

1 **Seasonal variability of multiple leaf traits captured by**
2 **leaf spectroscopy at two temperate deciduous forests**
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19 **Keywords:** phenology, leaf physiology, foliar chemistry, carbon cycle, chlorophyll,
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31 **Abstract**

32 Understanding the temporal patterns of leaf traits is critical in determining the seasonality
33 and magnitude of terrestrial carbon and water fluxes. However, robust and efficient ways
34 to monitor the temporal dynamics of leaf traits are lacking. Here we assessed the
35 potential of using leaf spectroscopy to predict leaf traits across their entire life cycle,
36 forest sites, and light environments (sunlit vs. shaded) using a weekly sampled dataset
37 across the entire growing season at two temperate deciduous forests. The dataset includes
38 field measured leaf-level directional-hemispherical reflectance/transmittance together
39 with seven important leaf traits [total chlorophyll (chlorophyll a and b), carotenoids,
40 mass-based nitrogen concentration (N_{mass}), mass-based carbon concentration (C_{mass}), and
41 leaf mass per area (LMA)]. All leaf properties, including leaf traits and spectra, varied
42 significantly throughout the growing season, and displayed trait-specific temporal
43 patterns. We used a Partial Least Square Regression (PLSR) analysis to estimate leaf
44 traits from spectra, and found a significant capability of PLSR to capture the variability
45 across time, sites, and light environment of all leaf traits investigated ($R^2=0.6\sim 0.8$ for
46 temporal variability; $R^2=0.3\sim 0.7$ for cross-site variability; $R^2=0.4\sim 0.8$ for variability from
47 light environments). We also tested alternative field sampling designs and found that for
48 most leaf traits, biweekly leaf sampling throughout the growing season enabled accurate
49 characterization of the leaf trait seasonal patterns. Increasing the sampling frequency
50 improved in the estimation of N_{mass} , C_{mass} and LMA comparing with foliar pigments. Our
51 results, based on the comprehensive analysis of spectra-trait relationships across time,
52 sites and light environments, highlight the capacity and potential limitations to use leaf

- 53 spectra to estimate leaf traits with strong seasonal variability, as an alternative to time-
- 54 consuming traditional wet lab approaches.

55 1. Introduction

56 Leaf traits are important indicators of plant physiology (Wright et al. 2004), and
57 critical components in numerous ecological processes (Kattge et al. 2011). For example,
58 Leaf chlorophyll concentration represents the light harvesting potential and is related to
59 photosynthetic activity (Niinemets 2007; Laisk et al. 2009), while accessory pigments
60 such as carotenoids protect leaves from damage when exposed to excessive sunlight
61 (Demmig-Adams and Adams 2000). Leaf mass per area (LMA) describes plants'
62 investment to leaves in terms of carbon and nutrients to optimize sunlight interception
63 (Poorter et al., 2009). Carbon is one of the major elements in cellulose and lignin, which
64 are used to build the cell walls of various leaf tissues (Kokaly et al. 2009). Nitrogen is the
65 key element in both carbon fixation enzyme RuBisCO and chlorophyll (Evans 1989), and
66 thus plays an important role in modeling leaf and canopy photosynthesis (Bonan et al.
67 2012). The aforementioned leaf traits strongly depend on leaf developmental stages and
68 light environments (Yang et al. 2014; Lewandowska and Jarvis 1977; Poorter et al. 2009;
69 Wilson et al. 2000). Thus, capturing the spatial and temporal variations of these leaf traits
70 is necessary to understand terrestrial ecosystem functioning (Schimel et al. 2015).

71 Despite the importance and increasing interests in the temporal and spatial
72 variability of these (and many other) leaf traits, the capacity to monitor these traits over
73 seasons has not progressed accordingly. Wet chemistry analysis of these leaf traits is
74 considered to be the standard method, yet the destructive and time-consuming protocols
75 do not allow for rapid and repeated sampling (including of the same leaves). On the other
76 hand, field spectroscopy has shown promise in the augmentation of the traditional
77 approaches (Asner and Martin 2008; Serbin et al. 2014). Despite this promise, many
78 previous efforts that predict leaf traits using spectroscopy only focused on mature sunlit

79 leaves (e.g., Asner and Vitousek 2005; Ustin et al. 2004; Wicklein et al. 2012; but see
80 Sims and Gamon (2002)) and have not explored the ability to track the continuous and
81 developmental changes of leaf traits throughout the growing season. The temporal
82 dimension of the spectra-trait relationship has mostly focused on leaf chlorophyll
83 concentration (Belanger et al. 1995; Dillen et al. 2012; Shen et al. 2009), while it is
84 largely unknown for other important leaf traits like nitrogen, carbon concentration and
85 LMA. Moreover, the availability of high temporal resolution (~weekly) datasets on
86 important leaf traits and spectra is limited. These data would be very useful for assessing
87 the utility of leaf spectral properties (i.e. reflectance) for estimating the temporal
88 variability of leaf traits, as well as scaling to broader regions and informing modeling
89 activities.

90 Leaf traits not only change with time, but also with the light environments, such
91 as the sun-lit or shaded light condition and the accompanying changes in microclimate,
92 affect leaf traits (Ellsworth and Reich 1993; Niinemets, 2007), as a consequence of
93 underlying fundamental evolutionary and ecophysiological constraints (Terashima et al.
94 2001). For example, shaded leaves display lower chlorophyll a to b ratio and higher LMA
95 compared with sunlit leaves (Niinemets, 2007). As such, it is important to not only
96 explore trait variation in space but also as in the vertical dimension to better capture
97 ecosystem responses to global change.

98 Three categories of methods to estimate leaf traits from leaf spectral properties
99 (i.e., reflectance and transmittance) are spectral vegetation indices (SVIs), statistical
100 inversion methods exploiting the full wavelength (400 – 2500 nm), and leaf radiative
101 transfer models like PROSPECT (Jacquemoud and Baret 1990), which are limited to a

102 few leaf traits and thus are not the focus of this study. SVIs are typically calculated using
103 the reflectance from two or three wavelengths (Huete et al. 2002; Richardson et al. 2002;
104 Sims and Gamon 2002). With proper calibration across a diverse range of vegetation
105 types, SVIs can yield relatively robust models (Féret et al. 2011). While statistical
106 methods such as Partial Least Square Regression (PLSR) modeling has become more
107 popular in recent years with the availability of high-resolution spectra and increasing
108 computational power (Asner and Martin 2008; Couture et al. 2013; Wold et al. 2001).
109 Although both being widely used, these methods have not been thoroughly assessed,
110 especially with respect to the robustness of PLSR models across time and different light
111 environments (but see Serbin et al., 2014).

112 Here we aim to assess the ability of leaf optical properties to track temporal
113 variability of a suite of leaf traits across sites and different light environments. We
114 collected a dataset of ~weekly-sampled leaf traits [including total chlorophyll (and
115 chlorophyll a and b), carotenoids, mass-based nitrogen concentration (N_{mass}), mass-based
116 carbon concentration (C_{mass}), and LMA] along with *in situ* directional-hemispherical
117 reflectance/transmittance during the growing season at two temperate deciduous forests.
118 We first presented the temporal variations of leaf traits and spectra, and then highlight the
119 ability of leaf spectra to track temporal variability of leaf traits. We investigate the
120 robustness of the PLSR across season, sites, and growth environments. We further
121 explore the optimal field sampling strategy. Finally, we conclude by discussing the broad
122 implications of our study.

123

124 2. Study area and methods

125 2.1. Study sites

126 Our field sampling was conducted in two temperate deciduous forests located in
127 the northeastern United States. The first site, on the island of Martha's Vineyard (MV,
128 41.362N, 70.578W), is a white oak (*Quercus alba*) dominated forest with a forest age of
129 80-115 years after natural recovery from abandoned cropland and pasture (Foster et al.
130 2002). Mean annual temperature is 10°C, and annual precipitation is about 1200 mm
131 from 1981 to 2010 (Yang et al. 2014). Site 2, Harvard Forest (HF, 42.538N, 72.171W),
132 has two dominating deciduous tree species: red oak (*Quercus rubra*) and red maple (*Acer*
133 *rubrum*), with a few scattered yellow birch (*Betula alleghaniensis*). The forest age is 70-
134 100 years. The annual mean temperature is about 7.5°C (Wofsy et al. 1993), and the
135 annual precipitation is 1200 mm. Remote sensing studies suggested that the start of
136 season in Martha's Vineyard is about 10-20 days later than that of HF (Fisher and
137 Mustard 2007; Yang et al. 2012).

138 2.2. Measurements of leaf spectral properties and traits

139 We conducted two field campaigns to collect leaf traits at Martha's Vineyard and
140 Harvard Forest. In 2011, weekly (biweekly in August) sampling of leaves throughout the
141 growing season (June - November) was conducted at the Martha's Vineyard on three
142 white oak trees. For each sampling period, we cut two fully sunlit branches (each having
143 ~6 leaves) and one shaded branch using a tree pruner. The spectral properties of the
144 leaves were immediately measured (see below). Then the leaves were placed in a plastic
145 bag containing a moist paper towel, and all the samples were kept in a cooler filled with
146 ice until being transferred back to the lab for further measurements. In 2012, the same
147 weekly (biweekly from mid-July to late August) measurements in Harvard Forest were

148 made on five individuals (two red oaks, two red maples and one yellow birch) from May
149 to October. For each tree, two sunlit and one shaded branch were collected each time.

