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Ecological applications of near infrared reflectance spectroscopy – a tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance

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Abstract Many ecological studies rely heavily on chemical analysis of plant and animal tissues. Often, there is limited time and money to perform all the required analyses and this can result in less than ideal sampling schemes and poor levels of replication. Near infrared reflectance spectroscopy (NIRS) can relieve these constraints because it can provide quick, non-destructive and quantitative analyses of an enormous range of organic constituents of plant and animal tissues. Near infrared spectra depend on the number and type of C—H, N—H and O—H bonds in the material being analyzed. The spectral features are then combined with reliable compositional or functional analyses of the material in a predictive statistical model. This model is then used to predict the composition of new or unknown samples. NIRS can be used to analyze some specific elements (indirectly – e.g., N as protein) or well-defined compounds (e.g., starch) or more complex, poorly defined attributes of substances (e.g., fiber, animal food intake) have also been successfully modeled with NIRS technology. The accuracy and precision of the reference values for the calibration data set in part determines the quality of the predictions made by NIRS. However, NIRS analyses are often more precise than standard laboratory assays. The use of NIRS is not restricted to the simple determination of quantities of known compounds, but can also be used to discriminate between complex mixtures and to identify important compounds affecting attributes of interest. Near infrared reflectance spectroscopy is widely accepted for compositional and functional analyses in agriculture and manufacturing but its utility has not yet been recognized by the majority of ecologists conducting similar analyses. This paper aims to stimulate interest in NIRS and to illustrate some of the enormous variety of uses to which it can be put. We emphasize that care must be taken in the calibration stage to prevent propagation of poor analytical work through NIRS, but, used properly, NIRS offers ecologists enormous analytical power.

Key words Near infrared reflectance spectroscopy · Leaf chemistry · Plant-herbivore interactions · Food intake · Decomposition

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Introduction

The analysis of nutrient concentrations in samples of plant and animal tissues is an integral part of many ecological studies. These analyses (e.g., total nitrogen, carbohydrates and lipids) are frequently so time-consuming and expensive that sampling strategies adopted are less than ideal. For example, variations of nutrients within individuals of a plant species often may be an important component of animal foraging that is ignored in many studies simply because the extra analytical work cannot be accommodated.

Another typical problem for the analyst involves the large amount of sample required for some analyses; these may be difficult to obtain (e.g., from seedling plants). Alternatively, the analyst may have to bulk replicate samples to get sufficient material for all necessary analyses and again compromise an ideal experimental design.

Often ecologists are forced to use a range of indirect measures in place of a more suitable direct measure of

the attribute of interest. This situation has often arisen in studies of the foraging behavior of folivorous primates and marsupials (Waterman et al. 1980). Typically, investigators measure the concentrations of a diverse range of elements and attributes such as "fiber" when what is actually needed is an estimate of relative food intake by the animal (Poppi 1996; A. McIlwee, I. Lawler and W.J. Foley unpublished work).

Near infrared reflectance spectroscopy (NIRS) offers ecologists enormous flexibility in meeting these sorts of challenges. The analysis of multiple constituents of plant tissues can be made rapidly in a single operation using NIRS technology (up to 150 samples can be processed in a day by a single operator) and with minimal sample preparation (Marten et al. 1989). The use of NIRS is low-cost (savings of at least 80% of normal laboratory costs are easily achievable; Aragones 1997) and amenable to small sample sizes (multiple analyses can be obtained on as little as 0.2 g of material; Aragones 1997). Finally, NIRS analysis is non-destructive and produces no chemical wastes, which should be an important consideration for all ecologists.

Use of NIRS has been adopted enthusiastically by many agricultural and manufacturing industries. Those concerned with evaluating and improving the nutritive value of feedstuffs for livestock are some of the biggest users (see Shenk and Westerhaus 1994). Some NIRS methods have been established for more than 20 years and have achieved "official" status (e.g., AOAC 1990). Much of the early use of NIRS centered on exploiting the technique as a time and cost-effective method of nutrient analysis. However, in recent years, there have been many innovative uses of NIRS and it is becoming an increasingly accepted tool for routine analysis in many research areas.

Many of the analytical techniques used in ecology are based on those used in agricultural industries, but NIRS has been little used in ecological studies (but see Brooks et al. 1984; Joffre et al. 1992; Malley et al. 1996; Nilsson et al. 1996 for notable exceptions). NIRS offers an excellent opportunity for ecologists to escape the constraints of laboratory analyses on the design of their investigations and to apply many laboratory-based measurements to free-ranging animals.

In this article we summarize some recent studies using NIRS in ways that we believe will be useful to ecologists. Our aim is to stimulate interest amongst ecologists in NIRS, to bring together a scattered literature, and to widen the applications of the methodology. We are less concerned with describing detailed methodologies for applying the technique to different materials that might be of interest to experienced users of the technique. In our own work (nutritional ecology of herbivores), we have found that NIRS has allowed us to focus our research on landscape-scale questions and to apply laboratory findings to the field. Perforce, many of the examples we cite are related to our own interests, but we stress that our belief is that the potential applications of NIRS are enormous.

Basis of the technique

The composition of plant or animal tissues is ultimately reflected in the types of bonds between the atoms or groups of atoms (functional groups) that make up those tissues and information about these functional groups can be sought through many different forms of spectroscopy. When a sample of organic material is irradiated, the bonds continually vibrate, which causes stretching and bending. This in turn causes a type of wave motion within the bond at a frequency that is characteristic of the functional group. The frequencies of the incident light that match the frequencies of the vibrational waves are absorbed whereas other frequencies are reflected or transmitted. The process is the same as that which allows us to see colored objects that intercept white light (Van Kempen and Jackson 1996).

Near-infrared radiation (750–2500 nm) (Fig. 1) is absorbed mainly by C—H, N—H and O—H bonds (Osborne et al. 1993) which are the primary constituents of the organic compounds of plant and animal tissues. The chemical constituents of the tissue determine the nature and number of bonds present and therefore the wavelengths and amount of light that is absorbed. Therefore, the spectrum of light that is reflected from the sample contains detail on the chemical composition of that material (Shenk et al. 1992; Shenk and Westerhaus 1994).

