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Impact of roots, mycorrhizas and earthworms on soil physical properties as assessed by shrinkage analysis

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S U M M A R Y

Soil biota such as earthworms, arbuscular mycorrhizal fungi (AMF) and plant roots are known to play a major role in engineering the belowground part of the terrestrial ecosystems, thus strongly influencing the water budget and quality on earth. However, the effect of soil organisms and their interactions on the numerous soil physical properties to be considered are still poorly understood. Shrinkage analysis allows quantifying a large spectrum of soil properties in a single experiment, with small standard errors. The objectives of the present study were, therefore, to assess the ability of the method to quantify changes in soil properties as induced by single or combined effects of leek roots (*Allium porrum*), AMF (*Glomus intraradices*) and earthworms (*Allolobophora chlorotica*). The study was performed on homogenised soil microcosms and the experiments lasted 35 weeks. The volume of the root network and the external fungal hyphae was measured at the end, and undisturbed soil cores were collected. Shrinkage analysis allowed calculating the changes in soil hydro-structural stability, soil plasma and structural pore volumes, soil bulk density and plant available water, and structural pore size distributions. Data analysis revealed different impacts of the experimented soil biota on the soil physical properties. At any water content, the presence of *A. chlorotica* resulted in a decrease of the specific bulk volume and the hydro-structural stability around 25%, and in a significant increase in the bulk soil density. These changes went with a decrease of the structural pore volumes at any pore size, a disappearing of the thinnest structural pores, a decrease in plant available water, and a hardening of the plasma. On the contrary, leek roots decreased the bulk soil density up to 1.23 g cm^{-3} despite an initial bulk density of 1.15 g cm^{-3}. This increase in volume was accompanied with a enhanced hydro-structural stability, a larger structural pore volume at any pore size, smaller structural pore radii and an increase in plant available water. Interestingly, a synergistic effect of leek roots and AMF in the absence of the earthworms was highlighted, and this synergistic effect was not observed in presence of earthworms. The structural pore volume generated by root and AMF growth was several orders of magnitude larger than the volume of the organisms. Root exudates as well as other AMF secretion have served as carbon source for bacteria that in turn would enhance soil aggregation and porosity, thus supporting the idea of a self-organization of the soil–plant–microbe complex previously described.

Keywords: Shrinkage analysis (ShC), Soil porosity, Earthworms, Arbuscular mycorrhizal fungi (AMF), Plant root, Soil structure

Introduction

As an interface between lithosphere and atmosphere, the soils control the earth water budget *via* its physical properties which determine the runoff and infiltration fractions. Water quality is strongly influenced by infiltration through the soil as well. These properties result from the equilibrium between constituents, soil life, and external factors, which vary on different time and space scales. Characterizing and predicting soil physical properties and their changes with time as a function of these factors are essential. Therefore, integrated approaches aiming at better understanding interactions between physical and biological processes in the soil and with the aboveground system are encouraged (e.g. Young and Crawford, 2004).

Many authors described soil–biota interactions based on the hierarchical aggregation model developed by Tisdall and Oades (1982) who emphasized the importance of bacteria, fungi and roots in soil aggregation (Brussaard et al., 2007; Dorioz et al., 1993; Feeney et al., 2006; Jastrow and Miller, 1991; Six et al., 2004). For example, the physical habitat of soil bacteria in terms of aggregate (Caesar-TonThat et al., 2007; Mummey et al., 2006; Ranjard and Richaume, 2001) or porosity (Feeney et al., 2006) was investigated and led to the concept of a *self-organization of the soil–microbe complex* (Young and Crawford, 2004). This concept assumes that soil structure initially defines microbial communities

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but is in turn modified through active microbial activities that alter pore geometry and stability. In addition, roots are known to modify the soil porosity and aggregation *via* direct entanglement of particles, the creation of biopores or secretion of glue-substances sticking particles together as reviewed by Angers and Caron (1998). Similar to plant roots, AMF are a very important component of the soil system. They influence soil aggregation by binding and enmeshing soil particles into larger aggregates. They also secrete a glycoprotein called glomalin that act as a glue-substance (see Rillig and Mummey, 2006 for a review). Earthworms, as ecosystem engineers (Lavelle et al., 1997), play subsequently a very important bioturbation role on soil structure through numerous ways such as casting and burrowing activities, and the transit of the soil through their digestive system, thus promoting the formation of the organo-mineral complex (Brown et al., 2004).

Describing the various effects of soil life on soil physical properties is a challenge. First, because of the numerous properties to be determined, e.g. soil volume or density, soil structure and structural stability, soil pores and aggregate size distributions, water retention curve (WRC), hydraulic conductivity, mechanical properties, each property requiring a specific characterization technique. Second, because the determination must be accurate enough to assess the changes, while most physical properties have a large variability (e.g. Gascuel-Odoux, 1987; Nielsen et al., 1973; Sisson and Wierenga, 1981; Vauclin, 1982).

The recent development of soil shrinkage analysis might overcome these limitations with determining in a single experiment many soil physical properties, namely hydro-structural stability (Schaffer et al., 2008), soil structural and plasma pore volumes, pore volume change, soil water holding capacity and water retention curves (WRC) (Boivin et al., 2006a; Braudeau et al., 1999, 2004) with small standard errors (Boivin, 2007). Soil shrinkage was defined as the soil specific volume change with water content (Haines, 1923). It has been used to assess soil structural stability and soil pore volume (Boivin et al., 2006a; Braudeau et al., 1999, 2004). Shrinkage analysis is based on the simultaneous and quasi-continuous measurement of soil shrinkage curve (ShC) and WRC (Boivin et al., 2004), and the analysis of the ShC with XP (for exponential) model (Braudeau et al., 1999) or the equivalent PS (for Pedostructure) model (Braudeau et al., 2004). XP/PS models are based on the assumption that there is a dual pore system in the soil (Braudeau, 1988b; Braudeau et al., 2004). Fitting XP model equations on an experimental ShC allows determining the volume, air and water content of these two pore systems at any soil water content, and the slopes of the shrinkage domains which can be considered as measures of the soil hydro-structural stability (Schaffer et al., 2008). It has been shown that the two pore systems quantified by shrinkage analysis are the plasma pores and the structural pores, respectively, long ago characterized by micromorphologists (Brewer, 1964). Plasma pores are made of the soil colloids (SSSA, 2008) and assumed to shrink like a clay paste, i.e. with no air entry on most of the water content range. The structural pores are made of biopores, lacunar voids between plasma and skeleton, and cracks. Structural pores are assumed to be semi-rigids, hence air entry is partly compensating the loss of water in the structural pores, when the soil is drying.

Shrinkage analysis has been applied to assess the impact of clay content and clay type on soil properties (Boivin et al., 2004), the impact of soil organic carbon on soil physical properties (Boivin et al., 2009), and the impact of trafficking on soil pore properties (Boivin et al., 2006b; Schaffer et al., 2008). Shrinkage analysis, however, has not been used yet to assess the physical impact of soil biota on soil.

The objective of this study was to test the potential of shrinkage analysis to assess changes in soil properties as induced by three model organisms, namely leek roots (*Allium porrum* L.), an arbuscular mycorrhizal fungus (*Glomus intraradices* Schenk and Smith) and an earthworm (*Allolobophora chlorotica* Savigny) in a microcosm experiment.

