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Improving the design of long-term monitoring experiments in forests: a new method for the assessment of local soil variability by combining infrared spectroscopy and dendrometric data

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Abstract

• **Key message** Near- and mid-infrared spectroscopy allows for the detection of local patterns of forest soil properties. In combination with dendrometric data, it may be used as a

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Contribution of the co-authors E.A realized the field and laboratory work, conducted the data analyses, and wrote the paper. L.S.A, M.B, and B.Z designed the experiment, realized the field work supervised the work, and coordinated the research project. PhS developed the algorithms to analyze NIR and MIR spectra.

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prospective tool for determining soil heterogeneity before setting up long-term forest monitoring experiments.

• **Context** Forest soils and stands generally exhibit higher spatial heterogeneity than other terrestrial ecosystems. This variability needs to be taken into account before setting up long-term forest monitoring experiments to avoid multiple interactions between local heterogeneity and the factors tested in the experiment.

• **Aims** We hypothesized that raw near- and mid-infrared spectra can be used as an integrated proxy of a large set of soil properties. The use of this method, in combination with dendrometric data, should provide a quick and cost-effective tool for optimizing the design of experimental forest sites.

• **Methods** We assessed the local soil heterogeneity at 11 experimental sites in oak and beech stands, which belong to a new forest long-term ecological research (LTER) network. We used near- and mid-infrared spectroscopy in soil and litter samples. The spectra were subjected to principal components analyses (PCA) to determine the intra-site variability of the soil and litter layers.

• **Results** Based on mapped PCA coordinates and basic dendrometric data, it was possible to design the experiment and minimize the interactions between the treatment layout and the tested variables. The method was validated with chemical analyses of the soil. No interaction was detected at the set-up of the experiment between the treatment layout and chemical soil properties (C, N, C/N ratio, pH, CEC, Al, Mg, P₂O₅, Fe, Mn, Na, and K).

• **Conclusion** Near-infrared (NIR) and mid-infrared (MIR) spectroscopy is a useful tool for characterizing the overall heterogeneity of soil chemical properties. It can be used without any preliminary calibration. In combination with dendrometric data, it provides a reliable method for optimizing LTER plots in different types of ecosystems.

Keywords Forest long-term ecological research · Local soil variability · Experimental design · Near-mid-infrared spectroscopy

1 Introduction

The current environmental context for forest ecosystems is mainly characterized by relevant global changes, in terms of land uses, energetic consumption, climate, and atmospheric depositions. These changes and their integration into research programs and forest policies occurred very recently, during the second half of the last century. The need to observe and evaluate the impacts of these major changes on forest ecosystems is highlighted in both the short and long term. These preoccupations led to the establishment of several long-term ecological research (LTER) experimental networks around the world, especially in the forest ecosystems: the Detritus Input and Removal Treatments experiment (Nadelhoffer et al. 2004), the CIFOR network (Nambiar et al. 2004), the Long-Term Soil Productivity network in North America (Powers et al. 2005), or the long-term monitoring networks in Fennoscandia (Smolander et al. 2010). To anticipate the evolution of ecosystem impacts, certain forest management practices (FMP) are often added to understand their effects on forest ecosystem functioning and to test the interaction between FMP and global changes. Furthermore, identifying the responses at an ecosystem scale requires sufficient surface areas and, consequently, an adapted size of the experimental sites (Fayle et al. 2015). Especially in the case of soil science studies in forestry domains, the experimental sites are mostly designed at a stand scale or higher (e.g., a watershed of several squared km).