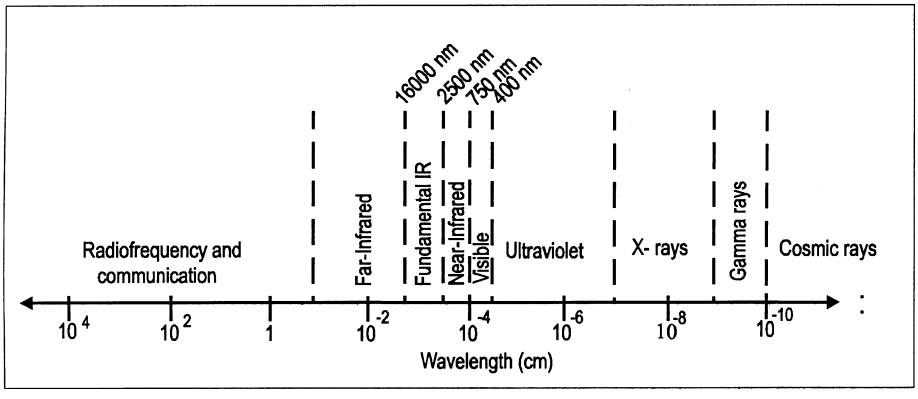

Fig. 1 The electromagnetic spectrum showing the position of near-IR

However, the peaks in an NIR spectrum are not distinct or sharp because they consist of overtones and combinations from primary absorptions in the mid-infrared region and also because some of the light is scattered (Shenk and Westerhaus 1993b). Consequently, there are few if any regions of the NIR spectrum where absorbance can be due to only one type of functional group and direct interpretation of the spectral absorbances is very difficult for complex mixtures (Fig. 2). Given the limited number of functional groups present in plant and animal tissues (due to limited numbers of metabolic pathways) an analyst could assume that absorbance in the spectral region characteristic of the amide bond of a protein is, in fact, largely due to the protein in the tissues. However, one could never be certain about this (see Fig. 2) and so NIRS relies on applying statistical tests to that assumption. In other words, the analyst derives a statistical model that tests the intensity of the relationship between a particular absorbance and an independent laboratory assay of protein content in a range of different plant or animal tissues. Since a different group of plant or animal tissue might contain different functional groups that also absorb in the spectral region of interest, the soundness of the relationship between spectral absorption and laboratory assays should be assessed for each type of tissues of interest. Near infrared analysis is thus an indirect or secondary method that estimates chemical composition by comparing spectra with samples of known composition (Shenk et al. 1992; Shenk and Westerhaus 1994). This procedure is known as calibration.

In practice, the analyst develops a multivariate statistical model to describe the relationship between the NIR spectral absorbances and the chemical components or characteristic of interest (Shenk and Westerhaus 1993b). The statistical model is then used to predict the composition of unknown samples that are part of the same population. Samples which fall outside the population can be analyzed by traditional means and iteratively included in a new model (Shenk et al. 1992; Shenk and Westerhaus 1994). These procedures are discussed in more detail below.

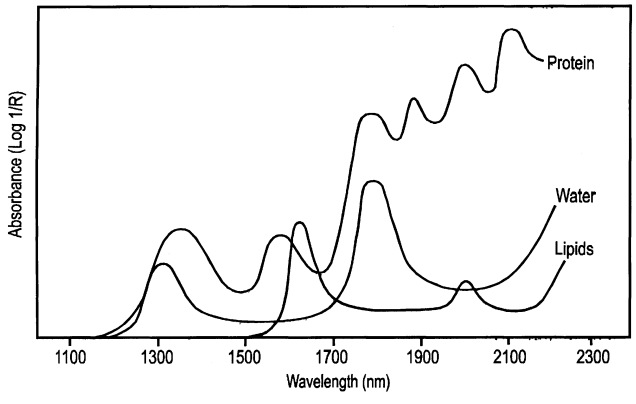

Fig. 2 Broad and overlapping peaks attributable to different constituents are characteristic of a near infrared reflectance spectrum (adapted from Osborne et al. 1993; Givens et al. 1997)

Mechanics of NIR spectroscopy and sample presentation – importance of particle size

A NIR spectrophotometer consists of a light source, a means of selecting particular wavelengths (NIR spectroscopists traditionally use wavelengths (nm) rather than wave-numbers as favoured by those working in the mid IR) within the spectrum, a detector for collecting the reflected radiation and a signal and data processing computer (Fig. 3). The sample (which in most current applications is dried and ground – but NIRS can be used with any other sample form – see section below on analysis of fresh forages), is usually packed manually into a sample cup which has an optical grade quartz glass cover on one side. Care is required to ensure that the sample is spread evenly and packed to a consistent degree of compression. The sample cups can be small ring cups of between 20 and 50 mm diameter or alternatively larger cells that hold up to 50 g of coarser material. These are then placed in the spectrometer and the reflectance spectra collected. In some applications, fiber optics are used to bring the light to a remotely presented sample and the information is either conducted back along the optic cable to the detector and microprocessors, or captured by remotely placed detectors and conveyed electronically to the microprocessor.

Particle size of the sample has a major effect on the NIR reflectance spectrum (Casler and Shenk 1985; Windham 1987). Increasing particle size results in an increased apparent path length for the incident light and so the measure of reflectance also increases (Fig. 4). Consistent particle size between samples is therefore important and analysts using NIRS techniques with dried, ground samples need to be aware of the performance of their laboratory grinders (Shenk and Westerhaus 1993b). The fineness of the particles is less important than the distribution of sizes and many grinders simply cannot produce a uniform particle size. Depending on the nature of the samples being ground, analysts may have to regularly replace impellers and other grinding parts more frequently than expected to ensure that this optimum is maintained.

