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Nutritional analysis of sagebrush by near-infrared reflectance spectroscopy

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ABSTRACT

Sagebrush (*Artemisia* spp.) habitat in the Intermountain West is one of the most endangered ecosystems in North America due, in part, to fire, climate change, and anthropogenic disturbances. However, restoration efforts rarely consider the dietary quality of sagebrush that is conserved or restored despite growing evidence that it is an influential parameter explaining habitat use by many important wild and domestic herbivores. The objective of this study was to evaluate the capacity of near-infrared reflectance spectroscopy (NIRS) to measure and monitor the dietary quality of sagebrush. Leaf samples were collected from two sagebrush species over two seasons and three sites in Idaho, USA. We developed calibration equations for crude protein (CP), dry matter digestibility (DMD), 1,8-cineole (cineole), and total polyphenolics. The coefficient of determination (r^2) and ratio of performance to deviation (RPD) were 0.93 and 3.5 for CP, 0.83 and 1.8 for DMD, 0.64 and 1.5 for cineole, and 0.64 and 1.6 for total polyphenolics. These results indicate that NIRS may offer a rapid, noninvasive, diagnostic tool for assessing dietary quality of sagebrush, but future research should explore the potential for development of improved prediction equations and *in situ* analysis of sagebrush dietary quality with field spectroscopy.

1. Introduction

Sagebrush (*Artemisia* spp. L.) dominates approximately 62 million ha of the western United States (West and Young, 2000), but is declining at an increasing rate, due, in part, to fire, climate change, and human activities (Meinke et al., 2009; Davies et al., 2011). Many herbivores eat sagebrush, including wild ungulates (e.g., mule deer [*Odocoileus hemionus*], pronghorn [*Antilocapra americana*], and elk [*Cervus canadensis*]) (Torstenson et al., 2006), livestock (e.g., sheep [*Ovis aries*]), and species that are of conservation concern (e.g., Gunnison sage-grouse [*Centrocercus minimus*], greater sage-grouse [*Centrocercus urophasianus*], and pygmy rabbits [*Brachylagus idahoensis*]). Herbivores select specific accessions or patches of sagebrush on the landscape, and individual sagebrush plants within a patch are preferentially browsed by herbivores (Behan and Welch, 1985; Frye et al., 2013; Arkle et al., 2014). In many cases, a higher concentration of crude protein (CP) and lower concentration of plant secondary metabolites (PSMs) such as monoterpenes and polyphenolics explain diet selection by herbivores (Frye et al., 2013; Ulappa et al., 2014). Therefore, land use and climate changes that affect the abundance and dietary quality of sagebrush (Karban et al., 2006; Beck et al., 2012) can influence the use of sagebrush by native herbivores and livestock for both cover (Camp et al., 2013) and food (Frye et al., 2013; Ulappa et al., 2014).

In particular, changes in climate are expected to change not only the abundance and distribution of sagebrush, but also the dietary quality of remaining sagebrush (Forbey et al., 2013). For example, rises in CO_2, ultraviolet light, temperatures, and drought are predicted to increase concentrations of PSMs and decrease concentrations of CP (Robinson et al., 2012), reducing the dietary quality of

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sagebrush for foraging herbivores. Rising temperatures also are predicted to increase abundance of insects (Currano et al., 2010) that could increase defoliation of sagebrush. Increased damage by insects limits the growth and reproduction of sagebrush (Takahashi and Huntly, 2010) and causes an induction of PSMs in sagebrush and surrounding plants through interplant volatile communication (Karban et al., 2000). Finally, PSMs are predicted to become more toxic to herbivores with increasing temperatures (Forbey et al., 2013; Dearing, 2013).

