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THE CHARACTERISTICS OF PORAL SPACE AS HABITAT OF SOYBEAN ROOTS AND *Rhizobium* NODULES

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ABSTRACT

The objective of the paper was to emphasize the characteristics of the poral space (quantified according to their size and shape) of an intensely irrigated soil, representing the habitat for soybean roots and *Rhizobium* bacteria nodulation. The studied site is located in Southern Bărăgan Plain, Perișoru region. The soil is Vermic Chernozem (according to SRTS-2012) formed in carbonate loess like deposits. The climate is temperate continental with an average annual temperature of 10.8°C, and an average annual precipitation of 480 mm. The poral space was quantitatively evaluated at microscopic scale, using image analysis on oriented soil thin sections with the help of an image-analyzer computer. The image analysis allowed the quantitative evaluation at microscopic scale of the undisturbed structure and the adjacent porosity, in 2-D dimension (in thin sections). The pores were divided according to their shape in three classes (regular, irregular and elongated pores), and further each shape class was divided into seven size classes according to the equivalent pore diameter (ranging between <100 and >1000 µm). The result showed, unspecific conditions in the studied Chernozem, thus: Ap horizon was more compacted than the underlined Apt, consequently the total porosity was lower (0.21 m^2m^{-2}) into the top Ap horizon, comparing to the underlined Apt (0.33 m^2m^{-2}). In agreement with the data obtained by means of image analysis (pores quantified according to their size and shape), the oriented thin sections gave a clear representation of the genesis, location and the characteristics of the poral space. The soybeans roots developed mainly into the areas with intense biological activity. The soil macro- and mesofauna proved to be good habitat builders for soybean roots and the *Rhizobium* nodules, even in the conditions of a droughty period with the irrigation application delay.

Keywords: soybean, *Rhizobium,* irrigation, image analysis, biodiversity.

INTRODUCTION

A sustainable agricultural system depends on good soil quality and crop performance; however, information is limited about the influence of long-term irrigation schedules on soil properties (Sun et al., 2018).

Arora et al. (2011) studying the combined effects of irrigation, deep tillage, and straw mulching regimes on soybean in relation to soil texture, concluded that soybean offers a diversification option in coarse - to medium-textured soils. However, its productivity in these soils is constrained by high soil mechanical resistance and high soil temperature during early part of the growing season. These constraints can be alleviated through irrigation, deep tillage and straw mulching.

Studying the effect of soil compaction on the root development of soybean plants (*Glycine max* L. Merrill *cv. San Baiba*), subjected to different treatments of irrigation frequencies, air porosity and shear stress of a sandy loam soil, Hossne et al. (2015) concluded that the soybeans root system was positively influenced by water content, more than compaction and the other variables under study.

Cameira (2003) showed that irrigation affected the macro-porosity and meso-porosity of the ploughed layer as evidenced by a decrease of 65% and 50%, respectively

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(in a maize cultivation experiment). This was attributed to the breakdown of fragile pores created by tillage. Furthermore, in the same experiment, seven irrigation events were found to induce a continuous reduction in macro-porosity until harvest when it increased, probably due to root development.

The total amount and the relative distribution of the different pore classes in the ploughed layer showed an important dynamic over the cropping season that leads to quite different values of bulk density, water retention curves, and near-saturated hydraulic conductivity (Sacco et al., 2012).

Soil porosity can be described using direct methods based on microscopic techniques and image analyses, or it can be described functionally using indirect methods based on the measurement of soil physical properties (Pagliai and Vignozzi, 2002).

Pore size distribution, together with pore shape and connectivity, influences the transport of dissolved and non-dissolved chemicals and gases and acts upon plant rooting and on the conditions for the life of all soil biota (Kutilek et al., 2006).

The objective of the paper was to emphasize the characteristics of the poral space (quantified according to their size and shape) of an intensely irrigated soil, representing the habitat for soybean roots and *Rhizobium* bacteria nodulation.

MATERIAL AND METHODS

The studied area is located in the central part of the Southern Bărăgan Plain (the Eastern part of the Romanian Plain), in Perișoru region (5.5 km south-west Perişoru village).

The climate is temperate continental, with long and warm summers and droughty periods in late summer and early fall.

The average annual temperature is 10.6°C and the average annual precipitation is 480 mm, while the evapotranspiration reaches 700 mm. De Martonne aridity index is 23. The water table is at >10 m depth, the moisture regime of the soil is ustic, while the soil temperature regime is mesic.

The soil is Vermic Chernozem according to SRTS - 2012 (Florea and Munteanu, 2012) and WRB-SR-2014 (update 2015), formed in carbonate loess like deposits.

The studied site is located in the steppe bioclimatic zone (Danubian steppe subzone).

