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Multivariate analyses of visible/near infrared (VIS/NIR) absorbance spectra reveal underlying spectral differences among dried, ground conifer needle samples from different growth environments

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Summary

• Absorbance of visible and near infrared (400–2500 nm) radiation by plant material is determined primarily by biochemical and structural components. We used three multivariate techniques to explore the spectral differences among dried, ground foliage samples of two conifer species from different montane growth environments (three elevations and two crown positions on three different mountains).
• Principal components analysis indicated underlying spectral patterns strongly related to species and crown position, and the derived components were correlated with the chemical composition of the samples. Discriminant analysis showed that it was possible to perfectly separate samples by species, but much more difficult to discriminate among different elevations, using just the spectral information. Samples from low and high elevation were well-separated, but mid elevation samples were frequently misclassified.
• Partial least squares regression produced results that were superior to those of discriminant analysis, in that all groups were better separated and there was less within-group variability.
• These approaches do not directly reveal the biochemical basis of the spectral differences. However, such methods provide a solid foundation for hypothesizing the overall degree of biochemical similarity among diverse samples. Thus, samples from different growth elevations appeared to be biochemically more similar than samples from different species or crown positions. Other potential applications are discussed.

Key words: balsam fir (Abies balsamea), conifer foliage, discriminant analysis, elevation, partial least squares (PLS) regression, principal components analysis, red spruce (Picea rubens), reflectance spectra.

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Introduction

For plant foliage, visible (VIS, 400–750 nm wavelengths) and near infrared (NIR, 750–2500 nm) absorbance (or, conversely, reflectance) spectra are the product of complex patterns of scattering and absorption by numerous structural and biochemical components. Pigments (e.g. chlorophylls and carotenoids) directly absorb visible light energy as the first step in photosynthesis, whereas other compounds (e.g. starch, protein, and carbohydrates) are characterized by NIR absorption signatures which are a consequence of stretching and vibration by the C-H, N-H and O-H groups of which they are composed. Interpretation of absorbance spectra is difficult at best, because although the spectral characteristics of the different compounds are unique, they are also broad and thus frequently overlap (Curran, 1989). However, the

information content of a sample's VIS/NIR spectrum is very high, because it provides a concise but very rich summary of the overall biochemical composition (Foley *et al.*, 1998).

There are a vast number of simple spectral indices that have been developed as indicators of foliar chlorophyll content, and quite often these work remarkably well, even on intact leaves (Richardson *et al.*, 2002). For other compounds, spectroscopic methods have been used in conjunction with extensive laboratory-based chemical analysis to calibrate empirical models for predicting the composition of a given sample (Curran, 1989). Although these methods were first developed by food chemists and agriculturists (Williams & Norris, 2001), they have also been used to a more modest degree by ecosystem ecologists and plant ecologists. Ecological applications include the determination of fiber constituents (Wessman *et al.*, 1988; Lacaze & Joffre, 1994; Bolster *et al.*, 1996), other biochemical compounds (Card *et al.*, 1988), and nutrient status (Hallett *et al.*, 1997; Gillon *et al.*, 1999a). A recent study by Gillon *et al.* (1999b) used similar methods to quantify a functional attribute (the litter decomposition rate constant *k*) on the basis of NIR spectral characteristics of a diverse array of litter types. However, despite the growing interest in using remote sensing to integrate from the leaf-level to ecosystem level (Gamon & Qiu, 1999), spectral analysis has really not yet received widespread acceptance by ecologists.

One reason for this may be that even after more than two decades of research, we are still at the stage where we cannot yet make a quantitative, or even qualitative, translation from the raw spectral pattern directly to the overall chemical composition without first calibrating some sort of empirical model. Therefore, we really don't yet know how to 'read' these spectra (Curran, 1989; Reeves, 1995). However, given the wide range of biochemical components that have been successfully predicted with NIR spectroscopy, it is clear that NIR spectra could potentially provide as much information about the chemical composition of a sample as if we were to perform a complete set of classical chemical analyses. This provides the foundation (and motivation) for the present work.

Here, we are operating under the assumption that there is a direct connection between the spectral pattern and the biochemical composition of a sample, and thus samples that differ in terms of biochemistry also have different VIS/NIR absorbance characteristics. Our hypothesis is that this spectral pattern is a signature that can be interpreted and used as a succinct, but quantitative, description of the chemical make-up. Even without conducting traditional chemical analyses, it should therefore be possible to determine the degree to which samples are biochemically similar or dis-similar on the basis of their spectral similarity (Gillon *et al.*, 1999a). In this regard, we must take a holistic view, and focus on the entire spectrum, rather than individual wavelengths. To this end, we use a variety of multivariate techniques (principal components analysis (PCA), discriminant analysis (DA), and partial least squares (PLS) regression – cluster analysis is omitted because it did not provide any significant insights) as tools with which we can begin to investigate and evaluate the overall spectral similarity of a set of samples. The research is framed in an ecophysiological context, in that samples from two conifer species, across a range of different growth environments (two crown positions and three growth elevations on three different mountains) are compared.

Methods

Overview of multivariate approaches

PCA is a data reduction technique whereby new composite variables (or components) are constructed as linear combinations of the original independent variables; usually, the first few components capture or explain most of the variation in the entire original data set (McGarigal *et al.*, 2000). With PCA, there is no attempt to describe relationships between the independent variables and any dependent variable. Thus the resulting ordination is unconstrained, in that PCA emphasizes the variation across all samples rather than minimizing the variation among similar samples (or those belonging to the same group). As a consequence, no artificial structure is imposed on the components. In previous studies, PCA has been used to differentiate the spectra of three categories of pine needles (fresh, falling, and litter; Gillon *et al.*, 1999a), and to compare the spectral characteristics of forages from different provenances of *Gliricidia* (Lister *et al.*, 2000). We use PCA to assess whether there are natural, underlying spectral differences between species or among samples from different growth environments.

