

Université de Montréal

Foliar spectra accurately distinguish the invasive common reed from co-occurring plant species throughout a growing season

Les spectres foliaires permettent de distinguer avec précision le roseau commun envahissant des espèces végétales co-occurentes tout au long d'une saison de croissance

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Ce mémoire intitulé

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espèces végétales co-occurrentes tout au long d'une saison de croissance*

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Résumé

Les espèces végétales envahissantes sont l'un des principaux facteurs de changement de la biodiversité dans les écosystèmes terrestres. Une détection précise et précoce des espèces exotiques est donc cruciale pour surveiller les invasions en cours et pour prévenir leur propagation. Présentement, les méthodes de surveillance des invasions biologiques permettent de suivre la propagation des envahisseurs à travers les aires de répartition géographique, mais une attention moindre a été accordée à la surveillance des espèces envahissantes à travers le temps. Les plateformes de télédétection, capables de fournir des informations détaillées sur les variations des traits foliaires dans le temps et l'espace, sont particulièrement bien placées pour surveiller les plantes envahissantes en temps réel. Les changements temporels des traits fonctionnels sont exprimés dans la signature spectrale des espèces par des caractéristiques d'absorption spécifiques de la lumière associés aux pigments photosynthétiques et aux constituants chimiques tous deux liés à la phénologie. Ainsi, les variations temporelles dans la réponse spectrale des plantes peuvent être utilisées afin de mieux identifier des espèces individuelles.

L'un des envahisseurs les plus problématiques au Canada est le roseau commun, *Phragmites australis* (Cav.) Trin. ex Steudel sous-espèce *australis*, dont la propagation menace la biodiversité des écosystèmes de zones humides en Amérique du Nord. Déterminer la période de l'année où cet envahisseur se distingue d'avantage, du point de vue spectral et fonctionnel, des autres plantes de la communauté serait centrale dans une meilleure gestion du roseau commun. Pour ce faire, nous avons utilisé des traits fonctionnels et une série temporelle de données spectrales foliaires à haute résolution au cours d'une saison de croissance à Boucherville, Québec, Canada, afin de déterminer la séparabilité spectrale de l'envahisseur par rapport aux espèces co-occurrentes et comment cette dernière varie à travers le temps. Nos résultats ont révélé que la spectroscopie foliaire a permis de distinguer le phragmite des espèces co-occurrentes avec une précision de plus de 95% tout au long de la saison de croissance – un résultat prometteur pour le futur de la télédétection des espèces végétales envahissantes.

Mots clés : spectroscopie foliaire, espèces végétales envahissantes, *Phragmites*, phénologie, zones humides.

Abstract

Invasive plant species are one of the main drivers of biodiversity change in terrestrial ecosystems. Accurate detection of exotic species is critical to monitor on-going invasions and early detection of incipient invasions is necessary to prevent further spread. At present, surveillance methods of biological invasions allow to track the spread of invaders across geographic ranges, but less attention has been given to invasive species monitoring across time. Remote sensing platforms, capable of providing detailed information on foliar trait variations across time and space, are uniquely positioned for monitoring invasive plants in real time. Temporal changes in foliar traits are expressed in a species spectral profile through specific absorption features related to variation in photosynthetic pigments and chemical constituents driven by phenology. Thus, variations in a plant's spectral response can be used to improve the identification of individual species.

One of Canada's most problematic invaders is the common reed, *Phragmites australis* (Cav.) Trin. ex Steudel subspecies *australis*, whose spread threatens biodiversity in wetland ecosystems in North America. Determining the time of year when the invader is spectrally and functionally more distinct from other plants in the community would be central to better management of common reed. To do so, we collected a time-series of foliar traits and high-resolution leaf spectral data over the course of a growing season at Boucherville, Quebec, Canada, to determine the spectral separability of the invader from co-occurring species and how its detection varies over time. Our results revealed that leaf-level spectroscopy distinguished *Phragmites* and co-occurring species with > 95% accuracy throughout the growing season – a promising result for the future remote detection of invasive plant species.

Keywords: leaf spectroscopy, invasive plant species, *Phragmites*, phenology, wetlands

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List of acronyms and abbreviations

VIS: visible

NIR: near infrared

SWIR: short wave infrared

SLA: specific leaf area

LMA: leaf mass per area

EWT: equivalent water thickness

PLSR: partial least square regression

PLSDA: partial least square discriminant analysis

RMSEP: root mean square error of prediction

RTM: radiative transfer model

UAV: unmanned aerial vehicle

Acknowledgements

“Today, however, we have to realize that a true ecological approach always becomes a social approach; it must integrate questions of justice in debates on the environment, so as to hear both the cry of the earth and the cry of the poor.”

Pope Francis, *Encyclical Letter Laudato Si: On care for our common home*

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Chapter 1

Introduction

Invasive plant species are one of the major drivers of biodiversity change in terrestrial ecosystems (Sala, 2000). Invasive species are those introduced to a novel environment with negative ecological, economic or social impacts (SCBD, 2001). Introduced species that do not spread substantially become naturalized but not invasive (Richardson & Pyšek, 2012). Therefore, invasive species are categorized as such in relation to their ability to sustain self-replacing populations over several life cycles, produce reproductive offspring, and their potential to spread considerably over long distances (Richardson & Pyšek, 2012). Biological invasions affect environmental systems at multiple scales ranging from evolutionary changes to organisms to altering whole ecosystems structure and function, and generate substantial costs to society (Ehrenfeld, 2010; Pimentel et al., 2005).

Accurate and early detection of invasive species is critical to implement effective control actions that prevent the spread or facilitate the eradication of invaders (Larson et al., 2020; Rupasinghe & Chow-Fraser, 2021). Field surveys and distribution maps are useful to identify existing biological invasions and monitor the distribution of invaders. However, to monitor the progression of invasions and predict where they are likely to occur in order to prevent them, comprehensive spatial and temporal information on plant functional traits is needed (Asner et al., 2015). At present, remote sensing platforms are uniquely capable of providing detailed spatial and temporal information on functional trait variations to monitor plant invasions in real-time and understand how plant communities and ecosystems are changing through time.

Functional leaf traits influence how light is absorbed, reflected or transmitted in different regions of the electromagnetic spectrum, affecting optical properties of leaves that can be detected using spectroscopy (Asner et al., 2014a). Thus, the interaction of foliage with solar radiation can be quantified from foliar spectra as patterns of leaf reflectance and transmittance express information that can be related to photosynthetic pigment concentrations, leaf anatomy, morphology and chemistry (Asner et al., 2014a; Cavender-Bares et al., 2017). As such, leaf reflectance spectra are a valuable tool for species discrimination and are increasingly being used

for invasive species detection (Bradley, 2014). Regardless of all plants being spectrally similar given that they are composed of the same spectrally active components, more functionally different and more distantly related species are more spectrally dissimilar (Price, 1994; Schweiger et al., 2018).

There are three main regions in the optical spectrum: the visible range (VIS; 400-700 nm), the near-infrared (NIR; 700-1100 nm) and the short-wave infrared (SWIR; 1100 - 2400) (Fig. 1.1). In the visible region, pigments absorb most of the incident light in the red and blue wavelengths, while reflecting most of the green (Ustin et al., 2009). Pigment concentrations affect the amount of light absorbed and structural changes related to the spatial distribution and packaging of chloroplasts affect the probability of light absorption within leaf tissues (Jacquemoud & Ustin, 2019). Absorption of non-pigment constituents such as water, nitrogen (N), cellulose and lignin have measurable absorption features in the NIR and SWIR spectral regions in wavelengths found beyond 700 nm (Kokaly et al., 2009). Absorption features of water are found throughout the electromagnetic spectrum, near 980, 1190, 1450, 1490 and 1920 nm (Kokaly et al., 2009; Yang et al., 2016) and those for nitrogen, cellulose and lignin are found near 1700, 2100 and 2300 nm (Curran, 1989; Kokaly et al., 2009). Nitrogen can be quantified from spectral measurements despite uncertainty on whether its detection is based on effects of N-containing compounds like proteins and chlorophylls (Jacquemoud et al., 1996), or the indirect effect of related leaf traits that influence overall patterns of scattering and reflectance such as leaf mass per area (LMA) and equivalent water content (EWT) (Curran, 1989; Jacquemoud et al., 1996; Kokaly et al., 2009). Cellulose and lignin, two important constituents of plant cell walls, contain common chemical bonds (C-H, N-H, C-O, O-H, etc.) in varying proportions that induce broad and overlapping absorption features in their spectra (Jacquemoud et al., 1996; Kokaly et al., 2009).

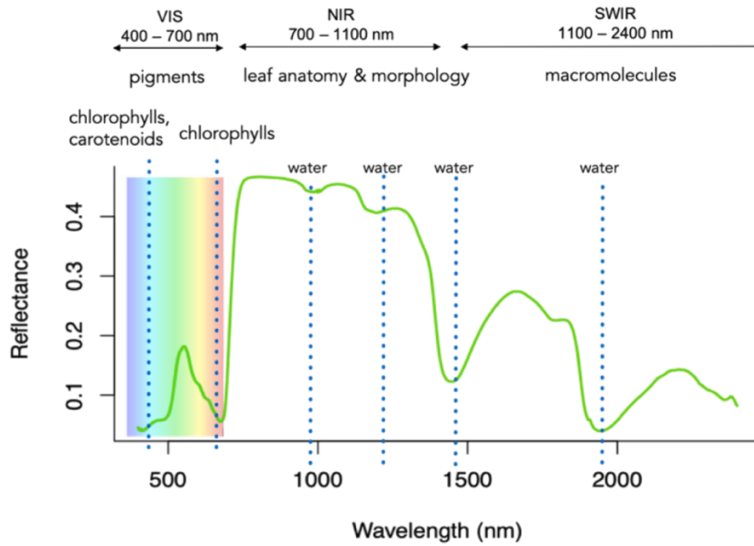


Figure 1.1. Electromagnetic reflectance spectrum and major absorption features (based on Cavender-Bares et al. (2017))

Varying concentrations of pigments and non-pigment constituents in plant leaves, changes the strength of the related absorption features directly through their individual absorption properties or indirectly through their relationship with leaf structural and biochemical attributes (Kokaly et al., 2009; Ollinger, 2011). For example, in the NIR region, plants are highly reflective because the effect of absorbers is weak or absent (Ollinger, 2011). Nevertheless, reflectance in this region is correlated with nitrogen and water content but is mostly affected by structural and anatomical parameters like mesophyll structure and leaf thickness that affect light scattering and the length of the path that light can travel inside a leaf (Jacquemoud & Ustin, 2019; Ollinger et al., 2008). On the other hand, reflectance in the SWIR region is determined mostly by the absorption of water and macromolecules like protein, cellulose and lignin as a result of vibrations and stretches of their chemical bonds (Curran, 1989; Kokaly et al., 2009). The relationship between spectral features and foliar traits varies considerably across the spectrum and the determination of leaf biochemical and physical properties from leaf spectra requires the use of particular bands and wavelength ranges (and corresponding absorption features) as different components of the spectrum are sensitive to different chemical constituents or leaf traits (Curran, 1989; Fourty et al., 1996; Kokaly et al., 2009). Important wavelengths for determination of traits do not always correspond to specific absorption features but instead could correlate with absorption features of other traits due to associations

among different traits or overlap in absorption features (Kokaly et al., 2009; Wang et al., 2020) (Table 1.1).

Detecting invasive species using remote sensing can be most successful when differences are maximized between the invader and the background community (Brym et al., 2011). This can happen for instance when the invader is the dominant growth form in a landscape and could be easily observed in aerial imagery (Andrew & Ustin, 2008); has a unique chemistry or biophysiology and could be distinguished using high spectral resolution (Asner & Vitousek, 2005) or has a unique phenology and could be better distinguished from native plants better during a specific time of the year (Bolch et al., 2020; Boyte et al., 2015). Spectrally detectable variations in chemistry, function and structure between invasive and native species present an opportunity to detect non-natives given that they often possess traits that are novel to the colonized environment and therefore in certain opportunities become more easily distinguished from non-invasive species (Bradley, 2014; Brym et al., 2011). Consistent dissimilarity in functional traits of invasive species relative to the native communities they colonize suggests that invasive species take advantage of an empty ecological niche in the novel environment (Emery, 2007).

Among mechanisms that promote invasiveness, extended leaf phenology (i.e. early leaf emergence or delayed senescence) has been proposed as a key feature driving events of invasion in northern-latitude, deciduous ecosystems (Fridley, 2012; Rejmánek, 2013; Smith, 2013). Phenological differences or mismatches between natives and invasive species are useful to discriminate invaders but also has been suggested that they can influence the invasion success of exotic species in native communities (Wolkovich & Cleland, 2011). As important traits of foliage vary throughout the course of a growing season, phenology (i.e. the timing of periodic events such as flowering and leaf emergence/senescence) should be considered as important as physiological or chemical traits in invaded systems as a driver of ecosystem change or as a driver of invasion itself (Ehrenfeld, 2010; Fridley, 2012; Liao et al., 2008; Willis et al., 2010).

Table 1.1. Spectral regions and bands of importance for determination of leaf biochemical and physical properties from spectra (from Wang et al., (2020)).

	Wavelength Range (nm)	Important bands (and absorption features) for spectroscopic determination (nm)
N (%)	400 - 2400	460 (chl b), 530, 900, 1270, 1730 (cellulose, protein), 1980 (protein), 2060 (protein), 2170, 2240 (protein), 2300 (protein)
C (%)	1200 - 2400	775 (red-edge) 1130, 1200 (water, cellulose, starch, lignin), 1210, 1530 (starch), 1540 (starch, cellulose), 1730 (cellulose, protein)
Chlorophyll ab (mg g ⁻¹)	400 - 760	690 (red edge), 740 (red edge), 1660 (phenolics), 1710, 1730 (cellulose, protein)
Carotenoids (mg g ⁻¹)	400 - 760	720 (red edge), 1130, 1200 (water, cellulose, starch, lignin), 1315, 2260 (lignin), 2300 (protein)
Chlorophyll ab (mg m ⁻²)	400 - 760	740 (red edge), 1130, 1200 (water, cellulose, starch, lignin), 1510 (protein, nitrogen), 1980 (protein), 2280 (starch, cellulose)
Carotenoids (mg m ⁻²)	400 - 760	720 (red edge), 1130, 1200 (water, cellulose, starch, lignin), 1270
LMA (g m ²)	800 - 2400	940, 1150, 1180, 1190, 1200 (water, cellulose, starch, lignin), 1210, 1338, 1730 (cellulose, protein), 2100 (starch, cellulose), 2260, 2300 (protein, nitrogen)
EWT (cm)	800 - 2400	740 (red edge), 990 (starch), 1110, 1190 (water), 1200 (water, cellulose, starch, lignin), 1270
Cellulose (%)	1200 - 2400	1130, 1210, 1730 (cellulose, protein), 1980 (protein), 2040, 2060 (protein), 2240 (protein), 2250 (starch), 2260 (lignin), 2300 (protein)
Lignin (%)	1200 - 2400	720, 250, 770, 1270, 1670, 1980 (protein), 2210
Water	800 - 2400	980, 1130, 1140, 1160, 1330, 1510 (protein, nitrogen), 1520, 1530 (starch), 1540 (starch, cellulose)

Early leaf emergence or delayed senescence response is mediated by functional traits linked to phenology and resource-acquisition strategies (Papeš et al., 2013). Photosynthetic pigment concentrations, water content, specific leaf area (SLA) and nutrient concentrations are among a set of important traits that accompany morphological and chemical changes that occur as leaves transition from emergence to maturation and senescence during a growing season (Chavana-Bryant et al., 2017; Yang et al., 2016). Trait variations that are tied to phenology are expressed more strongly in spectral features in the VIS and NIR regions of the spectrum that have been shown to change in amplitude and shape throughout a growing season (Yang et al., 2016). In particular, the green peak, red-edge, NIR and water absorption features are highly age-sensitive spectral domains (Chavana-Bryant et al., 2017; Papeš et al., 2013).

