durability trade-off. The growth of leaves with either high water content or relatively high structure axis, however, seems to be related more strongly to light availability as the H₂0-ST spectrum was significantly influenced at all cover levels. Plants growing in Low cover invest in leaf defensive structure as light availability increases. This could potentially be linked with drier soil and a more extreme microclimate associated with locations removed from tree cover (De Frenne et al., 2019; Vetaas, 1992). This relationship is reversed at the Medium and High cover subsites, supporting the idea that variations in soil moisture and microclimate due to the presence of trees impacts the availability of water and, in turn, investment in relatively water-rich leaves (Vetaas, 1992). N-n was significantly impacted at all cover levels, although demonstrated inconsistent relationships with increases in light availability. At Low and High cover, increases in the amount of light led to increases in the amount of leaf %N. At Low cover, this relationship signals an intent to create fastgrowing leaves and supports the idea plants select relatively higher leaf %N when water and leaf litter are relatively limited.

Topographic wetness did not consistently impact leaf economic strategy. Neither the GR-DU nor H₂0-ST spectrums were influenced at any level of canopy cover. N-n at Low and High subsites were also unaffected, while they increased with wetness when cover was Medium. Despite this significance, the inconsistent influence of topographic wetness on leaf economics across all subsites suggest the wetness is not a key driver of leaf economic strategies. This could be due to the small amount of annual precipitation that occurs at the site. This finding, however, can not lead to

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conclusions relating to soil depth. Variation in soil depth could prove an important factor in the availability of soil nutrients and should be evaluated in future studies.

The exclusion of wetness as a key driver of leaf economic strategies indicates that the key drivers of leaf economic strategies at the CGOP are more related to anthropogenic variables than ecological ones. Land cover changes, such as the construction of roadways, seem to impact the availability of soil nutrients and moisture while fire suppression, which leads to increased canopy cover and changes to the local light regime, affects resource allocation relating to leaf structure, water content and growth. This evidence presents a clear picture as to how plants elect to invest available resources and highlights the influence that humans can have on leaf economics at the site level.

4.5 Conclusion

Leaf economic trade-offs that exist across biomes also exist within a single site (Wright et al., 2004). Specifically, the investment in either fast growing or structurally sound leaves explained the largest amount of trait variation across subsites regardless of canopy cover. The construction of leaves with either high water content or enhanced structure also played a considerable role in explaining the variation in trait manifestation, although this economic trade of tended to change in relation to the level of surrounding canopy cover. Leaf %N also played an important role in explaining the variation in leaf traits throughout space. All three major economic trade-offs were spatially autocorrelated, suggesting that immediate environmental factors could be driving leaf economic strategies. The distance of a plant from the nearest road or tree crown, as well as the local light regime, impacted resource allocation and highlighted the trade-offs of key leaf functional traits between to growth and structure.

These findings demonstrate that anthropogenic activities which alter traditional land cover composition or fire regimes can influence leaf economics. This research provides a methodology that can be used to monitor two of the fundamental prairie-oak ecosystem characteristics outlined by Fuchs et al. 2001 as critically threatened. In the next chapter, I present a second, unique methodology for monitoring the impact of anthropogenic activities on these same ecosystem characteristics using continent-wide models to predict leaf traits of *Q. garryana*.

Chapter 5: Intraspecific trait variation in relation to anthropogenic activities²

5.1 Background and Motivation

Research presented in this chapter continues bridging the first and second knowledge gaps presented by Fuchs et al. 2001 by evaluating the impacts of habitat fragmentation and fire suppression on prairie-oak ecosystems through the lens of a single species. Intraspecific trait variation (ITV) has been largely underrepresented in spectroscopic studies of natural ecosystems as many ecological studies assume that interspecific variation is much larger than intraspecific variation (Albert et al., 2012). This assumption inherently ignores the variation present between individuals of the same species by averaging their trait values into a species mean. Recent studies have shown that ITV exists and is an important factor when evaluating plant functional traits at the community-level (Albert et al., 2010; Asner and Martin, 2011; Siefert et al., 2015; Zagajewski et al., 2017).

Correct interpretation of plant health is especially important in fragmented ecosystems that contain high-levels of biodiversity, making spectroscopy-based ITV increasingly relevant for the management of these areas. The conventional approach for understanding the effects of these modifications in the ITV of a species across a single site would include complete soil and vegetation sampling over multiple years, as well as long-term plot monitoring (Asner and Martin, 2009; Ustin et al., 2009; Zhao et al., 2018). Considering the expense and time associated with these tasks, it is unlikely that this approach is feasible for the majority of land managers. It is necessary, then, to identify methodologies that are both cost- and time-efficient, while still providing relevant information.

² Content presented in this chapter has been adapted from:

Hacker, P. W., Coops, N. C., Townsend, P. A., & Wang, Z. (2020). Retrieving foliar traits of *Quercus garryana* var. *garryana* across a modified landscape using leaf spectroscopy and LiDAR. Remote Sensing, 12(1). https://doi.org/10.3390/RS12010026

Here I address ITV using *Q. garryana* in British Columbia, Canada as a test species. In general, functional traits of the genus *Quercus* vary significantly based on environment, suggesting that ITV may be present at the site-level where significant variation in human manipulation of the environment is present (Petry et al., 2018; Violle et al., 2009). *Q. garryana* has considerable conservation value and utility to this study as a consequence of its role in biodiversity, known variability throughout its range and status as a flagship species for prairie-oak savannas (Fuchs, 2001).

In this research, leaf-level spectroscopy, ALS and pre-determined PLSR coefficients are used to explore the intraspecific functional trait variation of *Q. garryana* in association with anthropogenic landscape modifications across a single site. Specifically, I explore three research questions:

- Does intraspecific functional trait variation exist between *Q. garryana* experiencing different land use regimes
- 2. Do *Q. garryana* experiencing the same land use regime exhibit functional trait variation in relation to the spatial distribution of anthropogenic landscape modifications.
- 3. How can the analysis of functional trait variation within a single species can be used to examine the functional diversity of a site as well as indirectly sense the effects of land use?

5.2 Materials and Methods

5.2.1 Data Overview and ALS processing

The data used to conduct the analyses for this research was collected in the July 2018 field campaign. A DSM was created from the ALS point cloud using the CanopyModel function in FUSION (Forest Service of the U.S. Department of Agriculture, Seattle, WA, USA, 2014). The DSM was used to select 30 individual *Q. garryana* trees for sampling with five additional individuals selected from both the agricultural and natural meadow sub-sites to enable comparison between the agriculture and meadow sites (n = 40). Lifeform metrics of height, maximum crown width and height to crown base were calculated from the DSM using the 'lascanopy' tool provided by LAStools (rapidlasso GmbH, Gilching, DE, 2018) in ArcGIS Pro (ESRI, Redlands, CA, USA, 2018). The 'lascanopy' tool uses the values of each cell with the DSM raster to derive the aforementioned lifeform traits and presents them in a single attribute table (rapidlasso GmbH, Gilching, DE, 2018). Diameter at breast height (DBH) was measured in the field.

5.2.2 Leaf spectroscopy and PLSR

Spectroscopy of leaves from 40 *Q. garryana* individuals was conducted during the 2018 field campaign. The specifics of this data acquisition are highlighted in Section 2.3. This study utilizes PLSR leaf models and coefficients derived by Serbin 2012 using a dataset of various genera, including *Quercus*, from across the northern United States (Serbin, 2012; Wang et al., 2020) (Serbin, 2012; Wang et al., 2020). Chemical analysis of the sampled leaves was completed in order to validate the model (Serbin, 2012; Wang et al., 2020). The model was created using a similar fashion to those of other PLSR models designed for functional trait identification (Python, 2018) (Wang et al., 2020, 2016). PLSR models for eleven functional traits were developed. The PLSR models were then applied to our spectra to provide trait estimates. This model was selected in favor of the index - based method as it employs the entire spectral signature of each sample and encompasses many genera, making it applicable for non-*Quercus* studies. This latter point is important as it enables the transference of methodologies employed in this study.

5.2.3 Principal component analyses

Functional traits of interest were separated into three categories based on their functional role: lifeform, leaf growth and leaf structure. Traits in the leaf growth and leaf structure categories were derived solely using coefficients from pre-existing, validated PLSR models (Table 10) (Serbin, 2012). Deriving functional traits in this manner highlights the ability of spectroscopy to provide information relating to a multitude of relevant plant traits. The functional traits in the lifeform category were measured principally using ALS (Table 10), again demonstrating the power of advanced remote sensing techniques. The PCA of each group enabled the trait with the highest amount of variation to be identified and its relationship with the other traits in the group to be extracted and analyzed. In total, 14 plant functional traits were evaluated in this study (Table 10).

Table 10. Categories of functional morphological traits used to analyze the variance between leaf samples and the agricultural, CDF and road anthropogenic landscape modifications.

