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DIMENSION AND STRUCTURAL TRAITS OF SOIL MICROPORES IN CULTIVATIONS DIFFERING IN THE DURATION OF ORGANIC MANAGEMENT

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Abstract

The objectives of this study were to investigate: a) whether the duration of organic farming influences the dimension, shape and geometry (fractal dimension) of soil micropores (<50μm) and b) for relationships of morphometric traits with certain chemical and biochemical soil variables (microbial biomass, N- and C-mineralization rates, NH_4^+, NO_3^-, organic C and N, extractable P, Mg^{+2}, K^+, Ca^{+2}). We compared soil micromorphometric traits (area, perimeter, compactness, solidity, eccentricity) among fields with different duration of organic farming (2 (O2), 3 (O3), 5 (O5), and 6 years (O6)): these were planted with *Asparagus officinalis* and one was conventional cultivation (CV). No significant differences were observed among the morphometric traits of all fields. However, the soil of the older organic areas (O6, O5 and O3) was characterized by small-sized pores (<10 μm) while the newest (O1) and the conventional field were characterized by medium-sized micropores (10-20 μm). The fractal dimension D_2 of the larger pores was found to be significantly higher in O2 and O3 fields, indicating larger outline irregularity for these particular pores. Higher fractal dimension could be related to more heterogeneous distribution of the microbial community in space. All micropores were correlated with the concentration of soil mineral nutrients (Mg^{+2}, K^+, Ca^{+2}). In the small pore size category (≤10 μm), N-microbial and NO_3^- concentrations, parameters involved in the nitrogen cycle, were found to be correlated to the structure characteristics. Taking into account that the three older organic fields are characterized mainly by small sized pores (≤10 μm), it is suggested that improved soil quality is mainly related with the N-cycle.

Key words: fractal dimension, image analysis, micromorphometric traits, soil thin sections

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1. Introduction

The unfavorable effect of long lasting conventional farming on soil structural characteristic is well documented (e.g. Marks and Soane, 1987) and many authors recommend the employment of alternative farming practices to encounter harmful effects (Pagliai et al., 1989; Shipitalo and Protz, 1987). Soil porosity is generally considered an efficient parameter of soil structure. Specifically, the size, the shape and the connectivity of soil pores determine important soil processes such as water and mineral transportation, soil aeration etc. (Ringrose-Voase, 1990; Youngs and Leeds-Harisson, 1990). Moreover, regarding pore size, the soil canal network constitutes the habitat for a large array of soil fauna and microbes (Elliott and Coleman, 1988).

To study soil micromorphometric traits, techniques of image analysis are broadly used especially for comparing the effects of alternative farming practices on soil structure (Bui et al., 1989; Kooistra et al., 1985; Livingston et al., 1985; Shipitalo and Protz, 1987). Most relevant studies focus on soil porosity (Pagliai et al., 1984, Ringrose-Voase et al., 1990), as well as on void fractal dimension (Mandelbrot, 1982). Hatano and Booltnik (1992) have

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achieved to formulate a model adequately describing the relationship between pore perimeter and surface.

The change in soil quality because of management practice is a current topic of interest. There is growing evidence that organically cultivated systems exhibit improved soil quality than conventional ones (Droogers and Bouma, 1996; Monokrousos et al., 2006). However, very few studies have been conducted regarding the changes that organic farming might induce to the soil structure during the conversion period from conventional to organic farming and afterwards. Among them, Gerhardt (1997) and Papadopoulos et al., (2006) concluded that the management methods associated with organic farming induce the formation of an ameliorated soil structure, which is porous, better developed and has increased organic matter content and soil biota presence and activity. Based on literature review, more studies have been conducted on pores sized >50 μm in equivalent diameter, whereas our research specifically focused our focused on the study of soil micropores (<50 μm) on which relevant information is limited (Menendez et al., 2005; Pachepsky et al., 1996). These micropores play an important role for agriculture as they are responsible for water storage in the soil (Pagliai et al., 1981), form the soil microaggregates, allowing the growth of root hairs, and also form protective micro-habitats which will allow the increase of soil microbial biomass (Heijen and Van Veen, 1991). Thus, changes of morphological traits and numbers of soil micropores in organically cultivated fields can be regarded as a clear symptom of improved soil structure (Greenland, 1981).