This mechanism may constitute an advantage for invasive species by enhancement of resource competition via nutrient pre-emption and extended carbon assimilation (Liao et al., 2008; Wang et al., 2013). Alternatively, it has been proposed that invasive species tend to be more responsive than natives to warming trends that have lengthened the duration of spring or autumn growth (Willis et al., 2010), suggesting that rather than an advantageous intrinsic competition

ability, invader performance is higher in avoiding environmental filtering (Asner et al., 2006; Fagúndez & Lema, 2019). In either case, prolonged greenness in plants is underpinned by a range of biochemical and physiological characteristics that control processes like photosynthesis and nutrient and water cycling that determine how an ecosystem functions (Keddy, 1992; McGill et al., 2006). Physiological investments in photosynthesis, light harvesting, and water and nutrient acquisition are tied to a plant's structural investments and are therefore resource-constrained at the ecosystem level (Wright et al., 2004). Given that environments with high and low abundance of resources are prone to invasion, albeit the former being more susceptible (Davis et al., 2000; Funk, 2013), management of invasive species and prevention of their spread is critical to prevent deleterious effects on ecosystem functioning and loss of biodiversity on all natural systems.

Developing accurate, temporally and spatially explicit records of invasion is a high priority in the field of invasion biology (Mack et al., 2007). Although traditional field sampling methods have thus far provided detailed information on plant functional traits and species distribution, their implementation requires a significant investment of time and human resources (Drake et al., 2003). In addition, ground-based surveys cannot comprehensively cover large areas and therefore have limited capacity to translate ecological understanding to broad geographical scales (Wang & Gamon, 2019). On the other hand, spectral information of moderate and high spectral resolution can be acquired relatively rapidly and frequently over large ranges, which is of central importance for effective management approaches (Asner et al., 2015; Mack et al., 2007; Rupasinghe & Chow-Fraser, 2021).

As conservation and control efforts regarding biological invasions represent a considerable cost to society, the use of drones, aircraft and satellites that capture spectral differences in plant species at the ecosystem level, allow the monitoring of biological invasions at large geographical and temporal scales in a repeatable manner at a reduced cost and time investments compared to traditional monitoring approaches. This has the potential to transform ecosystem monitoring through time and invasive species monitoring to better direct monetary and management efforts of conservation strategies mitigating the economic and ecological impact of invasive plant species.

***Phragmites australis*: a highly invasive plant species**

The common reed (*Phragmites australis*) is a cosmopolitan species of grass with a wide geographic distribution (Chambers et al., 1999). It is present in every continent except Antarctica and has a complex history of introductions that make it a model species for studying biogeographical aspects of plant invasions (Meyerson et al., 2009; Pyšek et al., 2019). To this date, it is one of the most studied plant species due to perceived threats to ecosystem services upon establishment. Where it is introduced, it quickly becomes problematic as its spread results in loss of habitat, reduction in species richness and biodiversity, and alterations to biogeochemical cycles (Chambers et al., 1999).

Phragmites australis is a species complex that is made up of dozens of distinct genetic lineages, of which at least four are present in North America (Lambertini et al., 2008). Fossil records show that *Phragmites* has been present in tidal and non-tidal ecosystems for thousands of years, and for a long time it was considered to be a minor component of wetland plant communities. However, over the last 200 years, the relative abundance and distribution of *Phragmites* throughout the United States and Canada have increased dramatically (Chambers et al., 1999). Its recent invasive nature has been attributed to a cryptic invasion of a non-native Eurasian lineage introduced sometime in the 1800s, *Phragmites australis subsp. australis* (or haplotype M in the literature (Saltonstall, 2002)). Today, it is widespread in all of mainland United States and Southern Canada and is considered as an indicator of wetland disturbance (Saltonstall, 2002).

The expansion of *Phragmites* has been explained as a result of eutrophication, disturbance (human and non-human) and increased genetic variation (Chambers et al., 1999; Guo et al., 2013). The introduced lineage is an aggressive competitor that outperforms native lineages of *Phragmites* in mixed populations (Mozdzer et al., 2013) and rapidly excludes co-occurring plant species upon invading a marsh, transforming diverse wetlands into low-diversity common reed stands (Chambers et al., 1999; Meyerson et al., 2000). As a result, invaded ecosystems experience reduction in biodiversity and in habitat for certain types of wetland fauna (Chambers et al., 1999). Some characteristics of *Phragmites*' ecology that make it a successful invader are its dense and rapid growth, high biomass production and retention (alive and dead), and the use of vegetative and sexual reproduction (Albert et al., 2015; Meyerson et al., 2000; Pyšek et al., 2019).

Phragmites uses seeds for establishing populations over large distances and vegetative reproduction to expand established stands and populations locally (Albert et al., 2015). Seed germination occurs in wet (non-flooded) bare soils. Germination and subsequent seedling establishment require mean daily temperatures above 10°C for a period of at least 120 days (Tougas-Tellier et al., 2015). Low water-level conditions during summer or areas with anthropogenic disturbance favor the establishment of new colonies (Hudon et al., 2005). These conditions will likely be amplified by the effect of climate change on altering flooding regimes in wetlands, especially in the St. Lawrence-Great Lakes hydrographic system as high evaporation levels during winter due to a lack of extended ice cover (Croley II et al., 2003) could cause a reduction in the water supply from the Great Lakes into the St. Lawrence River which will increase the extent of suitable germination grounds for *Phragmites* (Tougas-Tellier et al., 2015). Germination and settlement of other plant species within established *Phragmites* stands is inhibited on account of high accumulation of above and below ground biomass that reduces available light penetration, limits access to resources and prevents growth from other species (Meyerson et al., 2000).

The nutrient-use and allocation strategies of *Phragmites* are a result of the set of functional traits it displays, and the functional traits of an exotic species in the context of a native community determine its potential to become a successful invader (Asner et al., 2006; Brym et al., 2011). With regard to *Phragmites*, previous studies have shown that it outperforms co-occurring species on account of having superior performance-related traits (such as specific leaf area (SLA), chlorophyll content and N content) (Meyerson et al., 2000; Mozdzer & Zieman, 2010). In addition, an extended growing season, grants a competitive advantage of enhanced resource acquisition to *Phragmites* over native species (Farnsworth & Meyerson, 2003; Mozdzer & Zieman, 2010; Park & Blossey, 2008; Saltonstall & Stevenson, 2007). The mechanism of extended leaf phenology is common among invaders and is an important driver of ecosystem change, as the timing of periodic events (such as budburst or senescence) impacts how species interact with one another and their environment, and therefore play a major role in structuring plant communities (Smith, 2013).

Numerous invasive species colonizing northern latitude ecosystems tend to leaf out earlier or retain leaves longer relative to native plants (Fridley, 2012; McEwan et al., 2009; O'Connell & Savage, 2020). Extended leaf phenology is thought to grant invasive species an advantage over

natives by enhancing access to light and resources and increasing carbon gains over a growing season (Liao et al., 2008). Where the invasive *Phragmites* exists, shoot emergence occurs early over a growing season and its leaves remain green and persist longer relative to other species on account of delayed senescence in the fall and therefore enjoys longer growing seasons than co-occurring species (Farnsworth & Meyerson, 2003; Mozdzer & Zieman, 2010; Park & Blossey, 2008; Saltonstall & Stevenson, 2007). Owing to this unique strategy, variations in traits affected by phenology could make it functionally more distinct from the native community at the beginning or the end of the growing season due to prompt leaf emergence and maturation or a late start of senescing.

Absorption features related to pigment concentrations and other chemical constituents that describe foliar traits and their variation over time can be expressed in light reflectance and transmittance patterns across hundreds of contiguous spectral bands within the visible, near infrared and shortwave infrared regions of the electromagnetic spectrum (Asner et al., 2014a; Yang et al., 2016). Thus, spectral signatures of vegetation beyond providing a unique make-up of a plant's condition, can also be used to differentiate individual species (Asner et al., 2014b). At present, emerging approaches for surveying and monitoring the spread of invasive use remote sensing techniques that rely on spectral differences between native and invasive species for their accurate detection (Bradley, 2014), and a number of studies have found critical time windows that capture the maximum differentiation between the invaders and the background community due to differing phenology (Boyte et al., 2015; Bradley & Mustard, 2005) and climatic conditions (Asner et al., 2006), providing key opportunities to detect them.

Plant species detection using spectroscopy

With remotely sensed data, there are trade-offs between spatial extent (size of the image), spatial resolution (pixel size), spectral resolution (number and range of visible and infra-red bands) and temporal resolution (frequency of data acquisition) that must be considered when choosing a particular approach of remote sensing platform (Bradley, 2014). As there is no sensor that can achieve high spatial, spectral and temporal coverage over a broad spatial extent, the choice of remote sensing approach will always be limited by trade-offs in these domains (Bradley, 2014).

Species-level mapping with imaging spectroscopy often requires high spatial and spectral resolution (Asner et al., 2017). Remote sensing platforms of high spatial and spectral resolution achieve reasonable to high success in species-level identification in wetlands: PROBE-1 80% accuracy to detect *Phragmites* and *Typha* (Lopez et al., 2004); HyMap 85% accuracy in identifying 55 different species (Zhang & Xie, 2013); AVIRIS 95% accuracy to detect leafy spurge (Williams & Hunt, 2004). As well, moderate spectral and high spatial resolution sensors achieve high accuracy at mapping invaders: WorldView > 90% accuracy in *Phragmites* detection (Rupasinghe & Chow-Fraser, 2021). The downside of these platforms is that their relative high cost makes them unsuitable for frequent monitoring and routine mapping which is necessary for invasive species mapping in wetlands due to frequently changing environmental conditions brought about by the dynamic nature of these ecosystems that influence species detection (Andrew & Ustin, 2008; Pengra et al., 2007).

Freely available and frequently acquired satellite imagery with high spectral resolution provide a means to achieve constant monitoring of extensive land coverage to produce accurate distribution maps of invaders (Huang & Asner, 2009; Zhang & Xie, 2013). *Phragmites* has been mapped with accuracy of over 80% using hyperspectral (Pengra et al., 2007) and multispectral data (Rupasinghe & Chow-Fraser, 2021; Tougas-Tellier et al., 2015). Rupasinghe & Chow-Fraser (2019) used a time series of freely available multispectral imagery with moderate spatial resolution and achieved accuracies as high as 89% to map *Phragmites* with images acquired during summer and fall seasons. They identified the best phenological state to differentiate *Phragmites* from co-occurring species to occur during the months of July to September. As a result of prolonged greenness of the leaves, high concentrations of chlorophylls and high water content, *Phragmites*' reflectance signal is most unique during this period of time when compared with co-occurring

vegetation in two wetland ecosystems in southern Ontario, Canada (Rupasinghe & Chow-Fraser, 2019). The study also revealed that reflectance of bands in the green, NIR and SWIR of the spectrum contributed most to the unique signal that resulted in higher classification accuracy with respect to co-occurring species, showing that images acquired in a particular phenological state can increase accuracy in classification of a target species (Rupasinghe & Chow-Fraser, 2019).

Nonetheless, considering the patch size of the invader relative to sensor resolution, platforms with moderate spatial resolution (< 10 m) prevent the detection of sparsely distributed patches, low density patches and small patches of *Phragmites* (< 2 m²) (Lopez et al., 2004; Pengra et al., 2007; Rupasinghe & Chow-Fraser, 2019, 2021; Tougas-Tellier et al., 2015). Detection of small patches and incipient populations at early stages of invasion is critical for successful invasion control given that management and eradication of large stands of *Phragmites* is considerably difficult as mechanical and chemical treatment are most effective when populations are small and sparsely distributed (Larson et al., 2020). In fact, in many cases, mechanical control cannot be implemented when the invaded area is too large (Gilbert et al., 2014; Rupasinghe & Chow-Fraser, 2021). In addition, multispectral data of moderate spectral resolution could make the precise identification of unique spectral and absorption features difficult as multispectral devices collect discontinuous information of a few absorption bands (Garg, 2020), whereas hyperspectral imagers acquire information in hundreds of contiguous spectral bands throughout the electromagnetic spectrum (400-2500 nm) in which there are numerous absorption features and information that could be meaningful in regards to traits that have not been measured or are so far unknown to be important (Cavender-Bares et al., 2016; Goetz, 2009).

With the acquisition of contiguous spectral bands, a reflectance spectrum can be derived for each pixel in a spectral image, which makes possible to create atmospheric correction factors from the data themselves and for each pixel as well (as atmospheric transmission changes across the image as a result of elevation and water vapour differences) (Goetz, 2009). This capability is not available with multispectral imagers and as a consequence, data acquisition is restricted to exclusive atmospheric windows. Moreover, contiguous sampling of spectral bands allows the use of least square techniques for statistical analyses and the use of correlation techniques to compare pixel spectra with spectral data bases improving the signal-to-noise ratio of the data by the square

root of the number of bands used in the analysis in contrast to comparisons of position of absorption bands with multispectral data (Asner & Martin, 2008; Clark et al., 2003; Goetz, 2009).

When canopy-level data is unavailable, leaf-level hyperspectral data can be scaled up to the canopy level with the use of radiative transfer models (RTMs) to extract biophysical parameters of vegetation from spectra (Féret & Asner, 2011; Jacquemoud et al., 1995). A radiative transfer model is a physically based model of photon transport inside leaves developed using the laws of optics (Jacquemoud et al., 1996). RTMs relate light absorption and scattering to biochemical constituents to better the understanding of the interaction of light with leaves (Féret & Asner, 2011). These models can be used in the forward mode to calculate reflectance and transmittance properties and in inversion to estimate leaf biophysical properties from high spectral resolution reflectance data (Jacquemoud et al., 1995, 1996). Promising results of retrieving canopy-level leaf biochemistry by inversion of RTMs using leaf-level spectral data has been documented mostly for agricultural fields and tropical forests (Asner & Martin, 2008; Asner et al., 2015; Jacquemoud et al., 1995, 1996) but as well for other ecosystems in Canada (Kalacska et al., 2015).

Although shifting from leaf-level spectroscopy to remote sensing approaches involves significant challenges, understanding how biochemicals are systematically expressed in hyperspectral measurements is a necessary condition to harness the potential of imaging spectroscopy to go beyond estimation of biophysical parameters to detection of taxonomic and genetic diversity at the canopy-level (Asner & Martin, 2008; Cavender-Bares et al., 2016). Therefore, this field of research warrants further exploration in multiple ecosystems and ecological contexts and demonstrates the importance of fine-scale leaf-level spectral measurements to interpret remote sensing data collected at broad geographic scales.

Objectives

In the context of invasion by the invasive common reed (*Phragmites australis* subsp. *australis*) in wetlands in the province of Québec, Canada, my master's project aims to test the ability of leaf-level hyperspectral data to detect the invader from co-occurring species and evaluate whether the accuracy of detection changes over the course of a growing season.

My hypothesis was that *Phragmites* would be more distinct spectrally towards the end or the beginning of the growing season from other co-occurring species in the invaded community due to variations in functional traits affected by extended leaf phenology that can influence the invader's reflectance signal in a way that it stands out from the background community. Alternatively, in the absence of major phenological differences, the invasive *Phragmites* should still be distinguished from co-occurring species if it is consistently chemically different from them.

With the collection of a time-series of leaf traits and spectral data from five different species (including the invasive *Phragmites*) collected from June to September of 2019, this project answered the following questions: (1) how do foliar traits of co-occurring species change over the course of a growing season? (2) are species functional differences captured in spectra? (3) can the invasive *Phragmites* be discriminated from others using leaf spectra? (4) does species discrimination using spectroscopy vary during a growing season?