150 Directional-hemispherical leaf reflectance and transmittance were measured
151 immediately after the sampling using a spectroradiometer (ASD FS-3, ASD Inc. Boulder,
152 CO, USA; spectral range: 300-2500 nm, spectral resolution: 3 nm@700 nm, 10
153 nm@1400/2100 nm) and an integrating sphere (ASD Inc.). The intensity of light source
154 in the integrating sphere decreases sharply beyond 2200 nm, with the signal in 2200-2500
155 nm being noisy (ASD Inc., personal communications), and thus is excluded from the
156 spectral-leaf traits analysis below.

157 The measured leaf traits include total chlorophyll concentration (including
158 chlorophyll a and chlorophyll b, $\mu\text{g}/\text{cm}^2$), carotenoids ($\mu\text{g}/\text{cm}^2$), leaf mass per area (LMA,
159 g/m^2), nitrogen concentration by mass (N_{mass} , %), and carbon concentration by mass
160 (C_{mass} , %). Each branch was divided into two subsets. One subset was used to measure
161 pigment concentrations. To measure the chlorophyll and carotenoids concentration, three
162 leaf discs ($\sim 0.28 \text{ cm}^2$ each) were taken from each leaf using a hole puncher, and then
163 ground in a mortar with 100% acetone solution and MgO (Asner et al. 2009). After an 8-
164 minute centrifugation, the absorbance of the supernatant was measured using a
165 spectrophotometer (Shimadzu UV-1201, Kyoto, Japan). Chlorophyll a, b and carotenoids
166 concentrations were calculated using the readings from 470, 520, 645, 662 and 710 nm
167 (Lichtenthaler and Buschmann 2001). The other subset (3 leaves) was scanned using a
168 digital scanner (EPSON V300, EPSON, Long Beach, CA, USA), and oven-dried (65°C)
169 for at least 48 hours for quantification of leaf dry mass. LMA was calculated based on the
170 following equations:

171

$$LMA = W_{dry} / A_{leaf}$$

172 where W_{dry} is leaf dry mass weight, A_{leaf} is the leaf area calculated from the scanned leaf
 173 using ImageJ (Schneider et al. 2012). Dried leaves were then ground and analyzed for
 174 N_{mass} and C_{mass} with a CHNS/O analyzer (FLASH 2000, Thermo Scientific, Waltham,
 175 MA, USA).

176

2.3. Methods to estimate leaf traits using leaf spectral properties

177

178 We used two categories of methods to estimate leaf traits based on leaf spectral
 179 properties: vegetation indices that utilize the reflectance from two wavelengths, and
 180 statistical methods that exploit the information from the full leaf spectrum.

180

181 Based on extensive datasets from various types of biomes and plants, Féret et al.
 182 (2011) established polynomial relationships between SVIs and total chlorophyll
 183 concentration, carotenoids and LMA (Table 1). We also obtained the best estimate of a, b,
 184 and c using our own dataset (see below for the division between training and validation
 185 dataset).

185

186 **Table 1** Simple Vegetation Indices (SVI) used in this study. These indices were
 187 calibrated using extensive datasets (Féret *et al.* 2011). Leaf traits were calculated based
 188 on a polynomial relationship: leaf trait = $a \times \text{index}^2 + b \times \text{index} + c$.

Leaf traits	Index	Coefficients		
		a	b	c
Chl ($\mu\text{g}/\text{cm}^2$)	$(R_{780}-R_{712})/(R_{780}+R_{712})$	40.65	121.88	-0.77
Car ($\mu\text{g}/\text{cm}^2$)	$(R_{800}-R_{530})/(R_{800}+R_{530})$	8.09	11.18	-0.38
LMA (g/cm^2)	$(R_{1368}-R_{1722})/(R_{1368}+R_{1722})$	-0.1004	0.1286	-0.0044

188

212 the corresponding wavelength. Generally wavelengths with VIP value larger than 1 are
213 considered being important (Mehmood et al. 2012).

214

215 2.4. Robustness of PLSR models and scenarios for field sampling design

216 To examine the robustness of PLSR models across time, light environment, and
217 sites, we designed the following scenarios. In all the scenarios, we used leaf traits and
218 spectra of a subset of the whole dataset (e.g., leaf samples that are collected during only a
219 certain period of time, or a certain level of light environment) to build PLSR models, and
220 test the performance of the models against the remaining dataset.

221 For this we created five scenarios to examine how the timing of leaf sampling
222 affects predictability of seasonality of leaf traits. Leaf traits and spectra in the first three
223 scenarios were sampled only for the spring, summer, and fall, respectively. We defined
224 these three seasons based on variations in total chlorophyll concentration: days before
225 total chlorophyll reached a plateau in the mid-season were defined as spring; days when
226 total chlorophyll started to decrease were defined as fall; days between spring and fall
227 were defined as summer. The last two scenarios were that leaf traits and spectra were
228 sampled monthly or biweekly (instead of weekly as in the full dataset). We then use the
229 PLSR trained with leaf samples in the above scenarios to predict the leaf traits of the
230 entire dataset. There are two reasons to choose the whole dataset for validation: 1) the
231 whole dataset captures the temporal variability of leaf traits, which is the goal of this test;
232 2) it is necessary to have the same validation dataset to test the performance of these five
233 scenarios. Performance of these sampling strategies was measured by calculating the
234 RMSE and R^2 .

235 We also explored our capacity to develop a generalized approach for capturing
236 seasonality in leaf traits with spectral observations. Two tests were conducted to examine
237 the robustness of PLSR models at different light environment and sites. Test 1 used sunlit
238 leaf traits and spectra to train a PLSR model, which was then used to predict shaded leaf
239 traits with corresponding spectra. We then switched the training and validation datasets
240 so that shaded leaves were used to train PLSR model which sunlit leaves were used to
241 validate. Test 2 divided the entire dataset into two subsets by geographic location: we
242 used Martha's Vineyard dataset to calibrate the model, and Harvard Forest dataset to
243 validate, and vice versa.