Although efforts to restore higher quality sagebrush habitats are increasing, these efforts rarely consider how restoration and management activities may influence the dietary quality of sagebrush. For example, most restoration efforts focus on planting Wyoming big sagebrush (*Artemisia tridentata* subsp. *wyomingensis* Beetle & Young) (McAdoo et al., 2013), and do not also include planting of dwarf species such as black sagebrush (*A. nova* A. Nels.) and low sagebrush (*A. arbuscula* Nutt.). However, Frye et al. (2013) showed that that black sagebrush was selected by free-ranging sage-grouse over Wyoming big sagebrush when both species co-occurred, and Rosentreter (2005) reported that these dwarf species were more nutritious for sage-grouse and other herbivores. Furthermore, when sagebrush is reseeded after fire, survival is low (Arkle et al., 2014) and the sagebrush that survives (McAdoo et al., 2013) may not include the species preferred by herbivores. Other restoration efforts such as prescribed fires or application of herbicides (Beck et al., 2012) often do not consider how these activities might influence the dietary quality of remaining sagebrush. One reason for this oversight is that measuring and monitoring the nutritional and chemical traits of plants is difficult to achieve over large landscapes. Managers currently lack the tools necessary to measure dietary constituents at the same spatial and temporal scales at which changes in habitat or management activities are occurring. For example, measuring plant quality requires collecting many plant samples throughout the year and detailed laboratory nutritional and PSM quantitative analysis, both of which are prohibitively time-consuming and expensive.

Management and restoration of sagebrush habitat may benefit from a rapid, predictive tool that can assess dietary quality of sagebrush both in the lab and in the field and decrease plant sample collection and processing tasks. One approach is the use of near-infrared reflectance spectroscopy (NIRS) to measure plant constituents that define dietary quality for herbivores, such as CP and PSM concentrations in plants (Foley et al., 1998; Roberts et al., 2004; Mitchell et al., 2012; Youngentob et al., 2012). NIRS has been widely used in food science and agriculture to predict CP (Abrams et al., 1987; Jensen et al., 2012), digestible energy (Abrams et al., 1987; Griggs et al., 1999), monoterpenes (Steuer et al., 2001; Wilson et al., 2002), and polyphenolics (Schulz et al., 1999; Flinn et al., 1996), among other dietary constituents that may be important to herbivores. Therefore, NIRS provides a more rapid and potentially cost-efficient approach to evaluate diet quality of specific species of sagebrush compared to laboratory methods (Foley et al., 1998). Moreover, NIRS has the potential to help map the distribution of sagebrush species with different chemical constituents at larger spatial scales (Clark and Roberts, 2012). This ability could help predict and monitor habitat use by herbivores, as was demonstrated in the koala (*Phascolarctos cinereus*)/eucalypt (*Eucalyptus microcorys* F. Muell.) system in Australia (Moore et al., 2010). Further, knowledge of sagebrush dietary quality may allow managers to prioritize conservation of higher quality sagebrush, or select seeds from these plants for restoration following disturbances such as fire. Current laboratory instrumentation and chemometrics software enable rapid and repeatable measurements of plant dietary quality. Field spectroscopy for similar purposes is evolving and has not yet been implemented widely (Starks and Brown, 2010). Therefore, our study objectives were to evaluate NIRS with respect to its 1) viability as a predictive tool of sagebrush dietary quality; and 2) accuracy of prediction of dietary quality with a single equation for two species (big sagebrush and black sagebrush) across three study sites and two seasons.

2. Methods and materials

2.1. Study sites

We conducted research at three sites in Idaho, US. The “Camas” site (43°14′28″N, 114°19′04″W, elevation 1472 m) is a ~300 ha area located north of Shoshone, Idaho, in Blaine County. This site is dominated by Wyoming big sagebrush, but also includes low sagebrush. During this winter study (October 2009 to March 2010), Camas had high temperatures averaging 2.6 °C, low temperatures averaging −9.2 °C, and mean annual precipitation of 226 mm. The “Leadore” site (44°41′57″N, 113°17′12″W, elevation 1942 m) is a ~225 ha area near Leadore, Idaho, in Lemhi County, which is also dominated by Wyoming big sagebrush and included black sagebrush. During our study, Leadore had high temperatures averaging 3.0 °C, low temperatures averaged −10.1 °C, and mean annual precipitation was 170 mm. The main browsing on sagebrush at the Camas and Leadore sites is by pygmy rabbits (see Ulappa et al., 2014). The “Browns Bench” site (42°11′31″N, 114°46′11″W, elevation 1550–1750 m) is a ~19 000 ha area located in Twin Falls County in south central Idaho. This site is dominated by black sagebrush, but also includes low sagebrush, hybrids between black and low sagebrush, and Wyoming big sagebrush. During our study, Browns Bench had high temperatures averaging 8.1 °C, low temperatures averaged −2.6 °C, and mean annual precipitation was 306 mm. The main browsing on sagebrush at the Browns Bench site is by sage-grouse (see Frye et al., 2013).