For example, in the current forest LTERs, the study areas are approximately 3.5 to 4 ha per site for detecting responses at the ecosystem scale (Nambiar et al. 2004; Hope 2006; Smolander et al. 2010; Helmisaari et al. 2011). In such areas, experimental plots have variations in soil properties (chemical, biological, and physical) and local topography, which have an impact on the vegetation characteristics and dynamics. In forest soils, for example, there is a high horizontal spatial variability of chemical and physical soil parameters, even at small scales (Zhou et al. 2010). From the perspective of a long-term monitoring of FMP impacts on ecosystem functioning, it is essential to have ways of characterizing and taking into account the pre-existing ecosystem variability, in terms of the soil properties and the vegetation cover heterogeneity. Variability at the site scale must be taken into account before implementing the FMP to ensure that there is no interaction between the FMP and the main ecosystem properties in the establishment of the network. An efficient approach frequently used to determine the soil variability in agronomy consists of crossing geostatistical data (DEM, etc.) with the results of principal components analyses from a chemical

description on the sampling grid (Nykänen et al. 2008; Sanchez et al. 2014). However, due to the costs of soil analyses, the authors have to focus on very few variables, such as soil moisture, phosphorus, nitrogen, carbon, or pollutant contents (Marchant et al. 2009; Lei et al. 2012).

Considering the increasing amount of interdisciplinary research and the number of parameters used to monitor in current LTER, along with the high cost of physical and chemical soil analyses, it seems necessary to develop an efficient tool that will integrate all these variables. Infrared spectroscopic approaches could be used to record all the necessary information and to provide an overview of local heterogeneity. Indeed, infrared reflectance spectrometry is a non-destructive physical analysis (Cécillon et al. 2009; Stenberg et al. 2010; Bellon-Maurel and McBratney 2011) that is frequently used to determine the chemical and physical properties of soil, in particular, the soil organic matter properties (Barthès et al. 2008; Ludwig et al. 2008). This method is used to accurately predict carbon and nitrogen concentrations (He et al. 2005; Brunet et al. 2007; Cécillon and Brun 2007), soil texture, or cation exchange capacity. Near-infrared spectrometry (NIRS) is used to predict carbon or nitrogen stocks, whereas mid-infrared spectrometry (MIRS) is a suitable method for evaluating organic matter composition (Ludwig et al. 2008; Tatzber et al. 2011). In arable soils, infrared spectroscopy was also used to predict the markers of soil biological activities, including enzyme activities, such as cellulase and phenoloxidase (Albrecht et al. 2008), the respiration rate, microbial biomass C, and the ergosterol content (Soriano-Disla et al. 2014; Ludwig et al. 2015). Soil nematode communities (Barthès et al. 2011) and earthworm activities can be predicted with a fingerprinting approach using near-infrared spectroscopy.

Consequently, infrared technologies are frequently used to characterize and map soil properties (Cécillon et al. 2009) related to agronomic or pollution issues at the stand to regional scale (for example, AMSR2 satellite imagery in Temimi et al. (2010)). The relationship between infrared spectra and the property of interest is calibrated using multivariate statistical approaches on a subsample, and the model is applied to the whole area from the infrared spectra measured on all samples to predict and monitor several soil variables, such as soil organic carbon (Vohland et al. 2011), soil nutrients (Gholizadeh et al. 2013), metal contaminants (Chodak et al. 2007; Horta et al. 2015), or indices of productivity in eucalyptus forests (Bikindou et al. 2012). To our knowledge, most of these studies integrated a calibration step between the spectra and chemical data (Lamsal 2009; Viscarra Rossel et al. 2010; Muñoz and Kravchenko 2011).

The basic idea of this study is that the variability of the spectra reflects the overall heterogeneity of the biological, chemical, and physical properties of the soil and thus can be used as such without preliminary calibration with the variables of interest. In a preceding study, Odlare et al. (2005) found that

NIR spectral analysis provides a better description of soil spatial variations than the soil reference variables at the field scale, i.e., in a 3.2-ha experiment plot using the results from principal component analyses realized on the spectral data. Here, we propose to extend this result, and our hypothesis is that raw NIR and MIR spectra can be used as an integrated proxy of many soil properties. This method, in combination with dendrometric data, would be a rapid and a cost-effective way to optimize the design of experimental forest sites. We tested this hypothesis on forest LTERs, which exhibit substantial spatial heterogeneity (Smithwick et al. 2005).

