RESEARCH ARTICLE

Using leaf functional traits to remotely detect *Cytisus scoparius* (Linnaeus) Link in endangered savannahs

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Abstract

Identification of invasive plant species must be accurate and timely for management practices to be successful. Currently, *Cytisus scoparius* (Scotch broom) is expanding unmonitored across North America's west coast, threatening established ecological processes and altering biodiversity. Remote detection of leaf functional traits presents opportunities to better understand the distribution of *C. scoparius*. This paper demonstrates the capacity for remotely sensed leaf functional traits to differentiate *C. scoparius* from other common plant species found in mixed grassland-woodland ecosystems at the leaf- and canopy-levels. Retrieval of leaf nitrogen percent, specifically, was found to be significantly higher in *C. scoparius* than each of the other 22 species sampled. These findings suggest that it may be possible to accurately detect introduced *C. scoparius* individuals using information collected from leaf and imaging spectroscopy at fine spatial resolutions.

Introduction

The introduction of invasive plant species to an ecosystem can drastically alter diversity and threaten ecosystem processes, such as soil water dynamics and nutrient availability (Shaben and Myers 2010; Albert et al. 2012; Slesak et al. 2016; Carter et al. 2018). In the past 200 years, humans have expanded across the planet and enhanced the

capacity of plant species to migrate (Zerega et al. 2004; Olivares et al. 2019). Some species have transited oceans for agricultural production, while others simply provide ornamental value (Hawkes and Francisco-Ortega 1993; Bossard and Rejmanek 1994). *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; Richardson et al. 2000; Odom et al. 2003; 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 nitrogen-rich leaves of nitrogen-fixing plant species, such as *C. scoparius*, are relatively more productive than 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, Garry oak (*Quercus garryana* Douglas ex Hook) savannahs provide habitat for several endangered plant and animal species (Bjorkman and Vellend 2010). In Canada, the footprint of this ecosystem has been reduced by urban and agricultural expansion to less than 5% of its original area (MacDougall et al. 2004). 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 (Fogarty and Facelli 1999; Carter et al. 2018). In mixed grassland-woodland 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 (Odom et al. 2003; Hill et al. 2016). 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 mixed grassland-woodland 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 mixed grassland-woodland ecosystems and has yielded success in a variety of other ecosystems (Asner et al. 2008; Niphadkar and Nagendra 2016; Große-Stoltenberg et al. 2018). Essentially, spectral information is acquired across several narrow bands and modelled with a measured plant functional trait, such as leaf nitrogen percent (%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 remotely piloted aircraft systems (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 mixed grassland-woodland plant species should be demonstrated at the leaf- and canopy-level. The aim of this study is to identify leaf functional traits of *C. scoparius* that are significantly different from other grassland-woodland species at the leaf- and canopy-levels through four hypotheses:

1. 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').
2. Significant differences of predicted leaf-level functional trait values remain between *C. scoparius* and Site species.
3. Significant differences of predicted canopy-level functional trait values remain between *C. scoparius* and Site species.
4. Alterations in illumination conditions do not impact the significance of predicted canopy-level trait differences.