2.2. Sample collection

To obtain reference samples for NIRS calibration, we collected representative leaf samples from individual sagebrush plants at all three sites in fall and winter, and additionally from the Camas site in summer. We focused sampling in the winter to better capture nutritional quality when sagebrush comprises up to 99% of pygmy rabbits’ diet (Shipley et al., 2006). At the Camas site, leaf samples from Wyoming big sagebrush were collected in November 2009 ($n = 50$) and in May and June 2010 ($n = 54$). At the Leadore site, leaf samples from Wyoming big sagebrush were collected in October 2009 ($n = 54$). At the Browns Bench site, the collection of black sagebrush leaf samples occurred in February and March 2010 ($n = 22$). Extra samples were collected at the Camas site in May and June 2010 to test the effects of sample drying and particle size and removal of spectral water regions on NIRS analysis (see Fig. S1 and Table S1, available online). Those results showed that prediction accuracy of chemical composition of coarsely ground samples (preparation described below) was better for dry than for wet material, and similar among dry coarsely ground and finely-ground for CP but better for dry finely-ground samples for total polyphenolics. We subsequently performed NIRS analyses with dry coarsely ground samples.

2.3. Sample preparation and nutritional analysis

To prepare sagebrush samples for nutritional analyses and NIRS, we separated leaves from stems by freezing the samples with dry ice, dislodging the leaves, and then removing the stems, dead leaves and debris. We then coarsely ground each sample in liquid nitrogen with a mortar and pestle to an average particle size of

approximately 2 mm. Subsamples were taken for NIRS analysis and for chemical analysis. A subsample was stored at −20 °C for monoterpene and total polyphenolic analysis while the remaining sample for chemical analysis was dried to constant weight at 50 °C to determine dry mass. The dried sample was analyzed for total CP and dry matter digestibility (DMD). Digestibility was performed with an *in vitro* digestion assay (DeGabriel et al., 2008; Ulappa et al., 2014) which subtracted average post-digestion dry matter (DM) amounts from average pre-digestion DM to calculate DMD. We used the combustion method (Dairy One Forage Labs, Ithaca, New York) to quantify protein concentration in subsamples before (pre-digest sample) and after (post-digest sample) digestion.

2.4. Plant secondary metabolite analysis

To obtain reference chemistry of PSMs for nutritional analysis and NIRS, we analyzed monoterpene concentration of 1,8-cineole (cineole) of wet subsamples using gas chromatography. For each sample ~0.10 g (wet weight) of sample was extracted for 24 h at room temperature in 1 mL of methylene chloride (HPLC grade) spiked with an internal standard of fenchone (>98% purity, CAS# 4695-62-9, not naturally present in sagebrush samples) at a concentration of 50 μg mL^{-1}. We then dried each sample for 1 h with 0.25 g of anhydrous sodium sulfate (granular). Extracts were removed, filtered through glass wool and processed by a HP 7673 controller and sampled by a HP 6890 series II injector. Two μL of each sample were injected into the HP 5890 series II gas chromatograph with flame ionization detector. The stationary phase was a DB-5 Agilent silica column (30 m × 0.25 mm) with a 0.25 μm coating. The initial oven temperature was 40 °C for 2 min, then increased at 3°min^{-1} until reaching 60 °C, then increased at 5°min^{-1} until 120 °C, and finally increased at 20°min^{-1} to 300 °C and was held for 7 min. Injector and detector temperatures were 250 °C and 300 °C, respectively. The make-up and carrier gases were helium. The inlet pressure was 80 kPa with a flow rate of 1.00 mL min^{-1}. Monoterpene retention times and peak areas were calculated by HP ChemStation version B.01.00 and their identities were verified using co-chromatography with standards. Monoterpene concentrations were expressed as μg of compound in fenchone equivalents per g DM of plant sample (μg g^{-1} DM).