2 Materials and methods

2.1 Experimental sites

This study was carried out at 11 experimental sites that belong to the MOS (Matières Organiques des Sols) network (Akroume 2014). Five beech stands (*Fagus sylvatica* L.) and six oak stands (*Quercus petraea* Matt. Liebl.) were selected in lowland production forests located in the northern half of France. The stands were selected to cover an age just before their current annual maximum increment (CAI), i.e., at an age between 30 and 60 years old (Table 1), and to avoid local constraints such as severe slopes or occurrence of hydromorphic soils. When available, the soil types and textures have been described from two pits (1 m deep) using the World Reference Base for Soil (WRB 2014).

This network is intended for long-term monitoring of the effects of intense biomass exportations on soil fertility, tree growth and health, and soil biodiversity (macro-, meso-, and microfauna). It requires experimental sites that have a study area that is sufficient to determine ecosystem responses.

The total area of a single experimental site is approximately 2–3 ha. We defined 12 subplots of 40 m × 40 m within each site. Four treatments corresponding to four levels of biomass exportations will be studied at each experimental site. The planned treatments, as described in Akroume (2014), are as follows: (1) a control (stem only harvesting), (2) logging with residue removal, (3) logging with residue and forest floor removal, and (4) logging with residue removal and wood ash fertilization. They will be repeated three times per experimental site and their layout on each site was randomly drawn.

2.2 Soil sampling and dendrometric measures

At each site, we sampled the leaf litter and soil in a 50 × 50 cm² every 20 m before the onset of the in situ experiments. After the forest floor was collected, the mineral soil was sampled at three depths: 0–5, 5–10, and 10–20 cm. In each soil layer, three cores were collected and pooled to obtain a single composite sample per soil layer and point. According to the site

configuration, this 20-m sampling grid provided approximately 70 points per site, with a total of 280 samples per site for the four layers (leaf litter and the three soil depths). The sample point locations were recorded in the field using a Trimble Geo 5T (Trimble Navigation Ltd., Sunnyvale, CA, USA) terminal (accuracy of 0.5–1 m in forests) and plotted using the free GIS software Qgis 1.8.0 (<http://www.qgis.org/fr/site/>). We measured the circumference at breast height and the dominant height (H_0 , height of the three largest trees) every 40 m to describe the forest stands. The trees were measured inside a 6-m-diameter circle, except for the trees with a circumference above 20 cm, which were measured inside a 10-m-diameter circle. These measurements provided tree density (number of stems per ha), index of fertility (dominant height), the basal area (m² per ha), and the species composition.

2.3 Mid–near-infrared spectroscopic analysis

The soil and forest floor litter samples were sieved through a 4-mm mesh and dried at 30 °C for 5 days before grinding. These ground samples were dried again at 30 °C for 24 h before infrared analysis. They were subjected to infrared (9997 to 2200 cm⁻¹) and mid-infrared (5000 to 550 cm⁻¹) scanning using a HTS-XT Bruker spectrometer (Vertex 70, NIR-MIR-MCT, Bruker Corporation, Billerica, MA, USA); the two sensors overlapped in the 2200- to 5000-cm⁻¹ region. We used the full range provided by each sensor, and the infrared scanner recorded the absorbance spectra for each sample.

2.4 Chemical analyses

We pooled the soil and litter samples per layer to obtain a single bulk sample for each 40 m × 40 m plot delimited in the field. This resulted in 12 bulked samples for each site (3 repetitions by treatment) and a total of 512 bulked samples that corresponded to the 11 experimental sites × 4 treatments × 3 repetitions per treatment (except for the oak Compiègne site with only 2 repetitions) × 4 studied layers (litter + 3 soil depths). The carbon and nitrogen concentrations of the 512 bulked samples were measured using a Thermoquest elemental analyzer (NCS2500, EA/NA 1110).