Methods and materials

Study site

Leaf material for 23 plant species was collected in and around a mixed grassland – woodland savannah within the Cowichan Garry Oak Preserve (CGOP; 48°48'29.85"N, 123°37'54.34"W) between May 4–19, 2019 (Fig. 1). Located near Duncan, British Columbia, Canada, this site harbours more than 61 plant species and a variety of other wildlife, including the red listed Western Bluebird (*Sialia mexicana*; IUCN Least Concern). The 23-plant species were selected based on a variety of criteria, including widespread abundance, known North American range and interest to local manag-
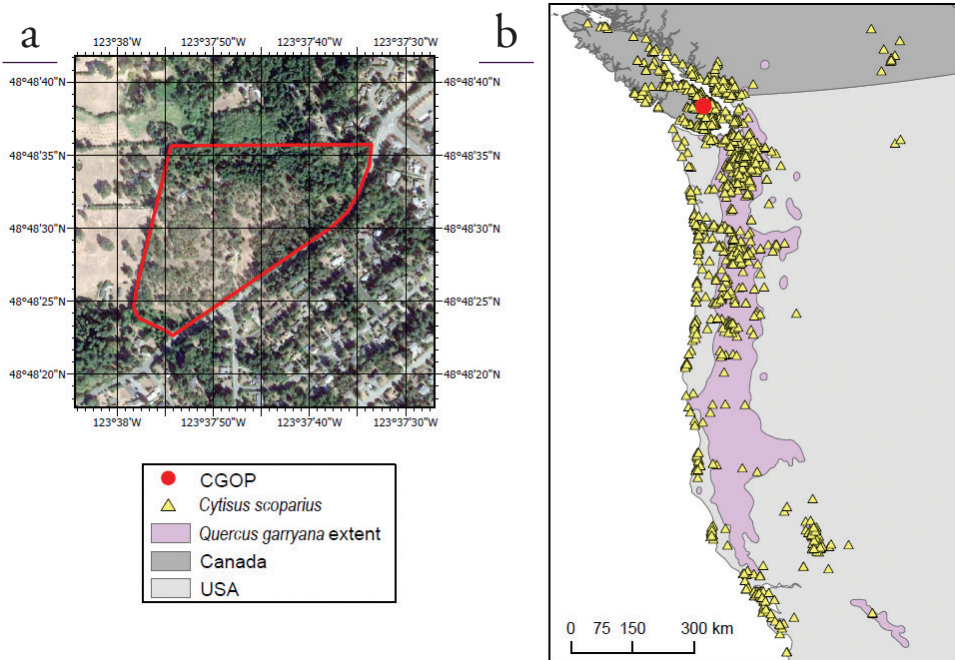
Figure 1. True color composite Imagery of **a** the Cowichan Garry Oak Preserve (CGOP) and **b** the extent of *Quercus garryana* (Little 1971) and locations of *Cytisus scoparius* (https://doi.org/10.15468/dl.dfdv48) individuals along North America's west coast.

ers. These mixed grassland-woodland ecosystems, often called Garry oak savannahs, are considered endangered in Canada as the percentage of near-natural habitat is less than 5% of its original footprint (MacDougall et al. 2004; Bjorkman and Vellend 2010). 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).

Target species

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 (Parker 1997; Carter et al. 2018). Growing quickly and reaching heights exceeding three meters, this invasive shrub faces few barriers upon introduction (Parker 1997).

Leaf trait evaluation

Chemical and spectral measurements

A total of 14 traits were measured across 23 unique plant species and four plant life forms. All leaf samples taken are considered to be from sunlit positions. Chemical evaluation of chlorophyll *a* + *b* (Chl*ab*) and carotenoids (Car), as well as leaf dry matter content (LDMC), equivalent water thickness (EWT) and %N were conducted following standards presented by the Canadian Airborne Biodiversity Observatory (CABO) (Laliberté 2018; Ayotte et al. 2019; Girard et al. 2019). 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 Spectra Vista Corporation (SVC) DC-R/T integrating sphere to measure 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 2018a, 2018b). 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 samples 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 partial least squares regression (PLSR), a statistical method well-suited for modelling datasets with high dimensionality, such as those created from spectroscopy. The data was split into training (70%) and test (30%) sets. This methodology models the relationship between spectral reflectance values recorded by leaf spectroscopy and measured leaf chemistry to enable the accurate prediction of leaf functional traits (Haaland and Thomas 1988). PLSR modelling has successfully predicted leaf traits in tropical forests and temperate grasslands from spectroscopy data, highlighting its cross-biome utility and capacity to evaluate large, highly-correlated datasets (Curran 1989; Asner and Martin 2009; Feilhauer et al. 2017). 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 (RTM) 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. We 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, PROSA-IL simulations were conducted at a variety of solar zenith angles spanning 20 – 70° 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 to conduct partial least squares regression and 'hsdar' (Lehnert 2020) enabled the use of PROSAIL.

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 1, Fig. 2). 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 1). 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 (Fig. 2). Leaf-level %N was accurately predicted using PLSR (R^2 = 0.70, NRMESP = 17%) (Table 2, Fig. 3). 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).