Materials and methods

Experimental setup, plant, mycorrhiza and earthworm

The organo-mineral horizon of an Anthrosol (IUSS, 2006) was collected at the botanical garden of Neuchâtel (Switzerland). The soil is a carbonated loamy soil (45.3% sand, 28.0% silt and 26.7% clay), containing 20.7% (w:w) carbonates, 2.0% (w:w) total organic carbon and showing a pH_{KCl} of 7.8. The CEC per kg of soil was 21.3 $cmol_c\ kg^{-1}$. A compartmental microcosm design was set up. It consisted of a PVC tube (35 cm height and 15 cm internal diameter) separated vertically into two equal parts by a nylon mesh (25 μm) to separate the individual effect of AMF from the root effect as roots could not pass through the mesh.

The soil was air-dried, sieved to 2 mm size aggregates, homogenized and gamma-ray sterilised (between 42 and 82 kGy) prior to repacking in each side of microcosms with six successive 5 cm thick layers of soil remoistened at 22% water content. Microcosms had a final bulk density of 1.15 $g\ cm^{-3}$. Afterwards, a 20 ml soil suspension (100 g of soil dispersed in 1000 ml of autoclaved distilled H_2O and filtered on 11 μm paper) was added to re-inoculate the sterilized soil with microorganisms, but without AMF (Koide and Li, 1989).

We applied a factorial design with three factors and one replicate of each treatment. The treatments were all the possible combinations of the presence/absence of three factors, thus involving four repetitions of each factor. These factors were leek (*A. porrum* var. Mercure, 18 days old, sown in sterilised conditions), AMF (*G. intraradices,* 30 g of spores and hyphae per microcosm), and endogeic earthworms (*A. chlorotica*, five individuals of equal biomass (1.3 ± 0.1 g) added in each side of the microcosm). This corresponds to a density of 650 individuals m^{-2} which is 3.4 times higher than the density found for a single endogeic species (*Aporrectodea caliginosa*) sampled in a maize crop according to Le Bayon and Binet (1999). We selected an endogeic species because this kind of earthworms inhabit the organo-mineral soil horizon feeding on the soil organic matter closely linked to the mineral matrix; as a matter of fact, they consume more soil than other ecological categories to fulfil their nutritional requirements and consequently largely burrow within the upper centimetres of the soil (Lee and Foster, 1991; Capowiez, 2000).

The microcosms were kept 35 weeks in a climate chamber under the following conditions: photoperiod 16/8 h (day/night), temperature 18 ± 2 °C, 50% humidity. Irrigation was performed twice a week using a modified Hoagland's nutrient solution without phosphorus (Milleret et al., 2009) in order to promote the AMF-plant symbiosis. Every three weeks, each microcosm was weighted and adjusted to equal soil water content with deionised water.

Sampling

After 35 weeks, undisturbed soil cores of approximately 100 cm^3 volume were removed from each side of the microcosm (i.e. with or without roots) for soil shrinkage curve (ShC) and water retention curve (WRC) measurement.

Shrinkage analysis

Quasi-continuous ShC and WRC were determined on undisturbed sub samples of approximately 100 cm^3. The equipment and methods used are the same as presented in Boivin et al.

(2004) and Schaffer et al. (2008). Briefly, we wetted the soil samples with deionised water by applying a water potential of −1 kPa with respect to the centre of the samples.

During drying, the samples were placed on electronic balances (0.01 g precision) contained in a thermostatic chamber at 20 °C. Calibrated displacement transducers (resolution of 1 μm) were used to measure changes in sample height during drying. Tensiometers (ceramic cups; length 2.0 cm, diameter 0.2 cm) connected to pressure transducers were inserted in the middles of the samples to measure the water potential (resolution of 1 hPa). Weight, height and water potential were recorded at intervals of 5 min until the sample weights reached constant values, which took about 4 days. Then, the dry sample volumes were determined by means of hydrostatic weighing with the plastic bag method described by Boivin et al. (1990), and the samples were dried in an oven at 105 °C for 24 h to obtain the dry weight.

Changes in sample height were converted to changes in specific bulk sample volume by

$$V = V_E \times \left(\frac{H}{H_E}\right)^3, \quad (1)$$

where the exponent 3 denotes isotropic shrinkage (e.g. Boivin, 2007), V_E and H_E are the specific bulk volume and height at the end of the experiment, and V and H are the bulk volume and height during the experiment.

The XP model equations (Braudeau et al., 1999) were fitted to the experimental shrinkage data by a non-linear simplex method (Chen and Saleem, 1986) to determine the coordinates of the transition points between the shrinkage domains (Fig. 1), namely shrinkage limit (SL), air entry (AE), the dry point of structural porosity (ML), and the maximum swelling of the plasma (MS). The slope of the structural shrinkage domain K_{str} was calculated as:

$$K_{str} = \frac{[V(\mathrm{ML}) - V(\mathrm{MS})][\exp(1) - 1]}{[W(\mathrm{ML}) - W(\mathrm{MS})] - K_{Bs}[\exp(1) - 2]}, \quad (2)$$

where V_{ML}, W_{ML}, V_{MS} and W_{MS} are the volume and water content of the soil at MS and ML, respectively and K_{Bs} the slope of the basic domain calculated as:

$$K_{Bs} = \frac{V_{AE} - V_{ML}}{W_{AE} - W_{ML}} \quad (3)$$

where V_{ML}, W_{ML}, V_{AE} and W_{AE} are the volume and water content of the soil at ML and AE, respectively.

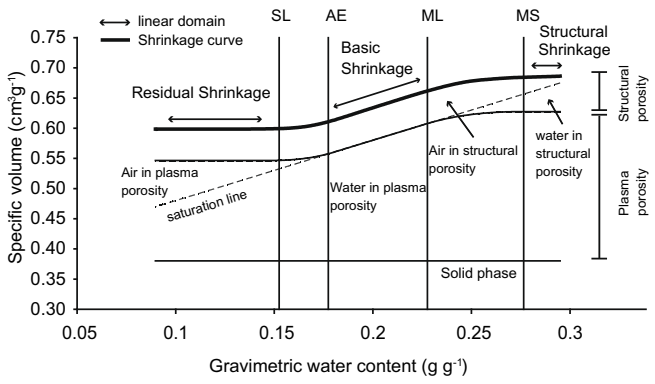

Fig. 1. Example of a shrinkage curve with the transition points (SL, shrinkage limit; AE, air entry; ML, macro-porosity limit; MS, maximum swelling), linear domains (residual shrinkage, basic shrinkage, structural shrinkage), and cumulated calculated specific volumes (from bottom to top: solid phase, water in plasma porosity, air in plasma porosity, water in structural porosity, air in structural porosity and bulk soil volume that is shrinkage curve) with saturation 1:1 line.

Using the XP model equations for the plasma porosity given by Braudeau and Bruand (1993), we then calculated the specific plasma porosity, V_p (in $cm^3\ g^{-1}$ of soil), and the plasma water content, W_p (in $g\ g^{-1}$ of soil). The specific air content of the plasma, A_p, was calculated as

$$A_p = V_p - W_p, \quad (4)$$

The specific structural porosity, V_s, was calculated as

$$V_s = V - V_p - \rho^{-1}, \quad (5)$$

where ρ^{-1} is the specific volume of the solid phase (set to $1/2.65\ cm^3\ g^{-1}$). The specific water content of the structural porosity, W_s, was calculated as

$$W_s = W - W_p, \quad (6)$$

where W is the total gravimetric water content. The specific air content of the structural porosity, A_s, was calculated as

$$A_s = V_s - W_s, \quad (7)$$

Bulk density was calculated as the inverse of the specific bulk volume, V. Plant Available Water (AW in $g\ g^{-1}$) content was determined as the difference between W at MS and AE, and Easily Available Water (EAW in $g\ g^{-1}$) was calculated as the difference between W at MS and ML (Braudeau, 1988a).

