

# Predicting Tree Species Origin of Soil Organic Carbon with Near-Infrared Reflectance Spectroscopy

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Near-infrared reflectance spectroscopy (NIRS) and partial least squares regression were used to develop prediction models for identifying the species of origin of soil organic C (SOC) in semiarid montane forests of quaking aspen (*Populus tremuloides* Michx.) and mixed conifers in Utah. Artificial mixtures of mineral soils (0–15 cm) sampled under pure aspen and pure conifer cover ( $n = 415$ ) at four locations were divided into a calibration-validation set ( $n = 265$ ) for model development and an independent validation set ( $n = 150$ ) to test model robustness. Models in the 10,000 to 4000  $\text{cm}^{-1}$  spectral region were developed separately with original soil spectra (OS) and organic matter spectra (OM) using the full and truncated (10th–90th percentile) sample sets. The OS models performed better than OM models, and the best OS models showed good prediction ability at the validation step, with  $R^2 = 76\%$ , ratio of standard deviation of reference value to standard error of prediction (RPD) = 2.1 for aspen SOC, and  $R^2 = 74\%$ , RPD = 2.0 for conifer SOC. Model performance decreased at independent validation ( $R^2 = 33\text{--}49\%$ , RPD = 1.2–1.6), probably due to unaccounted variability of site-specific factors in SOC chemical composition within and among aspen and conifer soils. Current models are still somewhat limited for accurately predicting contributions of aspen vs. conifers in independent samples. More detailed site information, such as texture, mineralogy, geology, and land use history is needed to improve models so that they can be used to provide insight into SOC properties changes along a continuum of aspen to conifer forests in the western United States.

Abbreviations: CM, Cedar Mountain; C-V, calibration-validation; DLL, Deseret Land and Livestock; FB, Franklin Basin; IV, independent validation; MM, mineral matrix spectra; NIRS, near-infrared reflectance spectroscopy; OS, original spectra; OM, organic matter spectra; PLSR, partial least squares regression; RMSEP, root mean square error of prediction; RPD, ratio of standard deviation of reference value to standard error of prediction; SOC, soil organic carbon; TOC, total organic carbon; TWDEF, T.W. Daniel Experimental Forest.

Quaking aspen is a major species in montane ecosystems of the semiarid region of western North America, occurring predominantly as a pioneer species that is replaced by conifers in later stages of succession (Mueggler, 1985). Fire suppression and ungulate browsing is believed to have caused a loss of aspen cover during the last century (Bartos and Campbell, 1998). Although changes in aspen cover may be within the range of historical fluctuation (Kulakowski et al., 2004), a shift toward coniferous species may modify soil physical, chemical, and biological properties (Ayres et al., 2009), including soil organic C (SOC) dynamics and  $\text{CO}_2$  emissions into the atmosphere.

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Previous studies in montane forests of western North America have found that aspen stands store more SOC in the first 60 cm of the mineral soil than adjacent conifer stands (Woldeselassie et al., 2012). The results further indicated that SOC in aspen soils has slower microbial turnover than conifer soils (i.e., more stable SOC) and that aspen have a higher proportion of SOC associated with mineral surfaces (Woldeselassie et al., 2012), which increases the residence time of SOC (von Lützow et al., 2007). Differences in input, litter chemical composition, and environmental soil conditions following conifer encroachment can also affect SOC dynamics (Olsen and Van Mieghroet, 2010; Woldeselassie et al., 2012). Soil organic C storage and its properties under mixed stands may not necessarily be predicted through linear interpolation between SOC contents and the properties of pure aspen and conifer stands. Being able to distinguish the vegetation type legacy on SOC (i.e., the contribution of aspen- and conifer-derived portions of SOC) would greatly contribute to our understanding of changes in SOC storage and dynamics as aspen transitions to mixed aspen–conifer forests. This challenge may be addressed using near-infrared reflectance spectroscopy (NIRS). To our knowledge, no study has used NIRS to distinguish the relative contributions of tree species belonging to different forest types (broadleaved vs. conifers) to SOC in the mineral soil.

Near-infrared reflectance spectroscopy is an empirical, non-destructive, inexpensive, and rapid technique that is commonly used in the food and chemical industries and agricultural science to simultaneously determine the concentration of various organic and inorganic components. Therefore, NIRS may be an appropriate technique for analyzing chemically heterogeneous SOC. In soil science, NIRS has been applied to predict organic C and N concentrations in agricultural soils (Dalal and Henry, 1986; Morra et al., 1991), the relative abundance of functional groups (Terhoeven-Urselmans et al., 2006), the concentrations of C, N, and P in litter at different stages of decomposition (Gillon et al., 1999), or SOC fractions (Coûteaux et al., 2003; Cozzolino and Morón, 2006). Near-infrared reflectance spectroscopy is based on the absorption of infrared radiation (800–2500 nm) by C–H, N–H, and O–H bonds (Foley et al., 1998) as found in organic and inorganic constituents of plant and soil materials. Thus, the near-infrared reflectance (NIR) spectrum of a material can be interpreted as the overall chemical composition of the soil organic matter (Palmborg and Nordgren, 1996; Coûteaux et al., 2003). When NIRS is combined with chemometrics, it is possible to develop prediction models for NIR-active constituents of known concentrations. As opposed to the characterization of individual compounds by wet chemical analyses, NIRS permits the determination of the chemical composition of heterogeneous samples and does not produce chemical wastes (Cozzolino and Morón, 2006). Gruselle and Bauhus (2010) used NIRS successfully to predict the species of origin of the forest floor in mixtures of European beech (*Fagus sylvatica* L.) and Norway spruce [*Picea abies* (L.) Karst.]. Few studies have used NIRS to distinguish the vegetation origin of

organic matter in mineral soils. Mineral soil is defined in this study as soil material distinct from the O horizon and litter and containing <20% (w/w) of SOC (Soil Survey Staff, 2010) and will hereafter be referred to as *soils*. Coûteaux et al. (2003) were able to predict  $^{13}\text{C}$  and  $^{15}\text{N}$  derived from labeled wheat (*Triticum aestivum* L.) straw with NIRS models 3 yr after the straw was added to coniferous forest soils. Michel and Ludwig (2010) used NIRS models to predict C derived from  $\text{C}_3$  and  $\text{C}_4$  plants in pools from the RothC model. Ertlen et al. (2010) used NIRS to discriminate soils originated under grassland or forests. These studies indicate that NIRS can be used to differentiate the origin of SOC by land use type and plants differing in metabolic pathways. Moreover, they invite the hypothesis that NIR spectra can reflect the types of vegetation and their relative contribution to the SOC concentration in the mineral soil where components of plant (aboveground and belowground) litter have been recycled into microbial biomass and/or result from advanced decomposition.

We investigated whether NIRS and chemometrics can be used to predict the concentration of SOC in soil derived from aspen and coniferous species using soils sampled directly under aspen and conifer canopies at different locations in Utah. We further wanted to test whether the legacy of vegetation on SOC could be predicted in the presence of the mineral matrix of the soil or whether it was necessary to remove the influence of the mineral matrix on NIR spectra before NIRS model development.