I used analyses of variance (ANOVA) and Tukey HSD multiple comparison tests to evaluate foliar trait differences among species through time and the significance of the term time:species interaction. To evaluate whether foliar traits can be predicted accurately from leaf spectra, I used partial least square regression (PLSR) analyses. PLSR approach uses the continuum spectra as a single measurement rather than performing band by band analyses (Asner & Martin, 2008). PLSR models are built using leaf traits as the response variable and spectral data as explanatory variables. It is designed for high-dimensional datasets in which explanatory variables are multicollinear, as is the case with spectral bands, and its utility lies in the fact that it establishes a quantitative link between spectral bands and functional and chemical traits (Asner & Martin, 2008; Asner et al., 2014a). Lastly, to evaluate whether the invasive species can be discriminated from others, I built species classification models using partial least square discriminant analyses

(PLSDA) for each week of sampling. PLSDA is used for predictive and discriminative modelling with high dimensional datasets (Cavender-Bares et al., 2016; Schweiger et al., 2020). It trains a classification model from reference samples of spectra from different classes maximizing group differences and then it applies the model to unknown samples and gives for each sample the probability that it belongs to one of the classes. The accuracy of each model was compared to assess whether the ability of leaf spectra to distinguish the invader and co-occurring species changed throughout the course of the growing season.

With the use of hyperspectral data across time, this project will build on previous work in the detection of the invasive *Phragmites* (Pengra et al., 2007; Rupasinghe & Chow-Fraser, 2019, 2021; Samiappan et al., 2017; Tougas-Tellier et al., 2015). In particular, I expect that the foliar traits associated with the distinct phenological state of the invader over a growing season reported by Rupasinghe & Chow-Fraser (2019), that occurs during late summer and early fall (Rupasinghe & Chow-Fraser, 2019), will aid in the accurate classification of *Phragmites* with respect to the co-occurring species. In addition, I expect that with the use of the full spectrum, this study will provide sufficient resolution to classify the invader with > 80% accuracy. Lastly, this project will contribute to efforts of conservation action by providing a high-quality and detailed hyperspectral library for an invaded ecosystem of great interest in Canada as a tool for biodiversity preservation and management of wetlands benefiting conservation practitioners in Canada and beyond.

The results of my research project will be presented in the following chapter of this thesis in the form of a scientific article. The article titled “Foliar spectra accurately distinguished the invasive common reed from co-occurring plant species throughout a growing season” will be submitted to the journal of *Remote Sensing in Ecology and Conservation* for publication. The co-authors of the article and their respective contributions are listed below; all authors will contribute to the final version of the manuscript before submission for publication.

Juliana Pardo: development of ideas, data analysis, interpretation of results and writing original draft of the manuscript

Xavier Guilbeault-Mayers: consultation in statistical analyses and collaboration in interpretation of results, development of ideas and theoretical concepts

Madeleine Trickey-Massé: development of ideas, sampling design and methodology, led data collection

Anna K. Schweiger: provided the script for statistical analyses and consultation in interpretation of results, development of ideas and theoretical concepts

Etienne Laliberté: development of ideas, sampling design and methodology, consultation in interpretation of results, collaboration on revisions of the manuscript

Chapter 2

Foliar spectra accurately distinguish the invasive common reed from co-occurring plant species throughout a growing season

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Introduction

Human activities alter plant communities worldwide and facilitate biological invasions by intentional and accidental movement of species (Sala, 2000). An invasive species is defined as a non-native species that spreads rapidly and can have negative ecological, environmental, and economic effects on habitats where it has been introduced (Masters & Norgrove, 2010). Invasions often have long-term direct and indirect effects that might only become apparent after invaders are well established and have acquired large ranges and population sizes (Masters & Norgrove, 2010; Richardson & Pyšek, 2012). Accurate detection of invasive species is critical to understand their origins and monitor their distribution throughout the invasion process. Attempts to eradicate introduced species are most likely to succeed when detection of incipient populations occurs early in the invasion process. Tools for early detection are currently lacking but are critical to improve our ability to manage invasive species, prevent their spread and facilitate their eradication.

For plants, surveillance of biological invasions has relied on ground-based methods such as field surveys, updated inventories and distribution maps (Drake et al., 2003). Emerging approaches for surveying and monitoring the spread of invasive species include developments and advances in molecular biology (Saltonstall, 2002), chemical ecology (Macel et al., 2014), spatial analysis combined with invasion risk modeling (Bradley, 2013) and remote sensing using spectral data (Asner et al., 2008). In the field of invasion biology, the need for standardized and well-replicated monitoring studies that achieve comprehensive mapping over the long-term has been long recognized (Blossey, 1999). At present, remote sensing platforms can be used to identify location, cover, biomass and traits of invaders throughout large geographic ranges and across temporal scales in a rapid and repeatable manner (Ollinger, 2011). With routine monitoring, these tools can be used to identify sources of invasive species, routes of spread, predict invasion processes and document temporal changes in ecosystems (Asner et al., 2015; Bolch et al., 2020). However, in contrast to land cover classification which can be achieved using low to moderate spectral and spatial resolution data (Arino et al., 2012), successful species detection depends on high spectral and spatial resolution data (Asner et al., 2017).

Spectral data, which describes the interaction of vegetation with sunlight across the electromagnetic spectrum (i.e. patterns of light absorbed, transmitted and reflected at different wavelengths), is a valuable tool for species discrimination and is increasingly being used to map invasive species (Bradley, 2014; Cavender-Bares et al., 2017). Leaf reflectance spectra are aggregate indicators of plant chemistry, physiology and internal and external structure that affect light absorption and scattering (Asner et al., 2014a; Kokaly et al., 2009). Regardless of all plants being spectrally similar given that they are composed of the same spectrally active components, more functionally different and more distantly related species are more spectrally dissimilar (Price, 1994; Schweiger et al., 2018). With regard to invasive species, this presents an advantage for their detection given that they often possess traits that are novel to the colonized environment which makes them more easily distinguished from non-invasive species.

As important traits of foliage vary throughout the course of a growing season, phenology (i.e. the timing of periodic events such as flowering and leaf emergence/senescence) is as important as physiological or chemical traits in invaded systems as a driver of ecosystem change or as a driver of invasion itself (Ehrenfeld, 2010; Fridley, 2012; Smith, 2013). As leaves transition through

different growth stages (i.e. juvenile, maturity and senescence), accompanying morphological and chemical changes affect their optical and spectral properties. Spectral properties that describe traits strongly tied to phenology, are expressed in variations in amplitude and shape in spectral features mostly in the visible (VIS) and near-infrared (NIR) regions of the spectrum (Knipling, 1970; Yang et al., 2016). In particular, the green peak, red-edge, NIR and water absorption features are highly age-sensitive spectral domains (Chavana-Bryant et al., 2017; Papeš et al., 2013). Phenological differences or mismatches between natives and invasive species timely captured using spectroscopy could present an opportunity to distinguish non-native species and monitor incipient invasions.

One of Canada's most prominent invaders is the common reed, *Phragmites australis* spp. *australis*; hereafter *Phragmites* (Chambers et al., 1999; Guo et al., 2013). *Phragmites* was introduced to the Eastern United States and southern Canada in the 19th century (Chambers et al., 1999). Today it is found in wetlands and along the edges of water bodies (Guo et al., 2013). *Phragmites* spreads along transport corridors competing with native species and transforming diverse communities into low-diversity common reed stands, which constitutes a threat for biodiversity of wetland ecosystems (Lelong et al., 2007). It can establish itself from rhizome fragments and by sexual reproduction, and grows rapidly, forming tall and dense colonies (Albert et al., 2015). Its high productivity (above and belowground) coupled with the retention of live and dead tissue, alters nutrient cycles and flooding regimes, affecting wetland functioning and impacting wetland fauna (Meyerson et al., 1999, 2000, 2009). At present, climate models predict that the suitable but so far uninvaded area is considerably greater than the currently invaded one, which constitutes a threat for biodiversity of wetland ecosystems (Tougas-Tellier et al., 2015). Therefore, the spread of the common reed is a major conservation challenge and its management and eradication are a conservation priority (Gilbert et al., 2014).

Dissimilarities in phenological patterns and functional traits of invasive species relative to native species, suggest that invasive species might take advantage of empty ecological niches in novel environments (Brym et al., 2011; Emery, 2007). In particular, extended leaf phenology (i.e. early leaf emergence or delayed senescence) has been proposed as a key feature driving events of invasion in northern-latitude, deciduous ecosystems (Fridley, 2012; Rejmánek, 2013; Smith, 2013). Variation in functional traits driven by phenology can thus make invasive species more distinct

from the native community at the beginning or at the end of the growing season. Therefore, detection of invaders should be facilitated when the differences in chemistry, function and structure that are affected by phenology and can be detected spectrally, are maximized between the invader and the background community. Alternatively, invasive and co-occurring plant species might still be distinguished spectrally from each other even in the absence of major phenological differences if invasive species consistently differ chemically from natives (van Kleunen et al., 2010). Thus, understanding chemical and phenological differences among invasive and co-occurring plant species is important to improve remote sensing of invasive plants using spectroscopy.

In this study, we characterized the seasonal changes in foliar traits and spectra in *Phragmites* and co-occurring species to determine whether the invasive species can be discriminated using leaf spectra and how its detection varies over the course of a growing season. We predict that differences in foliar traits among *Phragmites* and the other species in the community will be most pronounced towards the end or the beginning of the growing season due to *Phragmites*' extended leaf phenology (Mozdzer & Zieman, 2010; Park & Blossey, 2008). Further, we expect that leaf spectra capture these differences such that *Phragmites* can be spectrally differentiated from the other species using spectral species identification models. Determining the degree to which seasonal variation in foliar traits is captured spectrally will allow to determine the optimal time for the spectral detection of *Phragmites* (i.e. when the invasive is more distinct from co-occurring species and therefore better discriminated using spectroscopy). This information will be critical for detecting *Phragmites* remotely and will improve early detection and facilitate eradication.

Methods

Site and species selection

We conducted fieldwork in Parc des Îles-de-Boucherville (45.5967° N, 73.4700° W) and Parc de la Frayère (45.6454° N, 73.4435° W) located in Boucherville, Québec in Canada. The province of Québec is characterized by a humid continental temperate climate with four distinct seasons (Gérardin & McKenney, 2001). Temperatures vary between 5 and 20°C in summer and from -25 to 10°C in winter. The total annual precipitation of rain and snow fluctuates among the regions of the province and reaches between 500 and 1,200 mm annually (Doyon et al., 2008).

Over six one-week periods between June and September 2019, we measured leaf spectral and foliar traits of five different species: *Bromus inermis* Leysser, *Phalaris arundinacea* Linnaeus, *Phragmites australis* (Cavanilles) Trinius ex Steudel subsp. *australis*, *Solidago* sp. Linnaeus and *Typha latifolia* Linnaeus; hereafter: *Bromus*, *Phalaris*, *Phragmites*, *Solidago* and *Typha*. Although *Phalaris arundinacea* is a non-native genotype colonizing wetlands in North America, we refer only to *Phragmites* as invasive in this study given that the presence of *Phalaris* is much less harmful than the invasive *Phragmites*. As well, we note that the subspecies of *Phragmites australis* that colonizes our study area is composed entirely of the European invasive lineage (*Phragmites australis* (Cavanilles) Trinius ex Steudel subsp. *australis*) and not the native subspecies (*Phragmites australis* subsp. *americanus*) that is found in North America (including parts of southern Québec) (Albert et al., 2015; Tougas-Tellier et al., 2015).

Sampling sites were selected to minimize environmental variation among sampling locations allowing us to focus on temporal trait changes across species. We sampled plants from the same patch throughout the growing season to minimize differences among patches by species.

Leaf sampling and chemical analyses

A total of 300 plants from five species were sampled at six different time-points (approximately every three weeks) during summer 2019 to capture the seasonal variation of traits and spectra among co-occurring species throughout a growing season. Leaf samples of 10 individuals per species were collected each day of every sampling week (except for *Typha* in the week of July 8th when we sampled 12 individuals and in the week of July 29th, we sampled 8

individuals), resulting in a total of 50 individuals per week and 60 individuals per species at the end. Fully expanded, sunlit and healthy-looking leaves were selected for spectral and foliar trait measurements. Leaves used in foliar trait analyses were weighed upon collection and rehydrated for at least 12 h before being weighed again and scanned for total leaf area using a CanonScan LIDE 220 scanner (Canon, Brampton, Canada) and the software WinFOLIA Reg 2016b (Regent Instruments Inc., Québec, Canada). The scanned leaves were oven-dried (72 h at 65 °C) and weighed to determine leaf dry matter content and leaf water content based on rehydrated and dry weights (Laliberté, 2018). Approximately 12 g of fresh leaves without petioles were dried and grounded to a fine powder using a cyclone mill (2-mm mesh) for chemical analyses. Total carbon (C) and nitrogen (N) concentrations (% dry mass) in ground leaf samples were determined using an elemental analyzer (Ayotte et al., 2019). Carbon fractions in ground leaf samples were measured using a ANKOM 2000 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA). Concentration (% dry mass) of soluble C, hemicellulose, cellulose and lignin were determined by sequential digestion and inorganic recalcitrant materials (% dry mass) were determined from residues ashed in a muffle furnace (Ayotte & Laliberté, 2019). Photosynthetic pigments were determined from leaf disks stored at -80 °C (Girard et al., 2019). Concentrations of chlorophyll *a*, chlorophyll *b*, and total carotenoids (mg g⁻¹) were extracted with methanol and measured with a spectrophotometer (SPECTROstar® Nano, BMG LABTECH, Guelph, Canada) using a plate reader. Additional leaf material from the bulk sample was used to keep herbarium vouchers of every individual sampled.

Spectral measurements

Foliar spectral measurements were performed using a Spectra Vista Corporation (SVC) DC-R/T integrating sphere fitted to a portable full-range field spectroradiometer (SVC HR-1021i, Spectra Vista Corp. Poughkeepsie, NY). Six mature, fully-developed, healthy looking leaves per individual were selected following Canadian Airborne Observatory (CABO) protocols for spectral measurements for large and narrow leaves (Laliberté & Soffer, 2018a, 2018b). Reflectance measurements were corrected for stray light and referenced to a calibrated Spectralon® disk. Sensor overlap in the spectra were removed and a 1-nm linear interpolation applied. The foliar spectra were trimmed to the 400-2400 nm range and smoothed with a Savitzky-Golay filter with different parameters for each region of the electromagnetic spectrum: visible (VIS), near-infrared

(NIR), short-wave infrared (SWIR). After completing spectral measurements, leaf disks for pigment analyses were punched out from the selected leaves for spectroscopy using a 7-mm cork borer. Disks were immediately placed in an Eppendorf tube inside an ice cooler box before being transferred to a -80 °C freezer.

Statistical analyses

Leaf spectra through time

Partial least squares discriminant analysis (PLSDA) was used to assess the ability of leaf spectra to distinguish co-occurring plant species through time (model 1) and to distinguish the invasive *Phragmites* from co-occurring species (model 2). PLSDA is used for predictive and discriminative modelling with high dimensional datasets (Cavender-Bares et al., 2016; Schweiger et al., 2020). It trains a classification model from reference samples of spectra from different classes, maximizing group differences, and then it applies the model to unknown samples and gives for each sample the probability that it belongs to one of the classes. Using leaf-level reflectance, the dataset was iteratively separated into calibration (reference) and validation (prediction) data, using a 60:40 (1) and 70:30 (2) split per class for model calibration and validation, respectively. The models were run with 50 iterations and the optimal number of components was chosen based on significant differences in the Kappa score using Tukey tests (Girard et al., 2020).