After ShC analysis, the undisturbed samples were broken up to measure the dry root weight in each soil sample (see below, root and AMF size distribution).

Structural pore size distribution

The simultaneous weight and tensiometer measurements were used to determine the water retention curves. Shrinkage analysis allowed calculating the plasma and structural pores water retention curves, by using the W_s and W_p values at any soil water content. We converted the structural pores water retention curves into the structural pore size distributions of equivalent cylindrical pores using the Jurin–Laplace equation (e.g. Lawrence, 1977). Since only the structural pores allow air entry in the tensiometer reading pressure range, we did not apply the procedure to the plasma pores.

Root and AMF size distribution

Specific root volume per class of root diameter (750, 375, 175 and 75 μm) in the microcosms were measured as follows. First, the dried root weight per class of root diameter was measured on the root network remaining in the microcosms after the soil core sampling as described by Blouin et al. (2007). Briefly, the roots were dried at 50 °C and cut in a variable speed rotor mill (Fritsch, Laval Lab Inc., Canada) with a 2 mm sieve in order to obtain pieces of 2 mm length. Roots were placed on a sieve shaker at continuous agitation for 20 min with five successive sieves (1 mm, 0.5 mm, 0.25 mm, 0.1 mm and 0.05 mm).

A potential problem with the employed method is that roots of a smaller size may stick on a larger sieve size because the roots fell horizontally on the sieve. We, therefore, tested the method for the leek root system by visual observation of the root diameter with a binocular. We observed homogeneous root fragments within each class of diameter which allowed us to apply the method of Blouin et al. (2007).

Root fraction in each sieve was then weighted and the ratio of the weight of each root diameter fraction on the total root weight was calculated. This ratio was used to calculate the dry root weight of each root diameter fraction contained in the soil cores from the total root weight of the cores, by taking into account the root weight in the undisturbed samples used for ShC analysis.

In parallel, fresh root fragments were individually weighed, scanned at high resolution and dried at 50 °C overnight. The fresh and dry length, diameter, surface area and volume of the scanned fragments were subsequently calculated using an image analysis program (Image J v.1.40, National Institute of Health, USA). This allowed to determine the following regression ($r^2 = 0.73$, $P < 0.001$, $n = 76$) in order to convert dry weight (DW) to dry volume (DV):

$$DV = 2.146\,DW + 0.002, \quad (8)$$

The dry volume was thereafter converted in fresh volume (FV) by using the following equation ($r^2 = 0.79$, $P < 0.001$, $n = 76$):

$$FV = 1.551\,DV + 0.001, \quad (9)$$

The fresh volume per class diameter was finally divided by the soil core weight to have the Specific fresh Root Volume (SRV) per gram of soil ($cm^3\,g^{-1}$) for each class diameter.

The Specific AMF external Hyphal Volume (SHV) per gram of soil was calculated using the hyphal length density (HLD) ($m\,g^{-1}$) measured as described in Milleret et al. (2009). HLD was determined by using an aqueous extraction and a membrane filter technique modified after Jakobsen et al. (1992). Briefly, three replicates of a 4 g soil sample were dispersed in a sodiumhexametaphosphate solution ($35\,g\,l^{-1}$) and shaken for 30 s (end–over–end). After 30 min, the suspension was decanted quantitatively through a 40 μm sieve to retain hyphae, roots and organic matter, transferred with 200 ml of deionised water into a 250 ml flask and shaken vigorously by hand for 5 s. After 1 min, 4 × 1 ml aliquots (10 s interval) were taken and pipetted onto Millipore RAWG02500 membranes (Millipore, Bedford MA, USA). The filter was finally stained in 0.05% Trypan Blue. HLD was estimated with a gridline intersect method at 250× magnification (Newman, 1966). HLD measurements allowed calculating the length of external hyphae in the cores. Combined with the mean hyphal diameter (15 μm), we therefore calculated the specific AMF external hyphal volume.

Statistical analysis

We performed the statistical analyses with R 2.6.0 (R Development Core team, 2007). We used three-way analyses of variance with the presence/absence of leek roots, AMF and earthworms as independent variables. The effects of independent variables were considered to be significant if the probability of the null hypothesis was $\leqslant 0.05$.

Results

Biological activity

The quantification of the biological activity is reported in details in (Milleret et al., 2009). Throughout the experiment, no AMF colonization was observed in non-AMF-treated sample. Leek plants were heavier and better developed with AMF and earthworms had a high activity. Earthworms reproduced actively, leading up to 49 individuals in the AMF + Earthworm + Leek treatment. This is tenfold greater than the five initial earthworms introduced at the beginning of the experiment. Their average biomass increased therefore from 1.32 to 2.99 g. In the other treatments, the number of earthworms at the end of the experiment varied between 11 and 24 individuals and the mean biomass was similar when comparing the start and the end of the experiment (around 1.3 g). This was explained by the presence of juveniles with a low biomass (about 0.01 g per individual).

Table 1 shows the specific fresh root volume (SRV) per gram of soil ($cm^3\,g^{-1}$) per class of diameter as well as the total SRV and the specific AMF external hyphal volume. Roots occupied a total volume ranging from 2.6E−04 to 1.6E−03 $cm^3\,g^{-1}$ of soil and AMF occupied a total volume ranging from 1.1E−03 to 1.5E−03 $cm^3\,g^{-1}$ of soil.

Shrinkage analysis

The bulk soil shrinkage curves and the parameters of the fitted XP model are presented in Fig. 2 and Table 2, respectively. According to the precision of the transducers we used, the water contents were determined with a $10^{-4}\,g\,g^{-1}$ resolution, soil and structural pore volumes with a 0.01 $cm^3\,g^{-1}$ resolution, and plasma pore volumes with a $10^{-4}\,cm^3\,g^{-1}$ resolution. Moreover, the coefficients of variation of the shrinkage parameters as determined on neighboring soil samples collected in a field were estimated as below 10% (water contents) and 3% (volumes) by Boivin (2007), thus giving an overestimation of the standard errors associated with our measurements performed on homogenized soils. The changes commented below are larger than the corresponding errors (Table 2).

The samples with the AMF, Leek or AMF + Leek treatments presented a larger specific volume than the control. On the contrary, Earthworm treatment resulted in smaller specific volume of the soil than the control. Interestingly, the shrinkage curves of the control and the treatment containing Earthworm + AMF + Leek roots had a similar specific volume and water content range (Fig. 2).