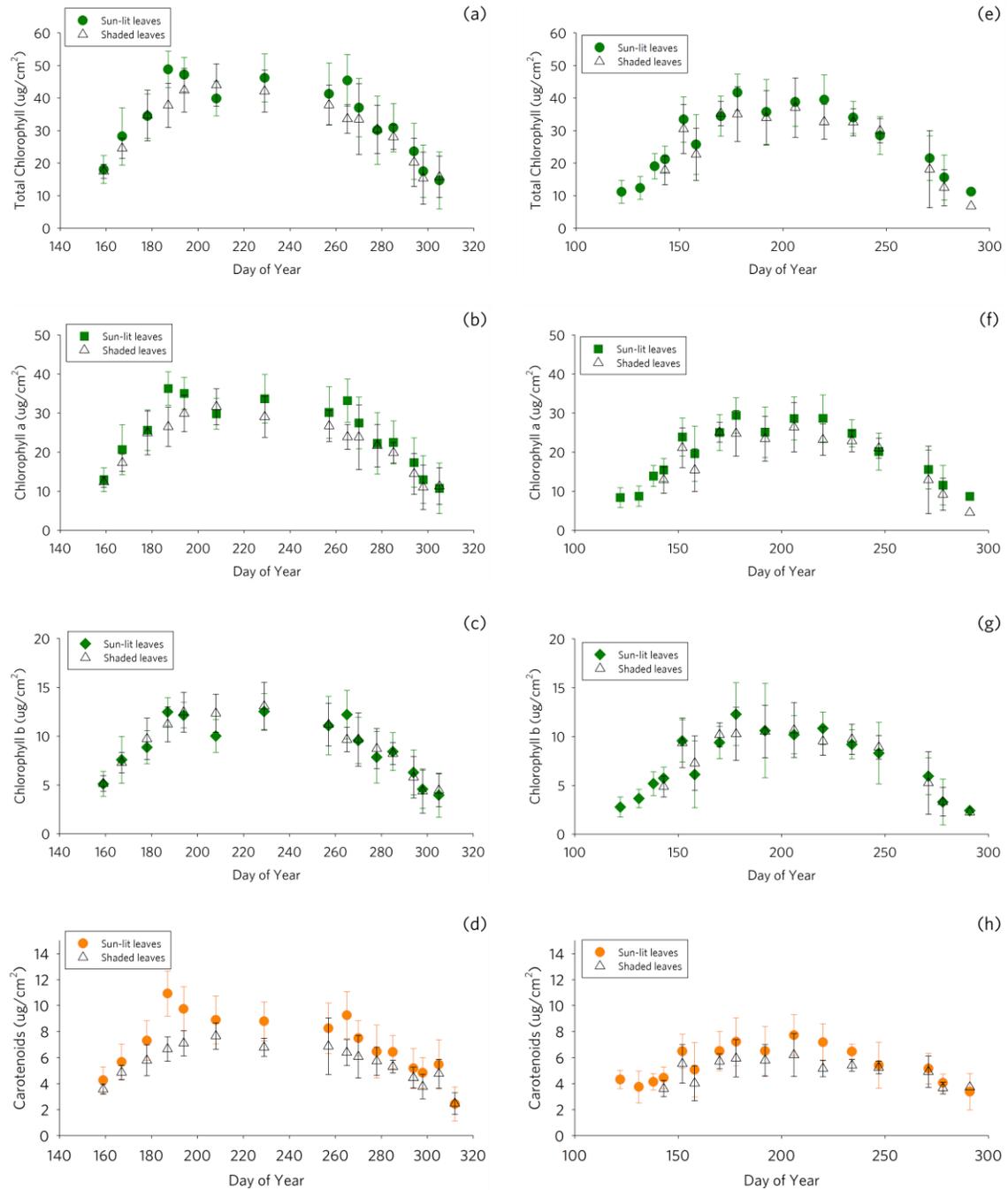
244 3. Results

245 3.1. Temporal and spatial variability of leaf traits

246 All leaf traits displayed significant temporal variations throughout the growing
247 season (Fig.1 and 2). Overall, pigments from both sites have similar bell-shaped
248 trajectories, despite being sampled from different species and locations within the
249 canopy. Chlorophyll and carotenoids concentration rapidly increased from $\sim 10 \mu\text{g}/\text{cm}^2$ at
250 the beginning of the season, and then stabilized around $\sim 50 \mu\text{g}/\text{cm}^2$ and $\sim 40 \mu\text{g}/\text{cm}^2$ in
251 Martha's Vineyard and Harvard Forest respectively during the summer followed by a
252 decline in the fall to $10 \mu\text{g}/\text{cm}^2$ before leaf shedding. The Harvard Forest samples were
253 from three different species, and showed much larger variability compared with Martha's
254 Vineyard, especially for the shaded leaves (Fig.1 e-h). The carotenoids concentration was
255 $\sim 3 \mu\text{g}/\text{cm}^2$ at the beginning/end of the season and $\sim 10 \mu\text{g}/\text{cm}^2$ at the peak season. The
256 total chlorophyll concentration relative to the carotenoids concentration (Chl/Car)
257 increased during the early seasons. In the fall, though both chlorophyll and carotenoids

258 decreased, Chl/Car decreased steadily, as a result of faster decline of chlorophyll relative
 259 to the carotenoids (Fig. S1a).

260

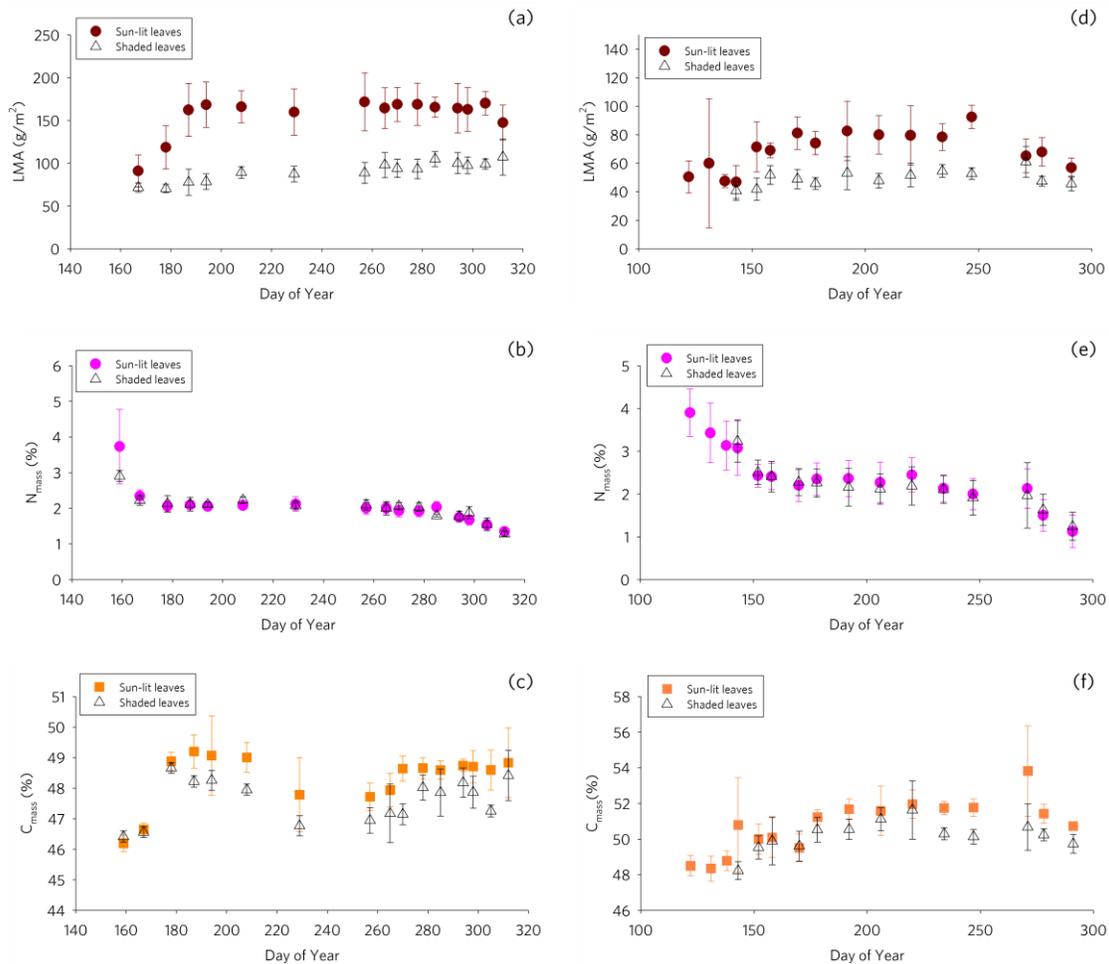


261

262 **Figure 1** Seasonal patterns of pigments of sunlit (diamonds) and shaded (open triangles)
 263 leaves from two deciduous forests. Martha's Vineyard, year 2011: (a) Total chlorophyll;

264 (b) chlorophyll a; (c) chlorophyll b; (d) carotenoids. Harvard Forest year 2012: (e) Total
 265 chlorophyll; (f) chlorophyll a; (g) chlorophyll b; (h) carotenoids. Each dot is the mean
 266 value of all the samples collected that day. Error bars are standard deviations.

267



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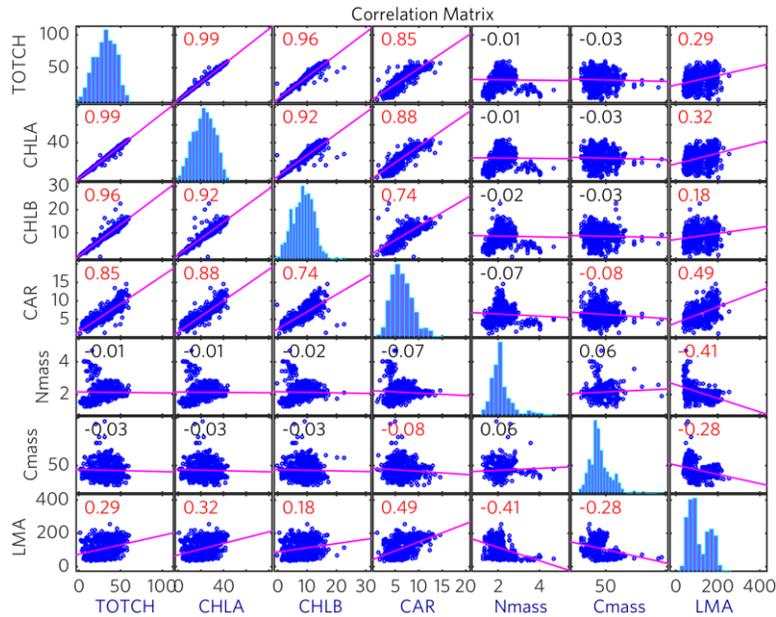
269 **Figure 2** Seasonal patterns of biochemical and biophysical properties of sunlit (closed
 270 symbols) and shaded (open symbols) leaves from two deciduous forest sites. Martha's
 271 Vineyard, year 2011: (a) Leaf mass per area (LMA); (b) mass-based nitrogen
 272 concentration (N_{mass}); (c) mass-based carbon concentration (C_{mass}). Harvard Forest, year
 273 2012: (d) LMA; (e) N_{mass} ; (f) C_{mass} . Each dot is the mean value of all the samples
 274 collected that day. Error bars are standard deviations.

275 The remaining three leaf traits (LMA, N_{mass} , and C_{mass}) displayed different
 276 seasonal patterns compared with leaf pigments (Fig. 2). For example, LMA rapidly
 277 increased in the spring, but showed only a minor decline by the end of the measurement

278 period. N_{mass} was higher (~4-5%) at the start of the season, and remained stable around 2%
279 during the summer, followed by ~1% decrease in the fall, presumably caused by nitrogen
280 resorption (Eckstein et al. 1999). Similar to LMA, C_{mass} accumulated 2-4% in the spring
281 and stabilized for the rest of the growing seasons around 50%. The rapid increase of
282 LMA in the spring was accompanied by a similar increase of C_{mass} and decrease of N_{mass} ,
283 which all ended at the same time (DOY ~194 in Martha's Vineyard, and DOY ~170 in
284 Harvard Forest).

285 Mean annual values of leaf traits from Martha's Vineyard were significantly
286 different from those at Harvard Forest (Table 2). For example, leaf chlorophyll in
287 Martha's Vineyard is $5.64 \mu\text{g}/\text{cm}^2$ (17.5%) higher than that from Harvard Forest ($p <$
288 0.0001). LMA in Martha's Vineyard showed much larger variation than that from
289 Harvard Forest, and the mean LMA was $39.85 \text{ g}/\text{m}^2$ (37.5%) higher than that from HF.
290 Similar situation applies to all other leaf traits except for C_{mass} , for which value at HF
291 were higher than the traits at MV.