Functional traits through time

Differences in functional traits among species through time were evaluated using ANOVA. Whenever significant differences were observed ($P \leq 0.05$), Tukey HSD multiple comparison tests were performed. Significance of the time:species interaction was evaluated as well. A level of significance of $P \leq 0.05$ for this interaction term means trait differences among species varied through time, or that temporal patterns in trait variation differed among species.

Functional traits captured by spectra

Partial least squares regression (PLSR) analysis was used to predict foliar traits from spectra. PLSR is designed for high-dimensional datasets in which explanatory variables are multicollinear as is the case with spectral bands. It establishes a quantitative link between spectral signatures and chemical and functional traits. The dataset was separated into calibration (reference)

and validation (prediction) groups using a 70:30 split, respectively. The models were trained with 50 iterations, using leaf traits as the response and spectral data as the explanatory variables, respectively. The prediction residual error sum of squares (PRESS) statistic was used to prevent overfitting and to identify the optimal number of PLSR components (Asner & Martin, 2008; Asner et al., 2014a). Model accuracy was assessed using the root mean square standard error of prediction (RMSEP) and regression coefficient (R^2). The variable importance of projections (VIP) metric was calculated on the final models to identify regions of the spectrum that were significant to the prediction of the foliar traits studied (Wold et al., 2001).

All statistical analyses were performed in the R statistical environment (*R Core Team 2020*). The following packages in R were used for data processing and statistical analyses: *signal* (Signal developers, 2015), *caret* (Kuhn, 2019), *pls* (Mevik et al., 2020), *effects* (Fox et al., 2020), *spectrolab* (Meireles et al., 2018).

Results

The foliar spectral signatures of all species differed across much of the spectral range through time (Fig 2.1 and S2.1). For instance, *Phragmites* exhibited higher reflectance than other species in the VIS region (spectral domain: 400-700 nm) during August and September, particularly in the blue wavelengths (~450 nm; Fig 2.1 e and f). In the NIR region (spectral domain: 700-1400 nm), *Bromus* showed consistently lower reflectance while *Typha* showed consistently higher reflectance than co-occurring species. In contrast to the NIR reflectance, *Typha* showed the lowest reflectance in the SWIR regions 1 & 2 (spectral domains: SWIR1 1400 – 1880 nm & SWIR2 1800-2400 nm). *Phragmites* SWIR reflectance during mid-season (June and July) was second lowest to *Typha* (Fig 2.1 a, b, c). *Bromus* showed the highest temporal variability in reflectance across the spectrum, particularly in the VIS region during July and August (Fig S2.1 c, d, e).

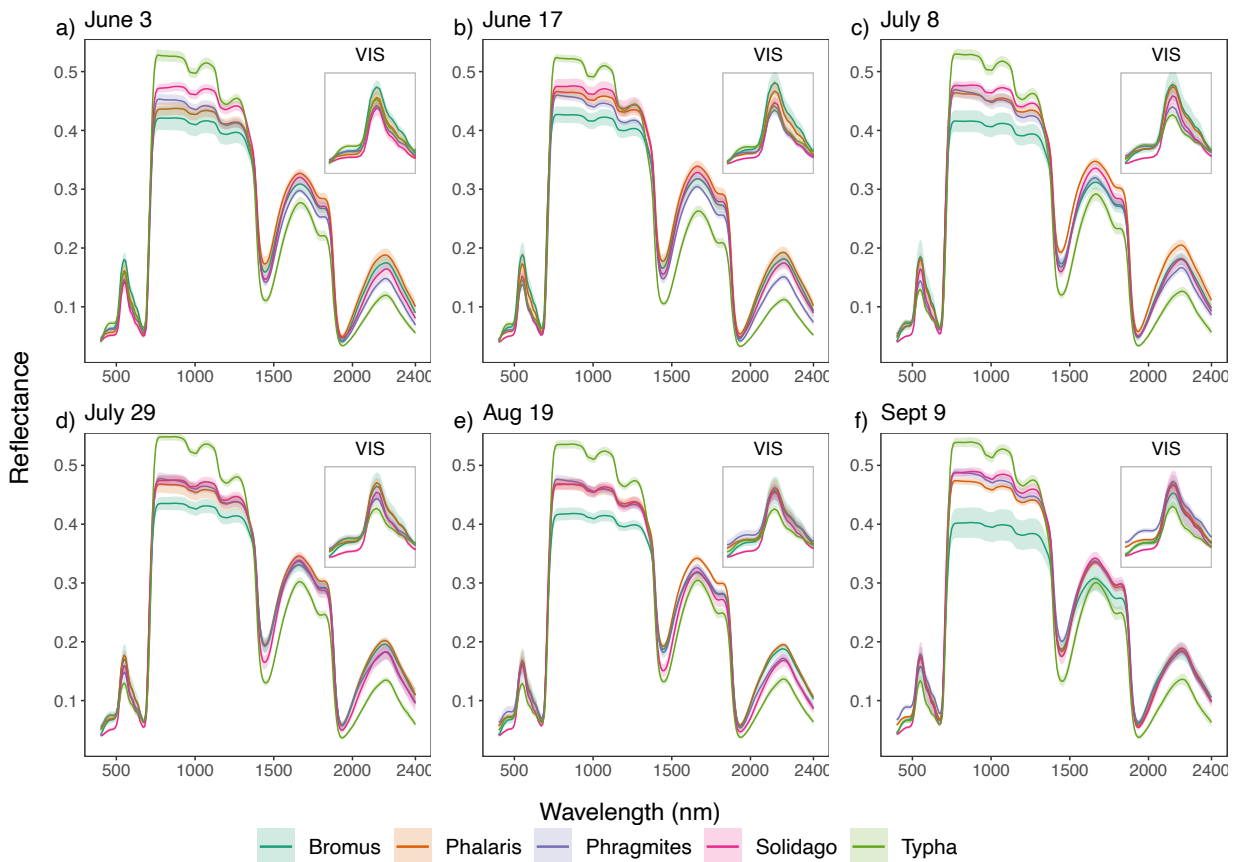


Figure 2.1. Reflectance spectra (mean \pm standard deviation) of the studied species throughout the growing season (June to September) of 2019. Insets in each panel (a-f) show the visible (VIS) range in detail.

Classification accuracy of our PLSDA models was over 95% for all plant species measured from June to September 2019 (Fig 2.2). Discrimination of the invasive *Phragmites* from co-occurring species based on foliar spectra was highly accurate throughout the growing season as well, with mid-June resulting in perfect classification of *Phragmites* (Fig 2.2 and Table 2.1). The PLSDA model for the beginning of June showed slightly higher sensitivity (highest rate of true positive classification for *Phragmites*) but slightly lowest specificity (lowest rate of true negative classification for *Phragmites*) (Table 2.1). The highest specificity was obtained during late mid-season on the week of July 29th (Table 2.1). The rate of false negatives (i.e. misclassification of *Phragmites*) for PLSDA models was lowest in mid-June and consistently low across the growing season (Fig 2.2). Absolute wavelength loadings of PLSDA models revealed their contribution to species differentiation across time (Fig 2.3 and S2.2). The VIS and NIR regions of the spectrum were of particular importance for accurate classification as they consistently had high relative loadings in PLSDA models from June through September (Fig S2.2). Bands in the SWIR regions had higher relative loadings from the end of June and beginning of July (late beginning through mid-season).

Foliar functional traits and carbon fractions displayed significant temporal variations among species (Figs. 2.4 and 2.5). The main observed patterns in leaf functional traits through time were: Nitrogen (N) concentrations (%) were highest in *Phragmites* during the growing season but also showed a marked and consistent drop through time, while N concentrations for the other species (except *Solidago*) increased modestly in August (Fig 2.4 a). *Phragmites* and *Typha* had high carbon (C) concentrations (%) throughout the growing season, followed by *Solidago* (Fig 2.4 b). *Typha* exhibited higher concentrations of pigments than all other species throughout the growing season (Fig 2.4 c, d, e). All species showed a decline in leaf pigments from June to August, consistent with chlorophyll breakdown as the leaves start to senesce (Fig 2.4 c, d, e). *Solidago* had the lowest concentrations of pigments and showed a slightly sharp decline towards the end of the season (Fig 2.4 c, d, e). Leaf mass per area (LMA) was highest in *Typha* and followed by *Phragmites* (Fig 2.4 f). All species increased gradually in LMA through time as leaves developed through time (Fig 2.4 f). *Typha* exhibited the highest equivalent water thickness (EWT) throughout the season (Fig 2.4 g). This was consistent with lower reflectance in the SWIR regions, suggesting high water content and high light absorption in those ranges (Fig 2.1), despite a gradual decline in EWT from July to September which we also found for all other species (Fig 2.4 g). Physical characteristics of leaf

structure were important as well, as thick-leaved *Bromus* showed consistently lower reflectance in the NIR region, where reflectance is affected by leaf anatomy and morphology; while *Typha*, that has more slender leaves, and presumably less compact mesophyll structure, showed consistently higher reflectance, indicating high internal light-scattering (Fig 2.1).

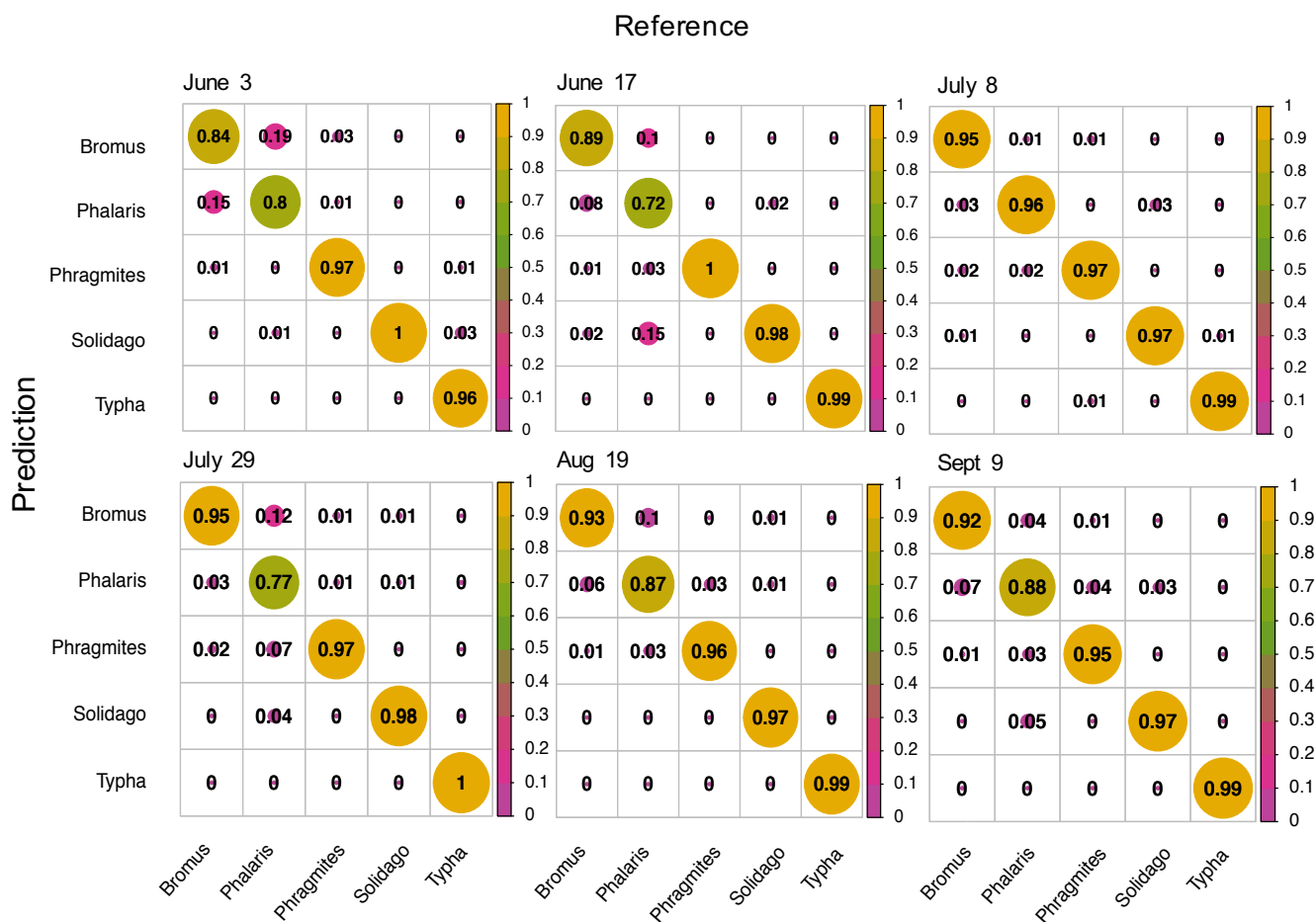


Figure 2.2. Classification accuracy per species across time in Model 1 of PLSDA. Number of components of PLSDA per week: June 3 - 4 components; June 17 - 4 components; July 8 - 7 components, July 29 - 7 components; Aug 19 - 8 components; Sept 9 - 5 components.

Table 2.1. Specificity (true positive rate of classification) and Sensitivity (true negative rate of classification) to detect Phragmites from all other species (PLSDA Model 1).

	Jun 3	Jun 17	July 8	July 29	Aug 19	Sept 9
Sensitivity	0.98	0.96	0.96	0.92	0.96	0.96
Specificity	0.02	0.04	0.04	0.08	0.04	0.04

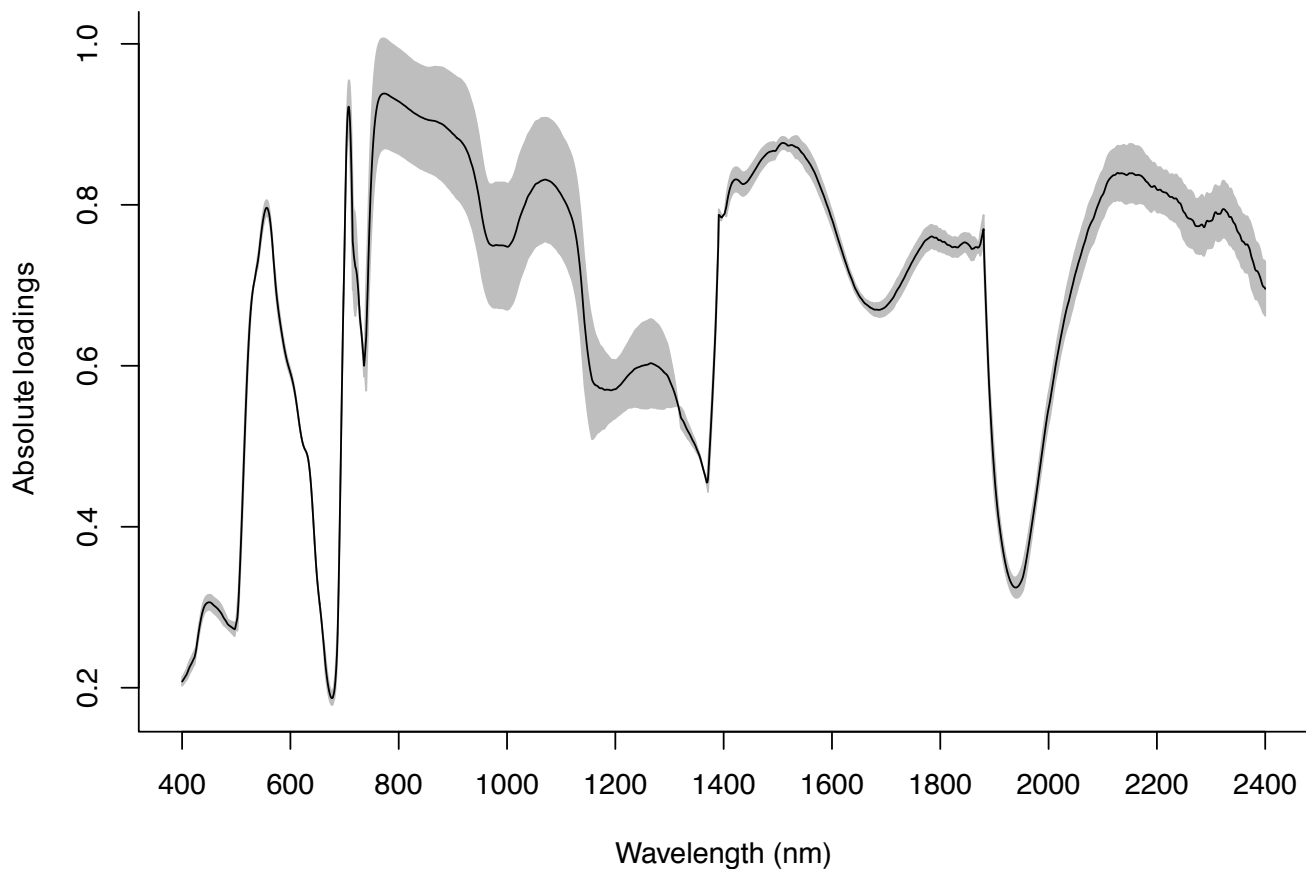


Figure 2.3. Absolute wavelength loadings of PLSDA models. Shown here are loading for the week of July 8th, see Fig S2.2 for all weeks from June to September.

