

APPLICATION OF FOURIER INFRARED SPECTROSCOPY METHOD FOR QUANTIFICATION OF FOLIAR LITTER COMPOSITION

Iona Skranda, Aldis Butlers

Latvian State Forest Research Institute "Silava", Latvia
ilona.skranda@silava.lv, aldiss.butlers@silava.lv

Abstract. Foliar litter has an important role in the nutrient cycle of the forest ecosystem. Various litter fractions have different chemical composition and decomposition rate, which determines the dynamics of soil nutrient input. In addition, the proportion of litter fractions varies within the year and inter-annually depending on both biotic and abiotic factors. Therefore, knowledge of the composition of litter fractions is important for the accurate modelling of nutrient cycling. This study aimed to evaluate the application of the Fourier transform infrared spectroscopy (FTIR) method for the quantification of litter fractions to increase the efficiency of acquiring such data by avoiding the need for manual sorting of litter fractions. Mid-infrared diffuse reflectance Fourier transform spectrometer (MIR-DRIFTS) was calibrated and the elaborated quantification methods were validated by using 152 fine-milled foliar litter samples with a known composition of four fractions: pine needles; all needles and leaves; twigs; rest of litter, including seeds, flowers, and insects. Samples used were collected at three Scots pine (*Pinus sylvestris* L.) dominated stands over eight years. The performance of elaborated MIR-DRIFTS methods was evaluated by estimating bias from reference value and standard deviation of results obtained. The estimated combined uncertainty of litter fraction quantification results by the MIR-DRIFTS method is in a range from 12% to 15%.

Keywords: FTIR, diffuse reflectance, litterfall fractioning.

Introduction

Foliar litterfall plays an important role in the biogeochemical cycle impacting the amount of elements and organic matter that are returned to the soil each year, thereby significantly affecting nutrient availability in the soil and forest productivity [1]. To estimate soil influx of various elements by litter decomposition it is necessary to consider both the chemical composition and quantity of the litter. Different litter fractions have different qualitative and quantitative properties [2], including rates of decomposition, e.g. cones and twigs have the slowest breakdown due to lignified parts [3]. Therefore, it is beneficial to assess not only the total biomass but also to investigate various fractions of litter in the studies of the forest ecosystem aimed to gain deeper insight into element cycling. Another important variable of the canopy used in various studies is the stand leaf area index. However, to accurately measure the total leaf area produced by deciduous species and determine the contribution of each species to the overall amount, collecting litterfall throughout the year and sorting it by species is likely the most precise method [4]. Thereby, utility of the information on litter fractions is certain, however, acquiring such data can be hindered due to laborious work of manual litter sorting.

Fourier transform infrared spectroscopy (FTIR) can be used to investigate and link spectral properties with characteristics of foliar litter [5]. Various previous studies have provided evidence that FTIR in combination with multivariate statistics can be an applicable method for the identification and characterisation of various biomass materials indicating a potential of the suitability of the method to study also foliar litter composition. FTIR has been demonstrated to be a feasible method for rapid, cost-effective and non-destructive method for evaluation of the chemical composition of lignin, cellulose and hemicellulose in juvenile and mature wood of Scots pine [6] and other tree species [7; 8]. It is found that FTIR spectroscopy is able to not only recognize various tree species but also differentiate wood samples based on their growth conditions [9; 10] and provide biochemical profiles in spectra acquired usable for the chemotaxonomy of flowering plants [11]. The capabilities of the FTIR method have been discovered also in obtaining information from samples with complex matrices that contain a variety of materials, for example, to distinguish the woody materials from which the pellet is composed [12], as well as to quantify the composition of plant functional types and species in composite root samples [13; 14].

This study aimed to evaluate the application of mid-infrared diffuse reflectance Fourier transform spectroscopy (MIR-DRIFTS) for quantification of different fractions (pine needles; all needles and leaves; twigs; rest of litter, including seeds, fruits and flowers) composition in a foliar litter of Scots pine (*Pinus sylvestris* L.) dominated forests.

Materials and methods

In this study, a Bruker Invenios-S spectrometer equipped with an HTS-XT accessory was utilized. Calibration and validation of the litter fraction mass proportion prediction models were performed by Bruker OPUS software using air-dried litter reference samples with known fractional composition.

