



Application of near infrared reflectance spectroscopy (NIRS) to assess some properties of a sub-arctic ecosystem

Caroline Stolter^{a,*}, Riitta Julkunen-Tiitto^b, Jörg U. Ganzhorn^a

^aDepartment of Animal Ecology and Conservation, Biozentrum Grindel, Martin-Luther-King Platz 3, 20146 Hamburg, Germany

^bDepartment of Biology, P.O. Box 111, University of Joensuu, 80101 Joensuu, Finland

Received 27 January 2005; accepted 31 May 2005

KEYWORDS

Animal–plant interactions;
Plant chemistry;
Salix phylicifolia;
Willows;
Browsing;
Decomposition;
Nitrogen;
Fibre;
Phenolics;
Alces alces;
Landscape level

Summary

Investigations of temporal and spatial variation of chemical properties of ecosystem components on a landscape level are hampered by the need to analyze large numbers of samples. Near infrared reflectance spectroscopy (NIRS) might provide a useful tool to overcome this problem. Here we investigated the possibilities and limitations to quantify the chemical composition of different plant parts and ecological properties with the help of NIRS. For this, we addressed the following questions:

- (1) Can NIRS-models be used to quantify different primary compounds (nitrogen, fibre), groups of secondary compounds (condensed tannins, total phenolics) as well as specific phenolic components (e.g., salicin) in leaves, twigs and litter of *Salix phylicifolia*?
- (2) Can NIRS be used to predict ecological properties such as moose browsing on willow or the decomposition rate of leaf litter?

NIRS predicted the different primary compounds and grouped secondary compounds in different plant material with high accuracy. Results were inconsistent for specific phenolics. For ecological properties (moose browsing, litter decomposition rate) NIRS-models had high coefficients of determination. But tests of the models with a second independent set of samples (independent-data-set-test: IDS-test) showed that the predicted values were too low even though they were ranked correctly.

Based on these results, the application of a second independent test is recommended. In the present study this second validation indicated inconsistencies in the NIRS-models that had not been revealed by the conventional validation procedures used to develop the models (test-set and cross-validation). According to

*Corresponding author. Tel.: +49 40 42838 3869; fax: +49 40 42838 5980.
E-mail address: caroline.stolter@web.de (C. Stolter).

the present results NIRS represents a suitable and cost-effective tool to measure primary and groups of secondary components of plant material. Application to specific phenolic compounds requires more elaborate testing. The successful reconstruction of moose browsing and the prediction of litter decomposition rate show that NIRS offers new possibilities for ecological applications.

© 2005 Gesellschaft für Ökologie. Published by Elsevier GmbH. All rights reserved.

Zusammenfassung

Die Erfassung zeitlicher und räumlicher Unterschiede von chemischen Eigenheiten der Komponenten eines Ökosystems wird durch die Notwendigkeit der Analyse großer Probenzahlen erschwert. Dies gilt insbesondere für Untersuchungen auf ökosystemarer Ebene. Nah-Infrarot-Spektrometrie (NIRS) könnte bei der Überwindung dieses Problems helfen. In dieser Arbeit wurden die Möglichkeiten und die Grenzen sowohl bei der Erfassung von chemischen Inhaltsstoffen in unterschiedlichen Pflanzenteilen als auch bei der Erfassung von ökologischen Parametern mit NIRS untersucht. Hierzu wurden folgende Fragen bearbeitet:

- (1) Kann NIRS zur quantitativen Erfassung verschiedener primärer (Stickstoff, Rohfaser) und sekundärer Inhaltsstoffe (kondensierte Tannine, Gesamtphenole) genutzt werden, und ist es zudem möglich, spezifische Phenole mit NIRS in unterschiedlichem Pflanzenmaterial zu erfassen?
- (2) Können mit Hilfe von NIRS ökologische Parameter bestimmt werden? Ist es z.B. möglich, den Elchverbiß an Weiden zu rekonstruieren oder die Kompostierungsrate von Laub vorherzusagen?

Zur Untersuchung dienten Blätter, Zweige und Laub der Weide *Salix phylicifolia*. Mit hoher Genauigkeit konnte der Gehalt an primären und sekundären Inhaltsstoffen (kondensierte Tannine, Gesamtphenole) mit NIRS vorhergesagt werden. Die Ergebnisse zur Bestimmung von spezifischen Phenolen waren jedoch widersprüchlich. Die entwickelten NIRS Modelle zur Bestimmung ökologischer Parameter wiesen einen hohen R^2 -Wert auf, jedoch lagen die Ergebnisse bei der Anwendung dieser Modelle auf einen unbekanntem Datensatz zu niedrig. Aufgrund unserer Ergebnisse wird die Verwendung eines zweiten unabhängigen Tests (independent-data-set-test) zur Evaluierung der NIRS Modelle empfohlen. In der vorliegenden Untersuchung wurden durch diesen Test Unzulänglichkeiten der entwickelten NIRS Modelle aufgedeckt. Diese waren durch die üblichen Validierungsmethoden zur Methodenerstellung (Kreuz- und test-set-Validierung) nicht ersichtlich. Aufgrund der vorliegenden Ergebnisse kann NIRS zur effektiven und kostengünstigen Analyse von primären und sekundären Pflanzenstoffen in unterschiedlichem Pflanzenmaterial eingesetzt werden. Die Verwendung von NIRS zur Bestimmung spezifischer Phenole bedarf weiterer Versuche. Die erfolgreiche Rekonstruktion von Elchverbiß und die Vorhersage der Kompostierungsrate von Weidenlaub zeigen, dass NIRS neue Anwendungsmöglichkeiten bei ökologischen Fragestellungen bietet.

© 2005 Gesellschaft für Ökologie. Published by Elsevier GmbH. All rights reserved.

Introduction

Quantification of spatial and temporal variations in plant productivity and chemical quality are prerequisites for a better understanding of the composition and dynamics of ecosystem processes, such as plant–herbivore interactions (e.g., Ostfeld & Keesing, 2000; contr. to Brockman & van Schaik, 2005). Plant characteristics and changes in these characteristics due to herbivory can affect individuals at the same trophic level or spread to trophic levels above and below with subsequent changes of community processes (reviewed, e.g., by Dicke &

Hilker, 2003; Rostás, Simon, & Hilker, 2003; contr. to Tschardtke & Hawkins, 2002). Analyses of these processes on an ecosystem or on the landscape level are rare (e.g., Eber, 2001). Primarily, this is due to the complexity of possible interactions. In addition, extrapolations from case studies to landscape level require enormous numbers of analyses to account for individual variation within species.

Near infrared spectroscopy (NIRS) provides a tool that allows processing and quantifying chemical or ecological properties of large sample numbers (Foley et al., 1998). NIRS has been used successfully in agricultural studies to estimate diet quality, the

composition of diets, or digestibility (deBoever, Vanacker, & deBrabander, 2003; Leite & Stuth, 1995; Lyons & Stuth, 1992; Pearce, Lyons, & Stuth, 1993; Stolter, Ball, Julkunen-Tiitto, Lieberei, & Ganzhorn, in press; Walker, Clark, & McCoy, 1998; Xiccato et al., 1999, 2003), and to assess properties of decomposition (Bouchard, Gillon, Joffre, & Lefeuvre, 2003; Gillon, Joffre, & Ibrahima, 1999). It is an accepted standard method in the Pharmacopöa Europaea (1997) and the USP (United States Pharmacopeia, 2002) for the determination of the quality of pharmaceutical products. It has been successfully used for the determination of soil and peat properties (Chang, Laird, Mausbach, & Hurburgh, 2001; McTiernan, Garnett, Mauquoy, Ineson, & Couteaux, 1998) and in paleolimnological and limnological studies for sediment and water analyses (Korsman, Nilsson, Öhman, & Renberg, 1992; Korsman, Nilsson, Landgren, & Renberg, 1999; Malley, Röncke, Findlay, & Zippel, 1999; Nilsson, Dabakk, Korsman, & Renberg, 1996; Rosén, Dabakk, Renberg, Nilsson, & Hall, 2000). However the use of NIRS in studies on a landscape level has been restricted to study food selection criteria and chemical habitat patchiness for marsupial folivores in Australian eucalypt forests (Lawler, Foley, Eschler, Pass, & Handasyde, 1998; Lawler, Foley, & Eschler, 2000; McIlwee, Lawler, Cork, & Foley, 2001). Similar analyses are lacking for other ecosystems.

In the present paper we investigate the possibilities and limitations to quantify chemical properties of plant parts relevant at various trophic levels with the help of NIRS. The analyses were performed with items from the willow *Salix phylicifolia* growing in the northern Taiga. Description of the chemical properties of plants as bases for community processes on the landscape level seems promising at higher latitudes due to the reduced species diversity and thus a reduced set of possible animal plant interactions (Bryant & Kuropat 1980; Bryant 1981).

S. phylicifolia is an abundant plant in riparian areas, mires and wetlands of the Swedish taiga. Willows are important food plants for moose (*Alces alces*) as one of the major herbivores (Stark, 2001) as well as for other mammalian herbivores and folivorous insects (Elmqvist, Ericson, Danell, & Salomonson, 1987; Nyman & Julkunen-Tiitto, 2000; Tahvanainen, Julkunen-Tiitto, & Lavola, 1985; Tahvanainen, Helle, Julkunen-Tiitto, & Kettunen, 1985; Zvereva, Kozlov, Niemelä, & Haukioja, 1997). Changes in plant chemistry lead to changes on different trophic levels, influencing herbivores, their predators (Sipura, 1999; Suomela, Suominen, & Törvi, 1997; Zvereva & Rank, 2003) and decom-

posers (Bardgett, Wardle, & Yeates, 1998; Hättenschwiler & Vitousek, 2000; Suominen, Danell, & Bergström, 1999).

Using twigs and leaves from *S. phylicifolia* we investigated the utility of NIRS to address questions concerning distinct chemical concentrations or general ecological properties of items.

(1) Distinct chemical concentrations:

- Is it possible to quantify different primary compounds (nitrogen, fibre), groups of secondary compounds (condensed tannins, total phenolics) in leaves and twigs of *S. phylicifolia* by NIRS? As a new application for NIRS analyses we tried to quantify specific phenolic components in plant material of *S. phylicifolia*.
- Can NIRS be used to quantify concentrations of different chemicals in litter of *S. phylicifolia* before and after decomposition?

(2) General ecological properties:

- Can the browsing degree (feeding damage) in the previous winter by moose (*Alces alces*) be reconstructed based on NIR spectra of the plant parts (twigs) grown in the subsequent vegetation period?
- Since litter decomposition (mass loss) should be different in plants with different chemical characteristics: Can NIRS-models predict the decomposition rates?

