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SCALING UP SAGEBRUSH CHEMISTRY WITH NEAR-INFRARED SPECTROSCOPY AND UAS-ACQUIRED HYPERSPECTRAL IMAGERY

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ABSTRACT:

Sagebrush ecosystems (*Artemisia* spp.) face many threats including large wildfires and conversion to invasive annuals, and thus are the focus of intense restoration efforts across the western United States. Specific attention has been given to restoration of sagebrush systems for threatened herbivores, such as Greater Sage-Grouse (*Centrocercus urophasianus*) and pygmy rabbits (*Brachylagus idahoensis*), reliant on sagebrush as forage. Despite this, plant chemistry (e.g., crude protein, monoterpenes and phenolics) is rarely considered during reseeding efforts or when deciding which areas to conserve. Near-infrared spectroscopy (NIRS) has proven effective in predicting plant chemistry under laboratory conditions in a variety of ecosystems, including the sagebrush steppe. Our objectives were to demonstrate the scalability of these models from the laboratory to the field, and in the air with a hyperspectral sensor on an unoccupied aerial system (UAS). Sagebrush leaf samples were collected at a study site in eastern Idaho, USA. Plants were scanned with an ASD FieldSpec 4 spectroradiometer in the field and laboratory, and a subset of the same plants were imaged with a SteadiDrone Hexacopter UAS equipped with a Rikola hyperspectral sensor (HSI). All three sensors generated spectral patterns that were distinct among species and morphotypes of sagebrush at specific wavelengths. Lab-based NIRS was accurate for predicting crude protein and total monoterpenes ($R^2 = 0.7$-0.8), but the same NIRS sensor in the field was unable to predict either crude protein or total monoterpenes ($R^2 < 0.1$). The hyperspectral sensor on the UAS was unable to predict most chemicals ($R^2 < 0.2$), likely due to a combination of too few bands in the Rikola HSI camera (16 bands), the range of wavelengths (500-900 nm), and small sample size of overlapping plants (n = 28-60). These results show both the potential for scaling NIRS from the lab to the field and the challenges in predicting complex plant chemistry with hyperspectral UAS. We conclude with recommendations for next steps in applying UAS to sagebrush ecosystems with a variety of new sensors.

1. INTRODUCTION

Sagebrushes (*Artemisia* spp.) are the dominant vegetation covering over 40 million ha of the western United States (Renwick et al., 2018), but have declined due to increased wildfires, conversion to cheatgrass (*Bromus tectorum*), and juniper (*Juniperus* spp.) encroachment. Sagebrush are an important source of food and cover for wildlife and livestock. For example, Greater Sage-Grouse (*Centrocercus urophasianus*) and pygmy rabbits (*Brachylagus idahoensis*) specialize on sagebrush, consuming as much as 99% for their winter diet (Wallestad and Eng, 1975; Green and Flinders, 1980). Sagebrush leaves contain a complex mixture of plant chemicals to protect against herbivory, including volatile monoterpenes and phenolics, but are also a good source of crude protein. This chemistry is highly variable among and within sites (Robb, 2020, Olsoy et al., 2020) and influences diet and habitat selection by wild herbivores at varying spatial scales (Frye et al., 2013; Ulappa et al., 2014; Fremgen-Taratino et al., 2020).

To better understand plant-herbivore interactions, we need to map this plant chemistry across the landscape. The broad distribution of sagebrush across the western United States has been coarsely mapped (e.g., LANDFIRE, GAP, NLCD), but these maps are at 30-m to 500-m spatial resolution and do not track finer-scale patterns in distinct species with phytochemical traits that matter to herbivores (Fremgen-Tarantino et al., 2021). Several remote sensing techniques show promise in filling the gap between broad-scale distribution maps and plant-scale chemistry. One sensor technology for predicting plant and leaf-scale chemistry is near-infrared spectroscopy (NIRS). The spectral signatures measured with NIRS depend on the number and type of C—H, N—H, and O—H chemical bonds, and can be related to plant defensive and nutritional chemistry (Foley et al., 1998; Moore et al., 2010; Robb, 2020).

