



# Determination of rice root density at the field level using visible and near-infrared reflectance spectroscopy



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## ABSTRACT

To improve our understanding of biomass allocation and soil carbon cycling in paddy fields, information on the root biomass of rice is fundamental. The commonly used conventional methods to study root biomass are difficult and time-consuming. In this study, visible and near-infrared (Vis–NIR) reflectance spectroscopy (350–2500 nm) was evaluated for the rapid analysis of rice root density from the flat, horizontal surface of a soil core section. A total of 123 intact soil cores (0–40 cm depth, with a 8.4 cm diameter) were collected from paddy fields in Yujiang, China, and the Vis–NIR reflectance was measured in the laboratory at two depths (5 and 10 cm). Three multivariate regression methods (principal component regression, PCR; partial least square regression, PLSR; and support vector machine regression, SVMR) were used to develop models to predict root density, and these models were compared to determine the one with the most accurate predictions. Based on the comparisons with both cross-validation and independent validation data sets, the SVMR model outperformed the PCR and PLSR models. The predictions of root density with the SVMR model were more accurate (coefficient of determination [ $R^2$ ] = 0.88; root mean square error [RMSE] = 4.28; ratio of standard deviation to RMSE [RPD] = 2.83; ratio of performance to inter-quartile distance [RPIQ] = 3.23; Slope = 0.80) than those with the PCR model ( $R^2$  = 0.81; RMSE = 5.58; RPD = 2.17; RPIQ = 2.47; Slope = 0.74) or the PLSR model ( $R^2$  = 0.82; RMSE = 5.22; RPD = 2.32; RPIQ = 2.65; Slope = 0.76), based on 73 independent validation samples. In conclusion, the Vis–NIR spectra acquired from the intact soil cores were used to accurately predict rice root density. Therefore, Vis–NIR spectroscopy combined with SVMR calibrations has the potential to detect root densities in paddy soils under field conditions.

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## 1. Introduction

As the concentration of carbon dioxide in the atmosphere increases, increasing the carbon stocks in agricultural soils to mitigate climate change and to also improve soil quality has received new attention (Lal, 2004; Bolinder et al., 2007). The carbon stocks in soils at both short- and long-term time scales are determined by the difference that exists between inputs from aboveground and belowground biomass and outputs through erosion and decomposition of plant material and soil organic matter (SOM) (Almagro et al., 2010). In cropping systems, because most of the aboveground biomass of field crops is harvested for grain, feed, fiber, or biofuels, the belowground biomass (particularly crop roots) becomes the primary contributor to soil carbon stocks (Mokany et al., 2006; Bolinder et al., 2007). Patterns of aboveground biomass distribution in terrestrial ecosystems are reasonably well understood, whereas the understanding of patterns of belowground biomass distribution remains limited; this limited understanding

is essentially due to methodological difficulties associated with collecting and measuring root biomass (Vogt et al., 1998; Mokany et al., 2006). Therefore, belowground biomass is a major source of the uncertainties in large-scale biomass estimations and soil carbon cycles (Bolinder et al., 2007). Rice is the staple food for approximately 50% of the global population, and paddy fields occupy 12% of the global cropland area (FAOSTAT, 2013). China is one of the major rice producers with more than 25 million hectares of paddy fields in production, which are 29% of the cultivated lands of China and 23% of those of the world (Ji et al., 2015). Consequently, precise estimates of rice root density are essential to improve understanding of root turnover and the influence on soil carbon dynamics.

Root biomass has been assessed previously with a range of direct and indirect methods, including soil excavation, sequential soil coring, in-growth cores, rhizotrons, minirhizotrons, and whole-system budgets (Jackson et al., 1996; Vogt et al., 1998; Bolinder et al., 2007; Almagro et al., 2010). However, these approaches are tedious and labor-intensive because roots must be separated from soils or because specific instrumentation is required (e.g., transparent tubes, digital photographic equipment and a flatbed scanner). Moreover, a general bias is typically found in the results from these different methods because of the method or

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the characteristics of the roots under observation (Jackson et al., 1996; Vogt et al., 1998). Currently, the measurement of root biomass has not been standardized for different ecosystems (Vogt et al., 1998), and therefore, accurate estimations of root biomass remain problematic and a technical challenge.