A second level of pooling was performed on the soil layers. The three repetitions per treatment were combined, resulting in 132 samples, where the depths represented the repetitions for each treatment (11 experimental sites × 4 treatments × 3 soil layers). Figure 1 summarizes the different steps of sample pooling.

Complementary analyses to C and N were conducted on these 132 samples. The pooling permitted a reduction in the number of samples and resulted in homogenized combined samples by block. The chemical analyses were carried out at the INRA laboratory of Arras. Phosphorus was measured using the Duchaufour and Bonneau method (1959), and

Table 1 Characterization of stand, soil, and geological material for the 11 experimental sites established in deciduous forests

Forest/code	Age (years)	Species	Coordinates		Elevation (m)	Average basal area (m ² /ha)	Dominant height (m)	Stem density (nb/ha)	Geological material	Humus type	Soil texture	Soil type (WRB 2014)
			GPS Lambert93	dirE								
FD Ban d'Haro/BAN	35	Beech	6783156.6	938890.7	350	36.3	15.6	3565	Lower Muschelkalk Sandstone	Mesomull	Loam	Stagnic Dystric Cambisols
FD Champenoux/CHAMP	40	Oak	6852020.9	945689.4	280	27.0	16.3	1745	Pliensbachian marl	Eumull/mesomull	Clay-loam	na
FD Compiègne/CompCHE	30	Oak	6915274.9	695540.9	180	19.7	13	12154	Middle Lutetian limestone	Mesomull	Sandy-loam	Eutric Cambisols
FD Compiègne/CompHET	30	Beech	6914427.8	695219.1	185	13.5	20	446	Middle Lutetian limestone	Mesomull	Sandy-loam	Stagnic Luvisols
FD Darney/DAR	35	Beech	6779652.2	936520.6	500	23.8	18.3	2898	Lower Muschelkalk Sandstone	Mesomull/oligomull	Sandy clay loam	Dystric Cambisols
FD Fontainebleau/FONT	40	Oak	6810531.1	682549.1	135	23.5	12	1878	Quaternary silt	Moder	Sand	Eutric podzol
Gaillfontaine/GAILL	40	Beech	6952055.8	602895.7	290	25.3	16.5	849	Upper Jurassic limestone	Eumull/mesomull	Silty-loam	Eutric Cambisols
FD Prieurés Grosbois/GRO	40	Oak	6599998.1	698904.5	319	20.9	16.4	1528	Autunian (Asselian)	Dysmull	Sandy-loam	na
FC Reichshoffen/REICH	40	Oak	6882801.78	1041673.4	270	21.3	16.5	1473	Lower Muschelkalk marl	Mesomull/dysmull	Silt	na
FD Tronçais/TRON	50	Oak	6614238.3	678776.2	270	25.6	17.16	1473	Trias sandstone	Mesomull/dysmull	Sandy-loam	na
FD Verrière du Grosbois/VERR	55	Beech	6683070.9	948588.4	585	24.6	21.6	458	Argovian Chert clay	Mesomull/oligomull	Silty clay loam	Dystric Luvic Cambisols