We observed a significant effect of Earthworm on the mean slope of the structural shrinkage (K_{str}, $P < 0.05$) (Table 2). The mean slope of the structural shrinkage was 0.26 ($SE = 0.03$) with earthworms whereas the slope was 0.07 ($SE = 0.02$) without earthworm (Fig. 3a). The specific bulk volume (V) at SL and MS was significantly decreased by Earthworm ($P < 0.05$) and increased by Leek roots ($P < 0.05$) (Table 2). The bulk soil density (ρ), calculated as the inverse of the specific bulk volume, was therefore increased by earthworms at SL and MS and decreased by leek roots (Fig. 3b). The structural porosity was increased by Leek roots, AMF, and their combined effect and decreased by Earthworm at SL, AE, ML and MS points (Table 2). Accordingly, Earthworm and Earthworm + AMF treatments reduced the plant available water

Table 1
Specific fresh Root Volume (SRV) per class of diameter ($cm^3\,g^{-1}$), total SRV ($cm^3\,g^{-1}$), Specific AMF external Hyphal Volume (SHV in $cm^3\,g^{-1}$) and air filled pore volume at water saturation ($cm^3\,g^{-1}$) for each of the eight microcosms. SHV was calculated based on the hyphal length density ($m\,g^{-1}$) and the mean hyphal diameter (15 μm).

Leek	AMF	Earthworm	SRV per class of diameter ($cm^3\,g^{-1}$)				Total SRV ($cm^3\,g^{-1}$)	Total SHV ($cm^3\,g^{-1}$)	Air filled pore volume at water saturation ($cm^3\,g^{-1}$)
			750 μm	375 μm	175 μm	75 μm			
+	+	+	5.27E−05	9.92E−05	6.01E−05	4.86E−05	2.61E−04	1.13E−03	0.33
+	+	–	4.77E−04	8.31E−04	1.64E−04	1.30E−04	1.60E−03	1.54E−03	0.61
+	–	+	1.58E−04	2.56E−04	1.70E−04	2.46E−04	8.31E−04		0.33
+	–	–	1.01E−04	4.01E−04	2.06E−04	2.21E−04	9.28E−04		0.48
–	+	+						1.25E−03	0.10
–	+	–						1.05E−03	0.37
–	–	+							0.07
–	–	–							0.30

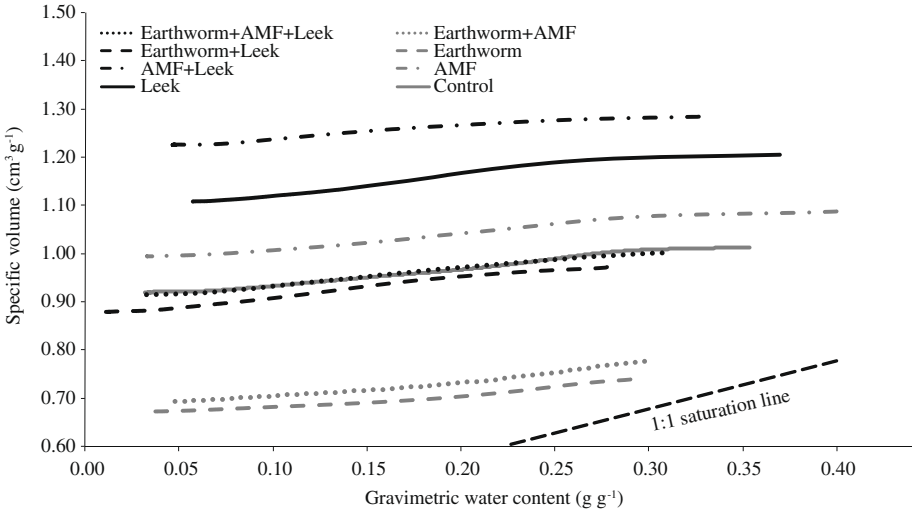

Fig. 2. Shrinkage curves of the eight treatments representing all the combinations of the presence/absence of the three factors: Leek roots (*Allium porrum*), Earthworms (*Allolobophora chlorotica*) and AMF (*Glomus intraradices*) with saturation 1:1 line (large dashed line). According to the experimental design, no confidence intervals can be given.

Table 2
Swelling capacity of the soil (SC) and the plasma (SC_p), slope of the structural shrinkage (K_{Str}), of the basic shrinkage (K_{Bs}), water content (W), soil specific volume (V) at the transitions points SL, AE, LM and MS, available water (AW), easily available water (EAW), and corresponding plasma (V_p) and structural (V_{st}) porosities as determined with fitting the XP model on the shrinkage curves established for each microcosm. E: Earthworm, A: AMF, L: Leek roots, C: control. Water contents are determined with a 10^{-4} g g^{-1} resolution, soil and structural pore volumes with a 0.01 cm^3 g^{-1} resolution, and plasma pore volumes with a 10^{-4} cm^3 g^{-1} resolution.

Bulk soil properties	Treatments							
	E + L	E	A + L	A	E + A + L	E + A	L	C
SC (%)	10.0	7.3	4.5	7.6	8.8	8.8	8.6	9.9
K_{Str} (cm^3 g^{-1})	0.178	0.279	0.105	0.075	0.258	0.309	0.023	0.061
K_{Bs} (cm^3 g^{-1})	0.462	0.467	0.365	0.393	0.395	0.555	0.538	0.380
W_{SL} (g g^{-1})	0.021	0.141	0.056	0.091	0.049	0.134	0.063	0.046
V_{SL} (cm^3 g^{-1})	0.879	0.688	1.226	1.004	0.916	0.712	1.108	0.920
W_{AE} (g g^{-1})	0.053	0.232	0.081	0.140	0.072	0.262	0.151	0.106
V_{AE} (cm^3 g^{-1})	0.888	0.715	1.230	1.018	0.921	0.759	1.140	0.934
W_{ML} (g g^{-1})	0.168	0.254	0.120	0.266	0.176	0.276	0.210	0.292
V_{ML} (cm^3 g^{-1})	0.941	0.725	1.245	1.068	0.962	0.767	1.172	1.005
W_{MS} (g g^{-1})	0.259	0.289	0.292	0.328	0.285	0.295	0.339	0.332
V_{MS} (cm^3 g^{-1})	0.967	0.738	1.281	1.080	0.997	0.774	1.203	1.011
AW (g g^{-1})	0.205	0.058	0.211	0.188	0.214	0.032	0.188	0.226
EAW (g g^{-1})	0.090	0.035	0.172	0.062	0.109	0.019	0.130	0.040
Calculated plasma V_p and structural porosity V_{st}								
V_p (SL) (cm^3 g^{-1})	0.040	0.194	0.070	0.119	0.062	0.209	0.114	0.081
V_{st} (SL) (cm^3 g^{-1})	0.462	0.117	0.779	0.507	0.477	0.126	0.616	0.462
V_p (AE) (cm^3 g^{-1})	0.053	0.232	0.081	0.140	0.072	0.262	0.151	0.106
V_{st} (AE) (cm^3 g^{-1})	0.457	0.106	0.772	0.501	0.472	0.119	0.612	0.451
V_p (ML) (cm^3 g^{-1})	0.168	0.254	0.120	0.266	0.176	0.276	0.210	0.292
V_{st} (ML) (cm^3 g^{-1})	0.395	0.094	0.747	0.424	0.409	0.113	0.584	0.336
V_p (MS) (cm^3 g^{-1})	0.206	0.269	0.192	0.292	0.222	0.284	0.264	0.308
V_{st} (MS) (cm^3 g^{-1})	0.384	0.091	0.712	0.411	0.398	0.113	0.561	0.325
SCp (%)	415.35	38.93	173.16	144.21	256.47	36.13	130.78	281.53

(AW) and easily available water (EAW) (Table 2). The compacting effect of earthworms was not mitigated by AMF, was partly mitigated by leek, and almost fully mitigated by AMF + Leek. Interestingly, the increase in structural volume observed with the Leek + AMF treatment was larger than the addition of the structural volume increase observed with the single Leek and AMF treatment, thus revealing a synergistic effect, particularly at lower water content.