Visible and near-infrared reflectance spectroscopy (Vis–NIR) is a physical, nondestructive, rapid, reproducible, and low-cost method to characterize materials according to their reflectance in the wavelength range from 350 to 2500 nm (Bellon-Maurel and McBratney, 2011; Conforti et al., 2015). The absorption bands observed in Vis–NIR spectra are a measure of the vibrations of C–O, O–H, C–H, and N–H bonds, in addition to the overtones and combinations of these vibrations (Viscarra Rossel et al., 2006). Vis–NIR has been used in the laboratory as an effective alternative for the prediction of various soil properties including SOM, total N, pH, cation exchange capacity (CEC), and soil texture (Ben-Dor and Banin, 1995; Brown et al., 2005; Viscarra Rossel et al., 2006; Vasques et al., 2008, 2009, 2010; Bellon-Maurel and McBratney, 2011; Conforti et al., 2015). Vis–NIR has also been used successfully to analyze plant materials (Curran, 1989; Foley et al., 1998; Serrano et al., 2002; Stenberg et al., 2004; Chodak, 2008), such as their chemical compositions (Elvidge, 1990; Gillon et al., 1993, 1999; Coûteaux et al., 2005; Parsons et al., 2011), proportions in mixtures of roots (Roumet et al., 2006; Lei and Bauhus, 2010), and crop residue cover (Daughtry, 2001; Serbin et al., 2009). Vis–NIR has been used to estimate the plant root density in pasture and dryland soils (Rumbaugh et al., 1988; Picon-Cochard et al., 2009; Kusumo et al., 2010, 2011), but there is no published study reporting on the Vis–NIR prediction of rice (*Oryza sativa* L.) root density in paddy soils.

Because of the broad and overlapping bands in the Vis–NIR spectra, multivariate statistics are required to mathematically extract complex absorption patterns and to correlate these patterns with reference values for calibration (Martens and Næs, 1989; Stenberg et al., 2010). Partial least square regression (PLSR) is one of the most commonly used techniques (Vasques et al., 2008; Viscarra Rossel and Behrens, 2010; Conforti et al., 2015). However, other multivariate methods are also used (Chang et al., 2001; Vasques et al., 2008; Mouazen et al., 2010; Bellon-Maurel and McBratney, 2011; Vohland et al., 2011), for example, stepwise multiple linear regression (SMLR), principal component regression (PCR), and nonlinear techniques such as artificial neural networks (ANN), regression trees (RT), and support vector machine regression (SVMR). Specifically for soils, the Vis–NIR spectra provide large sets of potential predictor variables; however, the benefit of covering the complete spectra with high spectral resolution is compromised by multi-collinearity and noise (Vohland et al., 2011). Therefore, the selection of the multivariate calibration method and the ability of the method to interpret the spectra data are essential factors for successful predictions (Mouazen et al., 2010).

This study was conducted in Yujiang County of Jiangxi Province to (i) determine whether Vis–NIR spectroscopy could predict root density in cores collected from paddy fields and (ii) compare the performance of three multivariate calibration models (PCR, PLSR, and SVMR) for the estimation of rice root density from Vis–NIR spectra. Finally, effective wavelengths for were explored to predict root density.

## 2. Materials and methods

### 2.1. Study area and soil sampling

Yujiang County (116°41′–117°09′ E, 28°04′–28°37′ N) is located in the transition zone from the northeastern hilly area to the Poyang Lake Plain in Jiangxi Province, China, and covers 927 km<sup>2</sup>. The hills and plains cover 78% and 22% of the county, respectively. The dominant parent materials include red sandstone, shale, river alluvium, and Quaternary red clay (Soil Survey Office of Yujiang County, 1986). The soils developed from these parent materials are predominantly red soils (acrisols; IUSS Working Group WRB, 2014) and paddy soils (anthrosols;

IUSS Working Group WRB, 2014), which together occupy over 90% of the total area in the county. In the area from which the soil cores were collected, the farmland was 87% paddy field. The major cropping system is double-season rice that is planted by seedling throwing or manual transplanting.