Leaf Growth	Leaf Structure	Lifeform		
Carbon (%)	Cellulose (%)	DBH (cm)		
Carotenoid area (mmol/m ²)	Fiber (%)	Height (m)		
Carotenoid mass (ng/mg)	Lignin (%)	Max Crown Width (m)		
Chlorophyll <i>ab</i> area (mmol/m ²)	LMA (g m ²)	Height to Crown base (m)		
Chlorophyll <i>ab</i> mass (ng/mg)				
Nitrogen (%)				

The values of each PC were extracted for all leaf samples in this study in order to enable their use as input values for statistical analysis. Normality and equal variance tests were completed in R using base functions of the software (R Core Team, 2018). Independence was achieved as the *Q*. *garryana* individuals in this study were not sampled more than once.

5.2.4 Statistical analyses

Statistical assumptions were completed to determine if the functional trait estimation data were parametric. Upon finding the data were parametric the Kruskal-Wallis rank sums test was used to evaluate ITV between the agricultural and meadow sites. ITV in spatial relation to the road and CDF anthropogenic landscape modifications was evaluated using Kendall's Tau rank correlation coefficient test. To ensure that ITV was not spatially significant at random within the meadow a randomization test was completed. Each sample was given a random distance before Kendall's Tau was completed. This was iterated 1000 times and the means of the resulting Tau estimates, zstatistics and p-values were recorded (Table 11). It was determined that the samples used in this study did not have spatially significant ITV in comparison to the samples evaluated at random distances.

Table 11. Mean z- and p-values resulting from the randomization test. The insignificant p-values suggest that intraspecific trait variation (ITV) does not exist at random within the meadow.

Group	Z	p-value
Lifeform	0.01	0.497
Leaf growth	-0.045	0.512
Leaf Structure	-0.013	0.508

5.2.5 Software

Road, RGB and ALS data were all processed in ArcGIS Pro (ESRI, Redlands, CA, 2018). ALS data were manipulated using the LAStools ArcGIS toolbox (rapidlasso GmbH, Gilching, DE, 2018). Spectroscopic data were analyzed using a combination of ASD's ViewSpecPro software and R (Analytical Spectral Devices Inc., Boulder, CO, USA; R Core Team, 2018). Statistical analysis was completed using the R package 'prcomp' (R Core Team, 2018).

5.3 Results

5.3.1 Leaf-level spectroscopy

Mean spectral signatures were created for the agricultural, meadow and sample population leaf samples. Clear differences in the reflectance values are observed in association with various absorption features, and therefore functional traits (Figure 11). Spectral variation is especially notable from 500–650nm (Figure 11a), wavelengths dominated by pigments, and 1400nm to 1700nm (Figure 11b), wavelengths dominated by water absorption as well as leaf structural compounds and proteins (Curran, 1989).



Figure 11. Mean spectral signatures of Agricultural (n = 5), Meadow (n = 5) and Sample (n = 30) population from (a) 500–650nm and (b) 1400–1700nm. Dotted lines signify the range of variation in the spectral signature evaluated.

5.3.2 Model performance

PLSR model evaluation indicated that 10 of the 11 functional traits evaluated were well estimated by the PLSR model with predictive accuracies ranging ($R^2 = 0.30$ to 0.95; Table 12) (Serbin, 2012; Wang et al., 2020). Upon evaluation of the PLSR results, five meadow trees were removed from the analysis as the range of at least one of their functional trait estimations was outside the valid range of the model. The final sample population used for the analysis was 27 sample, 3 natural meadow and 5 agricultural *Q. garryana* individuals (n = 35).

Table	12.	Mean an	d Standaro	deviation	of the	10 functional	traits	calculated	using partia	least	squares
regres	ssior	n (PLSR).									

Trait	Mean Value	Standard Deviation
Carbon (%)	50.082	0.219
Carotenoid Area (mmol/m ²)	181.786	3.383
Carotenoid Mass (ng/mg)	1266.166	38.087
Cellulose (%)	15.916	0.972
Chlorophyll <i>ab</i> Area (mmol/m ²)	589.166	24.965
Chlorophyll <i>ab</i> Mass (ng/mg)	7529.724	251.585
Fiber (%)	48.806	2.032
Lignin (%)	24.252	1.320
Nitrogen (%)	2.209	0.086
LMA (g/m^2)	139.895	13.114

5.3.3 PCA: ITV between agricultural and meadow sub-sites

The loadings of PC1 from each functional trait category were determined for the analysis of variation between the agricultural sub-site and the rest of the CGOP (Table 13). From the lifeform group, DBH had the largest influence (-0.989) on PC1, which explained 99% of the variation. This suggests that DBH has the largest amount of variation between samples. Chlorophyll *ab* Mass (-0.989, 99% of variation) and LMA (0.995, 68% of variation) had the largest influence on the leaf growth and leaf structure functional categories respectively. All variables in the lifeform and structure PC1s are negatively correlated, while all variables in leaf growth's PC1 are positively correlated (Table 13).

Table 13. PCA 1 completed using all leaf samples (agricultural and natural meadow sub-sites) collected at the CGOP in July 2018. The loadings from the first principal component for each functional trait category are shown as it accounted for \geq 68% of the variations in the analysis. Variations are presented in brackets.

PCA 1					
Lifeform	PC1 (>0.99) Leaf growth		PC1 (>0.99)	Leaf Structure	PC1 (0.68)
DBH	-0.995	Chlorophyll <i>ab</i> mass	0.989	LMA	-0.995
Height	-0.086	Carotenoid mass	0.137	Lignin	-0.068
Height to crown base	-0.043	Chlorophyll <i>ab</i> area	0.053	Fiber	-0.068
Max crown width	-0.031	Carotenoid area	0.005	Cellulose	-0.027
		Nitrogen	< 0.0001		
		Carbon	-<0.0001		

The Kruskal-Wallis test yielded significant results (alpha = 0.05) for the lifeform (<0.001), leaf growth (p = 0.05) and structure (p = 0.01) functional categories (Table 14). This indicates significant variation between functional traits of individuals at the two land use sites. The large Chisquare (χ 2) value associated with the lifeform group indicates that there is large variation between the agricultural and non-agricultural individuals. Leaf growth has a χ 2 of 3.80, which is ten times less than that of the lifeform group, indicating that although significant, the variation in leaf growth traits between sub-sites is considerably lower (Table 14). The χ 2 of structure is 6.25, suggesting that leaf structure is more variable between sub-sites than leaf growth, but less variable than lifeform (Table 14).

	Chi-Square (χ ²)	p-Value
Lifeform	38.24	< 0.001
Leaf Growth	3.80	0.05
Leaf Structure	6.25	0.01

Table 14. Results of the Kruskal-Wallis test evaluating the differences between agricultural and nonagricultural leaf samples.

Due to the nature of the Kruskal-Wallis test, it is not possible to identify how each functional trait category differs between land-use from this analysis alone. To determine this, the statistical mean of the functional trait with the highest loading in each of the functional trait categories must be examined. Table 15 shows that DBH, chlorophyll *ab* mass and LMA are lower in the meadow sub-site compared to the agricultural sub-site.

Table 15. Statistical means of the trait with the largest loading value in the PC1 of each functional trait category for the agricultural and meadow sub-sites of the CGOP.

Trait	Functional Trait Category	$\begin{array}{l} \text{Agricultural} \\ (n = 5) \end{array}$	Meadow (n = 30)
DBH (cm)	Lifeform	125.14	32.12
Chlorophyll <i>ab</i> mass (mmol/m ²)	Leaf growth	9463.48	7207.43
LMA (g/m^2)	Leaf Structure	170.85	134.74

5.3.4 PCA 2: ITV in spatial relation to anthropogenic landscape modifications

PCA 2 (Table 16), which did not include the *Q. garryana* leaf samples from the agricultural site, differed from the PC1 values of PCA 1 (Table 16). DBH (-0.987, 98% of variation), chlorophyll *ab* mass (-0.989, 99% of variation) and LMA (-0.999, 90% of variation) still accounted for the largest amount of variation in the lifeform, leaf growth and leaf structure functional categories respectively. The fact that the same functional traits were dominant in the PC1s of both PCA 1 and PCA 2 indicates that they are more variable than other traits across the species, regardless of context.

Table 16. PCA 2 completed using only non-agricultural leaf samples collected at the CGOP in July 2018. The loadings from the first principal component for each functional trait category are shown as it accounted for \ge 90% of the variations in the analysis. Variations are presented in brackets.