Unoccupied aerial systems (UAS) have emerged as a viable option for habitat mapping of vegetation and chemical traits at moderately large extents (Anderson and Gaston, 2013; Manfreda et al., 2018). Additionally, UAS can mount a variety of sensors such as multispectral, thermal, and hyperspectral cameras (Adão et al., 2017, Gerhards et al., 2019, Messina and Modica 2020), and are flexible, cheap, and mobile to deploy across the landscape (Koh and Wich, 2012). Previous work has shown UAS-based sensors can map shrub structure (Cunliffe et al., 2016; Olsoy et al., 2018), but relatively little work has been done to predict phytochemicals in sagebrush. Recent attempts at landscape mapping of diet quality involved classifying sagebrush structural morphotypes with unique chemical profiles, but relied on regression kriging (Olsoy et al., 2020), a type of spatial interpolation requiring a large amount of leaf sampling and laboratory analysis that does not directly predict plant chemical concentrations. NIRS (Olsoy et al., 2016; Robb, 2020) and airborne hyperspectral sensors (Mitchell et al., 2012b) have potential to link near- and short-wavelength infrared signals to plant chemistry. Recent technological advances have miniaturized hyperspectral sensors and allowed

for UAS platforms to capture high-resolution imagery at these longer wavelengths.

In this study, our objective was to evaluate NIRS and hyperspectral UAS for predicting plant chemistry in sagebrush and classifying sagebrush species and morphotypes. To accomplish this, we generated equations for plant chemistry with near-infrared spectroscopy collected in both the lab and the field. Next, we tested whether a UAS-based hyperspectral sensor could predict those same plant chemicals across landscapes.

2. METHODS

2.1 Study Site

We conducted research at the "Cedar Gulch" study site (lat 44°41'57"N, long 113°17'12"W, elevation 1885-1925 m), a ~155 ha area near Leadore, Idaho, in Lemhi County (Figure 1). Average temperatures in January were –6.9 °C, 14.9 °C in June, and the site received 32.8 cm precipitation annually (WRCC, 2016). The dominant vegetation at Cedar Gulch was Wyoming big sagebrush (*A. t. wyomingensis*), which occurs both on mounds with relatively deeper soils (on mound) where individual plants are large and short-statured "dwarf" patches of sagebrush in the matrix between mounds where the soil is shallower. The dwarf patches were primarily low-growing Wyoming big sagebrush (dwarf Wyoming) mixed with black sagebrush (*A. nova*). These morphotypes differed in both structural characteristics (Olsoy et al., 2018), thermal properties (Milling et al., 2018) and forage quality (Olsoy et al., 2020).

Figure 1. Flight footprints with inset map showing location in Idaho, USA.

2.2 Near-infrared Reflectance Spectroscopy

The ASD FieldSpec 4 spectroradiometer was used to measure continuous near infrared wavelength reflectance from 350 nm to 2500 nm in all the sagebrush samples under both laboratory and field conditions. In the lab, each ground dried sagebrush sample was placed in a sealed clear plastic bag and spread homogeneously on a black countertop with no countertop surface visible through the biomass. After calibrating and optimizing the ASD FieldSpec 4 to a pure white reflectance spectralon plate according to standard protocol in the user manual, it was then used to measure the reflectance of each sagebrush sample. Thirty replicate scans were collected for each sample. The instrument was recalibrated and optimized every 15 samples. In the field, we used an 8-degree FOV attachment held 0.5 m above the plant during each scan leading to a footprint of approximately 7 cm with white reflectance calibration every 5 scans or after every other scan if light conditions were changing.

2.3 Unoccupied Aerial System Flights

A portion of the study area was flown using a SteadiDrone Hexacopter UAS (SteadiDrone, Cape Town, South Africa) in June 2016. Four flights were conducted with a flight height of 25 m for approximately 20 minutes each covering 0.36-0.45 ha (Figure 1). We collected hyperspectral imagery of each flight area using the Rikola HSI (Senop Oy, Oulu, Finland) hyperspectral camera. The Rikola HSI camera collects spectra for each pixel within the range of 500-900 nm with 16 programmable bands for any increment within that range (Mozgeris et al., 2018a). For this study, we used a band combination from 550-849 nm (~20 nm increments).