Plasma pores

All the plasma volumes are close to the control except the treatments Earthworm and Earthworm + AMF (Fig. 4). In these cases, the plasma air entry values occurred at larger soil water content than with the other treatments. Moreover, Table 2 shows that $V_{p,SL}$ is more than two times larger for the Earthworm and Earthworm + AMF treatments compared to the other treatments, leading to a smaller swelling capacity of the plasma (SCp). The observed differences in volumes, however, are small compared to the volume changes observed on the structural pores with the different treatments.

Structural pore size distribution

Fig. 5 presents the cumulated volume of the structural pore size distribution, corresponding to the water saturated structural pores.

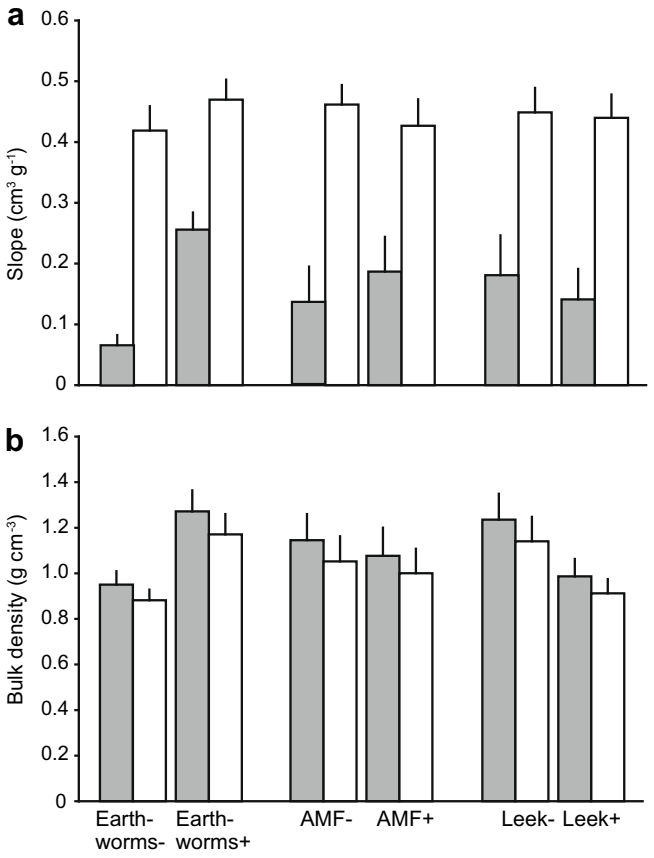

Fig. 3. Isolated effect of the three factors (earthworms, *Allolobophora chlorotica; AMF, Glomus intraradices* and Leek roots, *Allium porrum*), as illustrated by calculated average values of (a) the slope of the structural shrinkage (grey) and the slope of the basic shrinkage (white) and (b) the bulk soil density at SL (grey) and MS (white), with or without the factor in the corresponding treatment. Bar represents mean + *SE* as calculated from ANOVA.

The structural pore volumes of the Earthworm and the Earthworm + AMF treatments were similar (Fig. 5a), smaller at every pore size than the control, and the smallest radius of structural pores were larger with Earthworm than with the control. The smaller structural pore radius was 11 μm for the control, 16 μm for the Earthworm treatment and 25 μm for the Earthworm + AMF treatment. Only the Earthworm + AMF + leek roots treatment had a structural pore volume larger at every pore size than the control, and the smallest structural pore size were 3 μm radius. The cumulated pore size distributions of the different treatments including leek roots are presented in Fig 5b. The structural pore volumes with plant roots were higher than the control at any pore size, and the smallest structural pore radii were always smaller than the control. Fig. 5c shows the structural pore size distribution of the treatments containing AMF. As previously described in Fig. 5a, the curve of the AMF + Earthworm treatment was below the control curve. The three other treatment curves showed larger volumes than the control. The volumes were larger with the AMF and the AMF + Leek treatments than with the Earthworm + AMF + Leek. Unlike leek roots, AMF alone did not generated smaller structural pores than the control.

The results above are enforced by the values of the air filled structural pore volumes at water saturation (−10 hPa), corresponding to the coarser (larger than 150 μm) structural pore volume (Table 1). The leek + AMF, AMF, and Leek treatments showed a greater air filled structural pore volume than the control and a smaller air filled pore volume for Earthworm and Earthworm + AMF treatments. Compared with the air filled structural pore volumes, the root or AMF volumes in the soil was about 3 orders of magnitude smaller.

Discussion

Shrinkage analysis revealed a different impact of the investigated soil biota on the soil physical properties.

At any water content, the presence of earthworms resulted in a decrease of the specific bulk volume and in a significant increase in the bulk soil density. The physical changes induced by *A. chlorotica* included a decrease of the structural pore volumes at any pore size, a disappearing of the smallest structural pore radii, a decrease in plant available water, and a hardening of the plasma. Thus, we

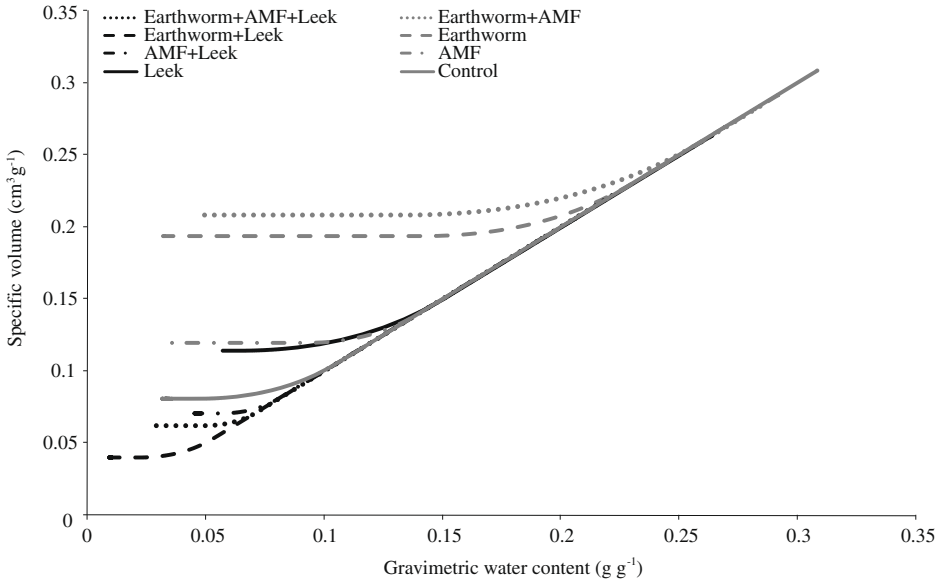

Fig. 4. Plasma shrinkage curves from the samples of the eight treatments representing all the combinations of the presence/absence of the three factors: leek roots (*Allium porrum*), earthworms (*Allolobophora chlorotica*) and AMF (*Glomus intraradices*). Specific volumes and gravimetric water contents are expressed in cm^3 and g per gram of soil, respectively. According to the experimental design, no confidence intervals can be given.