PCA 2					
Lifeform	PC1 (0.98)	Leaf growth	PC1 (>0.99)	Leaf Structure	PC1 (0.90)
DBH	-0.987	Chlorophyll ab mass	-0.989	LMA	-0.999
Height	-0.138	Carotenoid mass	-0.139	Fiber	0.013
Height to crown base	-0.069	Chlorophyll <i>ab</i> area	-0.045	Lignin	0.018
Max crown width	-0.033	Carotenoid area	-0.007	Cellulose	<-0.001
		Nitrogen	<-0.001		
		Carbon	<-0.001		

A significant correlation was identified between the PC1 loadings of lifeform and leaf growth functional categories and the distance to both the CDF and road anthropogenic landscape modifications using Kendall's Tau (Table 17). The leaf structure functional category showed a suggestive relationship association with the CDF anthropogenic landscape modification, but not the road (Table 17). Leaf growth's relationship with CDF is the strongest in comparison with lifeform, while leaf structure has a suggestive rather than significant relationship (Table 17). When associated with the road, lifeform has a less significant relationship compared to leaf growth, with leaf structure's relationship being insignificant (Table 17). For both the road and CDF anthropogenic landscape modifications the leaf growth functional category has the largest significant association (Table 17). Table 17. Associations between the functional traits with the largest loading in PC1 of each functional trait category and the distance to CDF and road for all non-agricultural Q. garryana individuals (n = 30). The dataset of each trait is arranged based on the individual's distance to the (a) CDF and (b) road anthropogenic landscape modifications, starting at 0 m. Quartiles are the median value of the first quartile (25%), the median (50%) and the third quartile (75%) of each dataset. Significance was calculated using Kendall's Tau.

Distance from CDF (m)				
Trait	First Quartile (25%)	Median Value (50%)	Third Quartile (75%)	p-value
DBH (cm)	30.50	27.70	22.0	< 0.001
Chlorophyll <i>ab</i> mass (ng/mg)	6674.25	6777.98	7035.07	< 0.001
LMA (g/m²)	135.14	131.35	130.38	0.069
	Distance from Road (m)			
Trait	First Quartile (25%)	Median Value (50%)	Third Quartile (75%)	p-value
DBH (cm)	22.00	27.70	29.00	0.004
Chlorophyll <i>ab</i> mass (ng/mg)	7608.99	7208.69	7028.97	0.002
LMA (g/m^2)	131.22	131.35	131.48	0.243

5.4 Discussion

5.4.1 Remote sensing of intraspecific trait variation

The accurate use of remote sensing technologies to estimate plant functional traits has been well documented (Asner and Martin, 2016; Kerr and Ostrovsky, 2003). Physiological traits, such as leaf pigment content, can be derived from spectroscopy, while morphological traits, such as plant height, can be measured from ALS (Asner and Martin, 2009; Sibona et al., 2017). This research uses a combination of these two methodologies to derive 14 functional traits from 40 individual tress of the same species. Spectroscopy at the leaf level provided the spectral information required to apply PLSR coefficients acquired from open-source, pre-validated models (Table 12) (Serbin, 2012). The functional trait estimations resulting from this analysis were within the acceptable range of the validated model, demonstrating that validated PLSR models are transferable (Table 12) (Serbin, 2012). This is significant as the cost and time required to collect and validate PLSR models is considerable.

The ALS data used to estimate morphological functional traits in this study was obtained freely from the Municipality of North Cowichan. With a density of 16 points/m², this dataset provides excellent representation of tree crowns and allows for the accurate derivation of traits like tree height (Sibona et al., 2017). Due to the open-source nature of this ALS data considerable time and cost were avoided when compared to measuring these attributes in the field.

The efficiency of acquiring remotely sensed and open-source data makes it possible to derive accurate functional traits within weeks of collecting leaf level spectral information. This reality means that land managers could have access to quantifiable information about functional traits across their site within a timeframe appropriate to undertake meaningful action. To make use of this potential, the focus of our research was on a relatively underrepresented area of functional trait analysis: intraspecific variation. This research demonstrates the ability of remotely sensed technologies to enable the analysis of intraspecific functional trait variation with respect to anthropogenic landscape modifications and identify the main drivers of functional diversity across the site.

Visual examination of the mean spectral signatures of the meadow, agriculture and sample populations indicates that the agricultural and meadow trees vary in multiple areas of the electromagnetic spectrum between 350 nm and 2500 nm. Separation between the signatures in Figure 11a from 500 nm to 600 nm suggests that there are differences in the pigments associated with leaf growth, such a chlorophyll and anthocyanin, between individuals living in the two land use types (Curran, 1989; Curran et al., 2001). Variation in structural components, such as cellulose or lignin, and possibly proteins, is suggested by differences in the reflectance values between 1400nm

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and 1700nm in Figure 11b (Asner and Martin, 2009; Curran et al., 2001). This simple examination of the mean spectral signatures from two sub-population in relation to the mean signature of the sample population supports further examination of the intraspecific variation of *Q. garryana* through the two hypotheses of this study. The use of leaf-based spectroscopy and derivation of traits is replicable and can be applied across species, enabling an efficient exploration of variance for managers interested in evaluating the ITV in a species of concern.

5.4.2 Model performance

In order to derive traits, I utilized a large North American database of plant spectral, pigment and chemical traits, including samples from a range of oaks, the key genus in this study. These coefficients are derived from PLSR models with validation R2 ranged from 0.30–0.95, while validation nRMSE ranged from 4.6–19.15% (Serbin, 2012; Wang et al., 2020).Only δ 15N was removed from the analysis due to low validation R2 (<0.30). The ten traits determined through PLSR demonstrated reasonable mean values and standard deviations (Table 12). By using this model, this study demonstrates that a multi-species, pre-validated dataset can be used to explore intraspecific variation within a single population. This is important as it suggests a single, multigenera dataset can be used to estimate plant functional traits within a population and across a variety of landscapes.

5.4.3 Functional trait categories

The results of both PCA 1 and PCA 2 determined that the greatest variation in lifeform, leaf growth and leaf structural functional trait categories was found in each of their PC1s, with DBH, chlorophyll *ab* mass and LMA having the highest influence respectively. The PC1 of each functional category explained over 68% of the variation between samples, so it was used exclusively in the statistical analysis. However, it is also possible to evaluate the presence of ITV for a desired trait by

using the values of the principal component in which said trait explains the highest proportion of the variance. This could prove useful for managing the drivers of a specific trait across a landscape.

5.4.4 Functional trait variation due to land use

Results support the hypothesis that functional trait variation exists in *Q. garyana* of the same population experiencing different land use pressures. These results are consistent with other studies exploring intraspecific variation through spectroscopy (Albert et al., 2010; Curran et al., 2001). The Kruskal-Wallis rank sums test found a significant level of variation when comparing each of the functional trait groups of individuals persisting in an agricultural environment with those living throughout the rest of the CGOP. Specifically, traits related to lifeform (p = <0.001) and leaf structure (p = 0.01), such as DBH and LMA respectively, had higher values in the agricultural subsite, while individuals from the meadow had higher values for leaf growth (p = 0.05) (Figure 12). Increased LMA values for individuals in the agricultural sub-site could indicate that these trees are growing thicker leaves to increase their resistance to stress, such as management practices that include the removal of competitors, the addition of fertilizers and irrigation.

Lifeform functional traits proved to be the most variant between sites (p < 0.001, $\chi 2$ of 38.24), with mean DBH being significantly lower in the meadow (32.12 cm) compared to the agricultural (125.14 cm) sub-site (Table 14). Management practices such as the removal of competitors could be a possible reason for this. These findings suggest that it is important to acknowledge the impacts of land use change on the functional strategies related to overall tree size and shape. The leaf growth functional trait category also showed significant differences between the agricultural and meadow sub-sties (p= 0.05, $\chi 2 = 3.80$), with *Q. garryana* individuals in the meadow (7207.43 ng/mg) exhibiting lower chlorophyll *ab* mass values that those in the agricultural (9463.48 ng/mg) sub-site (Table 15). These variations could be due to the consistent addition of fertilizers to the soil over the last century. ITV related to leaf structure was also proven significant (p < 0.001, $\chi 2$)

= 6.25), with LMA in the agricultural sub-site (170.85 g/m2) being larger than LMA in the meadow (134.74 g/m²) sub-site. This suggests that functional traits such as LMA can vary based on land use techniques, recognizing that LMA is indicative of overall plant health and a leaf's investment in photosynthesis versus longevity (Dechant et al., 2017).



Figure 12. Schematic depicting the relative increase or decrease in values for the traits with the highest PCA loadings from lifeform (DBH, purple), leaf growth (Chlorophyll ab mass, green) and leaf structure (LMA, blue) functional categories in relation to each anthropogenic landscape modification. Arrows are arranged in accordance with Table 15 for the agricultural vs. meadow analysis and on the Tau values in Table 17 for the CDF and road analyses. Coastal Douglas-fir forest (CDF) and Road values are measured on a continuous scale of distance (m) starting at the anthropogenic landscape modification border (0 m) and ending at 248 m and 273 m respectively. Values associated with the Agriculture anthropogenic landscape modification are Boolean.

5.4.5 Functional trait variation in relation to landscape modifications

Significant correlations of ITV were found with respect to the spatial relationship of Q. garryana individuals and both CDF and road anthropogenic landscape modifications, supporting the second hypothesis of this study (Table 17). Kendall's tau rank sum test also found that functional trait categories are affected differently by each of the anthropogenic landscape modifications and suggests that each modification invokes unique environmental changes. This supports the findings of other studies that anthropogenic landscape features can alter the functional strategies of plants (Johnson et al., 2017).