We analyzed total polyphenolic concentration of wet subsamples using a colorimetric assay (Ainsworth and Gillespie, 2007). Each sample was extracted in 95% methanol for 24 h. From the supernatant, 50 μL of sample was diluted 1:5 and then mixed with 200 μL of Folin-Ciocalteu reagent (20%) and 800 μL of 700 mM sodium bicarbonate in distilled water. The color intensity was immediately measured using a BioTek Synergy MX multi-mode plate reader (BioTek, Winooski, Vermont) at an absorbance of 765 nm at room temperature. Values were expressed as μM of compound in gallic acid equivalents per g DM of plant sample (μM g^{-1} DM).

2.5. NIRS analysis

To obtain NIRS spectra for calibration equations, we scanned dried subsamples with a scanning monochromator (NIRSystems Model 5000, FOSS North America, Eden Prairie, MN). Paired scans were averaged and absorbance spectra were recorded as the reciprocal log of reflectance (log [1/R]) at 2 nm increments over 1100–2498 nm. We combined the samples from all three sites in the winter with the summer samples from Camas into a single population to increase the sample size and maximize inference across time (seasons), space (sites), and species. Mean spectra for each sample population are shown in Fig. 1. Calibration sets were paired with laboratory measurements and spectral data of all

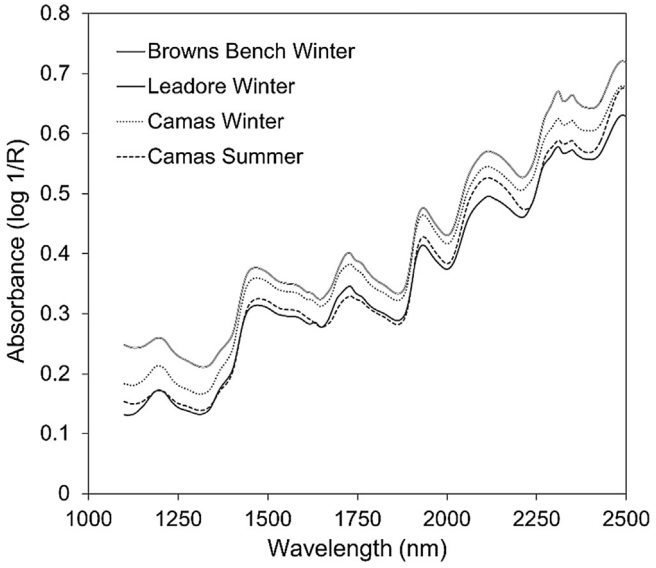

Fig. 1. Mean near-infrared reflectance spectra of samples of sagebrush leaves collected from two species (Wyoming big sagebrush [*Artemisia tridentata* subsp. *wyomingensis* Beetle & Young] and black sagebrush [*A. nova* A. Nels.]) at three study sites (Camas, Leadore, and Browns Bench) in Idaho, US, over two seasons (winter and summer).

samples ($n = 180$) for CP and total polyphenolics, and of all winter samples from all three sites ($n = 126$) for DMD and cineole. A set of 20 randomly selected samples was removed from the calibration set for subsequent external validation of the performance of prediction equations developed with the remaining calibration samples (ASTM, 2012) (Table 1).

We determined relationships between compositional constituents and NIRS spectra according to Shenk and Westerhaus (1991) with modified partial least squares regression using WinISI 4.5 chemometrics software (Infrasoft International LLC, State College, Pennsylvania). Prediction equations were developed using every eighth wavelength over 1108–2492 nm following 1,4,4–transformation of spectral data, representing the order of the derivative of raw spectral data and numbers of 2-nm data points over which the derivative and a running smooth were calculated. We used two outlier elimination passes and standard normal variate with detrending to reduce variance associated with light-scattering effects. Internal cross-validation was used to calculate standard error and coefficient of determination of cross-validation (SECV and 1-VR). The 20 external validation samples were used to

Table 1
Descriptive statistics of lab-measured chemistry for calibration and validation data sets of crude protein (CP), dry matter digestibility (DMD), 1,8-cineole (cineole), and total polyphenolics. Leaf samples were pooled across three study sites (Camas, Leadore, and Browns Bench) in Idaho, US, two seasons (winter and summer), and two species (Wyoming big sagebrush [*Artemisia tridentata* subsp. *wyomingensis* Beetle & Young] and black sagebrush [*A. nova* A. Nels.]).

