Relation between chemical indices of soil and earthworm abundance under chemical fertilization

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ABSTRACT

The study intended to establish how the dynamics of earthworms (Oligochaeta: Lumbricidae) changes in soil (abundance, biomass), under conditions of mineral fertilization with nitrogen and phosphorous in four different doses, in a 33-year experimental placement in the west of Romania, in wheat-soybean-maize-barley rotation. The soil indices taken into study were: pH, humus, total nitrogen, phosphorous and potassium. Statistical connections between the studied factors were realized using the dispersion analysis ANOVA and the SPSS Software (Statistical Package for the Social Sciences). The study showed an increase of earthworm abundance and biomass under conditions of chemical fertilization with nitrogen and phosphorous. The highest number of earthworms was recorded in the treatment with the largest dose of nitrogen fertilizer (by 85.85% higher compared to the control treatment). The greatest positive influence on earthworm abundance and biomass was manifested in humus and total nitrogen. The greatest negative influence on earthworm abundance was found in pH factor, while phosphorous content of soil exerted the greatest negative influence on earthworm biomass.

Keywords: earthworms; abundance; biomass; nitrogen and phosphorous fertilization

Among the organisms with their living activity in soil, the earthworms are recognized for their important role regarding the improvement of physical and chemical characteristics of soil, and thus increasing its fertility (Aina 1984, Edwards and Bohlen 1996, Abdul Rida and Bouché 1997). Knowing their dynamics in soil under the influence of different technological treatments, or as a result of fertilizers application, it is very important for soil fertility conservation (Aira et al. 2006, Asawalam 2006).

The influence of various inorganic fertilizers on earthworms was studied since 1982 by Edwards and Lofty in three long-term experiments and two short-term field experiments, one on grass and one on wheat, in conditions of annual fertilizer treatments with various forms of inorganic nitrogen (48, 96, 144 and 192 kg N/ha). The results of these researches showed that earthworms were more numerous in plots treated with inorganic nitrogen than in untreated plots. There was a strong positive correlation (r = 0.9825) between the amounts of inorganic N applied and populations of earthworms. Plots receiving inorganic nitrogen had the largest populations of earthworms (Edwards and Lofty 2002).

The effects of long-term use of nitrogenous fertilizers were studied with 3 different amounts (60, 120 and 180 kg N/ha/year) from 6 different sources (mineral ammonium sulphate, nitrochalk, synthetic sulphur-coated urea, organic-coated urea, isobutylidenediurea, and ureaformaldehyde) in uncultivated turfgrass on loamy sand soil. Results showed that mineral ammonium sulphate and sulphur-coated urea drastically decreased earthworm numbers and biomass and lowered pH of soil, nitrochalk had minor effects on pH and earthworms, and the effects of isobutylidenediurea, ureaformaldehyde and organic-coated urea remained intermediary. The study distinguished that endogaeic species of earthworms such as *Aporrectodea* spp. were more strongly affected than the epigaeic group of *Lumbricus* spp., with the exception of *A. caliginosa tuberculata* (Ma et al. 1990).

Other studies evaluated the effect of 14 years of treatment with NPKMg fertilizer compared to the

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manure fertilization on earthworm populations, on a clay loam (Humic Gleysol) situated at St-Lambert, Quebec. The chemical fertilization was carried out over a 4-year crop rotation of silage corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and canola (*Brassica campestris* L.). Earthworm sampling was done in fall 1991 under corn culture. The results established that mineral fertilizer application had no significant effect on earthworms, and compared to mineral fertilizer treatment, long-term application of manure increased earthworm populations (Estevez et al. 1996).

Numerous studies concerning the evolution of earthworm abundance and biomass focused on differences that appear between organic fertilization of soil and mineral fertilization. Whalen et al. (1998) found that abundance and biomass of earthworm communities in corn agroecosystems were significantly affected by the type of fertilizer treatment. Thus, plots amended with manure supported less earthworm number than inorganically fertilized plots (14.5% less individuals of *Lumbricus* spp. in the case of nitrogen fertilization against 18.6% in organic fertilization).