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) (Fig. 4). 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 to that of the 22 Site species (W = 1653, p-value = 1.003e-04) (Fig. 5). Alterations in viewing geometry did not compromise the significant differences found between canopy predicted relative %N of *C. scoparius*

Table 1. 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
Camassia leichtlinii (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	*Quercus garryana* 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
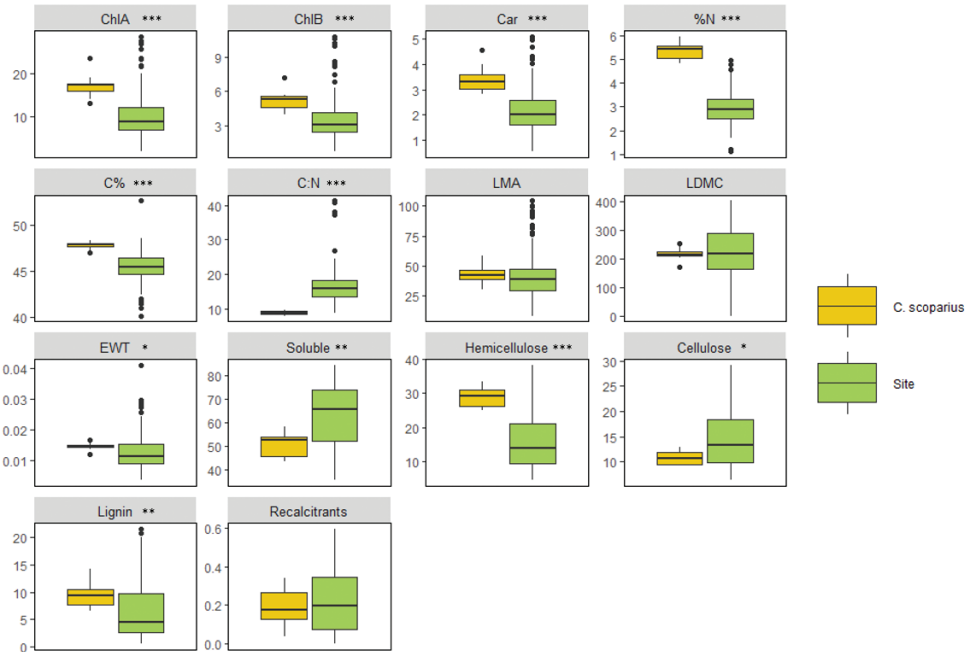
Figure 2. Comparisons of 14 functional traits between *C. scoparius* and Site species. Boxplots depicting the differences between *C. scoparius* (yellow) and 22 other "Site" plants (green) for 14 leaf functional traits using a Mann-Whitney test, 11/14 of which are significantly different. The level of significance is denoted in the banner of each facet (* <= 0.05, ** <= 0.01, *** <= 0.001).

Table 2. 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), normalized-RMSEP (NRMSEP) and the number of components, or latent variables).

| Trait	R^2	RMSEP (NRMSEP)	Components
Chlorophyll *a* (mg/g)*	.54	3.25 (31%)	7
Chlorophyll *b* (mg/g)*	.56	1.16 (33%)	8
Carotenoids (mg/g)	.36	0.68 (31%)	4
Nitrogen (%)*	.70	0.5(17%)	4
Carbon (%)	.48	0.99 (2%)	6
C:N*	.71	2.98 (18%)	4
Leaf mass per area (g/m^2)*	.67	10.34 (25%)	6
Leaf dry matter content (mg/g)*	.69	48.64 (22%)	7
EWT*	.85	0.002 (16%)	4
Solubles (%)	.41	9.78 (16%)	4
Hemicellulose	.36	6.43 (40%)	4
Cellulose*	.59	3.96 (27%)	4
Lignin	.46	3.64 (55%)	4
Recalcitrants	.28	0.12 (56%)	4

