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Factors controlling water-stable macroaggregation of Kastanozems exposed to water erosion

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Abstract

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The longterm agricultural use of Kastanozems in Bulgaria caused deterioration of soil structure, depletion of soil organic matter content and intensive water erosion on sloping lands. One of the indicators of these processes is the significant reduction of the water stability of soil aggregates. The aim of this study was to assess the influence of chemical, biochemical, biological and physical soil properties on the aggregate stability of Kastanozems on sloping lands. The topsoil layers of seven different in position, land cover and tillage variants (V) were investigated: noncultivated site under forest (V1); newly ploughed area (V2); high part of the slope with inclination 7.5^{o} (V3); middle part of the slope with inclination of 5^{0} with sowing of milk thistle (*Silybum marianum*)(V4); low part of the slope with deposition of eroded material (V5); maize sowed across the slope under erosion control technology combined with surface mulching with manure (V6); maize sowed along the slope under conventional technology (V7). The highest content of water stable aggregates (> 0.25 mm) was 41.9% observed at V1. The water stability of aggregates in V2 and V3 decreased by 40%, which corresponded to the same degree of decrease in total organic carbon. In the other variants the aggregate fraction 1-3 mm contained almost no water stable aggregates, although the content of organic carbon was higher due to overlay of the eroded material (V4, V5, V7) or to the applied manure (V6). Formation of water stable macro aggregates of eroded particles is very difficult when sloping lands are cultivated. The strongest positive relationship was found between water stability of macro aggregates and the content of microbial carbon, which in turn depended on the amount of actinomycetes and fungi. The instability of soil aggregate led to the reduction of the available water capacity of all variants, which was most evident in the site with deposition of eroded material (V4) and less pronounced in the variant with mulching with manure (V6).

Keywords: water stability of aggregates; soil water erosion; soil microorganisms

Introduction

The stability of soil aggregates in water is one of the indicators of soil structure (Six et al., 2000). When unstable soil aggregates disintegrate, porosity and infiltration decrease, finer particles and microaggregates are transported by surface water runoff, which leads to increase the intensity of runoff and soil erosion and subsequently to water deficiencies and stress for plants (Zhang & Horn, 2001).

Soil micro aggregates create physical protection of organic matter from microbiological decomposition (Six et al., 2004). The knowledge for the stabilization and protection mechanisms of soil carbon in structural aggregates is useful for the development of appropriate management practices for soil structure improving, increasing of the amount of organic carbon (Xie et al., 2017; Mustafa, 2020), and soil fertility and mitigating climate change (Bronick & Lal, 2005).

In soils containing carbonates of alkaline earth elements, their role in aggregation is mediated by the content of organic matter. At low soil organic matter content, macro aggregate stability is enhanced by carbonates (Boix-Fayos et al., 2001).

Soil structure is a result of the interaction of many factors, such as soil genetic properties, mineral composition, texture, concentration and composition of organic matter, soil pH, microbiological activity, ion exchange, elements stocks, hydraulic regime, as well as vegetation and land management (Six et al., 2004; Bronick & Lal, 2005; Kodesova et al., 2009; Regelink et al., 2015; Zhou et al., 2020).

The reduction of water stability of soil aggregates and the deterioration of the soil structure after prolonged use for crop production is well manifested in Kastanozems (Dilkova, 2014). In combination with the undulating relief of the Danube plain, this creates conditions for water erosion and additional loss of organic matter (Rousseva et al., 2015). Long-term experiments in the experimental station near to the village of Trastenik, Ruse region, showed that soil losses by water erosion under row crops varied between 7.6 and 15.7 t ha^{-1} y^{-1}, and under small grains – from 3.8 to 7.8 t ha^{-1} y^{-1}, which was classified as moderate and moderate to high actual risk of water erosion (5–20 t ha^{-1} y^{-1}) (Kuncheva, 2019).

The aim of the present study is to determine the influence of chemical, biochemical, biological and physical parameters on the water stability of macro aggregates (> 0.250 µm) of topsoil layers of Kastanozems at different position and management of sloped lands.