## MATERIALS AND METHODS

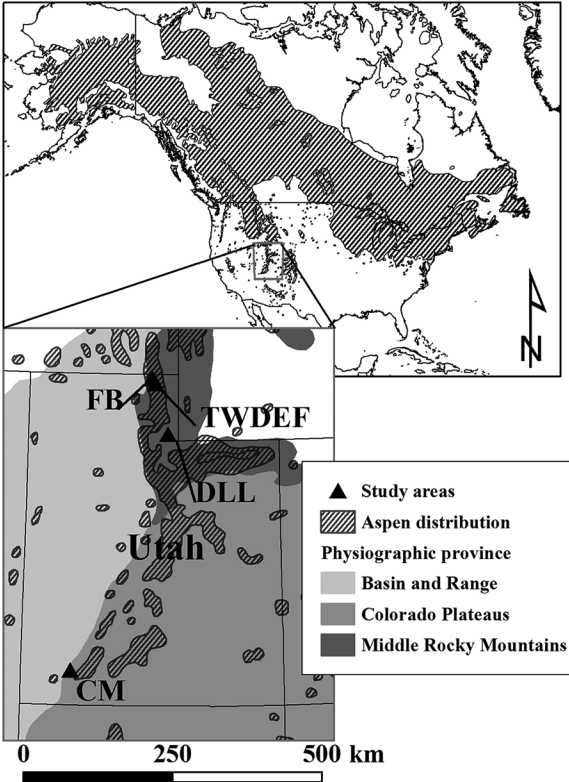
### Study Areas and Land Use History

Four study areas located in northern Utah (Franklin Basin [FB], T.W. Daniel Experimental Forest [TWDEF], and Deseret Land and Livestock [DLL]) and in southern Utah (Cedar Mountain [CM]) were sampled between 2007 and 2011 to capture the broad range of physical settings encompassed by aspen (Fig. 1). The FB, TWDEF, and DLL sites are located in the physiographic province of the Middle Rocky Mountains and CM is located on the Kolob Terrace, within the Colorado Plateau (Fig. 1) (Fenneman and Johnson, 1946). These are montane or subalpine ecosystems, with elevations ranging from 1770 to 3200 m (Table 1). The climate is characterized by cold winters and hot, dry summers. Annual precipitation across the sites ranges between 812 and 1197 mm (Table 1), decreasing from north to south. Precipitation occurs mainly as snow. Average temperatures of the hottest month are fairly similar across all study areas, ranging between 14.0 and 16.4°C (Table 1). Average temperatures of the coldest month decrease toward the north (Table 1), ranging from −3.8°C at CM to −10.0°C at TWDEF. The geology differs somewhat across the study areas: soils in CM developed mainly on sedimentary (shale, sandstone, or limestone) and igneous rock (basalt, basic or intermediate igneous rock) (Soil Survey Staff, 2014); at TWDEF and DLL, the parent material is derived from Wasatch conglomerate (Woldeselassie et al., 2012); and at FB, soils developed on sedimentary rock (limestone or quartz-

ite sandstone) (Kusbach, 2010). In our study areas, aspen was present as large pure stands or in patches embedded in mountain meadows, shrublands, or conifer forests. In mixtures, aspen was associated with a variety of coniferous species (Table 1). The understory vegetation under aspen stands commonly consists of diverse grasses, forbs [*Delphinium × occidentale* (S. Watson) S. Watson or *Achillea millefolium* L.], legumes (*Lupinus* spp.), and shrubs (*Symphoricarpos oreophilus* A. Gray, *Ribes* spp.), and are denser than under conifer stands, which often have bare soils or sparse grasses, forbs, and shrubs.

Complete soil profile descriptions for the plots at DLL and TWDEF can be found in Woldelessie et al. (2012). Soil profile descriptions were not available for the sites in CM and FB. However, Kusbach (2010) described several other soil pedons under aspen and conifer stands at FB, and as reported by Woldelessie et al. (2012), soils under aspen generally have a thick and pronounced A horizon (~30–50 cm) and are classified as Mollisols. Conifer soils have a shallower and lighter A horizon (~5–30 cm) and are commonly classified as Alfisols but also as Entisols or Inceptisols (Table 1).

Documentation on land use for the western United States is anecdotal before the 1900s, and for most of the 20th century there is a paucity of land use cartography for our study areas; thus, information on historical vegetation had to be derived from the literature. Rogers et al. (2011) characterized the transition in aspen communities in the last 150 yr in the Bear River Range, where FB and TWDEF are located, as being dominated by mixed and conifer stands in the early 1800s, with subsequent expansion of pure aspen stands during the end of the 19th century due to a shift in climatic conditions coupled with disturbances associated with the European settlement (e.g., timber extraction, sheep grazing, high-intensity fires). During the 20th century, fire suppression, cattle and sheep grazing, and a moist climate contributed to the natural succession toward mixed and conifer stands (Rogers et al.,



**Fig. 1. Location of study areas in Utah relative to the physiographic provinces defined by Fenneman and Johnson (1946) and aspen habitat distribution by Little (1971): Franklin Basin (FB), T.W. Daniel Experimental Forest (TWDEF), Deseret Land and Livestock (DLL), and Cedar Mountain (CM).**

2011). Similarly, grazing and intensive logging of accessible conifer stands were the main land uses in DLL in the late 1800s and early 1900s. Aspen communities at CM are presumably stable stands that self-regenerate continuously or through gap-phase regeneration (Kurzel et al., 2007), while conifer stands are found in the edges of the plateau and northern slopes. Intensive sheep graz-

**Table 1. General characteristics of the study areas.**

Study area†	Latitude	Longitude	Elevation range	Mean annual precipitation	MMT‡	mMT§	Common soil orders	Coniferous species¶	Sources
			m	mm	— °C —				
FB	41°56' N	111° 34' W	1770–3030	1197	16.4	–6.9	Mollisols and Alfisols	subalpine fir Douglas-fir limber pine	Kusbach (2010) NRCS (2013)
TWDEF	41°51' N	111°30' W	2600	950	14	–10	Mollisols and Alfisols	Engelmann spruce subalpine fir	Olsen and Van Miegroet (2010) Woldelessie et al. (2012)
DLL	41°8' N	111°14' W	1889–2700	910	16	–5	Mollisols, Entisols, Aridisols and Inceptisols	subalpine fir Douglas-fir	Woldelessie (2009) NRCS (2013)
CM	37°31' N	113°8' W	1800–3200	812	15.5	–3.8	Mollisols and Alfisols	subalpine fir Douglas-fir white fir	McNab and Avers (1994) Evans (2010) Rogers et al. (2010) NRCS (2013)

† FB, Franklin Basin; TWDEF, T.W. Daniel Experimental Forest; DLL, Deseret Land and Livestock; CM, Cedar Mountain.

‡ MMT, maximum mean monthly temperature.

§ mMT, minimum mean monthly temperature.