292 Sunlit leaves contained more total chlorophyll and carotenoids (Fig. S2) and the
293 carotenoids to the total chlorophyll ratio was significantly higher for sun-lit leaves
294 comparing with shaded leaves (Martha's Vineyard, $p < 0.0001$; Harvard Forest, $p =$
295 0.0182). Chlorophyll a/b was also significantly larger for sunlit leaves in both sites (MV,
296 $p < 0.0001$; HF, $p < 0.0001$). Similarly, LMA and C_{mass} values were significantly higher
297 in the sun-lit leaves versus shaded foliage, with the only exception of N_{mass} , in which both
298 sun-lit and shaded leaves were indistinguishable throughout the two seasons (Fig. 2b).



299

300 **Figure 3** Correlation matrix of all the leaf traits. Histograms of each leaf traits are on the
 301 diagonal positions. Number on each subplot indicates R^2 (Red means $p < 0.05$). See Table
 302 2 for units.

303 **Table 2** Annual mean values and standard deviation of leaf traits at two sites (stars
 304 indicate the p-values of t-test between the values of leaf traits from two sites: ***:
 305 $p < 0.0001$; **: $p < 0.01$; *: $p < 0.05$).

Leaf traits	Units	MV	HF
Total Chl ($\mu\text{g}/\text{cm}^2$)***	$\mu\text{g}/\text{cm}^2$	31.74 (12.17)	26.19 (9.29)
Chl a ($\mu\text{g}/\text{cm}^2$)**	$\mu\text{g}/\text{cm}^2$	23.19 (8.81)	18.92 (6.70)
Chl b ($\mu\text{g}/\text{cm}^2$)**	$\mu\text{g}/\text{cm}^2$	8.70 (3.31)	7.48 (2.73)
Car ($\mu\text{g}/\text{cm}^2$)**	$\mu\text{g}/\text{cm}^2$	6.16 (2.28)	5.59 (1.33)
N_{mass} (%)**	% (unitless)	2.17 (0.50)	2.03 (0.50)
C_{mass} (%)***	% (unitless)	48.34 (1.24)	51.12 (0.87)
LMA (g/cm^2)***	g/m^2	106.29 (45.04)	66.44 (15.56)

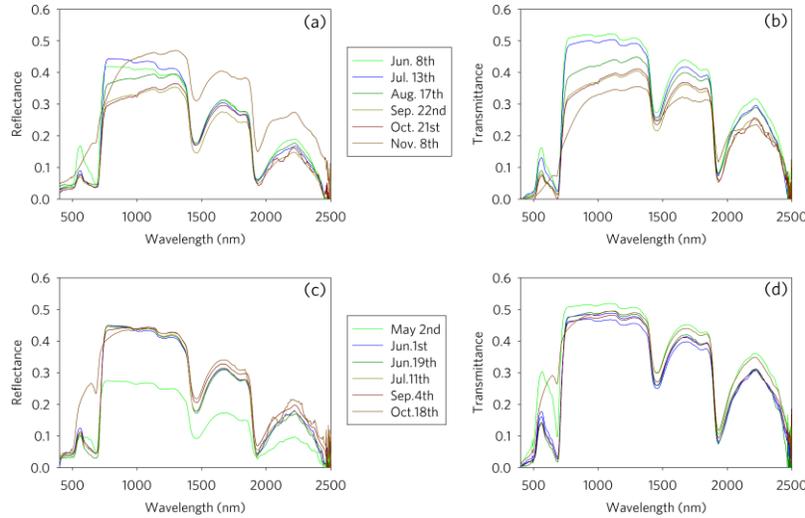
306

307 A linear regression analysis highlighted various levels of correlation among leaf
308 traits (Fig. 3). Close correlation was found among leaf pigments: total chlorophyll
309 concentration was highly correlated with carotenoids concentration ($R^2 = 0.85$),
310 suggesting a tight coupling among those pigments throughout the growing season despite
311 the faster decrease of chlorophyll concentration during the senescence (Fig. S1). For the
312 entire dataset (across all sunlit and shaded leaves from different species), N_{mass} was
313 weakly correlated with pigments. LMA showed positive correlation with all pigments
314 while a negative correlation was observed with N_{mass} and C_{mass} .

315

316 3.2. Seasonal variability of leaf spectral properties

317 The full leaf reflectance and transmittance spectrum showed significant variability
318 in both amplitude and shape (Fig.4). The visible (VIS, 400 – 700 nm) and near infrared
319 (NIR, 700-1000 nm) changed dramatically throughout the season, while shortwave
320 infrared (SWIR, 1000-2500 nm) was relatively stable. Data from Martha's Vineyard
321 showed larger variability in NIR compared to Harvard Forest.



322

323 **Figure 4** Examples of leaf directional-hemispherical reflectance and transmittance
 324 measured on (a, b) Martha’s Vineyard and in (c,d) Harvard Forest.

325 Fig.S3 shows the seasonal variations of individual bands. The R, G, and B
 326 reflectance at both sites showed a U-shape pattern (Fig. S3a, S3c): all of them decreased
 327 in the beginning of the season; and increased in the end of the season after a stable
 328 summer. The NIR from Martha’s Vineyard showed a consistent decline in the mid-
 329 summer and then increased in the fall, while the NIR from Harvard Forest was relatively
 330 stable throughout the season. Leaf transmittance at each band had similar patterns as the
 331 reflectance (Fig. S3b, S3d).

332 3.3. Comparisons of methods of leaf traits estimation

333 We compared two categories of methods to estimate leaf traits from leaf spectra.
 334 Overall, PLSR consistently outperformed the SVIs in estimating leaf traits, showing an
 335 improved performance when the SVIs were trained by the original datasets or our own
 336 dataset (Table 3). The PLSR models using leaf reflectance (PLSR_{ref} hereafter) had
 337 slightly better performance compared with those using leaf transmittance (PLSR_{tra}

338 hereafter) when assessed with the independent dataset. For different leaf traits, the
339 performance of these methods varied, as described in details below.

340 Leaf chlorophyll from the validation dataset was well estimated by $PLSR_{ref}$
341 (Fig.5. $R^2 > 0.70$ and $NRMSE < 10\%$). The SVI for chlorophyll showed slightly larger
342 prediction error ($0.5 \mu\text{g}/\text{cm}^2$) compared with $PLSR_{ref}$ and $PLSR_{tra}$ (Table 3). The two
343 components of chlorophyll (chl a and b) were also well captured by the $PLSR_{ref}$ approach
344 with $NRMSE$ less than 10% and R^2 of 0.73 and 0.66 respectively. Similarly, carotenoids
345 were estimated relatively well by $PLSR_{ref}$ and $PLSR_{tra}$ ($R^2 > 0.65$) but the SVI for
346 carotenoids had higher 30% higher RMSE comparing with $PLSR_{ref}$.

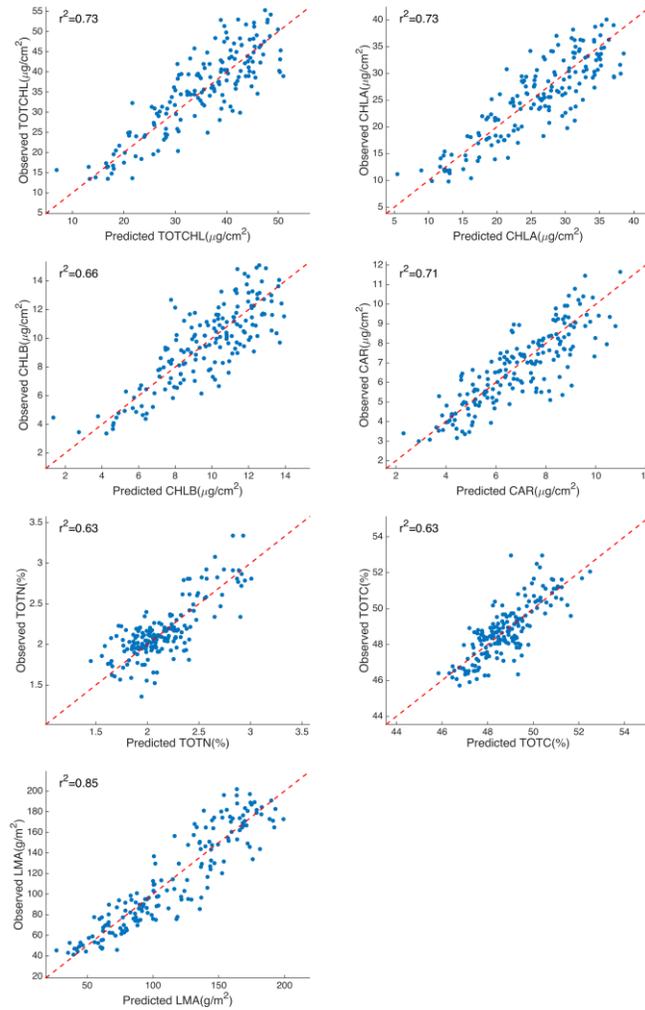
347 N_{mass} was well captured by leaf spectra especially with the reflectance dataset
348 (Fig.5. $R^2 > 0.6$ and $NRMSE < 5\%$). Similarly, both $PLSR_{ref}$ and $PLSR_{tra}$ explained ~60%
349 of the variance in C_{mass} ($R^2 > 0.6$ and $NRMSE < 7\%$). PLSR also displayed a strong
350 capacity to predict LMA ($R^2 = \sim 0.80$ and $NRMSE < 9\%$), where the SVI for LMA could
351 not capture more than 20% of the variation in LMA and more than double the RMSE of
352 $PLSR_{ref}$ mainly due to a saturation effect (data not shown).