In the whole area, the natural vegetation was replaced by crops. The weeds are specific for the steppe bioclimatic zone: *Echinochloa crus-galli, Cynodon dactylon, Agropyron cristatum, Agropyron repens, Bromus arvensis, Cirsium arvense, Solanum nigrum, Matricaria chamomilla.*

The soil profile (Figures 1a and 1b), dug into a soybean (*Glycine max* L. Merrill) plot (Figure 1b), was described in the field and afterwards, undisturbed micromorphological samples (Figure 1c) were collected for making soil thin sections.

The undisturbed soil samples were air drayed in the laboratory and impregnated with epoxidic resins. Oriented thin sections (25-30 μm) were made from each sample after resin hardening. All thin sections were studied with the optical microscope in PPL (plane polarized light). The terminology used for micromorphological description was according to Bullock et al. (1985).

Image analysis was performed on oriented soil thin sections with the help of an image-analyzer computer. The instrument was adjusted to measure pores higher than 50 μm. The pores were measured according to their shape, which is expressed by a shape factor (perimeter^2/4π*aria).

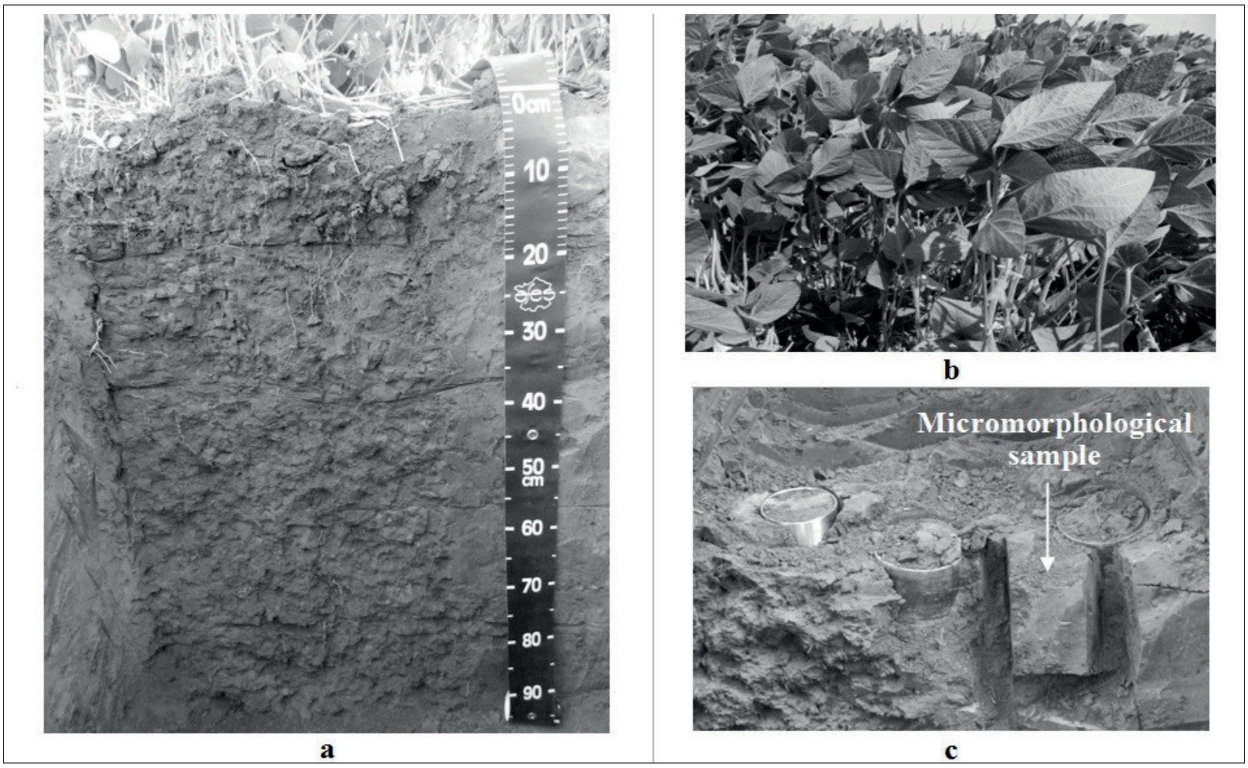

Figure 1. The soil profile (a); soybean crop in the studied plot (b); micromorphological sample (c)

RESULTS AND DISCUSSION

In order to characterize the soil porosity as a soybean root environment (Figures 2a and 2b) and as a habitat for *Rhizobium* bacteria living in the nodulations developed on soybean roots (Figure 2a), the porosity was measured by image analysis at microscopic level (on oriented thin sections – Figure 2c).