Lifeform traits changed significantly depending on an individual's distance to the CDF (p = <0.001) (Table 17). DBH, for example, decreased as distance to the CDF increased, suggesting that individuals are shifting their lifeform strategies based on the association with this competing ecosystem (Figure 12). The growth functional trait category (p < 0.001) also shows a significant relationship to the distance of an individual *Q. garryana* from the CDF and suggests that leaf chlorophyll increases as distance from the CDF increases. Correlation between the leaf structure (p = 0.069) functional trait category and the CDF is suggestive, implying that *Q. garryana* individuals may also change their leaf structure based on proximity to competitors, with leaf structural values associated with stress tolerance decreasing with distance (Figure 12) (Bjorkman and Vellend, 2010; Fuchs, 2001). This is interesting as leaf structure is related to a leaf's defensive capabilities and longevity (Asner et al., 2014; Aubin et al., 2016; Kitajima et al., 2016). These findings suggest that individuals closer to the CDF have a higher stress tolerance, which could be caused by a more favorable microclimate compared to individuals living in the open meadow.

Similar correlations were found between the functional trait category of each sample and their distance to the main road (Table 17), supporting the hypotheses that intraspecific functional

trait variation exists in relation to the spatial distribution of various anthropogenic landscape modifications. Lifeform (p = 0.004) values decreased as distance from the road increased, while leaf growth (p = 0.002) values increased (Figure 12). This could be due to changes in the nutrient composition of the soil closer to roads, with runoff and de-icing agents like sodium chloride (NaCl) affecting leaf growth (Amrheln et al., 1992; Forman and Alexander, 1998). The relationship between the leaf structure functional trait category (p = 0.243) and the road anthropogenic landscape modification was found to be insignificant. The associations between the three functional trait categories and the road anthropogenic landscape modification was inverse to the relationship of those groups with the CDF. This suggests that the influence of the road and CDF affect the lifeform, leaf growth and leaf structure strategies of *Q. garryana* in different manners. If this is the case, managers should deploy mitigation efforts for each anthropogenic influence separately, such as salt loading in the winter or excess ground water due to runoff from the road, rather than prescribing a site-wide plan (Forman and Alexander, 1998). The presence of significant intraspecific variation also suggests that Quercus trees should be considered individually when using functional traits to determine biodiversity metrics as the values of many traits vary in association with different anthropogenic landscape modifications.

In a biodiversity management context, where this project has key implications, our results imply that functional traits of *Q. garryana* should be considered on an individual-plant basis. When mapping functional traits across a site, for example, intraspecific variations among trees may provide insight relating to the relative functional diversity of one sub-site in relation to another. This information could be used to determine which treatment is necessary at specific locations as well as provide general, site-level context to other biodiversity metrics such as species diversity and species composition across sub-sites.

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5.5 Conclusions

Overall, the results presented in this study support the hypotheses that intraspecific functional trait variation exists within a population experiencing different land uses and in relation to various anthropogenic landscape modifications. This study also successfully begins to bridge the knowledge gap between intraspecific functional trait variations at the landscape- and site-levels. The methodology presented in this research provides a simple, replicable and relatively inexpensive baseline on which future studies examining functional trait variation can expand. For example, the coefficients used to estimate each of the leaf growth and leaf structure functional traits were determined by conducting PLSR on a spectral database containing various different genera, rather than *Quercus* alone. This variety suggests that examination of intraspecific trait variation is possible for the other genera, or species, represented in the database. For many land managers, this could improve efficiency as they would not be required to create and validate their own spectral datasets.

The findings of this chapter confirm that anthropogenic activities influence the spatial distribution of leaf functional traits across prairie-oak ecosystems. In combination with Chapter 4, research presented in this chapter adds valuable information relating to the ability of remotely sensed leaf functional traits to evaluate the impact of fundamental threats to the health of these ecosystems, namely habitat fragmentation and fire suppression. In the following chapter, I explore the potential for leaf functional traits to address the third, and final, threatened primary ecosystem characteristic outlined by Environment Canada in 2001: biotic integrity.

Chapter 6: Identification of invasive plant species using functional traits³

6.1 Background and Motivation

The focus of this chapter is to explore the ability of predicted leaf functional traits to evaluate the presence of invasive plant species. Presented by Fuchs et al. (2001) as the third fundamental ecosystem characteristic of prairie-oak ecosystems under significant threat from anthropogenic activities, research relating to the identification of invasive plant individuals is of high importance as their introduction to an ecosystem can drastically alter diversity and threaten ecosystem processes, such as soil water dynamics and nutrient availability (Albert et al., 2012; Carter et al., 2018; Shaben and Myers, 2010; Slesak et al., 2016). In the past 200 years, humans have expanded across the planet and enhanced the capacity of plant species to migrate (Olivares et al., 2019; Zerega et al., 2004). Some species have transited oceans for agricultural production, while others simply provide ornamental value (Bossard and Rejmanek, 1994; Hawkes and Francisco-Ortega, 1993).

Cytisus scoparius (L.) Link, or Scotch broom, is one such transplant. A nitrogen-fixing legume introduced to numerous countries around the globe, *C. scoparius* has proven adept at establishing in climatically temperate regions of North America, Australia and New Zealand (Downey and Smith, 2000; Odom et al., 2003; Richardson et al., 2000; Slesak et al., 2016). Initially transported from its native range in northern Africa and Europe, *C. scoparius* was a preferred decorative shrub of New World colonists due to its low-maintenance and striking yellow flowers (Fuchs, 2001). The nitrogenrich leaves of nitrogen-fixing plant species, such as *C. scoparius*, are relatively more productive than

³ Content presented in this chapter has been adapted from:

Hacker, P. W., & Coops, N. C. (2022). Using leaf functional traits to remotely detect *Cytisus scoparius* (Linnaeus) Link in endangered savannahs. 71, 149–164. https://doi.org/10.3897/neobiota.71.76573

non-nitrogen-fixing species and make them adept at invading ecosystems with favourable environmental conditions (McKey, 1994; Richardson et al., 2000).

One such ecosystem exists along the west coast of North America. Defined as a mixed grassland-woodland, prairie-oak savannas provide habitat for several endangered plant and animal species (Bjorkman and Vellend, 2010). Increased anthropogenic interaction has also introduced a variety of invasive plant species that could destabilize traditional plant assemblages (Fuchs, 2001).

C. scoparius presents a variety of challenges to native plant species (Shaben and Myers, 2010). As a nitrogen-fixing species, it can alter soil chemistry, an unseen change that has the potential to disturb nutrient cycling (Carter et al., 2018; Fogarty and Facelli, 1999). Prairie-oak ecosystems, the fast-growing nature of *C. scoparius* competes well against native shrubs, forbs and graminoids that maintain relatively slower growth strategies (Shaben and Myers, 2010). Over time, this can result in a shift in species diversity and further uproot traditional ecosystem processes (Carter et al., 2018). The dense soil seed bank created by reproducing *C. scoparius* individuals, which begins approximately 4 years post-establishment, virtually ensures perpetual species presence and renders removal of reproducing individuals irrelevant (Downey and Smith, 2000).

Despite its damage to natural ecosystems, programs monitoring the spread of this species are not common. Initial analysis of multispectral satellite and airborne imagery has confirmed that large, dense *C. scoparius* patches can be observed during spring bloom, however a more reliable method of year-round identification at finer spatial scales is needed for realistic removal efforts (Hill et al., 2016; Odom et al., 2003). A common issue faced by previous studies relates to the availability of relevant imagery. Odom et al. (2003) used high-spatial resolution airborne imagery and manually delineated *C. scoparius*, which was both cost and time intensive. In contrast, Hill et al. (2016) used satellite imagery with a relatively coarse spatial resolution (Landsat Thematic Mapper, 25m after resampling)
and automated classification of reflectance. Unfortunately for prairie-oak ecosystems, such mapping techniques may only prove relevant upon the large-scale establishment of *C. scoparius*, at which point removal efforts are redundant.

Continuing improvements in both the platforms and sensors used for remote landscape classification present a variety of options for monitoring *C. scoparius* presence. The estimation of foliar functional traits across a site using remote sensing techniques presents an opportunity to identify invasive species like, *C. scoparius*, in prairie-oak ecosystems and has yielded success in a variety of other ecosystems (Asner et al., 2008a; Große-Stoltenberg et al., 2018; Niphadkar and Nagendra, 2016) Essentially, spectral information is acquired across several narrow bands and modelled with a measured plant functional trait, such as leaf %N, to generate a predicted trait value for each pixel in an image. This methodology has proven successful at remotely identifying unique plant species in both tropical and temperate climates and lends well to analyses conducted at a range of spatial scales (Asner and Martin, 2009; Wang et al., 2019). The continued improvement of hyperspectral imaging sensors on RPAS, or drones, and airplanes presents another opportunity to estimate plant functional traits at relatively small spatial scales over large areas (Asner et al., 2016; Van Cleemput et al., 2018).