Conservation tillage, addition of nutrients either in organic or inorganic form for adequate crop production, and favourable soil moisture are all important in maintaining earthworm abundance and microbial activity in long- and short-term studies (Jordan et al. 2004).

The effect of chemical fertilization with NPK on number, biomass and coprolite weight of earthworms compared to organic fertilization with cattle manure showed in some cases that positive results are obtained when both types of technologies (chemical and organic) are applied (Tiwari 1993).

Several studies demonstrated that the application of chemical fertilizers as pulverization or powder can have disastrous effect on earthworm populations. Fertilizers with nitrogen create acidific conditions in soil, which is fatal for earthworms (Reinecke and Reineke 2004).

The present study intended to establish how dynamic of earthworms (Lumbricidae) changes in soil (abundance, biomass), in conditions of mineral fertilization with different doses (four) of nitrogen and phosphorous, in wheat culture.

MATERIAL AND METHODS

The experimental field belongs to OSPA Timiş (Office of Pedological and Agrochemical Studies from Timiş County, Romania), and the experimental soil was classified as a Calcic Luvisol according to the FAO System. The GPS coordinates of the sampling site are: latitude 45°85'31''N, longitude 21°16'81''E, Universal Transverse Mercator (UTM): ER17. The experimental device represents long term experiments (33 years) of application of chemical fertilizers with nitrogen and phosphorous, in a stationary regime, in wheat-soybean-maize-barley rotation (*Triticum aestivum* L.-*Glycine hispida* (Mnch)-*Zea mays* L.-*Hordeum vulgare* L.). Earthworm extraction from soil was realized in 2008 in wheat culture in control treatment and in the experimental treatments organized in three repetitions, using the formaldehyde solution 2%, according to specific methodology enounced by the standard ISO 23611-1/2006: Soil quality-Sampling of soil invertebrates, part 1-Hand-sorting and formaldehyde extraction of earthworms. It must be mentioned that this method can expel from soil only the earthworms located in the first 40 cm of soil. The inorganic fertilizers used for 33 years were NH_4NO_3 and superphosphate with 36% P_2O_5. The fertilizers were applied as follows:

Wheat and barley: half of nitrogen dose in autumn before seeding, at soil preparation and the other half in spring in vegetation; the phosphorous was applied in autumn before ploughing;

Soybean: nitrogen was applied in vegetation, after examination of plant nodules; phosphorus was applied in autumn before ploughing.

Maize: nitrogen was applied in spring at soil tillage, before seeding; the phosphorus was applied in autumn, before ploughing.

The experimental doses of fertilizers were established in the following amounts: 100 kg N/ha + 44 kg P/ha ($N_{100}P_{44}$); 150 kg N/ha + 44 kg P/ha ($N_{150}P_{44}$); 200 kg N/ha + 0 kg P/ha ($N_{200}P_0$); 200 kg N/ha + 44 kg P/ha ($N_{200}P_{44}$).

The pedological conditions and soil profile were described according to the FAO System. The physical and chemical analyses of soil were realized in 2008, according to the following methodologies:

the soil reaction (pH values) was established by the potentiometric method in aqueous suspension (pH_{H_2O}), ratio soil: solution 1:2.5;

total nitrogen content of soil: by the Kjeldahl method, digested with H_2SO_4 at 350°C, catalytic agents potassium sulphate and copper sulphate;

plant-available phosphorus and potassium from soil were determined by the spectrophotometry and flame spectrometry methods, respectively, in acetate-lactate ammonium solution (AL) at 3.7 pH, by the Egner-Riehm-Domingo method;

total organic carbon (humus) was determined according to the Walkley procedure (1947), by rapid dichromate oxidation;

Soil for chemical analyses was sampled following the general requirements of ISO 10381/2002 – parts 1 and 4. For each plot, a randomized sampling template with a grid of 100 cells was used. The sampling cells were selected at random, so that no cells are less than six-cell distance each other. Within each cell, three sampling points were established.