Materials and methods

Study site

The study was carried out in northern Lapland (Abisko, Sweden, 68°21'N, 18°49'E) from 2002 to 2003. The area is located in the northern Taiga vegetation zone (tree line about 600 m a.s.l. with an annual mean temperature of -1.1°C). The study area comprises an area of 50 km² with altitudes ranging from 389 to 408 m a.s.l. Willow thickets at lower elevations consist of *S. phylicifolia*. The willows chosen for analyses, had been sampled from different areas (wetlands, mires, riparian areas, lake shore). In different willow thickets 61 individuals of *S. phylicifolia* were marked, damage due to browsing by moose was determined and they were sampled for chemical and NIRS analyses as well as for decomposition studies. In all cases the samples were taken from the upper part of the willows (top shoots or high side branches).

Sampling of plant material

(1) Foliage and twigs

Sampling 1: Shortly after the snowmelt in 2002 (6–11 June 2002) browsed (leftovers) and unbrowsed twigs from the previous winter (2001/2002), and new leaves from June 2002 were sampled. The leaf samples were divided into leaves from browsed and unbrowsed twigs.

Sampling 2: In summer (18–22 July 2002) we sampled newly grown twigs and leaves from these twigs. Samples were also divided into new growth from browsed and unbrowsed twigs (browsing in winter 2001/2002).

Sampling 3: In autumn (15–23 October 2002) twigs grown in 2002 were sampled.

(2) Samples for the determination of the browsing intensity

At the beginning of October 2002 we collected annual top shoots (new growth, sampling 3) of browsed twigs from different willows with known browsing intensity (measured in spring 2002).

(3) Litter samples

Litter was collected from the ground under the different willows shortly after leaf fall at the beginning of October 2002. Damaged leaves (e.g., by folivorous insects) were avoided.

Chemical analyses

The samples were dried at 30 °C in a drying chamber and ground with a water-cooled mill to a fine, homogenous powder. Samples were analysed for nitrogen (Kjeldahl), condensed tannins (butanol-method; Oates, Swain, & Zantovska, 1977), and total phenolics (Folin & Ciocalteu, 1927). Fibre contents (neutral-detergent-fibre [NDF] and acid-detergent-fibre [ADF]) were determined with an ANKOM Fibre Analyser.

Specific phenolic compounds (including flavonoids and phenolic glycosides) of twigs and leaves of *S. phyllicifolia* were analysed with RP-HPLC (reversed phase high performance liquid chromatography; Julkunen-Tiitto, Rousi, Bryant, Sorsa et al., 1996; Stolter et al., in press; Tegelberg, Veteli, Aphalo, & Julkunen-Tiitto, 2003). The following components were identified and used for the development of NIRS-models: ampelopsin, galloca-

techin, myricetin-galactoside and glucuronide, (+)catechin for twigs and leaves combined. Additionally to these compounds, salicin, disalicortin, picein, and triandrin were analyzed for twigs. For models referring only to leaves we used ampelopsin, myricetin-glucuronide and (+)catechin. Concentrations of the components were expressed relative to standards used previously (Julkunen-Tiitto & Sorsa, 2001; Stolter et al., in press; Tegelberg et al., 2003).

Browsing intensity

The extent of winter browsing (browsing degree) was measured in 2002 shortly after snow melting as the percentage of twigs affected by browsing per individual tree at heights between 90 and 180 cm (Stolter et al., in press). Trees browsed by mountain hares (*Lepus timidus*) and trees, which showed morphological signs of hybridization (e.g. hairy leaves), were excluded.

Litter decomposition

The method of sample preparation and litter bed set up followed that of Cornelissen (1996) modified by Quested et al. (2003). The litter used consisted of undecomposed leaves of *S. phyllicifolia*. Litter bags (size 10 × 7 cm; mash size 0.3 mm) contained 0.69 ± 0.01 g of dried litter. Every leaf used was cut into halves and put into two different litter bags. This resulted in two litter bags per willow. Each litter bag contained leaves from only one willow. These bags were placed in two separate litter beds, so that both litter beds contained bags of all willows. The litter beds (wooden frames on a layer of grid stones; depth: 10 cm) were installed in a fenced tree nursery at the Abisko Scientific Research Station. The litter bags were covered with a layer of litter from a willow stand, mainly consisting of willow and birch leaves (*Betula pubescens*) and watered at the beginning. A 2 cm mesh was fixed over each litter bed to prevent disturbance by vertebrates. Incubation started in October 2002. The bags were removed after 1 year and the litter samples were extracted from the bags. After removal of extraneous materials the samples were dried at max. 30 °C and weighed. The decomposition rates (% mass loss) of the paired samples from the two litter beds were highly correlated (Spearman correlation: $r_s = 0.78$, $P < 0.001$, $n = 61$).

Near infrared spectroscopy (NIRS)

The results of the wet chemistry were used to calibrate NIRS as described by Foley et al. (1998).

For the NIRS the milled, homogenous samples were dried at 30 °C overnight and kept in an exsiccator to ensure that each sample had the same moisture content. NIR spectra were measured with a FT-NIR Spectroscopy Vector 22/N (Bruker GmbH, Germany). Spectra were scanned for each sample in duplicate (NIR-wavelength: 2500–800 nm \approx wave numbers: 12,500–4000 cm^{-1}) (Bruker Analytik GmbH, 1998).

Distinct chemicals

NIRS-models were developed for the concentrations of NDF, ADF (both fibre fractions only for leaves and twigs), nitrogen, total phenolics and condensed tannins in twigs, leaves and litter, as well as in decomposed litter. For leaves and twigs so-called “mixed” models were developed. These

models included spectra from leaves and twigs. NIRS-models for specific phenolics were developed for leaves and twigs separately.

Ecological properties

The browsing degree was linked to NIR spectra of the willows using twigs sampled in October 2002. The decomposition of leaf litter (mass loss) was correlated with the NIR spectra of the corresponding litter sample.

Developing NIRS-models

NIRS-models were generated with the Quant 2-method using partial least squares (PLS) regression with the software Opus NT Version 2.02 (Bruker GmbH, Germany). Spectral data were not

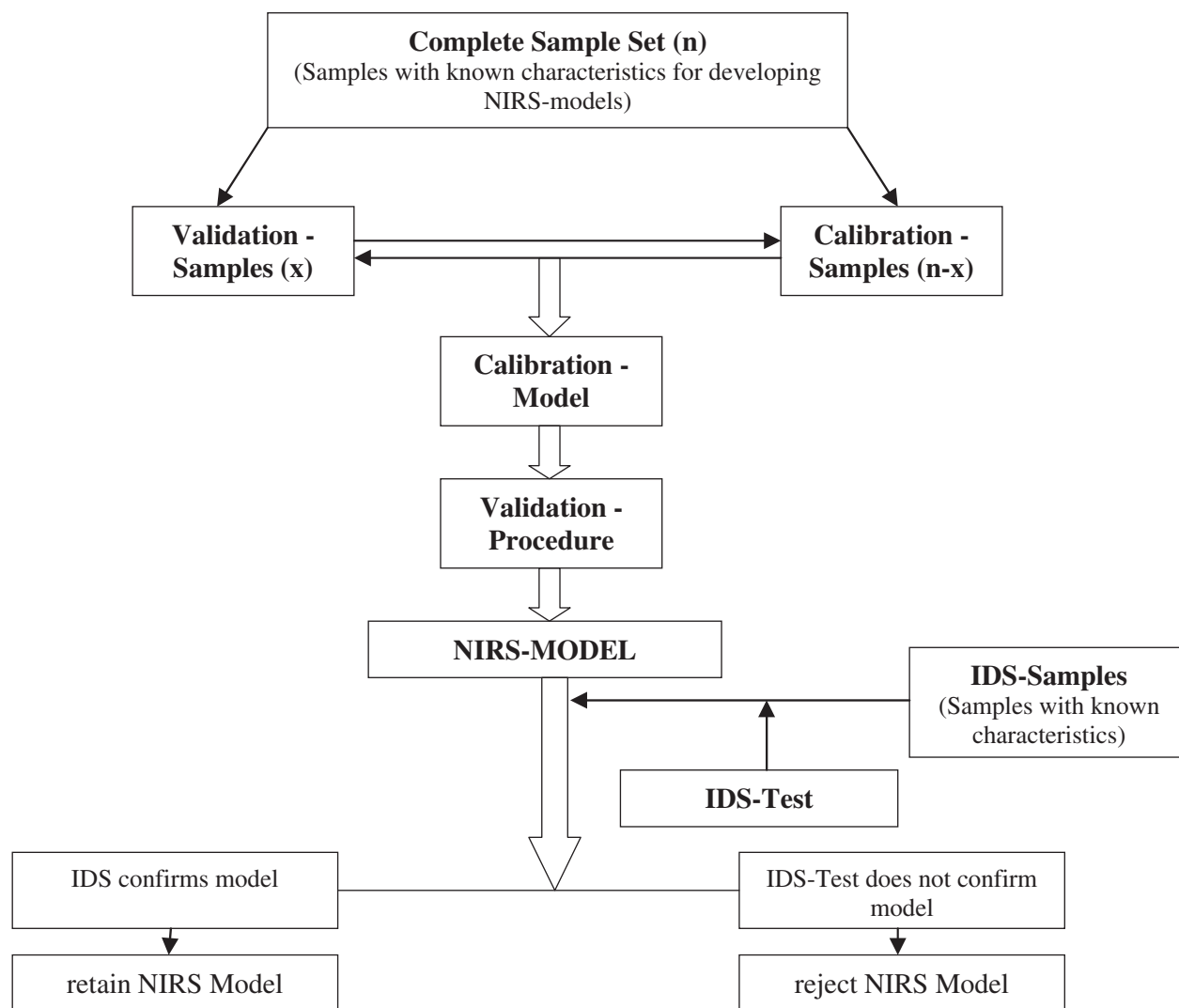


Figure 1. Diagram for the development of NIRS-models.

transformed prior to model development. For the models we selected samples in a way to maximize the variation of chemical and ecological properties in the samples used to generate the models.

We used two different validation methods to test the accuracy of the developed NIRS-models (Fig. 1; Beyer, 2003; Chang et al., 2001; McIlwee et al., 2001):

(1) Cross-Validation "c" (jack-knifing, internal validation)

To do this, one sample, the validation sample x , was removed from the data set at a time, while the remaining samples were used for calibration. This procedure was repeated for the whole data set until each sample had been removed once.

(2) Test-Set-Validation "ts" (external validation)

The complete data set was divided in two sets (this was done automatically and at random by the computer software Quant 2, Bruker Optics).

One set was used to develop the model (calibration set x). The other samples ($=$ validation samples $n-x$) were used to validate the model generated by the calibration set.

Optimization of models

We developed several models for the prediction of the chemical and ecological properties of the samples. All models were developed with test-set and cross-validation, and afterwards optimized in an iterative way, using the software Quant 2 (Bruker Optics). Of the various models we used the model with the highest coefficient of determination (R^2) between the analyzed and predicted concentrations in the samples (Table 1). Outliers in concentration or spectral outliers identified as such by the program Quant 2 were eliminated from the development of the model.