353

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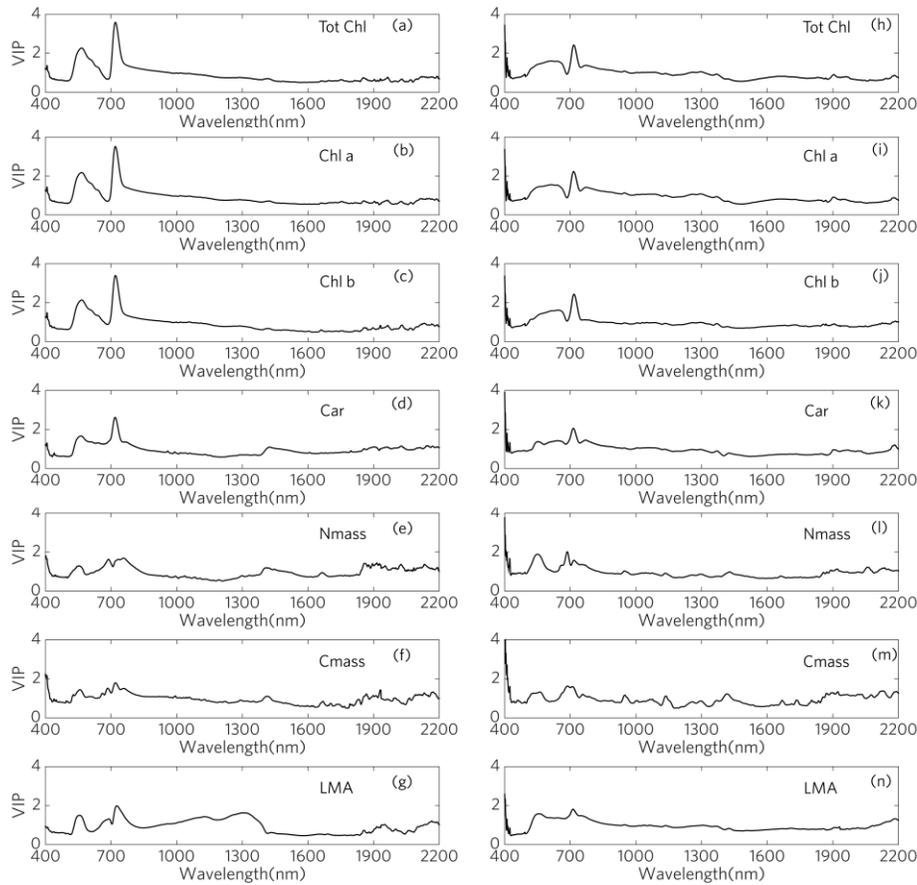
355 **Table 3** Comparisons among methods in terms of the goodness-of-fit (RMSE, NRMSE and R^2) for the dataset at both Martha's
 356 Vineyard and Harvard Forest. PLSR_{ref} indicates models using reflectance dataset to predict leaf traits. PLSR_{tra} indicates models using
 357 transmittance dataset to predict leaf traits.

Leaf traits	RMSE (NRMSE)				R^2			
	Simple indices (Féret <i>et al.</i> 2011)	Simple indices (this dataset)	PLSR _{ref}	PLSR _{tra}	Simple indices (Féret <i>et al.</i> 2011)	Simple indices (this dataset)	PLSR _{ref}	PLSR _{tra}
Total Chl ($\mu\text{g}/\text{cm}^2$)	5.93	6.04	5.48 (0.09)	5.62 (0.10)	0.71	0.71	0.73	0.64
Chl a ($\mu\text{g}/\text{cm}^2$)			3.99 (0.09)	4.14 (0.09)			0.73	0.68
Chl b ($\mu\text{g}/\text{cm}^2$)			1.62 (0.07)	1.82 (0.08)			0.66	0.58
Car ($\mu\text{g}/\text{cm}^2$)	1.53	1.54	1.07 (0.08)	1.20 (0.09)	0.39	0.40	0.71	0.68
N_{mass} (%)			0.22 (0.05)	0.24 (0.05)			0.63	0.54
C_{mass} (%)			0.93 (0.07)	0.95 (0.07)			0.63	0.71
LMA (g/cm^2)	40.6	39.7	18.11 (0.08)	19.01 (0.09)	0.20	0.19	0.85	0.79



358

359 **Figure 5** Comparisons between the observed leaf traits and predicted traits from PLSR_{ref}.
 360 For detailed statistics refer to Table 2 and 3. Observations are from the independent
 361 validation dataset selected using the Kennard-Stone method. The red dashed lines are 1:1
 362 line.



363

364 **Figure 6** Relative importance of each wavelength in Variable Importance on Projection
 365 (VIP). VIP values from PLSR_{ref} and PLSR_{tra} are on the right and left, respectively.

366 The VIP values of PLSR show the relative importance of each wavelength in
 367 predicting leaf traits (Fig.6). Visible and near-infrared wavelengths were important to the
 368 prediction of leaf pigments; there are three peaks (400, 550 and 730 nm) that are related
 369 to the chlorophyll absorption in the red (620-750 nm) and blue (400-450 nm), and
 370 reflection in the green (495-570 nm). The two components of chlorophyll (a and b) were
 371 also mainly contributing to the red/NIR region (600-750 nm), and the main contributing
 372 bands for chl b shifted towards green comparing to those for chl a (Fig. 6b and 6c) (Ustin
 373 et al. 2009). Carotenoids have a similar VIP curve comparing with the chlorophyll, with
 374 one distinction: the VIP values for carotenoids between 650 nm and 700 nm are relatively
 375 higher to those of chlorophyll.

376 Comparing with the pigments, N_{mass} , C_{mass} and LMA have relatively smooth VIP
377 curves. For N_{mass} , wavelengths around 700 nm and beyond 1900 nm are important to the
378 prediction of N_{mass} , presumably because the visible region is controlled by pigments and
379 nitrogen is an important component in leaf pigments, and the SWIR region near 2000 nm
380 is controlled by protein absorption features (Kokaly et al. 2009). Both C_{mass} and LMA
381 were related to the leaf structure and were largely contributing to the reflectance at NIR
382 and SWIR.

383 3.4. Robustness of the PLSR approach across time, sites and light environment

384 We examined the performance of the PLSR_{ref} models under five scenarios where
385 different sampling strategies were applied. The performance of the PLSR models
386 generally improved in the order of spring, fall, summer, monthly, and biweekly (Table 4).
387 As expected, more sampling throughout the season (and the increasing size and
388 representativeness of the calibration dataset) increased R^2 and reduced RMSE. When
389 comparing the three seasons, summer-only sampling yielded higher model performance
390 relative to the other two seasons, yet the improvements from scenarios 2 (summer-only)
391 to monthly (scenario 4) were not as obvious for pigments as much as N_{mass} , C_{mass} and
392 LMA. Sampling biweekly (scenario 5) largely improved the performance of PLSR,
393 especially for N_{mass} and C_{mass} (R^2 increased from <0.4 to ~ 0.6).

394 Examining the seasonal patterns of predicted and observed leaf traits reveal time-
395 dependent performance of each scenario. In spring-only scenario where leaf samples only
396 from the spring were used for PLSR calibration, all leaf traits during the first four weeks
397 of the growing seasons were well estimated. However, fall season leaf traits were
398 overestimated except for LMA in Martha's Vineyard (Fig. S4m). By contrast, in the fall-

399 only scenario, spring and summer leaf traits were underestimated except for C_{mass} (Fig.
400 S5k). Our summer-only scenario showed a better ability to capture the seasonal patterns
401 of leaf traits, only underestimated the N_{mass} peak in the early spring at Harvard Forest
402 (Fig. S6j). The monthly sampling scenario improved estimation of all leaf traits, in which
403 the improvement on estimating LMA was the most obvious (R^2 from 0.26 in the summer
404 case to 0.76 in the monthly sampling case, Fig. S7m, S7n). Biweekly sampling scenario
405 appeared to produce a satisfactory result for all the leaf traits studied here (Fig. S8).

406 PLSR_{ref} models trained using sunlit leaves explain 35%-70% of the variability in
407 shaded leaves with highest R^2 for pigments while lowest R^2 for C_{mass} (Fig. S9, Table S1).
408 However, PLSR_{ref} was less accurate for leaf traits like LMA in terms of RMSE (Fig.
409 S10m), for which the difference between sun-lit and shaded leaves was significant (Fig.
410 2). Similarly, PLSR_{ref} models trained with shaded leaves were able to predict the sunlit
411 leaf traits, but with lower model performance compared to when trained with sunlit
412 foliage. Depending on the leaf traits, the variability explained by PLSR ranges from 35%
413 to 70% (Fig. S10m).