Before air- or spaceborne analyses can be conducted, however, significant differences in both foliar functional traits and spectral reflectance between *C. scoparius* and other common prairieoak plant species should be demonstrated at the leaf- and canopy-level. The aim of this research is to identify leaf functional traits of *C. scoparius* that are significantly different from other prairie-oak species at the leaf- and canopy-levels through four research questions:

1. Is the measured value of at least one leaf functional trait of *C. scoparius* is significantly different than that of the 22 other site species sampled (henceforth referred to as 'Site')?

- Do significant differences of predicted leaf-level functional trait values remain between *C*. *scoparius* and Site species?
- Do significant differences of predicted canopy-level functional trait values remain between *C. scoparius* and Site species?
- 4. How do alterations in illumination conditions do not impact the significance of predicted canopy-level trait differences?

6.2 Materials and Methods

6.2.1. Data and Target Species

The data used in this research was collected during the May 2019 field campaign. I use leaf reflectance and chemical data collected for 23 common prairie-oak plant species to generate models capable of predicting leaf functional traits. The PROSAIL radiative transfer model (RTM) is also used to scale trait predictions of all 23-plant species to the canopy level and test whether predicted trait values of *C. scoparius* differ from 22 other plant species of varying lifeform.

C. scoparius presents a unique challenge to Garry oak ecosystems due to its ecology. Labelled "invasive" due to profuse seed production and capacity for year round growth, this shrub faces limited competition from native plant species and is capable of altering soil chemistry through nitrogen fixation (Shaben and Myers, 2010; Slesak et al., 2016). Upon establishment in a non-native environment, the spread of *C. scoparius* can be limited by a lack of compatible pollinators, but in general has shown strong capacity to alter plant diversity through native species exclusion and non-native recruitment (Carter et al., 2018; Parker, 1997). Growing quickly and reaching heights exceeding three meters, this invasive shrub faces few barriers upon introduction (Parker, 1997).

6.2.2 Leaf trait evaluation

Chemical and spectral measurements

A total of 14 traits were measured across 23 unique plant species and four plant lifeforms. All leaf samples taken are considered to be from sunlit positions. Chemical evaluation of chlorophyll a+b (Chlab), Car, LDMC, EWT and %N were conducted following CABO protocols (Ayotte et al., 2019; Girard et al., 2019; Laliberté, 2018). Due to a lack of normality in the distribution of measured trait values for multiple species the Mann-Whitney (Wilcoxon) test was used to determine if *C. scoparius* exhibits significantly different trait values from the other 22 plant species sampled (Milton, 1964). Leaf spectroscopy was conducted using a SVC DC-R/T integrating sphere and on 6 leaves from each individual plant samples (n = 201), with the number of samples ranging from 3- 10 per species, and followed CABO standards (Laliberté and Soffer, 2018). Reflectance values from 400 – 2400 nm were used in analyses after undergoing vector normalization and a Savitzky-Golay filter to enhance differences in spectral shape and reduce noise, respectively. All leaf sample underwent spectroscopy within 6 hours of collection and bulk leaf samples were chilled until chemical analyses began.

Modelling functional traits

Individual leaf traits were modelled using PLSR. The data were split into training (70%) and test (30%) sets. A Shapiro-Wilks test found the leaf-level chemical data to be non-parametric, so an independent 2-group Mann-Whitney test was used to determine if significant differences existed between the leaf functional traits of *C. scoparius* and the 22 Site species evaluated at the measured and predicted leaf-level.

Canopy-level modelling

Radiative transfer models are important methods of simulating the spectral reflectance of vegetation (Asner et al., 2011; Féret et al., 2017). There are generally two spatial scales at which models are designed: leaf and canopy. I employed the canopy-level RTM PROSAIL to simulate canopy spectra from an airborne imaging spectrometer using four measured chemical values obtained from 201 plant samples of all 23 species (Jacquemoud et al., 2009). The PLSR models developed using leaf-level spectra and chemical values were applied to the simulated spectra created by PROSAIL to predict relative trait values at the canopy-level.

The four traits used as input arguments for the PROSAIL algorithm were Chl*ab*, Car, LDMC and EWT. To determine the if canopy-level predicted traits react to changes in illumination geometry, such as different flight dates and latitudes, PROSAIL simulations were conducted at a variety of solar zenith angles spanning $20 - 70^{\circ}$ at 1-degree intervals. The functional trait models derived from PLSR were then applied to these spectra to generate predicted trait values at the canopy-level. An independent 2-group Mann-Whitney test was used to determine if the predicted trait values of *C. scoparius* were significantly different from predicted trait values of the Site species.

Software

All data manipulation was conducted in R (R Core Team 2021). The package 'spectrolab' was used to organize and manipulate data obtained through leaf spectroscopy (Meireles and Schweiger, 2021). The 'pls' package (Liland et al., 2021) was used conduct partial least squares regression and 'hsdar' (Lehnert, 2020) enabled the use of PROSAIL.

6.3 Results

An independent 2-group Mann-Whitney test determined that 11 of the 14 measured traits exhibited a significant difference between *C. scoparius* and the 22 Site species (Table A8, Figure 13).

Of these, %N (W = 1908, p-value = 1.08e-07) and carbon-nitrogen ratio (C:N; W = 15, p-value = 1.61e-07) demonstrated the largest differences (Table A8). The mean measured %N value for *C. scoparius* and Site species were 2.93% and 5.37%, respectively. Mean measured C:N values for *C. scoparius* and Site were 8.94 and 16.66, respectively. Due to the overlap in measured C:N values between *C. scoparius* and Site species, as well as the complexities introduced by measuring two traits compared to one, only %N was used in this study. Leaf-level %N was accurately predicted using PLSR ($R^2 = 0.70$, NRMESP = 17%) (Table 2, Figure 14). This is within the acceptable range of model accuracy presented in the literature and confirms its suitability for analyses (Asner and Martin, 2016; Wang et al., 2019).



Figure 13. Comparisons of 14 functional traits between C. scoparius and Site species. Boxplots depicting the differences between C. scoparius (yellow) and 22 other "Site" plants (green) for 14 leaf functional traits using a Mann-Whitney test, 11/14 of which are significantly different. The level of significance is denoted in the banner of each facet (* <= 0.05, ** <= 0.01, *** <= 0.001).

Leaf Nitrogen Percent



Figure 14. PLSR prediction plot. Comparison of the measured and predicted leaf nitrogen percent (%N) for 23 plant species at the Cowichan Garry Oak Preserve.

The use of the %N PLSR model to predict foliar %N from leaf spectral signatures determined that the leaf-level predicted %N values of *C. scoparius* and the 22 Site species were significantly different (W = 1910, p-value = 1.02e-07) (Figure 15). The significant functional difference displayed by *C. scoparius* at the leaf-level remained at the canopy-level as testing determined that relative %N of *C. scoparius* at the canopy-level was different than that of the 22 Site species (W = 1653, p-value = 1.003e-04) (Figure 16). Alterations in viewing geometry did not compromise the significant differences found between canopy predicted relative %N of *C. scoparius* and Site species (20° : W = 1653, p-value = 0.0001; 45° : W = 1653, p-value = $.0001; 70^\circ$: W = 1652, p-value = .0001026) (Figure 17).



Figure 15. Measured and predicted leaf %N. Comparison of measured and predicted leaf N% of C. scoparius (yellow) and 22 Site species of various lifeforms (Site; green) sampled at CGOP.



Figure 16. Measured leaf %N and predicted canopy N%. Comparison of the measured leaf-level and predicted canopy-level %N for C. scoparius (yellow) and 22 other plant species (Site; green) sampled at the CGOP in May 2019. Note the that y-axis scale varies, with the relative %N values predicted by PROSAIL being negative. This occurs as a result of using the relatively lower reflectance values generated by PROSAIL with a PLSR model developed using leaf-level spectra.



Figure 17. Predicted relative %N compared between C. scoparius and Site species using various solar zeniths. Boxplots demonstrating the difference between the PROSAIL predicted relative %N for C. scoparius (yellow) and Site species (green) using different solar zeniths (20 degrees, 45 degrees and 70 degrees).

6.4 Discussion

Mapping the spatial extent of invasive plant species is a key component of managing biodiversity at any scale. In North America, the invasion of *C. scoparius* populations are destabilizing the traditional species composition of plant communities, especially in prairie-oak ecosystems (Fuchs, 2001; Shaben and Myers, 2010). Previous monitoring efforts have mapped *C. scoparius* through observing yellow inflorescence from multi-spectral satellite imagery and, although effective at mapping well established populations, precludes removal efforts of young, unestablished individuals (Hill et al., 2016; Odom et al., 2003).