Table 1. Abbreviations for the description of NIRS-models (optimization procedures and validation) and associated tests

Abbreviation	
Ts	Validation of the NIRS model by test-set validation
C	Validation of the NIRS model by cross validation
n_c	Number of calibration samples
n_v	Number of validation samples
OL	Number of outliers eliminated from the developing procedure
R^{2a}	Coefficient of determination of the developed NIRS-models
Rank	Number of partial-least-squares-factors used for describing the spectrum
RMSEP ^b	Root of the mean square error of the prediction based on the test-set validation
RMSECV ^b	Root of the mean square error of the prediction of cross validation
Optim./Valid	Optimisation and validation procedures for NIRS-models
D1 ^c	First derivative
MMN ^c	Min-max-normalization
MSC ^c	Multiple scatter correction
COE ^c	Constant offset elimination
SL ^c	Subtraction of a straight line
VN ^c	Vector-normalization
IDS	Additional test of the model using an independent data-set (IDS)
n_i	Number of IDS-samples
r^2	Coefficient of determination based on linear regression
% Diff	Percentage of difference between NIRS results and the results obtained by standard methods or field measurements. Results from standard methods were set as 100%.

^aThe coefficient of determination R^2 for the NIRS model is based on the multiple coefficient of correlation for partial least squares (PLS) regression (Beyer, 2003). We used R^2 for NIRS-models to distinguish between results of NIRS-models and the results of the IDS-test (linear regression, r^2). R^2 is calculated as: $R^2 = (1 - \sum(y_i^{\text{meas}} - y_i^{\text{predict}})^2 / \sum(y_i - y_m))^2 * 100$ with y^{meas} = value measured with standard method or gained from field experiment; y^{predict} = predicted value, y_m = mean value.

^bRMSEP = RMSECV = $\sqrt{\frac{1}{M} \times \sum_{i=1}^M (y_i^{\text{meas}} - y_i^{\text{predict}})^2}$ for RMSEP y^{meas} , y^{predict} are values of the test-set, for RMSECV y^{meas} , y^{predict} are values of the data-set for cross validation.

^cOptimization procedures, used for the normalization of the spectra. MSC compensate for particle size effects by removing the effect of light scatter from the spectrum; for details see Bruker Optik (1998).

Evaluation of the models

During the optimization different calculation procedures were applied to achieve the best fit of the model (highest R^2). To test the accuracy of our models we used an independent set of samples with known characteristics ("Independent-Data-Set" IDS; Fig. 1). Samples of the IDS had not been used neither for calibration nor for validation of the original model. Samples used for the IDS test were within the range of concentrations of chemical or ecological properties of our models.

The samples were scanned and their characteristics were predicted with the NIRS-models developed as described above. The properties of this IDS sample (concentrations of chemicals, ecological properties) predicted by the NIRS-models were compared with the properties measured by standard chemical analyses or actual field measurements. Since samples were scanned in duplicate resulting in two predicted values per sample, we took the mean of these values for the IDS test. The goodness of fit (IDS-test) was evaluated using linear regression and subsequent F -test (ANOVA) [Sigma Stat 2.0].

The match between the values predicted by NIRS and conventional analyses was also expressed as the difference in % (%Diff) between the results obtained by NIRS and the results from standard methods (chemicals) or field measurements (browsing, litter decomposition). For the IDS-test we used only NIRS-models with $R^2 > 0.62$ (for abbreviations see Table 1). Even though this threshold is very low and should have low or qualitative predictive power, we included those models, because the purpose of the IDS-test was to test the power of the models and to identify their limits or inconsistencies.

Results

Numbers of spectra used for the development of the models ranged from 24 to 70 for the calibration-set when using test-set-validation (= 12–35 samples) and from 38 to 114 for the calibration-set when using cross-validation (= 19–57 samples). Test-set validations resulted more often in higher R^2 values for the models than cross-validations. Therefore test-set validation was used more often (18 models) than cross-validation (11 models). Twenty-four of the 29 models reached $R^2 > 0.62$. For four specific phenolics in twigs of *S. phyllicifolia* (gallocatechin, myricetin-galactoside, (+)catechin, disalicortin) and for myricetin-glucuronide in

leaves of *S. phyllicifolia*, we could not develop models beyond our threshold of $R^2 > 0.62$. These models were not tested any further. The results of four IDS-tests were negative (three models using cross-validation, one model using test-set-validation) for the determination of specific phenolics in plant material.

Subsequently we first report the tests of NIRS applications for chemical components and then describe the results for the tests of NIRS applications for ecological properties. A summary of the number of samples used and their chemical or ecological properties is given in Table 2.

Primary plant chemicals and groups of secondary components in twigs and leaves ("mixed" models)

Models for NDF, ADF, nitrogen, total phenolics and condensed tannins were developed in "mixed" models which combined spectra for twigs and leaves of *S. phyllicifolia* in one data set. For all compounds test-set-validation delivered the best results (Table 3, Fig. 2), with the highest R^2 for the nitrogen model ($R^2 = 0.99$).

The tests of the models with an independent data-set (IDS) showed that the prediction by NIRS was significantly correlated with the results from the chemical analyses for all models ($p < 0.001$). The best match with the IDS was obtained for ADF with $r^2 = 0.99$, a slope of 1.00, and a mean difference between predicted and measured values of 0.29%.

Specific phenolics in different plant parts ("mixed" models and separate models for twigs and leaves)

For the prediction of specific phenolic compounds in *S. phyllicifolia*, "mixed" models (consisting of twig and leaf samples) as well as separate models for both plant parts ("twig" models, "leaf" models) were developed.

"Mixed" models (twigs and leaves) for specific phenolics

We used five different specific phenolics (phenolic glucosides, flavonoids) occurring in *S. phyllicifolia* for the development of "mixed" models with NIRS (Table 4, Fig. 3). Consistent results with high qualitative value were achieved for ampelopsin ($R^2 = 0.97$). For myricetin-galactoside and myricetin-glucuronide NIRS-models had lower predictive values ($R^2 = 0.67$ and 0.74, respectively).

Table 2. Chemical characteristics for plant material taken from *Salix phylicifolia* to assess the development of different NIRS-models

	Chemical properties standard analyses					Ecological properties						
	NDF	ADF	Nitrogen	Phenolics	Tannins	Litter decomp.	Browsing degree	Picein	Disalicyrtin	Salicin	(+)Catechin	Triandrin
"Mixed" Models	41.87 ± 18.39 (n = 50) 16.41–68.51	30.54 ± 16.02 (n = 50) 0.24–52.44	1.86 ± 1.083 (n = 50) 0.74–3.85	4.76 ± 2.23 (n = 50) 1.66–9.33	3.27 ± 1.47 (n = 50) 1.12–7.53							
"Litter" Models (fallen, fresh leaves)			1.13 ± 0.30 (n = 45) 0.65–1.71	4.80 ± 2.00 (n = 43) 1.31–8.42	2.02 ± 1.31 (n = 44) 0.29–6.46	19.51 ± 3.77 (n = 25) 14.14–28.26						
"Decomp. Litter" Models (decomp. leaves)			1.44 ± 0.34 (n = 45) 0.81–1.99	1.13 ± 0.58 (n = 45) 0.44–3.17								68.22 ± 30.29 (n = 25) 4.70–92.27
"Twig" Models												
<i>Specific phenolics</i>												
	Ampelopsin	Gallocatechin	Myricetin- Galactoside	Myricetin- Glucuronide	(+)Catechin	Salicin	Disalicyrtin	Picein				
"Mixed" Models	63.31 ± 75.12 (n = 39) 0.40–260.31	1.00 ± 0.61 (n = 70) 0.00–2.57	0.97 ± 0.58 (n = 38) 0.16–2.22	2.59 ± 2.72 (n = 38) 0.04–9.55	2.13 ± 1.18 (n = 20) 0.43–3.99							
"Twig" Models	2.41 ± 1.84 (n = 20) 0.40–6.45	0.81 ± 0.58 (n = 50) 0.00–2.57	0.61 ± 0.27 (n = 30) 0.16–1.23	0.12 ± 0.11 (n = 49) 0.00–0.41	2.32 ± 1.20 (n = 20) 0.29–4.28	2.49 ± 1.26 (n = 50) 0.43–8.44	0.35 ± 0.35 (n = 49) 0.00–1.44	2.32 ± 2.03 (n = 60) 0.07–8.54				14.66 ± 9.81 (n = 35) 0.32–35.33
"Leaf" Models	127.42 ± 58.74 (n = 19) 36.74–260.31			4.28 ± 2.06 (n = 30) 0.36–7.90	1.43 ± 1.02 (n = 37) 0.08–3.55							

Values are means ± standard deviations, samples size and the range of concentration. For standard analyses and chemical properties means are given in (%), for specific phenolics in (mg/g dw).

Table 3. “Mixed” model performance of NIRS-models for different compounds in leaves and twigs of *Salix phylicifolia*

Compound	NIRS-models						IDS-tests					
	Optim., valid.	R^2 , rank	RMSEP	n_c	n_v	OL	n_i	%Diff.	R^2	Slope	F	P
NDF	D1+MSC ts	0.97 4	3.07	25	24	1	30	1.91	0.98	0.97	617.35	***
ADF	D1+MSC ts	0.98 4	1.91	25	24	1	30	0.29	0.99	1.00	1778.10	***
Nitrogen	D1+VN ts	0.99 6	0.09	25	23	2	30	1.47	0.97	0.93	432.15	***
Phenolics	D1 ts	0.98 8	0.31	25	25	-	30	1.59	0.96	0.96	293.91	***
Tannins	D1+MSC ts	0.89 3	0.41	25	23	2	30	0.44	0.83	0.61	64.10	***

Spectra were scanned for each sample in duplicate. Statistics for the IDS data sets are based on linear regression and F -test (ANOVA: $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$). The slope b is based of the equation $y = a + b \cdot x$ (where y is the NIRS predicted and x is the measured value). For abbreviations see Table 1.

In this case the regressions calculated with the IDS were significant but not convincing because of the low slopes $\ll 1$. The model for gallicocatechin reached only $R^2 = 0.64$ with the test-set validation, the IDS-test was not significant. For (+)catechin cross-validation was used because of the small number of samples ($n = 19$) resulting in $R^2 = 0.83$. As for gallicocatechin the IDS-test was not significant.

“Twig” models for specific phenolics

We developed NIRS-models for nine different phenolic compounds of willow twigs (Table 5, Fig. 4). No acceptable models could be developed for four components (gallicocatechin, myricetin-galactoside, (+)catechin and disalicortin). These models were not tested further (best models for these compounds are shown in Table 5). The model with the highest R^2 was achieved for ampelopsin using cross-validation ($R^2 = 0.80$). Even though the IDS-test was significant the difference between the concentrations predicted by the NIR model and the actual measurements (% Diff) for this model was very high.