In this paper we studied micromorphometric traits of the void phase in four organic areas of *Asparagus officinalis* (L), using image analysis of soil thin sections. The areas differed regarding the age of organic farming, so as to check for. We explore questions relating as to whether the age of organic farming influences the dimensions (surface, equivalent diameter and perimeter), the shape (eccentricity, solidity and compactness) and the fractal dimension of soil micropores. Moreover, in an attempt to associate micromorphometric traits with soil biochemistry, we further sought for correlations between the whole set of values relating to void phase with the corresponding set of values of certain biochemical variables.

2. Material and methods

2.1. Study area and sampling

The study area is located in Kria Vrisi 60 km north-west of Thessaloniki, northern Greece. The parent rock consists of Alluvial deposits (Holocene) (IGMR, 1983). In order to evaluate the influence of management practices on the soil profile, five undisturbed vertical soil samples were collected with cylindrical boxes (3 cm diameter, 4.5 cm high) from each sampling area. The samples were taken in September 2003 from fields planted with the perennial *Asparagus officinalis* (L). All fields were of similar soil texture (Monokrousos et al., 2006). Five organically cultivated fields and a conventional one was sampled. The organic fields represented a gradient in relation to the duration of organic cultivation; 2 (O2), 3 (O3), 5 (O5) and 6 (O6) years. Those cultivated organically for 2 and 3 years were considered as fields in transition (Stanhill, 1990). All organic fields were nearby to each other within a continuous field area of about 30.000 m^2. They were conventionally cultivated for several years before conversion to organic field. The conventional field (CV) covered an area of 9.000 m^2 and was located almost 500 m away from the organic ones. It was cultivated conventionally for more than 6 years.

2.2. Soil thin-section preparation

The samples were air-dried for a week and then were dried in an oven at 35^0C for 48 h. The first step included the placement of the boxes in a polyester resin system applied under vacuum in the resin impregnation process (Crystic17449, MEKP, Accelerator ‘G’; Scot Bader) (Sole et al., 1992). The resin droplets penetrated the soil pores by capillarity until each soil sample was fully saturated with resin. Impregnation time was 7 h and the hardening period 6 weeks. The resinous soil blocks were allowed to dry over a period of 3 months, after which a thick section was cut, mounted on a glass slide and polished to a thickness of 20-25 μm. Then, the soil blocks were bonded to polished glass slides (110 mm - 75 mm - 3 mm) with a standardized mass of epoxy (Epoxy 301; Logitech), then cut and lapped. Thin sections were then mechanically polished using 3 mm diamond pasted.

2.3. Image acquisition and thin section porosity analysis

To study the pore and soil particle distribution the soil blocks were photographed using a digital camera coupled with a polarizing microscope. Images (752 x 582 pixels) were taken using a camera with an objective magnification of 20x. Three replicates were obtained from each block.

The use of polarized microscopy allowed the discrimination of soil pores and matrix by color. Minerals are represented by various colors, the organic material by brown color, while the resin inside the pores by black color. The segmentation and classification of the digital image to patches was based on these different colors as well as on differences on the texture. Each soil section is considered a landscape image and for image analysis the eCognition program was used. The digital photos were transformed from vector to raster and the results were processed using Matlab. The following pore micromorphometric traits were estimated: area, perimeter, equivalent diameter, eccentricity, compactness and solidity. The first three parameters stand for pore dimensions, while eccentricity, compactness and solidity for the shape of pores. Eccentricity is equal to the ratio of the distance between the foci of the ellipse to the length of its major axis. Eccentricity values range between 0 and 1 (zero eccentricity corresponds to a circle while unity corresponds to a line). To estimate compactness, we used (Eq. 1)

$$C = \frac{(perimeter)^2}{area} \quad (1)$$

Finally, solidity is equal to the percentage of the pore which is within a convex hull. The relationship between area (A) and perimeter (P^2) is widely used in soil micromorphology to characterize the shape of pores (Murphy et al., 1997). In this paper we employed Swartz (1980) shape index (Eq. 2):

$$F=4\pi A/P^2 \quad (2)$$

This index is used to classify pore shapes into elongated or planar ($F<0.2$), irregular ($0.2<F<0.5$) and rounded ($F>0.5$) pores (Bouma et al., 1977, Mermut et al., 1992). The pores were further subdivided into three size classes (class 1: ≤10 μm, class 2: 10-20 μm, class 3: 20-50 μm) according to either the equivalent pore diameter for rounded and irregular pores and their width for elongated pores (Pagliai et al., 1983).