Constituent	Set	n	Range	Mean	SD
CP	Calibration	154	7.3–17.2	12.2	1.64
(% DM[a])	Validation	20	10.3–17.6	12.8	1.79
DMD	Calibration	106	69.4–84.0	76.7	2.44
(% DM)	Validation	20	68.7–84.7	76.1	4.35
Cineole	Calibration	99	0–12627	3519	3036
(μg g^{-1} DM[b])	Validation	20	473–14470	4158	3720
Total polyphenolics	Calibration	154	33.8–189.7	111.8	26.0
(μM g^{-1} DM[c])	Validation	20	58.8–248.4	109.2	45.5

[a] Units are in percent dry matter.
[b] Units are in μg of fenchone equivalents per g DM of plant sample.
[c] Units are in μM of gallic acid equivalents per g DM of plant sample.

calculate the standard error of validation (SEV), coefficient of determination (r^2), slope, and bias among predicted and actual laboratory reference values, and ratio of performance to deviation (RPD, calculated as standard deviation/SEV) according to Williams (2001). Values of RPD ≥3 indicate good predictive power.

3. Results and discussion

Our evaluation indicates that CP of sagebrush forage can be estimated accurately with NIRS, but DMD, cineole and total polyphenolics had lower prediction accuracy. We tested prediction accuracy of NIRS relative to known laboratory values for CP, DMD, cineole, and total polyphenolics. Externally-validated prediction accuracy was best for CP, which was the only constituent to closely match the 1:1 line (Fig. 2A). Further, CP prediction equations had the highest validation r^2 of 0.93 and the highest RPD of 3.5 (Table 2). The range in CP concentration of 7.3–17.6% in Wyoming big sagebrush leaves (Table 1) was wider than that observed by Mitchell et al. (2012) for leaves of Wyoming and basin big sagebrush (*A. t.* Nutt. subsp. *tridentata*) in Idaho, US. Welch and McArthur (1979) found mean CP for Wyoming big sagebrush of four populations to be 11.8% in winter, which is similar to what we found in our 3 populations of Wyoming big sagebrush ($\bar{x}$ = 12.5%, σ = 1.7). The results for CP compared favorably with standard error of prediction (SEP) of 0.8 and RPD of 6.5 for CP from diverse species of hay (Abrams et al., 1987), SEP of 1.0 and RPD of 3.6 for combined clipped timothy (*Phleum pratense* L.). Results for CP also compared favorably with SECV of 0.8–1.2 and 1-VR of 0.93–0.98, for broad populations of forage grasses and legumes in equations used by commercial forage testing laboratories (NIRS Forage and Feed Testing Consortium, 2014) and SECV of 0.6, 1-VR of 0.99 and RPD of 9.2 for 287 calibration samples of the shrub tagasaste (*Chamaecytisus proliferus* (L. f.) Link; Flinn et al., 1996). Although our instrumentation and development of predictive equations differed somewhat from that of Mitchell et al. (2012) for CP in 36 sagebrush samples, our calibration R^2 of 0.94 was slightly higher than their value of 0.86.

Prediction accuracy for DMD was moderate with a validation r^2 of 0.83 and RPD of 1.8 (Table 2) and compared well with other studies, suggesting NIRS can reliably determine some forage quality constituents. Results for DMD of SEV of 2.5 and validation r^2 of 0.83 were similar to SECV of 1.6–3.1 and 1-VR of 0.85–0.95 from a variety of cool-season grasses and legume samples (Griggs et al., 1999), and SECV of 2.7–4.2 and 1-VR of 0.84–0.85 for forage grasses and legumes in equations used by commercial forage testing labs (NIRS Forage and Feed Testing Consortium, 2014). Results for NIRS-predicted values also compared very favorably with SEP of 3.0 for multiple species of hay (Abrams et al., 1987). Predictive strength of DMD from sagebrush in this study as indicated by RPD of 1.8, however, was lower than the threshold of 3.0 suggested by Williams (2001) and the value of 2.8 observed by Abrams et al. (1987) for hay, perhaps as a function of differing reference methods or more variable particle size in our samples.