DA is another data reduction technique in which composite variables (or canonical functions) are derived as linear combinations of the original independent variables (McGarigal *et al.*, 2000). We based our DA on the components derived by PCA (Nilsson *et al.*, 1994; Kemsley *et al.*, 1995), rather than individual wavelengths, in order to reduce the likelihood of over-fitting the data, and to avoid the problems known to be associated with stepwise selection of individual wavelengths (Grossman *et al.*, 1996). The objective of DA is to establish relationships between a dependent grouping variable and the original independent discriminating variables. By contrast to PCA's maximization of variation across all samples, DA effectively minimizes the variation among samples from the same group, and maximizes the variation between samples from different groups. Previous applications of DA have included classifying plant material from closely related species or subspecies on the basis of spectral properties (Kemsley *et al.*, 1995; Atkinson *et al.*, 1997).

PLS regression is a technique whereby factors (similar to those in PCA) are derived by taking into account the variation in the spectral data that is relevant for explaining variation in the characteristics of interest in the original samples (Williams & Norris, 2001). PLS is one of the standard methods used to

develop calibration equations to predict the chemical composition of a sample from NIR spectra (Bolster *et al.*, 1996; Reeves & Van Kessel, 2000; Reeves, 2001). Here, instead of chemical data, we use PLS to try to predict to which group (species, crown position, mountain or elevation) a particular sample belongs (which is perhaps a more complex question than predicting the concentration of a single chemical). As with DA, knowledge of the sample qualities (i.e. group membership) is used to derive an empirical, predictive model, and so both PLS and DA are more directed than PCA. However, with this targeted approach, we may be able to uncover more subtle spectral differences than would be revealed by PCA.

There are several assumptions underlying each of the above procedures, and when these assumptions are not met, the results must be interpreted with care, and it must be kept in mind that statistical inference is likely invalid (Williams, 1983). Because the spectral data we use in this paper may not meet some of the most basic assumptions (in particular, multivariate normality), we use these multivariate techniques for exploratory data analysis, rather than statistical testing (Williams, 1983).

Study sites and sample collection

Study sites were located on three mountains in the northeastern United States, Whiteface Mt. (Adirondack Mountains, New York), Mt. Mansfield (Green Mountains, Vermont), and Mt. Moosilauke (White Mountains, New Hampshire). Sites and sampling methodology are described in greater detail by Richardson (2003), but a brief overview will be given here. Foliage samples were collected from red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea* [L.] Mill.) trees at three different elevations on each mountain: near the bottom edge of the spruce-fir forest; at the tree line (or transition from forest to *krummholz*); and within the highest patches of *krummholz*. These are denoted henceforth as low, mid, and high elevation, respectively; there was typically 300 m elevation between low and mid sites, and 100 m between mid and high sites. Two transects were run on each mountain (thus a total of six plots per mountain). At each plot, foliage was taken from two crown positions on each tree. These represented 'sun leaves' and 'shade leaves.' Three trees of each species were sampled per plot, but like samples (e.g. the three red spruce sun samples) at each plot were pooled. This sampling scheme yielded a total of 72 samples (2 species × 2 crown positions × 3 elevations × 3 mountains × 2 transects per mountain = 72). Sampling was conducted on Mt. Moosilauke in early July, Whiteface Mt. in late July–early August, and Mt. Mansfield in late August. Only fully mature needles from the previous year's growing season were collected. Chemical analyses of these samples (Richardson, 2003, in press) demonstrated that there were strong differences in nutrient concentrations both between species and crown positions, but not among elevations, whereas fiber concentrations differed

most between species, somewhat among elevations, and little (except for cellulose) between crown positions. Pigment content differed between species, and changed rapidly with elevation for sun needles but not shade needles.

Sample preparation and analysis

Because water within leaf tissues has strong absorption bands in the NIR that overlap with (and even mask) those of important biochemical components (Lacaze & Joffre, 1994; Foley *et al.*, 1998), and because cellular structure and surface waxes can have a strong effect on reflectance from intact leaves, needle samples were first oven dried at 70°C (Wessman *et al.*, 1988) and then ground to a fine powder in a coffee grinder in order to minimize these effects. Samples were held in a rotating sample cup and scanned at VIS and NIR wavelengths (400–2498 nm) using a scanning monochromator (Model 6500, FOSS–NIRSystems, Silver Spring, MD, USA) equipped with Si (400–1098 nm) and PbS (1100–2498 nm) detectors. Pseudo-absorbance spectra were collected as $\log(1/R)$ where R = reflectance. Data were collected every 2 nm (1050 data points) at a nominal bandwidth of 10 nm, but only every fifth data point (210 data points) was used for this analysis.

Spectra were processed (e.g. standard normal variate transformation and detrending (Barnes *et al.*, 1989), as well as first and second derivatives using a gap width of 8 data points) with GRAMS/386 PLSPlus software (Galactic Industries Corp., Salem, NH, USA). Both particle size distribution (which may be irregular, depending on the type of grinder used) and density of sample packing are known to have effects on the spectral properties (e.g. scattering and baseline shift) of a sample (Foley *et al.*, 1998; Williams & Norris, 2001), but the effects of these and other artifacts are significantly reduced by these transformations (Wessman *et al.*, 1988), which can also enhance small but critical differences among otherwise similar spectra (Reeves, 1995). For example, the first derivative transformation removes confounding offset variations, whereas the second derivative transformation removes confounding linear trends, and both reveal small differences in shape among spectra (Williams & Norris, 2001).

Statistical analysis was conducted using SAS 6.12 (SAS Institute, Cary, NC, USA), except for PLS regressions which were conducted in GRAMS/386.