Material and Methods

Site description

The study was conducted in the experimental field near the village of Trastenik (43.478°N, 25.901°E, H = 114 m a.s.l), Ruse region, on a slope with southeastern exposure on Kastanozem. The disturbed soil samples were taken from the topsoil (0-15 cm) of seven variants, reflecting different positions and management of sloped land: V1 deciduous forest; V2 newly plowed area, after forest; V3 the upper part of the slope with an inclination of 7.5°; V4 milk thistle (*Silybum marianum)* grown in the middle part of the slope with an inclination of 5°; V5 milk thistle, grown at the base of the slope on deposition of eroded soil material; V6 maize (*Zea mays*) grown across the slope with applying surface mulching with manure; V7 maize grown along the slope, under conventional technology.

Laboratory analyses

Particle-size fractions from 2 mm to 0.063 mm were determined by sieving and from 0.063 to 0.001 mm by the pipette method after chemical dispersion of air-dry grounded soil sample (< 2 mm) with 25 cm^3 of sodium pyrophosphate ($Na_4P_2O_7$) 30 g l^{-1} (ISO 11277, 2009). Textural fractions of sand (2-0.063 mm), silt (0.063-0.002 mm) and clay (< 0.002 mm) were determined for applying the textural classification of IUSS Working Group WRB (2015).

Soil mineralogical composition was determined on two soil fractions: sand (2-0.063 mm) and silt + clay (< 0.063 mm) after removal of soil organic matter on air-dry sample from the variant V7 without chemical pretreatment. XRD analysis was carried out with XRD Diffractometers D2 PHASER.

The distribution of dry-sieved aggregates in aggregate size classes (> 10 mm, 10-5, 5-3, 3-1, 1-0.25, < 0.25 mm) was determined by manual dry sieving of air-dried soil (about 500 g) using a set of sieves arranged from top to bottom with decreasing size of the openings in the following order 10, 5, 3, 1 and 0.25 mm. The proportion of each aggregate class (DSA) was calculated relative to the summed total weights of all aggregate size classes. Mean weight diameter (MWD, mm) of the fraction less than 10 mm was calculated.

The water stable aggregates were determined by the method of Savinov, modification of Vershinin and Revut (Revut & Rode, 1969). Four soil samples were prepared for wet sieving: one composite ($F_{0.25-10}$) ample (20 g) by taking equal quantity (5 g) of air-dried aggregates from four fractions: 10–5, 5–3, 3–1, and 1-0.25 mm; three replicate samples (20 g each) with air dried aggregates from a single fraction 3–1 mm (F_{1-3}). The wet sieving was done by the device of Savinov an hour after direct immersion of the air-dried soil aggregates sample into water (slaked pretreatment). The device consists of nest of sieves (i = 5, 3, 1 and 0.25 mm), which remained immersed in water during the whole procedure. The nest of sieves was shifted 10 cm up and dropped down by their own weight 10 times, and then additionally washed 5 times the last two sieves. The remaining soil aggregates on each sieve (i) were collected, dried at 105°C, weighed and expressed as a proportion (Pi) of the size class to the weight of aggregates sample placed on the sieve prior to wet sieving. The correction for aggregate-sized sand content was done according to Six et al. (2000):

$$WSAi = Pi - Si, \tag{1}$$

where WSAi – is the proportion of water stable aggregate of size class i,

Pi is the proportion of the aggregates remaining on the sieve to the weight of aggregates placed on the sieve prior to wet sieving, Si is the proportion of sand with size i in aggregates of size i after disruption.

The soil water retention at suction pF 2.5 was measured on soil aggregates size 2-5 mm by 5-Bar Pressure Plate Extractor (Soilmoisture Equipment Corp.) with 1 Bar Pressure Plate ceramic cell. Soil water retention at suction 1500 kPa (pF 4.2 – Wilting Point, WP) was determined using pressure membrane (cellulose) apparatus (Soilmoisture Equipment Corp.) according to ISO 11274:1998. The available water holding capacity (AWHC) was determined as the difference between water retained at pF2.5 and pF4.2:

$$AWHC = W_{pF2.5} - W_{pF4.2} \quad (2)$$

Water content at pF 5.6 was determined using vapor pressure method with controlled 75% relative humidity in desiccators containing saturated solution of NaCl,

Total soil organic carbon content (SOC,%) was determined by the modified Tjurin's method [dichromate digestion at 125ºC, 45 min, in the presence of Ag_2SO_4 and $(NH_4)_2.SO_4.FeSO_4.6H_2O$ titration, phenyl anthranilic acid as an indicator (Filcheva & Tsadilas, 2002; Kononova, 1966)]. The soil organic matter composition was determined by the method of Kononova & Belchikova (Kononva, 1966). Spectrophotometric analysis at 465 nm, 665 nm wave lengths were used to determine the extinction of humic acids. Mineral nitrogen concentration was determined by the method of Kjeldal. Available forms of phosphorus and potassium were determined and assessed as described by Nikolova et al. (2014), Soil pH of samples was measured in water suspension 1:2.5 by pH meter.