¶ Subalpine fir [*Abies lasiocarpa* (Hook.) Nutt.], Douglas-fir [*Pseudotsuga menziesii* (Mirbel) Franco], limber pine (*Pinus flexilis* E. James), Engelmann spruce (*Picea engelmannii* Parry ex Engelm.), white fir [*Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr.].

ing since the European settlement may have profoundly modified the structure of aspen stands (Rogers et al., 2010) and caused a shift in understory composition from forb to graminoid domination (Bowns and Bagley, 1986).

## Sampling Design

We used two sampling designs: the first sampling campaign (2007) was done at the plot level; subsequently (2009–2011), points were sampled along transects to capture the influence of a single tree on soil properties under its canopy. In July 2007, a total of six paired plots (20 by 20 m) (designated as plots in Table 2) were located at TWDEF and in two small watersheds named Upper Frost and Bear Canyon at DLL (DLL Frost and DLL Bear hereafter), under either a conifer- or aspen-dominated overstory. Each pair of plots had similar conditions of elevation and slope, and the plots were located between 10 and 100 m from each other (Woldeselassie et al., 2012). After removing the litter layer (when present), five soil cores (5-cm diameter) were

taken to a depth of 15 cm in each plot and combined into one composite sample per plot. Soil sampling was done by depth rather than by horizon, but consisted entirely of the A horizon under aspen and the A horizon with some portion of the underlying B, AB, or E horizon under some conifers (Olsen and Van Miegroet, 2010; Woldeselassie et al., 2012). A second sampling design (designated as transects [T] in Table 2) was applied in the fall of 2009 at three sites (DLL Bear, DLL Frost, and TWDEF) close to the 2007 plots. Three transects were laid out at each location, and within each transect, two soil cores (0–15 cm) were sampled beneath conifer or aspen canopy, after removal of the litter layer, and composited on site. The elevation, slope, and aspect were similar along each transect. In addition, four sites at CM (CM1, CM2, CM57, and CM111) and two sites at FB (FB1 and FB2) were sampled using the transect method in the fall of 2010 and 2011, respectively. In CM and FB, soil cores were taken to a depth of 15 cm and the middle section (5–10 cm) was used in subsequent analyses. Because SOC characteristics

**Table 2. Aspen and conifer soil organic C (SOC) concentrations and texture of end member soils (i.e., aspen and conifer soils) and range of concentrations in the artificial mixtures.**

Study area†	Site‡	Transect or plot	Artificial mixtures no.	End member SOC		Soil texture		Artificial mixture SOC range	
				Aspen	Conifer	Aspen	Conifer	Aspen	Conifer
				— g C kg <sup>-1</sup> soil —				— g C kg <sup>-1</sup> soil —	
CM	CM1	T1	0	64.8	—§	loam	—		
	CM1	T2	0	81.1	—	loam	—		
	CM1	T3	0	74.2	—	loam	—		
	CM2	T1	0	39.2	72.4	silt loam	silt loam		
	CM2	T2	20	41.7	49.6	loam	silty clay loam	4.2–36.6	2.8–42.2
	CM2	T3	30	66.2	84.7	NA¶	clay loam	4.1–53.4	3.4–66.4
	CM57 (IV)	T2	22	53.8	35.9	sandy clay loam	sandy clay loam	2.4–46.3	1.4–31.7
	CM57 (IV)	T3	20	30.2	24.3	loam	loam	2.2–24.1	0.9–19.4
	CM111	T1	20	24.1	17.3	sandy loam	sandy loam	1.8–21.2	0.7–14.9
	CM111	T2	0	14.0	—	sandy loam	—		
FB	CM111	T3	20	10.9	14.1	sandy loam	sandy loam	1.1–8.7	0.6–11.1
	FB1	T1	30	62.2	55.9	loam	clay loam	2.8–49.1	2.5–41.9
	FB1	T2	30	48.6	61.7	silt loam	loam	2.0–39.0	1.6–57.8
	FB1	T3	0	43.1	49.5	loam	silt loam		
	FB2 (IV)	T1	25	70.6	37.8	NA	silty clay loam	3.2–57.7	1.1–31.3
	FB2 (IV)	T2	0	54.6	43.4	silty clay loam	clay loam		
	FB2 (IV)	T3	25	50.6	49.2	silty clay loam	silty clay loam	6.4–42.2	1.8–39.2
DLL	DLL Frost	Plot	40	24.2	37.3	loam	silt loam	1.2–20.6	1.5–33.5
	DLL Frost	T1	0	20.2	19.0	sandy loam	loam		
	DLL Frost	T2	0	38.1	27.5	sandy loam	loam		
	DLL Frost	T3	0	35.4	36.3	loam	loam		
	DLL Bear (IV)	Plot	40	35.4	28.8	loam	loam	1.7–30.3	1.1–25.9
	DLL Bear (IV)	T1	0	43.3	66.1	loam	loam		
	DLL Bear (IV)	T2	0	27.5	25.7	sandy loam	loam		
	DLL Bear (IV)	T3	0	69.2	28.7	loam	sandy loam		
TWDEF	TWDEF	Plot	40	24.3	26.6	sandy clay loam	sandy loam	1.3–20.7	1.0–24.0
	TWDEF	T1	0	43.9	44.3	loam	loam		
	TWDEF	T2	0	46.8	—	loam	loam		
	TWDEF	T3	0	33.7	42.8	clay loam	clay loam		
Total data set			362	10.9–81.1	14.1–84.7			1.1–57.7	0.6–66.4

† CM, Cedar Mountain; FB, Franklin Basin; DLL, Deseret Land and Livestock; TWDEF, T.W. Daniel Experimental Forest.

‡ (IV), sites included in the independent validation set. All other sites were used for calibration–validation.

§ No conifer sample available, due to absence of pure conifer stands at CM1 and processing error of the samples at CM111 and TWDEF.

¶ NA, texture data not available due to insufficient sample.



change with depth (bulk density increases, SOC concentration and particulate organic matter content decrease), we considered the middle section to represent average properties of the entire 0- to 15-cm core (Román Dobarco and Van Miegrout, unpublished data, 2012). We refer to soils sampled under pure aspen or pure conifer canopies from paired plots or transects as end members.