The soil biochemical variables employed in this paper are described in Table 1. These data relate to the sampling period of September 2003 and were presented in Monokrousos et al. (2008).

2.4. Statistical analyses

A correspondence analysis was used to ordinate samples from various sampling areas with regard of their relative frequencies of the three pore size categories (≤10 μm, 10-20 μm, 20-50 μm). To estimate the fractal dimension the linear model (Eq. 3) was fitted on data:

$$Log\ P = \alpha + bLog\ S \quad (3)$$

where: *P* stands for perimeter, *S* is surface, *a* is elevation and *b* is slope.

Following Pachepsky et al. (1996) a piecewise nonlinear regression model has been fitted. The algorithm estimated parameters a_1 and b_1 for data up to the breakpoint and a_2 and b_2 for data beyond the breakpoint. To further seek for statistical differences among slopes and elevations an Analysis of Covariance (ANCOVA) was performed. Finally, a Canonical Analysis was performed to assess the relationships between the pore micromorphological traits and the soil biochemical variables.

3. Results and discussion

As it was revealed by estimating the Swartz index, the large elongated pores contribute 64-72% to soil porosity, whereas the small rounded pores participate with 12-17% (Fig. 1).

Pores were discriminated in three pore size categories (≤10 μm, 10-20 μm, 20-50 μm) according to their equivalent diameter. In Table 2 are presented the average values of morphological traits in all sampling sites for each pore size category. No significant differences were revealed in relation to management type (organic vs conventional farming) in dimension and shape measurements, for any of the three pore size categories. A One-Way ANOVA accompanied by a Bonferroni test showed that elongated pores occupy larger void surfaces in organic plots of age 2 and 3 years.

The pores were further subdivided into three classes according to the equivalent pore diameter for the rounded and irregular pores and their width for the elongated pores up to the size of 50 μm. The percentage contribution of the three size categories is shown in Fig. 2. The contribution of the small sized pores in the total void of the soils is higher than the other two categories, varying between 72-77%.

Table 1. Mean values (± standard error) of soil chemical and biochemical variables

Biochemical variables	***Mean values (±SE)***
pH	7.83 ± 0.09
Microbial C (μg g^{-1})	170.79 ± 8.68
Microbial N (μg g^{-1})	9.41 ± 0.32
NH_4^+ (μg g^{-1})	20.43 ± 0.79
NO_3^- (μg g^{-1})	34.04 ± 1.17
N-mineralization rate (μg $g^{-1}d^{-1}$)	0.26 ± 0.03
C-mineralization rate (μg $g^{-1}d^{-1}$)	166.64 ± 13.08
Organic C (mg g^{-1})	12.53 ± 0.89
Organic N (mg g^{-1})	1.06 ± 0.05
C/N ratio	12.02 ± 0.92
P extractable (μg g^{-1})	33.81 ± 3.73
Mg^{+2} (mg g^{-1})	0.31 ± 0.03
K^+ (mg g^{-1})	0.63 ± 0.09
Ca^{+2} (mg g^{-1})	0.22 ± 0.01

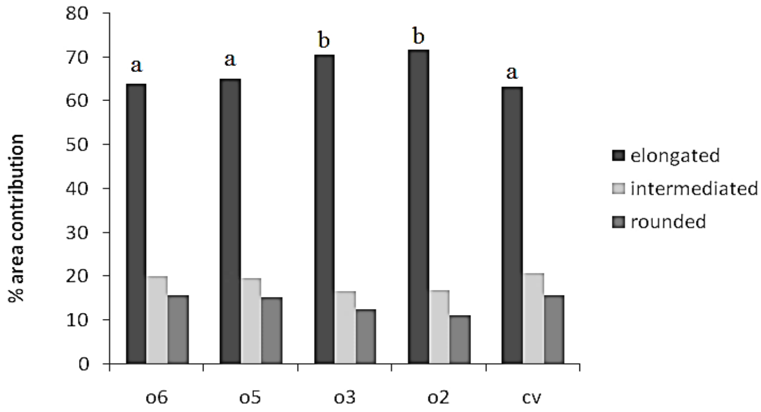

Fig. 1. Percentage contribution of the three pore shape categories in surface area in all sampling areas. Different letters correspond to statistically significant differences, as indicated by ANOVA, between the % contribution of the elongated pores of the sampling areas ($p<0.001$)

