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Near infrared reflectance spectroscopy (NIRS) could be used for characterization of soil nematode community

Bernard G. Barthès [a,*], Didier Brunet [a], Bodovololona Rabary [b], Oumar Ba [c], Cécile Villenave [a]

[a] IRD, UMR Eco&Sols (INRA-IRD-Montpellier SupAgro), 2 place Viala, bâtiment 12, 34060 Montpellier Cedex 2, France
[b] FOFIFA, URP SCRID (CIRAD, FOFIFA, University of Antananarivo), BP 230, Antsirabe 110, Madagascar
[c] IRD, UMR Eco&Sols, LEMSAT, Centre IRD-ISRA, route des Hydrocarbures, BP 1386, Dakar, Senegal

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ABSTRACT

Studying soil nematofauna provides useful information on soil status and functioning but requires high taxonomic expertise. Near infrared reflectance (NIR) spectroscopy (NIRS) has been reported to allow fast and inexpensive determination of numerous soil attributes. Thus the present study aimed at assessing the potential of NIRS for determining the abundance and diversity of soil nematodes in a set of 103 clayey topsoil samples collected in 2005 and 2006 from agricultural soils in the highlands of Madagascar.

The morphological characterization of soil nematofauna involved extraction through elutriation then counting under binoculars and identification at family or genus level using microscopy, on ca. 150-g fresh soil samples. Taxa were assigned to five trophic groups, namely bacterial feeders, fungal feeders, obligate plant feeders, facultative plant feeders, and omnivores and predators (together). In addition, four ecological indexes were calculated: the Enrichment index, Structure index, Maturity index, and Plant parasitic index.

Oven-dried (40 °C) < 2-mm sieved 5-g soil subsamples were scanned in the NIR range (1100–2500 nm), then spectra were fitted to nematofauna data using partial least square regression. Depending on the sample set considered (year 2005, year 2006, or both years), NIRS prediction of total nematode abundance was accurate (ratio of standard deviation to standard error of cross validation, i.e. RPD $\geq$ 2) or acceptable (RPD $\geq$ 1.6). Predictions were accurate, acceptable, or quasi-acceptable (RPD $\geq$ 1.4) for several of the six most abundant taxa, and to a larger extent, for most trophic groups (except facultative plant feeders); but they could not be made for taxa present in a small number of samples or at low abundance. By contrast, NIRS prediction of relative abundances (in proportion of total abundance) was poor in general, as was also the prediction of ecological indexes (except for the 2006 set). On the whole, these results were less accurate than NIRS predictions of soil attributes often reported in the literature. However, though not very accurate, NIRS predictions were worthwhile considering the labor-intensity of the morphological characterization. Most of all, NIRS analyses were carried out on subsamples that were probably too small (5 g) to allow representative sampling of nematofauna. Using larger samples for NIRS (e.g. 100 g) would likely result in more accurate predictions, and is therefore recommended. Scanning un-dried samples could also help improve prediction accuracy, as morphological characterization was carried out on samples not dried after sampling.

Examining wavelengths that contributed most to NIRS predictions, and chemical groups they have been assigned to, suggested that NIRS predictions regarding nematofauna depended on constituents of both nematodes and preys' food. Predictions were thus based on both nematofauna and soil organic properties reflected by nematofauna.

1. Introduction

Soil nematodes possess several characteristics that allow their utilization as bio-indicators of soil functioning (Bongers and Ferris, 1999): they are abundant in most terrestrial ecosystem whatever the climatic area and the vegetation (Yeates, 2003); they have high taxonomic diversity and high functional diversity in relation to soil processes (Ekschmitt et al., 2001); moreover, several nematofaunal indexes linked to soil functioning have been developed and have proven to be powerful tools for characterizing the soil food-web in ecosystems and agrosystems (Bongers, 1990; Ferris et al., 2001).

* Corresponding author. Tel.: +33 4 99 61 21 36; fax: +33 4 99 61 21 19.
E-mail address: bernard.barthes@ird.fr (B.G. Barthès).

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Indeed, nematodes belong to different trophic groups that are present at several levels of the food-web: microbivorous (bacterial feeders and fungal feeders), plant feeders (obligate or facultative ones), omnivores, and predators of other nematodes. Thus nematode community structure integrates a lot of information on the soil micro-food-web: microbial compartment (fungal and bacterial parts), microfauna and mesofauna, which are responsible for the decomposition and mineralization of soil organic matter hence nutrient release (Freckman et al., 1997; Ekschmitt et al., 2001; Villenave et al., 2004; Sanchez-Moreno and Ferris, 2007).

Characterization of soil nematofauna involves extraction from soil samples by elutriation then counting and identification to genus or family level under microscope (ISO, 2007). This method, if not expensive, is time-consuming and requires expertise in taxonomy, which impedes the study of large numbers of samples. Molecular methods are being developed (Perry and Jones, 1998; Floyd et al., 2002); though they are currently used to detect some specific taxa or in population dynamics studies, they are not sufficiently efficient yet to allow quantification of all taxa present in soil (Donn et al., 2008). To our knowledge, spectrometric approaches have not been tested to date.