All experimental sites were sampled during the spring of 2013

na not available

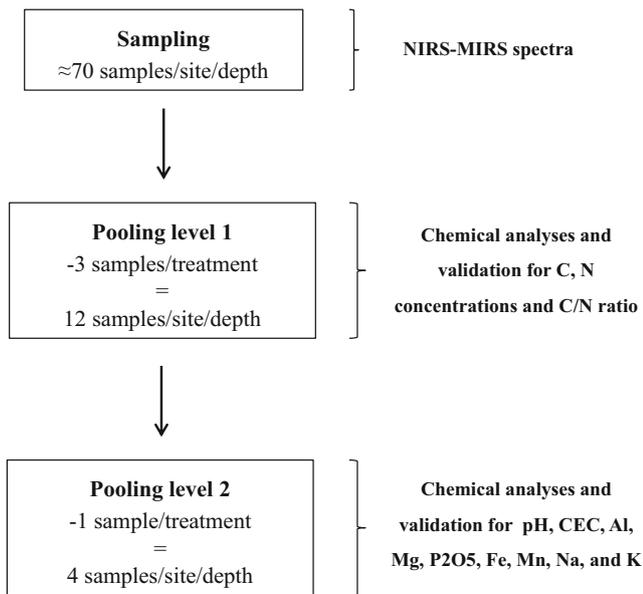


Fig. 1 Schematic plan of the different pooling steps for the spectral and the chemical characterization of soils. Around 70 samples per depth were collected on each site and used for the infrared spectroscopy analyses. A first pooling level provided 12 samples/site/depth which have been used to C and N analyses. The second pooling level provided four samples/site/depth used for measuring the other soil properties (pH, CEC, Al, Mg, P₂O₅, Fe, Mn, Na, and K) analyses

exchangeable elements (Ca, K, Mg, Na, Mn, Al, Fe) were extracted using cobaltihexammine chloride (Orsini and Rémy 1976). Furthermore, the pH (H₂O and KCl) was measured, and the CEC was estimated at soil pH after extraction with cobaltihexammine chloride. The soil chemical characteristics are given in table S1.

2.5 Statistical analyses of NIRS–MIRS data

For each of the 11 sites, the spectral data were saved as a matrix. Data analyses were conducted on the first derivate after standard normal transformation to accentuate spectral patterns (Reeves et al. 2002). Statistical analyses were carried out using SAS 9.2 software (SAS Inc., Cary, NC, USA).

The spectral data were then subjected to a principal components analysis (PCA) to determine the intra-site variability of the soils. For each of the 11 sites, the data set was composed of approximately 70 individuals (soil samples) and between 2566 (MIR) and 4044 (IR) variables. In all cases, the two first axes of the PCA were sufficient to explain more than 90 % of the variance. Then, the PCA coordinates of the main components and the dendrometric characteristics for each subplot were synthesized to a single data set.

2.6 Random drawing of the treatments' layout

The procedure comprises two steps: (1) for each site, the treatments were assigned randomly to the subplots; (2) then

interactions between the treatments' layout and variables mentioned in Section 2.5 were tested using ANOVA (proc GLM), with a significance threshold of 5 %. The two steps of the procedure were repeated until the analysis of variance did not reveal any significant effect between the treatments and the measured infrared (whatever the depth) or dendrometric data. The interactions between the treatments and spectral data were checked for all layers (soil and litter).

2.7 Validation of the method

Once the treatments have been assigned to each plot after the random drawing (as described in Section 2.6), it was necessary to cross-check with the chemical characteristics of the soil samples described in Section 2.4: if there was no interaction between the treatments' layout and the measured chemical properties, then the method is validated (i.e., the use of dendrometric variables and row NIRS spectra as synthetic surrogate of soil chemical properties allows to design efficiently experimental layout in forest ecosystems at low costs). This validation was performed on the first level of pooling (set of 512 samples) for the carbon and nitrogen concentrations and on the second level of pooling for the other chemical variables (set of 132 samples) (Fig. 1). For the first pooling, the interactions between the treatments and chemical properties were identified using nested proc GLM with a significance threshold of 5 %. The nested proc GLM permitted consideration of each soil depth separately.

In the second pooling data set, as the depths were used as replicates, the correlations existing between the different depths were taken into account in the “repeated measures anova” option in the SAS GLM procedure, taking sites as repetitions. To confirm the results, we also used mixed linear models with PROC MIXED in SAS. For each chemical element, the treatment effects were tested on each soil depth separately (fixed effects), and the 11 sites were taken as random effects parameters.