Results and Discussion

Absorbance spectra

Although there was some variation among samples, especially in the level of each curve, all absorbance spectra had generally the same shape (Fig. 1), with a spectral pattern similar to that previously shown for other species (e.g. red oak and white pine; Hallett *et al.*, 1997). In other words, prominent absorbance
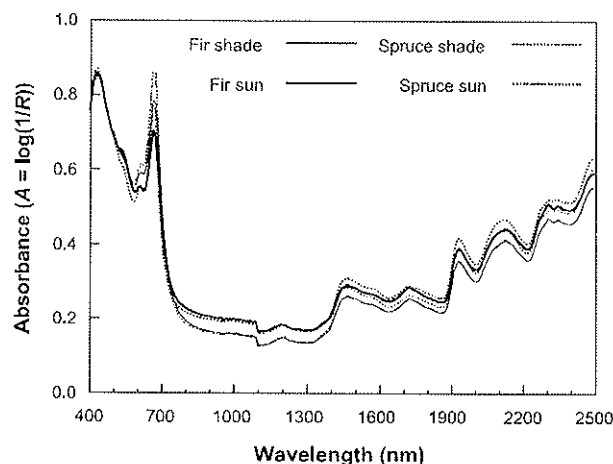
Fig. 1 Visible/near infrared (VIS/NIR) absorbance spectra for dried and ground conifer foliage from balsam fir (*Abies balsamea*) and red spruce (*Picea rubens*). Spectra represent the mean (*n* = 18) for each of four species × crown position combinations.
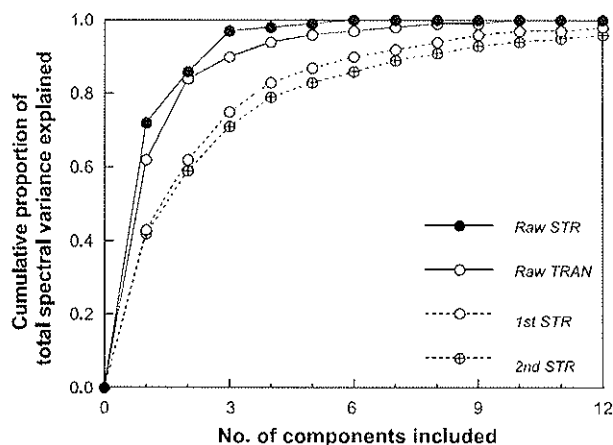

Fig. 2 Cumulative spectral variance explained by the first x principal components derived from analysis of four different sets of spectral data (visible/near infrared (VIS/NIR) absorbance of dried, ground conifer foliage).

peaks and troughs tended to occur at the same characteristic wavelengths across all samples. Absorbance was highest in the VIS, but tended to increase with increasing wavelength throughout the NIR. In the NIR, sun needle absorbance of both species was higher than that for shade needles. This contrasts with our previous observation that the VIS reflectance of fresh, intact sun needles (both species) is higher than that of shade needles (Richardson, 2003).

Principal components analysis (PCA) of absorbance spectra

PCA was conducted on a variety of different spectral sets, but we report the analysis for only four of these, as they provided the most meaningful results: the raw absorbance spectra, with no further transformation (*Raw STR*); the transformed raw absorbance spectra, processed with both multiplicative scatter and standard normal variate correction, as well as detrending (*Raw TRAN*); the first derivative spectra, with no further transformation (*1st STR*); and the second derivative spectra, with no further transformation (*2nd STR*). These same four spectral sets were used for all subsequent analyses.

Visual analysis of the eigenvalue scree plots (McGarigal *et al.*, 2000) indicated that the first three principal components were able to account for almost all of the total variance (97%, Fig. 2) of the *Raw STR* spectra. By contrast, for *Raw TRAN*, the first three components accounted for only 90% of the total variance. For both the *1st STR* and *2nd STR* spectra, the first three components accounted for less than three-quarters of the total variance, and even the eighth component was still making a modest contribution (≈ 2%) to the total variance (Fig. 2). Thus, more components were required to adequately summarize the *1st STR* and *2nd STR* spectra,

compared to either the *Raw STR* or *Raw TRAN* spectra. Two interpretations of this are possible: it could simply indicate that there is more noise in the derivatized spectra, but it may also reflect that the derivatized spectra actually contain more information than the raw spectra, since the derivatized spectra more precisely capture or describe subtle differences in spectral shape.

Underlying spectral patterns revealed by PCA

Fig. 3 shows that PCA revealed broad patterns in the spectra that indicate an underlying structure related to species and crown position. For example, for the *Raw STR* spectra, the two crown positions were roughly separated along the **Prin 1** axis (i.e. shade, **Prin 1** < 0; sun, **Prin 1** > 0). For *Raw TRAN*, the crown positions were well separated along **Prin 1**, and species was well separated along **Prin 2** (red spruce, **Prin 2** < 0; balsam fir, **Prin 2** > 0). For the derivatized data, *2nd STR* perfectly separated species along **Prin 1**, and roughly separated crown positions along **Prin 2**. These patterns can be traced back to variation in the original spectra: by looking at plots of the factor loadings (Fig. 4), it is possible to identify those spectral regions which contributed most to each principal component score. For the *Raw STR* spectra, **Prin 1** represented the overall NIR absorbance of the sample, as all wavelengths > 750 nm were weighted strongly, whereas for the *Raw TRAN* spectra, **Prin 1** represented a contrast of different spectral regions: 590–710 nm and 1490–2070 nm both had strong (absolute value of 0.6 or greater) negative weightings, whereas 750–1350 nm and 2270–2500 nm both had strong positive weightings. For the derivatized spectra, the factor patterns indicated a much more elaborate, oscillatory, component structure, with very clearly defined peaks.
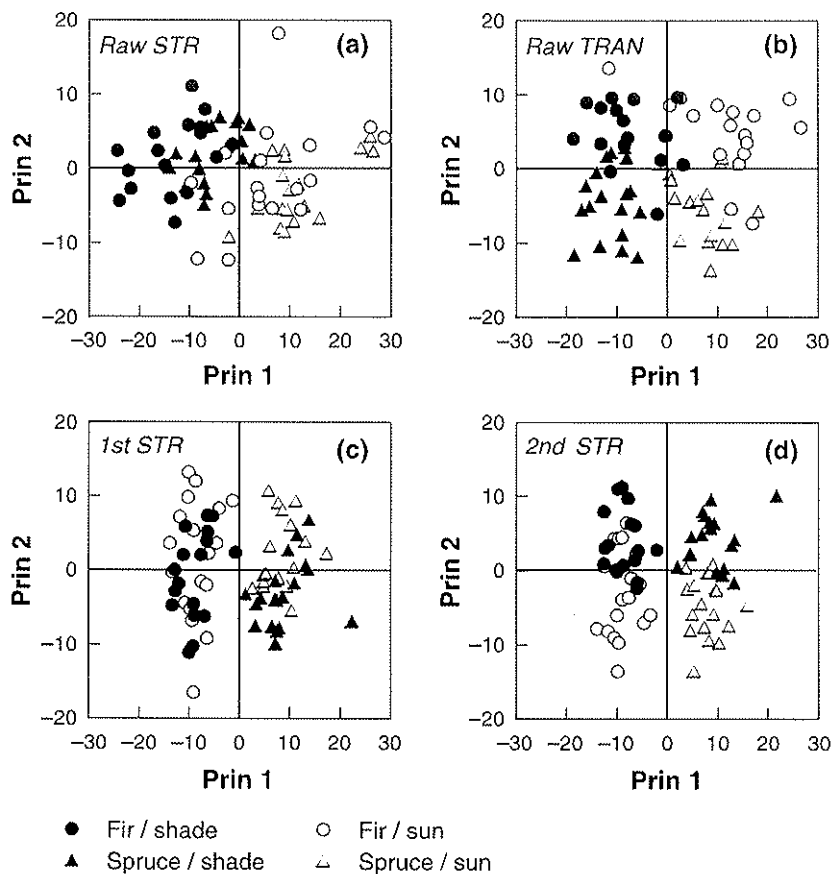
Fig. 3 Factor plot of first two principal components derived from visible/near infrared (VIS/NIR) absorbance spectra of dried, ground conifer foliage from balsam fir (*Abies balsamea*) and red spruce (*Picea rubens*). (a) Original, untransformed spectra (*Raw STR*); (b) Raw spectra processed with multiplicative scatter correction, standard normal variate transformation and detrending (*Raw TRAN*); (c) first derivative of untransformed spectra (*1st STR*); (d) second derivative of untransformed spectra (*2nd STR*). Filled symbols are used to denote shade foliage, open symbols denote sun foliage. Circles are used for balsam fir, and triangles for red spruce.