The amount of the main groups of microorganisms was determined on solid growing media by Koch's method: bacteria, spore-forming and non-spore-forming, actinomycetes, fungi, oligotrophic microflora, nitrogen-fixing and cellulose-decomposing. The microbial biomass was determined by the substrate-induced respiration method (SIR) according to Anderson and Domsch (1978) (Hope, 2008). The activity of the enzymes peroxidase (PO) and polyphenol oxidase (PFO) was assessed by the method of Galstian (1974) and expressed in relative units mg purpurogalin obtained per g absolutely dry soil for 30 min (mg PPG/g soil/30 min). The ratio of the activity of the two enzymes is used as the coefficient of humification (accumulation of humus) (PFO/PO).

Statistical analyses

Descriptive statistical analyses and one-way ANOVA were performed using STATGRAPHICS software. The geometric mean (d_g) and geometric standard deviation (s_g) of the particles size distribution were calculated as described in Bittelli et al., 2015.

Results

Soil texture and mineralogical composition

The soil textural fractions of the topsoils of the studied variants are presented in Figure 1. The contents of clay, silt and sand vary among the studied variants in the ranges 21-30%, 67-74%, 2-11%, correspondingly. Two texture classes are identified SiCL (V1) and SiL (V2 ÷ V7). The highest content (45% – 53%), is the coarse silt (10-50 µm). The next important fraction is the il (particles <1µm) (between 18 and 25%), which constitutes 77-87% of the clay fraction (<2 µm). The finest soil is under the forest (V1) with clay content of 29.7%. The coarsest texture is found in the sediment (V5), followed by the newly plowed site (V2) and the upper part of the slope (V3). Intermediate with similar particle size distribution are the other three sites (V4, V6 and 7V), located in the middle part of the slope. These differences are not significant as can be concluded from the values of the statistical parameters of the distribution – the geometric mean diameter of the particles (d_g) and geometric standard deviation (σ_g) (Fig. 1). The largest difference between d_g of variants with texture class SiL is 1.92 µm (between V5 and V7) and is of the same order as the smallest difference in d_g (2.0 µm) between the site classified as SiCL (V1) and V7, classified as SiL. The geometric standard deviations (σ_g) are lower

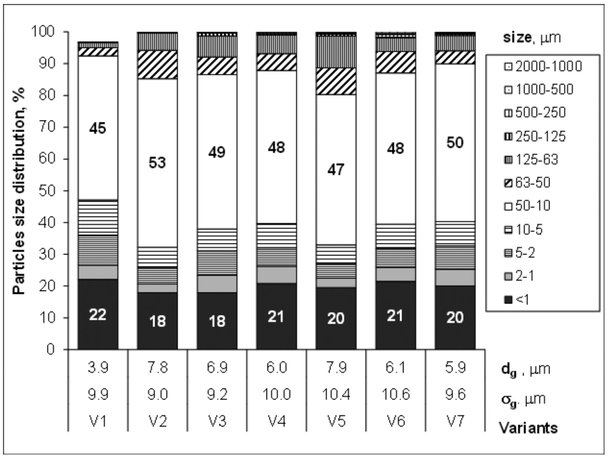

Fig. 1. Soil particle size distribution of topsoil layers of the studied variants: (V1) forest; (V2) newly plowed area after forest; (V3) upper part of a slope of 7.5º; (V4) milk thistle, middle part of a slope of 5o; (V5) milk thistle, sediment, foot of the slope; (V6) maize, grown under technology with surface mulching with manure; (V7) maize, grown under conventional technology, along the slope. d_g – geometric mean; s_g – geometric standard deviation

Table 1. XRD mineralogical composition of soil at variant V7 (maize grown along the slope, under conventional technology)

Minerals	>63 mm, %	<63 mm, %
Quartz (SiO_2)	23.4	15.6
Plagioclase [(Na,Ca)(Si,Al)$_4$O$_8$]	26.3	26.4
Orthoclase ($KAlSi_3O_8$)	10.6	12.2
Muscovite {$KAl_2[AlSi_3O_{10}](OH)_2$}	35.1	28.3
Amphibole {$Ca_2[Mg_4(Al,Fe)]Si_7AlO_{22}(OH)_2$}	1.1	2.8
Chloritte {$[Mg,Al,Fe]_6[Si,Al]_4O_{10}(OH)_8$}	3.1	8.5
Montmorrilonite [$(Na,Ca)_{0,3}(Al,Mg)_2Si_4O_{10}(OH)_2$•n($H_2O$)]	0.4	0.4

(9.0-9.2 µm) in V2 and V3 compared to the other variants in which they vary from 9.6-10.4 µm (Fig. 1).

