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## Abstract

Monitoring forest health is crucial to understanding function and managing productivity of forest systems. However, traditional estimates of tree health are time-consuming and challenging to collect because of the vertical and spatial scales of forest systems. This study evaluated the ability of a novel application of hyperspectral data to estimate foliar functional trait responses to multiple biotic and abiotic stressors and to classify different stress combinations. In a greenhouse environment, we exposed one-year-old black walnut (*Juglans nigra* L.) and red oak (*Quercus rubra* L.) seedlings to multiple stress factors, alone and in combination. We collected reference measurements of numerous leaf physiological traits and paired them with spectral collections to build predictive models. The resulting models reliably estimated most black walnut and red oak leaf functional traits with external validation goodness-of-fit ( $R^2$ ) ranging from 0.37 to 0.90 and normalized error ranging from 7.5% to 18.3%. Spectral data classified different individual stress groups well, but the ability of spectral data to classify stress groups depended on if the stress events were applied individually or in combination. High-dimensional spectral data can provide information about plant stress, improve forest monitoring in future predicted environments, and ultimately aid in management efforts in forest systems.

## Introduction

- Systematic monitoring of forest health is crucial to understanding the ecological function and managing the productivity of forest systems. Traditional estimates of tree health status are time-consuming and challenging to collect, because of both the vertical and spatial scales of forest plantations and natural forests. The development of a novel application of hyperspectral data can increase the efficiency of monitoring and provide more detailed information of forest health.
- The objectives of this study are 1) to determine the ability of hyperspectral data to estimate plant functional traits in responses to different stress events, alone and in combination, and 2) to determine the ability of hyperspectral data to classify different abiotic and biotic stress events.

## Materials and Methods

- Black walnut and red oak seedlings were exposed to the different combination of abiotic and biotic stressors, including fungal infection, drought, nutrient deficiency, and salt deposition in a greenhouse under a controlled environment.
- We measured numerous leaf functional traits (photosynthetic-, water-, and defense-related traits) using different analytical procedures.
- We paired these reference measurements with leaf spectral collections using Spectra Vista HR 1024i spectroradiometer (Spectra Vista Corporation, New York, USA).



### Trait retrievals

We built predictive models to estimate leaf traits as a function of leaf spectra using a Partial Least Square Regression approach.

### Spectral phenotyping

We used the permutational multivariate analysis of variance and the principal coordinates analysis to see if the spectral data can classify stress treatment groups.