The results of the correspondence analysis indicated that the first axis explain 84.32% of the samples inertia. Samples from O6, O5 and O3 areas were ordinated towards the left end point of the first axis. Moreover, the smallest pore size category (≤10 μm) was ordinated to the left, while the middle pore sized (10-20 μm) was ordinated in the middle and the largest sized (20-50 μm) on the right end point of this axis. These results indicate that the three oldest organically farmed areas are characterized by small sized (≤10 μm) pores while the newest organic area and the conventional one are characterized mainly by medium sized pores (10-20 μm).

The changes of microporosity due to different cultivation management are a very important for agriculture in general, because micropores determine the amount of water available to plants and the soil capacity for water storage (Pagliai et al., 1981). The micropores whose diameter is about 10 μm form the soil microaggregates, allowing the growth of root hairs and providing the ground for a good soil structure. Increase of micropores (0.2-50 μm) can be regarded as a clear indication of improved soil structure (Greenland, 1981). Soil structure is known to have a large influence on the population dynamics, ecology and activity of microorganisms (Stotzky, 1986). The small sized pores (≤10μm) form protective micro-habitats which are large enough to allow bacteria to enter but too small to be colonized by predating protozoa (Heijen and Van Veen, 1991). As shown the older organically cultivated fields in Krya Vrisi are characterized by the smallest pore size category and this is in accordance with the larger amounts of microbial biomass recorded in these soils (unpublished data). An increase of microbial biomass is a clear indicator of improved soil quality and therefore, it could be implied that the quality of soil structure is improved after three years of continuous biological cultivation (Fig. 3). Fractal dimension (D) describes the geometry of a pore rather than its shape. Dependencies of pore outline perimeter P on outlined area A are shown in Fig. 4a-e. In Table 3 are given the values of surface fractal dimension for voids as well as the corresponding breakpoints.

Table 2. Mean values (± standard error) of morphological traits in all sampling sites of the three pore categories, as these are shaped according to their size

	Area	*Perimeter*	*Eccentricity*	*Solidity*	*Compactness*
			≤10μm		
O6	31.76 ± 0.64	21.91 ± 0.39	0.82 ± 0.01	0.80 ± 0.01	15.77 ± 0.47
O5	30.75 ± 0.33	21.78 ± 0.48	0.83 ± 0.01	0.79 ± 0.01	15.99 ± 0.55
O3	29.54 ± 0.60	20.83 ± 0.46	0.81 ± 0.01	0.81 ± 0.02	15.26 ± 0.58
O2	31.63 ± 1.24	21.73 ± 0.89	0.81 ± 0.01	0.81 ± 0.02	15.57 ± 0.83
CV	31.74 ± 0.92	21.35 ± 0.92	0.81 ± 0.01	0.83 ± 0.12	15.01 ± 0.53
			10-20μm		
O6	166.27 ± 5.32	79.13 ± 2.43	0.83 ± 0.01	0.64 ± 0.02	39.58 ± 1.97
O5	153.44 ± 3.77	76.65 ± 1.95	0.82 ± 0.01	0.62 ± 0.02	40.02 ± 1.88
O3	148.95 ± 3.69	74.83 ± 3.41	0.83 ± 0.01	0.65 ± 0.02	39.45 ± 3.23
O2	149.69 ± 3.91	68.53 ± 1.03	0.82 ± 0.01	0.68 ± 0.01	32.78 ± 1.18
CV	151.59 ± 3.37	69.57 ± 1.78	0.81 ± 0.01	0.68 ± 0.01	33.59 ± 2.12
			20-50μm		
O6	768.71 ± 16.53	281.09 ± 15.93	0.84 ± 0.01	0.54 ± 0.03	108.53 ± 10.67
O5	741.76 ± 31.84	267.18 ± 17.26	0.80 ± 0.01	0.54 ± 0.02	103.71 ± 11.44
O3	759.72 ± 45.63	283.77 ± 26.79	0.83 ± 0.01	0.55 ± 0.03	113.29 ± 16.38
O2	687.11 ± 25.02	235.96 ± 12.38	0.84 ± 0.01	0.56 ± 0.02	85.53 ± 6.88
CV	753.30 ± 20.05	240.11 ± 10.89	0.84 ± 0.01	0.59 ± 0.02	81.52 ± 6.56