For both pooling datasets, our hypothesis was validated if no treatment effect was detected on the measured soil chemical properties and the dendrometric data when the treatment layouts were determined using only the infrared spectra and stand characteristics.

3 Results

3.1 NIRS/MIRS spectra and principal component analyses

For all sites, the two first components of the PCA explained more than 95 % of the total variance of infrared spectral data. The coordinates of the first two components were mapped on the sampling grid, which revealed the occurrence of a local variability at the plot scale for the leaf litter and the three soil

depths that were sampled. According to the experimental site, some spatial heterogeneity, soil homogeneity, or gradients of soil characteristics were highlighted (Fig. 2).

3.2 Definitive treatments' layout

The procedure described in Section 2.6 allowed to overcome the local heterogeneity of the soil and forest stands, or existing gradients revealed by infrared reflectance spectroscopy. The four treatments were settled on each experimental site accordingly to this result (Fig. 3). On average, four random drawings were necessary to obtain a suitable treatment arrangement (i.e., with no effect on dendrometric variables and spectra). When the ANOVA procedure detected treatment effects, they could have been due to soil variations, dendrometric characteristics, or a combination of both types of parameters.

3.3 Validation of the repeated random drawing

The nested analysis of variance exhibited no significant effect between the treatment layout and the carbon ($p=0.956$) and

nitrogen concentrations ($p=0.986$). The ANOVA result was also non-significant for the C/N ratio ($p=0.059$). This result confirmed that there was no link between the local variations in the carbon and nitrogen concentrations or the C/N ratios and the treatment design at the initial stage.

For all the other variables, the GLM procedure with "repeated measure anova" confirmed that the three soil depths were strongly correlated. The partial correlation coefficients were always highly significant and varied between 0.70 and 0.99 ($p < 10^{-4}$). Furthermore, there was no effect between the treatment layout and the different variables; for all elements, the GLM procedure exhibited no significant effect ($p > 0.90$).

The additional validation by a mixed linear model (PROC MIXED), considering the treatments and soil depths as fixed effects and the experimental sites as random effects, permitted us to test each soil layer independently. It confirmed this result and validated the absence of any initial interaction between the treatments and the soil chemical properties for each soil layer ($p > 0.05$).

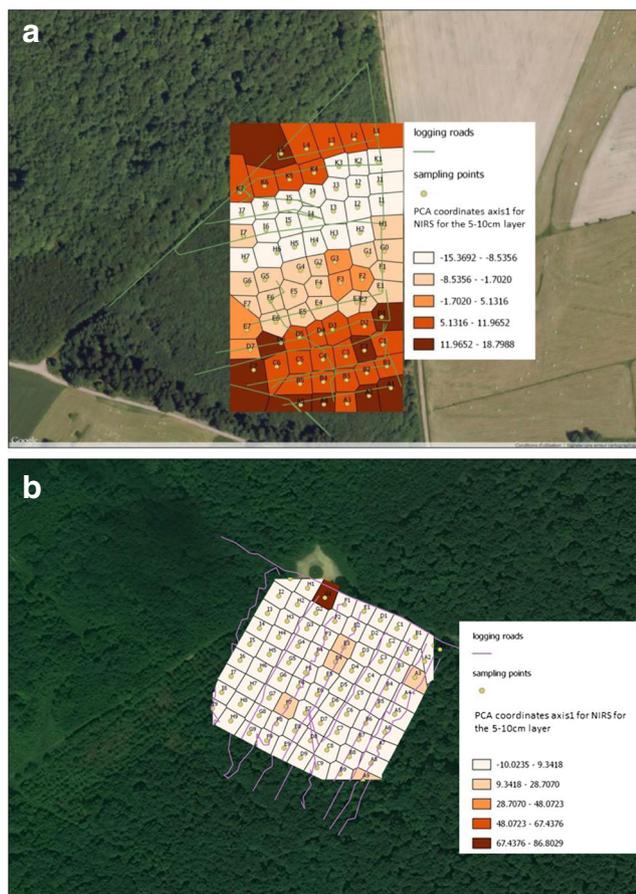


Fig. 2 Map of the axis 1 coordinates of the PCA for the infrared reflectance spectral data in the 5- to 10-cm layer. The Darney experimental sites exhibits spatial gradient variability (a) whereas the Compiègne beech site reveals an overall homogeneity (b)