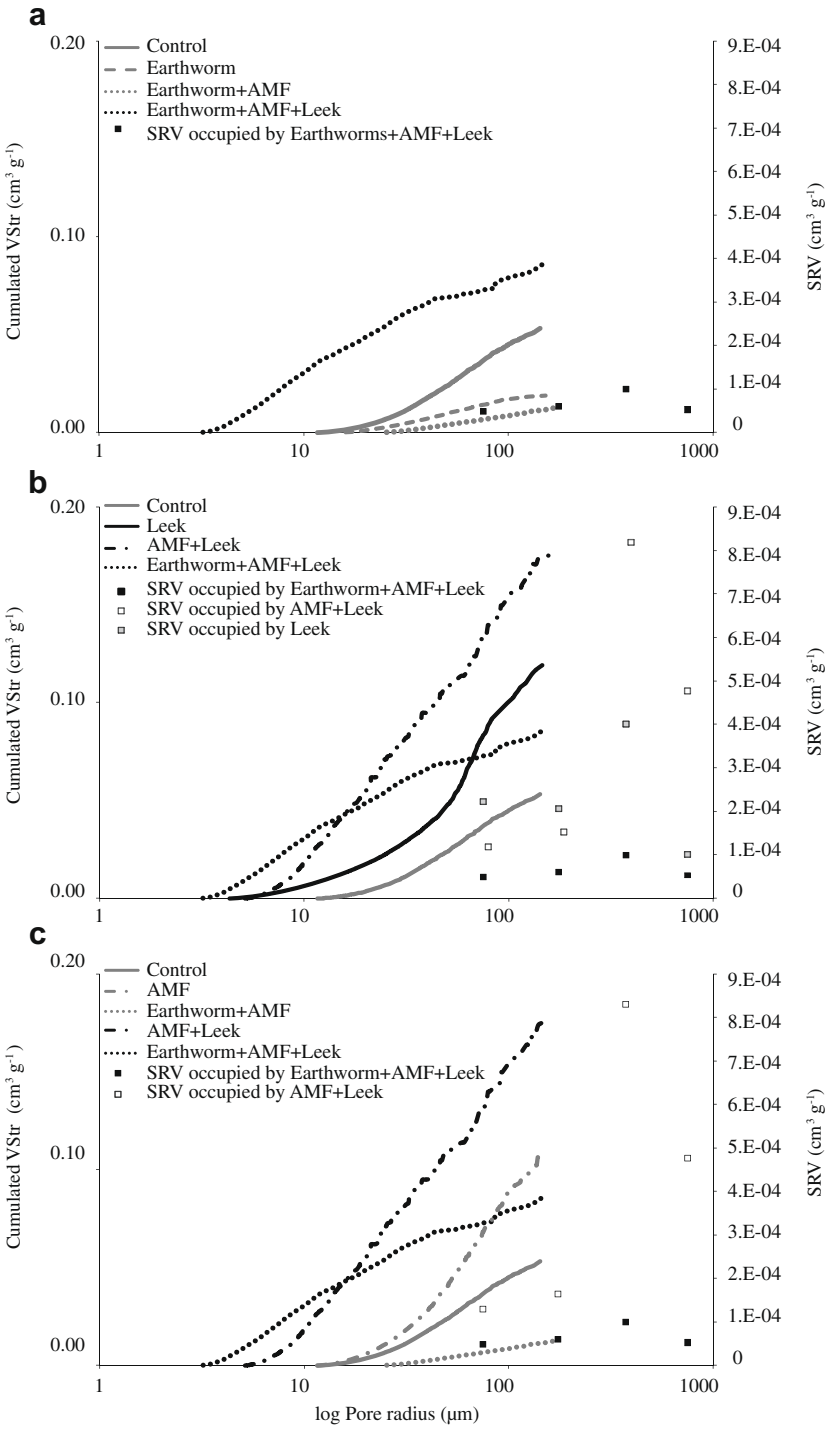

Fig. 5. Cumulated structural pore volume vs. equivalent pore diameter for (a) Earthworm treatments, (b) Leek roots treatment and (c) AMF treatments. Pore volumes are calculated with the Jurin–Laplace law for pores smaller than 150 µm (−10 hPa). Air filled pore volumes at −10 hPa are presented in Table 1. Squares represent the specific root volume (SRV) per class of root diameter. Data of the Earthworm + Leek treatment not available.

demonstrated that *A. chlorotica* compacted the soil. Soil compaction by earthworms was described by some authors with tropical endogeic earthworms (see Blanchart et al., 2004 for a review). In particular, the authors suggest that in Amazonia the compaction effect was induced by rapid changes in land use and mainly due to the proliferation of an endogeic species called *Pontoscolex corethrurus* during the reconversion of forests to pasture (Chauvel et al., 1999). To some extent, our experiment produces similar conditions, as the only earthworm species introduced was an endogeic species. Blanchart et al. (1997) described two functional groups within endogeic earthworms: compacting and decompacting species. They suggest that the presence of both types of earthworms is necessary to maintain the natural soil structure. If one or both types of earthworm are excluded from the soil, the initial structure is greatly affected.

We also observed an increase of the slope of the structural shrinkage (K_{str}) with *A. chlorotica*, thus indicating a decrease in the hydro-structural stability of the soil upon drainage of the structural pores (Schaffer et al., 2008) in the presence of earthworms. This is in agreement with the findings of Milleret et al. (2009)

based on six replicated measurements of the structural stability with the wet-sieving method on the same experiment. The percentage of water stable macro aggregates (i.e aggregates > 250 μm measured in the 1–2 mm size class) was significantly decreased with earthworms. To our best knowledge it is the first time that wet sieving aggregate stability and hydro-structural stability are measured on the same samples. The good agreement between the two methods seems promising and suggests further comparison.

Finally, the changes observed on plasma swelling may be attributed to more rigid particles (Tessier, 1980; Tessier et al., 1992) that is a hardening of the plasma by earthworms, which was not observed with Earthworm combined with Leek root treatments.

Although soil compaction attributed to endogeic earthworms was already reported (see above), our findings are largely in contradiction with the current knowledge of earthworm impacts on soil physical properties. Many studies highlighted a positive effect of earthworms on soil structure, soil aggregation or soil water infiltration (Edwards and Bohlen, 1996). In particular it has been demonstrated that earthworms enhance soil stability, especially when casts are ageing and drying (Shipitalo and Protz, 1989). We can comment on this apparent discrepancy as follows. First, most studies focused on anecic species and compared surface casts with the bulk soil. It is likely that our results apply specifically to some endogeic species. The casts of *A. chlorotica* are not deposited at soil surface, thus limiting ageing of the casts upon drying cycles. Second, in natural conditions earthworms population is a mix of all ecological categories (i.e. anecic, epigeic and endogeic), and soil physical properties result therefore from a complex equilibrium. The effects of functionally different earthworm species on soil aggregation have been studied and the results highlighted that different earthworm species differently affected the incorporation of fresh organic matter and soil stability, and that interactive effects between different earthworm species must be considered (Bossuyt et al., 2006). In our experiment, one species only was used. Third, the earthworm density we used was high for endogeic species alone compared with field observed earthworm density. We applied this density to emphasize the effect of the selected earthworm, as applying a field relevant density for *A. chlorotica* alone would have led to negligible effect at microcosm scale. Our results draw, therefore, the attention to the possible effect of one single species proliferating, as described in a particular field case by Blanchart et al. (2004). This also underlines the interest for further research on earthworm species interactions. The general case of multi-earthworm species in microcosm experimental conditions using shrinkage analysis in temperate soils remains, therefore, to be experimented.