Solidago exhibited the highest concentrations of soluble carbon and lowest concentrations of hemicellulose and cellulose throughout the growing season than the rest of the species (Fig 2.5 a, b, c). Lignin concentrations were highest for *Solidago* and *Typha*, with *Typha* showing a gradual increase from the beginning of the season, while *Solidago* showed a sharp decline from the initially high concentrations until both species maintained similar concentrations from July onward (Fig 2.5 d). *Phragmites* showed slightly higher concentrations of hemicellulose from the middle of the season onward (Fig 2.5 b). Inorganic recalcitrant materials increased consistently from the middle of the season onward for *Bromus*, *Phalaris* and *Solidago*, while *Phragmites* and *Typha* maintained stable concentrations throughout the growing season (Fig 2.5 e).

Foliar functional traits and carbon fractions were predicted with moderate to high accuracy from spectral data using partial least squares regression (PLSR) analysis (Figs 2.6 and 2.7; Tables 2.2 and S2.1). Regression coefficients (R^2) for PLSR models ranged from 0.36 to 0.92. Traits that were predicted with the highest accuracy ($R^2 \geq 0.80$) were EWT, LMA and concentrations of cellulose and soluble C (Fig 2.6 i and j; Fig 2.7 a and c). Traits predicted with the lowest accuracy ($R^2 \leq 0.50$) were concentrations of carbon, carotenoids, lignin and recalcitrants (Fig 2.6 b, e, f; Fig 2.7 d and e). All traits were predicted with relatively high precision (RMSEP < 30%) by PLSR models except for lignin and recalcitrants (RMSEP values of 47% and 64%, respectively). Lastly, regions of particular importance for foliar trait predictions were consistent with known features of absorption reported in the literature for each trait studied (Asner et al., 2011; Curran, 1989; Kokaly et al., 2009; Ustin et al., 2009; Wang et al., 2020) (Fig S2.3).

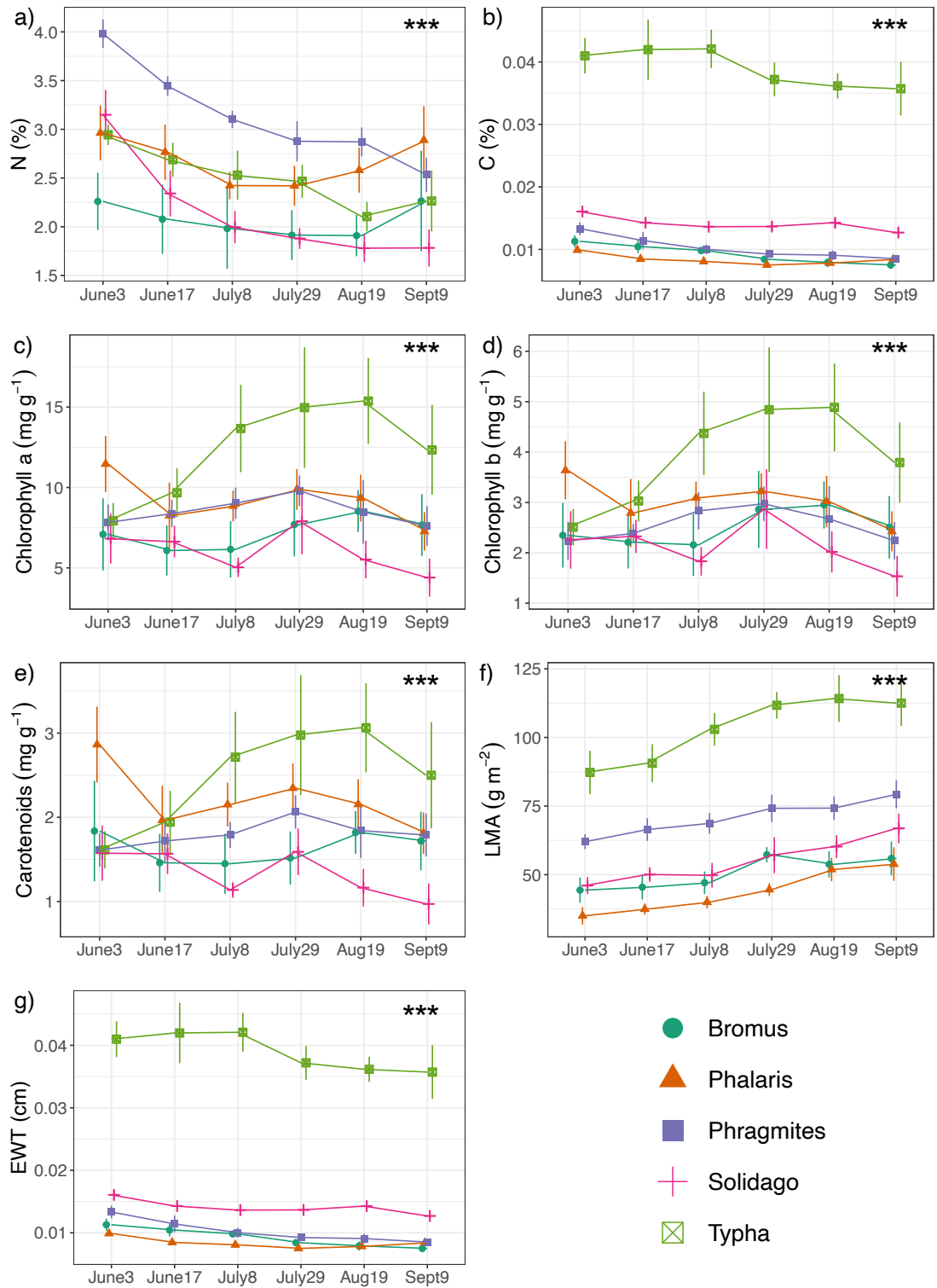


Figure 2.4. Temporal changes in leaf functional traits by species. Asterisks indicate the statistical significance of the time:species interaction terms in linear models (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$). Each symbol is the mean value for every trait measured each week. Error bars are 95% confidence intervals. LMA: leaf mass per area; EWT: equivalent water thickness.

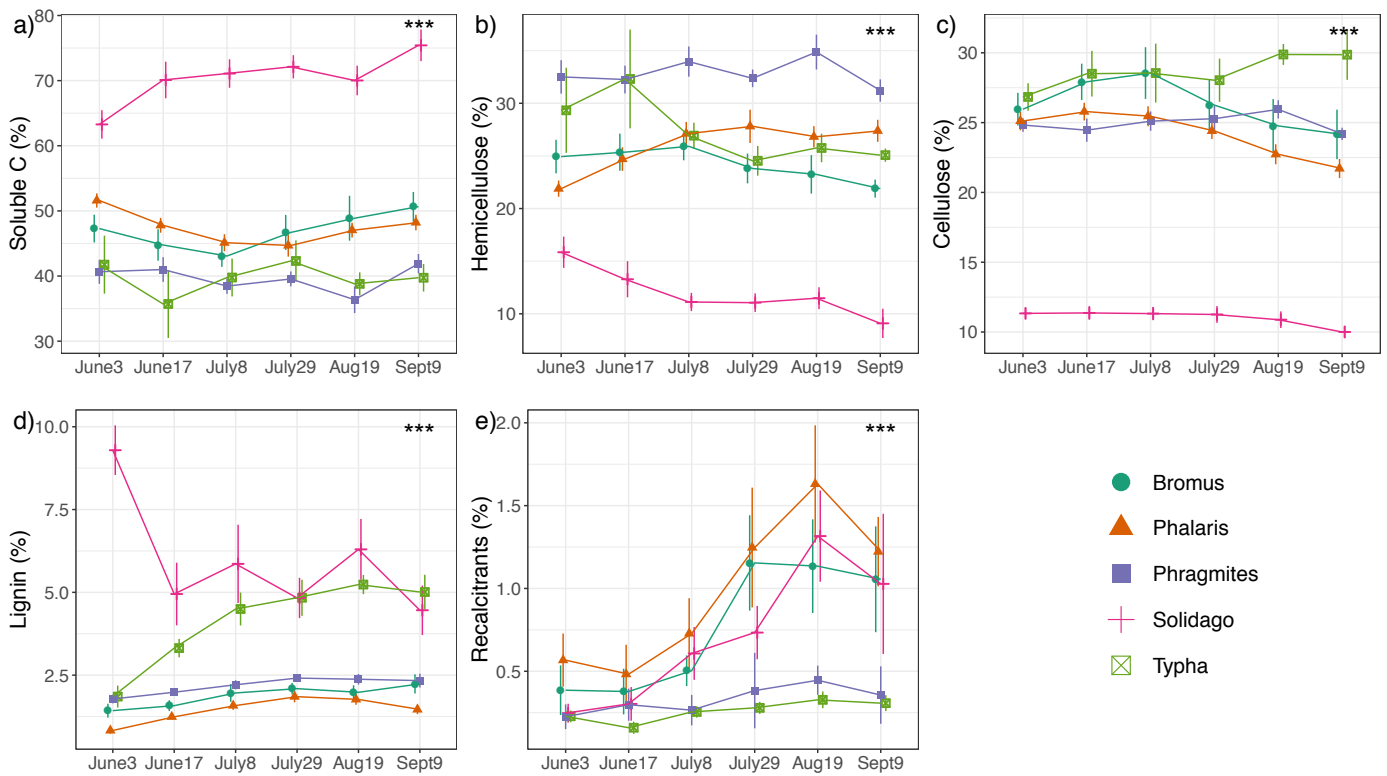


Figure 2.5. Temporal changes in leaf carbon fractions by species. Asterisks indicate the statistical significance of the time:species interaction terms in linear models (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$). Each symbol is the mean value for every trait measured each week. Error bars are 95% confidence intervals.

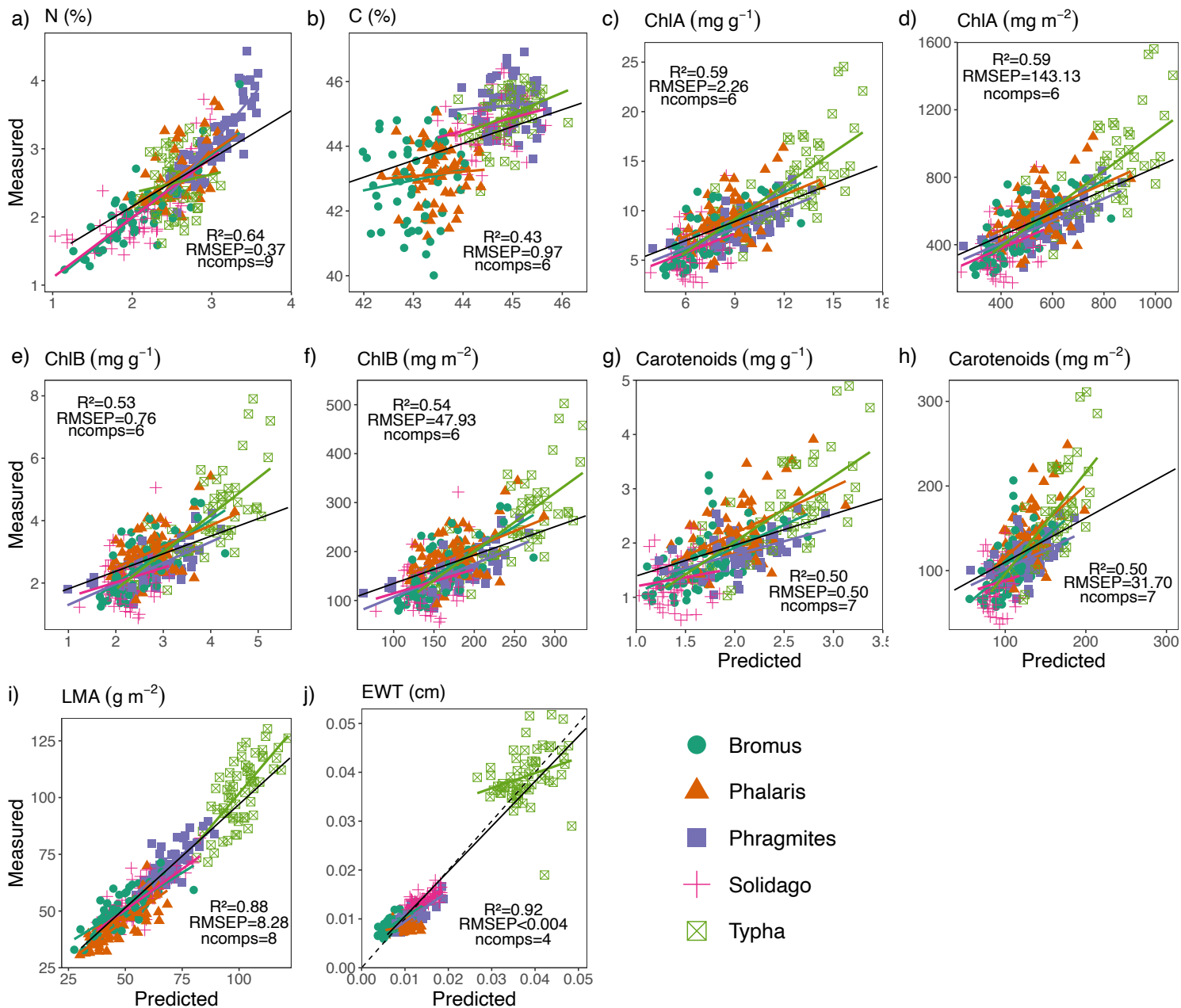


Figure 2.6. Relationships between measured and predicted leaf chemical and structural properties using leaf-level spectral data. Dashed lines indicate a 1:1 relationship, black solid lines indicate the linear relationship between measured and predicted values for all species and colored solid lines indicate the linear relationship between measured and predicted values per species. Measured values are on the y-axes; predicted values on the x-axes. Error bars indicate the mean standard deviation of the predictions. Statistics presented in the graphs are validation metrics. R^2 is the regression coefficient. RMSEP is the mean root mean square error of prediction as a percentage of the leaf trait (%). Ncomps is the number of components per PLSR model. ChIA: chlorophyll a; ChIB: chlorophyll b; LMA: leaf mass per area; EWT: equivalent water thickness.