This research demonstrated that *C. scoparius* is distinguishable from other common prairie-oak plants based on leaf functional traits, rather than bloom color. Multiple *C. scoparius* leaf traits were significantly different from those of 22 other plant species evaluated, with %N proving the most different. This is unsurprising as *C. scoparius* is a nitrogen-fixing legume and is likely to have leaves that are relatively nitrogen-rich (Große-Stoltenberg et al., 2018; McKey, 1994). Such differences can

lead to competitive advantages in photosynthetic capacity for nitrogen-fixers, which may in part explain the success *C. scoparius* has experienced at establishing beyond its traditional range in the Mediterranean (Große-Stoltenberg et al., 2018; Shaben and Myers, 2010). These findings are consistent with research in tropical and dune ecosystems, and strengthen the idea of using leaf %N to detect invasive plant species in a variety of environments (Asner et al., 2008a; Große-Stoltenberg et al., 2018). It should be noted, however, that the use of leaf %N to map nitrogen-fixers is dependent on the absence of other nitrogen-fixing species that present similar leaf %N to the target species.

The leaf-level PLSR model used to predict leaf %N explained 70% of the total variance between measured and predicted values while demonstrating a normalized error of 17%. The use of only four components suggests that this model is well fit. Differences in measured and predicted leaf %N between *C. scoparius* and Site species promoted testing whether leaf %N was scalable from the leaf-to canopy-level. It is interesting to note that similar differences existed for C:N, suggesting that this trait could potentially be used to differentiate *C. scoparius* from Site species. This would, however, require the measurement of two traits, rather than one.

The RTM canopy model PROSAIL was used to simulate canopy reflectance of *C. scoparius* and Site species, and determined that significant differences in %N scale from the leaf to canopy. This scalability suggests that this method could be used for the detection of individuals that have recently been introduced. There are currently no civilian satellite programs capable of providing this type of data at the required spectral and spatial resolution, meaning that the imagery must be acquired from airborne sensors. Some studies have demonstrated that imagery collected from drone-based sensors can accurately map shrubland vegetation (Prošek and Šímová, 2019) or predict functional traits in the arctic (Thomson et al., 2021), but questions remain surrounding the capacity of these methods to differentiate small individuals in species-rich ecosystems (>20 species per 1 m²), such as prairie-oak ecosystems. It may be possible, however, to generate a new nitrogen-index by selecting only bands common in multi-spectral sensors (Heim et al., 2019) or correlate pre-existing multispectral remote sensing indices with the measured leaf %N values, eliminating the need for hyperspectral data collection and reducing the cost of both data acquisition and processing.

6.5 Conclusion

The significant differences in measured and predicted leaf %N between *C. scoparius* and 22 other plant species common in Canadian prairie-oak savannas suggest that remote detection of *C. scoparius* is possible. This concept is supported by the up-scaling of leaf traits using the radiative transfer model PROSAIL, which demonstrated that the aforementioned differences in leaf %N scale from the leaf- to the canopy-level. Successful scaling, in turn, proves that *C. scoparius* could be detected based on its relatively high leaf %N, given that remote sensing technologies have the required spectral and spatial resolutions to small, individual plants.

Technological advances have made RPAS more affordable, allowing them to become a common platform used for the collection of imagery with fine spatial resolution in a variety of ecosystems (Arroyo-Mora et al., 2019; Sankey et al., 2018). The recent development of RPAS-based imaging spectrometers compliments the findings of this study and suggests that land managers could deploy these sensors prior to the bloom period of *C. scoparius* across prairie-oak ecosystems in order to identify areas that may contain young individuals. Considering the capacity for *C. scoparius* to alter soil chemistry, encourage establishment of other invasive plant species and outcompete native species, the ability to detect unestablished populations through leaf functional traits presents an interesting monitoring opportunity that could prove effective in a variety of ecosystems across the globe.

Chapter 7: Conclusions

7.1 Dissertation Objectives

The objectives of this dissertation were to address knowledge gaps relating to the capacity for remote sensing technologies to monitor prairie-oak ecosystems by:

- Evaluating the accuracy of remotely sensed leaf functional trait predictions using spectroscopy
- Mapping leaf functional traits and assessing the impact of anthropogenic activities, including land use change and the subsequent habitat fragmentation, as well fire suppression, on their spatial distribution
- Using leaf functional traits to identify invasive plant species

These objectives were achieved by answering the following research questions.

How are the predictions of plant functional traits affected by the inclusion of reflectance from multiple species in a single reflectance curve?

Chapter 3 presents evidence that spectral mixing can significantly affect the prediction accurate of leaf functional traits depending on the number of species contributing leaf reflectance information and the composition of those species leaves. The impact of spectral mixing can also be negligible, further complicating analyses of spectrally mixed datasets. These results suggest that the spatial resolution of imagery used for leaf trait prediction should be no larger than the footprint of the smallest plant individual expected in the dataset. In prairie-oak ecosystems, such a pixel size should be 3 cm² or less. Although possible using RPAS, such imagery often covers relatively small areas of the landscape and can limit the size of the study area. The intrinsic challenges of mapping leaf traits in highly biodiverse savannas demonstrated by this research imply that spectral mixing should be considered during study design and support further research related to this issue.

How do anthropogenic activities influence the spatial distribution of intraspecific plant functional traits?

Research presented in Chapter 4 demonstrates that anthropogenic activities can impact the spatial distribution of plant functional traits. The effects of habitat fragmentation and fire suppression include land use change and the encroachment of closed-canopy Coastal Douglas-fir forests, both of which have been correlated to the distribution of leaf traits across the landscape. These results provide land managers with quantitative evidence supporting the reintroduction of prescribed burning and cessation of land use change in and around prairie-oak ecosystems. This research also promotes further investigation into the impacts of anthropogenic activities on nonplant species reliant on these ecosystems.

How are the functional traits of a single plant species affected by anthropogenic activities?

The evaluation of intraspecific functional traits on *Q. garryana* leaves presented in Chapter 5 identifies the impact that anthropogenic land use change, namely the creation of roadways and transitions to agriculture, as well as the removal of prescribed burning can have on a single plant species. This research builds on the findings in Chapter 4 to underscore the need to mitigate further anthropogenic activities in prairie-oak savannas and emphasizes the potential for intraspecific leaf traits to be used to monitor their impact.

What is the capacity for plant functional traits to distinguish invasive plant species?

The establishment of invasive species within prairie-oak ecosystems is a key threat to established plant communities and the ecosystem characteristics, including soil nutrient and moisture content, that underpin them. Chapter 7 provides evidence that leaf functional traits could be used for the identification of *C. scoparius*, a Mediterranean shrub that has invaded multiple continents. Leaf nitrogen content, specifically, is significantly higher in *C. scoparius* than the leaves of 22 other common plant species found in prairie-oak savannas and could enabled the detection and removal of young individuals before they are able to establish. The implications of this research span beyond the open spaces characterizing oak/prairies and encourage further research into the capacity to use leaf functional traits to detect *C. scoparius* in other ecosystems.

7.2 Innovations

This dissertation provides several innovations relevant to the prediction and spatial evaluation of leaf functional traits in prairie-oak savannas:

- The consequences of spectral mixing presented in **Chapter 3** have implications for any study using imaging spectroscopy in an environment in which the leaves of multiple plant species may be observed in a single data point. This work addresses a fundamental challenge of leaf trait prediction that has hitherto been uninvestigated.
- **Chapter 4** presents a novel methodology with which to evaluate the impact of anthropogenic activities on the spatial distribution of leaf functional traits and shows the strong influence these activities exert on prairie-oak ecosystems.
- **Chapter 5** introduces a methodology for evaluating variation in leaf functional traits within a single species using PLSR models generated at the continent scale, again highlighting the role of humans in the spatial distribution of functional traits.

- The capacity for remote sensing to distinguish *C. scoparius*, an invasive shrub, from 22 other common prairie-oak savanna plants species is presented in **Chapter 6.** These findings are globally relevant as *C. scoparius* continues to expand across the planet.
- Overall, this dissertation directly addresses the knowledge gaps stated by Fuchs et al. (2001) and reiterated by Dunwiddie and Bakker (2011) through the creation of methodologies and analyses capable of evaluating the impacts of habitat fragmentation and fire suppression on plant function, as well as monitoring the presence of invasive species in prairie-oak savannas.

7.3 Limitations

7.3.1 Study area and data curation

The use of a single prairie-oak savanna site is one limitation of this study. The lack of replication beyond the boundaries of this site precludes the scaling of my findings beyond the CGOP, which must be taken into consideration by land mangers using these results to guide actions at other sites. I acknowledge that scalability is a valuable component of any scientific pursuit and that the inclusion of multiple, comparable sites would have been useful. However, due to cost and time, such analyses were not undertaken and I believe that the lack of scalability does not reduce the importance of the findings presented herein.

7.3.2 Imaging spectroscopy

The use of imagery in this research introduces a variety of well documented challenges, including variations in atmospheric effects and solar illumination, to data collection and use. Multiple pre-processing protocols have been applied to minimize the impact of these challenges, but I acknowledge that it is impossible to fully remove the impact of these challenges (Arroyo-Mora et al., 2021).