NIRS-predicted values for CP with SEV of 0.5 supported the ability of this approach to distinguish between plants that are likely to be selected for browsing by animals and therefore could be used

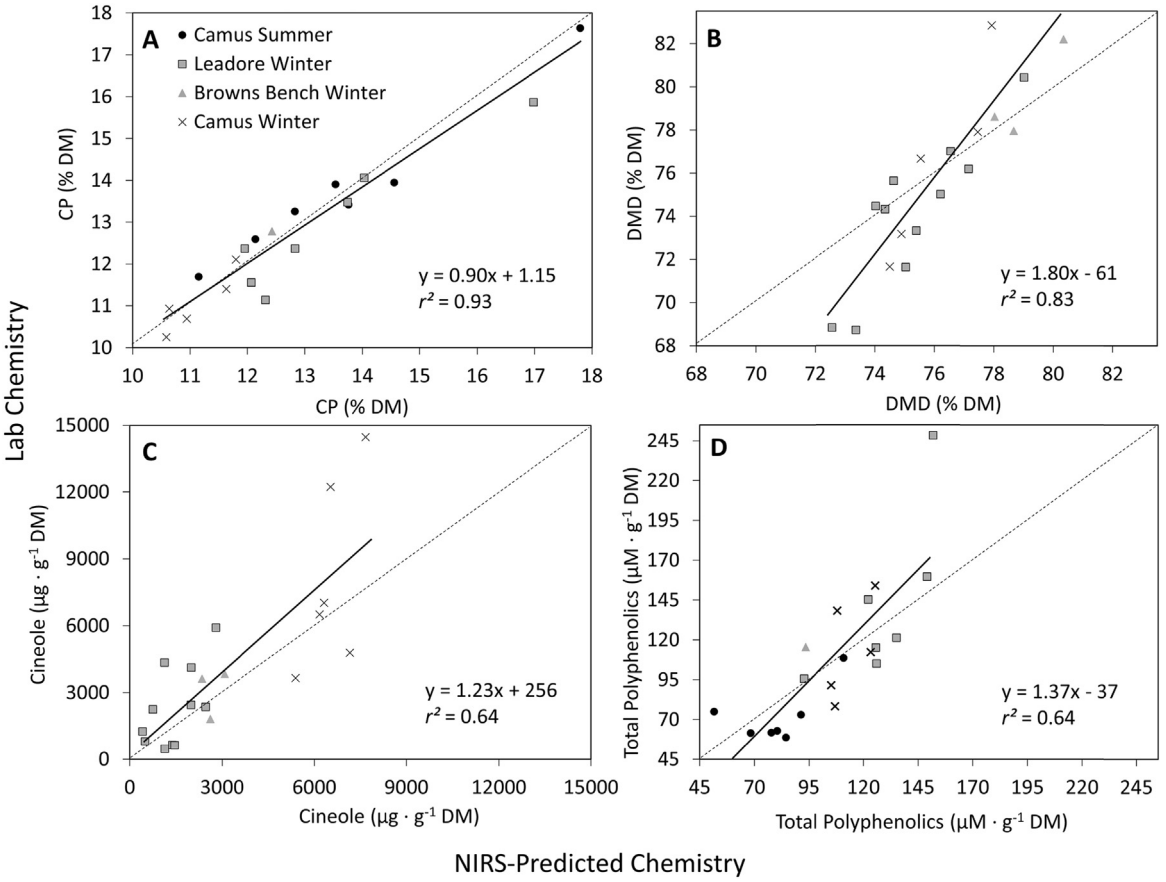

Fig. 2. Validation graphs showing near-infrared reflectance spectroscopy-predicted (NIRS-predicted) nutritional quality of sagebrush leaves regressed against lab-measured nutritional quality for **A)** crude protein (CP), **B)** dry matter digestibility (DMD), **C)** 1,8-cineole, and **D)** total polyphenolics. Samples of two species (Wyoming big sagebrush [*Artemisia tridentata* subsp. *wyomingensis* Beetle & Young] and black sagebrush [*A. nova* A. Nels.]) were collected at three study sites (Camas, Leadore, and Browns Bench) in Idaho, US, during two seasons (winter and summer). Dashed line indicates a 1:1 relationship, and solid line shows the validation regression fit.