Calibration procedures

The spectroscopic and chemometric principles used in developing calibration models are beyond the scope of this short review. Nonetheless it is appropriate to provide a broad outline of approaches which are most typically taken by analysts so that readers can appreciate the strengths and limitations of the whole technique. Those interested in specific procedures should consult some of the many excellent references on this subject (e.g., Shenk and Westerhaus 1991a,b, 1993; Smith and

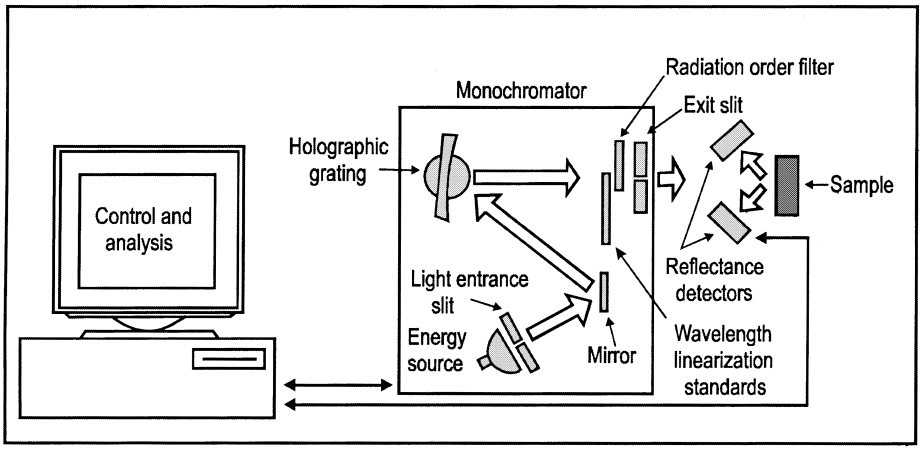

Fig. 3 Components of monochromator-based near-IR spectrometer

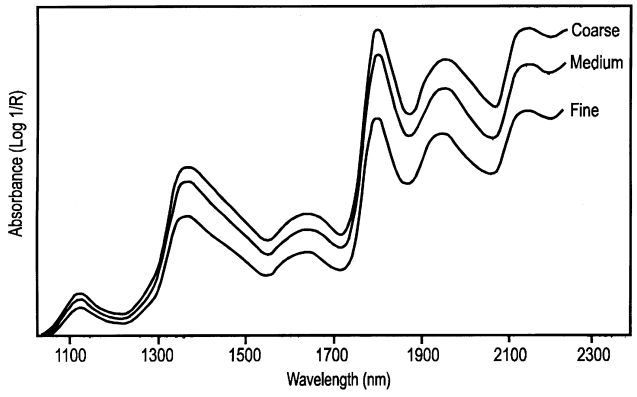

Fig. 4 The effect of particle size on spectral absorbance in the near-IR region (adapted from Shenk et al. 1992)

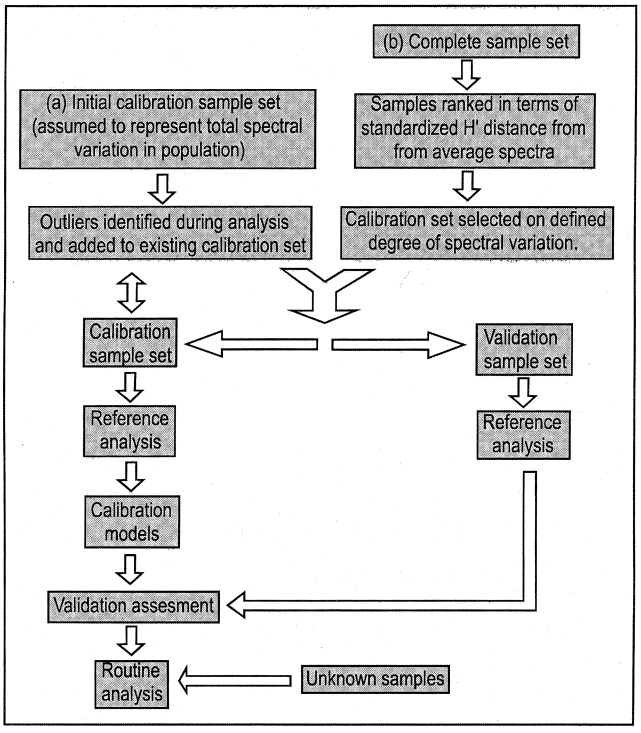

Fig. 5 Alternative strategies for the development of calibration equations needed for the routine analysis of unknown plant or animal samples

Flinn 1991; Osborne et al. 1993; Baker et al. 1994). Figure 5 summarizes the most common steps taken to develop calibrations for plant and animal tissues (Shenk and Westerhaus 1993b).

The essence of any calibration procedure is to ensure that the range of spectral variation found in the whole population is represented in the samples selected for analysis for calibration development. Generally, the entire population is ranked in terms of distance from the average spectrum. Prior to ranking, a variety of mathematical treatments are applied to the spectra to emphasize only those data that are relevant. Particle size and structure of a sample can cause spectral differences that are unrelated to chemical composition. These transformations usually include scatter correction that may use standard normal variate procedures and mathematical derivative transformations.

Once the population has been structured in this way, samples with either extreme spectra or those that have very similar spectra are eliminated so that those remaining represent a defined degree of spectral variation. Various algorithms have been developed for doing this. The CENTER and SELECT algorithms of Shenk and Westerhaus (1991a) have proved among the most popular for forage scientists but a variety of other methods have been developed (Shenk and Westerhaus 1991b).

In theory selection of samples that cover the range of spectral variation in the data set should be sufficient but we believe that most ecologists would be more comfortable if the calibration set also covered the range of taxonomic variation or included all types of treatments applied in field experiments. For example, in our work on herbivore foraging in tropical savannas, a number of common grass species were not included in the original calibration set chosen by the SELECT algorithm. Consequently, we expanded the set by selecting samples

from our collection of those particular plant species (A.P. Woolnough and W.J. Foley, unpublished work).