414 PLSR_{ref} models trained using data from Harvard Forest (Test 1) were able to
415 capture 60~70% of variability of the pigments from Martha's Vineyard, except for N_{mass}
416 and C_{mass} (Table 5). Similar results were obtained from PLSR_{ref} trained using Martha's
417 Vineyard data (Test 2) and validated with HF data. VIP values for pigments in Test 1
418 were similar to those from Test 2. This is in stark contrast with VIP values for N_{mass} ,
419 C_{mass} , and LMA from both experiments. The locations of important wavelengths were
420 quite different between two tests (Fig. S11).

421
422

Table 4 Performance of all scenarios (spring, summer, fall, monthly, and biweekly) in terms of the goodness-of-fit (RMSE, R^2)

Leaf traits	RMSE					R^2				
	Spring	Summer	Fall	Monthly	Biweekly	Spring	Summer	Fall	Monthly	Biweekly
Total Chl ($\mu\text{g}/\text{cm}^2$)	8.64	6.64	7.23	6.32	5.66	0.60	0.70	0.72	0.73	0.77
Chl a ($\mu\text{g}/\text{cm}^2$)	5.97	4.75	5.25	4.65	4.15	0.63	0.72	0.72	0.73	0.78
Chl b ($\mu\text{g}/\text{cm}^2$)	2.73	1.92	2.06	1.89	1.69	0.48	0.67	0.69	0.69	0.73
Car ($\mu\text{g}/\text{cm}^2$)	1.71	1.31	1.29	1.22	1.12	0.48	0.65	0.69	0.69	0.73
N_{mass} (%)	1.62	0.42	0.51	0.37	0.29	0.08	0.36	0.07	0.36	0.62
C_{mass} (%)	1.59	1.71	1.74	1.26	1.03	0.20	0.21	0.19	0.39	0.56
LMA (g/cm^2)	61.13	27.17	24.86	21.78	18.76	0.13	0.71	0.75	0.79	0.85

423 **Table 5** Performance of PLSR reflectance models that were calibrated using data from one site and validated using data from the other
 424 site.

Leaf traits	RMSE		R ²	
	MV→HF	HF→MV	MV→HF	HF→MV
Total Chl (μg/cm ²)	6.17	7.44	0.72	0.67
Chl a (μg/cm ²)	4.39	5.29	0.73	0.69
Chl b (μg/cm ²)	1.85	1.99	0.68	0.66
Car (μg/cm ²)	1.19	1.54	0.59	0.59
N_{mass} (%)	0.56	0.72	0.29	0.20
C_{mass} (%)	2.89	2.90	0.10	0.23
LMA (g/cm ²)	35.62	59.45	0.60	0.72

425

426

427 4. Discussion

428 *4.1 Can we track the seasonality of leaf traits using leaf spectroscopy?*

429 Here we show that the seasonal variability of leaf traits can be captured with leaf
430 spectroscopy approaches (Fig.5, Table 3). All leaf properties (seven leaf traits and leaf spectra)
431 display seasonal dynamics that are also related to the location and microclimate (i.e., sunlit vs.
432 shaded, and the accompanying changes in humidity and temperature). The PLSR approach
433 explained 60%~80% of variability of these leaf traits in our study, supporting the hypothesis that
434 leaf spectra can capture the seasonal variability of leaf traits. Indeed, each leaf trait has its own
435 spectral fingerprint, as we have seen from the VIP values of PLSR models (Fig.7). Patterns of
436 VIP values were similar to previous studies (Asner et al. 2009; Serbin et al. 2014) and consistent
437 with our understandings of leaf physiology (Ustin et al. 2009). This is an important result as
438 collecting leaf spectra is much more time-efficient than traditional approaches and allows for
439 repeat sampling of the same leaves throughout the season. SVIs can be an alternative for the
440 estimation of total chlorophyll concentration when there are limits on available instruments or,
441 for example, using two-band LED sensors (e.g., Garrity et al. 2010; Ryu et al. 2010). The result
442 also has implications for the current and future use of field spectrometers that measure leaf or
443 canopy reflectance at high temporal frequency (e.g., Hilker et al. 2009). Our well-calibrated
444 model using PLSR can be used on leaf reflectance to track the seasonality of multiple leaf traits
445 in temperate deciduous forests.

446 The tests on the robustness of leaf spectra-trait relationships suggested that the overlap
447 between the training dataset and an independent validation dataset is important for a good
448 prediction. Summer mature leaves displayed higher pigments concentration and LMA, while

449 lower N_{mass} compared with young leaves (Fig. 1, Fig. 2). In addition, the corresponding leaf
450 spectra were significantly different (Fig. 4). Traditionally, the development of the leaf traits-
451 spectra relationship has been focused on a single time point, typically mid-season mature leaves.
452 We have shown here that if we apply an empirical relationship between spectra and traits derived
453 from one period (for example, summer) to another (spring or fall), leaf traits will likely be over
454 or under-estimated (Fig. S4-S6). Thus predicting leaf traits like N_{mass} , which has an obvious
455 seasonality, will not be well represented. However, we have also illustrated that with proper
456 calibration, we can adequately characterize the seasonality of a range of leaf traits, which is
457 critical for ecosystem monitoring and informing process modeling activities (Table 5).

458 VIP values as indicators of band importance can help to explain the prediction power of
459 PLSR models. For example, in the case of using PLSR trained use data from one site to predict
460 another (Test 1 & 2), VIP values of leaf pigments overlap well, indicating both sites share similar
461 wavelength regions (Fig. S11). As a result, cross-site prediction of leaf pigments showed
462 reasonable accuracy (Table 5). It also has important implications for the design of multi-band
463 sensors and imagers as it can select the wavelengths that are most useful for the leaf traits of
464 interest (Nijland et al. 2014; Ryu et al. 2010).

465 The variability of our seven leaf traits was not equally captured (Table 3). The absorption
466 features of pigments are well understood and clearly represented in the VIP value plots (Fig. 6).
467 While for C_{mass} and N_{mass} , although there have been studies on the possible linkage between
468 certain components in the leaves (e.g., protein, cellulose) and leaves' optical properties, the
469 impact on leaf spectra is less obvious comparing with that from the pigments (Kokaly et al.,
470 2009). This may partly explain the less accurate PLSR models for the C_{mass} and N_{mass} .

471 As expected, the PLSR approach, which can exploit the full spectrum information to
472 estimate leaf traits performed better than traditional SVIs (Table 3). While SVIs that calibrated
473 with extensive datasets displayed a similar performance to PLSR in estimating total chlorophyll
474 concentration, we observed significant difference for the carotenoids and LMA. Recalibrate SVIs
475 using our own datasets did not improve their performance. This suggests that the leaf traits
476 variability in our dataset was not fully captured by the SVIs, despite that our large dataset covers
477 ranges observed by others (Féret et al. 2011). Incorporating more datasets to the calibration of
478 simple indices could potentially improve the performance of these methods, but will not alleviate
479 the saturation issue that is pervasive when using simple SVIs, especially for LMA.

480 As the applications of leaf spectra-traits relationship become more common, we argue
481 that a standardized protocol to calibrate and validate PLSR-type models is needed. This includes
482 an independent validation dataset to avoid validating against the calibration dataset itself and a
483 method to choose the optimal number of PLSR components to prevent overfitting (Serbin et al.
484 2014). A globally relevant algorithm for leaf traits that can be used by ground spectral
485 observations (Hilker et al. 2010) or existing or planned satellite missions like HypsIRI (such as
486 <https://hyspiri.jpl.nasa.gov/>) hinges on a rigorously-tested method and on datasets covering a
487 wide range of variations in leaf traits.

488

489 *4.2 The implications for field sampling strategy*

490 The leaf traits time-series we presented showed the critical time windows to capture their
491 seasonality. Extensive field sampling is laborious and expensive and the continual question in
492 plant ecology is “how much is good enough?” Since the measurements of leaf spectral properties
493 are less labor-intensive (and non-destructive) compared with the measurements of most leaf traits,

494 we explored how many destructive measurements of leaf traits were needed to calibrate the
495 models using full leaf spectra. For example, LMA showed dramatic changes in the early season,
496 thus the sampling and calibration processes need to include the data at this stage. Similarly, N_{mass}
497 was relatively stable in the mid season, and most of the variations occurred in the early and end
498 of season, which makes the sampling at these time frames important. This explains why our
499 comparisons that only considered the variability of leaf traits in the summer showed much poorer
500 performance. Monthly and even biweekly sampling should be considered, at least for the four
501 temperate deciduous species examined in this study.

502

503 *4.3 Broad implications of using leaf spectroscopy for ecological studies*

504 Understanding the seasonality of leaf traits has recently gained attention as an effort to
505 improve our modeling of terrestrial carbon and water cycles (Bauerle et al. 2012; Grassi et al.
506 2005; Medvigy et al. 2013). For example, in the Community Land Model, N_{mass} and LMA
507 control the maximum rate of carboxylation, V_{cmax} , which is highly variable temporally and
508 across different species and light environments (Oleson et al., 2010). Our time-series of N_{mass}
509 capture two important features: (1) the seasonal peak at the beginning of the spring, suggesting
510 that nitrogen was allocated to the leaves early in the season. As leaves matured, other types of
511 elements such as carbon accumulated at a faster rate, resulting in an increase of C_{mass} relative to
512 N_{mass} ratio. (2) A decline of N_{mass} by the end of the season. N_{mass} and LMA was relatively stable
513 at both sites during the summer (Fig. 2a and 2b), thus leaf age does not appear to be affecting the
514 nitrogen concentration during the peak season (Field and Mooney 1983). This finding highlights
515 the importance of tracking the seasonality of leaf traits (Wilson et al. 2000), and our work
516 demonstrates that leaf spectroscopy can provide a rapid means to routinely measure leaf traits.