### Sample Preparation and Spectra Measurements

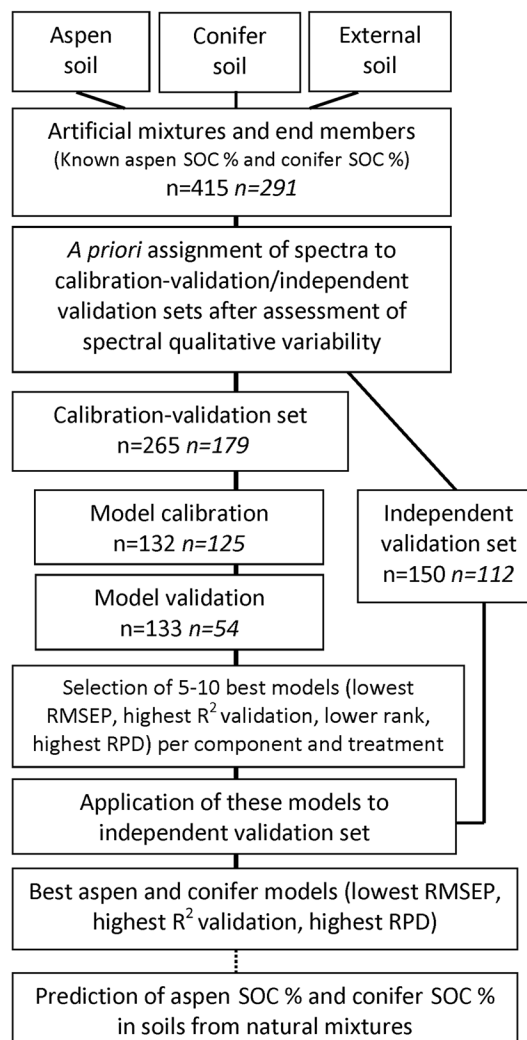
Soils were oven dried at 105°C, sieved through a 2-mm mesh, finely ground with mortar and pestle, and analyzed for total C, inorganic C, and total organic C (TOC) concentrations with a Skalar Primacs Analyzer (Skalar, Inc.). While oven drying may have induced some alterations (e.g., oxidation and loss of volatile organic C) in organic matter configuration, earlier laboratory comparisons between oven-dried and air-dried soils did not indicate changes in total C content (Román Dobarco and Van Miegrout, unpublished data, 2012). The texture of the original soil samples was determined using the pipette method.

Thirteen pairs of end members were used to generate 362 artificial mixtures in the laboratory (Table 2) by mixing known amounts of aspen and conifer soils in a 0 to 100% gradient. A third soil component (TOC = 48.7 g kg<sup>-1</sup> soil), hereafter called *external soil*, from a garden in Neustadt, Germany, was added (0–85% w/w) to avoid autocorrelation typical of simple two-component mixtures (as per Gruselle and Bauhus, 2010). Mixtures were created exclusively within paired plots or a transect to control for parent material and soil texture, although the texture differed somewhat between aspen and conifer soils in some pairs (Table 2). Pure aspen (*n* = 29) and pure conifer (*n* = 24) soil samples were included in the data set for a total of 415 samples (Table 2; Fig. 2). The spectra of end members and artificial mixtures are referred to as original spectra (OS) hereafter. The relative SOC concentration (g C kg<sup>-1</sup> soil) of each vegetation type (aspen or conifer) in the artificial mixtures was calculated as

$$\text{SOC}_{\text{Veg}} = \frac{\text{Weight}_{\text{Veg}} \times \text{TOC}_{\text{Veg}}}{\sum_{i=1}^3 \text{Weight}_{\text{Veg}_i}}$$

where SOC<sub>Veg</sub> is the relative SOC concentration of the vegetation type (aspen or conifer) for which the NIRS models were developed, Weight<sub>Veg</sub> is the weight (g) of a given vegetation type, TOC<sub>Veg</sub> is the C concentration (g kg<sup>-1</sup> soil) of the vegetation type in the source sample, and *i* = 1 to 3 for the three soils (aspen, conifer, and external soil) used in the mixtures.

Organic-free mineral matrix samples were obtained from an aliquot of the original soil samples using a modification of the NaOCl extraction protocol described by Kaiser et al. (2002). Briefly, NaOCl (6%) was added to the soil in a 50:1 (v/w) ratio, and the soil slurry was shaken at room temperature for a total 30 h, replacing the NaOCl two times (after 12 and 24 h). The remaining mineral material was rinsed at least four times with deionized water (44:1 v/w ratio) and centrifuged at 18,000 rpm. The samples were dried at 40°C and ground with mortar and



**Fig. 2. Methodology followed for the development and validation of near-infrared reflectance spectroscopy (NIRS) prediction models (modified from Gruselle and Bauhus, 2010). Sample size of the truncated data set in *italics* (i.e., spectra with reference values between the 10th and 90th percentiles); RMSEP, root mean square error of prediction at validation; RPD, ratio of standard deviation of reference values to standard error of prediction. The dashed line indicates the ultimate goal of NIRS models development.**

pestle (<1 mm) before spectral analysis. This extraction method effectively removes organic matter with minimal effects on the mineral structure, as discussed by Siregar et al. (2005). The spectra of the remaining organic-free mineral material is referred to as the mineral matrix spectra (MM) hereafter.

Near-infrared reflectance measurements and multivariate statistics were performed at the Institute of Silviculture of the University of Freiburg (Freiburg, Germany). Spectra from artificial mixtures, end members, and the mineral matrix were obtained with a Tensor 37 spectrometer (Bruker Optics GmbH). Samples were dried in the oven overnight at 40°C to eliminate any interference of water with the NIRS spectra. Absorbance was measured in 8 cm<sup>-1</sup> intervals across the range 12,000 to 3500 cm<sup>-1</sup> (833–2857 nm). The spectral region 10,000 to 4000 cm<sup>-1</sup> was actually used by the chemometric software OPUS (see details below) for calibration–validation because the regions

outside this range have limited utility in the calibration due to spectral noise (Locher et al., 2005). Five to eight spectra per sample (32 scans per spectrum) were obtained and an average spectrum for each sample was calculated with the software OPUS 6.5 (Bruker Optics GmbH), which is specific to the Tensor 37 spectrometer. Samples were shaken and well mixed between spectra acquisitions to ensure a mean spectrum representative of the sample variability (Gruselle and Bauhus, 2010).

The spectrum of the soil organic matter (OM) was obtained by subtracting the spectrum of the mineral matrix (MM) from that of its corresponding original sample (OS) using the OPUS software. For each pair of end members, an average mineral matrix spectrum was calculated using OPUS, assuming small variability in the mineral matrix of the soil among end member pairs within a given site or transect, and then subtracted from their site-specific artificial mixtures. Spectral subtraction to obtain OM improved NIRS prediction models for total N and N mineralization of soil samples (Russell, 2003) and has also been performed previously in Fourier-transform infrared spectroscopy (Ellerbrock and Kaiser, 2005).