Once sufficient samples have been selected, the traditional compositional analyses are performed on this subset. These may include measures such as concentration of nitrogen or protein, carbohydrates, lipids or even individual fatty acids and amino acids. However, as we describe in more detail below, other more integrative attributes may be measured such as the digestibility and intake of food by herbivores, grain yield during milling (Welsh et al. 1996), susceptibility to insect attack, or pulp yield in the paper industry. Given the importance of these assays or measures, it is vital that the analyst be confident of the degree of accuracy and precision of these analyses (see Conventional laboratory assays).

The actual model is then constructed by developing a regression equation between the spectral absorbances and the traditional laboratory analyses (Fig. 6) (Shenk and Westerhaus 1991c, 1993a, b). This involves any of a number of multivariate regression procedures including multiple linear regression (MLR), principal components regression (PCR) and partial least squares (PLS) regression. Although many early studies were performed with MLR, this approach uses only a few wavelengths whereas the other methods use the full spectrum. Greater computing power is one reason why a modification of the PLS procedures (MPLS) is one of the most popular regression procedures (Shenk and Westerhaus 1993b) but there are other even more sophisticated approaches available involving neural networks and wavelet theory. All these options are provided in various statistical packages but the analyst needs a clear view of the purpose of the calibration before choosing one of these methods and should operate within the bounds of standard practices (Anon 1995).

Validation of the calibration can be done in several ways. Previously the most common method used involved an independent set of samples for which compositional data was also available. In such cases, the calibrations derived from a "calibration data set" could be tested by predicting values in a "validation data set", the relationship between these being assessed by simple regression analysis. More recently, this approach has been superseded by a procedure called cross validation, a form of Monte Carlo simulation, in which the population is arbitrarily divided into a small number of groups and a prediction is made of the values for one group based on calibrations developed from the remaining groups. In turn, predictions are made for all groups with the average of predictions for all groups (Shenk and Westerhaus 1993a, b). Depending on the amount of computing power available, these "groups" may contain as few as one sample. This procedure is useful because all available chemical analyses for all individuals can be used to determine the calibration model without the need to maintain separate validation and calibration sets (Shenk and Westerhaus 1993a, b). Nonetheless, some analysts still maintain separate validation sets for verifying these models.

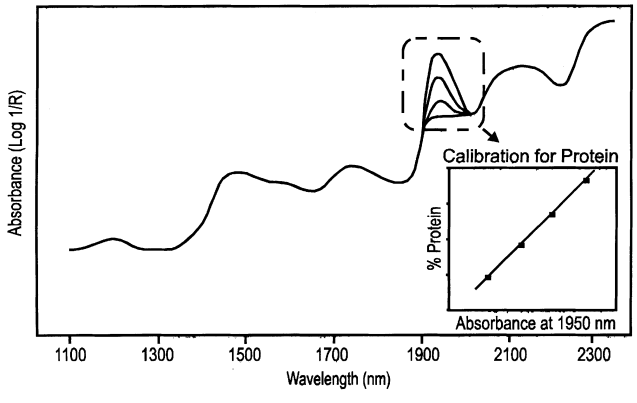

Fig. 6 Fictitious example illustrating how spectral information is correlated with analytical information in order to develop a calibration equation (adapted from Van Kempen and Jackson 1996)

Conventional laboratory assays – what NIRS has revealed

Most scientists who have embarked on studies using NIRS have found that conventional analyses involve as much work as the NIRS technique in providing an assessment of the calibration model. Because NIRS is so critically dependent on the quality of the data used to build predictive models, analysts often need to check and evaluate their assays in more detail than previously, as unknown sources of error become identified (Windham et al. 1988). NIRS can be used, to some degree, to provide an independent check of laboratory values. For example, in our laboratory we were confident from earlier work that we could predict total nitrogen in plant material with a high degree of accuracy, so when we obtained a set of samples and data from another laboratory that resulted in a poor calibration, we first questioned the accuracy of the original analyses. Reanalysis for total nitrogen in our laboratory with blind duplicates resulted in a much better calibration that doubled the precision of the NIRS analysis.

Some debate exists as to whether NIRS calibrations can be more accurate than the underlying reference method on which they are based. NIRS predictions contain both the underlying laboratory errors and NIR instrument errors, so it is unlikely that this could be the case. However, what may be most important is the kind of laboratory errors that are made. During the development of calibration models, most software packages will highlight erroneous mixes between spectra and laboratory values which can be of use in helping to identify potential sources of error. It is also possible, in theory, that unbiased but randomly inaccurate sources of error may be averaged out in large calibration sets. Whether a calibration is used for analysis depends very much on the discretion of the analyst. However, in practice, a good NIRS method should have a prediction error which is close to the standard error of the laboratory reference data.

Several studies have highlighted the fact that inter-assay variation can also be a potential problem when trying to construct calibration equations (e.g., Shenk et al. 1992; Shenk and Westerhaus 1994). This variability may need to be assessed by re-analyzing series of blind duplicate samples on successive occasions. Exchange of samples between laboratories conducting the same analyses benefits all parties.

Linking wavelengths to chemical constituents

Although the statistical models built on NIRS data are essentially empirical, users must realize that the wavelengths used in the equations relate to the underlying chemical composition. Ecologists will have greater confidence in NIRS if they can see that the wavelengths used in calibration models are those characteristic of the components of interest. For example, several studies have shown that the prediction of crude protein in temperate grasses depend on absorbances at 2120–2160 nm. The spectral region between 2100 and 2200 nm corresponds to N—H stretching in amide bonds (Shenk and Westerhaus 1993b) and so wavelengths of 2120–2160 nm should be suitable for estimating crude protein. Not surprisingly, many calibration equations for crude protein emphasize wavelengths in this region. Plant secondary metabolites (PSMs) such as phenolics are expected to have a major absorbance around 1650 nm (Flinn et al. 1996) and this wavelength has been used to separate forage samples containing high and low concentrations of phenolic constituents (Fig. 7; Flinn et al. 1996) but again we caution that lignin could also absorb in this region and so the 1650 nm spectral region is not necessarily diagnostic for phenolics. Calibration is required to make quantitative estimates.