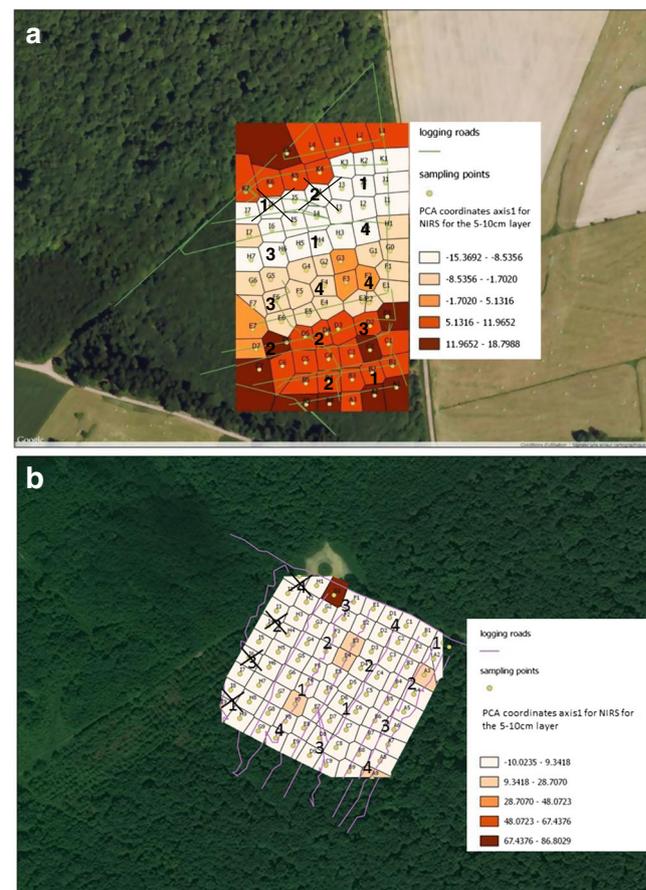


Fig. 3 Treatment layout at the Darney experimental site (a) and the Compiègne beech experimental site (b) overlapped with the map of the axis 1 coordinates of the PCA for the infrared reflectance spectral data. The *crossed areas* correspond to additional plots, which were not retained in the definitive experimental design

4 Discussion

This work aimed to develop a methodology for designing experimental sites in a long-term monitoring network where the soil organic matter is manipulated, such as in our context of increased forest harvesting. Then, for both the soil chemical measurements and the NIRS/MIRS spectral analyses, we focused on the topsoil (litter and the first 20 cm), which is supposed to be more affected by organic matter removal than the deeper layers (Sayer 2005; Thiffault et al. 2011). Indeed, some studies in *Eucalyptus* plantations have not shown any effect of litter removal after 2 years on the carbon and nitrogen concentrations in mineral soil located at a depth below 15 cm (Versini et al. 2014). Furthermore, nitrogen from leaf litter degradation is mainly incorporated between depths of 15 and 30 cm (D'Annunzio et al. 2008). In temperate broadleaf forests, similar monitoring shows that most of the nitrogen is incorporated in the top 5 cm of the soil after 2 years (Swanston and Myrold 1997; Zeller et al. 2001). This finding is why we deliberately chose not to consider the spatial variability in deeper soil layers, despite their relevance for tree growth. For all of the soil chemical properties studied here, the random drawing based on infrared data was validated because there was no significant effect of treatment on the soil characteristics. It is interesting to note that the random drawing was validated even for the C/N ratio and for some elements, such as Fe, Mn, Na, or K, whose concentrations are difficult to predict accurately by near- or mid-infrared spectroscopy (Bikindou et al. 2012; Kuang et al. 2012; Soriano-Disla et al. 2014). These results confirm that near- and mid-near-infrared spectra can be used as such in the design of experimental sites as a proxy of a large set of chemical properties of forest soils. This result is clearly new and generalizes the preceding study proposed by Odlare et al. (2005). In this study, we chose to focus only on chemical properties, even if we acknowledge that a relevant part of soil heterogeneity is driven by physical parameters. Complementary studies would be required to validate this method with physical characteristics, such as soil texture or structure.