517 Importantly, these results highlight that spectroscopy observations can provide key information
518 on the individual differences in multiple leaf traits that can feed into ecosystem models (Medvigy
519 et al., 2009) or be used to test key ecological questions (Rowland et al., 2015). In addition, this
520 emphasizes the important capability of monitoring ecosystem dynamics across a range of spatial
521 and temporal scales with hyperspectral observations from leaves, towers, as well as with new
522 instruments mounted on Unmanned Aerial Systems (UASs) and existing and future instruments
523 on piloted aircraft and satellite platforms (Yang et al., 2014; Yang et al., 2015; Asner and Martin,
524 2008; Hilker et al., 2010).

525

526 5. Conclusion

527 This paper presents a comprehensive study of the relationship between leaf spectra and
528 foliar traits across varying leaf developmental stages, sites, and light environment using a near
529 weekly dataset of seven leaf traits and spectra at two sites. A Partial Least Square Regression
530 (PLSR) modeling approach, after proper calibration with leaf traits from different times of the
531 season, showed a strong capacity to quantify the seasonal variation of leaf traits within and
532 across sites. The robustness of a PLSR model largely depends on the overlap of leaf trait ranges
533 between the calibration dataset and the dataset to be estimated, and extrapolation outside the
534 ranges of the calibration dataset can result in a significant error. We found that biweekly
535 sampling of leaf traits and spectra would provide a robust PLSR model to estimate the seasonal
536 variations of leaf traits. This work demonstrated the capability of leaf spectra to track seasonally-
537 variable leaf traits, and thus supports the use of automated field spectrometers, airborne and
538 satellite hyperspectral sensors to track leaf traits repeatedly throughout the season and across
539 broad regions (Roberts et al. 2012; Singh et al., 2015; Yang et al. 2015).

540

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558 **Reference**

559

560 Asner, G., & Martin, R. (2008). Spectral and chemical analysis of tropical forests: Scaling from
561 leaf to canopy levels. *Remote Sensing of Environment*, *112*, 3958-3970

562 Asner, G.P., Martin, R.E., Ford, A., Metcalfe, D., & Liddell, M. (2009). Leaf chemical and
563 spectral diversity in Australian tropical forests. *Ecological Applications*, *19*, 236-253

564 Asner, G.P., Martin, R.E., Knapp, D.E., Tupayachi, R., Anderson, C., Carranza, L., Martinez, P.,
565 Houcheime, M., Sinca, F., & Weiss, P. (2011). Spectroscopy of canopy chemicals in humid
566 tropical forests. *Remote Sensing of Environment*, *115*, 3587-3598

- 567 Asner, G.P., & Vitousek, P.M. (2005). Remote analysis of biological invasion and
568 biogeochemical change. *Proc Natl Acad Sci U S A*, 102, 4383-4386
- 569 Bauerle, W.L., Oren, R., Way, D.A., Qian, S.S., Stoy, P.C., Thornton, P.E., Bowden, J.D.,
570 Hoffman, F.M., & Reynolds, R.F. (2012). Photoperiodic regulation of the seasonal pattern of
571 photosynthetic capacity and the implications for carbon cycling. *Proceedings of the National
572 Academy of Sciences*, 109, 8612-8617
- 573 Belanger, M., Miller, J., & Boyer, M. (1995). Comparative relationships between some red edge
574 parameters and seasonal leaf chlorophyll concentrations. *Canadian Journal of Remote Sensing*,
575 21, 16-21
- 576 Bonan, G.B., Oleson, K.W., Fisher, R.A., Lasslop, G., & Reichstein, M. (2012). Reconciling leaf
577 physiological traits and canopy flux data: Use of the TRY and FLUXNET databases in the
578 Community Land Model version 4. *Journal of Geophysical Research: Biogeosciences*, 117,
579 G02026.
- 580 Chapin, F.S., Matson, P.A., & Vitousek, P.M. (2011). *Principles of terrestrial ecosystem
581 ecology*. (2nd Edition ed.). Springer
- 582 Couture, J.J., Serbin, S.P., & Townsend, P.A. (2013). Spectroscopic sensitivity of real-time,
583 rapidly induced phytochemical change in response to damage. *New Phytologist*, 198, 311-319.
- 584 Curran, P.J. (1989). Remote sensing of foliar chemistry. *Remote Sensing of Environment*, 30,
585 271-278
- 586 Demmig-Adams, B., & Adams, W.W. (2000). Photosynthesis: Harvesting sunlight safely.
587 *Nature*, 403, 371-374
- 588 Dillen, S.Y., de Beeck, M.O., Hufkens, K., Buonanduci, M., & Phillips, N.G. (2012). Seasonal
589 patterns of foliar reflectance in relation to photosynthetic capacity and color index in two co-
590 occurring tree species, *Quercus rubra* and *Betula papyrifera*. *Agricultural and Forest
591 Meteorology*, 160, 60-68
- 592 Eckstein, R.L., Karlsson, P.S., & Weih, M. (1999). Leaf life span and nutrient resorption as
593 determinants of plant nutrient conservation in temperate-arctic regions. *New Phytologist*, 143,
594 177-189
- 595 Ellsworth, D.S., & Reich, P.B. (1993). Canopy structure and vertical patterns of photosynthesis
596 and related leaf traits in a deciduous forest. *Oecologia*, 96, 169-178
- 597 Elvidge, C.D. (1990). Visible and near infrared reflectance characteristics of dry plant materials.
598 *International Journal of Remote Sensing*, 11, 1775-1795
- 599 Evans, J. (1989). Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia*, 78,
600 9-19
- 601 Evans, J. (1989b). Partitioning of nitrogen between and within leaves grown under different
602 irradiances. *Australian Journal of Plant Physiology* 16(6): 533-548.

603 Féret, J.-B., François, C., Gitelson, A., Asner, G.P., Barry, K.M., Panigada, C., Richardson,
604 A.D., & Jacquemoud, S. (2011). Optimizing spectral indices and chemometric analysis of leaf
605 chemical properties using radiative transfer modeling. *Remote Sensing of Environment*, *115*,
606 2742-2750

607 Field, C., & Mooney, H.A. (1983). Leaf age and seasonal effects on light, water, and nitrogen
608 use efficiency in a California shrub. *Oecologia*, *56*, 348-355

609 Fisher, J., & Mustard, J. (2007). Cross-scalar satellite phenology from ground, Landsat, and
610 MODIS data. *Remote Sensing of Environment*, *109*, 261-273

611 Foster, D., Hall, B., Barry, S., Clayden, S., & Parshall, T. (2002). Cultural, environmental and
612 historical controls of vegetation patterns and the modern conservation setting on the island of
613 Martha's Vineyard, USA. *Journal of Biogeography*, *29*, 1381-1400

614 Garrity, S.R., Vierling, L.A., & Bickford, K. (2010). A simple filtered photodiode instrument for
615 continuous measurement of narrowband NDVI and PRI over vegetated canopies. *Agricultural
616 and Forest Meteorology*, *150*, 489-496

617 Grassi, G., Vicinelli, E., Ponti, F., Cantoni, L., & Magnani, F. (2005). Seasonal and interannual
618 variability of photosynthetic capacity in relation to leaf nitrogen in a deciduous forest plantation
619 in northern Italy. *Tree Physiol*, *25*, 349-360

620 Hilker, T., Coops, N.C., Coggins, S.B., Wulder, M.A., Brown, M., Black, T.A., Nesic, Z., &
621 Lessard, D. (2009). Detection of foliage conditions and disturbance from multi-angular high
622 spectral resolution remote sensing. *Remote Sensing of Environment*, *113*, 421-434

623 Hilker, T., Nesic, Z., Coops, N.C., & Lessard, D. (2010). A New, Automated, Multiangular
624 Radiometer Instrument for Tower-Based Observations of Canopy Reflectance (Amspec II).
625 *Instrumentation Science & Technology*, *38*, 319-340

626 Huete, A., Didan, K., Miura, T., Rodriguez, E.P., Gao, X., & Ferreira, L.G. (2002). Overview of
627 the radiometric and biophysical performance of the MODIS vegetation indices. *Remote Sensing
628 of Environment*, *83*, 195-213

629 Jacquemoud, S., & Baret, F. (1990). PROSPECT: A model of leaf optical properties spectra.
630 *Remote Sensing of Environment*, *34*, 75-91

631 Kattge, J., Díaz, S., Lavorel, S., Prentice, I.C., Leadley, P., Bönsch, G., Garnier, E., Westoby,
632 M., Reich, P.B., Wright, I.J. et al., (2011). TRY - a global database of plant traits. *Global
633 Change Biology*, *17*, 2905-2935

634 Kennard, R.W., & Stone, L.A. (1969). Computer Aided Design of Experiments. *Technometrics*,
635 *11*, 137-148

636 Killingbeck, K.T. (1996). Nutrients in Senesced Leaves: Keys to the Search for Potential
637 Resorption and Resorption Proficiency. *Ecology*, *77*, 1716-1727

638 Kokaly, R.F., Asner, G.P., Ollinger, S.V., Martin, M.E., & Wessman, C.A. (2009).
639 Characterizing canopy biochemistry from imaging spectroscopy and its application to ecosystem
640 studies. *Remote Sensing of Environment*, 113, S78-S91

641 Laisk, A., Nedbal, L., & Govindjee (2009). *Photosynthesis in silico: understanding complexity*
642 *from molecules to ecosystems*. Springer Science & Business Media

643 Lewandowska, M., & Jarvis, P. (1977). Changes in chlorophyll and carotenoid content, specific
644 leaf area and dry weight fraction in Sitka spruce, in response to shading and season. *New*
645 *Phytologist*, 79, 247-256

646 Lichtenthaler, H.K., & Buschmann, C. (2001). Chlorophylls and Carotenoids: Measurement and
647 Characterization by UV-VIS Spectroscopy. *Current Protocols in Food Analytical Chemistry*:
648 John Wiley & Sons, Inc.