The semiquantitative analysis of soil mineral composition at V7 revealed the dominance of micas (muscovite), feldspars (plagioclase, orthoclase), and quartz (Table 1).

The dominance of hydromica in primary minerals is typical for Kastanozems.

Chemical properties

The content of total organic carbon (TOC) is high (1.8-1.9%) in the soil under forest (V1), sediment (V5) and areas with maize grown with surface mulching with manure in the middle of the slope (V6) (Table 2). In the sediment samples this is due to the deposition of specific humus fractions with the surface water runoff. Medium SOC content (1.17-1.25%) was found in variants V2, V4 and V7. SOC was low (0.91%) at the top of the slope (V3).

The composition of the soil organic carbon in all variants is of fulvic-humic type (1 <Ch:Cf < 2.0) (Table 2). The amount of humic substances extracted with pyrophosphate

Table 2. Chemical properties of the studied variants: (V1) forest; (V2) newly plowed area after forest; (V3) upper part of a slope of 7.5°; (V4) milk thistle, middle part of a slope of 5°; (V5) milk thistle, sediment, foot of the slope; (V6) maize, grown under technology with surface mulching with manure; (V7) maize, grown under conventional technology, along the slope

Properties	(V1)	(V2)	(V3)	(V4)	(V5)	(V6)	(V7)
Content and composition of soil organic carbon							
SOC, % soil	1.76±0.05	1.17±0.05	0.91±0.04	1.25±0.08	1.90±0.07	1.80±0.04	1.21±0.03
C extr., % soil C extr , %SOC	0.52±0.03 29	0.23±0.01 19	0.16±0.01 18	0.39±0.02 32	0.37±0.02 19	0.54±0.03 30	0.34±0.03 28
Ch, %soil	0.30±0.02	0.13±0.02	0.09±0.01	0.20±0.01	0.20±0.02	0.36±0.02	0.18±0.01
Cf, %soil	0.22	0.10	0.07	0.19	0.17	0.18	0.16
Ch:Cf	1.4	1.2	1.3	1.1	1.2	2.0	1.2
C unextr., %	1.24	0.94	0.75	0.86	1.53	1.26	0.88
E4/E6	3.92	3.77	3.35	3.55	3.69	3.88	3.76
clay/SOC	17	18	26	21	12	14	21
Physicochemical and agrochemical properties							
$CaCO_3$, %	3.0	2.4	15.3	4.7	5.5	6.5	7.3
pH	7.7±0.03	8.3±0.01	8.2±0.21	8.1±0.04	7.7±0.06	7.8±0.02	7.9±0.14
EC, µS/cm	198.4±6.2	70.6±3.5	104.6±14.4	79.9±17.5			
N min, mg/kg	70.3±3.8	14.6±1.2	89.0±6.4	59.1±2.5	59.5±1.8	167.6±7.8	48.0±2.3
P_2O_5, mg/100 g	9.1±0.4	7.4±0.2	4.7±0.1	6.6±0.3	22.0±1.4	31.1±0.6	5.3±0.1
K_2O, mg/100 g	55.4±1.7	39.8±0.2	44.6±1.2	30.8±0.1	51.0±0.7	60.6±1.5	26.5±1.4

are the highest (0.5%) in the samples from V1 and V6, which constitutes 30% of the total organic carbon. Despite the high amount of organic matter, which is translocated by the surface runoff in the sediment (V5), there is not observed high level of soluble humic substances, but a larger amount of insoluble carbon at this variant (Table 2).

According to some authors (Dexter et al., 2008; Jensen et al., 2019), the ratio of clay to total organic carbon expresses the capacity of the soil to stabilize carbon and has a stronger relationship with the stability of the soil structure. The data show that the clay/SOC ratio is above 10 (Table 2), which according to Dexter et al. (2008) is a criterion that the soil is not sufficiently saturated with carbon and reduced stability of the soil structure can be expected in all variants.

