



Development of a novel image analysis procedure to quantify biological porosity and illuvial clay in large soil thin sections



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ABSTRACT

Among other driving forces, climate change may lead to significant modifications of soil properties through variations of the intensity and dynamics of a number of soil processes including the especially sensitive and ubiquitous processes of bioturbation and illuviation. Progress toward the quantification of these processes needs to be made as a first step in order to ascertain or predict the impact of climate change. In this study, we develop, and validate through an exhaustive accuracy assessment, a digital 2D image analysis method adapted to large soil thin sections, leading to the quantification, characterization and classification of pores and illuvial clay features considered diagnostic of bioturbation and illuviation, respectively. The need to consider large soil thin sections (14 × 6 cm) stems from observations that areas from 684 mm² to more than 5 000 mm² are needed to obtain representative measurements of fragments of illuvial clay (papules) and porosity associated with earthworms respectively. Whereas the soil heterogeneity in thin sections of such a large size prohibits the use of classical global and colorimetric image analysis procedures, we succeeded to quantify and characterize the porosity and illuvial clay features with accuracies as high as 96% for porosity and 92% for illuvial clay by carefully considering different levels of soil organization from aggregates to individual illuvial clay features. Quantification of the size and the shape of pores and of illuvial clay features makes it possible to develop morphological classifications distinguishing pores of biological origin (earthworms or roots) from others, or clay coatings and infillings from papules. All these data are particularly useful to better characterize underlying soil processes. Results suggest that the proposed methodology provides an accurate and representative quantification of biological porosity and illuvial clay features in large thin sections. We argue that the use of this approach could be extended to study and quantify bioturbation and illuviation intensities at the profile scale.

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1. Introduction

In the last few decades, a number of authors have argued that climate change and the associated modifications of man's use of landscapes have already affected the rate and direction of Holocene pedogenesis, leading to significant alterations of soil properties (e.g., Macphail et al., 1987; Richter, 2007). Among them, the amount and the distribution with depth of the clay-sized fraction is of particular interest as this fraction is a key-compartment of soils involved in most of soil functions (Cornu et al., 2012). In temperate climates, where translocation processes generally prevail over the formation/dissolution/transformation ones (Cornu et al., 2012), distribution with depth

of the clay-sized fraction is principally governed by bioturbation and eluviation/illuviation processes.

By increasing litter or crop residues, as well as root mass and organic matter content, climate change may stimulate the activity of soil macrofauna (i.e., bioturbation), with consequent increases in infiltration rate and bypass flow due to the larger numbers of biopores (Brinkman and Sombroek, 1996; Rounsevell et al., 1999). In addition, the expected increase in precipitation over the next 50–100 years in northern Europe (IPCC, 2001) might result in an increasing amount of water infiltrating into soils and thus in more intense lixiviation or eluviation (Rounsevell et al., 1999). In that context, Montagne and Cornu (2010), using agricultural soil drainage as an analogy to climate change, have suggested that eluviation intensity, i.e., the amount of fine particles up to 10 μm that are transferred vertically, increases as a result of climate change.

To understand better the possible effect of climate change on bioturbation and eluviation/illuviation processes, especially given the fact that i) they are both sensitive to climate change, and ii) eluviation has

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proven able to respond faster than initially expected (Montagne and Cornu, 2010), it is crucial to be able to quantify their intensity precisely (Rounsevell et al., 1999). In that respect, morphological studies present a great interest (Montagne et al., 2007). Bioturbation process can be identified and quantified by analyzing the extent of the porosity associated with burrowing activity (Bruneau et al., 2004; Lamandé et al., 2003) or by measuring the extent of casting activity (Davidson, 2002; Davidson and Grieve, 2006). By quantifying illuvial clay features such as clay coatings in soil thin sections, Jongmans et al. (1991), McKeague et al. (1980), Miedema and Slager (1972) and Sauzet et al. (2016) have generated several quantitative data about the degree of illuviation.

Starting in the 1940s, research on quantitative soil micromorphology has been based either on visual estimates with reference to appropriate empirical charts of (e.g., Bullock et al., 1985; Thompson et al., 1990) or on counting procedures. Point counting used to be the most common procedure to determine frequency (modal analysis) but it is considered tedious and error prone (Aydemir et al., 2004; Marschallinger, 1997). Both quantitative techniques tend to be too operator dependent (Ulery and Drees, 2008; Zhang et al., 2014). With the 1970s, digital image processing has been developed and used for quantitative micromorphology as it seems to be potentially superior over point counting in accuracy and speed of data extraction (Grove and Jerram, 2011). Image analysis methods moreover afford distinct advantages over conventional microscopic ones in that they can provide a range of previously unobtainable quantitative information including measurements of morphological aspects and spatial relationships of different soil components (Bielders et al., 1996; Bryant and Davidson, 1996; Gargiulo et al., 2013; Taina and Heck, 2010).

While porosity has been studied extensively, and researchers have succeeded in characterizing and classifying pores according to different morphological attributes, only three studies deal with the characterization and quantification of clay coatings by image analysis to our knowledge (Protz and VandenBygaart, 1998; Terribile and Fitzpatrick, 1992, 1995). None of these articles, however, proposes a detailed and quantitative characterization of illuvial clay features.