Each thin section was analyzed with the help of an image analyzer to measure total porosity and to characterize pores according to their *shape* and *size*.

Total porosity is the average of the data obtained by measuring the porosity of two fields of 12/21 mm in each soil thin section (Figure 2c).

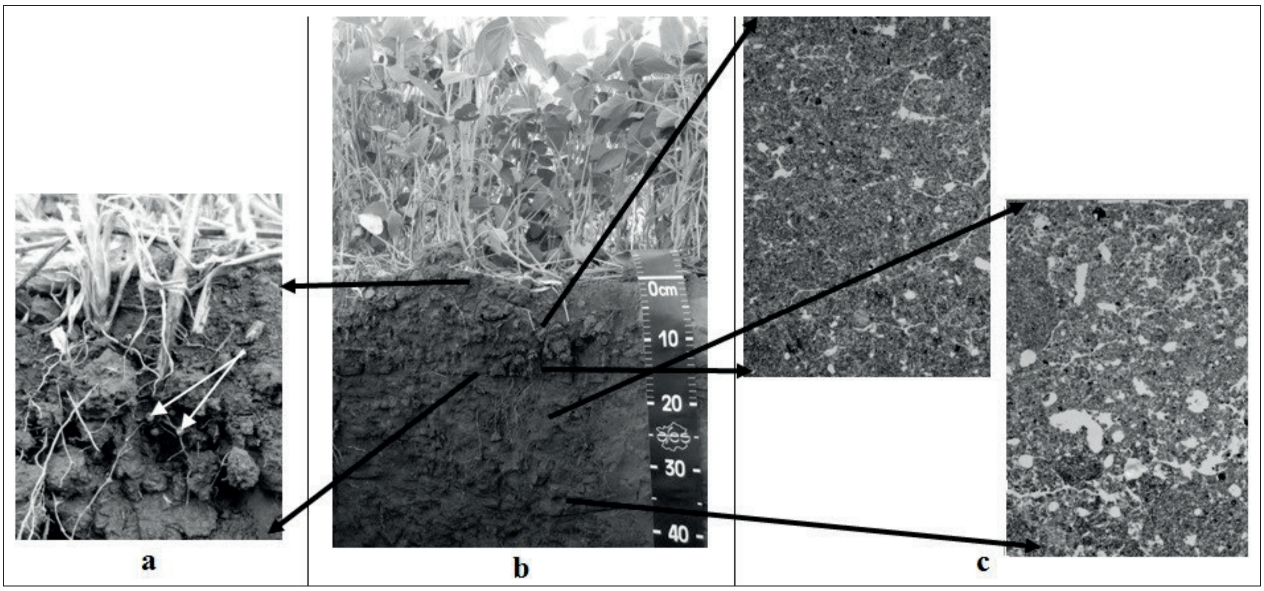

Figure 2. a) Nodules developed on soybean roots; b) soil profile under soybean; c) thin sections from the active layer of the soybean roots (corresponding to Ap and Apt horizons)

The pores were divided according to their *shape* in three classes (which broadly correspond to those defined by Bouma et al. in 1977), as follows:

- regular pore – having a regular shape, and being represented by the pores more or less rounded (with a shape factor of 1-2);
- irregular pores – having an irregular shape (with a shape factor of 2-5);
- elongated pores – pores represented mainly by the cracks/fissures (with a shape factor up to 5).

The pores of each shape class were further divided into *size classes* according to the equivalent pore diameter (e.c.d) for the pores of the first two classes (regular and irregular), and to the width for the elongated pores in the third class (Pagliai et al., 1995).

The fauna (soil architects) activity explored the soil horizons and generated structural elements and a poral space network with predominantly continuous and interconnected pores. The study of the biological pedofeatures (coprolites) and the bio-pores at micromorphological level could show the soil health, on which its physico-chemical characteristics as well as soil life depend.

The soil structure, together with the adjacent porosity permanently change, usually more often than seasonal, as a result of a rich and very active biodiversity, appropriate to a favourable environment, specific for a Chernozem.

Although soil porosity is an exchangeable parameter, the results of the image analysis can give useful information about the soil health.

In the studied soil, both image analysis (Figures 3a and 4a) and micromorphological observation (Figures 3b and 4b) reveal high differences between the horizons of the active layer of the soybean roots (corresponding to Ap and Apt horizons respectively).

The image analysis of the pore distribution (characterized by their size and shape - Figure 3a) showed in the top Ap horizon a total porosity of 0.21 m^2m^{-2}, distributed in all the seven size classes (<100 - >1000 μm).