Correlations between the component scores and the biochemical composition help to identify the chemical constituents (nutrients, fibers, and pigments; data from Richardson (2003)) underlying the spectral differences. Note that any number of additional compounds, for which we did not analyze (not only starches and sugars, but also secondary metabolites; Foley *et al.*, 1998), may further contribute to the observed spectral variability. For example, for the *2nd STR* spectra, **Prin 1** was very strongly negatively correlated with foliar N ($r = -0.92$; $P \le 0.001$) and chlorophyll ($r = -0.56$, $P \le 0.001$), but positively correlated with hemicellulose and cellulose ($r = 0.66$ and $r = 0.77$, respectively; both $P \le 0.001$). As described above, **Prin 1** for *2nd STR* separated the samples into two distinct species groups. Relating this back to the chemistry data, it was previously shown that balsam fir foliage had higher N and chlorophyll, but lower hemicellulose and cellulose, than red spruce (Richardson, 2003). These correlations demonstrate the fundamental connection between the spectral data and the sample tissue chemistry. However, they do not really provide a means for directly estimating tissue chemistry on the basis of the derived components.

Mixed-model analysis (see Richardson (2003) for details of model structure and specification) indicated that, in addition to species and crown position, the derived principal

components (even beyond just the first two components) could also be related to other experimental factors, such as elevation and mountain (Table 1). For example, for *2nd STR*, **Prin 2** varied significantly among elevations ($F_{2,10} = 14.2$, $P \le 0.01$): samples from low, mid, and high elevation had **Prin 2** means of 3.3 ± 0.9, 0.9 ± 0.9, and -4.0 ± 0.9, respectively. Similarly, for *Raw TRAN*, **Prin 2** varied significantly among mountains ($F_{2,10} = 13.8$; $P \le 0.01$): samples from Mansfield, Moosilauke and Whiteface had **Prin 2** means of 4.4 ± 1.0, -1.5 ± 1.0, and -2.8 ± 1.0, respectively.

These results clearly demonstrate that PCA, on the basis of absorbance spectra, ordinated the samples into naturally occurring (and, in some cases, quite distinct) groups. These groups, which might be considered 'spectral families' (Gillon *et al.*, 1999a), were strongly related to species and crown position, and more weakly related to elevation and mountain. In a similar vein, Nilsson *et al.* (1994) demonstrated that PCA of NIR spectra separated *Silene dioica* stems from rosette leaves along **Prin 1**, and, to a lesser degree, smut-infected tissue from uninfected tissue along **Prin 2**. Even without identifying the biochemical source of differences among samples, PCA nevertheless derives components that correlate with the overall characteristics of the samples in question. These components have a descriptive value (in that they
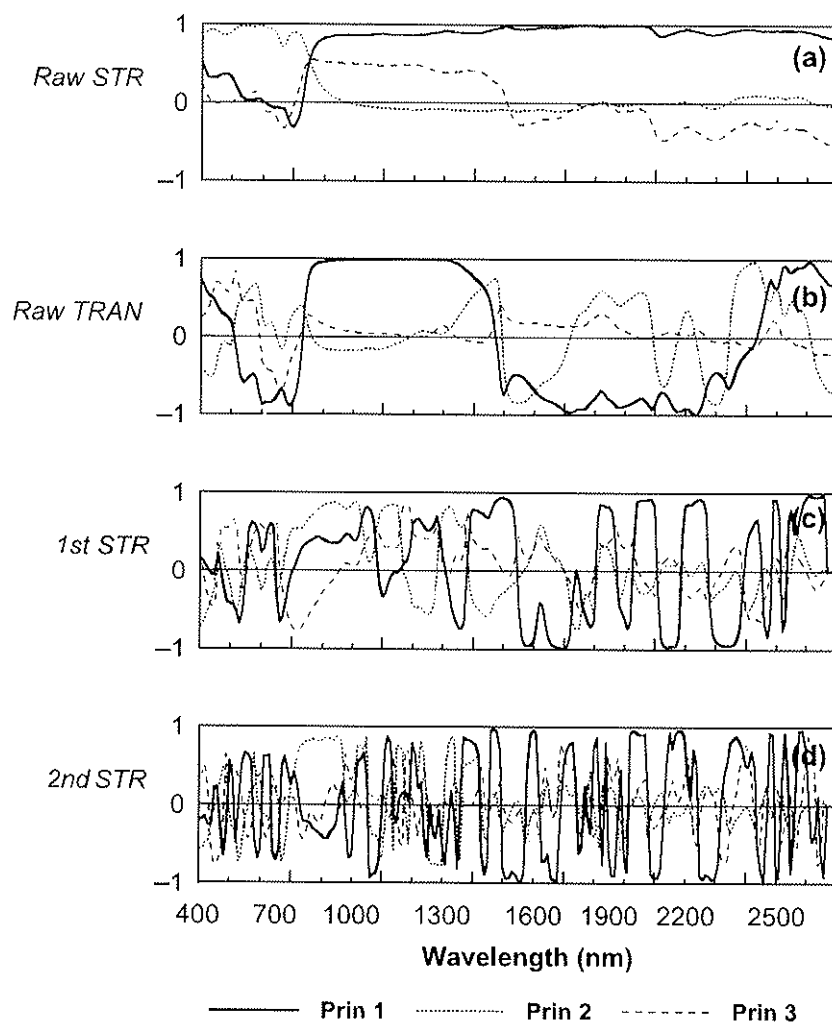
Fig. 4 Factor loading for the first three principal components for four different sets of spectral data (visible/near infrared (VIS/NIR) absorbance of dried, ground conifer foliage). (a) Original, untransformed spectra (*Raw STR*); (b) raw spectra processed with multiplicative scatter correction, standard normal variate transformation and detrending (*Raw TRAN*); (c) first derivative of untransformed spectra (*1st STR*); (d) second derivative of untransformed spectra (*2nd STR*).