In parallel with numerical and biomass study of earthworms, data were recorded on several chemical indices of soil such as pH, humus, total nitrogen, phosphorous, and potassium to establish their influence on earthworm dynamics, in conditions of inorganic fertilization with nitrogen and phosphorous.

The statistic connections between all researched factors were realized based on statistical methods, consisting in dispersion analysis ANOVA (Analysis of Variance). For statistical calculation, the SPSS software (Statistical Package for the Social Sciences) was used.

The physical and chemical characteristics of the Calcic Luvisol are presented in the Tables 1 and 2.

RESULTS AND DISCUSSION

The data recorded for all types of inorganic fertilization, namely, $N_{100}P_{44}$, $N_{150}P_{44}$, $N_{200}P_{44}$ and $N_{200}P_0$, showed an increase of earthworm number according to the nitrogen and phosphorous dose contained in the chemical fertilizers. The highest value was recorded in the treatment with the largest dose of nitrogen (N_{200}), 85.85% higher than the value found in the control treatment. The simultaneous application of the nitrogen and phosphorous increased the earthworm number in the treatment $N_{100}P_{44}$, where the earthworm number was by 57.23% higher than in the control treatment; values of earthworm number and biomass, related to chemical indices of soil, are presented as the mean below (Table 3).

The values of earthworm biomass indicated a different evolution compared to earthworm abundance, fact that could be explained by various degree of biological development (juvenile and adult organisms), which means that the applied dozes of inorganic fertilizers could influence the reproduction rate of earthworms, thus representing a possible aspect for a future study.

Concomitantly with data about numerical and biomass evolution of earthworms there were registered data regarding the total nitrogen and phosphorous content of soil, to study their influence on earthworm abundance and biomass, in conditions of inorganic fertilization with nitrogen and phosphorous (Table 3). The statistical study showed that the greatest positive influence on earthworm abundance was exerted by the factors of humus ($r = 0.667$) and total nitrogen ($r = 0.639$).

The values of correlation coefficients showed a major positive influence of humus factor on earthworm biomass ($r = 0.726$). Another factor

Table 1. The physical characteristics of the soil

Depth of pedological horizon (cm)	0–17	17–29	29–43
Sample depth (cm)	5–15	20–25	35–40
Rough sand (2.0–0.2 mm) (%)	0.6	0.5	0.5
Fine sand (international system) (0.2–0.02 mm) (%)	33.0	29.9	28.6
Dust (international system) (0.02–0.002 mm) (%)	30.1	31.7	27.7
Clay (< 0.002 mm) (%)	36.3	37.9	43.2
Physical clay (< 0.01 mm) (%)	52.2	55.4	56.4
Density (g/cm^3)	–	2.57	2.59
Apparent density (g/cm^3)	–	1.56	1.50
Total porosity (%)	–	39	42
Aeration porosity (%)	–	2	5
Compaction degree (%)	–	21	19
Resistance to standard penetration (kg/cm^2)	–	66	23
Blight coefficient (%)	–	15.3	15.8
Water field capacity (%)	–	23.7	24.6
Water total capacity (%)	–	25.0	28.0
Useful water capacity (%)	–	8.4	8.8
Hydraulic conductivity (mm/h)	–	40	180

Table 2. The chemical characteristics of the soil

Depth of pedological horizon (cm)	0–17	17–29	29–43
Sample depth (cm)	5–15	20–25	35–40
Total organic carbon (%)	2.42	2.35	2.23
N total (%)	0.120	0.115	0.109
P total (%)	–	–	–
C:N (%)	13.6	13.8	13.8
$CaC0_3$ (%)	–	–	–
pH in water (pH units)	5.80	6.35	6.70
P mobile (mg/kg)	22.5	11.7	2.7
K mobile (mg/kg)	157	126	153
Exchangeable bases (meq/100 g soil)	17.65	18.16	24.19
Ca^{2+}exchangeable (mmol/100 g soil)	6.75	7.25	–
Mg^{2+} exchangeable (mmol/100 g soil)	1.77	1.87	–
K^+ exchangeable (mmol/100 g soil)	0.42	0.33	–
Na^+ exchangeable (mmol/100 g soil)	0.19	0.19	–
H^+ exchangeable (mmol/100 g soil)	5.29	3.83	3.68
Ca^{2+} exchangeable (% of total)	56.9	64.1	–
Mg^{2+} exchangeable (% of total)	15.4	16.6	–
K^+ exchangeable (% of total)	1.8	1.5	–
Na^+ exchangeable (% of total)	0.8	0.8	–
H^+ exchangeable (% of total)	23.1	17.0	13.2
Base saturation (% of total)	76.9	83.0	86.8