Images acquired from the flights were pre-processed using the camera manufacturer software. Noise and vignetting were removed for image clarity, and digital number values (DN) were converted to radiance (W/(m^2 x srad x µm)) (Jakob et al., 2017; Mozgeris et al., 2018a). The Rikola HSI software aligns each image, but we found that the imagery had too much shift in between each band for the images to align properly (Mozgeris et al., 2018b). This shift was caused by the movement in the drone and an approximately 10 ms delay in shooting each band by the camera. Therefore, we photogrammetrically processed each band individually by flight using Agisoft Metashape (Agisoft LLC, St. Petersburg, Russia). Processing for each flight included ground control placement, image mosaicking, point cloud generation, digital surface model generation, and aligning chunks to create a 16-band orthomosaic.

After the hyperspectral orthomosaics were created, we used GPS points acquired from previous field surveys to identify individual plants and species from the images. Only plants with associated chemical data were used in the analysis. We extracted pixels representing unmixed spectral signatures of leaves and averaged by plant. After spectra were extracted, the values were standardized. Minimum values for each flight were calculated using the values closest to the lower 0.05%, and maximum values were calculated using the values closest to the upper 0.05% of the range of values. After maximum and minimum values were determined for each flight, the spectra were standardized with $(x\text{-min})/(\text{max-min})$, where x is the value of the spectra.

2.4 Lab Chemistry

After field NIRS scans and UAS flights were completed, we clipped leaf samples from each plant and kept the samples on ice until stored at –20 °C in the lab for later analysis. Leaves and stems were ground in liquid nitrogen (~2 mm) and immediately subsampled for crude protein and monoterpene analysis. For crude protein, a subset of 1-2 g of ground sample was dried at 64 °C to a constant dry weight (at least 48 h) and analysed for total nitrogen content at Dairy One Forage Labs (Ithaca, NY). Total nitrogen (%) values were converted to crude protein by multiplying each value by 6.25 (Robbins, 1983). For monoterpenes, a subset of 100 mg of sample was transferred to a headspace vial and analysed using headspace gas chromatography (Agilent 7694 Headspace Sampler, Agilent 6890 Series GC). See Robb (2020) and Olsoy et al. (2020) for more details on chemical analysis.

2.5 Statistical Analyses

We performed all statistical analyses with Camo Analytics Unscrambler chemometric software (Montclair, NJ, USA). For the laboratory and field collected NIRS dataset, the thirty

replicate reflectance scans were checked for outliers with Unscrambler's outlier detection algorithm and averaged to one spectral profile per sample. For laboratory samples, we converted each spectrum to absorbance values using a $\log_{10}(1/R)$ transformation, where R is reflectance. Spectral absorbance values were transformed by taking a 1st gap derivative every 1 nm. Laboratory spectra were truncated from 450 nm to 2350 nm. The distributions of response variables were checked for normality for all field and laboratory ASD samples. Unscrambler was then used to analyse spectra using partial least squares regressions (PLSR) between NIR spectral values (i.e., predictor variables) and plant chemistry (i.e., response variables) to produce NIRS-predicted chemistry. Each model was independently calibrated and validated using 20-fold cross-validation and results were downweighted to prevent overfitting of the models. The UAS samples were not downweighted, and leave-one-out cross-validation was utilized for the PLSR validation instead of 20-fold cross validation.
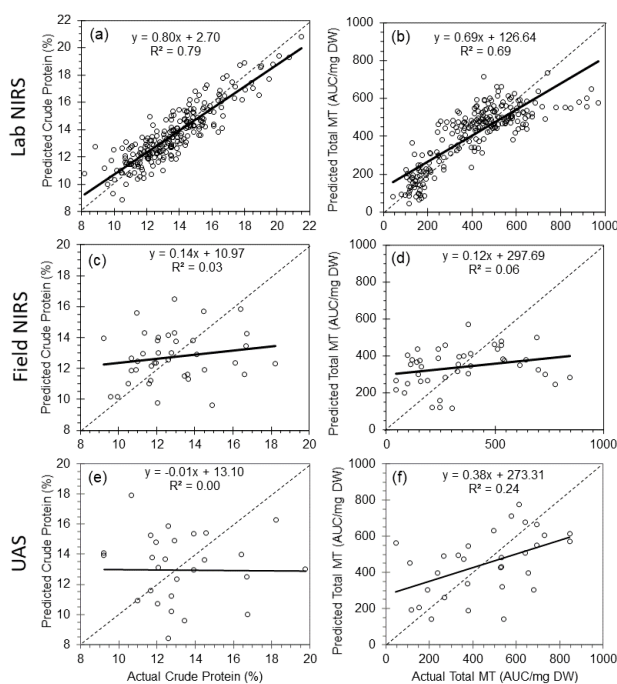

Figure 2. Cross-validation results from (a,b) lab-based near-infrared reflectance spectroscopy (NIRS), (c,d) field-based NIRS, and (e,f) unoccupied aerial system (UAS)-acquired hyperspectral predictions of crude protein (a,c,e) and total monoterpene concentration (b,d,f) at the Cedar Gulch study site in Idaho, USA.