The E4/E6 ratio is high for samples under forest (V1), surface mulching (V6) and sediment (V5) (Table 2). Higher values of this ratio are associated with lower molecular weight, lower aromaticity of humic acids, with the presence of more aliphatic chains, while variants with low content of organic carbon and humic acids, as the upper part of the slope 7.5^0 (V3) and the newly plowed area (V2) show higher degree of aromaticity and higher molecular weight.

The soil pH varies from slightly alkaline (7.7-8.2) to moderately alkaline (8.3) (Table 2). The change in soil reaction is due to the change of SOC, as can be concluded from the high correlations with soil organic matter and its constituents (Table 5). No statistical relationship was found between the soil pH with $CaCO_3$ content. The high content of carbonates on the ridge of the slope is evidence for the strong erosion of the surface layer and the discovery of richer in carbonate subsurface layers.

The electrical conductivity (EC) is the highest 339.0 µS/cm when applying surface mulching with manure (V6) (Table 2). It is significantly lower in forest (V1) 198.4 µS/cm and sediment (V5) 135.2 µS/cm. In the other areas the electrical conductivity is between 70.6 µS/cm (V2) to 104.6 µS/cm (V3).

The storage of available forms of phosphorus varies from very low (<6.0 mg /100 g) in the upper part of the slope (V3) and under the cultivation of maize along the slope by conventional technology (V7), low (between 6.1 and 12.0 mg/100g) in variants V1, V2 and V4, high (18.1-28 mg/100 g) in the eroded soil (V5) and very high (> 28 mg/ 100 g) in the variant with surface application of manure (V6). The amount of available forms of potassium is medium (V7), high (V2 and V3) and very high (V1, V5, V6), which can be explained by the mineral composition of the soil (Table 1). Mineral nitrogen varies from 14.6 mg/kg at V2 to 167.4 mg/kg at V6, depending on the crop and the applied fertilization.

Table 3. Microbiological parameters of the studied variants (V): (V1) forest; (V2) newly plowed area after forest; (V3) upper part of a slope of 7.5°; (V4) milk thistle, middle part of a slope of 5°; (V5) milk thistle, sediment, foot of the slope; (V6) maize, grown under technology with surface mulching with manure; (V7) maize, grown under conventional technology, along the slope. Cmic – carbon content in microbial biomass

Parameters	(V1)	(V2)	(V3)	(V4)	V(5)	(V6)	(V7)
Cmic,mg kg^{-1}	738.4±17.9	276.2±9.9	253.7±14.9	186.0±6.4	343.8±41.0	491.9±65.5	266.4±11.2
Main groups of spoil microorganisms in CFU (colony forming units) x 10^6 g^{-1} abs. dry soil							
Total number of saprophytic bacteria	72.6±2.0	385.2±35.1	7.4±0.6	7.6±0.6	1118.3±356.6	33.9±0.2	23.0±0.1
Spore forming bacteria	9.7±0.2	27.8±2.0	0.4±0.6	1.1±0.1	28.6±0.5	1.15±0.1	203.4±5.2
Oligotrophs	338.8±4.9	267.5±30.0	239.4±12.5	403.3±26.5	1679.3±120.6	172.5±15.3	90.4±5.8
Actinomycetes	1.57±0.07	0.86±0.04	0	0.11±0.01	0.55±0.04	1.04±0.10	0.30±0.60
Fungi	0.0049	0.0	0.0024	0.0008	0.0024	0.0029	0.0015
Bacteria on SAA (starch ammonia agar)	4.3±1.1	5.9±1.0	4.9±0.1	0	4.9±0.1	3.4±0.7	6.1±0.3
Nitrogen fixing bacteria	5.0±0.2	4.0±1.4	3.4±0.3	3.4±0.6	3.4±0.4	4.8±1.2	2.1±0.3
Cellulose decomposers	4.1±0.3	4.0±0.5	1.9±0.8	1.7±0.7	4.0±0.2	2.4±0.1	1.9±0.1
Enzyme activity, mg PPG/g soil/30 min							
Peroxidase (PO)	1.75±0.09	2.00±0.04	3.40±0.11	4.57±0.09	3.00±0.17	2.55±0.05	3.33±0.09
Polyphenol oxidase (PPO)	1.24±0.03	1.30±0.04	1.86±0.09	3.10±0.10	1.33±0.03	2.00±0.04	2.30±0.05
PPO/PO	0.71	0.65	0.55	0.68	0.44	0.78	0.69

Microbiological indicators

The microbiological analyzes show high amount of ammonifying bacteria in sediment (V5) and arable land after forest (V2), followed by forest (V1) and maize growing with applied surface mulching with manure (V6) (Table 3). This implies a higher intensity of mineralization in the eroded soil (sediment) and in the newly plowed areas (V2). Spore-forming bacteria are also high in sediment (V5) and arable land after forest (V2), and their number is the lowest in samples from the high part of the slope (7.5°) (V3).