For some experimental sites, in particular those with strong local variability of soil and stand characteristics, the mapping of the PCA coordinates on the spectral results revealed that the heterogeneity did not follow the same pattern across the four sampled layers. That is why it is essential to check the treatment effects using ANOVA on several depths and not only the upper layers. This point is important, not only for soil chemical properties but also for the soil microbial analysis, because soil fungi are also differentially distributed in the vertical soil profile (Dickie et al. 2002; Coince et al. 2013). Both soil variability and dendrometric characteristics have to be considered before implementing the treatments in situ, particularly when dendrometric data are very homogenous. In these cases, infrared data are the only parameters that permit the detection

of local soil variations, which could interact with the treatment layout. Finally, it is essential to notice that the infrared data were validated using chemical analysis but not using biological activity factors, whereas the local variability observed using infrared spectroscopy could also be linked to the heterogeneous distribution of soil organisms (Ludwig et al. 2015). Indeed, Terhoeven-Urselmans et al. (2008) reported a high correlation between NIRS/MIRS and microbial properties, as ergosterol and microbial carbon measurements. Moreover, Morris (1999) found that considerable spatial variability in fungal and bacterial biomass exists at the 1- to 10-cm scale. This author suggested that this variability could be adequately managed by sampling in a pattern, which takes into account components of this important biological variation.

Both near- and mid-infrared were taken into account for the 11 experimental sites. Mid-infrared spectra cover a larger range of frequencies and are more efficient to reflect the chemical properties of soil organic matter (Ludwig et al. 2008). They are preferentially used to predict organic components and exchangeable elements in litter and soil (Patzold et al. 2008), especially soil carbon, nitrate, metals, and microelements (Kuang et al. 2012), whereas near-infrared analyses associated with MIR reveal the physical properties of soil (Soriano-Disla et al. 2014). Near-infrared spectra are a good predictor of clay content, exchangeable K (Viscarra Rossel et al. 2006), and moisture content (Kuang et al. 2012). Evidence suggests that MIR spectroscopy provides an integrative overview of soil properties and will reveal more accurate local variations of existing gradients. Nevertheless, near infrared is still useful for identifying some variations and for crossing them with mid-infrared data for confirmation. Using both NIRS and MIRS analyses produces the most complete characterization of soil properties. Finally, Viscarra Rossel et al. (2006) emphasized the superior efficiency of MIR spectroscopy in the laboratory, whereas NIRS provides better results for in situ analyses, partly because of the sample preparation required for mid-infrared spectroscopy (Kuang et al. 2012).

5 Outlook and conclusions

NIRS–MIRS prospection is an accurate method for reflecting the local variability of forest soil for the main chemical variables. It allows for horizontal spatial heterogeneity to be overcome and limits the number of chemical analyses in the initial characterization of experimental sites.

Finally, NIR–MIR spectroscopy appears to be an efficient tool to describe the spatial heterogeneity of forest soil at the scale of a forest stand. Crossed with vegetation characteristics, it permits both belowground and dendrometric variability to be taken into account in the implementation of optimal experimental designs. It provides a reliable method that is

applicable for the optimization of LTER plots in different types of ecosystems.

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Compliance with ethical standards

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