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Application of near infrared reflectance (NIR) and fluorescence spectroscopy to analysis of microbiological and chemical properties of arctic soil

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Abstract

Applicability of near infrared reflectance (NIR) and fluorescence spectroscopic techniques was tested on highly organic arctic soil. Soil samples were obtained at a long-term climate change manipulation experiment at a subarctic fell heath in Abisko, northern Sweden. The ecosystem had been exposed to treatments simulating increasing temperature (open-top greenhouses), higher nutrient availability (NPK fertilization) and increasing cloudiness (shading cloths) for 15 years prior to the sampling. For each of the 72 samples from the 0 to 5 cm soil depth and 36 samples from the 5 to 10 cm depth, the wavelength range of 400–2500 nm (visible and near infrared spectrum) was scanned with a NIR spectrophotometer and fluorescence excitation-emission matrices (EEMs) were recorded with a spectrofluorometer.

Principal component analyses of the visible, NIR and fluorescence spectra clearly separated the treatments, which indicates that the chemical composition of the soil and its spectral properties had changed during the climate change simulation. Similarly to the results from the conventional analyses of soil chemical and microbiological properties, fertilization treatment posed strongest effects on the spectra. Partial least-squares (PLS) regression methods with cross-validation were used to analyse relationships between the spectroscopic data and the chemical and microbiological data derived from the conventional analyses. The fluorescence EEMs of the dried solid soil samples were moderately related to soil ergosterol content (correlation coefficient $r = 0.84$), bacterial activity analysed by leucine incorporation technique ($r = 0.78$) and total phospholipid fatty acid (PLFA) content ($r = 0.74$), but in general fluorescence provided inferior predictions of the chemical and microbiological variables to NIR. NIR was highly related to soil organic matter content ($r > 0.9$) and showed promising predictions of soil ergosterol content ($r > 0.9$), microbial biomass C, microbial biomass P, and total PLFA contents ($r = 0.78$–0.79).

These results suggest that especially NIR could be used to predict soil organic matter and fungal biomass. Since it is rapid and inexpensive, and requires little sample mass, it could be used as a 'quick and dirty' technique to estimate progression of the treatment responses in long-term ecosystem experiments, where extensive soil sampling is to be avoided.

Keywords: Fluorescence; NIR; Excitation-emission matrix; Spectroscopy; Arctic; Heath; Climate change; Soil organic matter; Microbial biomass; Ergosterol; Multivariate data analysis

1. Introduction

Near infrared reflectance (NIR) spectroscopy has been presented as a rapid, inexpensive, and precise technology, which can be applied to prediction of sample composition in ecological studies (Foley et al., 1998). In soil science, NIR

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has been used to assess e.g. clay content, specific surface area, cation-exchange capacity, moisture content, organic matter, and concentrations of organic and inorganic fractions of carbon and nitrogen (Morra et al., 1991; Ben-Dor and Banin, 1995; Islam et al., 2003; Velasquez et al., 2005). Some studies have also attempted to predict microbial and fungal biomass, and respiration in boreal (Palmborg and Nordgren, 1993; Fritze et al., 1994; Pietikäinen and Fritze, 1995) and temperate (Chodak et al., 2002; Ludwig et al., 2002; Chodak et al., 2003; Coûteaux et al., 2003) forest soils. Since silica, which constitutes a major fraction of the non-organic matter in soil, is transparent to infrared radiation, NIR applied on high organic matter soils is likely to yield different responses than applications on soils with high mineral content. However, measurements on forest humus suggest that NIR can also be applied on organic soil (Fritze et al., 1994; Pietikäinen and Fritze, 1995). Arctic soils, which harbor 14% of the total global soil organic carbon (Post et al., 1982) and which are experiencing rapidly advancing climatic warming (ACIA, 2005), receive more and more attention in climate change science, and therefore applicability of the NIR technique to this highly organic soil should be assessed.