summarize the spectral information) but perhaps also a functional significance, in that the derived components are, in effect, measured quantities which can be shown to differ among ecophysiologically distinct samples.

Discriminant analysis (DA)

PCA differs from both DA and PLS in that with PCA, underlying patterns can be revealed without any *a priori* knowledge of the sample characteristics. By contrast, for DA and PLS, analysis is conducted in relation to a dependent variable, which for DA must be a grouping variable, but for PLS could also be a continuous variable. The potential applications of these methods therefore differ. Both DA and PLS could be used to predict group membership of an unknown sample, but only once a suitable data set had been assembled and used to calibrate the predictive model.

For this study, DA was conducted on the different groupings (as defined by species, crown, elevation and mountain)

using the principal components derived above for the four spectral sets as independent variables. The first few components were not necessarily those that were of the greatest use in discriminating among groups (e.g., recall that for *2nd STR*, there was separation of crown positions along **Prin 2** but not **Prin 1**, see Table 1 and Fig. 3), and so we used a stepwise procedure, whereby at each step, the variable (i.e. component) which contributed most to the discriminatory power of the model was added to the model. The procedure was continued until a specified number of steps had been taken. To prevent overfitting, no more than eight variables were used in any single discriminating model, and we limited ourselves to selecting from the first 12 components of each spectral set, which together accounted for between 99.9% (*Raw STR*) and 95% (*2nd STR*) of the total variance (Fig. 2). Results of the stepwise analysis were then compared with those arrived at by using a discriminating model based on just the first four principal components.

The effectiveness of each discriminating model was judged using two criteria: the average squared canonical correlation

Table 1 P-values from statistical analysis of principal components derived from four sets of spectral data visible/near infrared (VIS/NIR) absorbance of dried, ground conifer foliage) in relation to experimental factors

Component	Prin 1	Prin 2	Prin 3	Prin 4	Prin 5	Prin 6
Raw STR (original, untransformed spectra)						
Species	**≤ 0.01**	0.62	**≤ 0.01**	**≤ 0.01**	0.32	**0.04**
Crown pos.	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	0.11	0.22	0.53
Elevation	**0.02**	0.26	**0.03**	**≤ 0.01**	0.29	0.18
Mountain	**≤ 0.01**	**≤ 0.01**	0.51	0.25	0.08	0.08
Raw TRAN (original spectra transformed with MSC, SNV and detrending)						
Species	**≤ 0.01**	**≤ 0.01**	**0.04**	**≤ 0.01**	0.06	**≤ 0.01**
Crown pos.	**≤ 0.01**	0.35	0.29	**≤ 0.01**	**≤ 0.01**	0.07
Elevation	**0.01**	0.14	**≤ 0.01**	0.12	**≤ 0.01**	0.84
Mountain	**0.03**	**≤ 0.01**	0.76	**0.04**	0.07	**≤ 0.01**
1st STR (first derivative of original spectra)						
Species	**≤ 0.01**	0.38	0.94	**0.03**	0.61	0.76
Crown pos.	0.71	**≤ 0.01**	**≤ 0.01**	0.25	0.13	0.42
Elevation	**0.04**	**≤ 0.01**	0.35	0.17	**≤ 0.01**	0.20
Mountain	**≤ 0.01**	**0.01**	**0.03**	0.09	**≤ 0.01**	**≤ 0.01**
2nd STR (second derivative of original spectra)						
Species	**≤ 0.01**	0.32	**0.04**	0.09	0.68	0.38
Crown pos.	0.06	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	0.13	**0.01**
Elevation	**0.05**	**≤ 0.01**	0.10	0.37	**≤ 0.01**	0.98
Mountain	**≤ 0.01**	0.11	**≤ 0.01**	0.16	**≤ 0.01**	0.06

The split-split-plot experimental design is described in text. Two species (red spruce (*Picea rubens*) and balsam fir (*Abies balsamea*)), two crown positions (sun foliage and shade foliage), three elevations (low, mid and high), and three mountains (Whiteface Mt., NY, Mt. Mansfield, VT, and Mt. Moosilauke, NH, USA). Factors significant at $P \leq 0.05$ are shown in bold.

(ASCC), which approaches 1 if the different groups are well separated (and 0 if the groups are not well separated), and the classification error rate (CER), as measured by a one-out cross-validation procedure (Foley *et al.*, 1998) whereby each sample was classified according to a model developed using all the other samples. This jackknife resampling procedure is considered a useful means of assessing the stability of the classification functions when the sample size is small (McGarigal *et al.*, 2000), as is the case here.