For myricetin-gluconide, picein, and triandrin NIRS-models with $R^2 > 0.62$ could be developed using test-set or cross-validation. The IDS-tests of these three models were highly significant even though the slopes were low. The results for salicin were inconsistent. Cross-validation resulted in an

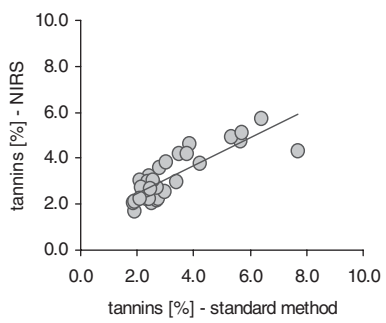
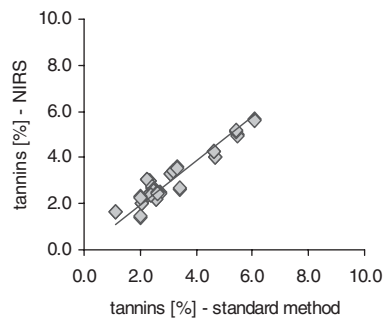
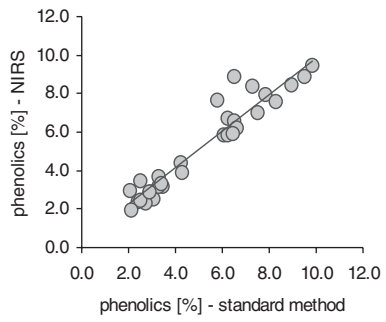
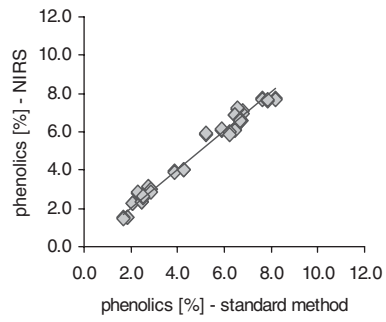
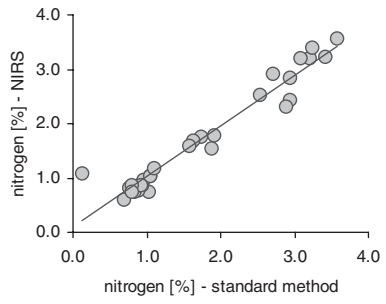
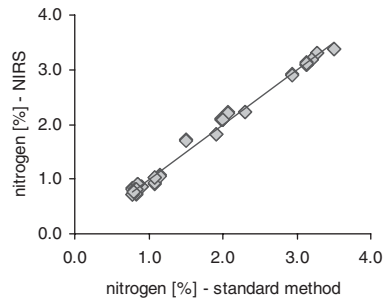
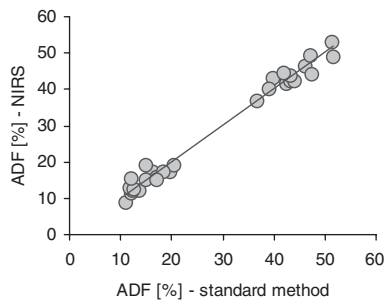
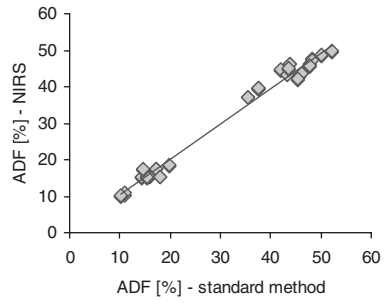
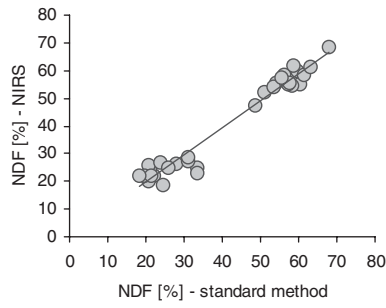
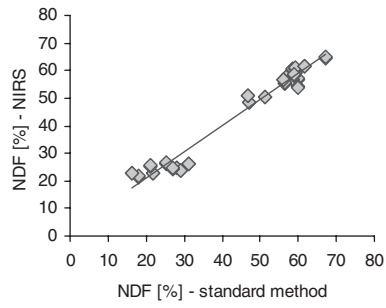
R^2 value of 0.78, but the IDS-test was not significant.

“Leaf” models for specific phenolics

We developed models for three different specific phenolics (ampelopsin, myricetin-gluconide, and (+)catechin) of leaves (Table 6, Fig. 5). For ampelopsin and (+)catechin the validation of the models was high (ampelopsin: $R^2 = 0.88$; (+)catechin: $R^2 = 0.92$). The IDS tests for these two models were significant for ampelopsin but not for (+)catechin. We could not develop models with high accuracy for myricetin-gluconide.

Analysis of the chemical composition of fallen (fresh) and decomposed leaves of *S. phylicifolia* used for decomposition experiments

The results of the NIRS applications for nitrogen and groups of phenolic components (“phenolics” and “tannins”) in fallen leaves of *S. phylicifolia* (Table 7, Fig. 6) as well as in the leaf material after decomposition (Table 8, Fig. 7) match the results obtained for leaves and twigs for the same components (Table 3). NIRS-models provide robust models for all three components. Models for “tannins” were not developed for the decomposed leaf material because tannin concentrations in decomposed leaf material were too low.



NIRS-Validation

IDS-Test

Table 4. “Mixed” model performance of NIRS-models for different phenolic compounds of leaves and twigs of *Salix phylicifolia* and their test with an independent data-set (IDS-test)

Compound	NIRS-models						IDS-tests						
	Optim., valid.	R^2 , rank	RMSEP, RMSECV	n_c	n_v	OL	n_i	%Diff.	r^2	Slope	F	P	
Ampelopsin	VN	0.97	11	19	20	–	13	0.86	0.95	0.77	106.88	***	
	ts	6											
Gallo-catechin	D1+VN	0.64	0.36	35	34	1	30	19.76	0.26	0.17	2.03	-	
	ts	2											
Myricetin-galactoside	VN	0.67	0.34	18	20	–	21	0.80	0.63	0.45	12.63	**	
	ts	2											
Myricetin-glucononide	D1+VN	0.74	1.35	18	20	–	21	11.27	0.73	0.43	21.89	***	
	ts	1											
(+)Catechin	D1+MSC	0.83	0.45	19	–	1	19	4.3	0.11	0.05	0.19	-	
	C	3											

For further explanations see [Tables 1 and 3](#).

In addition we developed models for nitrogen and total phenolics of “fresh” and decomposed litter resulting in $R^2 = 97.5$, $RMSEP = 0.06$ for nitrogen and $R^2 = 96.31$, $RMSEP = 0.359$ for phenolics with $n = 98$ (2 outliers). Best results were obtained with test-set-validation.

Analyses of ecological properties: browsing degree

NIRS-models were developed for the determination of the browsing damage caused by moose on willows in the previous winter. Therefore we estimated the degree of browsing damage in winter 2002 and sampled these willows in the subsequent autumn using the new growth of browsed twigs. The browsing degree was used to calibrate the NIR-spectra. We used test-set and cross validation. Test-set validation resulted in a higher coefficient of determination ($R^2 = 0.93$; [Table 9](#), [Fig. 8](#)). Thus, the standard NIRS validation indicated high predictive power. Based on the calculation of the linear regression between NIRS predictions and the actual measurements the second validation with IDS confirmed the general suitability of the NIRS model to reconstruct browsing damage.

However, the slope was very low, and the %Diff was high.

Analyses of ecological properties: decomposition rate

NIRS-models were developed for the determination of the decomposition rate (measured as mass loss over 1 year) of *S. phylicifolia* using 25 samples from both litter beds. The models were tested with test-set and cross-validation. Thirty-five samples were used for the IDS-test. The highest coefficient of determination was achieved by test-set-validation ($R^2 = 0.70$; [Table 10](#), [Fig. 8](#)). The IDS-test was significant, though the slope of the regression line was very low (slope = 0.23).

Discussion

Distinct chemicals: primary compounds and groups of secondary compounds

Suitable NIRS-models could be developed for the concentrations of different fibre fractions,

Figure 2. Results for “mixed” models for different primary and pooled secondary compounds in the twigs and leaves of *Salix phylicifolia*. Results of the validation procedure (left side): relationships between values measured by standard laboratory assays or field studies, and those predicted by using NIRS-models. Results of the IDS-test (right side) testing an independent sample set with known chemical characteristics: relationships between values measured by standard laboratory assays or field studies and values predicted by using NIRS-models. Dotted lines indicate trends which were not significant ($p > 0.05$), solid lines indicate significant relationships ($p \leq 0.05$).

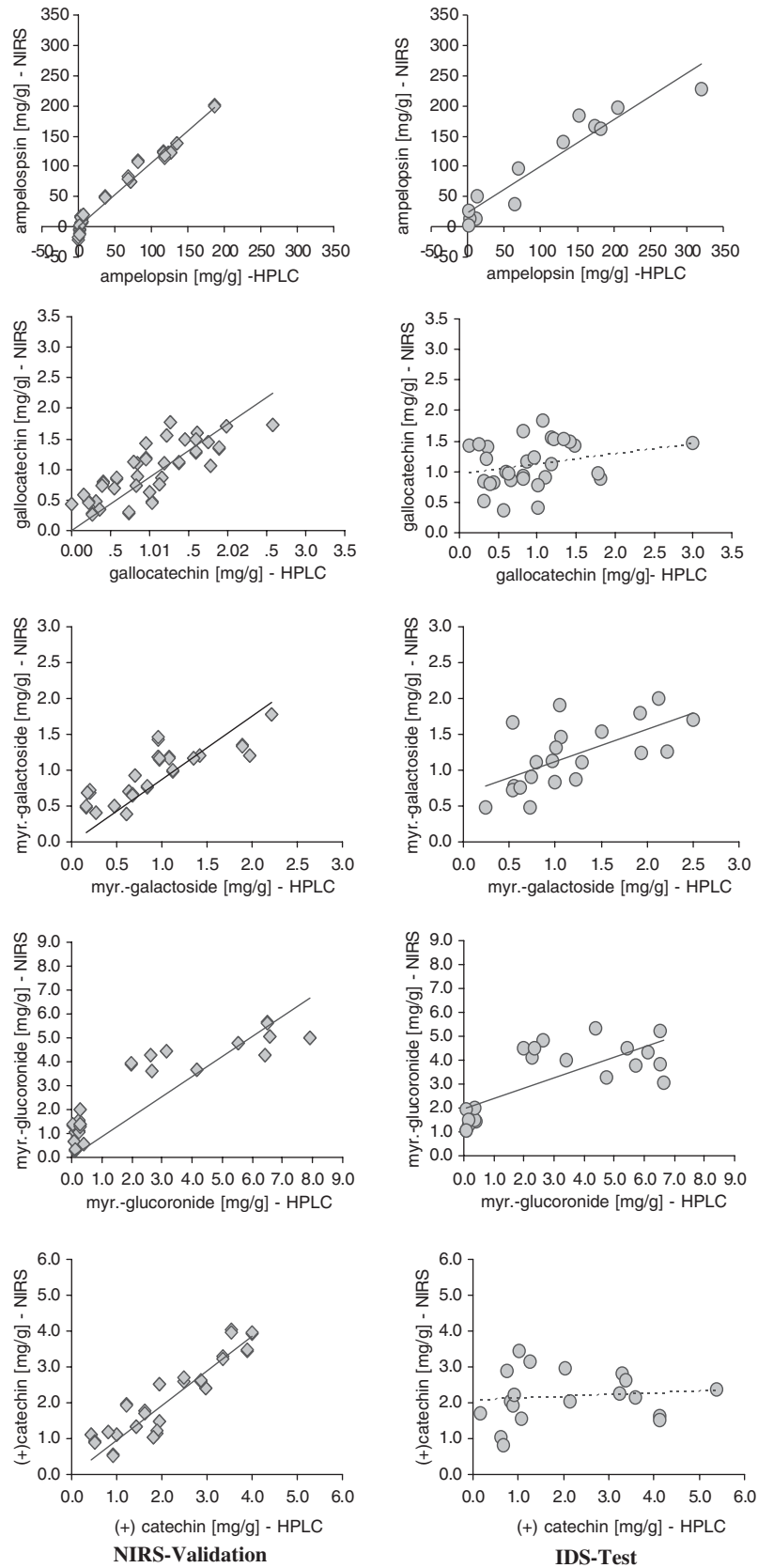


Figure 3. Results for “mixed” models for different specific phenolics in the twigs and leaves of *Salix phylicifolia*. Results of the validation procedure are shown on the left side, results of the IDS-test shown on the right side. For further details see Fig. 2.