Assignment of wavelengths for attributes such as dietary fiber, total digestibility, or food intake to particular chemical compounds is not so easy because these entities are either poorly defined chemically, depend on a great number of individual compounds or vary from forage to forage. One would expect however, that wavelengths for plant fiber estimation should correspond to regions where C—H bonds absorb and this is what has been found (Clark and Lamb 1991). Assigning wavelengths to components of interest remains an important research goal for many using NIR spectroscopy, particularly in agricultural industries because of the extensive databases that have already been built to describe aspects of plant quality on which producers are paid.

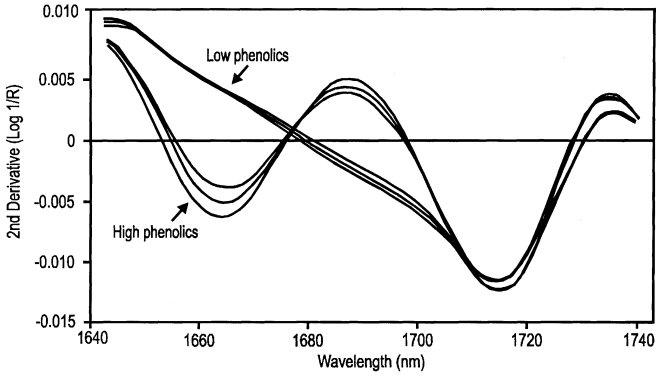

Fig. 7 Example showing the allocation of wavelengths to specific chemical bonds (taken from Flinn et al. 1996)

Ecological applications

The main purpose of this section is to highlight some of the recent innovative studies using NIRS that have potential applications to ecologists. From these studies, we synthesize implications for its possible use in the future. We conclude the review with a list of new challenges which NIRS might help to overcome. However, we stress that in many of these "new areas" much work needs to be done in testing the limitations and potential of NIRS before the technique can be universally adopted in these areas of research. Despite this, we are hopeful that given time and the growing enthusiasm and support for NIRS, that this will happen in most, if not all, of the fields mentioned below.

In assessing the potential use of NIRS in each of these areas, it is important to bear in mind that the main benefit of NIRS lies not as a replacement tool for traditional analyses, but rather as a complementary tool which can be used alongside conventional analyses to improve the efficiency and cost of studies, or alternatively to explore areas which are impossible using alternative means. As with the application of any technology into new areas, there is a potential danger to over-use or misapply NIRS and any predictions that are made should be carefully evaluated before they are applied. The following examples illustrate some of the current and emerging applications of NIRS in the field of ecology.

Resolution of complex mixtures

Measuring the botanical composition of different plant communities is integral to many ecological studies, especially those concerned with foraging in grasslands. In the past this information was obtained either by hand-sorting clipped quadrats, or by a variety of visual estimation. Hand sorting is slow and laborious and although visual estimation is rapid, there may be many unquantifiable errors (Coleman et al. 1985).

Shenk et al. (1979) originally showed that NIRS could be used to determine the proportion of legumes in legume-grass mixtures to an accuracy of about $\pm 10\%$ and several studies have attempted to expand this concept to more complex pastures (e.g., Coleman et al. 1990; Pitman et al. 1991; Atkinson et al. 1996). Artificial mixtures of

three to four different components were accurately predicted by Coleman et al. (1990), but subsequent work showed that the predictive equations were too sensitive to the source of material used to make up the mixtures. Single sources of each component led to acceptable calibrations whereas calibrations involving many sources of each component, as would occur with natural pastures, were not sufficiently robust for routine use. Nonetheless, acceptable calibrations have been generated from pasture samples that were meticulously separated by hand (Petersen et al. 1987; García-Criado et al. 1991).

NIRS has been used to assess the botanical diversity of grassland habitats by comparing the spectral similarity or "composition" between samples (Hill et al. 1988). For example, Atkinson et al. (1996) used NIRS to monitor changes in the composition of vegetation samples across time and space, which could be traced back to hand-sorted component species. In this study, NIRS was used as a discriminant tool which could be used to group samples at varying levels of detail, in order to identify the overall similarity in composition between samples (see section on Emerging applications for more detail).

A second example of how NIRS can be used to resolve differences in complex mixtures is a study by Downey and Boussion (1996) who tested the ability of NIRS to discriminate between different blends of coffee. They obtained a 95% successful resolution based on differences in spectra alone. These differences were presumed to be predominantly due to variation in the concentration of caffeine or another related alkaloid.

Overall, it seems that NIRS has the potential to save researchers substantial time and effort in monitoring the make-up of mixtures. From past studies, it is evident that calibrations for narrow or closed populations can be expected to yield more precise information than predictions across multiple landscapes and a range of environmental conditions. However, before this prospect is adopted, a greater effort needs to be devoted to understanding the factors that affect the robustness of the predictive equations in individual situations.

Determination of plant nutrients

By far the widest use of NIRS has been in the measurement of nutritional components in animal feeds (e.g., Norris et al. 1976; Brown et al. 1990; Coleman and Murray 1993). Components measured include total nitrogen, moisture, fiber (neutral and acid detergent fiber and acid lignin), starch, individual sugars, amino acids and plant tannins. To give but one example of the potential time and cost benefits of using NIRS to analyze such components, Jin et al. (1994) used NIRS to simultaneously measure concentrations of sucrose, glucose, fructose, citric acid, malic acid and vitamin C in intact strawberries. NIRS gave a similar accuracy to conventional HPLC techniques, but with the advantage of near-instantaneous, non-destructive and chemical-free analysis. The prediction of crude protein in grain remains the most common application of NIRS in agricultural industries but plant nutrients and carbohydrate fractions have been successfully predicted in a range of different shrub and tree leaves as well (Meuret et al. 1993; Martin and Aber 1994).

Plant secondary metabolites and anti-nutritional components

Components of several PSMs such as the hydroxyl groups of plant phenolics, have significant absorbances in the NIR region and successful calibrations have been developed for these constituents. For example, Windham et al. (1988) predicted the content of "total tannin" (Folin-Denis assay of 50% methanol extractives) with a coefficient of determination of 0.91 in *Lespedeza cuneata*. Given the errors of conventional laboratory assays for these poorly defined compounds these levels of accuracy may well be the best achievable. However, there is no reason to suspect that other types of tannin analyses would not yield calibrations that are satisfactory for most ecological studies (Roberts et al. 1993).