All four spectral sets did a good job of differentiating between the red spruce and balsam fir samples, even when only **Prin 1**–**Prin 4** were used (all ASCC ≥ 0.8, CER ≤ 1%; Table 2). For the *1st STR* and *2nd STR* spectra, the first component was the most important variable for discriminating between species, and indeed, near-perfect separation of species could have been obtained using only **Prin 1** for these two spectral sets (see Fig. 3). By contrast, for *Raw STR*, **Prin 3** was the most important and for *Raw TRAN*, it was **Prin 2**.

When eight variables were included in the stepwise model, each spectral set could be used to obtain good separation of sun needles from shade needles (all ASCC ≥ 0.8, CER ≤ 5%; Table 2). However, with only four variables included, the error rates rose somewhat, especially when the four variables were restricted to just **Prin 1** through **Prin 4** (CERs of 10% for three out of four spectral sets, Table 2).

The *2nd STR* results illustrate the relative ease with which different species and crown positions could be discriminated (Figs 3 and 5). In comparison, it was much more difficult to

discriminate among samples from different elevations, even with eight variables included. Error rates of more than 30% were the general rule, and those of 40% or greater were not uncommon (Table 2). Accompanying the high CERs were consistently low ASCCs (≤ 0.4). While there was no problem in separating high and low elevation samples, there was considerable overlap of low and mid samples, and mid and high samples (Fig. 5). For example, for the *2nd STR* spectra, in the cross-validation test, only one sample from high elevation was classified as low elevation, and only one low elevation sample was classified as high elevation. However, eight mid elevation samples were misclassified as low elevation samples, and four mid elevation samples were misclassified as high elevation samples. This is likely due to the fact that the midelevation samples were from sites that were spatially intermediate between the low and high elevation sites. The failure of DA to successfully differentiate among samples from different elevations is probably related to the biochemical similarity of foliage from different elevations but of the same species. The ability to discriminate among samples from different mountains was similar to that for elevations, suggesting that site-specific differences in leaf chemistry accounts for only a small portion of the spectral variability among samples.

Past efforts with DA have focused on differentiation among samples from closely related species. For example, Gong *et al.* (1997) used discriminant analysis of reflectance spectra (320–930 nm) to differentiate among several conifer species; reflectance of VIS wavelengths, which is primarily determined

Spectral set	Order of component selection	Best 8		Best 4		Prin 1–4	
		ASCC	CER	ASCC	CER	ASCC	CER
Raw STR (original, untransformed spectra)							
Species	3, 4, 1, 7, 6, 9, 5, 8	0.94	0%	0.84	0%	0.84	1%
Crown pos.	1, 2, 8, 3, 10, 4, 7, 9	0.86	4%	0.80	3%	0.80	6%
Elevation	4, 8, 6, 3, 10, 1, 2, 12	0.35	43%	0.25	41%	0.25	52%
Mountain	2, 10, 7, 6, 8, 9, 1, 5	0.65	15%	0.44	25%	0.23	40%
Raw TRAN (original spectra transformed with MSC, SNV and detrending)							
Species	2, 4, 6, 3, 5, 10, 1, 12	0.97	0%	0.82	0%	0.82	1%
Crown pos.	1, 5, 10, 4, 6, 11, 9, 7	0.87	1%	0.77	6%	0.76	10%
Elevation	5, 3, 1, 12, 4, 10, 2, 11	0.41	32%	0.32	35%	0.32	48%
Mountain	6, 2, 7, 12, 10, 4, 11, 5	0.67	19%	0.45	28%	0.22	41%
1st STR (first derivative of original spectra)							
Species	1, 4, 7, 2, 5, 12, 6, 10	0.98	0%	0.97	0%	0.97	0%
Crown pos.	3, 2, 7, 8, 9, 12, 5, 6	0.82	4%	0.74	7%	0.74	10%
Elevation	2, 5, 4, 9, 6, 10, 8, 3	0.40	38%	0.34	35%	0.33	46%
Mountain	5, 7, 2, 9, 4, 6, 11, 1	0.66	9%	0.42	25%	0.22	35%
2nd STR (second derivative of original spectra)							
Species	1, 4, 3, 6, 5, 7, 10, 2	0.98	0%	0.97	0%	0.97	0%
Sun/shade	2, 3, 9, 6, 4, 8, 7, 12	0.84	1%	0.75	6%	0.69	10%
Elevation	2, 5, 7, 9, 3, 10, 4, 11	0.41	38%	0.33	38%	0.31	49%
Mountain	3, 7, 5, 6, 9, 11, 4, 2	0.75	8%	0.56	24%	0.31	32%

Table 2 Results of discriminant analysis of principal components derived from visible/near infrared (VIS/NIR) absorbance spectra (four different spectral sets) of dried, ground conifer foliage in relation to sample characteristics

A stepwise routine was used to identify, in order, the principal components (selected from the first 12) which contributed most to the discriminating power of the model ('Best 8' and 'Best 4' columns). These results are compared with a model based strictly on the first four principal components ('Prin 1–4' column). ASCC is the average squared canonical correlation, CER is the classification error rate based on a one-out cross validation procedure.

by pigment content, was found to be of more use than that of NIR. This may be due to the fact that the limited portion of the NIR spectrum used by those authors contains little or no biochemical information. Atkinson *et al.* (1997) were able to successfully distinguish between two *Betula* species and their hybrids using discriminant analysis of VIS/NIR reflectance spectra, but wavelengths of less than 900 nm generally had low discriminating power. These authors found that models with 22 individual wavelengths had more discriminatory power than those based on principal component scores. Finally, Kemsley *et al.* (1995) found that mid infrared reflectance could be used to distinguish between *Coffea canephora* var. *robusta* and the more highly valued *C. arabica*. What differentiates the present study is that these results demonstrate that there are sample characteristics, which might be considered to be more subtle than species (such as crown position or growth elevation), to which DA can be successfully applied using spectral data. Clearly, there are numerous practical applications for this type of analysis. For example, this approach could potentially be used for the rapid differentiation between an exotic invasive population and native populations of the same species (cf. Lister *et al.*, 2000), which would be possible if the populations differed in their tissue chemistry. Ultimately, remote sensing could then be used for management purposes to map and monitor the extent or spread of the invasive populations.