On the contrary, leek roots decreased the bulk soil density despite an initial bulk density of 1.15 g cm^{-3}. This increase in volume was accompanied with a enhanced hydro-structural stability, a larger structural pore volume at any pore size, smaller structural pore radii and an increase in plant available water. The generated structural pore diameters were smaller and larger than the roots, volume of which was much smaller than the generated pore volumes. Leek root diameters were mostly in the range of pore diameter greater than 150 μm (Fig. 5), which corresponds to air-filled pores at −10 hPa. This result is in accordance with O'Keefe and Sylvia (1992) who showed that AMF and root hairs were of diameter that would allow them to penetrate pores that hold water at water contents less than field capacity while root would be excluded from these pores. The new structural pores were, therefore, not generated by the mechanical effect of root growth, but most likely by the induced microbial activity and the resulting self-organization of the soil–microbe complex (Feeney et al., 2006; Young and Crawford, 2004).

Regarding AMF, they induced a decrease in soil bulk density and structural pore volume analogous to that of roots though less pronounced. However, AMF alone did not develop small diameter structural pores. The size and volume of the generated structural pores were larger than the size and volume of the AMF, suggesting an indirect effect of the mycorrhizae.

The combined treatment revealed different interactions. Obviously, AMF could not mitigate the compaction induced by *A. chlorotica*, Leek roots partly mitigated the effect of the earthworm, and it is only AMF + leek root that allowed keeping soil physical properties close to the control. The effects of roots were identified as identical in all the treatments, in particular the generation of very small diameter structural pores. The structure generation due to AMF and roots revealed a positive synergistic effect at lower water content in agreement with the stimulation of plant growth by AMF. This is in accordance with studies demonstrating that the presence of plant have the greatest impact on structure generation, with AMF also contributing to accentuate soil stability (Hallett et al., 2009; Jastrow et al., 1998). The possibility of differential AMF response to soil compaction was described by Nadian et al. (1998). As the diameter of external hyphae is smaller than roots it may penetrate smaller pores and enhance the observed leek roots effect and stimulate plant exudates secretion, thus increasing bacterial activity.

Conclusions

In the present study, shrinkage analysis was successfully applied to the assessment of the physical impact of soil biota in soil microcosms. To our best knowledge, such application of shrinkage analysis was not reported previously. Although performed on a limited number of samples, the provided results seem promising for this kind of investigation. The advantages of the method are both the accuracy of the determination, thus revealing small changes, and the large spectra of properties determined, thus allowing a full description of small concomitant changes.

A compacting and destabilizing effect of *A. chlorotica*, and a de-compacting and stabilizing effect of AMF and leek roots were revealed. Interestingly, a synergistic effect of roots and AMF in the absence of the earthworm was also highlighted, and this synergistic effect was not observed in presence of the earthworm. Combining ShC and WRC analysis allowed comparing the structural pore size distribution in the sampled treatments. This analysis showed that the structural pore volume generated by root and AMF growth was several orders of magnitude larger than the volume of the organisms and that the new structural pore diameters were not the same as those of the organisms. We, therefore, show that these pores were not generated by mechanical intrusion of the biota in the soil. More likely, root exudates as well as other AMF secretion serve as carbon source for microorganisms that in turn enhance soil aggregation and porosity. These changes resulted in more porous and stable soils with larger plant available water induced by AMF and plant roots. Our results, therefore, support the idea of a self-organization of the soil–plant–microbe complex as previously suggested by Young and Crawford (2004).

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References

Angers, D.A., Caron, J., 1998. Plant-induced changes in soil structure: processes and feedbacks. Biogeochemistry 42 (1–2), 55–72.

Blanchart, E., Albrecht, A., Brown, G., Decaens, T., Duboisset, A., Lavelle, P., Mariani, L., Roose, E., 2004. Effects of tropical endogeic earthworms on soil erosion. Agriculture Ecosystems and Environment 104 (2), 303–315.

Blanchart, E., Lavelle, P., Braudeau, E., LeBissonnais, Y., Valentin, C., 1997. Regulation of soil structure by geophagous earthworm activities in humid savannas of Cote d'Ivoire. Soil Biology and Biochemistry 29 (3–4), 431–439.

Blouin, M., Barot, S., Roumet, C., 2007. A quick method to determine root biomass distribution in diameter classes. Plant and Soil 290 (1–2), 371–381.

Boivin, P., 2007. Anisotropy, cracking, and shrinkage of vertisol samples – experimental study and shrinkage modeling. Geoderma 138 (1–2), 25–38.

Boivin, P., Brunet, D., Gascuel-Odoux, C., 1990. Une nouvelle méthode de mesure de la densité apparente sur échantillons de sols non remaniés. Bulletin du groupe français d'humidimétrie neutronique et des techniques associées 28, 57–71 (in French).

Boivin, P., Garnier, P., Tessier, D., 2004. Relationship between clay content, clay type, and shrinkage properties of soil samples. Soil Science Society of America Journal 68 (4), 1145–1153.

Boivin, P., Garnier, P., Vauclin, M., 2006a. Modeling the soil shrinkage and water retention curves with the same equations. Soil Science Society of America Journal 70 (4), 1082–1093.

Boivin, P., Schaffer, B., Temgoua, E., Gratier, M., Steinman, G., 2006b. Assessment of soil compaction using shrinkage modelling: experimental data and perspectives. Soil and Tillage Research 88 (1–2), 65–79.

Boivin, P., Schaffer, B., Sturmy, W., 2009. Quantifying the relationship between soil organic carbon and soil physical properties using shrinkage modelling. European Journal of Soil Science 60 (2), 265–275.

Bossuyt, H., Six, J., Hendrix, P.F., 2006. Interactive effects of functionally different earthworm species on aggregation and incorporation and decomposition of newly added residue carbon. Geoderma 130 (1–2), 14–25.

Braudeau, E., 1988a. Quantitative assessment of soil structural quality by mean of shrinkage curves analysis. Comptes Rendus De L Academie Des Sciences Serie II 307, 1933–1936 (in French).

Braudeau, E., 1988b. General shrinkage curve equation for undisturbed soil samples. Comptes Rendus De L Academie Des Sciences Serie II 307 (15), 1731–1734 (in French).

Braudeau, E., Bruand, A., 1993. Détermination de la courbe de retrait de la phase argileuse à partir de la courbe de retrait sur échantillon de sol non remanié. Application à une séquence de sols de Côte-d'Ivoire. Comptes Rendus De L Academie Des Sciences Serie II 316 (5), 685–692 (in French).

Braudeau, E., Costantini, J.M., Bellier, G., Colleuille, H., 1999. New device and method for soil shrinkage curve measurement and characterization. Soil Science Society of America Journal 63 (3), 525–535.

Braudeau, E., Frangi, J.P., Mohtar, R.H., 2004. Characterizing nonrigid aggregated soil–water medium using its shrinkage curve. Soil Science Society of America Journal 68 (2), 359–370.