Table 2
Calibration and validation results for predicting the nutritional quality and chemistry of sagebrush using near-infrared reflectance spectroscopy. Leaf samples were pooled across three study sites (Camas, Leadore, and Browns Bench) in Idaho, US, two seasons (winter and summer), and two species (Wyoming big sagebrush [*Artemisia tridentata* subsp. *wyomingensis* Beetle & Young] and black sagebrush [*A. nova* A. Nels.]).

Constituent	Calibration			Internal cross-validation			External validation		
	N	Model terms	SEC[a]	R^2[b]	SECV[b]	1-VR[c]	SEV[d]	r^2[b]	RPD[e]
Crude protein	154	8	%DM[f] 0.4	0.94	%DM 0.5	0.91	%DM 0.5	0.93	3.5
Dry matter digestibility	106	5	%DM 1.6	0.59	%DM 1.7	0.49	%DM 2.5	0.83	1.8
1,8-cineole	99	2	μg g^{-1} DM[g] 1971	0.58	μg g^{-1} DM 2007	0.56	μg g^{-1} DM 2454	0.64	1.5
Total polyphenolics	154	6	μM g^{-1} DM[h] 15	0.69	μM g^{-1} DM 16	0.60	μM g^{-1} DM 28	0.64	1.6

[a] Standard error of calibration.
[b] Standard error of cross-validation.
[c] Proportion of variation in laboratory values accounted for in cross-validation.
[d] Standard error of validation ($n = 20$).
[e] Ratio of performance to deviation, calculated as standard deviation of reference laboratory values/SEV.
[f] Units are in percent dry matter.
[g] Units are in μg of fenchone equivalents per g DM of plant sample.
[h] Units are in μM of gallic acid equivalents per g DM of plant sample.

for diet selection studies. Our coarsely-ground samples may have greater particle size heterogeneity than is suggested by a mean particle size of ~2 mm. As indicated in our supplemental data (Fig. S1 and Table S1, available online) and also found by Griggs et al. (1999), accuracy of NIRS predications would likely improve for DMD if samples were more finely ground and dried before analysis.

Predictive strength for total polyphenolics and cineole by NIRS was weaker than for CP and DMD with validation r^2 of 0.64, and RPD values of 1.6 and 1.5, respectively (Table 2). RPD values less than 3.0 suggest poor predictive ability (Williams, 2001) and that NIRS may be more appropriate for preliminary screenings of sagebrush samples than for quantitative analysis of DMD, total polyphenolics, and cineole. Both cineole and total polyphenolics under-predicted samples with high PSM concentrations (Fig. 2C and D), suggesting the calibration models do not capturing all of the variation at the higher end. One potential reason for the lower accuracy in cineole prediction is that monoterpenes are volatile and thus lost during the process of drying samples prior to NIRS. Another possible reason is the limited ability of NIRS to detect concentrations of constituents below 0.1–1.0% (Westerhaus et al., 2004). However, in other studies, NIRS accurately predicted monoterpene concentrations in dried samples (Steuer et al., 2001; Wilson et al., 2002). This discrepancy may be caused by a larger sample size in other studies, or because some monoterpenes may be correlated with other non-volatile chemical attributes that can be detected by NIRS. In systems where these volatile chemicals are important, we recommend analyzing wet samples. However, this is a tradeoff because scanning wet samples could reduce prediction accuracy by NIRS (see Fig. S1 and Table S1, available online), because water absorption bands may obscure important spectral peaks related to proteins and other chemicals (Griggs et al., 1999).

The strength of predicting total polyphenolics with NIRS was similar to that found in other studies. Our results of 0.60 and 1.6 for 1-VR and RPD were similar to that reported by Schulz et al. (1999) in green tea (*Camellia sinensis* (L.) Kuntze) leaves where values for 1-VR and RPD were 0.67 and 1.7, and much lower than reported by Goodchild et al. (1998) in annual legume hays and straws for 1-VR and RPD of 0.95 and 4.5. Statistics from this latter study were based on a limited calibration set of 39 samples and 8 model terms, however, which suggests model overfitting. In a study with a calibration set of 227 samples, Flinn et al. (1996) were able to predict total polyphenolics in tagasaste plants with 1-VR of 0.96 and RPD of 4.7. The reference method for total polyphenolics in this case was a modification of one of the procedures of Price and Butler (1977), which includes a spectrophotometric measurement following treatment with $FeCl_3/K_3Fe(CN)_6$. Our results may reflect a limited sensitivity of our colorimetric reference method for analysis of total polyphenolics, as suggested by Schulz et al. (1999).