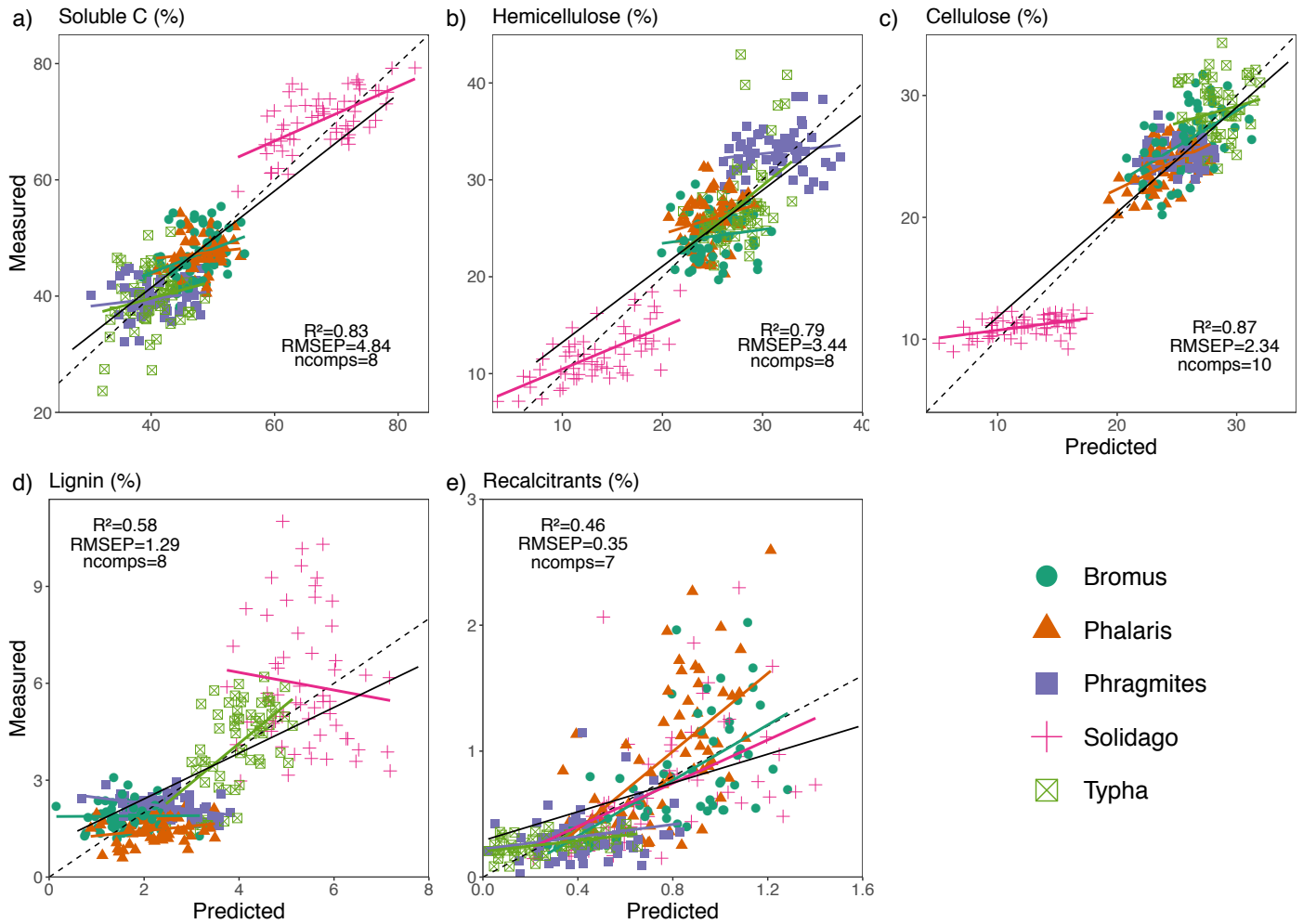


Figure 2.7. Relationships between measured and predicted leaf carbon fractions using leaf-level spectral data. Dashed lines indicate a 1:1 relationship, black solid lines indicate the linear relationship between measured and predicted values for all species and colored solid lines indicate the linear relationship between measured and predicted values per species. Measured values are on the y-axes; predicted values on the x-axes. Error bars indicate the mean standard deviation of the predictions. Statistics presented in the graphs are validation metrics. R^2 is the regression coefficient. RMSEP is the mean root mean square error of prediction as a percentage of the leaf trait (%). Ncomps is the number of components per PLSR model.

Table 2.2. Statistics for partial least square regression (PLSR) models predicting functional traits from leaf spectra ordered by descending R^2 for the validation dataset. The strength of the relationship (R^2) indicates the absolute importance of each parameter in determining the spectral properties of species. RMSEP is the root mean square standard error of prediction in original measurement units and RSMEP (%) is the root mean square standard error of prediction as a percentage of the mean leaf trait.

	Mean (\pm SD)	Wavelength Range (nm)	R^2	RMSEP	RMSEP (%)
EWT (cm)	0.02 (0.01)	800 - 2400	0.92	0.00	21.34
LMA (g m^{-2})	64.6 (23.4)	800 - 2400	0.88	8.28	12.82
Cellulose (%)	23.0 (6.5)	1200 - 2400	0.85	2.52	10.96
Soluble C (%)	48.7 (12.0)	1200 - 2400	0.80	5.40	11.08
Hemicellulose (%)	24.5 (7.5)	1200 - 2400	0.74	3.84	15.66
N (%)	2.51 (0.61)	400 - 2400	0.64	0.37	14.87
Chlorophyll a (mg m^{-2})	549.3 (221.0)	400 - 760	0.59	143.13	26.06
Chlorophyll a (mg g^{-1})	8.7 (3.5)	400 - 760	0.59	2.26	26.08
Chlorophyll b (mg m^{-2})	179.5 (69.1)	400 - 760	0.54	47.93	26.70
Chlorophyll b (mg g^{-1})	2.8 (1.1)	400 - 760	0.53	0.76	26.77
Carotenoids (mg m^{-2})	120.1 (44.2)	400 - 760	0.50	31.70	26.30
Carotenoids (mg g^{-1})	1.89 (0.70)	400 - 760	0.50	0.50	26.40
Lignin (%)	3.1 (2.0)	1200 - 2400	0.50	1.48	47.46
C (%)	44.2 (1.3)	1200 - 2400	0.43	0.97	2.20
Recalcitrants (%)	0.61 (0.49)	1200 - 2400	0.36	0.39	64.37

Discussion

Our study revealed a near-perfect capacity to distinguish the invasive *Phragmites* from co-occurring species using foliar reflectance spectra throughout June to September. As such, our results show that *Phragmites* is consistently different chemically from the community it is invading and therefore can be distinguished spectrally with high accuracy irrespectively of the timing of detection. Our study suggests a strong potential for detecting *Phragmites* using spectroscopy, but do not point toward an optimal time for detection during the growing season.

Previous studies using time-series datasets of seasonal changes in leaf traits and spectra found critical time windows that capture the maximum differentiation amongst co-occurring species due to differing phenology (Boyte et al., 2015; Bradley & Mustard, 2005; Rupasinghe & Chow-Fraser, 2019) and climatic conditions (Asner et al., 2006). Although our results do not point to a specific time frame for better detection using spectroscopy, we found evidence of spectral domains of particular importance that contribute to species discrimination and the accurate detection of *Phragmites*. In particular, the unique blue-green color of the invasive *Phragmites* is a key feature (even if subjective) to differentiate native and exotic forms of the invader in the field (Mozdzer & Zieman, 2010; Swearingen & Saltonstall, 2012) that is captured with precision in the foliar spectra, especially later in the season. The importance of leaf anatomy and morphology to differentiate species confirmed in the high contribution of the NIR reflectance for species spectral separability at the leaf level also holds at the canopy level (Ollinger, 2011), and makes *Phragmites* detection well-suited for remote sensing approaches (Rupasinghe & Chow-Fraser, 2021).

Phragmites showed early and significantly higher N allocation to leaves compared to other species. Nitrogen is a key element in the Ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco), the enzyme responsible for carbon fixation in plants (Chapin et al., 1987). An early allocation of N to leaves that could precede later enhanced carbon gains, given that as much as 30-50% of leaf N is tied to Rubisco, could be indicative of extended lead phenology in the invader pointing to enhanced carbon gains during a growing season (Chapin et al., 1987; Liao et al., 2008). At the same time, as N is also a key component of chlorophylls, the light-harvesting compounds in plants, a high allocation of N could translate to high concentrations of chlorophylls and consequently higher photosynthetic activity (Kokaly et al., 2009). However, C3 grasses, such as

Phragmites allocate about 70% of leaf N to molecules that support carbon fixation and only a small fraction to chlorophylls (Chapin et al., 1987; Evans, 1983), which explains the lack of a positive correlation between leaf N and chlorophyll content in our study (Fig 2.4 a, c and d). The range of biochemical and physiological characteristics that plants display in their functional traits have strong control over ecosystem functions given that they determine processes like photosynthesis and nutrient and water uptake (Keddy, 1992; McGill et al., 2006). In the case of *Phragmites*, its distinct phenology and accompanying traits grant the invader an advantage by enhancement of resource competition via nutrient pre-emption or extended carbon assimilation but is also an important driver of change in altering carbon and nitrogen cycles at the ecosystem level (Ehrenfeld, 2010; Liao et al., 2008).

Successful invasive species often have higher trait values for performance-related traits (Asner et al., 2006; Farnsworth & Meyerson, 2003; Fridley, 2012; van Kleunen et al., 2010). Such physiological investments in photosynthesis, light-harvesting and water and nutrient acquisition are tied to structural investments and are therefore resource-constrained at the ecosystem level (Wright et al., 2004). Plants that grow where resource availability is high, can afford a life strategy that promotes rapid carbon gains and fast growth supported by high value traits (high nutrient concentrations, photosynthetic-rates and specific leaf-area; SLA) (Reich, 2014; Wright et al., 2004). On the other hand, plants that grow in resource-limited environments display traits related to resource conservation and longevity (lower nutrient concentrations, lower photosynthetic rates and higher investment in structural and defense compounds) (Reich, 2014; Wright et al., 2004). It follows then, that *Phragmites* might benefit from having extended leaf phenology if it grows in nutrient-rich environments as having an extended period of nutrient acquisition allows it to allocate nutrients in belowground biomass and store them as temperatures drop (Meyerson et al., 2000). Simultaneously, it could be expected that it would outperform co-occurring species in terms of displaying superior functional traits. In the context of the plant community studied, *Phragmites* exhibited intermediate trait values (with the exception of N and hemicellulose), which might be indicative of *Phragmites*' strategy to succeed under conditions of high and low resource availability (Mozdzer & Megonigal, 2012). There is evidence that *Phragmites* has the ability to deploy different nutrient-use strategies facilitating successful invasion in both stressful and resource-rich environments, and suggesting that its invasiveness might intensify in the future (Mozdzer & Megonigal, 2012; Richards et al., 2006).

Long-term adaptation of plants to particular environmental conditions may impart phylogenetic patterns in their chemical traits (Kursar et al., 2009). As such, species that grow in similar environments and are closely related tend to be functionally similar. A strong taxonomic pattern in the functional response of plants could translate to taxonomic patterns in foliar spectral properties since certain regions of the spectrum are sensitive to the independent effect of biochemical and structural traits (Asner et al., 2014b; Meireles et al., 2020). This could complicate the spectral separability of closely related species or species with similar life/growth forms since they might show considerable overlap in spectrally detectable leaf traits (Schweiger et al., 2017). Our results indicate that the use of hyperspectral data at the leaf level captures sufficiently detailed chemical and structural characteristics to differentiate species within the same family of grasses (Poaceae: *Bromus*, *Phalaris*, *Phragmites*), within the same order (Poales: *Bromus*, *Phalaris*, *Phragmites* and *Typha*) and within the same herbaceous life/growth form. Differentiating species with the same habit is especially important with respect to *Phalaris*, *Phragmites* and *Typha*, for example. These species are often co-occurring and in terms of being tall, unbranched and forming dense monospecific stands with similar leaf arrangement, are often confused by the broader public and to some extent by remote sensing platforms as well (Rupasinghe & Chow-Fraser, 2021; Tougas-Tellier et al., 2015).

The ability to accurately detect (i.e. high sensitivity) a problematic species is desirable in an ecological context of invasion (Andrew & Ustin, 2008). However, the ability to reduce false negatives (i.e. high specificity) is more important from a conservation perspective so as to not miss the presence of an invasive individual which may eventually form a large population. Our PLSDA models showed high sensitivity throughout the growing season, reflected in the near-perfect classification of individual plant species that is consistent through time. However, specificity was higher during mid-growing season around the week of July 29th. This is consistent with Rupasinghe & Chow-Fraser (2019) that report *Phragmites*' most spectrally distinguishable phenological state to occur during the months of July and September (Rupasinghe & Chow-Fraser, 2019). Using a time-series of multispectral satellite imagery they achieved highest accuracies (89%) to map *Phragmites* with images acquired during late summer and early fall seasons as a result of prolonged greenness of the leaves, higher concentrations of chlorophylls and higher water content months of July-September (Rupasinghe & Chow-Fraser, 2019). The authors report as well that spectral bands

in the green, indicative of prolonged greenness, and NIR and SWIR regions contributed to the uniquely detectable signal of *Phragmites* during this period (Rupasinghe & Chow-Fraser, 2019).

Our study is consistent with the aforementioned results, and it contributes to a growing body of knowledge in the detection of the invasive *Phragmites*. Mainly, with the use of hyperspectral data we found that *Phragmites*' detection particularly in July would be advantageous on account of a higher probability to reduce the rate of false negatives according to our classification models. In addition, we identified a particular feature of interest in the higher reflectance in the blue wavelengths to distinguish the invasive *Phragmites* that was accurately detected by the spectra towards the end of the growing season, and with the use of high resolution spectral data, our models achieved consistently high classification accuracies for *Phragmites* of over 95% throughout the growing season.

The functional differences in chemistry and structure that influence the spectral response of individual species, are also well predicted by spectroscopy with moderate to high accuracy, as shown in our PLSR models. The traits that were best retrieved from leaf spectra (i.e. EWT, LMA, cellulose, soluble C and hemicellulose) include important chemical and structural constituents and describe fundamental processes that mediate light capture, CO₂ uptake and influence a variety of ecosystem processes (Reich, 2014; Wright et al., 2004). The less accurate spectroscopic determinations were for compounds involved in structure and defense like carbon and lignin and pigments such as chlorophylls and carotenoids which are critical for photosynthesis and excess light dissipation (Ustin et al., 2009). These results confirm that reflectance and transmittance patterns in leaf spectra are an expression of both the direct elemental and molecular composition of specific chemicals such as water, pigments, N, and C (Curran, 1989), making it possible to link plant functional and spectral responses (Schweiger et al., 2017).

Using imaging spectroscopy to detect and track the spread of invaders has the potential to transform invasive species monitoring. To date, most control actions and management efforts of invasive species have been reactive, but the future of the field is in prevention (Bolch et al., 2020). Spectral data that captures vegetation properties indicative of ecosystem functioning might aid in the early and accurate detection of invaders, track their spread, and inform management and conservation strategies to predict when and how fast invasions occur. Moreover, future hyperspectral Earth imaging satellite missions rely on the application of spectroscopy to remote

sensing techniques (Asner & Martin, 2008; Ustin & Middleton, 2021). Currently available satellite platforms offer potential to improve mapping of invasive species on a global scale at rather coarse spatial resolution (Bradley, 2014; Cawse-Nicholson et al., 2021). Nevertheless, such systems provide high temporal and spectral resolution, and our work shows that accurate detection in the spectral domain is possible.