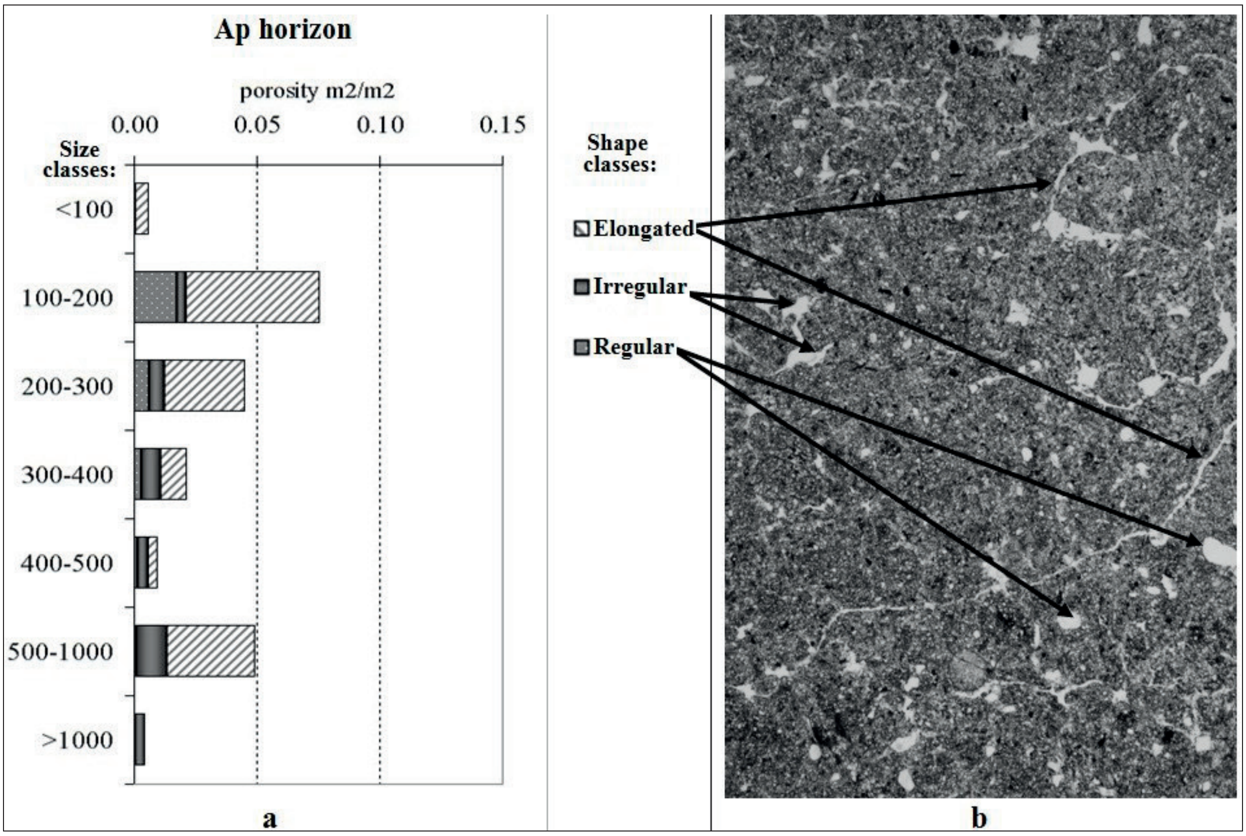

Figure 3. Ap horizon porosity: (a) Image analysis: soil porosity according to their shape and size; (b) Thin section: soil porosity generated by physico-mechanical processes and biological activity (according to their shape)

According to Pagliai (1988) and Pagliai et al. (1995), a soil is densely compacted when the total porosity is less than 0.10 m^2m^{-2}, moderately porous when the total porosity ranges from 0.10-0.25 m^2m^{-2}, and extremely porous when it ranges from 0.25-0.40 m^2m^{-2}, and extremely porous when the value is greater than 0.40 m^2m^{-2}.

In agreement with the data obtained from the image analysis, the Ap horizon of the studied Chernozem was moderately porous.

The dominant pores in this horizon were elongated (Figure 3a), with the width ranging from <100 µm to 1000 µm (size classes). However, the maximum value was reached in the 100–200 µm size class and gradually decreased to the 400-500 µm, to reach a second maximum into the size class of 500-1000 µm.

The micromorphological image of the thin section (Figure 3b) emphasized that the major part of the elongated pores, originated in the cracks, were generated by the physico-mechanical processes (as a result of soil drying). Nevertheless, the significant amount of the elongated pores was also generated by the biological activity: more exactly by the collapse of the channels filled with coprolites (biological pedofeatures), which currently are more or less integrated into the soil matrix and delimitated by the fine circular and semi-circular fissures (Figure 3b).

In the case of irregular pores, although they were significantly less numerous (comparing to the elongated), their sizes ranged from 100-1000 µm, and were distributed in all these six size classes (Figure 3a).