## Near-Infrared Reflectance Spectroscopy Models Development

Prediction models for aspen SOC and conifer SOC in the artificial mixtures were developed with partial least squares regression (PLSR), the most widely used method in chemometrics for multivariate calibration (Dunn et al., 2002; Locher et al., 2005; Cozzolino and Morón, 2006; Peltre et al., 2011). The methodology used to develop aspen SOC and conifer SOC prediction models is shown in Fig. 2 and involves a calibration-validation (C-V) step, followed by an independent validation (IV) step. The division of the whole spectral data set ( $n = 415$ ) into C-V and IV sets was done a priori based on a principal components analysis (PCA) on the raw spectra, which allowed examination of qualitative differences among the sites (as in Cozzolino et al., 2009). This approach was taken to ensure (i) that the environmental variability of all sample locations throughout Utah was represented in the C-V set as well as in the IV set, and (ii) that the largest possible spectral variability was represented in the C-V set. Sites assigned to the C-V set were CM1, CM2, CM111, FB1, DLL Frost, and TWDEF ( $n = 265$ ), while the IV set consisted of CM57, FB2, and DLL Bear ( $n = 150$ ) (Table 2). Screening of the C-V set showed that the concentrations of aspen SOC and conifer SOC were skewed, with more observations at low aspen or conifer SOC concentrations (aspen SOC skewness = 1.8, kurtosis = 3.4; conifer SOC skewness = 1.5, kurtosis = 2.0). Because few observations at higher concentration in the range will have high leverage during calibration, we considered two different data sets: the initial data set ( $n = 415$ ) and a spectral subset (i.e., a truncated data set) with reference values between the 10th and 90th percentiles for each component (aspen SOC and conifer SOC;  $n = 291$ ), with the corresponding C-V and IV sets containing 179 and 112 spectra, respectively. The truncated spectral data set reduced skewness and

was used to develop models for concentration ranges of 2.18 to 36.82 g C kg<sup>-1</sup> soil for aspen and 1.34 to 41.40 g C kg<sup>-1</sup> soil for conifers. The Kendall rank correlation coefficient between aspen SOC and conifer SOC was  $r = -0.18$  for the entire data set and  $r = -0.02$  for the truncated data set used in C-V, supporting the assumption that both components were independent from one another in both data sets.

Different mathematical treatments, embedded in the OPUS software, were systematically applied in the C-V step for both the entire and the truncated data sets. These were applied to normalize the C-V spectra before model calibration. The mathematical treatments were: no spectral preprocessing, straight line subtraction (SLS), vector normalization (VN), first derivative (FD) with 13 smoothing points, FD + SLS, and FD + VN. The SLS treatment causes a tilt in the recorded spectrum (Tripathi and Mishra, 2009), while VN entails mean centering and variance scaling and removes the multiplicative interferences of scatter and particle size, while FD removes the background and increases the spectral resolution (Cen and He, 2007). The full-range spectra within the C-V data set were divided at a 50:50 ratio into spectra for calibration ( $n = 132$ ) vs. validation ( $n = 133$ ) using the PCA technique with the program QUANT embedded in OPUS 6.5. For the truncated data set, 70% of the spectra were used for calibration ( $n = 125$ ) and 30% for validation ( $n = 54$ ).

Models for aspen SOC and conifer SOC were calibrated with OM and OS separately using the optimization routine in the program QUANT for OPUS 6.5, which provided models developed in the spectral regions presented in Tables 3 and 4.

Criteria of good performance of the models at the validation stage were: highest coefficient of determination ( $R^2$ ), lowest root mean square error of prediction (RMSEP), highest ratio of standard deviation of reference values to standard error of prediction (RPD), and low rank. The RPD classification proposed by Chang et al. (2001) is often used to assess the prediction ability of NIRS models for soil analysis: good models have  $RPD > 2$ , models with  $1.4 < RPD < 2$  could be improved with other calibration techniques, and models with  $RPD < 1.4$  are not reliable (Cozzolino and Morón, 2006). Between five and 10 best models per mathematical treatment and component were selected after validation. These models were then applied to the IV set (i.e., samples not included in model development), which was the final step in evaluating model performance and our ability to predict species-derived SOC.

## RESULTS AND DISCUSSION

At the C-V stage, the best aspen SOC model developed with OS for the initial data set had  $R^2 = 62\%$ ,  $RMSEP = 9.4$  g C kg<sup>-1</sup> soil, and  $RPD_{VAL} = 1.6$ , while the best conifer SOC model had  $R^2 = 54\%$ ,  $RMSEP = 10.8$  g C kg<sup>-1</sup> soil, and  $RPD_{VAL} = 1.5$  (Table 3). Models developed with OM for the initial data set performed worse than the OS models for both components (Table 3).

Models developed for the truncated data set with OS at the C-V stage yielded the best results, with  $R^2 = 76\%$ ,  $RMSEP =$

4.6 g C kg<sup>-1</sup> soil, and RPD<sub>VAL</sub> = 2.1 for the best aspen SOC model and  $R^2 = 74\%$ , RMSEP = 5.1 g C kg<sup>-1</sup> soil, and RPD<sub>VAL</sub> = 2.0 for the best conifer SOC model (Table 3). These models can thus be considered good for soil analysis, with an RPD above or near 2 (Chang et al., 2001; Cozzolino and Morón, 2006) and can be used to predict the concentration of both components in unknown soil samples from mixed aspen–conifer stands with similar history and physical characteristics. Contrary to our expectation, the best models for both components developed for the truncated data set with OM (i.e., with the mineral matrix

subtracted) did not improve the prediction ability ( $R^2 = 76\%$ , RMSEP = 4.1 g C kg<sup>-1</sup> soil, and RPD<sub>VAL</sub> = 2.0 for aspen SOC and  $R^2 = 70\%$ , RMSEP = 5.4 g C kg<sup>-1</sup> soil, and RPD<sub>VAL</sub> = 1.8 for conifer SOC; Table 3). They performed similarly to models developed for the truncated data set with OS at the C-V stage.

The best models developed with OS for the initial data set at the IV phase had  $R^2 = 49\%$ , RMSEP = 10.1 g C kg<sup>-1</sup> soil, and RPD<sub>IV</sub> = 1.6 for aspen SOC and  $R^2 = 33\%$ , RMSEP = 8.5 g C kg<sup>-1</sup> soil, and RPD<sub>IV</sub> = 1.2 for conifer SOC (Table 4). The performance of OS models at IV was noticeably less than

**Table 3. Aspen and conifer soil organic C (SOC) models developed in the calibration–validation phase with original spectra (OS) and organic matter spectra (OM).**

Type of spectra	Data set	Component	Concentration range	SD <sub>VAL</sub> †	Mathematical treatment‡	Range	Rank	R <sup>2</sup> <sub>VAL</sub> §	RMSEP <sub>VAL</sub> ¶	RPD <sub>VAL</sub> #
			— g C kg <sup>-1</sup> soil —			cm <sup>-1</sup>		%	g C kg <sup>-1</sup> soil	
OS	initial	aspen SOC	0–81.1	14.71	FD + SLS	7347.7–6676.5, 4829–3992	10	62	9.4	1.6
OS	truncated	aspen SOC	2.18–36.82	8.40	FD + VN	5440–4246.6	8	76	4.6	2.1
OS	initial	conifer SOC	0–84.7	16.14	FD + VN	4601.5–3999.8	9	54	10.8	1.5
OS	truncated	conifer SOC	1.34–41.40	9.85	SLS	6101.8–4597.6	9	74	5.1	2.0
OM	initial	aspen SOC	0–81.1	14.71	NSP	8751.6–7498.1, 6101.8–4597.6	8	55	9.9	1.5
OM	truncated	aspen SOC	2.18–36.82	8.40	FD + VN	7502–6800, 5450–4246.6	8	76	4.1	2.0
OM	initial	conifer SOC	0–84.7	16.14	VN	5349.7–4597.6	9	43	13.1	1.3
OM	truncated	conifer SOC	1.34–41.40	9.85	SLS	5450–4597.6	8	70	5.4	1.8

† Standard deviation of validation set.