7.3.3 Leaf functional trait prediction

The prediction of leaf functional traits from imaging spectroscopy in prairie-oak savannas faces the complications of spectral mixing presented in Chapter 3. The potential for multiple species to contribute reflectance information to the single spectral signature captured in an image pixel can render the predicted value of a trait inaccurate when the models used for prediction are generated using spectral signatures from a single species. However, this inaccuracy relative to the true value of a functional trait does not preclude the use of this information in an evaluation of other, similarly affected pixels. Rather, it highlights the necessity to pursue the evaluation of functional traits relative

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to one another. This fundamental truth has been considered in the study design of research presented in Chapters 3, 4 and 5 and therefore does not reduce the significance of the findings within.

7.4 Future Directions

7.4.1 Scalability

- Incorporate a variety of prairie-oak savannas sites into a single analysis
- Ground-truth species presence at a spatial resolution equal to or less than that of the imagery
- Generate functional trait models including all associated plant species from multiple site

Considering the immediate threats faced by prairie-oak ecosystems it is vital that research relating to plant and ecosystem health are undertaken at a variety of scales. Although important, the findings presented in this dissertation represent a single site and therefore neglect the larger mosaic of prairie-oak ecosystems on the west coast of North America. Multi-site studies of leaf functional traits would provide information relating to the impact of various anthropogenic activities on ecosystem health and enable a broader understanding of the effects of climate on the distribution of plant species and function.

At the site level, future research should ensure that each pixel generated from imaging spectroscopy can be matched with the species present, at least for small portions of the site (such as vegetation plots). The ability to confirm the species that compose a single pixel would enable a better understanding of the effects of spectral mixing. It could also enhance the accuracy of functional trait predictions by linking leaf chemistry of a single species with the spectral signature stored in a pixel, enabling PLSR models to be generated from the spectra captured by imaging, rather than leaf, spectroscopy

7.4.2 Data Curation

- Compile leaf-level spectral and chemical datasets including all associated plant species from multiple sites
- Collect imagery of prairie-oak savannas at various spatial resolutions across seasons from multiple sites

The evaluation of 23 common prairie-oak plant species, although useful for the analyses in this dissertation, represents only a small portion of the plant species associated with these ecosystems. Future scientific pursuits should aim to collect leaf-level spectral and chemical data for each relevant species. Not only would this information enhance the capacity to prediction leaf functional traits and species diversity across the landscape, it will also add substantial knowledge about an underrepresented ecosystem to the current global trait databases.

Collection of imagery at all scales, including airborne and spaceborne, across a broad variety of sensors and seasons will be vital to future research undertaking monitor the health and range of prairie-oak ecosystems. The survival of these ecosystems will rely on evidence of their continued demise at large-spatial scales and their revival will rely on the capacity to identify future sites. The identification of invasive species and the discernment of ideal planting and burning schedules are also reliant on the ability to evaluate plant species and functional metrics, guiding management activities.

7.4.3 Computation

• Incorporate advanced computation methods, such as cloud computing, to complete largescale analyses

The large amount of data that is required to conduct research in prairie-oak ecosystems poses a challenge to future analyses. Output from one imaging spectroscopy flight can contain millions of relevant pixels, suggesting that the analyses of numerous images from multiple sites will require the use of cloud computing. Google Earth Engine has demonstrated that satellite imagery can be used to monitor the state and change of Earth's forest over time and has the potential to enable the large-scale analyses required in future prairie-oak analyses (Gorelick et al., 2017; Hansen et al., 2013).

In the context of this dissertation, which focuses on a single site, the call to evaluate multiple sites may seem obvious, but is in fact complimentary. I have demonstrated here that anthropogenic activities are key drivers of leaf function, and therefore ecosystem function, but this is not new information. For decades scholars have published opinions suggesting that human activities are undermining the health of a vital ecosystem and have been ignored. This research adds quantitative and novel approaches to monitoring the health of prairie-oak ecosystems using remote sensing and directly implies that such studies are possible at much larger scales.

7.5 Closing statement

This dissertation addresses the fundamental knowledge gaps presented by Fuchs et al. (2001) in relation to prairie-oak savannas in British Columbia, Canada. I provide evidence that leaf functional traits can be predicted and mapped in highly diverse plant communities indicative of prairie-oak savannas using leaf and imaging spectroscopy. A major challenge relating to the accuracy of leaf trait prediction in these ecosystems is also quantitatively explored, the result of which can provide fundamental guidance for sampling strategies in similar environments. This research also confirms that anthropogenic activities influence the spatial distribution of leaf functional trait both within and among plant species. It is my hope that the knowledge gained from this dissertation will improve the capacity to monitor and manage these valuable systems, as well as promote further research.

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Appendix

Table A1. Produced by Curran (1989), this table described the wavelengths at which specific chemicals drive absorption. The interaction between photons and molecules that produce the absorption is explained in the second column, while considerations of potential challenges to measurement accuracy are presented in the fourth. (Continued on page133)

Wavelength (µ)	Electron Transition or Bond Vibration	Chemicals	Considerations	
0.43	Electron transition	Chlorophyll a	Atmospheric scattering	
0.46	Electron transition	Chlorophyll b	Atmospheric scattering	
0.64	Electron transition	Chlorophyll b		
0.66	Electron transition	Chlorophyll a		
0.91	C-H stretch, 3rd overtone	Protein		
0.93	C-H stretch, 3rd overtone	Oil		
0.97	O-H bend, 1st overtone	Water, starch		
0.99	O-H bend, 2nd overtone	Starch		
1.02	N-H stretch	Protein		
1.04	C-H stretch, C-H deformation	Oil		
1.12	C-H stretch, 2nd overtone	Lignin		
1.20	O-H bend, 1st overtone	Water, cellulose, starch, lignin		
1.40	O-H bend, 1st overtone	Water		
1.42	C-H stretch, C-H deformation	Lignin		
1.45	O-H bend, 1st overtone, C-H stretch, C-H deformation	Starch, sugar, lignin, water	Atmospheric absorption	
1.49	O-H bend, 1st overtone	Cellulose, sugar		
1.51	N-H stretch, 1st overtone	Protein, nitrogen		
1.53	O-H bend, 1st overtone	Starch		
1.54	O-H bend, 1st overtone	Starch, cellulose		
1.58	O-H bend, 1st overtone	Starch, sugar		
1.69	C-H stretch, 1st overtone	Lignin, starch, protein, nitrogen		
1.78	C-H stretch, 1st overtone	Cellulose, sugar, starch		
1.82	O-H stretch/C-O stretch, 2nd overtone	Cellulose		
1.90	O-H stretch/C-O stretch	Starch		
1.94	O-H stretch, O-H deformation	Water, lignin, protein, nitrogen, starch, cellulose	Atmospheric absorption	
1.96	O-H stretch/O-H bend	Sugar, starch	Rapid decrease in signal -	
1.98	N-H asymmetry	Protein	to-noise ratio of sensors	

2.00	O-H deformation, C-O deformation	Starch	
2.06	N=H bend, 2nd overtone/N=H bend/N- H stretch	Protein, nitrogen	
2.08	O-H stretch/O-H deformation	Sugar, starch	
2.10	O=H bend/C-O stretch/C-O-C stretch, 3rd overtone	Starch, cellulose	
2.13	N-H stretch	Protein	
2.18	N-H bend, 2nd overtone/C-H stretch/C- O stretch/C=O stretch/C- N stretch	Protein, nitrogen	
2.24	C-H stretch	Protein	
2.25	O-H stretch, O-H deformation	Starch	
2.27	C-H stretch/O-H stretch, CH2 bend/CH2 stretch	Cellulose, sugar, starch	
2.28	C-H stretch/CH2 deformation	Starch, cellulose	
2.30	N-H stretch, C=O stretch, C-H bend, 2nd overtone	Protein, nitrogen	
2.31	C-H stretch, 2nd overtone	Oil	
2.32	C-H stretch, CH2 deformation	Starch	
2.34	C-H stretch/O-H deformation/C-H deformation/O-H stretch	Cellulose	
2.35	CH2 bend, 2nd overtone, C-H deformation, 2nd overtone	Cellulose, protein, nitrogen	

Table A2. Partial Least Squares Regression model evaluation. Functional traits selected for hypothesis testing and their associated model performance metrics (R^2 , Root mean squared error of the predictor (RMSEP) and normalized-RMSEP (NRMSEP) and the number of components, or latent variables).

Trait	Components	R ²	RMSEP (NRMSEP)
Chlorophyll <i>a</i> (mg/g)*	7	0.54	3.25 (31%)
Chlorophyll <i>b</i> (mg/g)*	8	0.56	1.16 (33%)
Nitrogen (%)*	4	0.70	0.5(17%)
C:N*	4	0.71	2.98 (18%)
Leaf mass per area (g/m²)*	6	0.67	10.34 (25%)
Leaf dry matter content (mg/g)*	7	0.69	48.64 (22%)
EWT*	4	0.85	0.002 (16%)
Cellulose*	4	0.59	3.96 (27%)
Carotenoids (mg/g)	4	0.36	0.68 (31%)
Carbon (%)	6	0.48	0.99 (2%)
Hemicellulose	4	0.36	6.43 (40%)
Lignin	4	0.46	3.64 (55%)
Solubles (%)	4	0.41	9.78 (16%)
Recalcitrants	4	0.28	0.12 (56%)

Table A3. Percent of tree cover at each subsite calculated from the number of returns above 2m in the airborne laser scanning point cloud.