The irregular pores gradually increased from the lower to the higher size class, reaching a maximum into the 500-1000 µm size class (Figure 3a).

Irregular pores, less numerous than elongated but more abundant than regular ones, originated in the cannels and chambers filled with coprolites (biological pedofeatures) generated by the soil fauna activity (Figure 3b). These bio-pores are currently collapsed and the coprolites partially integrated into the soil matrix.

The regular pores reached the maximum values (Figure 3a) into the class 100-200 µm size class, missing in 2 size classes (<100 µm and >1000 µm, respectively).

The regular pores are mainly bio-pores that originated in the channels created both by roots and soil fauna.

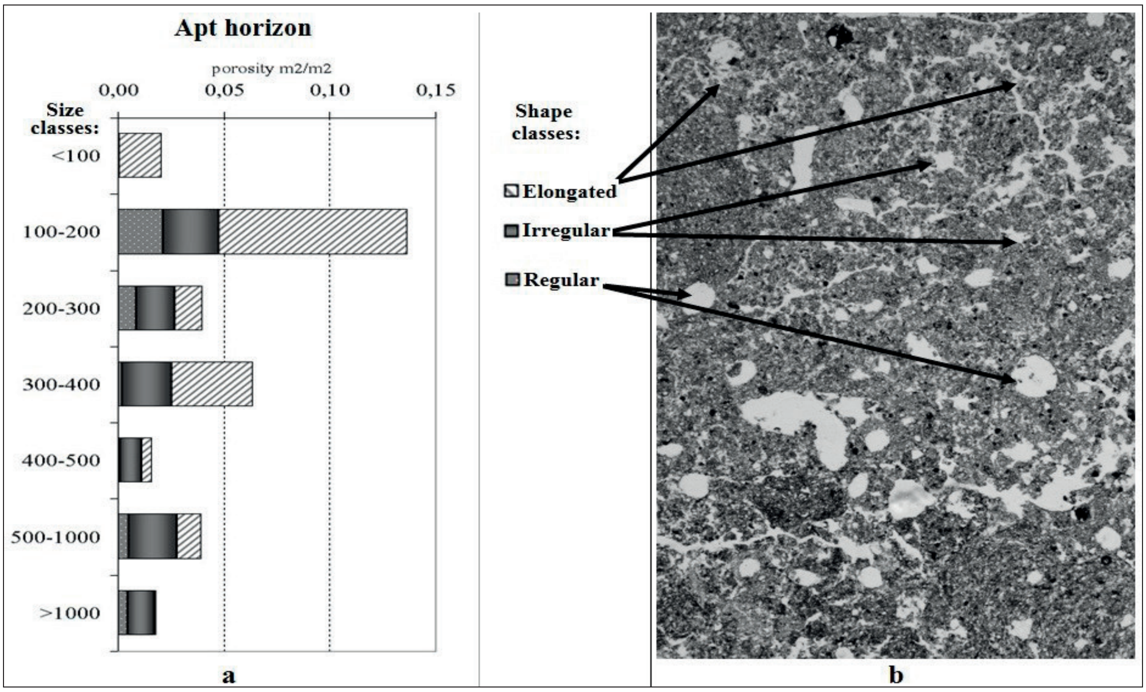

Figure 4. Aph horizon porosity: (a) Image analysis: soil porosity according to their shape and size; (b) Thin section: soil porosity generated mainly by biological activity (reflected by their shape)

Into the deeper Apt horizon, the number and the distribution of the pores quantified by the image analysis (Figure 4a) showed that total porosity (<100 - >1000 μm) increased considerably (comparing to the top Ap horizon), reaching 0.33 m^2m^{-2}.

Usually, in tilled soils, the surface Ap horizon has a higher porosity then the underlined Apt, which is more compacted and consequently with a lower porosity. In the studied plot, the results at the microscopic level, pointed out a higher porosity.

Irregular pores were very common within the Apt horizon, ranging from 100-1000 μm size class, and dominating the size classes of 100-200 μm, 300-400 μm, and 500-1000 μm respectively. Their proportion drastically decreased in the size class of 400-500 μm.

The irregular pores prevailed into the area highly explored by the soil fauna (Figure 4b), where vughy structure appeared. The vughy structure contained numerous irregularly shaped vughs, as a result of the partially collapse of the channels and chambers generated by soil macro- and mesofauna.

The voids had either rounded or ellipsoidal shapes (regular pores) with smooth walls, or irregular shapes (irregular pores) with smooth to rough walls (accommodated to un-accommodated). The pore walls smoothness (or roughness) strongly influences the air and soil solution circulation into the soil.

Regular pores (which typically include root channels) were present in the range of 100-1000 μm, being less numerous than the other types of pores.