‡ NSP, no spectral preprocessing; SLS, straight line subtraction; FD, first derivative; VN, vector normalization.

§ Coefficient of determination at validation.

¶ Root mean square error of prediction at validation.

# Ratio of SD<sub>VAL</sub> to the standard error of prediction at validation.

**Table 4. Statistics of model performance at the independent validation stage for aspen and conifer soil organic C (SOC) models developed in the calibration–validation stage with original spectra (OS) and organic matter spectra (OM).**

Type of spectra	Data set	Component	Concentration range	Mathematical treatment†	Range	Rank	SD <sub>IV</sub> ‡	R <sup>2</sup> <sub>IV</sub> §	RMSEP <sub>IV</sub> ¶	SEP <sub>IV</sub> #	RPD <sub>IV</sub> ††
			g C kg <sup>-1</sup> soil		cm <sup>-1</sup>		g C kg <sup>-1</sup> soil	%	—mg C g <sup>-1</sup> soil—		
OS	initial	aspen SOC	0–81.1	SLS	6761.4–6244.6, 5446.2–4007.5	10	14.2	49	10.1	8.7	1.6
OS	truncated	aspen SOC	2.18–36.82	VN	6101.8–4597.6	9	7.9	27	6.7	6.6	1.2
OS	initial	conifer SOC	0–84.7	FD	5222.4–4987.2, 4516.6–4285.2, 4134.8–4007.5	7	10.3	33	8.5	8.4	1.2
OS	truncated	conifer SOC	1.34–41.40	SLS	5349.7–4597.6	7	8.7	31	7.2	6.5	1.3
OM	initial	aspen SOC	0–81.1	SLS	6850.1–3999.8	8	14.2	44	10.5	10.4	1.4
OM	truncated	aspen SOC	2.18–36.82	FD + SLS	10001.3–7498.1, 6101.8–5446.2, 4601.5–4246.6	4	7.9	2	7.7	7.3	1.1
OM	initial	conifer SOC	0–84.7	FD + SLS	7085.4–6846.3, 5403.7–4397	7	10.3	3	10.1	10.1	1.0
OM	truncated	conifer SOC	1.34–41.40	FD + SLS	7725.7–5446.2, 4601.5–4246.6	5	8.7	9	8.3	6.9	1.3

† SLS, straight line subtraction; FD, first derivative; VN, vector normalization.

‡ Standard deviation of independent validation set.

§ Coefficient of determination at independent validation.

¶ Root mean square error of prediction at independent validation.

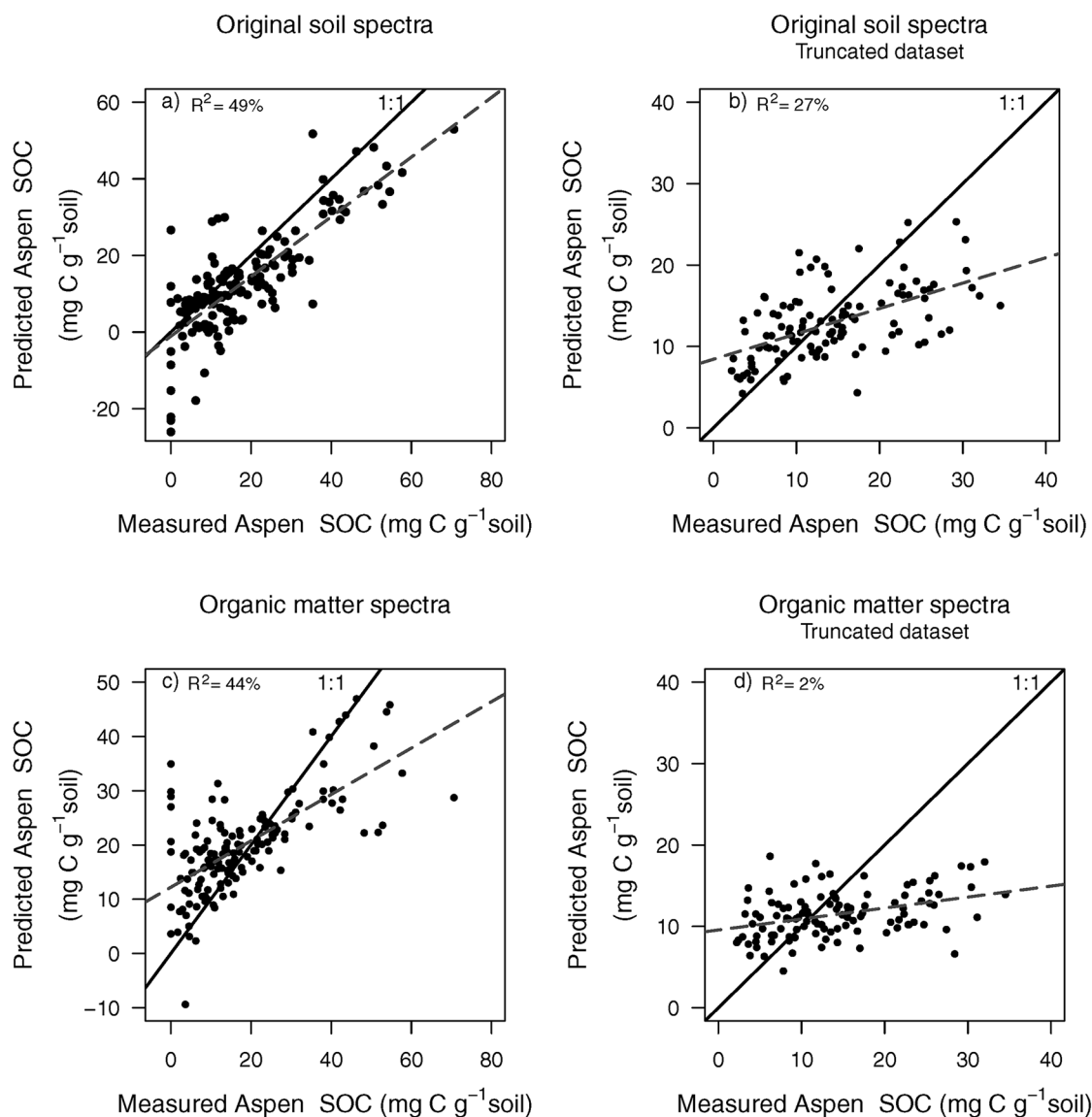
# Standard error of prediction at independent validation.