3. RESULTS

Overall, lab-based NIRS predicted plant chemistry more accurately than field-based NIRS. Crude protein was predicted best with lab-based NIRS ($r^2 = 0.79$), but poorly with field-based NIRS ($r^2 = 0.03$) and UAS-based hyperspectral ($r^2 = 0.00$). For both lab-based NIRS and UAS-based hyperspectral, total monoterpenes were predicted better than individual monoterpenes (Table 1, Figure 2).

Hyperspectral UAS showed promise in differentiating species (black sagebrush from Wyoming big sagebrush) and morphotypes within a species (dwarf Wyoming big sagebrush from large Wyoming big sagebrush) (Figure 3). There was consistent distinction between morphotypes within a species

(large and dwarf Wyoming) and between species (Wyoming and Black) at specific wavelengths (Figure 3f, R1-R3). Where Wyo more similar than black. Consistent differentiation of species with similar morphotype in shared spatial context (between mounds).
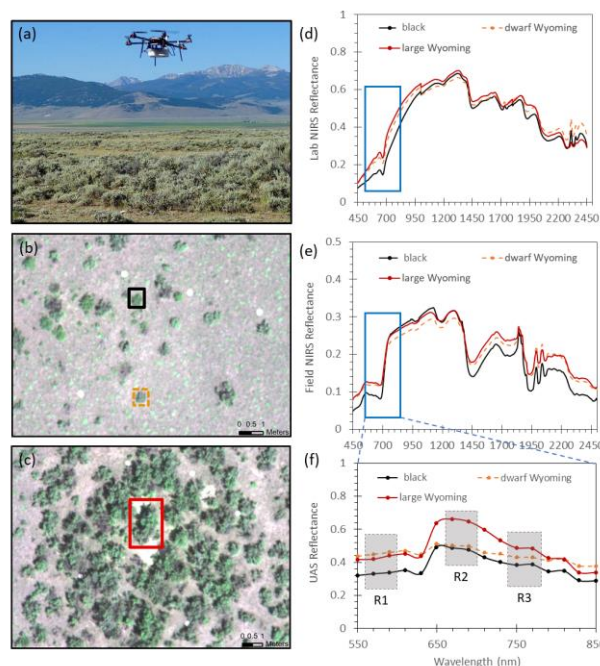

Figure 3. SteadiDrone Hexacopter UAS with a Rikola HSI camera (a). Examples of hyperspectral UAS imagery at a dwarf sagebrush patch (b) and on-mound big sagebrush (*Artemisia tridentata*) patch (c). Comparison of NIRS collected in the lab (d), NIRS in the field (e), and hyperspectral UAS (f). Colour of boxes and spectra represent species and patch type for black sagebrush (*A. nova)* in a dwarf patch (black lines), small morphotype of Wyoming big sagebrush in a dwarf patch (dotted orange lines), and large morphotype of Wyoming big sagebrush in an on-mound patch (red lines). Gray boxes in (f) showcase possible regions for classifying Wyoming from black (R1), large Wyoming from dwarf (R2), and all 3 classes from one another (R3).

4. DISCUSSION

Our lab-based NIRS results predicting crude protein were comparable to Olsoy et al. (2016) ($r^2 = 0.79$ compared to $r^2 = 0.93$) and Mitchell et al. (2012a) ($r^2 = 0.76$-0.86). The poor results with field-based NIRS and UAS hyperspectral are likely due to small sample size (< 50 samples). However, Mitchell et al. (2012b) had more success with airborne hyperspectral (HyMap, $r^2 = 0.56$) by minimizing the influence of bare ground, suggesting further processing of the data and a smaller field-of-view attachment on the NIRS could improve results. In the lab, extrinsic factors are controlled for by providing an external light source and drying and grinding the leaf samples to reduce the impact of particle size and water absorption features (Mitchell et al., 2012b; Olsoy et al., 2016). In the case of the hyperspectral sensor, previous studies have found the important wavelengths to be above 1000 nm for predicting nitrogen (Mitchell et al., 2012b), while the Rikola HSI camera has a range of 500-900 nm. A sensor matching more closely to the ASD such as the Headwall VNIR+SWIR (400-2500 nm) (Headwall Photonics, Inc., Fitchburg, MA, USA) would likely

Table 1. Calibration and validation statistics for near-infrared reflectance spectroscopy (NIRS) prediction of sagebrush phytochemistry at the Cedar Gulch study site in Idaho, USA.