The elongated pores were also frequent in this horizon, being within a larger range of sizes, 100-1000 μm (Figure 4a), and reaching two maximum values: into the 100-200 μm size class and 300-400 μm respectively.

The microscopic observation (Figure 4b) revealed that Apt horizon had an open structure with a rich poral space, consisting of fissures and many voids (more packing than interconnected), as a result of very active physico-mechanical and biological processes.

A good porosity is essential to the development of the soybean, being related to the need of adequate aeration for *Rhizobium*-soybean symbiosis activity.

In tilled soils, usually, the Apt horizon is more compacted (characteristics also showed by the Apt horizon definition: A ploughed compacted horizon) than surface horizon. The same characteristic was also reported in Perişoru area by Calciu et al. (2016). In contrast to the situation reported previously, our obtained results (both from image analyses and thin sections observations) emphasised a more compacted Ap horizon than the underlined Apt (Figure 5).

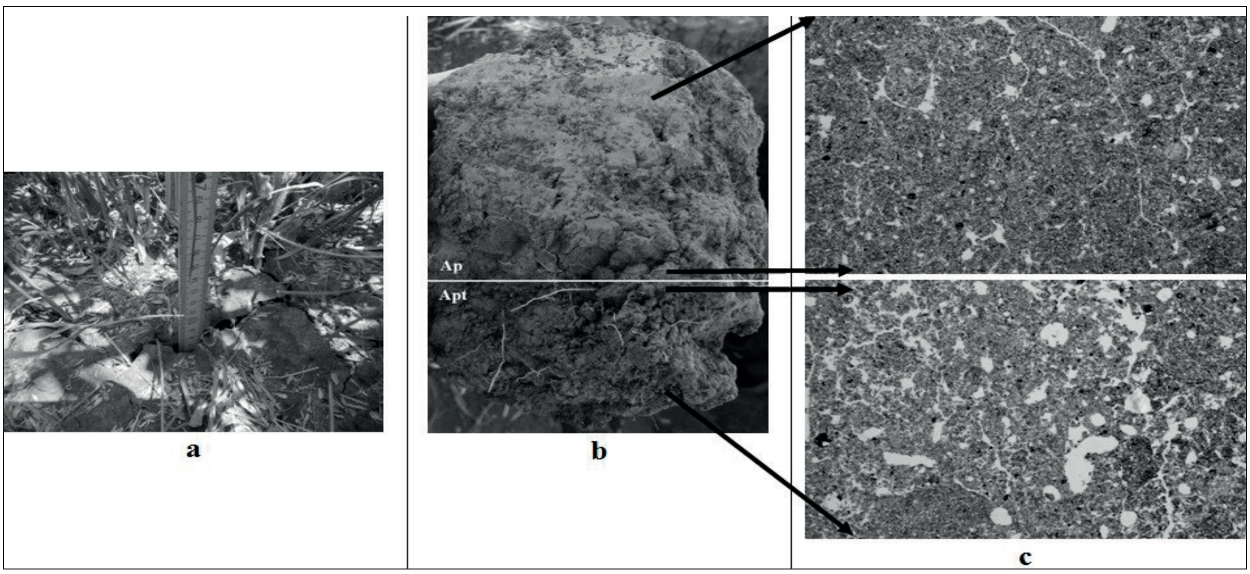

Figure 5. a) Deep (8-10 cm) cracks formed in the top soil; b) a cloud sampled from the border between the two horizons (Ap and Aph); c) corresponding thin sections

The higher compaction of the Ap horizon could be explained by the delay of the irrigation application (due to technical reasons).

The delay in implementing irrigation in a period with high temperatures, had considerable effects on soil; as a result relatively deep cracks formed on soil surface (Figure 5a), the clouds being high and compacted (Figure 5b), but showing clear differences between the two horizons.

This could also represent the soil self-defence, to preserve the small moisture reserve.

Irrigation decreased the period of soil drought (when fauna is usually resting). The favourable soil moisture regime maintained by irrigation, extended the active period of soil fauna and allowed an intense exploration of soil profile, and consequently a very active bioturbation process.

In these conditions, many coprolites from the A horizons contained calcium carbonate brought from the lower calcic horizons, showing that bioturbation process was active, soil fauna deeply explored the soil profile.

This emphasizes that structuring and pore generating processes are complex and had different intensities, as follows.

1) In Ap horizon -

Physico-mechanical processes < **Biological processes** < **Compaction processes**

2) In Apt horizon -

Compaction processes < **Physico-mechanical processes** < **Biological processes**

In the Ap horizon, fine fissures (both straight and circular or semi-circular - Figure 3b) were generated by the physico-mechanical processes (the dominant pores in the Ap horizon being elongated). The continuity of the matrix is also interrupted by the presence of the irregular pores (vughy type), generated mainly by mesofauna (both coprophagous and terrophagous), much more resistant to the conditions created by the advanced dryness of the soil profile.