†† Ratio of SD<sub>IV</sub> to SEP<sub>IV</sub>.

at C-V (Tables 3 and 4), with  $R^2 = 49$  vs.  $R^2 = 62\%$  for aspen SOC and  $R^2 = 33$  vs.  $R^2 = 54\%$  for conifer SOC. A  $RPD_{IV}$  of 1.6 indicated that the best aspen SOC model requires further improvement. The  $RPD_{IV}$  of the best conifer SOC model was worse than at the C-V stage ( $RPD_{IV} = 1.2$  vs.  $RPD_{VAL} = 1.5$ ) (Tables 3 and 4) and should be considered as not reliable for soil analysis. The models developed with OM for the initial data set at the IV stage were all classified as unreliable for soil analysis, especially for conifer SOC ( $R^2 = 3\%$ ,  $RMSEP = 10.1 \text{ g C kg}^{-1}$  soil, and  $RPD_{IV} = 1.0$ ) (Table 4). Furthermore, we observed a higher deviation of predicted vs. measured values from the ideal 1:1 line in the OM models for both aspen and conifer components (Fig. 3a vs. 3c and 4a vs. 4c, respectively), indicating less accuracy of predictions of OM models than the OS models. Moreover, predictions of these four models tended to underestimate the aspen SOC and conifer SOC in the higher range of concentrations. This may be due to the smaller sample size in the higher concentration range.

For the best aspen SOC and conifer SOC models based on the truncated data set, OS and OM models showed a lack of prediction ability at the IV stage, as the  $RPD$  for these models were all  $\leq 1.6$  (Table 4). The best OS model for aspen SOC on the truncated data set at the IV stage had an  $R^2 = 27\%$ ,  $RMSEP = 6.7 \text{ g C kg}^{-1}$  soil, and  $RPD_{IV} = 1.2$  and the best OS model for conifer SOC had an  $R^2 = 31\%$ ,  $RMSEP = 7.2 \text{ g C kg}^{-1}$  soil, and  $RPD_{IV} = 1.3$  (Table 4). The best OM models developed on the truncated data set at the IV stage had significantly lower  $R^2$  values ( $R^2 = 2\%$  for aspen SOC and  $R^2 = 9\%$  for conifer SOC) than OS models but similar  $RPD$  and  $RMSEP$  (Table 4). Furthermore, the best OM models underestimated the concentrations of aspen SOC and conifer SOC at the higher end of the concentration ranges and overestimated the aspen SOC and conifer SOC at the lower end of the ranges (Fig. 3d and 4d).

Of all the models developed in this study, the models developed with the truncated data set and OS offered the best results at the C-V stage. This may be due to a more homogeneous



**Fig. 3. Predicted vs. measured values of aspen soil organic C (SOC) for the independent validation set and the truncated data set, comprised of the 10th to the 90th percentiles of original reference values. The dashed line is the regression line; the solid line is the 1:1 line.**

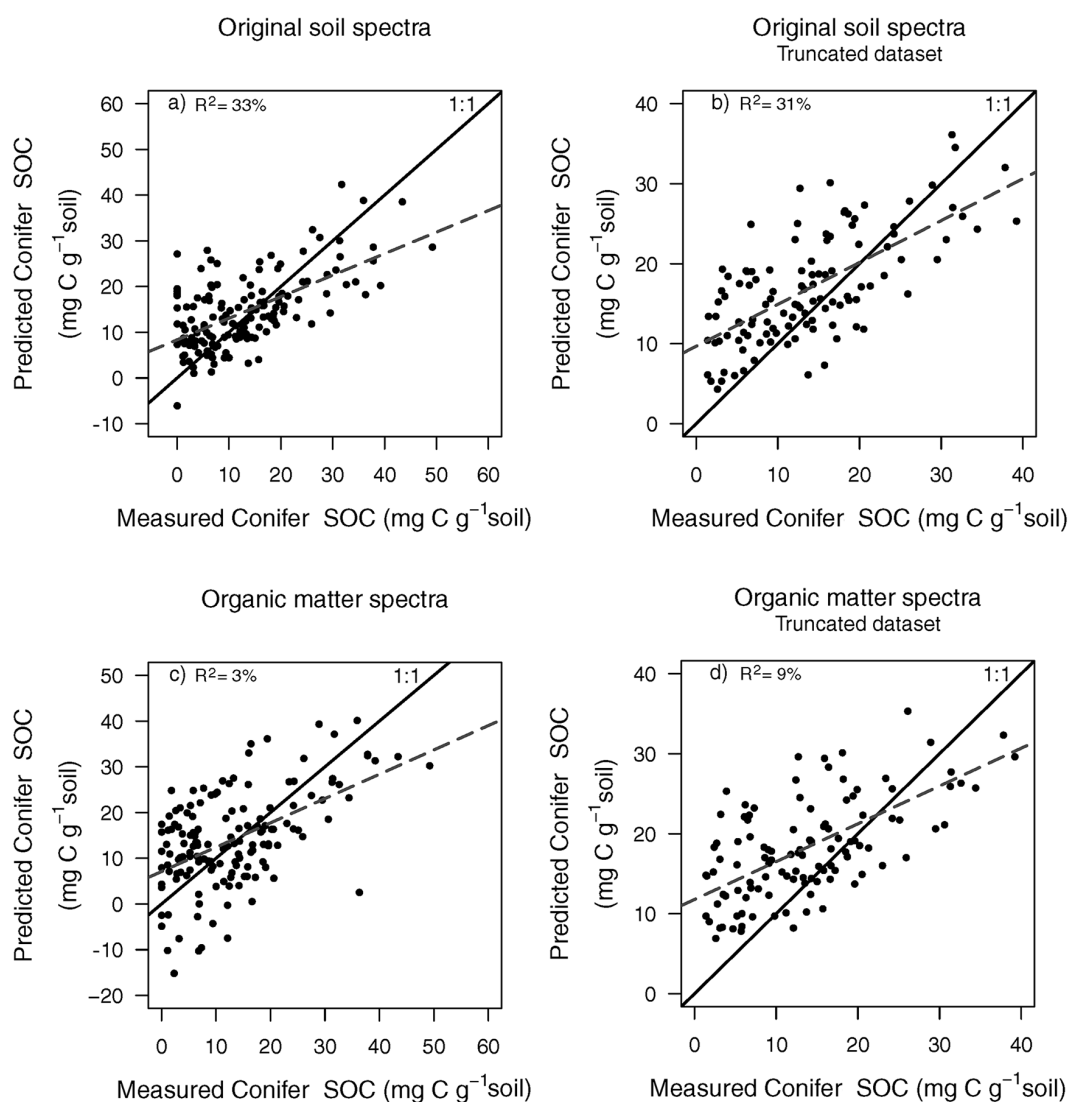


distribution of samples across the concentration range for which the calibrations were developed. A compact data set improved the fitting of the models in comparison to model calibration for the initial data set, which was affected by observations at the extremes of the concentration range ( $<2.18 \text{ g C kg}^{-1}$  soil for aspen and  $<1.34 \text{ g C kg}^{-1}$  soil for conifer;  $>36.82 \text{ g C kg}^{-1}$  soil for aspen and  $>41.40 \text{ g C kg}^{-1}$  soil for conifer). From a practical standpoint, our results indicate that the time-consuming organic matter removal from the soil samples before spectra acquisition is not necessary because it did not improve the prediction ability of our models.