Partial least squares (PLS) regression

For PLS, which is normally applied to continuous data, group membership was coded numerically, for example, shade (−1) vs sun (+1) foliage, or low (−1) vs mid (0) vs high (+1) elevation. The number of PLS factors used in the calibration was determined by the Prediction Residual Error Sum of Squares (PRESS) *F*-statistic from the one-out cross-validation procedure. Once the optimal number of factors was determined, a final calibration was developed using the results from the one-out cross validation.

For the sake of simplicity, we report only the PLS results for two spectral groups, *Raw STR* and *2nd STR*. Results using the derivatized data were consistently superior to those using the raw spectra; not only were fewer factors required for the derivatized data, but the resulting structure explained a higher proportion of the variance in the grouping data (i.e. higher R^2, see Table 3). This was especially true for elevation, for which PLS on the *2nd STR* spectra ($R^2 = 0.88$) worked much better than the *Raw STR* spectra ($R^2 = 0.79$). Overall, however, results were similar to, but in comparison superior, to those of DA. This can be seen by comparing Figs 5 and 6, and the ASCC and CER values in Table 2 with those in Table 3. For all grouping variables, PLS did a better job of both separating groups, and minimizing the scatter within groups, compared to DA. However, as with DA, PLS did a better job predicting
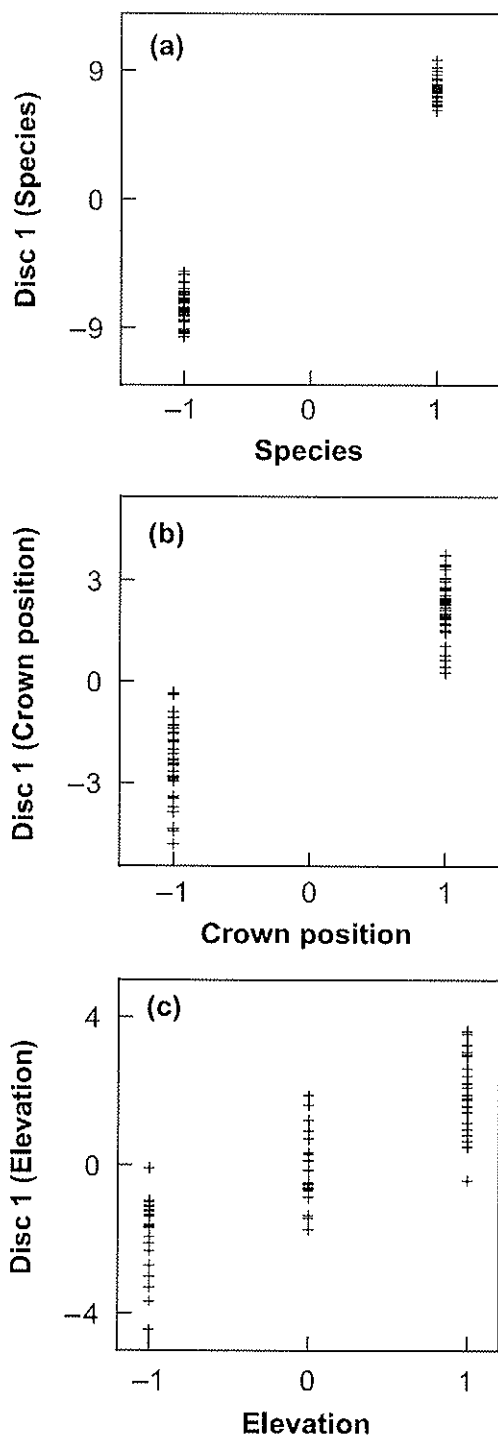
Fig. 5 Discriminant analyses based on visible/near infrared (VIS/NIR) absorbance spectra of dried, ground conifer foliage in relation to sample characteristics. The x-axes are coded as follows: (a) Species, red spruce (*Picea rubens*) = −1, balsam fir (*Abies balsamea*) = +1; (b) crown position, shade foliage = −1, sun foliage = +1; (c) elevation, low = −1, mid = 0, high = +1. The y-axes show the first canonical discriminant axis for each analysis.
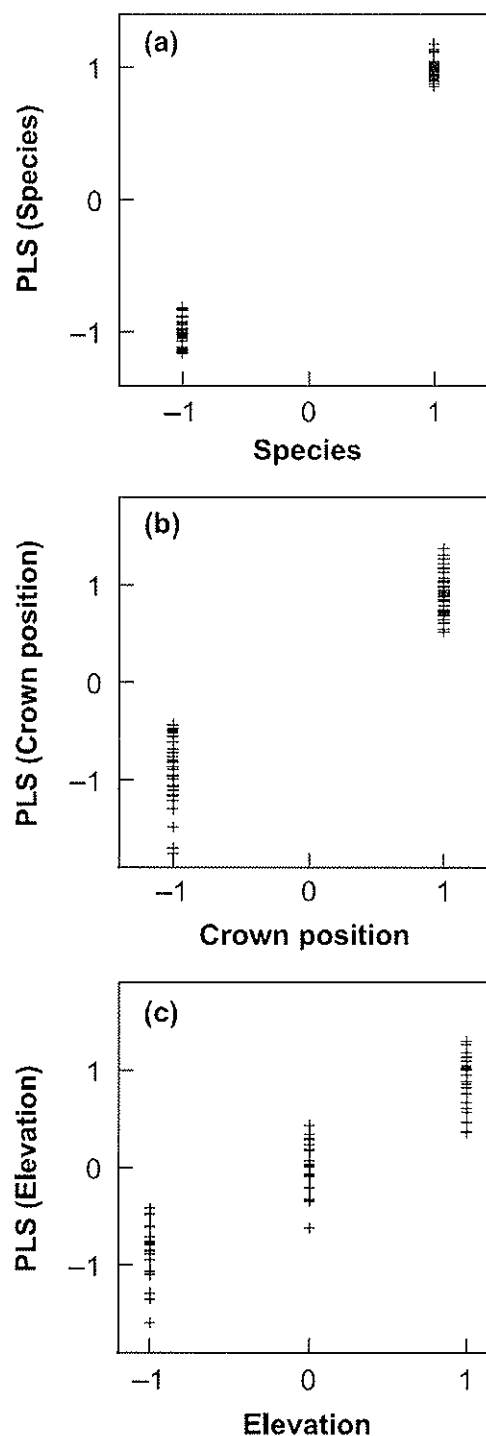
Fig. 6 Actual (x-axis) and predicted (y-axis) sample characteristics, based on partial least squares (PLS) regression of visible/near infrared (VIS/NIR) absorbance spectra of dried, ground conifer foliage. Axes are coded as follows: (a) species, red spruce (*Picea rubens*) = −1, balsam fir (*Abies balsamea*) = +1; (b) crown position, shade foliage = −1, sun foliage = +1; (c) elevation, low = −1, mid = 0, high = +1.