Litter samples used were collected from three Level II monitoring sites of Scots pine forests (*Myrtillosa* forest site type) located in Valgunde, Rucava and Taurene typical to hemiboreal conditions in Latvia [15]. Sample collection was conducted monthly between 2014 and 2021 in the scope of the International Cooperative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests). Annually collected samples were pooled by site and manually sorted by four fractions: pine needles; all needles and leaves; twigs; rest of litter, including seeds, flowers, and insects (henceforth referred to as the Other fraction). Sorted and fine-milled fractions representing the corresponding year and site were used to acquire eight samples with known fraction composition that was assigned randomly to avoid the effects of collinearity. As a result, 152 reference samples with known litter fraction composition were acquired.

Each reference sample was scanned by the spectrometer in three replicates using separate subsamples. Reference values for litter fractions were assigned to the corresponding spectra, and the acquired spectra library was divided into calibration and validation datasets in an 80:20 ratio, respectively. Multivariate calibration was performed to create litter composition prediction models by using partial least squares (PLS) regression analysis integrated into OPUS software by Bruker. Multiple attempts of calibration procedure were performed for each of the litter fractions to acquire various versions of the models predicting mass proportion (%) in the composite litter sample. To evaluate the performance of the prediction models, we used them to predict the composition of litter fractions using spectra from the validation dataset. The acquired results of each model version were then compared to the corresponding reference values to determine a correlation coefficient (r), root mean square error of prediction (RMSEP), mean value of deviation (Bias), standard error of prediction (SEP), residual prediction deviation as ratio of standard deviation to standard error of prediction (RPD). Model version for each of the predicted fraction with the lowest RMSEP and highest RPD was selected for further evaluation. Prediction results acquired by the selected models were corrected by linear regression equation showing a relationship between predicted and reference values of fraction proportion. Finally, the uncertainty of the elaborated litter fraction composition prediction methods was expressed by combining the accuracy and precision of the corrected prediction results, expressed as the root mean square bias from the reference value (RMSbias) and standard deviation (SD) of prediction result replicates, respectively. The quality indicators are expressed in the same unit as the predicted fraction proportion (as a percentage), when applicable.

Results and discussion

The highest quality litter fraction proportion prediction models, as indicated by the validation procedure, have a RMSEP ranging from 14.7 to 16.6%, depending on the fraction predicted (Table 1). Results acquired by these models have a strong correlation (r from 0.67 to 0.95) with the reference values of litter fraction proportion in the validation sample set. According to previous studies of infrared spectroscopy on biomass materials, prediction models having RPD values exceeding 1.5 are acceptable for screening, whereas those having RPD values between 2.0 and 2.5 have very good prediction power [16]. Our study demonstrates the limited capability of the FTIR spectroscopy method to accurately predict the proportion of the Other fraction, as evidenced by the RPD value of 1.1. While for fractions of all needles and leaves, pine needles and twigs RPD value ranging from 1.6 to 1.9 can be interpreted as an indicator for good prediction power of models developed.

Table 1

Quality indicators of prediction model versions with the highest prediction power

Fraction	r	RMSEP	Bias	SEP	RPD
All needles, leaves	0.94	15.8	7.5	13.9	1.7
Pine needles	0.95	14.7	7.9	12.8	1.9
Twigs	0.93	16.6	2.5	16.5	1.6
Other	0.67	16.5	5.3	15.7	1.1

The results of the linear regression analysis shown in Figure 1 indicate that applying the slope and intercept coefficients to the prediction results can reduce bias for pine needles and twigs fractions, as well as for all needles and leaves. However, for the Other fraction, a relatively larger data dispersion is observed, illustrating difficulties in predicting its proportion. This is also reflected in the RPD value.