For both components and spectral types, model performance decreased between the C-V and the IV stages, suggesting that factors other than SOC concentration and species of origin interfered with our analysis. Gruselle and Bauhus (2010) developed models to predict the contribution of beech and spruce in the forest floor, using material in various stages of decomposition and from different sites across the Black Forest (Germany). They were able to achieve a high degree of accuracy for both species

at the IV stage ( $R^2$  of 91% for beech and 90% for spruce). Compared with prediction models for the forest floor, our OS models for the soil at the IV stage showed  $R^2$  between 33 and 49%. We considered that differences in the composition of detritus inputs and microbial communities, as well as variability in biotic and abiotic characteristics within and among our aspen and conifer ecosystems could have contributed to this lower model performance.

Sources of organic matter in the mineral soil consist of litter-fall, dead roots, and rhizodeposits from trees and understory vegetation and their decomposition products. They all potentially influence soil NIR spectra through differences in organic matter chemistry, amount, and allocation within the soil profile. Lower  $R^2$  for SOC models than forest floor models most likely reflects the greater complexity emerging from interaction between soils and organic matter, as well as the presence of the mineral matrix with its own spectral signal (Viscarra Rossel and Webster, 2011). Also, we had greater success with aspen SOC models than with conifer SOC models. Nevertheless, differentiation of aspen



**Fig. 4.** Predicted vs. measured values of conifer soil organic C (SOC) for the independent validation set and the truncated data set, comprised of the 10th to the 90th percentiles of original reference values. The dashed line is the regression line; the solid line is the 1:1 line.

SOC vs. conifer SOC was possible due to initial differences in the amount and composition of litter, root, and understory inputs. Studies conducted in boreal forests have found that aspen and conifer species retain distinct chemical characteristics in the foliar litter after 6 yr of decomposition (Strukelj et al., 2012) and exhibited differences in fine root net primary productivity, root decomposition rates, and their relative contribution to total detritus input (Finér et al., 1997; Steele et al., 1997). Furthermore, the sensitivity of NIRS may explain the lower performance of conifer models than aspen models because the conifer soil samples were derived from stands representing multiple conifer species. Indeed, because NIRS is able to discriminate pine needles from different species (Espinoza et al., 2012), it is possible that having multiple conifer species increased the SOC chemical (and spectral) heterogeneity within the conifer component, thereby confounding the calibration of the conifer SOC model across all Utah sites.

We observed a relative clustering of spectra by site, as well as high spread among the spectra of pure aspen soils in the scores plot (data not shown), which can be attributed to two properties of aspen. First, aspen is a species of high ecological plasticity, and in the interior western United States alone, 35 plant community types have been described for pure aspen (Mueggler, 1988). Understory biomass and diversity is significantly higher under aspen stands than under adjacent conifer stands, possibly due to more favorable conditions of soil moisture, nutrients, and light in aspen stands (Mueggler, 1988; Stam et al., 2008). Hence, aspen soil NIR spectra are expected to be affected by the understory to a greater extent than conifer spectra. Second, it is plausible that aspen genetic diversity also contributed to greater spectral variability. Sexual reproduction in aspen is frequent in western U.S. landscapes (Mock et al., 2008; Long and Mock, 2012). Even within a single stand, clone diversity can be high (Hipkins and Kitzmiller, 2004; Mock et al., 2008; De Woody et al., 2009). The aspen genotype influences root growth (Fischer et al., 2006), foliar and litter chemistry, soil C and N concentrations, microbial enzymatic activity (Madritch et al., 2009), and microbial community structure (Madritch and Lindroth, 2011), all of which may be reflected in soil spectral properties.

Differences in microbial community structure, composition, and activity between aspen and conifer stands and among conifer species may also have contributed to the differentiation of SOC between aspen and conifer soils with NIRS. While we have no direct measurements on microbial community compositions for our sampling sites, soil fauna and microbial community structure have been shown to differ among aspen and conifer forests in boreal (Laganière et al., 2009; Royer-Tardif et al., 2010) and temperate semiarid environments (Ayres et al., 2009). Thus, if microbial communities associated with different species produce distinct assemblages of organic compounds, their legacy on SOC chemistry could be identifiable through NIRS.

Our initial assumption of an additive relationship among OS, OM, and MM (i.e.,  $OS = OM + MM$ ) was not supported. Clustering of spectra by site (data not shown) further suggested

that, apart from biotic factors, site-specific soil characteristics exerted some influence on NIR spectral properties as well. Indeed, NIR spectra can reflect the influence of soil type (Bartholomeus et al., 2008; Viscarra Rossel and Webster, 2011), soil texture (Van Waes et al., 2005), mineralogy (Vendrame et al., 2012), and soil development (Knadel et al., 2013). This presence of a latent site imprint on our spectra, including those controlled for MM, may also suggest selective or differential preservation of certain organic compounds, causing an indirect influence of the mineral matrix on SOC composition (e.g., Kaiser and Guggenberger, 2000). This is consistent with Schmidt et al. (2011), who proposed that SOC persistence is an ecosystem property that emerges from the interaction between the biological and physicochemical features of a given site. Woldeselassie et al. (2012) found that aspen soils had a greater fraction of mineral-associated SOC than conifer soils and suggested leaching and adsorption of litter decomposition products to the mineral matrix as the main pathways. Our soils consisted mostly of loams, but there were slight differences in texture among the sites, which ranged from sandy loams to silty clay loams (Table 2). Uneven representation of textural classes in the C-V and IV sets (Table 2) may thus have contributed to the lower performance of the models at the IV stage. Although we do not have mineralogy data for our study areas, differences in this aspect may have further contributed to the lower accuracy we achieved in our SOC models compared with forest floor models.

## CONCLUSIONS

The ecology of aspen and conifer forests in the interior western United States is closely linked to the disturbance regime, which has been intensely modified through land use changes since European settlement. Thus, the spectral properties of aspen and conifer soils do not solely reflect the influence of current overstory and understory diversity, soil microbial community, and soil texture and mineralogy but also carry with them the legacy of past land use. The complex interactions among site environmental conditions, forest dynamics, and historical land use all contribute to NIR spectral heterogeneity of soil samples, requiring a sufficiently populated spectral library to develop robust models that could be applied across montane forests in the western United States.

The good model performance ( $R^2 \sim 70\%$ ) of SOC models at C-V indicates that the contribution of vegetation to SOC can be predicted using the artificial mixtures method. However, to develop more powerful models at the IV stage (i.e., models with  $RPD > 2$ ), further work with NIRS models applied to aspen and conifer forests should consider (i) application of other chemometrics methods besides PLSR to OS, (ii) a more systematic testing of SOC spectra across a geographically broad aspen–conifer soils database, and (iii) stratification of the spectra data sets based on prior land use history and soil physical characteristics. Acquiring detailed information on historical vegetation cover for stratification purposes is specially challenging in regions with relatively recent land use records, such as Utah. These ecosys-

tems may experience further change in vegetation cover during the next decades due to land management and climate change that may alter SOC dynamics. Near-infrared reflectance spectroscopy may thus prove to be a useful tool in large-scale SOC accounting or the prediction of future SOC stock trajectories in these montane forests.

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