Brewer, R., 1964. Fabric and Mineral Analysis of Soils. John Wiley & Sons, New York.

Brown, G.G., Edwards, C.A., Brussaard, L., 2004. How earthworms affect plant growth: burrowing into the mechanisms. In: Edwards, C.A. (Ed.), Earthworm Ecology. CRC Press, Boca Raton, USA, pp. 13–49.

Brussaard, L., Pulleman, M.M., Ouedraogo, E., Mando, A., Six, J., 2007. Soil fauna and soil function in the fabric of the food web. Pedobiologia 50 (6), 447–462.

Caesar-TonThat, T.C., Caesar, A.J., Gaskin, J.F., Sainju, U.M., Busscher, W.J., 2007. Taxonomic diversity of predominant culturable bacteria associated with microaggregates from two different agroecosystems and their ability to aggregate soil in vitro. Applied Soil Ecology 36 (1), 10–21.

Capowiez, Y., 2000. Differences in burrowing behaviour and spatial interaction between the two earthworm species *Aporrectodea nocturna* and *Allolobophora chlorotica*. Biology and Fertility of Soils 30, 341–346.

Chauvel, A., Grimaldi, M., Barros, E., Blanchart, E., Desjardins, T., Sarrazin, M., Lavelle, P., 1999. Pasture damage by an Amazonian earthworm. Nature 398 (6722), 32–33.

Chen, D.H., Saleem, Z., 1986. A new simplex procedure for function minimization. International Journal of Modelling and Simulation 6 (3), 81–85.

Dorioz, J.M., Robert, M., Chenu, C., 1993. The role of roots, fungi and bacteria on clay particle organization – an experimental approach. Geoderma 56 (1–4), 179–194.

Edwards, C.A., Bohlen, P.J., 1996. Biology and ecology of earthworms. Chapman & Hall, London, UK. 426 pp..

Feeney, D.S., Crawford, J.W., Daniell, T., Hallett, P.D., Nunan, N., Ritz, K., Rivers, M., Young, I.M., 2006. Three-dimensional microorganization of the soil–root–microbe system. Microbial Ecology 52 (1), 151–158.

Gascuel-Odoux, C., 1987. Variabilité spatiale des propriétés hydriques du sol, cas d'une seule variable: revue bibliographique. Agronomie 7 (1), 61–71 (in French).

Haines, W.B., 1923. The volume-changes associated with variations of water content in soil. Journal of Agricultural Science 13, 296–310.

Hallett, P., Feeney, D.S., Bengough, A.G., Rillig, M.C., Scrimgeour, C.M., Young, I.M., 2009. Disentangling the impact of AM fungi versus roots on soil structure and water transport. Plant and Soil 314 (1–2), 183–196.

IUSS, Working Group WRB, 2006. World Reference Base for Soil Resources 2006, second ed. World Soil Resources Reports No 103. FAO, Rome.

Jakobsen, I., Abbott, L.K., Robson, A.D., 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium-subterraneum* L.1. Spread of hyphae and phosphorus inflow into roots. New Phytologist 120 (3), 371–380.

Jastrow, J.D., Miller, R.M., 1991. Methods for assessing the effects of biota on soil structure. Agriculture Ecosystems and Environment 34 (1–4), 279–303.

Jastrow, J.D., Miller, R.M., Lussenhop, J., 1998. Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biology and Biochemistry 30 (7), 905–916.

Koide, R.T., Li, M.G., 1989. Appropriate controls for vesicular arbuscular mycorrhiza research. New Phytologist 111 (1), 35–44.

Lavelle, P., Bignell, D., Lepage, M., Wolters, V., Roger, P., Ineson, P., Heal, O.W., Dhillion, S., 1997. Soil function in a changing world: the role of invertebrate ecosystem engineers. European Journal of Soil Biology 33 (4), 159–193.

Lawrence, G.P., 1977. Measurement of pore sizes in fine-textured soils – review of existing techniques. Journal of Soil Science 28 (4), 527–540.

Le Bayon, R.C., Binet, F., 1999. Rainfall effects on erosion of earthworm casts and phosphorus transfers by water runoff. Biology and Fertility of Soils 30 (1–2), 7–13.

Lee, K.E., Foster, R.C., 1991. Soil fauna and soil structure. Australian Journal of Soil research 29, 745–775.

Milleret, R., Le Bayon, R.C., Gobat, J.-M., 2009. Root, mycorrhiza and earthworm interactions: their effects on soil structuring processes, plant and soil nutrient concentration and plant biomass. Plant and soil 316 (1–2), 1–12.

Mummey, D.L., Rillig, M.C., Six, J., 2006. Endogeic earthworms differentially influence bacterial communities associated with different soil aggregate size fractions. Soil Biology and Biochemistry 38 (7), 1608–1614.

Nadian, H., Smith, S.E., Alston, A.M., Murray, R.S., Siebert, B.D., 1998. Effects of soil compaction on phosphorus uptake and growth of *Trifolium subterraneum* colonized by four species of vesicular–arbuscular mycorrhizal fungi. New Phytologist 140 (1), 155–165.

Newman, E.I., 1966. A method of estimating total length of root in a sample. Journal of Applied Ecology 3 (1), 139–145.

Nielsen, D.R., Biggar, J.W., Erh, K.T., 1973. Spatial variability of field-measured soil–water properties. Hilgardia 42 (7), 215–259.

O'Keefe, D.M., Sylvia, D.M., 1992. Chronology and mechanisms of p-uptake by mycorrhizal sweet-potato plants. New Phytologist 122 (4), 651–659.

R Development Core team, 2007. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ranjard, L., Richaume, A.S., 2001. Quantitative and qualitative microscale distribution of bacteria in soil. Research in Microbiology 152 (8), 707–716.

Rillig, M.C., Mummey, D.L., 2006. Mycorrhizas and soil structure. New Phytologist 171 (1), 41–53.

Schaffer, B., Schulin, R., Boivin, P., 2008. Changes in shrinkage of restored soil caused by compaction beneath heavy agricultural machinery. European Journal of Soil Science 59 (4), 771–783.

Shipitalo, M.J., Protz, R., 1989. Chemistry and micromorphology of aggregation in earthworm casts. Geoderma 45 (3–4), 357–374.

Sisson, J.B., Wierenga, P.J., 1981. Spatial variability of steady-state infiltration rates as a stochastic-process. Soil Science Society of America Journal 45 (4), 699–704.

Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil and Tillage Research 79 (1), 7–31.

SSSA (Soil Science Society of America), 2008. Glossary of soil science term 2008. Soil Science Society of America, Madison, WI, 88 p.

Tessier, D., 1980. The significance of shrinkage limits in clay materials (Kaolinites and Smectites). Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences Serie D 291 (4), 377–380.

Tessier, D., Lajudie, A., Petit, J.C., 1992. Relation between the macroscopic behavior of clays and their microstructural properties. Applied Geochemistry, 151–161.

Tisdall, J.M., Oades, J.M., 1982. Organic-matter and water-stable aggregates in soils. Journal of Soil Science 33 (2), 141–163.

Vauclin, M., 1982. Méthodes d'étude de la variabilité spatiale des propriétés d'un sol. Colloques S.H.F.-I.N.R.A., Avignon, pp. 9–45.

Young, I.M., Crawford, J.W., 2004. Interactions and self-organization in the soil–microbe complex. Science 304 (5677), 1634–1637.