The amount of actinomycetes is the highest in samples taken from forest (V1), followed by the variant with surface mulching with manure (V6). The same trend is observed in fungi, which are the largest number in these variants. Bacteria that consume mineral nitrogen and are associated with its immobilization (SAA – starch ammonium agar, bacteria) do not differ significantly among the different samples, but the highest amount is observed in the variant with conventionally grown maize, along the slope (V7).

The highest number of nitrogen-fixing bacteria, was found in samples from forest (V1), also in the variant with surface mulching (V6) and arable land after forest (V2). The same trend is observed in cellulose-decomposing microorganisms, as they are high number in the eroded soil (V5). The amount of nitrogen-fixing bacteria correlates with the content of mobile potassium in the soil (r = 0.82) and the quantity of actinomycetes (r = 0.80),

The carbon content in the microbial biomass is highest in the samples under forest (V1) – 738.4 mg kg^{-1} and in the application of surface mulching with manure (V6) – 491.9 mg kg^{-1}. The samples of eroded material at the base of the hill (V5) show a higher amount of microbial biomass compared to the middle part of the slope, which together with the high number of saprophytic bacteria suggests more intensive mineralization processes. The highest positive correlation was found between Cmic and the amount of actinomycetes (r = 0.90) and fungi (r = 0.94). The combined effect of these two groups of microorganisms on Cmic (mg/kg) can be predicted by multiple linear regression relationships with statistically proven coefficients (Eq. 3).

$$Cmic=72.6^{+} + 154.11^{++} \times X_1 + 84812.0^{++} \times X_2, \quad (3)$$
$$R^2 = 98.5\% \ (R^2adj = 97.8\%),\ SEE = 28.3 \text{ mg/kg},$$

where X_1 and X_2 are, respectively, the amount (10^6 CFU g^{-1}) of actinomycetes and fungi.

The highest values for the activity of the enzymes peroxidase and polyphenol oxidase were obtained in the samples from the middle part of the slope under milk thistle growing (V4), in the upper part of the slope 7.5° (V3) and in maize grown with surface mulching (V6) (Table 3). The lowest values of activity of the two enzymes were reported in the samples under the forest (V1), but the humification coefficient (PPO/PO) was second in value. The highest humification coefficient is 0.78 observed in variants with maize grown with surface mulching (V6). The lowest coefficient of humification is observed in the eroded soil (V4), compared to the other sites, which is evidence for the mineralization processes but not for the accumulation of organic matter. The activity of both enzymes is negatively correlated (r = -0.8) with the content of cellulose-decomposing microorganisms.

Water holding capacity

The hygroscopic water content, determined at 75% relative humidity ($W_{pF5.6}$), is highest 5.1% under forest (V1) (Table 4). The performed one-way analysis of variance shows that $W_{pF5.6}$ at variants V4, V5, V6 and V7 do not differ statistically from each other and it is 4.0% in average. Slightly lower (3.6%) are the values in the newly plowed area (V2) and in the upper part of the slope (V3) – 3.3%. This indicator strongly depends on the soil texture and the content of organic matter. The sediment (V5) has coarser texture, but with a higher content of organic matter, which is manifested in the lowest value of clay/SOC = 12, close to the criterion of soil saturation with organic matter (Table 2). This leads to an increase in the value of $W_{pF5.6}$ in V5 and it is equalized with variants V4, V6 and V7.