with positive influence on earthworm biomass was the total nitrogen content of soil ($r = 0.430$). Two analyzed factors led to a decrease of earthworm number and biomass, namely the factors pH ($r = –0.629$ and $r = –0.307$, respectively) and phosphorous ($r = –0.120$ and $r = –0.454$, respectively).

The obtained results showed that application of these types of fertilizers increased the earthworm number and biomass in soil, especially the treatment with nitrogen. Even the significance of correlations between earthworm biomass and total nitrogen content of soil is less relevant, it must be notified that a reason could be the earthworm age, which is an important factor that must be taken into account. Thus, there were sampled earthworms with various weights, which affected the biomass quantification, because the juveniles weight less than adult organisms.

Table 3. Mean values of earthworm abundance and biomass and chemical indices of soil under chemical fertilization with NP

Experimental variants/ fertilization dose	Earthworm abundance (individuals/m^2)	Earthworm biomass (g/m^2)	pH	Total organic carbon content of soil	Total nitrogen content of soil	Phosphorous content of soil	Potassium content of soil
				(%)		(ppm)	
N_0P_0	9.33	4.40	6.67	2.26	0.22	10.11	140.00
$N_{100}P_{44}$	14.67	2.67	5.95	2.16	0.27	36.00	129.50
$N_{150}P_{44}$	9.33	3.47	6.37	2.16	0.26	32.12	128.50
$N_{200}P_{44}$	13.33	3.60	6.49	2.10	0.27	32.09	125.00
$N_{200}P_0$	26.67	9.20	6.12	2.53	0.31	13.46	131.50
Standard deviation	8.23465	2.49148	0.28204	0.16873	0.04719	18.09886	8.64215
Homogeneity degree	56.14	71.34	4.53	6.97	16.00	52.54	6.4

Table 4. The influence of chemical indices of soil on earthworm abundance

Model	*R*	*R* Square	Adjusted *R* Square	Std. Error of the Estimate	Change statistics				
					R Square change	*F* change	*df1*	*df2*	Sig. *F* change
A	1	2	3	4	5	6	7	8	9
1	0.800[a]	0.640	0.497	5.84237	0.640	4.453	4	10	0.025
2	0.795[b]	0.632	0.532	5.63230	–0.008	0.223	1	10	0.647
3	0.791[c]	0.626	0.563	5.44156	–0.007	0.201	1	11	0.663

[a]predictors: (constant): total organic carbon, K, pH, P; [b]predictors: (constant): total organic carbon, K, P; [c]predictors: (constant): total organic carbon, P

The statistical study intended to find some possible models of influence exerted by chemical indices of soil on earthworm abundance under chemical fertilization with nitrogen and phosphorous. Three models of interest were found. Within these models, the simultaneous influence of all chemical factors on earthworm abundance was studied:
model 1: includes the study of the factors: total organic carbon, K, P, pH;
model 2: includes the study of the factors: total organic carbon, K, P;
model 3: includes the study of the factors: total organic carbon, P.

The correlation coefficients (*R*) and determination coefficients (*R* Square) for all three models showed that connection between factors is strong and direct. For the first model (which indicates the most significant connection between variables) the correlation coefficient is 0.800, for the second model the correlation coefficient is 0.795, and for the third model the correlation coefficient is 0.791 (Table 4).