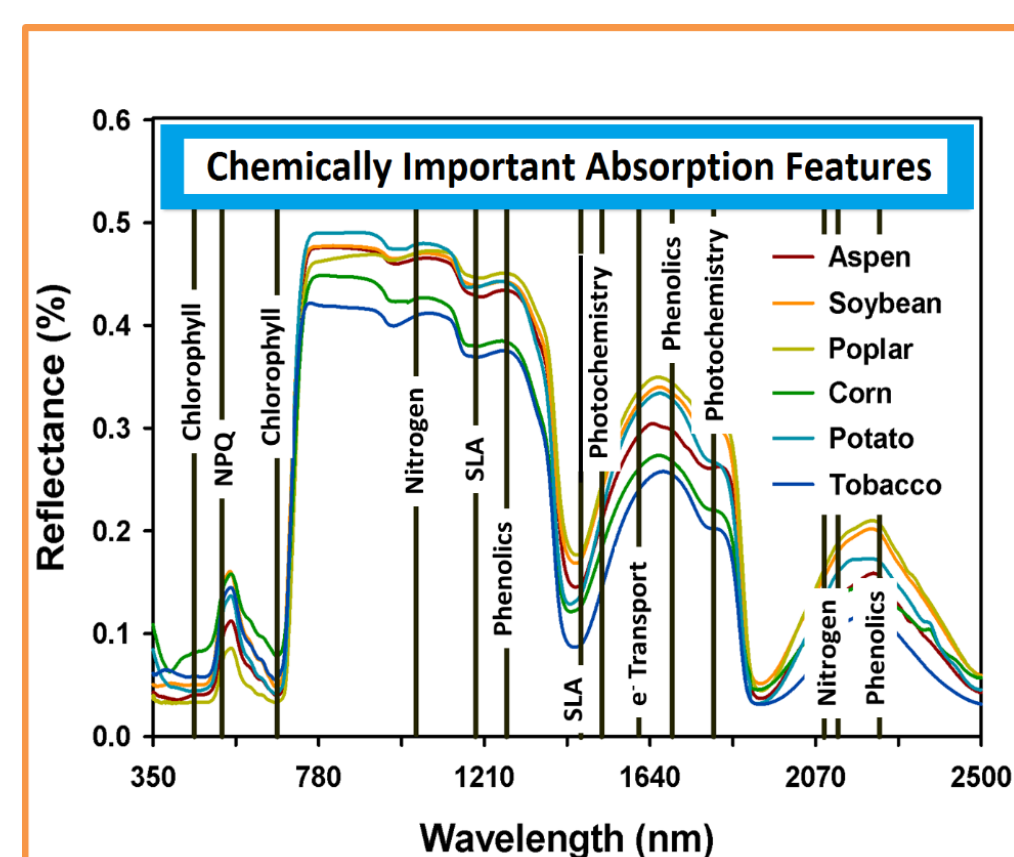


Figure 1. A typical leaf reflectance spectra (400–2500 nm) and chemically important spectral absorption features.

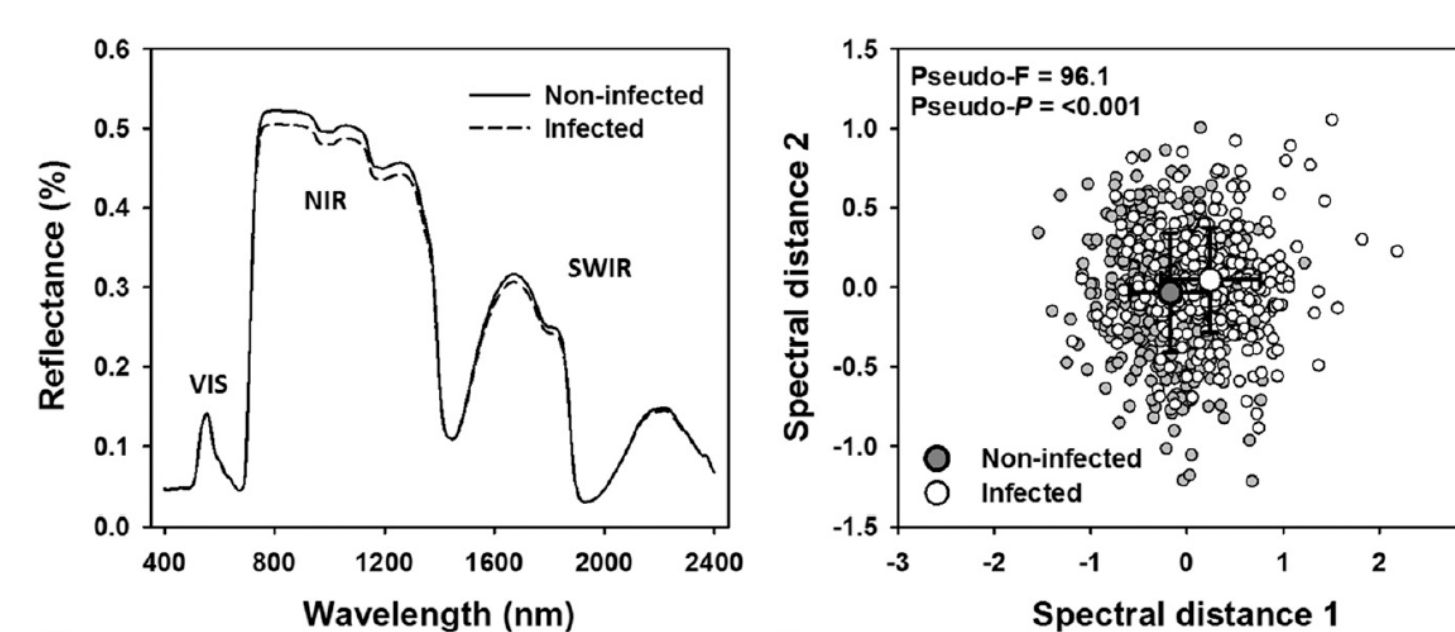
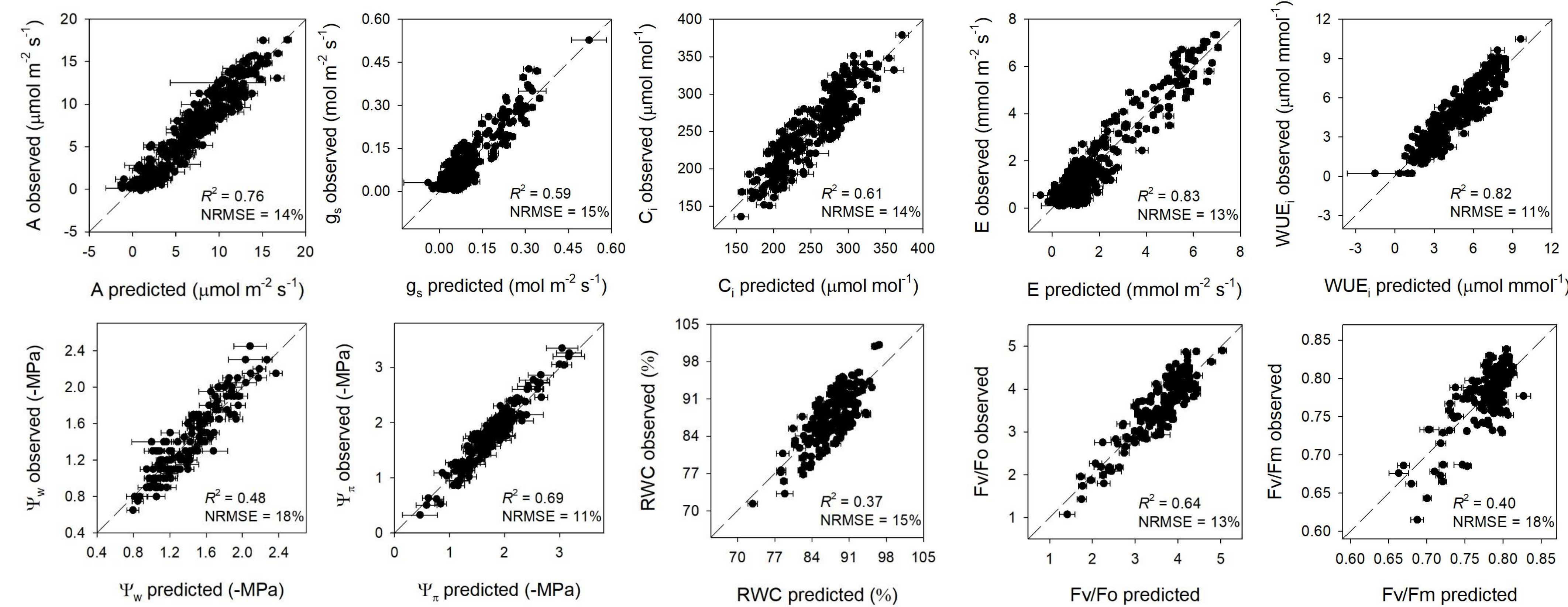