*accepted trait
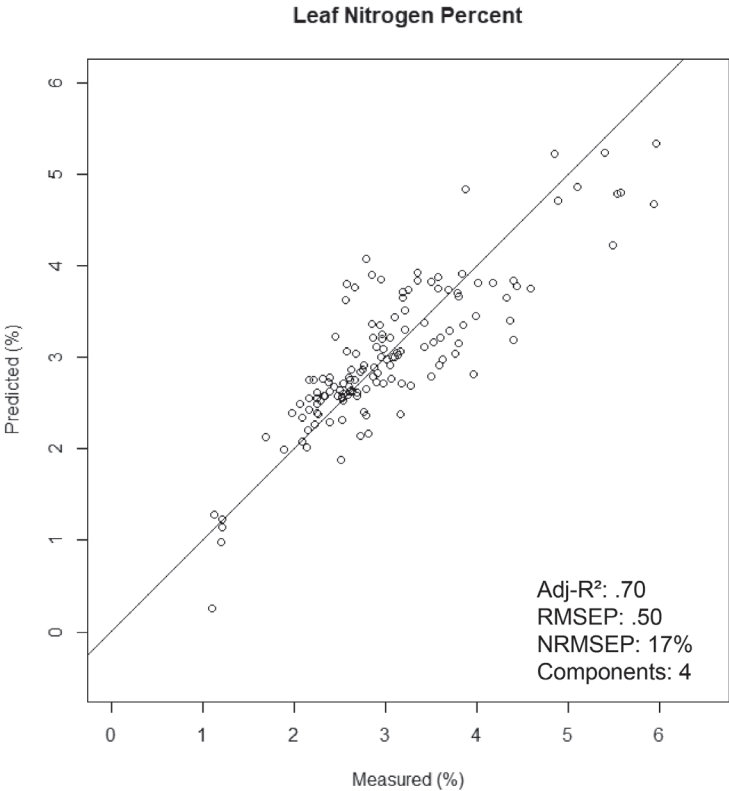
Figure 3. PLSR prediction plot. Comparison of the measured and predicted leaf nitrogen percent (%N) for 23 plant species at the Cowichan Garry Oak Preserve.
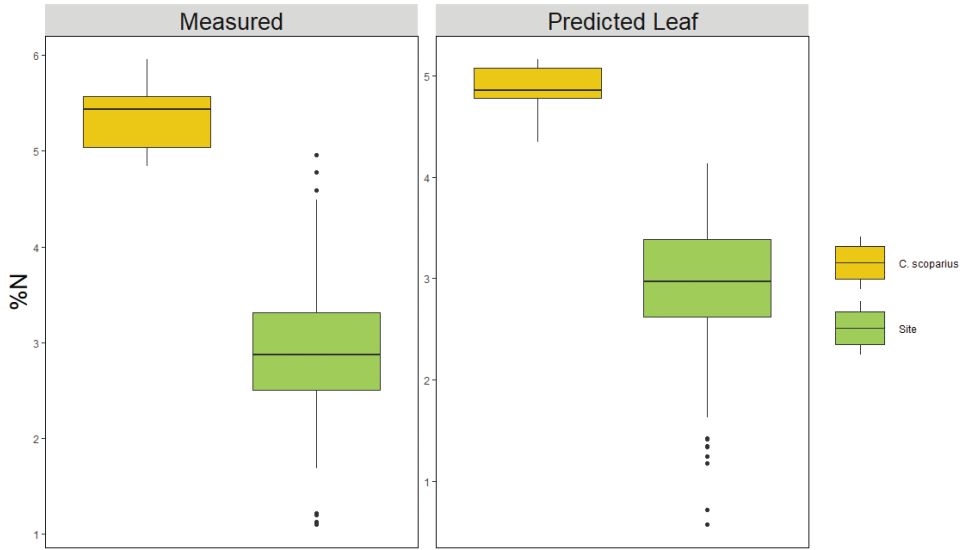
Figure 4. Measured and predicted leaf %N. Comparison of measured and predicted leaf N% of *C. scoparius* (yellow) and 22 Site species of various lifeforms (Site; green) sampled at CGOP.
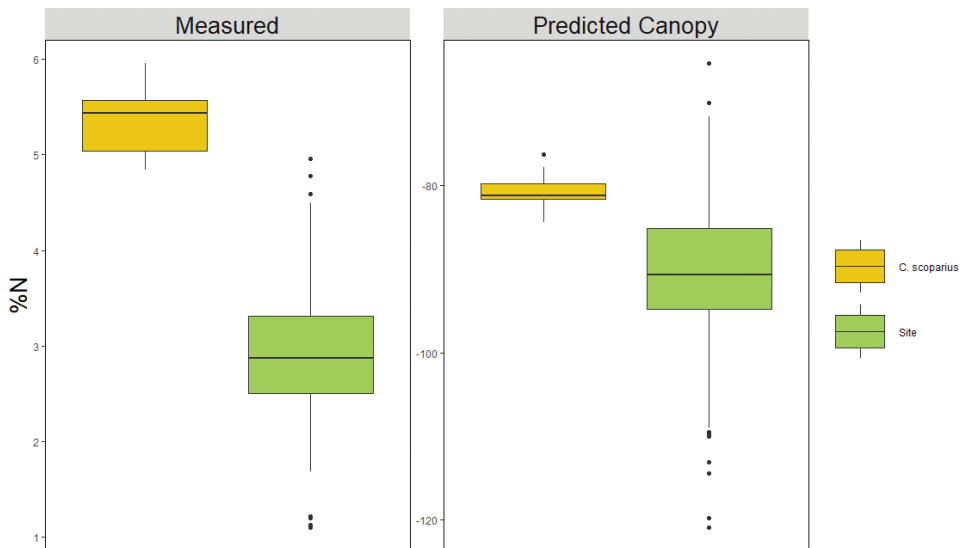