Under compaction, the biological pedofeatures (as channels and chambers), mainly filled with coprolites collapsed and the coprolites gradually integrated into the soil matrix.

The result was a close porosity, with fine fissures (both circular and semi-circular) that delimitated the coprolites from the surrounding matrix.

The new conditions created by the dryness, favoured the dominance of the mezofauna, much more resistant to harsh conditions than macrofauna (lumbricides). The collapse of smaller biological pedofeatures under compaction generates vughy structure (consisting of small irregular packing voids).

The presence of biological pedofeatures in different degrees of integration into the A horizons matrix (showing an active compaction process), together with the presence of calcium carbonate into the coprolites, underlined that: although the biological activity and bioturbation process were very active, the biological pedofeatures were poorly preserved due to a more active compaction process.

Both image analysis and micromorphological observations in thin sections showed that the compaction process was much more intense into the surface Ap horizon, than all other processes.

The soybean roots succeed to develop in these conditions, while the *Rhizobium* nodules were very rare in this horizon (Figure 2a).

Into the Apt horizon, the biological activity was very high (Figure 4b) and hindered the compaction occurrence, as present in the specific ploughed soils.

The soybean roots succeeded to develop in these conditions, while the *Rhizobium* nodules were rare in Ap horizon (Figure 2a).

The genesis and the evolution of the poral space of the active layer of the studied Chernozem (Ap and Apt) were lead by the three main processes: a) the spatial arrangement of the aggregates generated both by tillage and fauna; b) physico-mechanical processes (wetting and drying); c) compaction.

The above aspects showed that soil macro- and mesofauna activity (soil structure architects) had a major influence on the development of the soybean roots and implicitly on the process of *Rhizobium* nodules growth. Many soybean roots penetrated the soil through the channels created by soil fauna.

Although the analytical data of agro-pedological characterization of the studied Chernozem highlighted a number of aspects related to the medium-low vulnerability of the structural degradation processes with negative consequences on soil aeration and permeability, the micromorphological observation „visualized" important aspects related to soil biodiversity:

- the biological pedofeatures, although poorly preserved, showed the intense activity of soil fauna that continuously built key elements (bio-aggregates and bio-pores) for the good aeration processes and for a good water permeability of the studied soil despite the compaction;

- the studied Chernozem has a great potential for natural „self-restoration" of the structural elements and of the adjacent porosity, through soil biodiversity that continuously adapted to the new agro-ecosystem conditions.

The porosity was also subdivided in three classes (Figure 6):

1. fissures (>500 µm e.c.d. - equivalent cylindrical diameter);

2. transmission pores (50 - 500 µm e.c.d.);

3. storage pores (0.5 - 50 µm e.c.d.).

There was also a fourth class (according to Greenland (1977) of residual pores (<0.5 µm e.c.d.), but they are difficult to be measured. In accordance with Greenland classification, storage pores represent the water reservoir for plants and microorganisms, whereas transmission pores (elongated and continuous pores) are important in soil-water-plant relationship and in maintaining good structural conditions in soil.

Both Ap and Apt horizons had a porosity dominated by the transmission pores, with a diameter ranging between 50-500 µm (Figure 6). These pores were less abundant (0.16 m^2m^{-2}) in Ap horizon and registered an important increase (0.27 m^2m^{-2}) in Apt, where there were dominated by the regular pores (Figure 6), showing a good development of soybean roots. Transmission pores play an important role in the soil-water-plant relationship but also in maintaining good structural soil conditions (Pagliai et al., 1995).

Storage pores, or pores <50 µm e.c.d. (Figure 6) had significantly higher values (0.04 m^2m^{-2}) in the surface horizon Ap (due to a more active compaction process), compared to those in the underlying Apt horizon (0.01 m^2m^{-2}).

Pores having the equivalent diameter >500 µm reached the highest proportion (0.05 m^2m^{-2}) in Apt horizon, in contrast to Ap where the proportion was much lower (0.02 m^2m^{-2}).