649 Medvigy, D., S. C. Wofsy, J. W. Munger, D. Y. Hollinger, and P. R. Moorcroft. (2009)
650 Mechanistic scaling of ecosystem function and dynamics in space and time: Ecosystem
651 Demography model version 2. *Journal of Geophysical Research - Biogeosciences*, 114, G01002,
652 doi:[10.1029/2008JG000812](https://doi.org/10.1029/2008JG000812).

653 Medvigy, D., Jeong, S.-J., Clark, K.L., Skowronski, N.S., & Schäfer, K.V.R. (2013). Effects of
654 seasonal variation of photosynthetic capacity on the carbon fluxes of a temperate deciduous
655 forest. *Journal of Geophysical Research: Biogeosciences*, 118, 1703-1714

656 Mehmood, T., Liland, K.H., Snipen, L., & Sæbø, S. (2012). A review of variable selection
657 methods in Partial Least Squares Regression. *Chemometrics and Intelligent Laboratory Systems*,
658 118, 62-69

659 Niinemets U. 2007. Photosynthesis and resource distribution through plant canopies. *Plant Cell*
660 *and Environment* 30(9): 1052-1071.

661 Nijland, W., de Jong, R., de Jong, S.M., Wulder, M.A., Bater, C.W., & Coops, N.C. (2014).
662 Monitoring plant condition and phenology using infrared sensitive consumer grade digital
663 cameras. *Agricultural and Forest Meteorology*, 184, 98-106

664 Oleson, K.W., D.M. Lawrence, G.B. Bonan, et al., 2013: Technical Description of version 4.5 of
665 the Community Land Model (CLM). Near Technical Note NCAR/TN-503+STR, National
666 Center for Atmospheric Research, Boulder, CO, DOI: 10.5065/D6RR1W7M.

667 Ollinger, S.V. (2011). Sources of variability in canopy reflectance and the convergent properties
668 of plants. *New Phytologist*, 189, 375-394

669 Poorter, H., Niinemets, Ü., Poorter, L., Wright, I.J., & Villar, R. (2009). Causes and
670 consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist*, 182,
671 565-588

672 Richardson, A.D., Duigan, S.P., & Berlyn, G.P. (2002). An evaluation of noninvasive methods to
673 estimate foliar chlorophyll content. *New Phytologist*, 153, 185-194

674 Roberts, D.A., Quattrochi, D.A., Hulley, G.C., Hook, S.J., & Green, R.O. (2012). Synergies
675 between VSWIR and TIR data for the urban environment: An evaluation of the potential for the
676 Hyperspectral Infrared Imager (HyspIRI) Decadal Survey mission. *Remote Sensing of*
677 *Environment*, *117*, 83-101

678 Rowland, L., et al. (2015), Death from drought in tropical forests is triggered by hydraulics not
679 carbon starvation, *Nature*, *528*(7580), 119-122, doi:10.1038/nature15539.

680 Ryu, Y., Baldocchi, D.D., Verfaillie, J., Ma, S., Falk, M., Ruiz-Mercado, I., Hehn, T., &
681 Sonnentag, O. (2010). Testing the performance of a novel spectral reflectance sensor, built with
682 light emitting diodes (LEDs), to monitor ecosystem metabolism, structure and function.
683 *Agricultural and Forest Meteorology*, *150*, 1597-1606

684 Schimel, D., Pavlick, R., Fisher, J.B., Asner, G.P., Saatchi, S., Townsend, P., Miller, C.,
685 Frankenberg, C., Hibbard, K., & Cox, P. (2015). Observing terrestrial ecosystems and the carbon
686 cycle from space. *Global Change Biology*, *21*, 1762-1776

687 Schneider, C.A., Rasband, W.S., & Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of
688 image analysis. *Nature Methods*, *9*, 671-675

689 Serbin, S.P., Singh, A., McNeil, B.E., Kingdon, C.C., & Townsend, P.A. (2014). Spectroscopic
690 determination of leaf morphological and biochemical traits for northern temperate and boreal tree
691 species. *Ecological Applications*, *24*, 1651-1669

692 Shen, M., Chen, J., Zhu, X., & Tang, Y. (2009). Yellow flowers can decrease NDVI and EVI
693 values: evidence from a field experiment in an alpine meadow. *Canadian Journal of Remote*
694 *Sensing*, *35*, 99-106

695 Sims, D.A., & Gamon, J.A. (2002). Relationships between leaf pigment content and spectral
696 reflectance across a wide range of species, leaf structures and developmental stages. *Remote*
697 *Sensing of Environment*, *81*, 337-354

698 Singh, A., Serbin, S.P., McNeil, B.E., Kingdon, C.C., & Townsend, P.A. (2015). Imaging
699 spectroscopy algorithms for mapping canopy foliar chemical and morphological traits and their
700 uncertainties. *Ecological Applications*

701 Terashima, I., Miyazawa, S.-I., & Hanba, Y.T. (2001). Why are sun leaves thicker than shade
702 leaves?—Consideration based on analyses of CO₂ diffusion in the leaf. *J Plant Res*, *114*, 93-105

703 Ustin, S.L., Gitelson, A.A., Jacquemoud, S., Schaepman, M., Asner, G.P., Gamon, J.A., &
704 Zarco-Tejada, P. (2009). Retrieval of foliar information about plant pigment systems from high
705 resolution spectroscopy. *Remote Sensing of Environment*, *113*, S67-S77

706 Ustin, S.L., Roberts, D.A., Gamon, J.A., Asner, G.P., & Green, R.O. (2004). Using imaging
707 spectroscopy to study ecosystem processes and properties. *Bioscience*, *54*, 523-534

708 Wicklein, H.F., Ollinger, S.V., Martin, M.E., Hollinger, D.Y., Lepine, L.C., Day, M.C., Bartlett,
709 M.K., Richardson, A.D., & Norby, R.J. (2012). Variation in foliar nitrogen and albedo in
710 response to nitrogen fertilization and elevated CO₂. *Oecologia*, *169*, 915-925

- 711 Wilson, K.B., Baldocchi, D.D., & Hanson, P.J. (2000). Spatial and seasonal variability of
712 photosynthetic parameters and their relationship to leaf nitrogen in a deciduous forest. *Tree*
713 *Physiol*, 20, 565-578
- 714 Wofsy, S.C., Goulden, M.L., Munger, J.W., Fan, S.-M., Bakwin, P.S., Daube, B.C., Bassow,
715 S.L., & Bazzaz, F.A. (1993). Net Exchange of CO₂ in a Mid-Latitude Forest. *Science*, 260,
716 1314-1317
- 717 Wold, S., Sjöström, M., & Eriksson, L. (2001). PLS-regression: a basic tool of chemometrics.
718 *Chemometrics and Intelligent Laboratory Systems*, 58, 109-130
- 719 Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares,
720 J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J.,
721 Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets,
722 U., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas,
723 S.C., Tjoelker, M.G., Veneklaas, E.J., & Villar, R. (2004). The worldwide leaf economics
724 spectrum. *Nature*, 428, 821-827
- 725 Yang, X., Mustard, J., Tang, J., & Xu, H. (2012). Regional-scale phenology modeling based on
726 meteorological records and remote sensing observations. *J. Geophys. Res.*, 117, G03029
- 727 Yang, X., Tang, J., & Mustard, J. (2014). Beyond leaf color: comparing camera-based
728 phenological metrics with leaf biochemical, biophysical and spectral properties throughout the
729 growing season of a temperate deciduous forest. *Journal of Geophysical Research:*
730 *Biogeosciences*, 119, 181-191.
- 731 Yang, X., Tang, J., Mustard, J.F., Lee, J.-E., Rossini, M., Joiner, J., Munger, J.W., Kornfeld, A.,
732 & Richardson, A.D. (2015). Solar-induced chlorophyll fluorescence that correlates with canopy
733 photosynthesis on diurnal and seasonal scales in a temperate deciduous forest. *Geophysical*
734 *Research Letters*, 42, 2977-2987
- 735 Zhang, Y., Chen, J.M., & Thomas, S.C. (2007). Retrieving seasonal variation in chlorophyll
736 content of overstory and understory sugar maple leaves from leaf-level hyperspectral data.
737 *Canadian Journal of Remote Sensing*, 33, 406-415
- 738 Zhao, K., Valle, D., Popescu, S., Zhang, X., & Mallick, B. (2013). Hyperspectral remote sensing
739 of plant biochemistry using Bayesian model averaging with variable and band selection. *Remote*
740 *Sensing of Environment*, 132, 102-119