Less work has been directed at other PSMs. Clark et al. (1987a) developed a robust calibration to predict total alkaloids in larkspur (*Delphinium occidentale*) and lupine (*Lupinus leucophyllus*). Both morphine and nicotine have been predicted with a high degree of precision in poppies and tobacco (McClure and Williamson 1988), and glucosinolate concentrations have been reliably measured in rapeseed (Mika et al. 1997). In many of these studies, examination of spectral characteristics has revealed strong relationships between functional groups and specific wavelengths, potentially a valuable means of screening samples for the presence of these groups.

The wide range of plant types and chemistries involved in these examples gives confidence that NIRS can be used successfully to analyze large numbers of samples for PSMs. However, all these examples are of organic compounds and the ability of NIRS to predict functional aspects of PSMs such as astringency or protein-precipitating capacity is unknown.

Given the clear potential of NIRS to investigate the chemical composition of plants, we believe the technique has a wide application for investigating many ecological questions. Examples where NIRS might be particularly useful is in understanding the effects of resource availability on plant growth and chemical defense, the role of nutrition in influencing the population dynamics of herbivores and in understanding the specific foraging patterns of animals in relation to the nutritional heterogeneity and complexity of their surroundings.

Mineral analysis

The estimation of mineral elements by NIRS is usually dependent on the occurrence of those elements in either organic or hydrated molecules (Clark et al. 1987b; Vasquez de Aldana et al. 1995). For example, key

wavelengths used in detecting Mg were similar to the peaks of the chlorophyll spectrum and Ca peaks were similar to those of pure calcium pectate which may be a component of cell walls. Mineral analysis studies have suggested that K, P, Mg, Ca, S, Al and possibly Si exist in forms detectable by NIRS at least in some grasses and legumes (Clark et al. 1987b, 1989; Saiga et al. 1989).

Despite the reports of many satisfactory calibrations for mineral elements, it is difficult to apply their results to other plants because the form of organic molecule-mineral compounds can vary seasonally or among species and genera. This may lead to unstable calibrations, inconsistent results and difficulty in expanding calibrations beyond a well characterized population. For example, phosphorus in plants exists mainly in organic forms frequently as phytate, phospholipids and nucleic acids. The proportion of total P in this form varies between closely related cereals and forages and also seasonally (De Boever et al. 1994). Calibrations obtained for total P in forages were considered acceptable by Clark et al. (1987b) but were not successful in similar studies by Vasquez de Aldana et al. (1995). Caution needs to be exercised when using NIRS to analyze mineral elements. However, the technique can be useful for broad first approximations and for selecting samples for more accurate analyses.

Soil analysis

Due to the large inorganic fraction of material in soil, NIRS has not been as widely used for soil analysis (Meyer 1989). However from the few studies for which there are data, it is evident that at least for carbon and nitrogen, calibration equations in size-specific soil fractions can yield an accuracy similar to that recorded for plant tissues (Morra et al. 1991).

A potential application of NIRS for soil biologists is its usefulness in selecting soil samples from populations to maximize the variation in particular soil properties in a minimum subset of samples from the population (Stenberg et al. 1995). This is a powerful application of NIRS, as it allows one to select samples over the full range of variation in any one or combination of parameters. In this way, extreme or peripheral samples may be chosen which might otherwise fail to be represented in a sample set for analysis. In addition, a specific distribution of samples can be chosen in reference to a particular parameter. Soil parameters used by Stenberg et al. (1995) included clay content, cation exchange capacity, base saturation, and pH . Cost savings of 70% were achieved when the technique was compared to the most appropriate sampling strategy involving only wet chemistry analysis.

Prediction of functional attributes

NIRS has enormous potential as a holistic tool for investigating natural systems. Although reductionist approaches are useful in understanding how individual components of a system function, what is often more desirable is an understanding of how these connected parts function as a whole. For example, at times the prediction of chemical composition may be the ultimate aim of the analyst. However, in many situations ecologists are interested in more complex attributes and are constrained to use compositional measures as proxies for these more complex phenomena. Examples of complex attributes which have been examined using NIRS include features such as the susceptibility of plants to insect attack (Rutherford and Van Staden 1996), the yield of pulp and biological degradability of sawlogs (Wright et al. 1990; Hoffmeyer and Pedersen 1995) and the prediction of the nutritional quality of wild foods for free-ranging herbivores (A. McIlwee, I. Lawler and W.J. Foley, unpublished work).

Understanding the organization of complex systems is an important objective of ecology. However, because there is an almost unlimited variety of ways that biotic and abiotic components can be assembled into complex ecological systems, our understanding of the structure and dynamics of these systems has remained inherently limited due to traditional approaches that have thus far dominated ecology (Brown 1997). Many systems are inherently non-linear, which limits our ability to make ecological predictions. However, much time and effort can be saved by looking at these systems from a holistic viewpoint and deciding whether they are in fact capable of being modeled. NIRS offers the potential for a shift towards whole-system empirical modeling in several areas of ecology. This methodology can still be used alongside more formal experimental approaches to generate new insights into how complex systems function.

We see the prediction of functional attributes as being one of the most important applications of NIRS in ecology. Any attribute that is suspected of being influenced by the composition of plant or animal material could potentially be modeled in this way. As well as aspects of animal performance, NIRS could also be used to measure attributes of plant performance, such as innate mean relative growth rate (see Cornelissen et al. 1996). Although this is partly an empirical approach, it is similar to the many successful bioclimatic models that have been used to predict the limits of animal distribution. Below, we provide a range of examples which illustrate the power of NIRS.