The values of *F* test showed that for models 2 and 3 the null hypothesis is rejected, which means that for these two models the earthworm abundance will be simultaneously influenced by the factors of total organic carbon, phosphorous and potassium, with a probability of 95% (Table 5).

In conclusion, our study shows that under conditions of inorganic fertilization with nitrogen and phosphorous in different dozes, performed on a Calcic Luvisol in the west of Romania an increase of earthworm number and biomass was noticed compared to the control treatment with no fertilization. The results on earthworm abundance and biomass were correlated to several chemical indices of soil, namely total nitrogen and phos-

Table 5. Analysis of variance (ANOVA) regarding the influence of chemical indices of soil on earthworm abundance

Model		Sum of squares	*df*	Mean square	*F*	F_{table}	Sig.
A		1	2	3	4	5	6
	regression	608.000	4	152.000	4.453	5.59	0.025[a]
1	residual	341.333	10	34.133	–	–	–
	total	949.333	14	–	–	–	–
	regression	600.383	3	200.128	6.309	3.88	0.010[b]
2	residual	348.951	11	31.723	–	–	–
	total	949.333	14	–	–	–	–
	regression	594.007	2	297.003	10.030	4.67	0.003[c]
3	residual	355.327	12	29.611	–	–	–
	total	949.333	14	–	–	–	–

[a]predictors: (constant): total organic carbon, K, pH, P; [b]predictors: (constant): total organic carbon, K, P; [c]predictors: (constant): total organic carbon, P

phorous content of soil, in chemical fertilization (NP), whose values were found favourable (total nitrogen) or unfavourable (phosphorous) to earthworm abundance and biomass in soil. The study also showed that pH decreases the earthworm abundance and biomass.

We believe that inorganic fertilization during the period of 33 years led to pH alteration of soil, namely acidification, that negatively affects earthworms; they prefer soils with pH values around 6.5–7.5 (Edward et al. 1995, Edwards and Bohlen 1996). In all experimental plots we found lower pH values (5.95–6.49), except for the control treatment where the pH was 6.67 (Table 3). Furthermore, the positive correlation between earthworm abundance and biomass and total nitrogen content in soil indicate that it is a consequence of relation that exists between inorganic fertilization with nitrogen, crop increase and feeding behaviour of earthworms. Earthworms feed with organic matter, which is abundant in the fields with large crops; it provides favourable premises for their living. Moreover, the mucus secreted by earthworm tegument, indispensable for their life because it is involved in the respiration mechanism, contains a large amount of nitrogen, which explains their preference for soils rich in this chemical element.

Other researchers found similar or different results on this topic. Tiwari (1993) showed that applications of fertilizers with nitrogen and phosphorous caused significant increases in earthworm number and biomass in an oxisoil from India. As well, total earthworm biomass was also significantly greater in the manure-amended plots, compared to inorganic fertilizer-treated plots (150 kg N/ha/year). The biomass of juvenile and mature *Lumbricus* spp. was significantly greater in the manure-amended plots compared to the inorganic fertilizer-treated plots. The biomass of juvenile *Lumbricus* spp. ranged from 1.32 g to 6.89 g/m^2 in manure-amended plots and from 0.88 g to 4.02 g/m^2 in inorganic fertilizer-treated plots. Mature *L. terrestris* biomass was between 1.05 g and 8.21 g and between 0.81 g and 5.91 g/m^2 in the manure-amended and inorganic fertilizer-treated plots, respectively (Whalen et al. 1998). Similar results were obtained by Mathews et al. (2001), Smetak et al. (2007), and Bilalis et al. (2009). On the contrary, some studies found a significant reduction of earthworm population in certain conditions, such as chemically fertilized forest soil from Puerto Rico (Yang et al. 2007). Xiang et al. (2006) observed that under chemical fertilization, both the number of earthworm species and the quantity of individuals were significantly smaller than under other treatments, or even than under no fertilization.

Generally, it was observed that management practices that encourage earthworm abundance (like mineral fertilization with nitrogen and phosphorous) are the same as those assuring a good support for a large crop productivity necessary for sustainable agroecosystems.

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