Table 4. Soil water content (W, % by mass) at potentials pF5.6 ÷ pF2.5 of the studied variants (V): (V1) forest; (V2) newly plowed area after forest; (V3) upper part of a slope of 7.5°; (V4) milk thistle, middle part of a slope of 5°; (V5) milk thistle, sediment, foot of the slope; (V6) maize, grown under technology with surface mulching with manure; (V7) maize, grown under conventional technology, along the slope

Soil Water Content, %	(V1)	(V2)	(V3)	(V4)	(V5)	(V6)	(V7)
$W_{pF5.6}$,	$5.1^d \pm 0.1$	$3.6^b \pm 0.1$	$3.3^a \pm 0.03$	$4.1^c \pm 0.1$	$4.0^c \pm 0.1$	$4.1^c \pm 0.04$	$3.9^c \pm 0.02$
$W_{pF4.2}$	12.4	9.6	9.0	10.5	10.3	10.5	10.1
$W_{pF2.5}$	39.8	27.8	26.3	30.5	26.5*	34.0*	27.1
$W_{pF2.5} - W_{pF4.2}$		18.2	17.3	20.0	16.2	23.5	17.0

* analyzes were performed on aggregates with size <2 mm

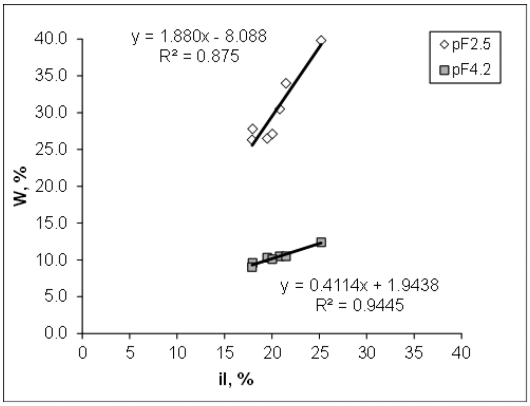

Fig. 2. Relationships between water contents (W, %) at potentials pF2.5 and pF4.2 and il (content of soil particles < 1 mm).

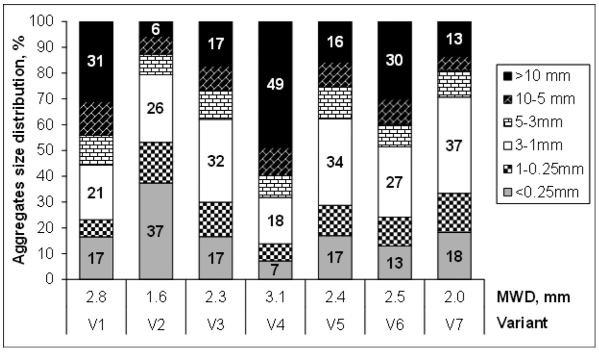

Fig. 3. Aggregates size distribution and mean weight diameter of dry aggregates (MWD) of the studied variants (V): (V1) forest; (V2) newly plowed area after forest; (V3) upper part of a slope of 7.5o; (V4) milk thistle, middle part of a slope of 5o; (V5) milk thistle, sediment, foot of the slope; (V6) maize, grown under technology with surface mulching with manure; (V7) maize, grown under conventional technology, along the slope

More significant is the variation in water retained at pF2.5 (Table 4), which is generally accepted as representative for field capacity. The dependence of $W_{pF2.5}$ on particles <1 mm (il) is better pronounced than the dependence of the water retention at pF 4.2 on il, which leads to overall increase of the available water capacity with il (Figure 2). At matric potential pF2.5, water retention ranges from 26-27% at variants V3, V5 and V7 to 39.8% at V1. The performed soil protection technologies and the applied mulching with manure lead to an increase of the water retention $W_{pF2.5}$ = 34%, which implies an increase of the water retention capacity by about 7% compared to the variant with maize, grown under conventional technology, along the slope (V7).

Relationships of physical indicators with chemical and microbiological indicators

Some of the correlations of physical with chemical and microbiological indicators are presented in Table 5.

Aggregate composition and water stability of soil aggregates

The content of agronomically valuable aggregates (0.25-10 mm) is lowest (44%) in V4, average (52-58%) in variants (V1, V2 and V6, and highest (66-68%) in V3, V5 and V7) (Figure 3). The tendency to formation of clods is best manifested in soil at variant V4, where the content of aggregates larger than 10 mm is 48% (Figure 3). The newly plowed area can be described as single grains, with small content of clods (6%) and significant content of aggregates <0.25 mm – 37%. The aggregate fraction 1-3 mm is about half of the agronomically valuable aggregates (0.25-10 mm), and the share of larger aggregates fraction (3-10 mm) in them is between 1/5 and 1/3 in most variants. Exceptions are V1 and V4, where the share of fraction 1-3 mm is smaller (2/5), and almost half (0.47 – 0.44) of agronomically valuable aggregates are larger (3-10 mm).