Phytochemical	Instrument	n	Calibration			Cross-validation				
			$RMSE_C$	SE_C	r^2	B_1	B_0	$RMSE_{CV}$	SE_{CV}	r^2
Crude protein (%)	Lab NIRS	236	0.96	0.96	0.82	0.80	2.7	1.03	1.04	0.79
	Field NIRS	40	2.16	2.19	0.12	0.14	11.0	2.44	2.47	0.03
	UAS	28	2.39	2.44	0.16	-0.01	13.1	3.47	3.54	0.00
Total monoterpenes (AUC/mg DW)	Lab NIRS	234	110	110	0.68	0.61	160	125	126	0.59
	Field NIRS	43	193	196	0.20	0.12	298	214	217	0.06
	UAS	31	166	169	0.44	0.38	273	203	206	0.24

have more success predicting crude protein and other plant chemistry across the landscape.

Total monoterpenes were predicted well by lab-based NIRS (r^2 = 0.69), and the UAS hyperspectral camera (r^2 = 0.24) performed better than regression kriging at Cedar Gulch reported by Olsoy et al. (2020) ($r^2 < 0.1$). Kokaly and Skidmore (2015) detected an absorption feature at 1.63 μm and attributed that to C—H bonds on phenols and aromatics such as terpenoids, suggesting that a camera with SWIR capabilities could better detect and predict sagebrush plant chemistry and could explain the better prediction with lab-based NIRS. The signal was weaker in wet leaves compared to dry leaves (Kokaly and Skidmore 2015), matching up with the results seen here with lab versus field-based NIRS and previous work in the lab by Olsoy et al. (2016).

Despite our finding that the Rikola HSI camera was unable to predict crude protein or total monoterpenes, it showed potential for classifying sagebrush species and morphotypes. These sagebrush species may be hard to distinguish based on structure from the ground or in the air and are often misclassified in land cover maps from satellite images (Fremgen-Tarantino et al., 2021), however, these species have important differences in phytochemicals and potential use by herbivores (Frye et al., 2013).

Next steps involve testing a hyperspectral sensor with 274 bands over a similar wavelength (Headwall NIR + LiDAR), and another sensor with bands into the SWIR (Headwall NIR + SWIR). Future work should take advantage of larger sample size, sites with more chemical diversity, and a better balance between species and chemo-types. Additionally, the continued success of NIRS in lab environments shows potential for scaling to the field to classify sagebrush and predict chemistry (Robb, 2020). We recommend using existing datasets (either NIRS or lab-based chemistry) to set up which bands are more important for the goal in hand (i.e., differentiation between chemotypes or predicting chemistry of interest) to select the sensor best suited to that purpose. Alternatively, UAS could be used by managers in exploratory work to determine what is differentiable from the air and to decide what should be sampled on the ground to test whether these spectral differences are chemical or physical (e.g., soil related), or whether sites may contain hybrid zones. In this way, UAS could iteratively serve as a tool for adaptive management in a changing world.

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APPENDIX

Table A1. UAS flight and orthomosaic information.

Flight	Photos	Altitude (m)	Resolution (cm)	XY error (cm)	Coverage (ha)
1	238	25.6	1.58	3.89	0.499
2	212	24.1	1.48	3.86	0.446
3	198	25.2	1.55	3.68	0.426
4	241	24.1	1.48	3.88	0.484

Table A2. Descriptive statistics for reference phytochemistry.

Phytochemical	Sensor	n	mean	sd	range
Crude protein (%)	Lab NIRS	236	13.6	2.3	8.2-21.5
	Field NIRS	42	12.8	2.2	9.2-18.2
	UAS	25	13.4	2.5	9.2-19.8
Total monoterpenes (AUC/mg DW)	Lab NIRS	232	409	191	44.1-970
	Field NIRS	45	346	216	44.1-846
	UAS	28	451	223	46.2-846