Contents lists available at ScienceDirect

### Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

# Assessment of the species composition of forest floor horizons in mixed spruce-beech stands by Near Infrared Reflectance Spectroscopy (NIRS)

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#### ARTICLE INFO

Article history: Received 24 August 2009 Received in revised form 10 March 2010 Accepted 12 March 2010 Available online 15 April 2010

Keywords: Forest floor Mixed-species forests Beech Spruce NIRS

#### ABSTRACT

How the mixture of tree species modifies short-term decomposition has been well documented using litterbag studies. However, how litter of different tree species interact in the long-term is obscured by our inability to visually recognize the species identity of residual decomposition products in the two most decomposed layers of the forest floor (i.e. the Oe and Oa layers respectively). To overcome this problem, we used Near Infrared Reflectance Spectroscopy (NIRS) to determine indirectly the species composition of forest floor layers. For this purpose, controlled mixtures of increasing complexity comprising beech and spruce foliage materials at various stages of decomposition from sites differing in soil acid-base status were created. In addition to the controlled mixtures, natural mixtures of litterfall from mixed stands were used to develop prediction models. Following a calibration/validation procedure, the best regression models to predict the actual species proportion from spectral properties were selected for each tree species based on the highest coefficient of determination ( $R^2$ ) and the lowest root mean square error of prediction (RMSEP). For the validation, the  $R^2$  (predictions versus true proportions) were 0.95 and 0.94 for both beech and spruce components in mixtures of materials at all stages of decomposition from the gradient of sites. The  $R^2$  decreased only marginally by 0.04 when models were tested on independent samples of similar composition. The best models were used to predict the beech-spruce proportion in Oe and Oa layers of unknown composition. They provided in most cases plausible predictions when compared to the composition of the canopy above the sampling points. Thus, tedious and potentially erroneous hand sorting of forest floor layers may be replaced by the use of NIRS models to determine species composition, even at late stages of decomposition.

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#### 1. Introduction

Forest floor properties such as structure, mass, and composition are very important for forests productivity and sustainability. These properties are highly variable in space (Yanai et al., 2003), especially in multiple-species forests. In addition to site conditions, the composition of forest canopies determines the amount and composition of litterfall (Prescott, 2002) and hence forest floor properties. Tree species composition influences forest floor mass through litterfall quantity and quality (Binkley and Giardina, 1998), litter decomposition rates (Swift et al., 1979; Aerts, 1997; Gartner and Cardon, 2004), quantities of nutrients cycled and their resulting availability (Prescott, 2002), soil microbial activity and composition (Brandtberg and Lundkvist, 2004), as well as the quantity and quality of soil organic carbon (Van Miegroet et al., 2005; Berg and Laskowski, 2006). In multi-species forests, the spatial heterogeneity in forest floor properties is very high (Aubert et al., 2006). Changes in forest floor species composition, both in vertical as well as horizontal direction, may be important for C storage in forest floors, nutrient cycling and small-scale soil biodiversity.

Increasing the proportion of broad-leaf species in coniferous forests is often thought to reduce forest floor mass through the addition of nutrient rich and faster decomposing leaf litter. However, leaf litter of broadleaved species may decay faster than that of conifers only for a short initial period (Prescott et al., 2000). In addition, broad-leaf tree species may produce as much or more humus, which can be regarded as the residue of litter decomposition, as conifers under comparable conditions (Berg and Eckbohm, 1991; Berg et al., 1996; Giardina et al., 2001). This is expressed in the limit value, a measure for the cumulative mass loss of plant litter until the decomposition has become very slow (e.g. rate of or lower than  $10^{-4}$ % per day, Coûteaux et al., 1998), hence the remaining material has become humus (Berg et al., 2003). Limit values of 74%





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and 59% have been reported for Norway spruce and European beech (Berg and Johansson, 1998), indicating that litter decomposition may be less complete in broadleaves than in conifers. Owing to the fact that litter of different species decomposes at different rates at different phases of the process and to an overall different degree, it is likely that, tree species composition differs in vertical direction between the different layers of the forest floor, which represent these different phases. For instance, the proportion of species A with easily decomposing litter and low residual mass declines with depth in the forest floor of a mixed-species stand, whereas the proportion of species B with slowly decomposing litter and high residual mass increases with depth. In situations, where initially easily decomposable litter of one species has a high residual mass (low limit value) and vice versa initially slowly decomposing litter of another species has little residual mass, there may be little or no net effect on the composition of the entire forest floor, while there may still be different proportions in the different forest floor layers. However, possible changes in species composition in forest floor profiles have not been well studied (Berg and Laskowski, 2006). To our knowledge, there is no study investigating whether or not the species composition forest floors differs among the Oi, Oe and Oa forest floor layers.