Grouping variable	Spectrum	# Factors	RMSD	R^2	ASCC	CER
Species	Raw STR	10	0.122	0.985	0.99	0%
	2nd STR	7	0.082	0.993	0.99	0%
Crown Pos.	Raw STR	7	0.339	0.885	0.88	0%
	2nd STR	6	0.292	0.915	0.91	0%
Elevation	Raw STR	10	0.374	0.788	0.39	17%
	2nd STR	7	0.281	0.881	0.44	7%
Mountain	Raw STR	9	0.255	0.901	0.45	3%
	2nd STR	5	0.237	0.915	0.45	4%

Table 3 Results of partial least squares (PLS) regression of sample characteristics against visible/near infrared (VIS/NIR) absorbance spectra of dried, ground conifer foliage

Sample characteristics were defined by grouping variables, which were coded numerically (for example, shade (–1) vs sun (+1) foliage, or low (–1) vs mid (0) vs high (+1) elevation). Results are shown for two sets of spectra, Raw STR (original, untransformed spectra), and 2nd STR (second derivative of the raw spectra). The 2nd STR spectra produced the best results of all four spectral sets tested. RMSD (root mean squared deviation) and R^2 are calculated for predicted values. Discriminant analysis-type statistics (ASCC, average squared canonical correlation, and CER, the classification error rate based on a one-out cross-validation procedure) were computed by running discriminant analysis on the predicted values from PLS regression.

species ($R^2 \approx 0.99$ for both spectral sets) than crown position ($R^2 \approx 0.9$ for both spectral sets). Unlike DA, PLS was able to differentiate between elevations and between mountains with a high degree of success (CER < 10% with the 2nd STR spectra). The reason that PLS performs better than DA for these analyses has to do with the way in which the variance of the spectral set is captured by the PCA factors vs the PLS factors. With both methods, the factors are linear combinations of the independent variables. However, with PCA, the first factor explains the maximum amount of variance in the original data, and the second factor, which is orthogonal to the first, explains the maximum amount of the remaining variance, and so on. These factors are not derived in relation to a dependent variable, and so the components used for different discriminant analyses will always be identical (i.e. the principal components), regardless of the grouping variable used. By contrast, PLS extracts the variance in the data set as it correlates with the quantity of interest. Thus the PLS factors depend entirely on the dependent variable used – for example, different PLS factors are derived for species and crown position.

We did not attempt to associate specific biochemical compounds with the PLS factors (factor weights and loadings are not shown here). However, the PLS results for mountain and elevation support the results of the mixed model analysis of the PCA components, namely that although less pronounced than the spectral differences among species or crown position, there are detectable spectral differences among samples from different mountains or elevations.

In future studies, PLS could be applied to develop a proxy (based on the spectral measurements) for a sample characteristic or trait which is difficult, time consuming, or costly to measure. This trait, which clearly need not be a continuous variable, could then be measured on a subset of the samples, a calibration equation developed, and the trait value predicted for the remainder of the samples.

Conclusion

The results presented here suggest that spectral (and thus presumably biochemical) differences are largest among samples from different species (i.e. spruce vs fir), and smallest among samples from different elevations or different mountains. These results agreed with our laboratory analysis of foliar nutrients and fiber content.

Thus, these multivariate techniques, in particular PCA, are proposed as exploratory methods well-suited to the holistic (in that the entire spectrum is utilized, rather than individual wavelengths) analysis of VIS/NIR data. Based on the derived principal components, generalizations can be made about the overall compositional similarity of different samples. Results indicated that the components derived from PCA were correlated not only with sample characteristics such as species and growth environment, but also with the chemical composition (nutrient, fiber and chlorophyll content) of the samples. DA and PLS can be used similarly in an exploratory manner to investigate whether groups of samples (i.e. experimental treatments) differ in terms of spectral properties (and hence chemical composition). Unlike PCA, however, DA and PLS can be further used to develop empirical models to actually predict sample characteristics. Although a set of known samples is required to first calibrate a model, the results presented here show that certain characteristics (such as species, crown position, and growth elevation) can be predicted with reasonable success on the basis of VIS/NIR absorbance.

What makes VIS/NIR spectral analysis a useful analytical tool is that each spectrum contains such a wealth of information. There are many instances where it may be more important to know whether there are biochemical differences among two sets of samples than it is to know exactly what causes these differences. This is where multivariate analyses of VIS/NIR spectra could be most suitably applied. In comparison, with

classical methods of chemical analysis, it is necessary not only to decide which analyses to perform, but also to consider practical constraints (e.g. time, money, and laboratory resources) that typically limit the number of analyses that can be conducted. If samples differ in lignin, but are only analyzed for chlorophyll and N, one might erroneously declare there to be 'no treatment effect' simply because the appropriate chemical analysis was not performed. With the sorts of multivariate analysis performed here, this is much less likely to be the case.

Although multivariate techniques applied in this manner do not provide any direct information about what chemical components actually cause the spectral differences, these approaches may still be useful as rapid and inexpensive screening tools. For example, from the original set of organic samples (foliage, leaf litter, seed mixtures, etc.), a subset of samples could be selected for further analysis by identifying those samples that are spectrally either most similar or most different. Or, if samples from two different experimental treatments were found to be spectrally similar, one might choose not to spend the time and money conducting laboratory analyses to look for chemical differences which don't exist. Furthermore, with more research, we may be better able to identify, based on spectral differences, the source of the chemical differences — from this, we could target the appropriate traditional analyses that would be required to conclusively document the chemical differences.

Acknowledgements

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