Table 5. “Twig” model performance of NIRS-models for different phenolic compounds of twigs of *Salix phylicifolia* and their test with an independent data-set (IDS-test)

Compound	NIRS-models			IDS-tests									
	Optim., valid.	R^2 , rank	RMSEP, RMSECV	n_c	n_v	OL	n_i	%Diff.	r^2	Slope	F	P	
Ampelopsin	COE c	0.80 9	0.79	20	–	–	15	48.94	0.79	0.77	21.44	***	
Gallo-catechin	D1+SG Ts	0.62 1	0.36	25	24	1	–	–	–	–	–	–	
Myricetin-galactoside	MMN c	0.55 4	0.16	28	–	2	–	–	–	–	–	–	
Myricetin-glucoronide	VN ts	0.77 3	0.05	24	25	–	14	2.97	0.84	0.59	27.65	***	
(+)Catechin	SL c	0.60 7	0.74	20	–	–	–	–	–	–	–	–	
Salicin	COE c	0.78 7	0.38	43	–	7	24	8.66	0.03	0.01	0.02	–	
Disalicortin	VN ts	0.34 9	0.28	24	25	–	–	–	–	–	–	–	
Picein	D1+SL c	0.63 10	1.14	57	–	3	25	1.72	0.75	0.56	30.15	***	
Triandrin	VN c	0.75 7	4.80	35	–	–	17	12.88	0.75	0.63	19.65	***	

For further explanations see [Tables 1 and 3](#).

nitrogen, total phenolics and condensed tannins in leaves, twigs, and litter of *S. phylicifolia*. The accuracy of the models was confirmed by an additional test with an independent dataset (IDS-test). These analyses revealed the high potential of near infrared spectroscopy to substitute conventional analyses. This has been illustrated before with samples from eucalypt forest, samples of bamboo or willows (Lawler et al., 1998, 2000; McIlwee et al., 2001; Ortmann in press; Stolter et al., in press).

From an economic point of view the models could be achieved with relatively small data sets used for the calibration. Thus, NIRS offers a suitable and efficient tool for the measurements of these primary and some groups of secondary plant components. McIlwee et al. (2001) estimated that application of NIRS-models reduced the time requirements by about 80% compared to standard analyses when applied to a single component. Since we were able to develop useful models for five components (two fibre fractions, nitrogen, total phenolics and condensed tannins) with two spectra per sample (time for scanning: 90s), the

savings in terms of chemicals and time are substantial.

Distinct chemicals: specific phenolics

Phenolics are secondary plant components known to play important roles in ecosystem processes such as defence against herbivory (Bryant, 1981; Karban & Baldwin, 1997; Stolter et al., in press), or they can act positively as feeding cues for leaf beetles (Kohlemainen, Julkunen-Tiitto, Roininen, & Tahvanainen, 1995). Changes in phenolic composition of the plant due to herbivory, damage or changes of abiotic factors (e.g. UV-radiation) might have effects across trophic levels, such as on other herbivores, soil chemistry and decomposers (Suominen et al., 1999; Tegelberg et al., 2003). Instead of using time and cost intensive HPLC, NIRS could offer a tool for rapid analyses of plant material, which could allow intensive monitoring on a landscape level.

Our results for the specific phenolics were inconsistent. Promising (though not always satisfying) models could be achieved for certain components

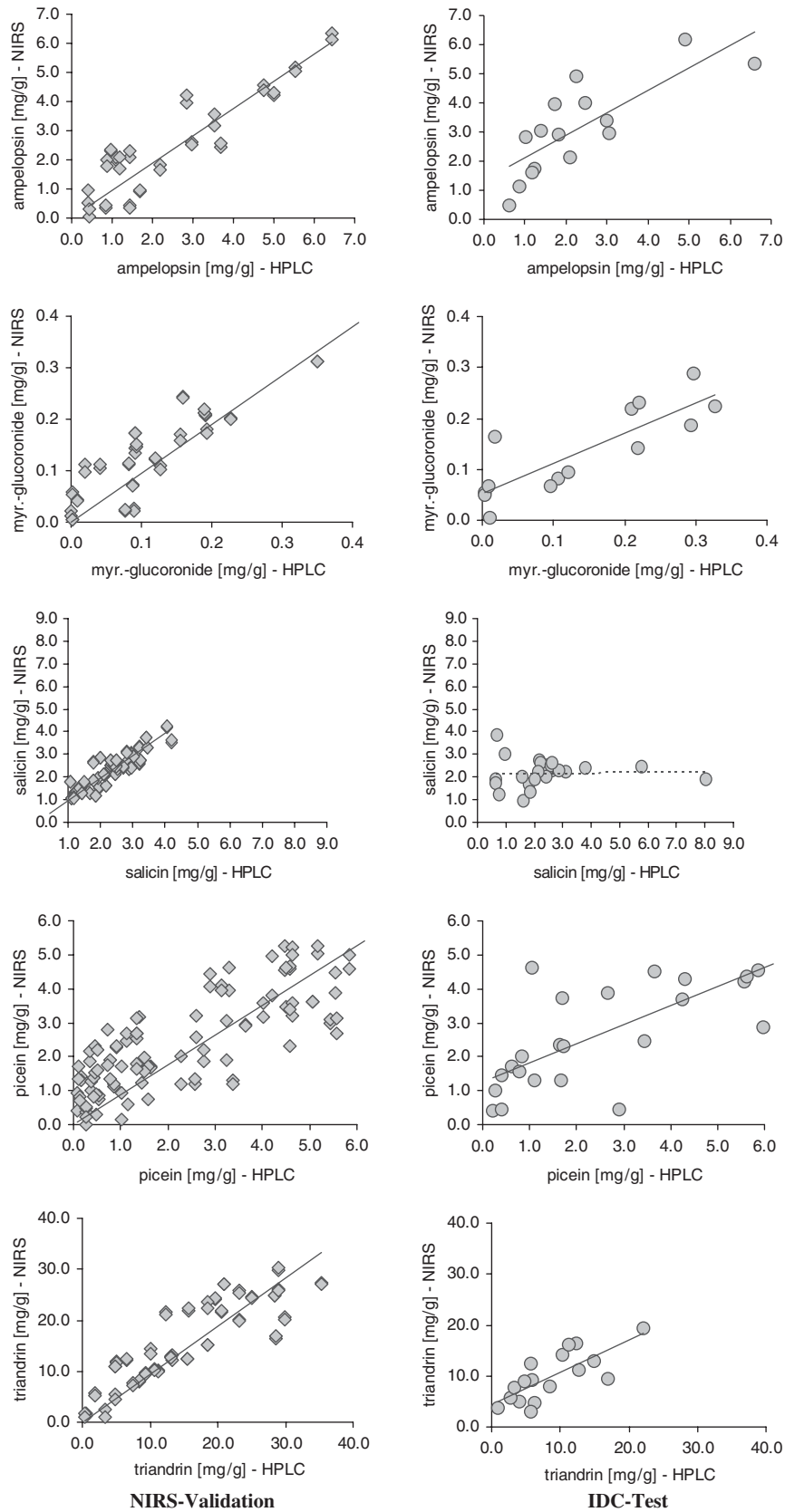
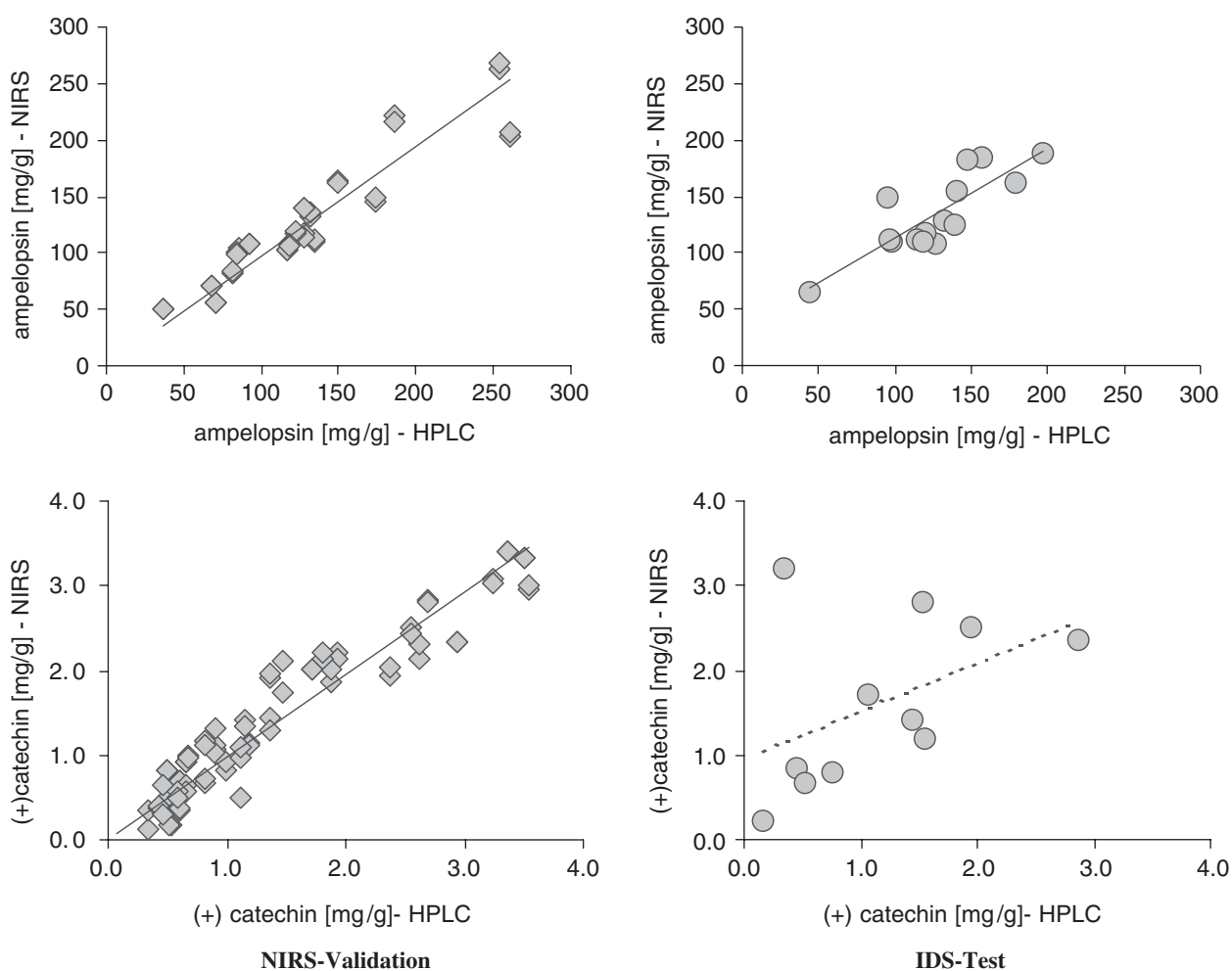


Figure 4. Results for “twig” models for different specific phenolics in the twigs of *Salix phylicifolia*. For further details see Fig. 2.