Figure 5. Measured leaf %N and predicted canopy N%. Comparison of the measured leaf-level and predicted canopy-level %N for *C. scoparius* (yellow) and 22 other plant species (Site; green) sampled at the CGOP in May 2019. Note that the y-axis scale varies, with the relative %N values predicted by PROSAIL 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.
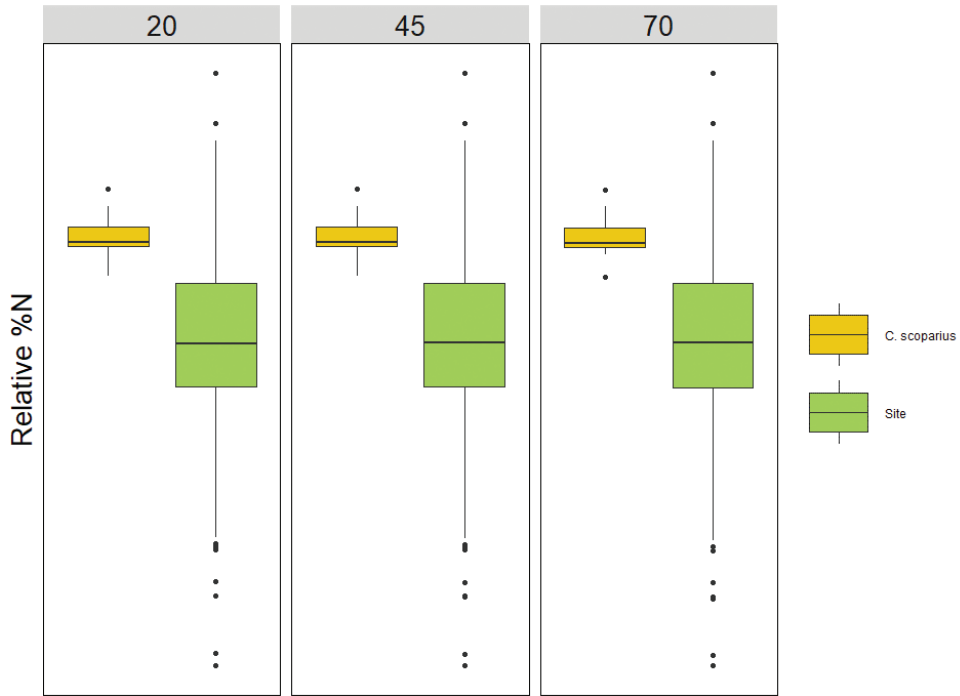
Figure 6. Predicted relative %N compared between *C. scoparius* and Site species using various solar zeniths. Boxplots demonstrating the difference between the PROSAIL predicted relative %N for *C. scoparius* (yellow) and Site species (green) using different solar zeniths (20 degrees, 45 degrees and 70 degrees).

and Site species (20°: W = 1653, p-value = 0.0001; 45°: W = 1653, p-value = .0001; 70°: W = 1652, p-value = .0001026) (Fig. 6).

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 is destabilizing the traditional species composition of plant communities, especially in mixed grassland-woodland 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 (Odom et al. 2003; Hill et al. 2016).

This paper demonstrated that *C. scoparius* is distinguishable from other common grassland-woodland 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 unsur-

prising as *C. scoparius* is a nitrogen-fixing legume and is likely to have leaves that are relatively nitrogen-rich (McKey 1994; Große-Stoltenberg et al. 2018). 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 (Shaben and Myers 2010; Große-Stoltenberg et al. 2018). 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. 2008; 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 fitted. 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 m^2), such as mixed woodland-grasslands. 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.

Conclusion

The significant differences in measured and predicted leaf %N between *C. scoparius* and 22 other plant species common in Canadian mixed woodland-grassland savannahs 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 dem-

onstrated 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 identify 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 (Sankey et al. 2018; Arroyo-Mora et al. 2019). 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 a mixed grassland-woodland 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.

Acknowledgements

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