Insect resistance

A good example of this approach is in studies of the resistance of different sugarcane cultivars to attack by stem borers. Rutherford and Van Staden (1996) developed a MLR model of NIR data to predict resistance of sugarcane cultivars to *Eldana saccharina*. They found that they could account for 54% of the variation in resistance but, more significantly, wavelengths in their model indicated that alcohols and carbonyl components

of the stalk surface wax correlated with an important part of this resistance. In this case, NIRS became not just a tool to build an empirical model but indicated an underlying mechanism that could be further investigated. This approach can save substantial time in targeting a research program to enhance resistance.

A second example where NIR has been used to predict a poorly defined attribute is the variable susceptibility of *Eucalyptus* trees to insect attack. Generally this is caused by variations in both primary and secondary chemistry of the foliage (Edwards et al. 1993) but the most important features have not yet been identified. However, it was still possible to develop a model to account for 88% of the variation in resistance to defoliation of individual trees based solely on the spectral characters of the foliage (W. Foley, P. Edwards, W. Wanjura and M. Matsuki, unpublished work).

Prediction of animal performance

Many studies postulate differences in the nutritional quality of the diets consumed by animals as a basis of different population densities, social organization, and evolutionary success. However, testing these hypotheses is limited by an inability to measure "nutritional quality" in free-living animals (Hanley 1997). Nutritional quality has most often been estimated indirectly by measuring the concentration of nutrients including nitrogen and fiber and PSMs such as condensed tannins. In most cases, whether tannins affect the intake or digestibility of foods in these species, or whether the crude protein is in a usable form or not is not known. In fact, crude measures of plant composition tells us little about how well a particular food will sustain an animal.

Nutritional quality is reflected in two parameters that can be measured in feeding experiments with captive animals; the level of food intake and the digestibility/metabolizability of that food. These estimates have been made by NIRS using both samples of the food being eaten (Redshaw et al. 1986; Givens et al. 1991, 1992) or alternatively from samples of the feces excreted (e.g., Lyons and Stuth 1992). Prediction of diet quality from feces samples means that it is the actual diet chosen by the animal that is being evaluated, not that which the ecologist decides is being eaten. Brooks et al. (1984) developed a number of calibrations for use with both food and feces samples of white-tailed deer but most innovations in this area have been from Stuth and coworkers (Lyons and Stuth 1992; Leite and Stuth 1994).

Use of feces samples to predict the quality of diets ingested by free-ranging herbivores means that the researcher need not know what the actual diet ingested by the animal comprised. Examination of feces has also been used to examine the digestive efficiency of herbivores on range of natural diets through a simple comparison of difference spectra between diet and feces (e.g., Coleman and Murray 1993). Although there are several assumptions that need to be addressed using this method, namely the effects of microbial residues and sloughed animal tissue, it does provide a simple and potentially valuable means of evaluating the nutritional value of foods and looking at the associative effects of food ingestion and nutrient assimilation.

When combined with geographic information systems, estimates of the nutritional quality of herbivore diets can become powerful tools for monitoring pastoral degradation at many scales. For example, J. W. Stuth (personal communication) has used NIRS to predict diet quality from feces samples of cattle and deer and by plotting the position of each feces sample has identified differences in the quality of different habitats to support these herbivores. Knowledge of the spectral stability of feces exposed to field conditions adds to its power as a management tool (Leite and Stuth 1994).

Isotope discrimination – water use efficiency and herbivore diets

Stable isotopes are used in many areas of ecology, including estimation of water use efficiency, separation of C4 and C3 plants and in studies of foraging and food webs. Modern isotope ratio mass spectrometry (IRMS) is highly accurate and precise, but it is relatively expensive, laborious, and time consuming. NIRS may be able to predict relative abundances of ^{13}C. For example, Okano et al. (1993) found that NIRS could predict abundance of ^{13}C in a range of C3 and C4 plants to within 97% of measured values. This degree of accuracy could be sufficient for many studies of herbivore foraging where the aim is to measure the proportion of tropical grass in the diet.

Clark et al. (1995) used NIRS to predict ^{13}C discrimination in a range of grasses and legumes so that they could select genotypes with high water use efficiency (WUE), based on the well-established relationship between water use efficiency and ^{13}C carbon ratios in plants (Salisbury and Ross 1992). They found that NIRS could identify up to 82% of samples with low WUE, and concluded that accuracy may be sufficient to identify promising genotypes in the early selection stages. Clearly NIRS is not expected to replace IRMS as a means of determining isotope abundance but we believe that NIRS may have a role in rapidly screening a large number of samples of plant tissues so that the more expensive analysis can be targeted on those samples that are of interest.

Litter decomposition studies

Mineral cycling in forest and aquatic ecosystems has been investigated using NIRS. The chemical composition of leaf litter is a key factor regulating its rate of breakdown and the speed and non-destructive nature of NIRS has been used by several groups. McClellan et al. (1991) showed that total N, C, and ash could be accu-

rately measured by NIRS as well as several components of plant cell walls. Following on from this, Gillian et al. (1993) showed that NIRS was able to directly predict the stage of decomposition (expressed as the percentage of ash-free litter mass remaining) without relying on indirect estimates based on chemical composition. Cornelissen and Thompson (1997) provide a range of potential functional attributes of living leaves (for both monocots and dicots) which can be used as a basis for predicting the decomposition rate of leaf litter. Such attributes could be used by people using NIRS to examine the impact of anthropogenic activities and environmental changes on decomposition processes.

Limnology and freshwater ecology

NIRS has been used to measure C, N, and P in suspended sediments and in seston by collecting the material on glass-fiber filters and then obtaining NIR spectra (Malley et al. 1993, 1996). Agreement between the NIRS measures and those obtained by traditional methods was excellent in both cases. However, the use of NIRS is not restricted to these types of compositional assays. Korsman et al. (1992) and Nilsson et al. (1996) have shown that NIRS can be used to predict the past chemical composition of lake water from models of the surface sediments and current water chemistry. A record of the current water chemistry is contained in the surface sediments and once a relationship is established between these parameters, past histories can be evaluated by taking further sediment samples. Nilsson et al. (1996) found that they could predict 83–85% of the variance in total phosphorus and pH, respectively, and 68% of the variance in total organic carbon. This approach could become a powerful tool for monitoring past events in water chemistry.