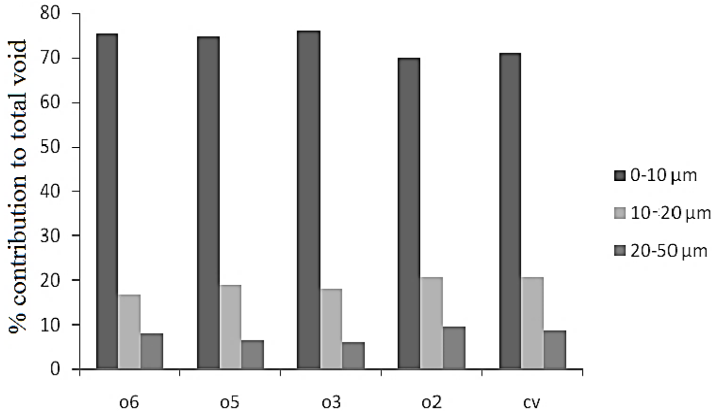

Fig. 2. Percentage contribution of the three pore size categories in the total void in all sampling areas

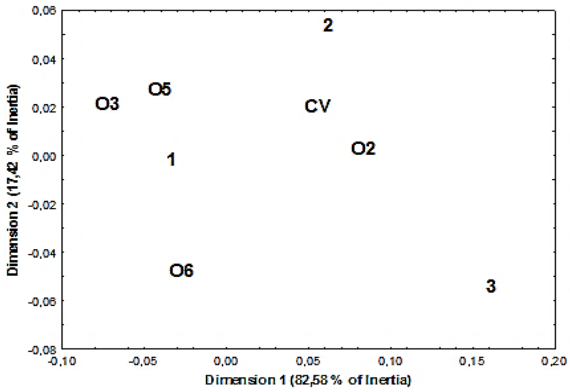

Fig. 3. Coordination of the pore size categories (1: ≤10 μm, 2:10-20 μm, 3: 20-50 μm) and the sampling areas (O6: 6 yrs, O5: 5 yrs, O3: 3 yrs, O2: 2 yrs and CV: conventional field) according to correspondence analysis

Furthermore, fractal dimension values reported for smaller voids (surface lower than that indicated by the corresponding breakpoint of the model) are lesser (1.112-1.272) than the fractal dimension values estimated for their larger counterparts (1.416-1.54) (Table 3). The fact that the fractal dimension D_1 for the small pores is closer to 1 indicates their rounded geometry.

Analysis of covariance showed that the elevations and the slopes of the relation for the smallest pores did not differ statistically among the areas (Table 4). Thus, no significant differences were detected between fractal dimensions of pore outlines among the sampling areas (Table 3). In contrast, elevations and slopes for larger voids were found to be affected significantly by the age of farming (Table 4). Fractal dimension D_2 is significantly larger in samples from the O3 and O5 areas than in samples from the other areas (Table 3). According to Kampichler and Hauser (1993) the variations in the fractal dimension may result in variations in the available habitat for different microbial species. The higher fractal dimension could be related to more heterogeneous distribution of the microbial community in space. Highest D_2 fractal dimension estimated for O3 and O5 indicating more favorable biological transformation processes as well as easy infiltration of water in case of water surplus and water retention under conditions of water deficit (Yakovchenko et al., 1996). Accordingly, it is concluded that more favorable conditions for soil humidity and biological transformations prevail in organic fields aged more than 2 years.

Table 3. Fractal dimensions values and breakpoint for soil samples from all sampling areas

	D₁	***D₂***	***Breakpoint***
O6	1.216	1.474^b	18.2 μm²
O5	1.272	1.540^a	20.0 μm²
O3	1.242	1.538^a	17.8 μm²
O2	1.166	1.416^b	19.1 μm²
CV	1.112	1.474^b	18.6 μm²