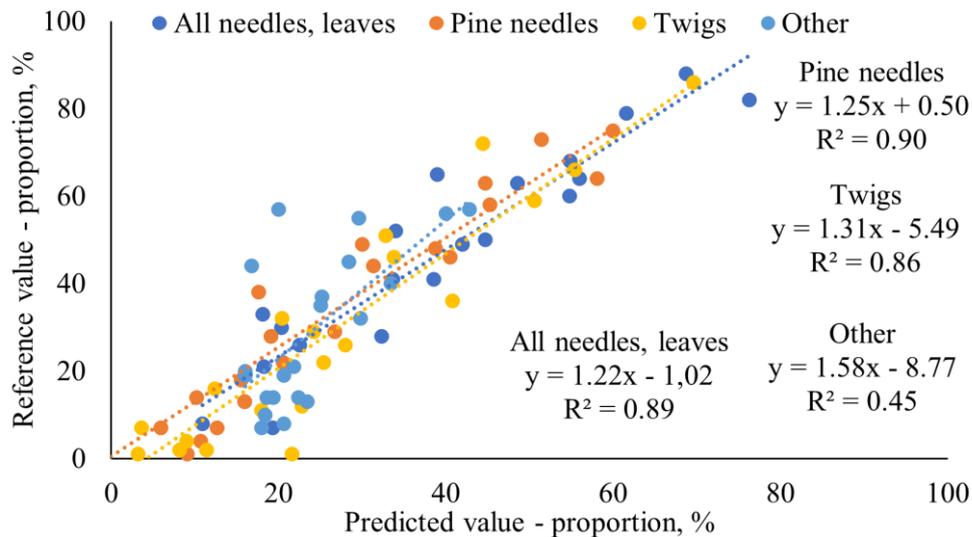


Fig. 1. Relationship between reference values and predicted values of the fraction proportion

RMSbias of prediction results before the correction was 14% for the Other fraction and ranged from 11% to 12% for the rest of the fractions. However, after applying slope and intercept coefficients to correct the results, the RMSbias reduced to 13% for the Other fraction and to a range of 8% to 10% for the remaining fractions (Fig. 2). Following the correction, the bias of proportion prediction for all needle and leaves fractions, and to pine needle and twig fractions, ranges from -19% to 22%. While, for the Other fraction, the bias ranges from -34% to 16%.

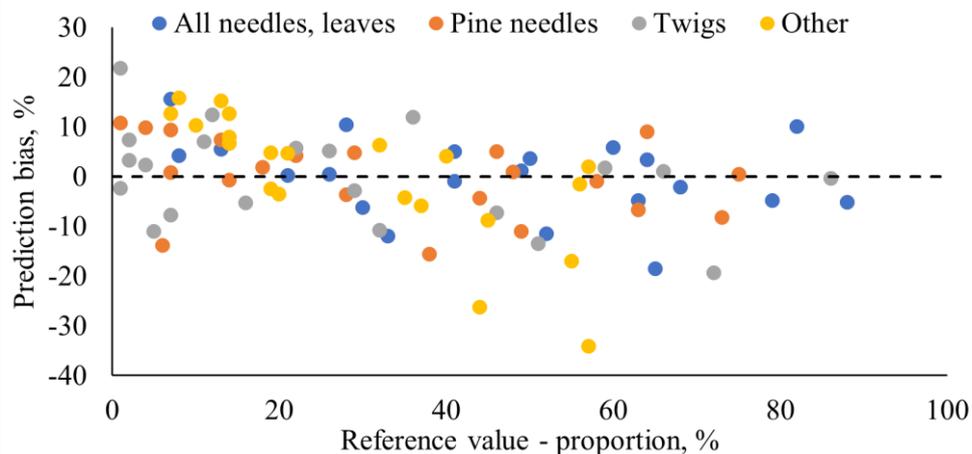


Fig. 2. Bias of corrected results of the prediction

Evaluation of prediction precision shows that the mean standard deviation of prediction results ranges from 7% to 12%, while minimum and maximum values of standard deviation range from 1% to 28% (Fig. 3). Slightly better prediction repeatability is achieved for the Other fraction than for the rest of fractions. As the comparability of spectra acquired by scanning different subsamples is likely related to the homogeneity of the sample, it highlights that poor prediction power for the Other fraction spectra is likely related to rather a sample matrix than introduced by sample preparation. The complexity of the Other fraction matrix can be attributed to the inclusion of various materials such as lichens, insects, and moss, as well as the addition of fine residues from other fractions. These factors have the potential to introduce errors in the calibration process.