Biochemical markers and rates of biological activity

NIRS can be used to detect biochemical markers, such as chitin (Roberts et al. 1994) which is used to estimate levels of fungal infection in crops and pastures. Direct estimates of fungal infection have also been made by NIRS (Roberts et al. 1988). Dysprosium (Dy), a pulse-dosed marker used to measure rates of digesta flow in herbivores, has also been successfully measured using NIRS in studies on cattle (Reeves and Glenn 1995). Although NIRS was capable of determining Dy in labeled forages, interference by a second co-marker limited the accuracy of predictions.

In addition to detecting markers, NIRS also has the potential to measure rates of biological activity. For examples Hall et al. (1991) used NIRS to measure bloodmeal size and its disappearance rate in live disease-carrying mosquitoes, NIRS has been used as an non-intrusive means of monitoring changes in blood composition (Hinckley et al. 1995; Delpy and Cope 1997), and Palmborg and Nordgren (1993) applied NIRS to the measurement of microbial biomass and activity of forest soils.

Analyses of high-moisture materials – prospects for analysis of fresh samples

Although some of the earliest applications of NIRS involved analysis of fresh meat samples, most applications currently involve dry and ground materials. Even this limited sample preparation increases analysis time and cost substantially. The imbalance between the number of applications for dried and fresh samples discredits the real advantages offered by the direct analysis of fresh materials.

Samples which contain substantial quantities of moisture present particular difficulties for NIRS. The hydrogen bonds in water absorb significant amounts of NIR radiation and result in broad peaks that obscure spectral information derived from other compounds (Abrams et al. 1988). Wet samples tend to be more heterogeneous and moisture can be expressed from the sample when it pressed against the glass window of the sample cell. A major difficulty is to generate samples for instrument standardization that will not degrade over time. Currently this is a serious weakness in applying NIRS to high-moisture materials (Berding and Brotherton 1996).

There is no single solution to these problems and each case has to be treated individually. The difficulties are not insurmountable since many robust calibrations have been developed for moist materials including dairy products, fish, fresh, intact fruits and vegetables, meats, and sugarcane (Blosser and Reeves 1986; Mathias et al. 1987). In calibration development for example, use of high absorbance spectral regions characteristic of high moisture materials are best avoided because of the non-linearity associated with these regions.

Emerging applications: challenges and prospects for the future use of NIRS

Use of NIRS as a discriminant tool – chemotaxonomy and beyond

Ecologists often wish to classify elements of populations into groups for a range of different reasons, even if it is as simple as determining whether an animal is one sex or another. NIRS has been successfully used in this fashion to predict whether pupae are male or female (Jin et al. 1995) and to predict whether individual seeds or grains have been infected by larval insects (Ridgway and Chambers 1996). In such applications, the spectral data are often acquired by fiber-optic probes that can be focused on very small targets such as single insect larvae.

NIRS is also finding use as a tool for discriminating among products from different provenances or of

different species. The wealth of data available in NIR spectra means that the digitized spectra are particularly suited to the variety of multivariate statistical procedures that are typically used in discriminant analyses. For example, Schimleck et al. (1996) acquired the NIR spectra of a range of timber samples. They were able to build models based on principal components analysis of NIR spectral data that successfully discriminated between woods from *Eucalyptus* and *Pinus*, between woods of different species of *Eucalyptus* and between woods of the same species of *Eucalyptus* grown at different sites. Other similar applications have involved identifying adulterated food products, such as contamination by fungi (Davies et al. 1987) or determining the infection levels of diseases such as anther smut disease in crops (Nilsson et al. 1994). These approaches can easily be transferred to ecological applications.

Discriminant analyses are very common in ecology and typically require substantial amounts of chemical or physical measurements to be successful. Collecting these can be time-consuming and expensive and we believe that many ecological applications could profit from using NIRS data as an input to discriminant procedures.

On-line processing and portable and hand-held NIRS

Most NIR spectrophotometers that are currently used for research are dedicated laboratory instruments which are housed in carefully controlled conditions. However, the greatest use of NIRS is as an on-line analytical tool in industrial plants ranging from sugar mills and wheat silos to pharmaceutical manufacturing plants (for examples see Bellon and Boisde 1989; Osborne et al. 1993; Schulz and Losing 1995). In these environments the instrumentation is exposed to much more rugged conditions and usually involved in the analysis of fresh, intact samples. This provides the potential for more exposed environmental monitoring.

In the future we can expect NIR instruments to be become smaller and more portable. There are already a number of hand-held instruments that have been used to measure the nitrogen concentration of the leaves of crop plants as an aid to fertilizer management. For example, Blakeney et al. (1996) successfully used a hand-held battery operated instrument for nitrogen measurements in rice leaves.

In the past year, portable instruments that can scan the whole NIR spectrum have become available. These instruments use a variety of fiber-optic probes and, coupled with the speed of advances in optics and microprocessor technology, should lead to even more robust portable instruments in the future that offer ecologists the possibility of real-time analyses of plant and animal composition in the field. In particular we could expect closer alliances to be formed between those interested in traditional airborne remote sensing and those interested in finer resolutions as well as advances in statistical processing which will allow a better description of spectral features.

Conclusion

Although we are very optimistic about the future of NIRS, we conclude with the warning that NIRS cannot turn poor analysts into good ones. Used uncritically, it can allow poor results to be propagated. Ultimately, the quality of NIRS-based predictions depends entirely on the quality of the measurements used to generate the statistical model and this must guide any analytical endeavour.

NIRS offers several special advantages such as speed of determination, minimal or no preparation of sample, non-destructive analysis, no consumption of reagents, and low costs of analysis. However, the capital cost of NIR spectrophotometers is high (ca. $US 40,000–80,000), so ecologists might first consider developing collaborations with existing users of NIRS in agricultural industries or research stations where the technique is widely used. In this way, they can evaluate the usefulness of the method for particular samples and analyses before committing to purchase. As NIRS moves forward into the future, we look forward to witnessing the integration of the technique into applied ecology.

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