Developing accurate early detection methods that target young individuals of *Phragmites* and incipient populations is critical for successful invasion control (Larson et al., 2020). In addition, time-series data that include more than one growing season allow characterizing the phenology of invasive species and the climate effects that influence their spread (Asner & Martin, 2008; Bolch et al., 2020; Yang et al., 2016). Lastly, invasive species monitoring with remote sensing is most successful when a proposed approach provides sufficiently high resolution in the spatial (pixel size), spectral (number and range of bands) and temporal (frequency of data acquisition) domains (Bradley, 2014). At the moment there is no sensor that achieves high coverage in all three, and in choosing an optimal remote sensing approach a trade-off along these axes must be carefully evaluated (Ollinger, 2011). Higher spectral resolution creates opportunities to differentiate subtle, yet significant, differences in chemistry and structure among species to discriminate them. For this reason, hyperspectral sensors in remote sensing platforms are commonly used for invasive plants detection. The downside is that typically they offer limited spatial and temporal coverage or can be costly to acquire (Bradley, 2014). The use of hyperspectral unmanned-aerial vehicles (UAV) of ultra-high spatial resolution provide a means to overcome limitations in the spatial domain as pixel size can approximate the width of a single leaf (> 5 cm) but the use of such platforms is still within the expensive range and require a large crew of people to be fully operational (Arroyo-Mora et al., 2019).

Conclusion

Our work illustrates the potential of leaf-level spectroscopy to translate information of reflected light into meaningful knowledge about a plant's functional traits response. We showed that leaf-level spectra have the potential to accurately detect an invasive species that is functionally different from co-occurring species throughout a growing season. Management of invasive species is critical from a conservation perspective considering the substantial ecological and economic costs associated with plant invasions, and in the face of a persistent threat to natural ecosystems due to accelerated global change, the need for rapid monitoring and effective control of invasive species is ever more important. At present, the only way to provide timely and frequent monitoring at large temporal and geographic scales is with the use of remote sensing techniques that rely on the application of spectroscopy, highlighting the importance of building high-quality and detailed spectral libraries for invaded ecosystems.

Chapter 3

General discussion

The global objective of this MSc thesis was to test whether an invasive species could be accurately distinguished from other co-occurring species throughout the course of a growing season using leaf-level spectra, and whether its detection changed throughout this period. The ultimate goal of this research is to contribute to detection by remote sensing and spatial monitoring of the invasive *Phragmites*.

The main chapter presented as a scientific article for publication explores variation in spectral and foliar traits of five different plant species found in the wetlands of Boucherville in Québec, Canada, including the invasive common reed *Phragmites australis* spp. *australis*. Throughout the development of this study the following questions were addressed: (1) how do foliar traits of co-occurring species change over the course of a growing season? (2) are species functional differences captured in spectra? (3) can the invasive *Phragmites* be discriminated from others using leaf spectra? (4) does species discrimination using spectroscopy vary during a growing season?

Our study showed that the invasive *Phragmites* can be distinguished from co-occurring species using foliar reflectance spectra nearly perfectly throughout June to September. Concurrently, based on the links that exist between plant spectra, plant form and function, our results show that *Phragmites* is consistently different chemically from its co-occurring species and therefore can be distinguished spectrally with high accuracy irrespective of timing of detection. Our results also revealed that leaf hyperspectral data capture sufficiently detailed information to differentiate not only the invader from the background community but also species within the same family (i.e: Poaceae: *Bromus*, *Phalaris* and *Phragmites*), within the same order (i.e: Poales: *Bromus*, *Phalaris*, *Phragmites* and *Typha*) and within the same herbaceous life or growth form. Moreover, our study confirmed the remarkable capacity for detecting *Phragmites* using spectroscopy thanks to its particular phenological characteristics that make it stand out from the background plant community, as previous studies have found (Rupasinghe & Chow-Fraser, 2019).

The invasion history of *Phragmites* in the United States and Canada, suggests that its current distribution is susceptible to expansion or shifting gradually over time, aided by the effects

of climate change (Guo et al., 2013). Therefore, predicting where and when invasions are likely to occur is critical to understand the ecological and evolutionary implications of the invasion and to plan appropriate control measures. Given the difficulty of eradicating introduced species like *Phragmites* after they have become established and acquired large ranges, there is an increasing need to develop better practices for early and accurate detection of invasive species (Gilbert et al., 2014; Larson et al., 2020). Especially, early detection of young individuals and incipient populations is critical management strategies including eradication efforts if control actions are to be effective and affordable to preserve biodiversity in wetland ecosystems.

An ideal monitoring program of invasive species at the ecosystem level takes into account different spatial, spectral and temporal scales. At present, the only way to obtain explicit and detailed information in these three domains is with the use high resolution sensors and remote sensing platforms that capture variation in functional traits at the individual level and document temporal changes in plant communities and at the ecosystem level. Thus, the coupling of high-resolution spectroscopy and remote sensing techniques with the use of drones, aircraft and satellites to detect and track the spread of invaders has the potential to transform invasive species monitoring.

Spatially and spectrally explicit distribution models are especially important for invasion risk analyses in order to predict future spread of invasive species. For example, using hyperspectral image data and a high-resolution light detection and ranging (LiDAR) digital elevation model, Andrew & Ustin (2009) mapped the invasive *Lepidium latifolium* in riparian and wetland areas in western US (Andrew & Ustin, 2009). The results showed that the current and predicted distributions indicate that the invader occurs in areas with low salinity and not prone to inundation (Andrew & Ustin, 2009). A recent study using remote sensing data of moderate spectral resolution, identified suitable germination grounds for the invasive *Phragmites* along the freshwater wetlands of the St Lawrence River in Québec, Canada (Tougas-Tellier et al., 2015). The resulting model suggests that current and future climate conditions create suitable germination grounds for *Phragmites* which could potentially offset the most extensive invasion of the common reed in North America (Tougas-Tellier et al., 2015). These studies showcase the utility of remote sensing in prevention as this information can inform conservation practitioners to better direct monetary and human efforts to implement better control actions in areas readily identified to be susceptible to future invasion in order to prevent them.

Beyond mapping invaders, a step needed to advance the role of remote sensing in canopy-level research lies in developing the quantitative linkages between foliar chemical and spectral properties, as the spectroscopy of canopies is driven primarily by the chemical composition of foliage (Asner et al., 2014a; Asner & Martin, 2009). A variety of foliar characteristics such as nitrogen, pigment content, water and even SLA have been linked to spectral measurements and retrieved with accuracy using leaf-level spectral reflectance (Sims & Gamon, 2002; Ustin et al., 2009). Canopy-level hyperspectral measurements have also been linked to plant chemistry, but it remains unclear as to how well leaf properties can be retrieved from spectral reflectance data acquired from airborne imaging spectrometers as many estimations are dependent on variable parameters like canopy structure, leaf angle of inclination, sun angle and atmospheric effects (Ollinger, 2011).

In some cases, variable canopy structure can decrease the sensitivity of remote spectral measurements to leaf chemicals (Asner, 1998; Jacquemoud et al., 1996). But on the other hand, due to multiple scattering in upper canopy layers, biologically driven covariance among leaf chemical and canopy structural properties, may enhance the apparent sensitivity of spectral data to certain foliar properties and leaf biochemicals (Asner & Martin, 2008; Barrett et al., 1993). Given the lack of generalization in procedures to retrieve and monitor changes in canopy composition, physiology and chemistry in natural systems, leaf-level work is needed for the development of remote sensing techniques as it provides a basis from which to test the potential gains and losses incurred when scaling up to the canopy level (Asner & Martin, 2008).

As the leaves are the most important plant surfaces interacting with solar energy, and the major ecological processes at the ecosystem-level like photosynthesis and primary production are related to nutritional status and growth conditions of vegetation, it is critical to understand the relationship between light absorption and scattering to biochemical constituents. Since quantifying multiple leaf properties is a challenge in any ecosystem, the fine-scale spectral library that our study provides constitutes a valuable contribution for conservation practitioners and biodiversity managers because it documents the chemical basis for the spectral differences we observed. Additionally, our results demonstrate potential for remote sensing applications with respect to what concerns the spectral domain. The use of full spectrum covering the 400 nm to 2500 nm spectral region increases the number of important spectral features available for analyses with respect to

multispectral data, but also allowed us to apply PLSR and PLSDA models that require the continuum spectrum as a single measurement rather than analyzing single band comparisons or band by band analyses (Asner & Martin, 2008; Schmidt & Skidmore, 2003). Our PLSR and PLSDA models showed that hyperspectral data capture sufficiently detailed information to accurately classify plants that might be taxonomically related or with similar morphological features, and that many of the foliar properties that underpin the differentiation among species can be accurately predicted from the spectra.

As advances in the modeling of pigments and biophysical parameters at the leaf level can be incorporated into models suitable for imaging spectrometers with the use of radiative transfer models (Jacquemoud et al., 1995, 1996), it is essential to develop a solid coupling of leaf radiative transfer models with canopy scale models for the successful retrieval of biochemical parameters from airborne and spaceborne platforms since most remote sensing of ecosystems is done at the canopy level (Ustin et al., 2009). In this respect, it is critical to acquire information about the spatial distribution of invasive species for invasion research. Maps of spatial distribution of invaders are useful for land managers to implement conservation and control plans in natural ecosystems and the spatial resolution of the data (which refers to the pixel size in an image) is a critical consideration as the ratio of spatial resolution relative to the size of the objects being classified (whether it is at the patch-level or individual plant and leaf-level) plays an important role in achieving adequate classification (Nagendra, 2001).

In general, high spatial resolution increases classification accuracy. However, with higher resolution, more and smaller pixels could increase spectral variance in the image, and as a result decrease spectral separability of target objects, whereas low spatial resolution can hardly discriminate objects on the ground (Nagendra & Rocchini, 2008). Remote sensing platforms of coarse spatial resolution like Landsat with a resolution of 30 m², can be very effective in detecting areas of large infestations but might be too coarse to detect incipient populations (Rupasinghe & Chow-Fraser, 2019). For example, Pengra et al. (2007) captured few if any pure pixels of the invasive *Phragmites* along a 7.5 km swath in a study area located in mid-western US using Hyperion with a resolution of 30 m². Given that early detection of individual plants and incipient populations is critical to accurately track *Phragmites* expansion and prevent further spread, high spatial resolution is critically important for its monitoring.

The use of hyperspectral unmanned-aerial vehicles (UAV) of ultra-high spatial resolution provide a means to overcome limitations in the spatial domain as pixel size can approximate the width of a single leaf (< 5 cm) (Arroyo-Mora et al., 2019). As an example, early results of the operation of the UAV- μ CASI system in three sites across Canada with different biophysical characteristics and conservation challenges (including two ecosystems threatened by the establishment of invasive species), found a close agreement between reflectance derived in the imagery and the reflectance acquired by field measurements, suggesting that such platforms can be used to address research questions in contexts that require spectral fidelity (Arroyo-Mora et al., 2019). Thus, the implementation of UAV-Hyperspectral imagers in ecological monitoring highlights the importance of supplementary field spectral data to verify fidelity of airborne spectral data and show potential to bridge the gap between field and airborne observations, yet the cost associated with acquiring and operating these platforms remains within the expensive range (Arroyo-Mora et al., 2019).

Alternative and less expensive platforms such as consumer-grade UAVs hold promise to detect individual small plants by remote sensing at high spatial and temporal resolution (Dandois et al., 2015). In a recent study, a UAV collecting geo-referenced high resolution (5 cm² pixels) visible imagery was employed to create a basin-wide distribution map of *Phragmites* along the Pearl River delta in Louisiana, US (Samiappan et al., 2017). An average of 85% accuracy in detection of the invasive *Phragmites* was achieved by applying a texture-based approach that differentiated *Phragmites* from all other land cover classes due to a particularity in roughness, granulation and regularity in the visible imagery (Samiappan et al., 2017). As well, UAVs equipped with high resolution RGB sensors and the use of computer vision algorithms (e.g. convolutional neural networks) could provide extremely detailed biophysical parameters at ultra-fine grain landscape scales (i.e. < 1 cm²) for individual plant detection (Cunliffe et al., 2016). In this case, detection would rely on structural parameters like differences in canopy architecture or spatial patterns rather than on spectral differences due to lower spectral resolution (given that RGB sensors are sensitive to only three bands in the VIS (Ashpure et al., 2019)). The results from our study showing that *Phragmites* could still be successfully distinguished from the surrounding vegetation from its higher reflectance in blue wavelengths, especially towards the end of the growing season, suggests that RGB imagery could potentially be used successfully to detect *Phragmites*, especially if its canopy architecture differs from other co-occurring species.

Moreover, the incorporation of phenology (i.e. the timing of periodic events such as flowering and leaf emergence or senescence) in invasion research is particularly useful for invasive species detection. Phenological differences or mismatches between native and invasive species have been documented in a variety of ecosystems and it has been suggested that they influence the invasion success of exotic species (Willis et al., 2010; Wolkovich & Cleland, 2011). The phenological uniqueness of certain invaders provides an outstanding opportunity for the characterization of spectral differences between the target species and the background vegetation (Boyte et al., 2015; Bradley & Mustard, 2005). Since the maximum differentiation between invaders and co-occurring species might occur only during a short period, and considering the potential high costs associated to the use of remote sensing platforms, before investing in an imaging spectroscopy campaign, it is important to get a sense of the expected efficiency of species discrimination and provisionally determine the best time to do so. For this purpose, the creation of spectral libraries of high temporal and spectral resolution is all-important as they can be used to address ecological and environmental question of various kinds.

In an ecological context of invasion by an invader with distinct phenology, the temporal resolution or the frequency of data acquisition becomes a critical consideration in order to capture such specific time-windows in natural ecosystems (Boyte et al., 2015; Bradley & Mustard, 2005; Willis et al., 2010). In the US, the invasion pattern of an invasive species of grass (*Bromus tectorum*) was remotely mapped using multirate AVIRIS data (Noujdina & Ustin, 2008). The detectability of the invader was more accurate with the use of multirate data in comparison with single-date imagery, and the authors attributed the success of mapping accuracy to clear spectral differences controlled by phenological dissimilarities between the invader and the surrounding vegetation (Noujdina & Ustin, 2008). Moreover, imagery acquired during key phenological events (such as blooming) or key phenological states (such as prolonged greenness in leaves) improves overall mapping accuracy of target species (Rupasinghe & Chow-Fraser, 2019).

High temporal resolution remote sensing data captures important aspects of ecosystem functioning to monitor changes in canopy composition, physiology, and chemistry through time. This has enormous value because understanding temporal dynamics of invasion can be useful to understand how an invader succeeds and to assess potential and real impacts of invasions in natural systems. In Hawaii, a time-series of Hyperion data was used to study the dynamics of rain forests

occupied by an invasive tree species (*Myrica faya*) and compared with forests occupied by a native one (*Metrosideros polymorpha*) (Asner et al., 2006). The authors were able to identify that warmer and drier times favor the spread of the invader which suggests that the invader's success might be determined by its ability to grow and increase in abundance under environmental stress, underscoring the importance of furthering our understanding of vegetation-climate interactions through time in a context of invasion (Asner et al., 2006).

Detailed spatial, temporal and spectral information on foliar trait variations and the scalability of their relationships to the canopy scale are much needed to monitor biodiversity at a global scale (Jetz et al., 2016). Deepening our understanding of spectral variation of vegetation is not only needed for biodiversity monitoring and trait mapping but is especially important considering current and future hyperspectral missions that rely on the application of spectroscopy to take the pulse of the planet everywhere and at all times, and constitute the only realistic way to document environmental changes and ecological processes, including biological invasions, at a global scale at a time when it is more urgent than ever (Ustin & Middleton, 2021).