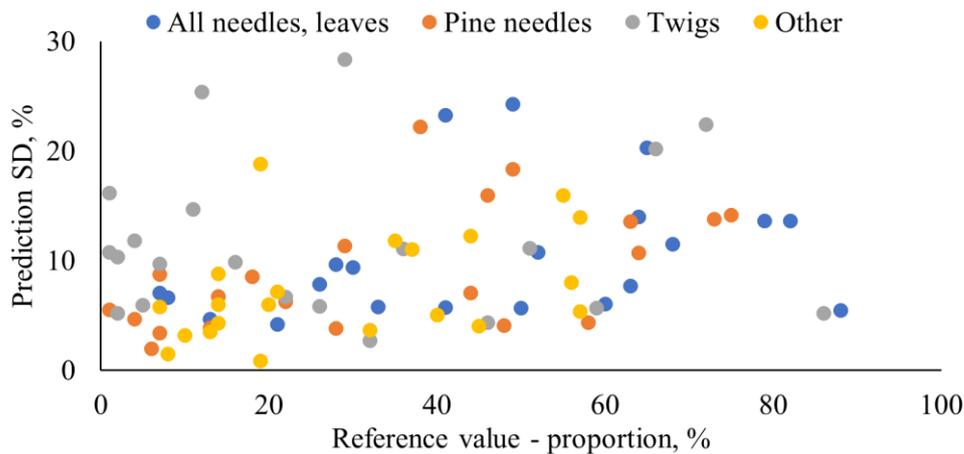


Fig. 3. Standard deviation of the prediction replicates

The results of prediction uncertainty estimation show that a higher prediction bias of a fraction's proportion does not necessarily imply also a relatively higher standard deviation. Therefore, the combined uncertainty of 15% estimated for the Other fraction does not differ significantly from the uncertainty of the proportion prediction of the other fractions, which range between 12% and 15% (Table 2). This is because the Other fraction had relatively better prediction repeatability.

Table 2

Correction coefficients and quality indicators of the corrected prediction results

Fraction	Slope	Intercept	RMSbias	SD	Combined uncertainty
All needles, leaves	1.22	-1.02	8	10	13
Pine needles	1.25	+ 0.50	8	9	12
Twigs	1.31	-5.49	10	12	15
Other	1.58	-8.77	13	7	15

The study results demonstrate that MIR-FTIR spectroscopy can be used for the quantification of litter fractions in fine milled foliar litter samples. Furthermore, considering the complexity of the sample matrix evaluated in this study, the prediction power of analysing samples could be further improved, as the sample size used in this study was small. In our study, we reached RMSEP of around 16%. A similar study aimed at developing FTIR spectroscopy models for predicting mass proportion in composite materials containing roots of various species and dimensions, representing within-site and between-site variation, could achieve better prediction power for root species with higher within-species heterogeneity. That study found the RMSE ranged from 1% to 11% for discriminating roots of graminoids, forbs, ferns, and woody roots. However, for different types of woody roots, the RMSE was higher, at 20% [13]. A similar observation is made in another study concluding that biomass samples of the same species can provide variable spectra as the chemical composition of the sample can be affected by the growing conditions even if the plants examined are genetically identical [17]. This demonstrates the influence of the diversity of properties of a specific fraction of biomass composite material on the accuracy of quantifying their proportion. In our study, we used pooled litter material from three sites with similar conditions to create artificial different proportion mixtures for both calibration and validation of the prediction models. Therefore, although the evaluation of the litter fraction proportion prediction power should be consistent when applying the method to samples collected from the same or similar Scots pine-dominated stands, as we accounted for some within-fraction spectral variation by creating artificial mixtures from samples collected in different years, the performance may decline if unknown samples collected under different conditions are used. Prediction power could be increased by including pure litter fractions in the calibration procedure to cover more of the natural variation of different fraction properties [13]. To apply the elaborated method to a wider range of samples, a validation procedure should be conducted using samples from sites of interest, or the calibration models

should be improved by incorporating more samples that are similar to the unknown samples intended for analysis.

Conclusions

1. MIR-FTIR spectroscopy is a promising method for accurately quantifying the composition of foliar litter fractions. With a good fraction composition prediction performance, as indicated by RPD values ranging from 1.6 to 1.9, this method has shown potential for accurately predicting the composition of fractions such as all needles and leaves, pine needles, or twigs.
2. The quantification of the proportion of fraction consisting of fine litterfall material, such as seeds, flowers, and insects, showed poor prediction power, with an RPD value of 1.1, highlighting that the success of using MIR-FTIR spectroscopy to quantify the composition of foliar litter fractions is dependent on the level of variation in their properties and corresponding infrared spectra.

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Author contributions

Conceptualization and methodology, A.B.; spectra acquiring, I.S.; calibration and validation, A.B. and I.S.; writing – original draft preparation, A.B and I.S.; writing – review and editing, A.B and I.S. All authors have read and agreed to the published version of the manuscript.

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