	Low	Medium	High
Tree cover (%)	39.99	42.86	47.16

Table A4. Loading and proportion of variance values for each principal component derived from analyses of eight functional trait layers at the Low cover subsite of CGOP.

Low Cover	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Proportion of Variance (%)	61.36	19.34	13.08	4.48	1.43	0.22	0.07	0.00
Chl a	0.44	0.18	-0.01	0.14	-0.03	0.45	0.70	0.24
Chl b	0.32	0.43	0.22	0.63	0.21	-0.36	-0.24	0.16
C:N	0.41	-0.32	-0.15	0.04	0.12	0.53	-0.60	0.23
%N	0.18	-0.07	0.85	-0.42	0.25	0.03	-0.01	0.00
EWT	-0.39	0.34	0.13	0.25	0.30	0.57	-0.08	-0.48
LDMC	0.39	-0.32	-0.24	0.03	0.53	-0.22	0.19	-0.56
LMA	-0.42	-0.21	-0.08	0.07	0.66	-0.04	0.16	0.55
Cellulose	-0.15	-0.64	0.35	0.58	-0.28	0.04	0.14	-0.09

Table A5. Loading and proportion of variance values for each principal component derived from analyses of eight functional trait layers at the Medium cover subsite of CGOP.

Medium Cover	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Proportion of Variance (%)	64.59	25.79	8.06	0.75	0.61	0.15	0.03	0.01
Chl a	0.42	0.16	-0.11	0.07	-0.16	0.21	0.78	-0.31
Chl b	0.38	0.32	-0.11	-0.40	-0.58	-0.27	-0.37	-0.18
C:N	0.38	-0.35	-0.05	-0.06	0.07	0.73	-0.39	-0.18
%N	0.27	0.22	0.89	-0.17	0.25	-0.01	0.00	0.01
EWT	-0.33	0.45	-0.01	-0.31	-0.22	0.55	0.11	0.47
LDMC	0.36	-0.38	-0.18	-0.53	0.20	-0.20	0.22	0.54
LMA	-0.43	-0.13	0.00	-0.66	0.19	0.01	0.13	-0.57
Cellulose	-0.20	-0.57	0.39	0.02	-0.67	0.02	0.16	0.06

Table A6. Loading and proportion of variance values for each principal component derived from analyses of eight functional trait layers at the High cover subsite of CGOP.

High Cover	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Proportion of Variance (%)	64.06	33.95	1.6	0.24	0.12	0.03	0.01	0
Chl a	-0.42	-0.19	0.06	0.09	-0.32	-0.22	0.39	0.68
Chl b	-0.42	-0.17	0.02	0.16	-0.30	-0.15	0.36	-0.73
C:N	-0.29	0.46	0.12	-0.24	0.08	0.69	0.38	0.02
%N	-0.40	-0.22	-0.71	-0.39	0.37	-0.04	-0.07	0.00
EWT	0.26	-0.49	-0.12	-0.35	-0.59	0.45	-0.09	-0.01
LDMC	-0.36	0.35	0.25	-0.49	-0.34	-0.28	-0.51	-0.04
LMA	0.43	0.12	0.02	-0.57	0.03	-0.41	0.55	-0.06
Cellulose	0.16	0.55	-0.63	0.26	-0.45	-0.06	0.02	0.03

Table A7	Global Moran's	I value for PCs 1	– 3 at low	medium and high (cover subsites
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Spatial Autocorrelation	PC1	PC2	PC3
Low	0.89	1.07	0.94
Medium	0.98	1.16	0.84
High	1.10	1.22	0.82

Table A8. Resulting p-values from Mann-Whitney tests comparing measured Cytisus scoparius nitrogen percent with the 22 Site species. The difference in %N between C. scoparius and each of the 22 other species is significantly different (p < 0.05). The number of individuals sampled per species is included in parentheses under their names.

Species	Nitrogen (%)	Species	Nitrogen (%)	
Berberis aquifolium Pursh (10)	1.08E-05	Lomatium utriculatum (Nuttall ex Torrey & A. Gray) J.J. Coulter & Rose (10)	1.08E-05	
Bromus sitchensis var. carinatus (Hooker & Arnott) R.E. Brainerd & Otting (10)	1.08E-05	Oemleria cerasiformis (Torrey & A. Gray ex Hooker & Arnott) J.W. Landon (10)	1.08E-05	
Bromus sterilis Linnaeus (6)	2.50E-04	Plectritis congesta (Lindley) de Candolle (10)	1.08E-05	
<i>Camassia leichtlinii</i> (Baker) S. Watson (10)	5.67E-06	Poa pratensis Linnaeus (10)	2.50E-04	
Camassia quamash (Pursh) Greene (10)	1.08E-05	Polystichum munitum (Kaulfuss) C. Presl (7)	1.03E-04	
Claytonia perfoliata Donn ex Willdenow (10)	1.08E-05	<i>Quercus garryana</i> Douglas ex Hooker (10)	1.08E-05	
Crataegus monogyna Jacquin (10)	2.50E-04	Rosa nutkana C. Presl (10)	1.08E-05	
Dactylis glomerata Linnaeus (10)	1.08E-05	Sanicula crassicaulis Poeppig ex de Candolle (10)	1.08E-05	
Festuca idahoensis Elmer (6)	2.50E-04	Sericocarpus rigidus Lindley (3)	0.007	
Holodiscus discolor (Pursh) Maximowicz (10)	1.08E-05	Symphoricarpos albus Poeppig ex de Candolle (10)	1.08E-05	
Lathyrus sphaericus Retzius (6)	2.50E-04	Vicia sativa Linnaeus (10)	4.33E-05	



Figure A1. Fractionated spectral signature of Vicia sativa at 25% (dark blue), 50% (light blue), 75% (light green) and 100% (dark green). These spectra are created by multiplying the pure spectral reflectance, or endmember, of V. sativa by a specific percent. The spectra "25% V. Sativa" was generated by multiplying the endmember values of all spectral bands by 0.25.



Figure A2. Boxplots presenting the differences in 14 trait values between grass (salmon), herb (green), shrub (blue) and tree (purple) lifeforms. Kruskal-Wallis one-way analysis of variance was used to evaluate the significance of trait variation between lifeforms. A pairwise Wilcox test was used to determine which specific lifeforms were different and highlighted that all lifeforms were significantly different from each other for all traits, except for two (carotenoid values for graminoid and tree species and leaf mass per area g m-2 values between graminoids and shrubs were not significantly different).









Figure A3. Changes in the predicted (a) chlorophyll a, (b) chlorophyll b, (c) nitrogen %, (d) carbon: nitrogen (C:N), (e) leaf dry matter content (LDMC), (f) equivalent water thickness (EWT) and (g) cellulose for four levels of percent target species presence (20, 40, 60 and 80%). 2- (n = 22), 3- (n = 110) and 4species (n = 409) mixes refer to the total number of species included in the mix. The horizontal red line represented the predicted trait value for the target species endmember. Horizontal black lines within each box indicate the median predicted trait value and the whiskers of each box represent the largest value equal to or within 1.5 times the inter-quantile .range. Mean trait values outside the 95% confidence interval (horizontal dotted lines) are significantly affected by spectral mixing.



Figure A4. Biplots of (a) PC1 and PC2 and (b) PC2 and PC3 of trait rasters for all subsites within the CGOP. Subsites are divided by cover (red = low, green = medium, blue = high).





(d)



Figure A5. Biplots of PCs 1 and 2 (a, c, e),as well as PCs 2 and 3 (b, d, f) for low (green), medium (blue) and high (red) cover subsites at CGOP.



Figure A6. Boxplots depicting the changes in values of PCs 1 - 3 at the Low, Medium and High Cover subsites in relation to the distance from the nearest tree. Values were sampled randomly within 1 m of the associated distance and binned. Regression lines (reg) are based on the linear model (Im) function in R using median values of each boxplot.



Figure A7. Boxplots depicting the changes in values of PCs 1 - 3 at the Low, Medium and High Cover subsites in relation to the distance from the nearest road. Regression lines (reg) are based on the linear model (Im) function in R using median values of each boxplot.



Figure A8. Boxplots depicting the changes in values of PCs 1 - 3 at the Low, Medium and High Cover subsites in relation to the amount of incoming solar radiation that a pixel is exposed to. Regression lines (reg) are based on the linear model (Im) function in R using median values of each boxplot.



Figure A9. Boxplots depicting the changes in values of PCs 1 - 3 at the Low, Medium and High Cover subsites in relation to topographic wetness. Regression lines (reg) are based on the linear model (Im) function in R using median values of each boxplot.