The paddy fields were sampled during the first 2 weeks of November 2014 (following rice harvest). A total of 123 geo-referenced sample sites were selected using a stratified sampling design based on different parent materials and rice varieties. At each site, an intact soil core (8.4 cm internal diameter and 40 cm long) was sampled using a closed sampler with a gasoline-powered percussion hammer (Eijkelkamp, Giesbeek, Gelderland, Netherlands). The soil cores were stored in sealed PVC tubes and transported to the laboratory. To remove some of the soil moisture, the cores were air dried on a laboratory bench for several days before the measurements.

### 2.2. Sample preparation and spectral measurement

Rice is a shallow-rooted crop with 90% of root biomass concentrated in the upper 15 cm soil layer, and about 75% of the roots were found in the top 10 cm soil (Sharma et al., 1987; Jackson et al., 1996). Therefore, to facilitate the cutting of slices, soil reflectance spectra were recorded on two horizontal surfaces (5 and 10 cm) of the soil core that were cut with a utility knife (Fig. 1a). The top 1.0 cm section (Slice A) of each surface was sliced to measure root biomass using a wet-sieve method (Almagro et al., 2010), and the soil slice was gently rinsed through a series of three successively smaller mesh sieves (2.0, 1.0, and 0.5 mm). The roots were handpicked from the sieves and placed in a tray. The remaining soil (<0.5 mm) was mixed with tap water, and the floating roots were decanted onto a 0.2-mm mesh sieve. The flotation procedure was repeated until no roots floated to the surface. The material collected on the 0.2-mm sieve was added to the tray, and any material not derived from roots was removed with tweezers. The root samples were first air dried in paper bags and were then oven dried at 70 °C to a constant weight. From each core surface, a further 1.0 cm section (Slice B) was cut and oven dried (105 °C for 48 h) to measure soil moisture content and bulk density. The root density of slice A was expressed as mg dry root g<sup>-1</sup> dry soil.

Immediately after the slices were removed, each flat surface was measured with an ASD FieldSpec 3 portable spectroradiometer (Analytical Spectral Devices, Boulder, Colorado, USA) with a spectral range of 350 to 2500 nm. The spectral resolution of the spectroradiometer was 3 nm for the region 350–1000 nm and 10 nm for the region 1000–2500 nm. The radiometer bandwidth was 1.4 nm from 350 to 1000 nm and was 2 nm from 1000 to 2500 nm. To acquire the diffuse reflectance spectra of the soil samples, an ASD high-intensity contact probe with a built-in light source (6.5 W halogen lamp) and a 2-cm-diameter circular viewing window was attached by a fiber-optic cable to the spectroradiometer (Fig. 1b). The probe was fixed horizontally to a burette stand with a clamp and was placed in direct contact with the soil surface to minimize the errors associated with stray light during measurements (Waiser et al., 2007). To measure the variation of each sample, nine replicate scans of each sample surface (with each integrating 10 observations) were conducted after adjustment of the location with a rotation of the soil core (Fig. 1c). The ninety readings were averaged to generate a representative spectral signature for each soil core. The sensor was calibrated with a Spectralon (Labsphere, North Sutton, NH) white reference for each soil core to convert the measured radiance values into relative reflectance values.

Additionally, samples with standardized root contents were prepared to explore the spectral differences between soil and rice roots, excluding the impact of moisture and particle size. Oven-dried soils (105 °C for 48 h) and rice roots (70 °C for 48 h) collected from the sample sites were mixed to create nine composite samples with root contents of 0, 1%, 2%, 3%, 4%, 5%, 8%, 10% and 100%. Both soils and roots were ground and passed through a 2-mm sieve before oven

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