NIR measures the vibrational transitions of molecular bonds. A broad range of molecules, including most organic compounds, absorb in the NIR range, and therefore NIR spectroscopy is a valuable analytical tool in the quantitative and qualitative analysis of organic matter. The strongest absorbers in the NIR region are the bonds O–H, such as in water, and bonds such as C–N, N–H and C=O, characteristic to organic matter. Samples are often measured without any pretreatments, which makes NIR a fast and non-invasive technique.

Compared to near infrared and visible wavelengths, fluorescence spectroscopy can better distinguish soil and crop residue samples from each other (Daughtry et al., 1995) suggesting that fluorescence could serve as a useful indicator of the amount of plant-derived material in soil. Most commonly fluorescence spectroscopy has been used to assess properties of humic and fulvic acids (Mobed et al., 1996) or quality of organic matter (Cannavo et al., 2004) in soil. However, fluorescence has been measured in water extracts of soil, whilst analysis of solid soil samples by this technique is uncommon.

Fluorescence spectroscopy measures the excitation of electrons from the ground state, and their return to the ground state. This is detected in the UV/VIS range of the spectra. Molecules, which exhibit fluorescence properties, are highly conjugated organic molecules and aromatic compounds with rigid molecular skeletons. Fluorescence spectroscopy is therefore typically used to detect proteins and other complex organic compounds. Samples are often measured in a solution, but novel improvements to the instruments enable measuring solid samples.

Our aim was to assess the applicability of NIR and fluorescence spectroscopy to predict microbiological and chemical soil constituents in an arctic soil with up to 95% organic matter. The soil samples were taken from a long-term climate change experiment simulating increased temperature, increased nutrient availability resulting from higher temperature, and increased cloudiness (e.g. Havström et al., 1993). We hypothesized that both NIR and fluorescence could separate the fertilized soil from the non-fertilized soil, because fertilization has strongly increased microbial biomass and altered microbial community composition (Rinnan et al., 2007), possibly as a result of increased abundance of vascular plants and changed vegetation composition (van Wijk et al., 2004). Secondly, we hypothesized that NIR could predict microbial biomass, because it has previously been predicted with high accuracy in boreal soils (Palmborg and Nordgren, 1993). Fluorescence was assumed to be related to the properties reflecting the amount of plant residues in the soil, such as soil organic matter.

2. Materials and methods

2.1. Site and sampling

Soil samples originated at a long-term field experiment in Abisko, northern Sweden (68°21′N, 18°49′E). The experiment, which was set up in 1989 (Havström et al., 1993), simulates the predicted changes in sub-arctic climate. The soil at the site has 12–15 cm deep organic horizon, and the vegetation is dominated by *Cassiope tetragona* (L.) D. Don. The samples were cored from six replicate plots with (1) passive open-top greenhouse warming simulating increased temperature, (2) NPK fertilization simulating increased nutrient availability, (3) shading treatment simulating increased cloudiness, combinations of (4) fertilization plus warming and (5) fertilization plus shading, and (6) unmanipulated control plots. This adds up to 36 plots (120 × 120 cm), which are described in detail by Havström et al. (1993).

Shortly, four soil cores were taken from each plot to 10 cm depth and divided into 0–5 and 5–10 cm depths. For the surface layer, the cores were combined into two subsamples per plot and for the deeper layer into one sample per plot, yielding altogether 108 samples for analyses. Roots and stones were removed, and the soil was homogenized by hand 15 min per sample. Root biomass was determined after oven-drying at 70 °C.

2.2. Spectroscopic analyses

For spectroscopic analyses the soil samples were oven-dried at 70 °C and finely ground. Each sample was analysed twice with re-packing. Prior to data analysis, these replicate measurements—and the separate measurements of the two subsamples of the surface soil—were averaged to yield one data point per field plot.

A NIRSystems 6500 NIR spectrophotometer (Foss Analytical, Höganäs, Sweden) was used to record reflectance spectra for a spectral range of 400–2500 nm at 2 nm