Limitations and future research questions

Given that it has been shown that leaf-level variation in foliar traits is detectable remotely and can be distinguished from canopy structure (Asner & Martin, 2008, p. 200; Asner et al., 2015; Townsend et al., 2013), we expect that the spectral library provided by our study would serve in future remote sensing studies of invaded ecosystems by the invasive *Phragmites*. Since we focused on leaf-level measurements of high temporal and spectral resolution, we expect that this study will be a valuable contribution to the remote sensing community. Nevertheless, the spatial scale, which is a critical aspect of remotely sensed data was not explored by us and therefore, the most immediate research question for future studies would be how to scale from leaf-level spectroscopy to imaging spectroscopy by applying the spectra-trait relationships reported in our study? In addition, spatial resolution is a critical consideration for future studies to address the early detection of incipient populations of the common reed to prevent further spread, especially considering that numerous remote sensing studies of detection of the common reed have shown that it is possible to detect the invader, but in general, most platforms fail to capture stands of small size ($< 2 \text{ m}^2$). Moreover, incorporating spectral variation by developmental stage, which was not addressed in this study, would greatly enrich this and future spectroscopy studies to address the detection of young individuals of *Phragmites* that would be suitable for eradication strategies.

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Supplements

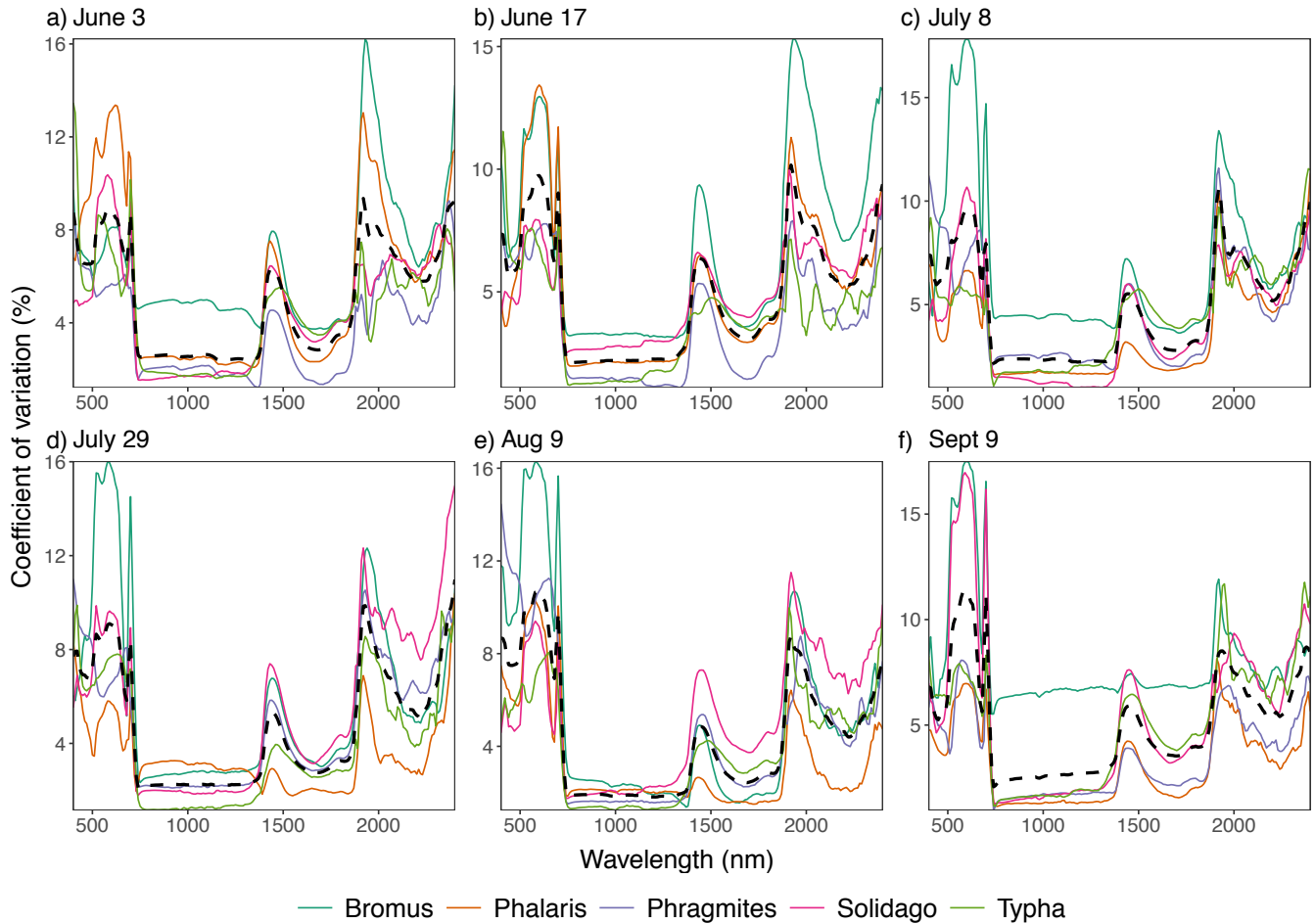


Figure S2.1. Spectral coefficients of variation (CV, %) across time. Colored lines represent CV across the spectral range (400-2400 nm) per species and the black dashed lines represents mean CV for all species studied.

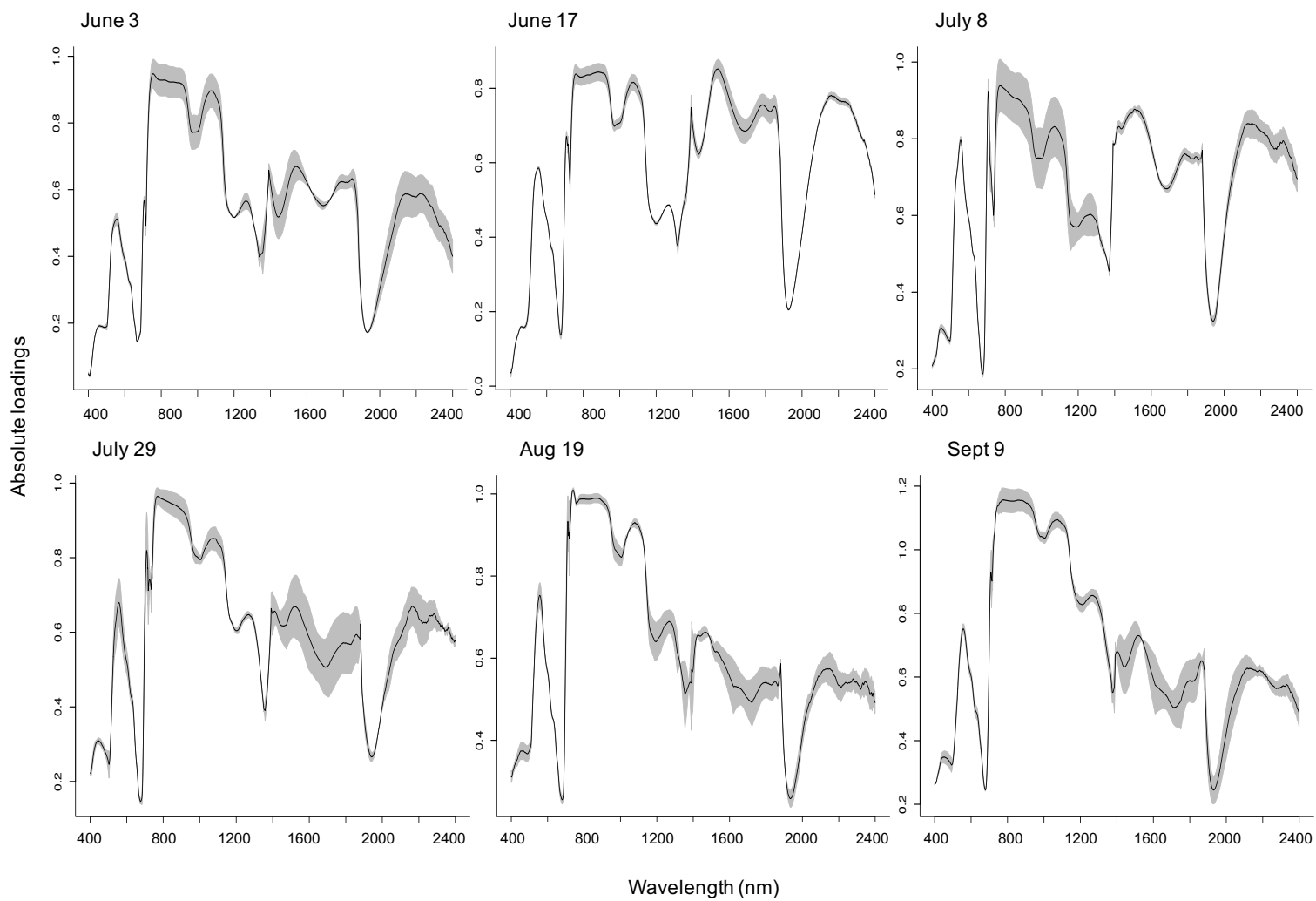


Figure S2.2. Absolute wavelength loadings of PLSDA model 1 through time.

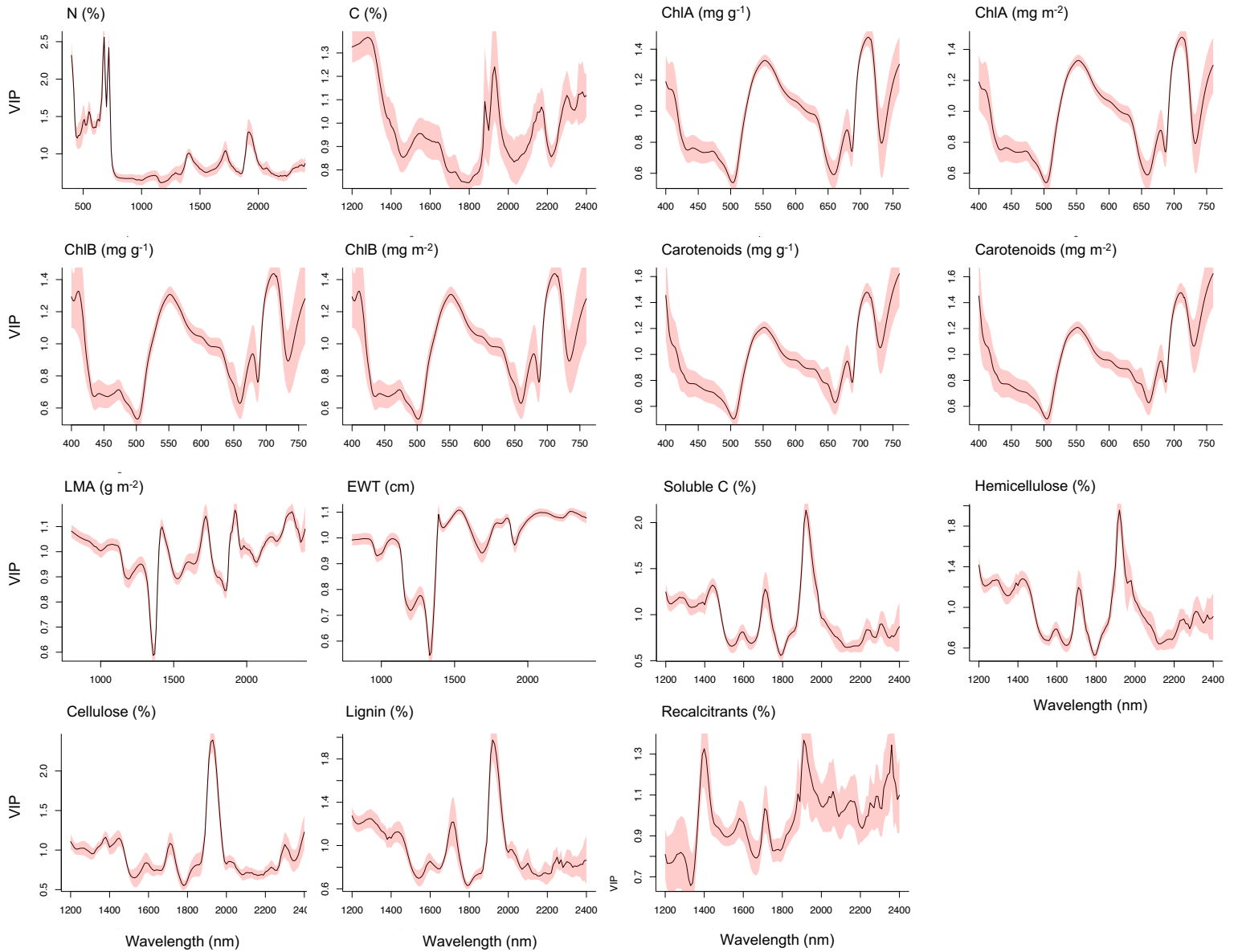


Figure S2.3. Variable importance of predictors (VIP) of wavelengths from PLSR models indicate their contribution to the predictions of the foliar traits studied.

Table S2.1. Statistics for partial least square regression (PLSR) models predicting functional traits from spectra ordered by descending R^2 for the calibration (cal) and validation (val) datasets. The strength of the relationship (R^2) indicates the absolute importance of each parameter in determining the spectral properties of species. RMSEP is the root mean square standard error of prediction in original measurement units. RSMEP (%) is the root mean square standard error of prediction as a percentage of the mean leaf trait. NRMSEP (%) is the normalized root mean square standard error of prediction (RMSEP/mean of measured values).

	Range	Mean (\pm SD)	Wavelength Range (nm)	Number of components	R^2		RMSEP		RMSEP (%)		NRMSEP (%)	
					cal	val	cal	val	cal	val	cal	val
EWT (cm)	0.01 - 0.05	0.02 (0.01)	800 - 2400	4	0.92	0.92	0.00	0.00	20.63	21.34	0.21	0.21
LMA (g m^{-2})	30.6 - 130.3	64.6 (23.4)	800 - 2400	8	0.91	0.88	7.15	8.28	11.06	12.82	0.11	0.13
Cellulose (%)	9.0 - 34.3	23.0 (6.5)	1200 - 2400	12	0.89	0.85	2.09	2.52	9.07	10.96	0.09	0.11
Soluble C (%)	23.7 - 79.3	48.7 (12.0)	1200 - 2400	8	0.83	0.80	4.89	5.40	10.03	11.08	0.10	0.11
Hemicellulose (%)	7.1 - 42.9	24.5 (7.5)	1200 - 2400	8	0.78	0.74	3.46	3.84	14.14	15.66	0.14	0.16
N (%)	1.23 - 4.43	2.51 (0.61)	400 - 2400	9	0.70	0.64	0.33	0.37	13.10	14.87	0.13	0.15
Chlorophyll a (mg m^{-2})	174.1 - 1560.9	549.3 (221.0)	400 - 760	6	0.61	0.59	137.60	143.13	25.05	26.06	0.25	0.26
Chlorophyll a (mg g^{-1})	2.7 - 24.5	8.7 (3.5)	400 - 760	6	0.60	0.59	2.17	2.26	25.07	26.08	0.25	0.26
Chlorophyll b (mg m^{-2})	55.9 - 502.5	179.5 (69.1)	400 - 760	6	0.55	0.54	45.96	47.93	25.61	26.70	0.26	0.27
Chlorophyll b (mg g^{-1})	0.9 - 7.9	2.8 (1.1)	400 - 760	6	0.55	0.53	0.73	0.76	25.66	26.77	0.26	0.27
Carotenoids (mg m^{-2})	36.8 - 311.2	120.1 (44.2)	400 - 760	7	0.52	0.50	30.37	31.70	25.19	26.30	0.25	0.26
Carotenoids (mg g^{-1})	0.58 - 4.89	1.89 (0.70)	400 - 760	7	0.53	0.50	0.48	0.50	25.29	26.40	0.25	0.26
Lignin (%)	0.6 - 11.0	3.1 (2.0)	1200 - 2400	8	0.58	0.50	1.29	1.48	41.31	47.46	0.41	0.47
C (%)	40.0 - 46.9	44.2 (1.3)	1200 - 2400	6	0.48	0.43	0.92	0.97	2.09	2.20	0.02	0.02
Recalcitrants (%)	0.02 - 2.59	0.61 (0.49)	1200 - 2400	7	0.45	0.36	0.36	0.39	58.51	64.37	0.59	0.64