Individual polyphenolic compounds such as coumarins fluoresce under UV light, and are predicted to be indicators of more palatable sagebrush for wildlife (Rosentreter, 2005). Isolating individual polyphenolic compounds, or classes of polyphenolics with known fluorescence, in addition to increasing the sample size, may improve NIRS prediction accuracy of polyphenolics, particularly those related to diet selection, and should be evaluated in future research. NIRS could be used to classify subspecies or hybridized morphotypes of sagebrush that vary in their fluorescence (McArthur et al., 1988). Furthermore, NIRS can be used to holistically evaluate palatability for wildlife (Moore et al., 2010), therefore reducing the need for relatively more costly lab analyses.

Predicted values of CP and DMD seem to be independent of site (Fig. 2A and B), whereas NIRS-derived equations for cineole and polyphenolics seem to be heavily affected by site (Fig. 2C and D) and may not be able to distinguish site-level differences in some PSMs. This site-dependence is clearest in the cineole predictions where Camas winter samples are mostly overestimated, whereas predictions for Leadore winter samples are largely underestimated (Fig. 2C). Additionally, higher PSM concentrations were underestimated with NIRS. The NIRS model could not distinguish between samples with lab values of 6000–15 000 μg g^{-1} DM cineole, and predicted those samples to be between 6000–7000 μg g^{-1} DM. Further, where total polyphenolics were high at Leadore in winter, samples with 145–245 μM g^{-1} DM total polyphenolics were not discriminated from each other with NIRS. Removing the largest outlier (>200 μM g^{-1} DM) improved the slope from 1.37 to 1.04, but the validation r^2 remained 0.64. Researchers and managers should be careful about predicting samples with extreme values (i.e., very high PSM concentrations) without sufficient reference samples to effectively train the calibration equations. Increasing the sample size of the calibration set may improve prediction accuracy by more completely representing all sources of variation (ISO, 2010).

4. Conclusions

This study demonstrates that NIRS can predict dietary constituents, specifically crude protein, of sagebrush important to many wild and domestic herbivores. The application of NIRS to predict

nutrients and chemicals in sagebrush or other plants requires an initial investment in instrumentation (cost range $45 000–100 000) and initial quantification of actual PSMs of interest from typically ≥ 120 samples in the laboratory (~$6–10 sample^{-1} for CP and $2–3 sample^{-1} for each PSM). However, after the predictive equations have been established and validated, analysis of vegetation by NIRS offers a faster and cheaper measure of diet quality compared to laboratory analysis. NIRS instruments are becoming increasingly available in the agricultural industry and therefore offer researchers greater opportunity to collaborate with industry to apply NIRS to other systems. Moreover, portable NIRS or other validated techniques for field spectral analysis may allow researchers to scale up analysis of diet quality to habitats and landscapes (Curran, 1989; Goetz, 2009; Kokaly et al., 2009). Much like geographic information systems have been used to locate and map areas of the sagebrush landscape that deserve the highest priority for conservation (Meinke et al., 2009), the development of NIRS to quantify nutrients and PSMs has the potential to locate, map, and allow managers to prioritize areas with sagebrush of higher food quality or select seeds from these plants for propagation following disturbance such as fire. NIRS provides great potential to map important dietary variables across the landscape, calibrated for the variable of interest (Foley et al., 1998; Ebbers et al., 2002), for a more complete picture of dietary factors driving landscape use by wild and domestic species.

NIRS can also be used to identify thresholds of nutrients and chemicals that influence habitat use by herbivores. These thresholds can then be used to identify the most palatable species or patches of sagebrush that might deserve additional protection with respect to road development, fire suppression, location of water troughs, energy development and other disturbances. Results offer an encouraging first step that supports the feasibility of laboratory NIRS analysis and potential field spectral analysis of the dietary quality of sagebrush across species, sites, and seasons. However, more sampling is required to predict more complex PSMs such as cineole and total polyphenolics, particularly of plants with extreme values. Further development of NIRS may provide land managers with a powerful tool for assessing and conserving critical habitats containing sagebrush that is the highest quality food for wild and domestic species.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jaridenv.2016.07.003.

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