Figure 2. Potato disease infected vs non-infected leaves and their different spectral profiles (Couture et al., Integrating Spectroscopy with Potato Disease Management, 2018, Plant Disease).

## Results and Discussion

### 1) Trait retrievals: Estimation of leaf functional traits



1) Most functional traits in response to stress treatment were well predicted by spectral data.

Figure 3. Observed vs predicted values of 10 key foliar functional traits of black walnut and red oak collected in 2018 and 2019. Partial least square regression was used for modeling with the whole spectrum (400–2400 nm). Error bars for predicted values represent the standard deviations generated from the 500 simulated models. For model performance evaluation, goodness-of-fit ( $R^2$ ) and NRMSE (Normalized Root Mean Squared Error) were presented. Dashed line is 1:1 relationship. Trait abbreviations: A, CO<sub>2</sub> assimilation rate;  $g_s$ , stomatal conductance;  $C_i$ , intercellular CO<sub>2</sub> concentration; E, transpiration rate; WUE<sub>i</sub>, instantaneous water use efficiency;  $\Psi_w$ , leaf water potential;  $\Psi_{\pi}$ , leaf osmotic potential; RWC, relative water content; Fv/Fo, maximum quantum efficiency of PSII; Fv/Fo, maximum primary yield of photochemistry of PSII.