Near infrared reflectance (NIR) spectroscopy (NIRS) is a physical non-destructive, rapid, reproducible and low-cost approach that characterizes materials according to their reflectance in the wavelength range between 800 and 2500 nm (Roberts et al., 2004). The analysis of NIR spectra relies on calibration, which in general is a multivariate regression procedure that expresses a given property, determined using a conventional method, as a function of absorbance at all or selected wavelengths of the NIR region. The calibration equation can then be used to predict that property on new samples from their NIR spectra only, the acquisition of which is time- and cost-effective (<1 min per sample, no consumables required). The application of NIRS to soil has been mentioned from the 1960s (Bowers and Hanks, 1965) and it has been used extensively to determine soil content in carbon and nitrogen (Al-Abbas et al., 1972; Chang et al., 2001; Barthès et al., 2006). It has also proven useful for characterizing soil fractions such as NMR species (i.e. alkyl, O-alkyl, carboxylic and aromatic C; Terhoeven-Urselmans et al., 2006), organic size fractions (Barthès et al., 2008) and microbial biomass (Palmborg and Nordgren, 1993; Chang et al., 2001), as well as microbial biomarkers based on phospholipid fatty acids (Zornoza et al., 2008) and microbial activities such as carbon and nitrogen mineralization (Palmborg and Nordgren, 1993; Chang et al., 2001; Terhoeven-Urselmans et al., 2006). However, few attempts have been made to apply quantitative NIRS for characterizing soil fauna.

The present study aimed at assessing the usefulness of soil NIR spectra for determining the abundance and functional diversity of soil nematodes in a clayey Ferralsol under soybean/rice rotation in the Madagascar highlands.

2. Materials and methods

2.1. Studied site and sample collection

The studied site was located at Bemasoandro, near Antsirabe, in the Madagascar highlands (19°47′ S, 47°06′ E, ca. 1600 m a.s.l.). The climate is altitude tropical; mean annual rainfall and temperature are 1300 mm and 16 °C respectively. The soil is developed on volcano–lacustrine alluvia (Raunet, 1981) and classified as andic Dystrustept (Soil Survey Staff, 2003). It is clayey, acidic (>70% clay and pH ≈ 5 in the topsoil) and includes kaolinite, and to a lesser extent, gibbsite, quartz, hematite, and goethite. The topsoil contains ca. 8–9% organic matter at 0–10 cm depth.

The studied experiment was installed in 1996 by the French agricultural research centre CIRAD (Centre de coopération internationale en recherche agronomique pour le développement), the Malagasy NGO TAFA (i.e. Land and development) and the FOFIFA (i.e. National centre of applied research for rural development). The cropping system was a soybean/rice rotation (one crop per year) with either manual ploughing using a large spade (called angady) or no tillage, crop residues being removed in the former case but returned to the soil surface as mulch in the latter. Each plot was divided into three subplots with either no inputs, bovine manure application (5 Mg ha^{-1} yr^{-1}), or both mineral fertilizer (70N–30P–40K for rice and 30N–30P–40K for soybean) and bovine manure application (5 Mg ha^{-1} yr^{-1}). The six tillage × input treatments were replicated three times, resulting in 18 elementary plots, 13.5 m^2 each. Further information on the site and experiment has been provided by Razafimbelo (2005).

Topsoils were sampled during the rainy season, in January 2005 under soybean and in February 2006 under rice (Villenave et al., 2009a). Three composite soil samples were collected at 0–5 cm depth toward the upper part, the middle and the lower part of every elementary plot, yielding 54 composite samples yearly. Each composite sample resulted from the grouping and thorough mixing of five neighboring samples, collected using 100-cm^3 cylinders. An aliquot of every composite sample was air-dried then gently crushed to pass a 2-mm sieve. Due to the loss of some samples, a total set of 103 samples was studied.

2.2. Morphological characterization of soil nematofauna

Soil nematofauna was analyzed following a standardized procedure ISO 23611-4 (ISO, 2007). For each of the 103 fresh samples, nematodes were extracted from approximately 150 g of wet soil by elutriation followed by an active pass through a filter for 48 h at room temperature, according to the Seinhorst method (Seinhorst, 1962); they were then counted using a binocular microscope. After fixing in a formalin–glycerol mixture and transferring to mass slides, the composition of soil nematofauna was determined at family or genus level through microscopic observation at 400× magnification. On average, 111 nematodes were identified per mass slide. The nematode taxa were then assigned to trophic groups modified from Yeates et al. (1993): bacterial feeders (BF), fungal feeders (FF), facultative plant feeders (FPF), obligate plant feeders (OPF), and omnivores and predators (OMPR). Nematodes were also allocated to colonization–persistence (c–p) classes following Bongers (1990): the colonization–persistence scale ranges from 1 (colonizers) to 5 (persisters); it varies within trophic groups, so that combination between trophic groups and c–p classes defines feeding guilds such as BF_1 (bacterial feeders with a c–p class of 1) and FF_4 (fungal feeders with a c–p class of 4). In addition, two nematode ecological indexes were calculated after Ferris et al. (2001):

$$\text{Enrichment index}: \quad EI = 100 \times \{e/(e+b)\}$$

$$\text{Structure index}: \quad SI = 100 \times \{s/(b+s)\}$$

where e, b, and s are the sum of weighted abundances of guilds BF_1 and FF_2 (enrichment component), BF_2 and FF_2 (basal component), and BF_{3-5}, FF_{3-5} and $OMPR_{2-5}$ (structural component), respectively. Also, the Maturity index (MI) and the Plant parasitic index (PPI) were calculated as $\Sigma v_i p_i$, where v_i is the c–p value assigned to the family i and p_i its relative abundance in the sample, considering only free-living nematodes for MI and plant-feeding nematodes for PPI (Bongers, 1990).