Table 6. “Leaf” model performance of NIRS-models for different phenolic compounds of leaves of *Salix phylicifolia* and their test by using an independent data-set (IDS-test)

Compound	NIRS-Models						IDS-tests					
	Optim., valid.	R^2 , rank	RMSEP, RMSECV	n_c	n_v	OL	n_i	% Diff.	r^2	Slope	F	P
Ampelopsin	D1+VN c	0.88 3	19.90	19	—	—	15	3.78	0.83	0.78	29.05	***
Myricetin-glucoronide	COE ts	0.57 5	1.26	15	14	1	—	—	—	—	—	—
(+)Catechin	SL c	0.92 6	0.29	37	—	—	11	40.54	0.47	0.57	2.60	—

For further explanations see Tables 1 and 3.

**Figure 5.** Results for “leaf” models for different specific phenolics in the leaves of *Salix phylicifolia*. For further details see Fig. 2.

in some plant parts, such as ampelopsin, myricetin-glucoronide and (+)catechin. But the models failed for other components, such as galocatechin or salicin.

Reasons for the inconclusive results could be unspecific responses to the NIR-wavelengths by these molecules, or interferences by attached molecules. The procedures of the software used

Table 7. Performance of NIRS-models for different compounds in fallen leaves of *Salix phylicifolia* used for decomposition experiments

Compound	NIRS-models			IDS-tests								
	Optim., valid.	R^2 , rank	RMSEP, RMSECV	n_c	n_v	OL	n_i	%Diff.	r^2	Slope	F	P
Nitrogen	MMN ts	0.98 10	0.04	15	30	–	11	2.62	0.97	1.00	149.05	***
Phenolics	SL c	0.97 6	0.32	42	–	1	15	7.43	0.90	0.77	57.52	***
Tannins	D1+MSC c	0.92 7	0.35	43	–	1	15	6.64	0.98	0.95	372.52	***

For explanations see Tables 1 and 3.

for model development selected frequencies which were either different for “mixed”, “leaf”, and “twig” models for the same component or they covered almost the whole spectrum, indicating that there were no prominent differences between spectra. This was a striking difference to the models developed for the primary components or the groups of secondary components listed above. Furthermore, some of the frequencies chosen by the procedures of development were on the “edge” of NIR, close to MIR (mid infrared). MIR-spectroscopy had been used successfully to determine total phenolics in wine (Edelmann, Diewok, Schuster, & Lendl, 2001). Another possibility could be that the concentrations of some of the components were simply too low or not variable enough to produce significant signals. For example, the concentrations for ampelopsin varied between 0.4 and 186.5 mg/g dry weight. For this component models could be achieved, while this was not possible for galocatechin which ranged only from 0 to 0.26 mg/g dry weight. Furthermore, the additional drying of the samples prior to the NIRS analyses might have destroyed some of the phenolics (Julkunen-Tiitto & Tahvanainen, 1989).

General ecological properties: browsing degree and decomposition

One of the advantages of working with NIRS is, that the spectrum covers a wide range of wavelengths and therefore has the potential to discover hidden information, which might be useful for answering ecological questions, such as the percentage of different plant species in mixed plant samples (Coleman, Christiansen, & Shenk, 1990).

In our studies the degree of browsing as well as the decomposition rate was reflected in the NIR-spectra. In both cases the second independent test of the model did reconstruct or predict the response variable correctly only qualitatively (i.e., the samples were ordered correctly according to the response variable but the absolute values deviated from the measured values by up to 50%). This might be due to low accuracy of the original measurements (such as measuring the degree of moose browsing on a specific willow in the field) or small number of samples used to develop the model. In contrast to our results Gillon et al. (1999) were able to predict litter decomposition in a standardized microcosm experiment for different litter. Thus, the lack of precision might be due to field conditions rather than due to the NIRS per se. Nevertheless, our results show that mass loss can be predicted from the initial quality of the litter to some extent.

Despite the general applicability of the models generated by the commercial software provided with NIR equipment, additional tests of the models with independent datasets are recommended, to reveal inconsistency in the developed models. For instance some of the models had a higher R^2 -value (e.g. model for (+)catechin), but their predictive power was lower, than models with lower R^2 (e.g. model for myricetin-galactoside). High R^2 can be linked to other criteria (e.g. a high number of PLS-factors (=rank) needed to describe the spectrum). In our study a high rank number sometimes lowered the predictive quality of the models (see Table 3) though this was not consistent for all models (see results for specific phenolics). Additional statistical tests have been described to examine this problem (Chang et al., 2001). In our

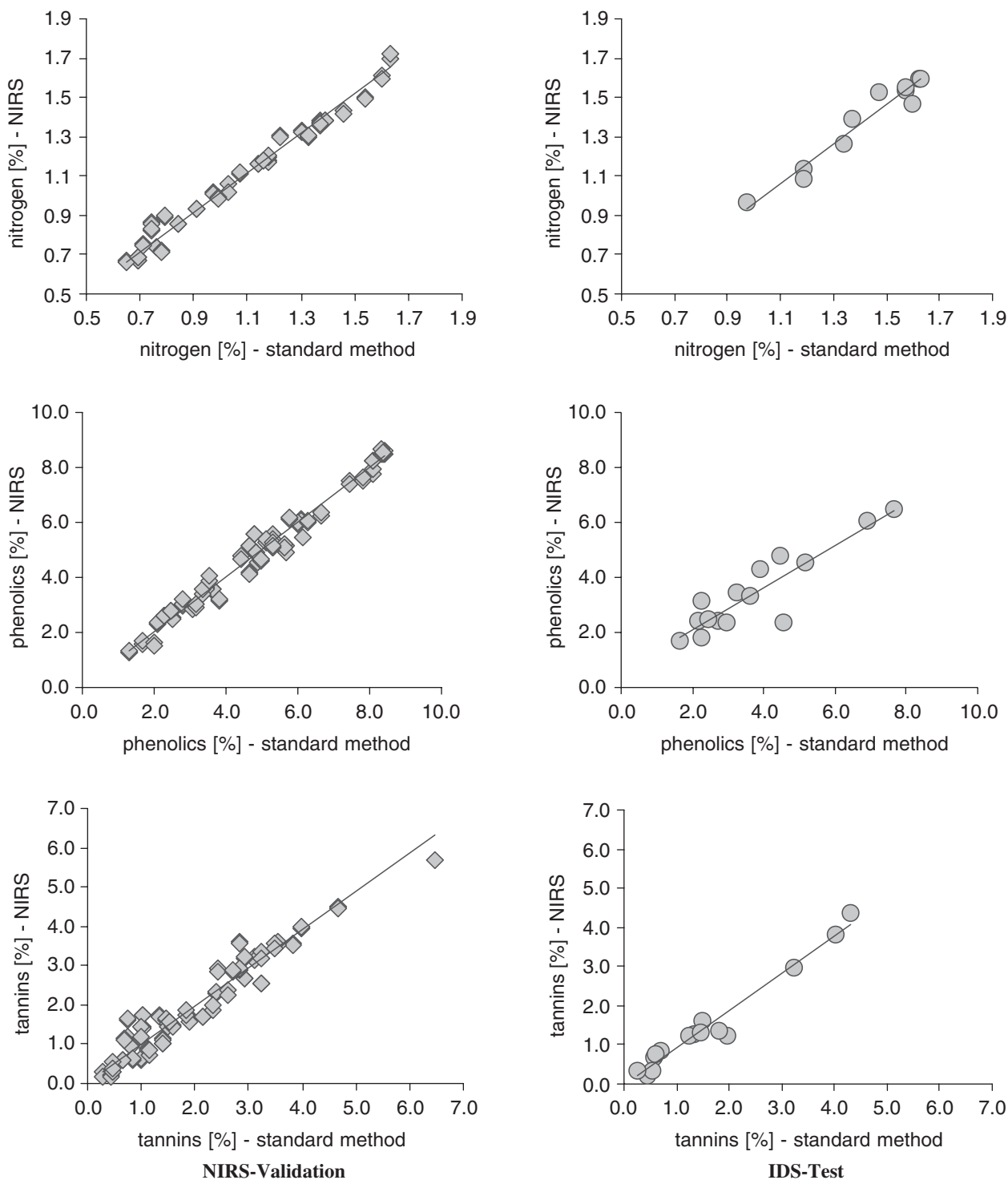


Figure 6. Results for “litter” models for nitrogen and pooled secondary compounds in litter of *Salix phylicifolia*. For further details see Fig. 2.

study test-set validation resulted in reliable models with high accuracy.