### 2) Spectral phenotyping: Detection of fungal diseases

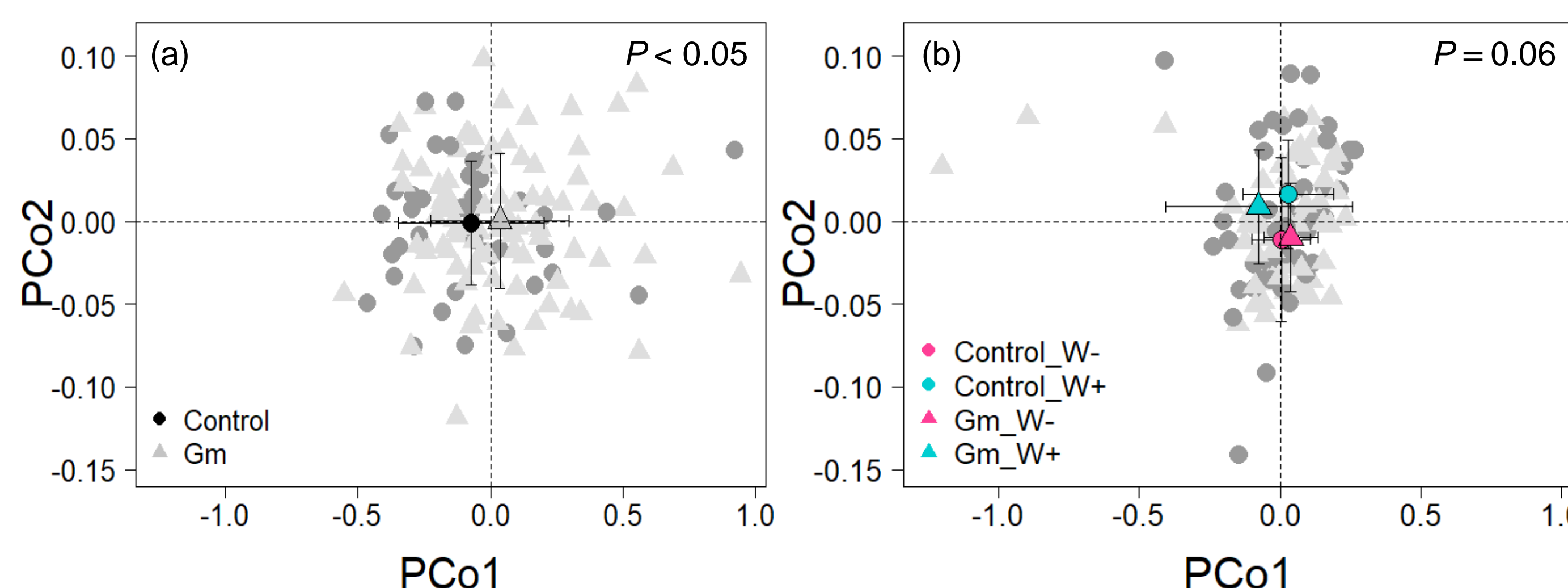


Figure 4. Scores (mean  $\pm$  standard error) for the first and second principal components from principal coordinates analysis of reflectance data (400 – 700 nm) collected from black walnut leaves. (a) Spectral data significantly discriminate control (circle) versus *Geosmithia mobida* (Gm) infected plant groups (triangle). (b) This change of spectral data to fungal treatment depended on water availability (W-: drought (pink), W+: well-watered (blue) group).

2) Spectral data identified the influence of fungal infection on trees, but the spectral profiles varied among soil type across time after fungal infection. In a follow-up experiment, water treatment had influence on the ability of spectral profile to detect fungal infection.

### 3) Trait retrievals + Spectral phenotyping

Table 1.  $P$  values of three-way analysis of variance for the effects of fungal infection ( $Gm$ ), soil type ( $S$ ), time ( $T$ ) and their interactions on predicted leaf traits of black walnut. Significant values ( $P < 0.05$ ) are shown in bold.  $df$ , Degrees of freedom.

Trait	$df$	A	$g_s$	$C_i$	E	WUE <sub>i</sub>	$\Psi_w$	$\Psi_{\pi}$	RWC	Fv/Fm	Fv/Fo
$Gm$	1	<b>0.018</b>	<b>0.020</b>	0.215	<b>0.016</b>	0.074	0.777	0.741	0.592	0.387	0.506
$S$	2	0.110	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.015</b>	0.243	0.454	0.603	0.853	<b>&lt;0.001</b>	<b>&lt;0.001</b>
$T$	1	<b>0.001</b>	0.428	<b>&lt;0.001</b>	0.075	<b>&lt;0.001</b>	0.443	0.211	0.052	<b>0.006</b>	0.064
$Gm \times S$	2	0.054	0.548	<b>0.016</b>	0.687	0.168	0.915	0.720	0.934	<b>0.039</b>	<b>0.008</b>
$Gm \times T$	1	0.375	0.787	<b>0.003</b>	0.717	<b>0.008</b>	0.742	0.370	0.668	0.526	0.426
$S \times T$	2	0.062	0.051	<b>0.014</b>	0.785	0.273	0.526	0.722	0.268	0.111	0.240
$Gm \times S \times T$	2	0.154	0.140	<b>0.009</b>	0.562	0.200	0.549	0.701	<b>0.043</b>	0.057	0.222

3) Spectrally predicted leaf trait responses varied among treatments of fungal infection, soil type, and time. We found significant fungal infection effects for gas-exchange related leaf traits.

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## Conclusions

- High-dimensional spectral data can provide information about plant stress, improve forest monitoring in future predicted environments, and ultimately aid in management efforts in forest systems.
- Future extensions of this work for predicting other physiological, biochemical and morphological leaf traits under abiotic and biotic stressors will contribute to upscaling from leaf to canopy and airborne measurements.