Table 4. Results of ANCOVA on the lines from which emerges the fractal dimensions D_1 and D_2 of the solid phase of the sampling areas

	p-values	
	D_1	***D_2***
Elevation (a)	0.076	0.0001
Slope (b)	0.057	0.0001

To correlate the set of biochemical variables with the set of micromorphometric variables of the voids a Canonical Correlation Analysis was employed for each pore size category separately. In all size categories only the first root of the analysis was statistically important, which accounts for the 87-96% of the between sets correlation. Furthermore, the analysis showed that the relationship between the analyzed sets was indeed linear, while no point clouds are depicted on the relevant graphs (not provided). Accordingly, the linear correlation is homogeneous along the whole range of values. For all pore classes, significant are those correlations between morphometric variables, which are describing the structure of the pores such as solidity and compactness, and biochemical variables such as N microbial, Ca^{+2}, Mg^{+2}, K^+ and the rate of C-mineralization. More specifically, in small sized pores (≤10 μm), a correlation exists between the pore solidity and compactness and the N-microbial, NO_3^-, K^+ and Ca^{+2}. In the middle size category (10-20 μm) the correlation is noticed between pore solidity and N-microbial, N mineralization rate, K^+, P extractable, Ca^{+2} and Mg^{+2} concentrations, while in the larger size category (20-50 μm) the correlation is between solidity and compactness and the rate of C-mineralization as well as the Mg^{+2} and K^+ concentrations. Pores with equivalent diameter of <50 μm are considered to be the storage pores of soil. The root hairs grow along these pore channels and sometimes modify and enlarge them, at the same time absorbing nutrients, water and oxygen via the adjoin soil material (De Willigen and Van Noordwijk, 1987).

The correlation between the availability of soil nutrients and the structure of voids could be explained by the fact that the latter is driven to a large extent by growing root hairs.

In the middle and larger (10-50 μm) pore size categories the correlation between the N- and C-mineralization rates with the structural morphometric traits indicate high microbial activity. Strong et al. (2004) also found that the decomposition rate of organic C is faster in medium sized pores with neck diameter 15-60 μm than in smaller ones. This is due to greater microbial activity since this region supports both microbial motility and best oxygen supply. Fungi have been found to be more abundant in this pore class (Strong et al., 2004) supporting larger numbers of fungal-feeding nematodes (Jones and Thomason, 1976, van der Linden et al., 1989). Thus the fast rate of decomposition of organic C in the 20-50 μm sized could be enhanced by the nematode activity (Table 5).