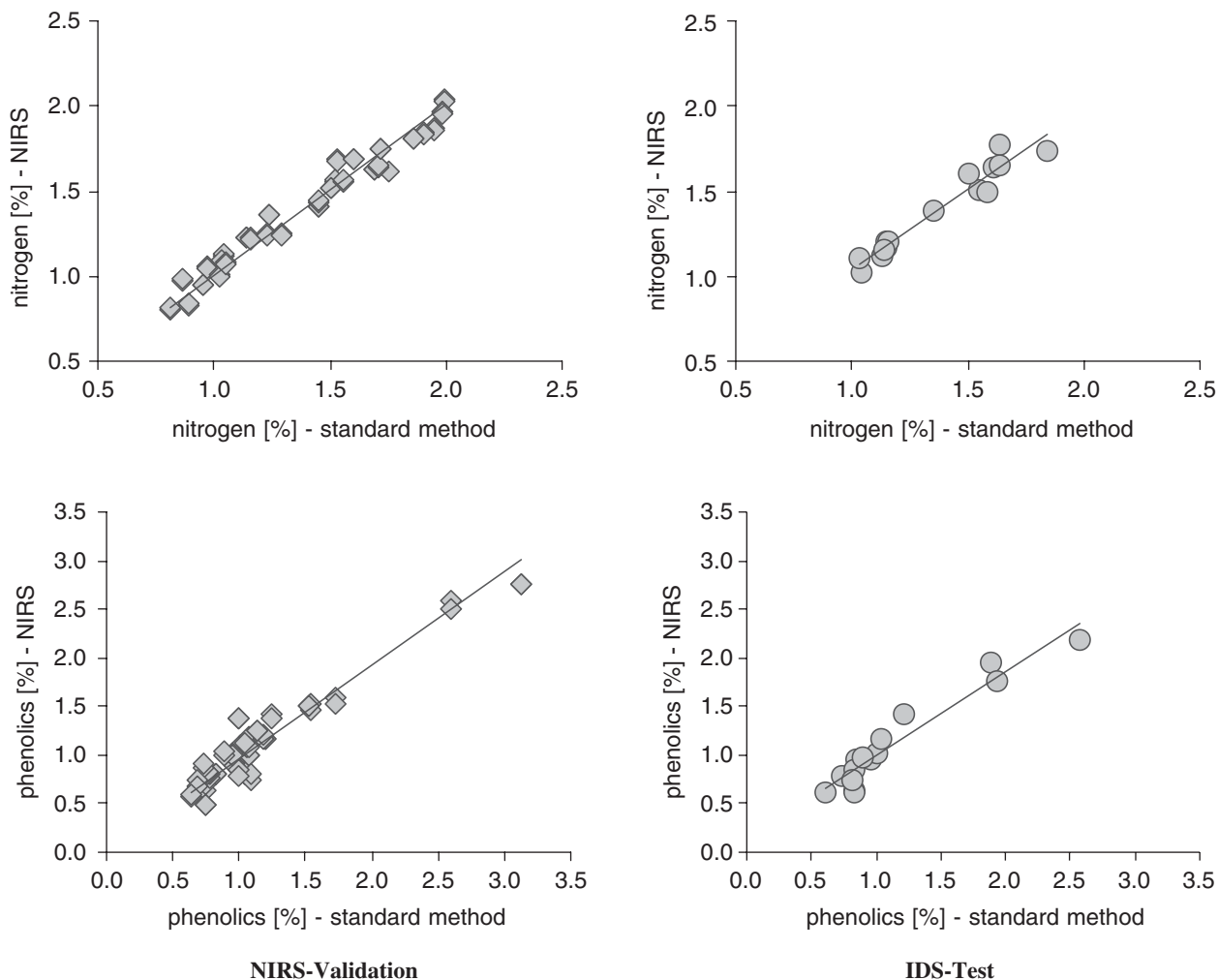
In conclusion, NIRS can be an economically interesting and an appropriate substitute for detailed

chemical analyses and for the analyses of general properties of plants in an ecosystem context. Certainly, the NIRS-models cannot be more accurate than the data used for their calibration and testing.

Table 8. Performance of NIRS-models for different compounds in decomposed leaves of *Salix phylicifolia* used for decomposition experiments

Compound	NIRS-models						IDS-tests					
	Optim., valid.	R^2 , rank	RMSEP, RMSECV	n_c	n_v	OL	n_i	%Diff.	r^2	Slope	F	P
Nitrogen	SL ts	0.97 5	0.07	16	29	—	15	1.27	0.97	0.94	213.49	***
Phenolics	D1+VN ts	0.92 8	0.152	16	29	—	15	1.89	0.96	0.86	161.27	***

For explanations see Table 1 and 3.

**Figure 7.** Results for “decomposed litter” models for nitrogen and total phenolics in decomposed litter of *Salix phylicifolia*. For further details see Fig. 2.**Table 9.** Performance of the NIRS-model for predicting the browsing damage on *Salix phylicifolia* caused by moose and their test by using an independent data-set (IDS-test)

Compound	NIRS-models						IDS-tests					
	Optim., valid.	R^2 , rank	RMSEP	n_c	n_v	OL	n_i	%Diff.	r^2	Slope	F	P
Browsing degree	COE ts	0.93 3	8.75	13	12	—	15	56.98	0.71	0.34	13.00	**

For explanations see Tables 1 and 3.

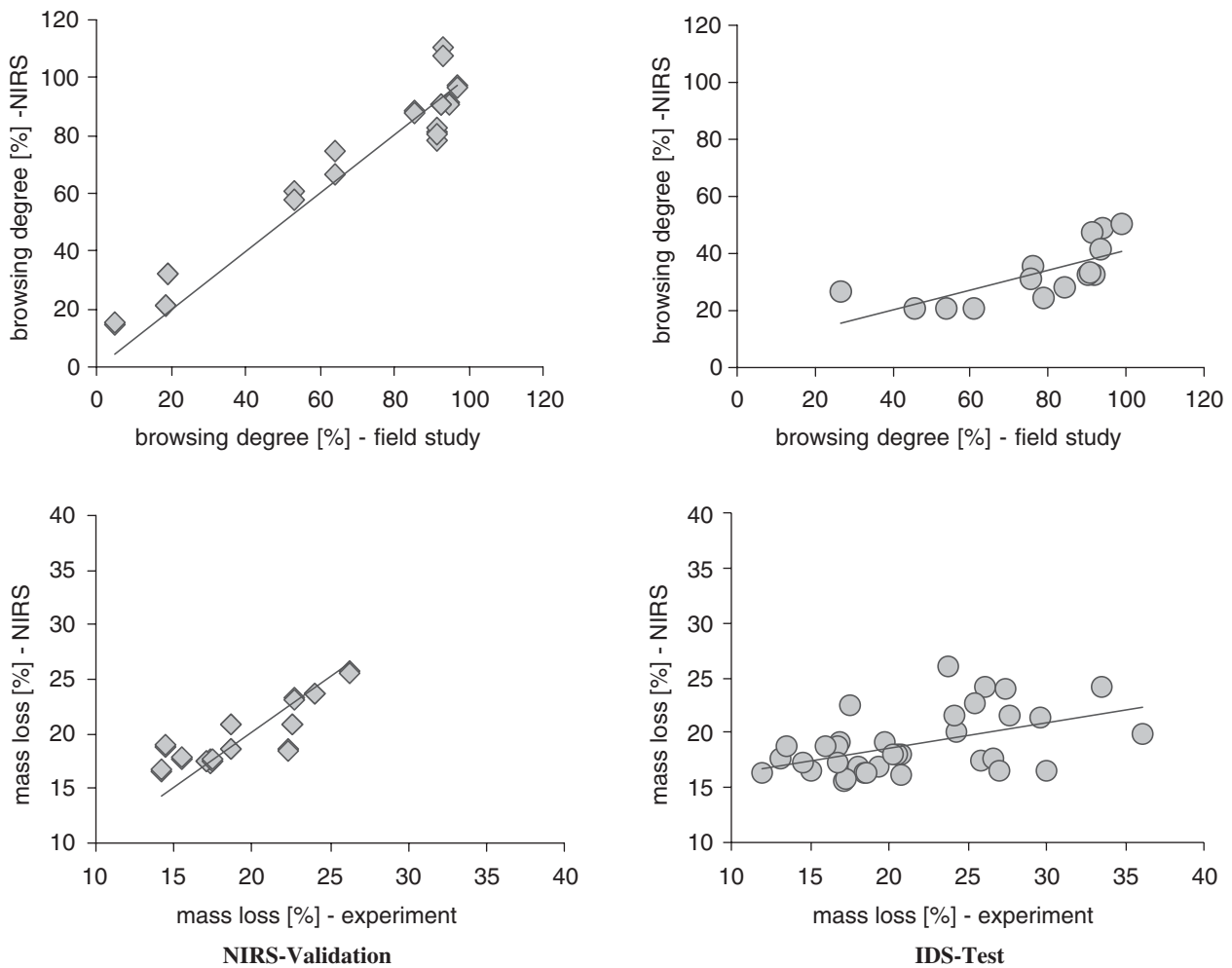


Figure 8. Results for models testing general ecological properties (browsing degree and decomposition rate (mass loss)) of *Salix phlyicifolia*. For further details see Fig. 2.

Table 10. Performance of the NIRS-model for predicting the decomposition rate [% mass loss] of *Salix phlyicifolia* and their test by using an independent data set (IDS-test)

Compound	NIRS-models						IDS-tests					
	Optim., valid.	R^2 , rank	RMSEP	n_c	n_v	OL	n_i	%Diff.	r^2	Slope	F	P
Mass loss	COE ts	0.70 3	2.02	12	13	—	35	11.55	0.49	0.23	10.29	**

For explanations see Tables 1 and 3.

Acknowledgments

We thank the staff of Abisko Scientific Research Station for logistical support, Outi Nousianinen and Irene Tomaschewski for technical support. Special thanks go to Helen Quedstedt from Stockholm University for the support with the decomposition experiment, to John Ball for helping to set up the moose browsing experiment, to Klemens Irmer

from Bruker GmbH (Germany) for his help getting the NIRS running. We thank two unknown reviewers for their helpful comments on the manuscript. The study was supported by the Deutscher Akademischer Austauschdienst (DAAD), the Deutsche Forschungsgemeinschaft (DFG Ga 342/11-1), the Graduiertenförderung and the University Foundation of Hamburg University and the Royal Swedish Academy of Science.