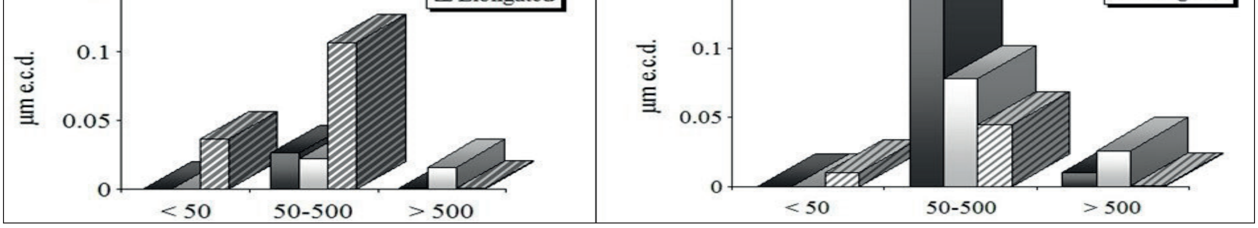

Figure 6. The storage pores, transmission pores and fissures into the Ap and Apt horizons

The porosity values (sizes) measured by image analysis together with the porosity shape observed in the thin sections draws a complete image of the environment of the soybean roots, in a critical period (of delayed irrigation). This layer (the soil skin) favours all the changes between soil and atmosphere (air, water, organic matter etc.).

The total porosity values (of the Ap horizon), corroborated with the values of each pore type (measured by their size and shape), emphasised a moderate-strong compacting of the surface Ap horizon of the Chernozem from Perişoru.

What is noteworthy for the studied soil profile is that the lack of water in a critical period of plant growth, led to soil compaction with the formation of very large (11 cm - Figure 5) structural elements, non-specific for a well-structured soils like Chernozem.

The advanced compactions of the soil, together with the advanced drought, inhibit soybean roots growth and nodulation.

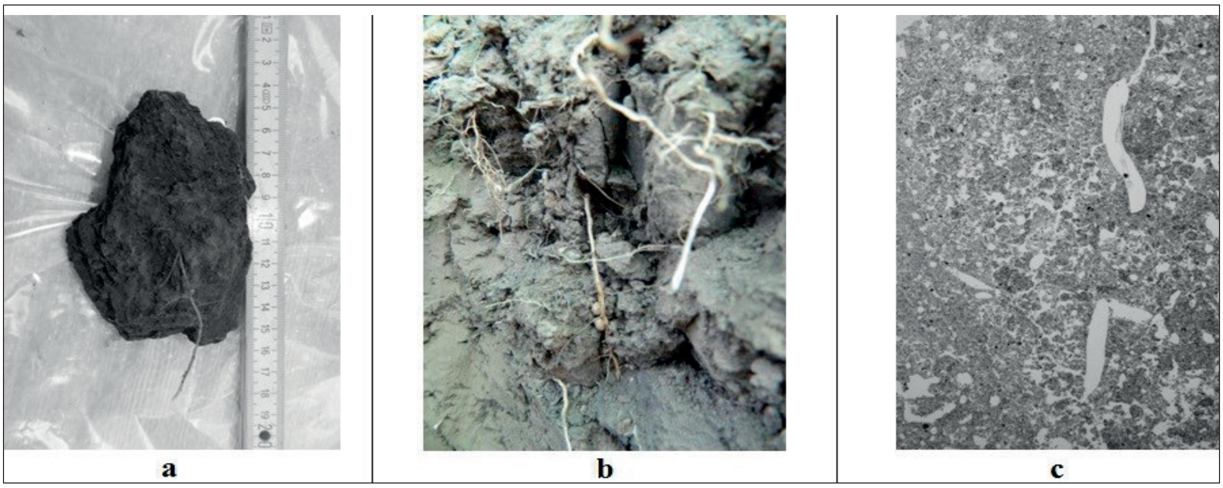

Figure 7. a) Structural element sampled from Apt horizon; b) nodules formed in a compacted soil; c) soybean roots preferential developed in areas with intense biological activity

In the compacted horizon, with large structural elements (Figure 7a), the number of nodules developed on soybean roots were much smaller.

At micromorphological level (in thin sections - Figure 7c) the observations showed that soybean roots prefer the areas with intense biological activity, and developed mainly into the bio-pores, where the average of the nodules per plant (Figure 7b) was 28.7 nodules/plant. In these areas, with a rich poral space, consisting of many interconnected bio-pores through which water and air circulate unhindered, both soybean roots and *Rhizobium* nodules were well developed.

CONCLUSIONS

The image analysis was a very useful tool in the study of the poral space genesis under the structural reconstruction of soil fauna activity.

The results of image analysis showed unspecific conditions in the studied Chernozem, thus: Ap horizon was more compacted (total porosity = 0.21 m^2m^{-2}) than the underlined Apt (0.33 m^2m^{-2}).

In agreement with the data obtained by means of image analysis (pores quantified according to their size and shape), the oriented thin sections gave a clear representation of the genesis, location and the characteristics of the poral space.

The soybeans roots developed mainly into the areas with intense biological activity.

The soil macro- and mesofauna proved to be good habitat builders for soybean roots and the *Rhizobium* nodules, even in the conditions of a droughty period with the irrigation application delay.

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