Forest floor classification keys commonly describe at least three layers (see Babel, 1971; Brêthes et al., 1995; Green et al., 1993; Arbeitskreis Standortskartierung, 2003; van Delft, 2004): (1) the Oi layer composed mostly of unaltered leaves or needles, (2) the Oe layer comprising fragments of leaves and needles with between 30 and 70% of fine substances, and (3) the Oa layer comprising organic materials (at least 70%) of unrecognizable origin (i.e. fine substances).

The quantification of species proportions in Oi-layer material is commonly carried out by hand sorting. This is impossible for the Oe- and Oa-layer materials. The time required to clean and weigh mixed forest floor material by species origin is very high. Moreover, the number of samples to process in order to get a good estimate of the species composition of the forest floor at the stand level is enormous. Therefore, new methods are required to replace tedious hand sorting and morphological examinations of mixed-species forest floors.

The determination of species origin and chemical composition of organic matter is a field in which Near Infrared Reflectance Spectroscopy (NIRS) has proven to be useful (see e.g. McLellan et al., 1991; Joffre et al., 1992; Coûteaux et al., 1998; Coûteaux et al., 2005; Kong et al., 2005; Ono et al., 2008). Near Infrared Reflectance Spectroscopy is an indirect and empirical technique routinely used in the food and chemical industry and in agricultural science for its rapidity and its suitability for analysing many constituents simultaneously (Osborne, 2001). Multivariate regression statistics are necessary to establish NIRS prediction models in order to relate spectral data to reference values prior to routine analysis. For example, NIRS has already been used to estimate the species composition and the chemical composition of husbandry animals' faeces (Decruyenaere

et al., 2003; Smith et al., 2001), the legume content in multispecies legume—grass mixtures (Locher et al., 2005), or the chemical composition of wine and its geographical origin (Liu et al., 2006; Cozzolino et al., 2008). However, to our knowledge, the quantification of the species composition of forest floors via NIRS has not been carried out before. Contrary to the most commonly accepted description of humic substances as macromolecules, Piccolo (2001) proposed that humic substances are spontaneous, random associations via weak bonds of small molecular weight organic molecules. Therefore, humic substances produced under different tree species might carry contrasting (species-specific) chemical properties. These may be discerned by their spectral properties using NIRS.

This study aimed at determining the species composition of mixed-species forest floor layers collected in spruce-beech forests using NIRS. We tested whether NIRS can replace the hand sorting procedure commonly used to determine the species composition of organic horizons.

The rationale behind these objectives is based on the observation that tree species growing on the same soil produce litter differing considerably in chemical and physical properties (Swift et al., 1979; Binkley and Giardina, 1998). Based on the above, we hypothesized that:

- leaf and needle litter from European beech and Norway spruce differ in chemical composition, thus in spectral properties, as well as that they differ from those of soil material and organic matter other than beech leaves and spruce needles. Therefore, the use of controlled mixtures of these materials is suitable to determine the species origin of natural forest floor layers using NIRS.
- 2. spruce needles produce more recalcitrant products of decomposition than beech and therefore spruce derived products of decomposition increase from the Oi to the Oe to the Oa forest floor layer, whereas beech derived decomposition products decrease.
- 3. the accuracy of NIRS predictions declines with increasing decomposition stage, since the litter of both species is processed, at least in part, by the same organisms, thus producing similar metabolites and residues regardless of the initial species origin of litter.

#### 2. Materials and methods

#### 2.1. Study sites

Nine study sites, all located in south-west Germany, were selected as to represent a wide range of soil conditions regarding pH and base saturation for spruce and beech (Table 1). Three sites were on limestone (Late Jurassic/Malm) at an elevation of 900 m a.s.l. in the Swabian Alb region (centred around 47°57′56N, 8°40′47E).

Table 1	
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Some site and stand characteristics at the study sites.

Site	Elevation (m)	Temperature (°C)	Precipitation (mm)	Soil type <sup>a</sup>	Basal area beech (m <sup>2</sup> ha <sup>-1</sup> )	Basal area spruce (m <sup>2</sup> ha <sup>-1</sup> )
S1	910	5-6	1080-1260	Inceptisol lithic eutrudept	15	27
S2	910	5-6	1080-1260	Inceptisol lithic eutrudept	14	17
S3	900	5-6	1080-1260	Inceptisol lithic eutrudept	14	17
S4	550-580	8-9	1080-1260	Inceptisol typic dystrudept	19	19
S5	700-750	8-9	1080-1260	Inceptisol typic dystrudept	12	32
S6	690-760	8-9	1080-1260	Inceptisol typic dystrudept	26	32
S7	390	8-9	900-1080	Inceptisol typic dystrudept	10	27
S8	790-810	7-8	1260-1440	Inceptisol typic dystrudept	11	32
S9	700-710	7-8	900-1080	Inceptisol typic dystrudept	18	34

<sup>a</sup> Soil type determination is based on the US soil taxonomy (Soil Survey Staff, 1999).

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