Due to technical difficulties during sampling and because manufacturing larger thin sections resulted in larger variations in thickness within single slides (Simpson et al., 2003), biological activity and illuviation have been generally quantified on small areas (e.g., 10×10 mm for Cassagne et al. (2008) or 10.7×6.4 mm for Bruneau et al. (2004)). Such sizes are in most cases smaller than the representative elementary areas (REA), leading to variability in the results (Bruneau et al., 2004). Some authors succeeded in quantifying porosity on larger images (70×50 mm for Hallaire et al. (1997) or 90×90 mm for Moran et al. (1988)) but they worked at a low resolution (90 to $176 \mu\text{m}$ per pixel), unsuitable for the study of a large part of the soil porosity and for most illuvial clay features. Clay coatings are indeed said to be 60 or $200 \mu\text{m}$ thick (Dalrymple and Theocharopoulos, 1987; Thompson et al., 1990). In order to have accurate and representative measurements, high resolution image mosaics covering a large contiguous area is required (Adderley et al., 2002; VandenBygaart and Protz, 1999).

In the work carried out to date, only a few authors have tried to validate their image analysis protocol. They have often compared their results with point counting ones (Grove and Jerram, 2011; Terribile and Fitzpatrick, 1995), whereas Protz et al. (1992) proposed direct visual assessment, and Terribile and Fitzpatrick (1992), a comparison with a reference image.

In that general context, the main objective of our study is to develop and validate a novel image analysis procedure in order to identify and quantify pores and illuvial clay features considered as diagnostic features of bioturbation and illuviation processes, respectively. The procedure is adapted to large thin sections (i.e., larger than the representative elementary area of the quantified parameters), and it is validated through an exhaustive accuracy assessment procedure commonly

used in remote sensing studies (e.g., Laba et al., 2008), and recently adopted in the analysis of soil images (Baveye et al., 2010). The proposed procedure is used subsequently to i) determine the REA for porosity and illuvial clay features, ii) quantify the size and the shape of illuvial clay features, and finally iii) characterize the spatial variability of porosity and illuvial clay at the meter scale.

2. Materials and methods

2.1. Study area, sampling and thin section preparation

The soil used in the research is classified as a Luvisol (WRB, 2014) of approximately 1.5 m depth that has developed on decarbonated quaternary loess deposits (more details in Sauzet et al., 2016). This Luvisol is under conventional agricultural management (CULT) with a maize-wheat crop succession and is part of the QualiAgro long-term field experiment located on the Plateau des Alluets (Yvelines, France). This soil presents the typical horization of a Luvisol according to WRB (2014), namely: Ap E Bt C. The ploughed layer contains 14% clay, 79% silt, and 7% sand whereas the Bt horizon exhibits the diagnostic clay content increase and is composed of 31% clay, 64% silt, and 5% sand. After having dug a soil pit down to 2 m in CULT in April 2011, a $150 \text{ mm width} \times 80 \text{ mm height} \times 50 \text{ mm depth}$ Kubiena box (see Sauzet et al., 2016) was used to extract an undisturbed soil sample in the core of the Bt-horizon (60–70 cm depth), to maximize diversity and to obtain a significant number of pores and illuvial clay features. This Kubiena box was gently oven-dried at 30°C for no more than two days and impregnated under vacuum with polyester resin. After polymerization, three thin sections of $140 \text{ mm width} \times 60 \text{ mm height}$, spaced 30 to 50-mm apart, and around $25\text{-}\mu\text{m}$ thickness, hypothesized to be of a larger size than the representative elementary areas of pores and illuvial clay features, and called CULT 60–70 R1, CULT 60–70 R2, CULT 60–70 R3, respectively, were prepared following the procedures of Guilloiré (1985). In order to analyze the spatial variability of our measurements, three supplementary Kubiena boxes were taken laterally at regular 0.5 m intervals in the soil pit, and in each case one thin section was manufactured. These additional thin sections are labeled as CULT 60–70_0.5, CULT 60–70_1, and CULT 60–70_1.5.

2.2. Image acquisition

Each thin section was photographed under transmitted light by using an optical microscope Leica DM5500B with an automated stage, combined with a digital camera. The automation of the picture taking was achieved by using the dedicated Power Mosaic software (Leica Microsystems, Wetzlar, Germany). The whole procedure of mosaicing over a duration of twenty minutes allowed 576 pictures to be taken for each thin section.

Eventually, we obtained 6 images of $22,024 \times 9224$ pixels with a spatial resolution of $5.3 \mu\text{m}$ and with a color depth of 2^{24} (RGB, i.e., Red, Green and Blue bands). Images were cropped in order to avoid any edge effect. Final images have a size of $21,725 \times 8924$ pixels, corresponding to an area of 5475 mm^2 .

2.3. Image analysis procedure

2.3.1. Porosity

As porosity clearly appears as contrasted areas in brightness, each RGB color image was transformed to a unique luminance band image (Figs. 1, 2a and b). The gray-level distributions obtained are bimodal, i.e., each image histogram is well structured by two well-defined peaks, related respectively to pore or solid space (Marschallinger, 1997). Automatic triangle thresholding (Fig. 1) was used to segment the image between voids and non-voids according to the typical bimodal configuration of the gray level histogram distribution (Zack et al., 1977). A binary image was then obtained with voids defined as 1 and

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