References

- Bardgett, R. D., Wardle, D. A., & Yeates, G. W. (1998). Linking above-ground and below-ground interactions: how plant response to foliar herbivory influence soil organisms. *Soil Biology & Biochemistry*, *30*, 1867–1878.
- Beyer, J. (2003). *Nahinfrarotspektroskopische Untersuchungen an pharmazeutischen Hilfsstoffen und festen Arzneiformen*. Doctoral Thesis. Bonn: Rheinische Friedrich-Wilhelm-Universität.
- Bouchard, V., Gillon, D., Joffre, R., & Lefeuvre, J. C. (2003). Actual litter decomposition rates in salt marshes measured using near-infrared reflectance spectroscopy. *Journal of Experimental Marine Biology and Ecology*, *290*, 149–163.
- Brockman, D. K., & van Schaik, C. P. (Eds.). (2005). *Primate seasonality: Implications for human evolution*. Cambridge: Cambridge University Press.
- Bruker Analytik GmbH. (1998). *Handbook Vector 22/N*. Germany: Ettlingen.
- Bryant, J. P. (1981). Phytochemical deterrence of snowshoe hare browsing by adventitious shoots of four Alaskan trees. *Science*, *213*, 889–890.
- Bryant, J. P., & Kuropat, P. J. (1980). Selection of winter forage by subarctic browsing vertebrates: The role of plant chemistry. *Annual Review of Ecology and Systematics*, *11*, 261–285.
- Chang, C. W., Laird, D. A., Mausbach, M. J., & Hurburgh, C. R., Jr. (2001). Near-infrared reflectance spectroscopy – principal components regression analyses of soil properties. *Soil Science Society of American Journal*, *65*, 480–490.
- Coleman, S. W., Christiansen, S., & Shenk, J. S. (1990). Prediction of botanical composition using NIRS calibrations developed from botanical pure samples. *Crop Science*, *30*, 202–207.
- Cornelissen, J. H. C. (1996). An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. *Journal of Ecology*, *84*, 573–583.
- deBoever, J. L., Vanacker, J. M., & deBrabander, D. L. (2003). Rumen degradation characteristics of nutrients in compounds feeds and the evaluation of tables, laboratory methods and NIRS as predictors. *Animal Feed Science and Technology*, *107*, 29–43.
- Dicke, M., & Hilker, M. (2003). Induced plant defences: From molecular biology to evolutionary ecology. *Basic and Applied Ecology*, *4*, 3–14.
- Eber, S. (2001). Multitrophic interactions: The population dynamics of spatially structured plant-herbivore-parasitoid systems. *Basic and Applied Ecology*, *2*, 27–33.
- Edelmann, A., Diewok, J., Schuster, K. C., & Lendl, B. (2001). Rapid method for the discrimination of red wine cultivars based on mid-infrared spectroscopy of phenolic wine extracts. *Journal of Agricultural & Food Chemistry*, *49*, 1139–1145.
- Elmqvist, T., Ericson, L., Danell, K., & Salomonson, A. (1987). Flowering, shoot production, and vole bark herbivory in a boreal willow. *Ecology*, *68*, 1623–1629.
- Foley, W. J., McIlwee, A., Lawler, I., Aragones, L., Woolnough, A. W., & Berding, N. (1998). 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. *Oecologia*, *116*, 293–305.
- Folin, O., & Ciocalteu, V. (1927). On tyrosine and tryptophane determination in proteins. *Journal of Biological Chemistry*, *27*, 627–650.
- Gillon, D., Joffre, R., & Ibrahima, A. (1999). Can litter decomposability be predicted by near infrared reflectance spectroscopy. *Ecology*, *80*, 175–186.
- Hättenschwiler, S., & Vitousek, P. M. (2000). The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology and Evolution*, *15*, 238–243.
- Julkunen-Tiitto, R., Rousi, M., Bryant, J. P., Sorsa, S., Keinänen, M., & Sikanen, H. (1996). Chemical diversity of several Betulaceae species: comparison of phenolics and terpenoids in northern birch stems. *Trends in Ecology and Evolution*, *11*, 16–22.
- Julkunen-Tiitto, R., & Tahvanainen, J. (1989). The effect of the sample preparation method of extractable phenolics of salicaceae species. *Planta Media*, *55*, 55–58.
- Julkunen-Tiitto, R., & Sorsa, S. (2001). Testing the effects of drying methods on willow flavonoids, tannins and salicylates. *Journal of Chemical Ecology*, *27*, 779–789.
- Karban, R., & Baldwin, I. T. (1997). *Induced responses to herbivory*. Chicago: University of Chicago Press.
- Kohlemainen, J., Julkunen-Tiitto, R., Roininen, H., & Tahvanainen, J. (1995). Phenolic glucosides as feeding cues for willow-feeding leaf beetles. *Entomologia Experimentalis et Applicata*, *74*, 235–243.
- Korsman, T., Nilsson, M. B., Landgren, K., & Renberg, I. (1999). Spatial variability in the surface sediment composition characterised by near-infrared (NIR) reflectance spectroscopy. *Journal of Paleolimnology*, *21*, 61–71.
- Korsman, T., Nilsson, M., Öhman, J., & Renberg, I. (1992). Near-infrared reflectance spectroscopy of sediments: a potential method of infer the past pH of lakes. *Environmental Science and Technology*, *26*, 2122–2126.
- Lawler, I. R., Foley, W. J., & Eschler, B. M. (2000). Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. *Ecology*, *81*, 1327–1338.
- Lawler, I. R., Foley, W. J., Eschler, B. M., Pass, D. M., & Handasyde, K. (1998). Intraspecific variation in Eucalyptus secondary metabolites determines food intake by folivorous marsupials. *Oecologia*, *116*, 160–169.
- Leite, E. R., & Stuth, J. W. (1995). Fecal NIRS equations to assess diet quality of free-ranging goats. *Small Ruminant Research*, *15*, 223–230.
- Lyons, R. K., & Stuth, J. W. (1992). Fecal NIRS equations for predicting diet quality of free-ranging cattle. *Journal of Range Management*, *45*, 238–244.
- Malley, D. F., Rönicke, H., Findlay, D. L., & Zippel, B. (1999). Feasibility of using near-infrared reflectance

- spectroscopy for the analysis of C, N, P and diatoms in lake sediments. *Journal of Paleolimnology*, *21*, 295–306.
- McIlwee, A. M., Lawler, I. R., Cork, S. J., & Foley, W. J. (2001). Coping with chemical complexity in mammal-plant interactions: Near-infrared spectroscopy as a predictor of Eucalyptus foliar nutrients and of the feeding rates of folivorous marsupials. *Oecologia*, *128*, 539–548.
- McTiernan, K. B., Garnett, M. H., Mauquoy, D., Ineson, P., & Couteaux, M. M. (1998). Use of near-infrared reflectance spectroscopy (NIRS) in paleoecological studies of peat. *The Holocene*, *8*, 729–740.
- Nilsson, M. B., Dabakk, E., Korsman, T., & Renberg, I. (1996). Quantifying relationships between near-infrared reflectance spectra of lake sediments and water chemistry. *Environmental Science and Technology*, *30*, 2586–2590.
- Nyman, T., & Julkunen-Tiitto, R. (2000). Manipulation of the phenolic chemistry of willows by gall-inducing sawflies. *Proceedings of the National Academy of Science of the United States of America*, *97*, 13,184–13,187.
- Oates, J. F., Swain, T., & Zantovska, J. (1977). Secondary compounds and food selection by the colobus monkey. *Biochemical Systematics and Ecology*, *5*, 317–321.
- Ortmann, S., Bradley, B. J., Stolter, C., & Ganzhorn, J. U. (in press). Estimating the quality and composition of wild animal diets – a critical survey of methods. In C. Boesch, G. Hohmann, M. Robbins (Eds.), *Primate feeding ecology*. Cambridge: Cambridge University Press.
- Ostfeld, R. S., & Keesing, F. (2000). Pulsed resources and community dynamics of consumers in terrestrial ecosystems. *Trends in Ecology and Evolution*, *15*, 232–237.
- Pearce, R. A., Lyons, R. K., & Stuth, J. W. (1993). Influence of handling methods on fecal NIRS evaluations. *Journal of Range Management*, *46*, 72–276.
- Pharmacopöä Europaea. (1997). *Europäisches Arzneibuch Ph.Eur.* Stuttgart: Deutscher Apotheker Verlag.
- Quested, H. M., Cornelissen, J. H. C., Press, M. C., Callaghan, T. V., Aerts, R., Trosien, F., Riemann, P., Gwynn-Jones, D., Kondratchuk, A., & Jonasson, S. E. (2003). Decomposition of sub-arctic plants with differing nitrogen economies: A functional role for hemiparasites. *Ecology*, *84*, 3209–3221.
- Rosén, P., Dabakk, E., Renberg, I., Nilsson, M., & Hall, R. (2000). Near-infrared spectrometry (NIRS): A new tool for inferring past climatic changes from lake sediments. *The Holocene*, *10*, 161–166.
- Rostás, M., Simon, M., & Hilker, M. (2003). Ecological cross-effects of induced plant responses towards herbivores and phytopathogenic fungi. *Basic and Applied Ecology*, *4*, 43–62.
- Sipura, M. (1999). Tritrophic interactions: Willows, herbivorous insects and insectivorous birds. *Oecologia*, *21*, 537–545.
- Stark, C. (2001). *Fraßpräferenzen von Elchen (Alces alces) unter besonderer Berücksichtigung der Weide*. Diploma Thesis. Hamburg: Universität Hamburg.
- Stolter, C., Ball, J. P., Julkunen-Tiitto, R., Lieberei, R., & Ganzhorn, J. U. (in press). Winter browsing of moose (*Alces alces*) on two different willow species: food selection in relation to plant chemistry and plant response. *Canadian Journal of Zoology*.
- Suomela, J., Suominen, O., & Törvi, M. (1997). Variation in quality of mountain birch and tea-leaved willow for mammal and insect herbivores: Differences among trees, among sites and between tree species. *Ecography*, *20*, 224–232.
- Suominen, O., Danell, K., & Bergström, R. (1999). Moose, trees, and ground-living invertebrates: indirect interactions in Swedish pine forest. *Oikos*, *84*, 215–226.
- Tahvanainen, J., Helle, E., Julkunen-Tiitto, R., & Kettunen, J. (1985). Phenolic glycosides govern the food selection pattern of willow feeding leaf beetles. *Oecologia*, *67*, 52–56.
- Tahvanainen, J., Julkunen-Tiitto, R., & Lavola, A. (1985). Phenolic compounds of willow bark as deterrents against feeding by mountain hare. *Oecologia*, *65*, 319–323.
- Tegelberg, R., Veteli, T., Aphalo, P. J., & Julkunen-Tiitto, R. (2003). Clonal differences in growth and phenolics of willows exposed to elevated ultraviolet-B radiation. *Basic and Applied Ecology*, *4*, 219–228.
- Tscharntke, T., & Hawkins, B. A. (Eds.). (2002). *Multi-trophic level interactions*. Cambridge: Cambridge University Press.
- United States Pharmacopeia. (2002). *USP25-NF20 Supplement, official monographs*. Rockville: The United States Pharmacopeial Convention, Inc.
- Walker, J. W., Clark, D. H., & McCoy, S. D. (1998). Fecal NIRS for predicting percent leafy spurge in diets. *Journal of Range Management*, *51*, 450–455.
- Xiccato, G., Trocino, A., Carazzolo, A., Meurens, M., Maertens, L., & Carabano, R. (1999). Nutritive evaluation and ingredient prediction of compound feeds for rabbits by near-infrared reflectance spectroscopy (NIRS). *Animal Feed Science and Technology*, *77*, 201–212.
- Xiccato, G., Trocino, A., DeBoever, J. L., Maertens, L., Carabano, R., Pascual, J. J., Perez, J. M., Gidenne, T., & Falcao-E-Cunha, L. (2003). Prediction of chemical composition, nutritive value and ingredient composition of European compound feeds for rabbits by near infrared reflectance spectroscopy (NIRS). *Animal Feed Science and Technology*, *104*, 153–168.
- Zvereva, E. L., Kozlov, M. V., Niemelä, P., & Haukioja, E. (1997). Delayed induced resistance and increase in leaf fluctuation asymmetry as responses of *Salix borealis* to insect herbivory. *Oecologia*, *109*, 368–373.
- Zvereva, E. L., & Rank, N. E. (2003). Host plant effects on parasitoid attack on the leaf beetle *Chrysomela lapponica*. *Oecologia*, *135*, 258–267.