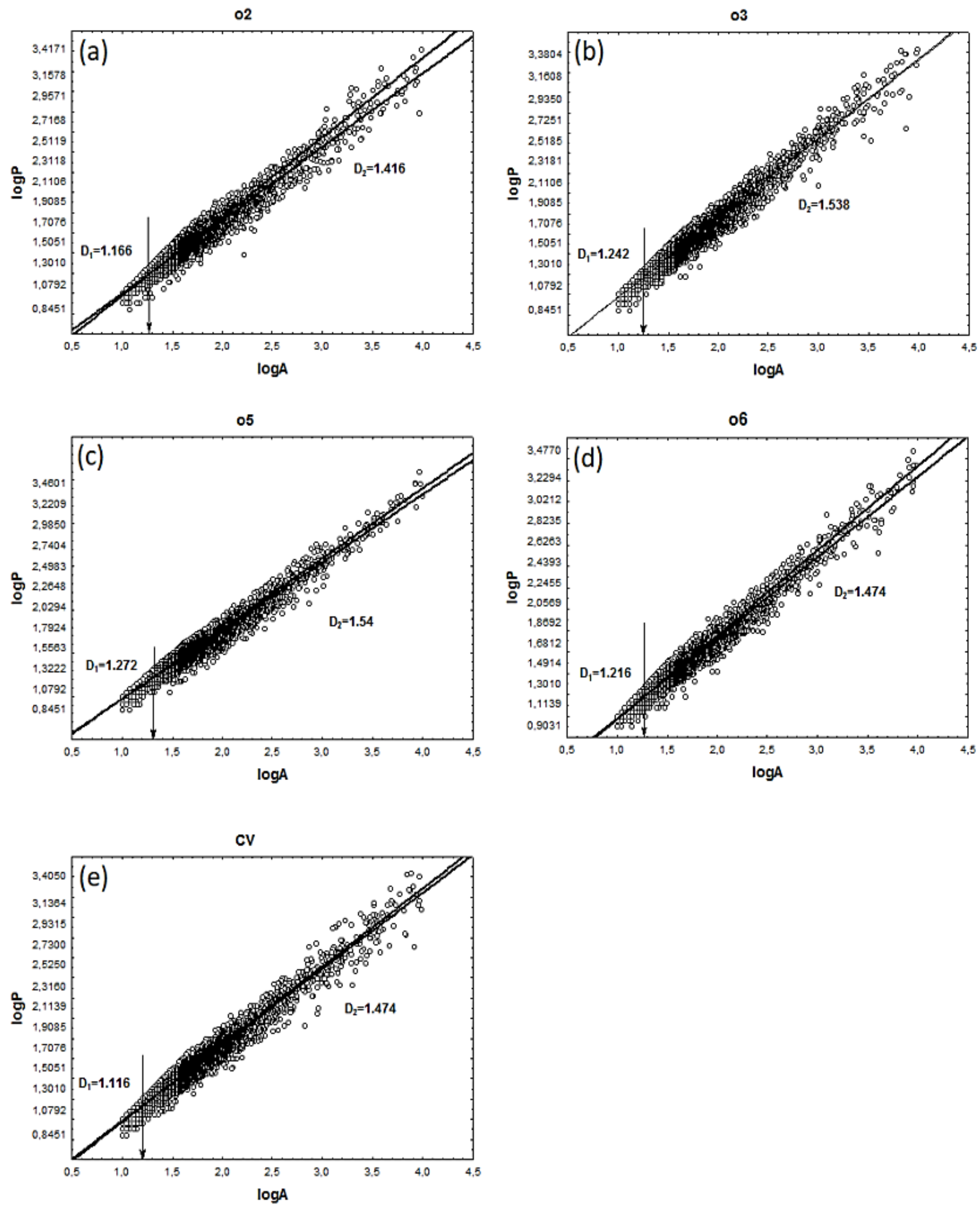

Fig. 4. Dependencies of the pore outline perimeter (P) on the outlined area (A) for samples from all sampling areas: (a) O2, (b) O3, (c) O5, (d) O6 and (e) CV

Table 5. Factor structure of canonical analysis for chemical and biochemical and morphometric variables

	Variables	***≤10 μm***	***10-20 μm***	***20-50 μm***
Morphometric	perimeter	0.272	-0.340	0.190
	eccentricity	-0.628	0.596	0.047
	solidity	-2.653	1.088	0.391
	compactness	-2.237	0.322	-0.801
	pH	0.153	0.052	0.101
Biochemical	C microbial	0.171	0.299	-0.226
	N microbial	-0.486	-0.386	-0.109
	NH_4^+	0.247	-0.109	-0.005
	NO_3^-	-0.306	0.001	0.232
	N-mineralization	-0.190	0.379	-0.153
	C-mineralization	0.050	0.180	-0.518
	C organic	-0.183	-0.132	0.199
	N organic	-0.213	0.190	0.261
	C/N ratio	-0.153	-0.109	0.152
	P extr	-0.252	-0.516	-0.387
	Mg^{+2}	0.043	-0.457	-0.660
	K^+	0.386	0.901	0.659
	Ca^{+2}	-0.380	-0.449	0.323

In the small pore size category (≤10 μm) N-microbial and the concentrations of NO_3^- are also correlated with the structure characteristics. It is noticeable that these biochemical parameters are involved in the nitrogen cycle. Consequently, it seems plausible that in this region there are dominant processes that transform nitrogen to absorbable by the root hairs forms. Taking into account that the older organic fields (O6, O5, O3) are characterized mainly by the small sized pores (≤10 μm), we could suggest that the improved soil quality in these field is rather related with the N-cycle.

4. Conclusions

The duration of organic farming is reflected on the dimension and structural characteristics of micropores. Areas organically cultivated for more than two years are mainly characterized by small sized pores (≤10 μm), while the soil of organic cultivations with duration of three and five years consist of pores with higher fractal dimension supporting a more heterogeneous distribution of microbes in space. In the small pore size category (≤10 μm), N-microbial and NO_3^- concentrations, parameters involved in the nitrogen cycle, were found to be correlated to the structure characteristics. All the changes related to the properties of soil microporosity took place after the 2-3 years' time period that is necessary for the conversion of a cultivation from a conventional to an organic one. These results support the idea that alternative cultivation practices, such as organic farming result in improved soil structure and quality.

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