Despite these features, the aggregate composition in most sites does not show structureless, which is most likely due to the binding role of carbonates at soil drying.

The amount of agronomically valuable aggregates ($DSA_{10-0.25}$) depends on the soil texture and correlates with the number of bacteria consuming mineral nitrogen and participating

Table 5. Correlation coefficients of the physical by the chemical and microbiological parameters

	Il (<1 µm)	Clay (<2µm)	SOC	Cext.	Ch	Cf	Cmic
pH	-0.69	-0.58	-0.87++	-0.82+	-0.77+	-0.79+	-0.64
Il (< 1 µm)	1	0.92+++	0.61	0.86++	0.79+	0.87++	0.84+
Clay (< 2 µm)	0.92+++	1	0.36	0.74+	0.68	0.77+	0.67
Fine silt (2-10 µm)	0.78+	0.85++	0.07	0.40	0.36	0.42	0.71
Silt (2-63 µm)	-0.36	-0.46	-0.47	-0.46	-0.39	-0.55	-0.11
$W_{pF2.5} – W_{pF4.2}$, %	0.90++	0.81+	047	0.76+	0.78+	0.64	0.86

in its immobilization (SAA bacteria). Expression of these dependences is a statistically justified (R^2 = 84.6%, R^2adj = 77.0%, SEE = 4.3%) multiple regression model (eq. 4)

$$DSA10\text{-}0.25 = 173.4^{+} - 1.946^{+} \times silt + + 4.524^{++} \times b\_SAA \quad (4)$$

There are significant differences in the water stability of the aggregates in the studied sites (Figure 4). As can be seen from the water stability of the aggregates forming the composite sample ($F_{0.25\text{-}10}$), the content of water stable aggregates (> 0.25 mm) is highest 44% under forest (V1), about 30% is in variants V2 and V3, and between 1 and 9% in the other variants V4, V5, V6 and V7) (Figure 4). In the sediment (V5) there is a certain amount of large water stable aggregates, most likely from translocated insoluble organic residue, which leads to a slightly higher value of the water stability index MWDR = 0.21. This effect is not observed in water stable aggregates of the 1-3 mm fraction ($F_{1\text{-}3}$) which allow to group the variants into three groups (Table 6). The highest content of water stable aggregates (> 0.25 mm) is 41.9% observed under forest (V1) (Table 6). The water stability of the

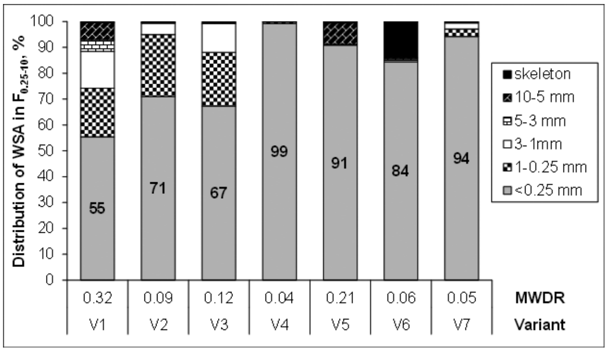

Fig. 4 Distribution of water-stable aggregates by size in the composite sample ($F_{0.25\text{-}10}$) and ratio of mean weight diameters after and before wet sieving (MWDR) of the studied variants (V): (V1) forest; (V2) newly plowed area after forest; (V3) upper part of a slope of 7.5o; (V4) milk thistle, middle part of a slope of 5o; (V5) milk thistle, sediment, foot of the slope; (V6) maize, grown under technology with surface mulching with manure; (V7) maize, grown under conventional technology, along the slope

aggregates decreases by 40% in the newly plowed area (V2) and in the upper part of the slope with inclination of 7.5° (V3), which correspond to the same degree of reduction of the total organic carbon (Table 2). In the other four variants, the fraction 1-3 mm ($F_{1\text{-}3}$) contains almost no water stable aggregates, despite the fact that in some of the variants the organic matter is increased by the overlaid eroded material (V5) or by the applied manure (V6) (Table 2).

Following the analysis of the obtained correlation coefficients of all independent variables and stepwise multiple regression analysis, it was found that the content of microbial carbon (Cmic, mg/kg), humic acids (Ch, % soil) and pH best describe the content (%) of water stable (> 0.25 mm) soil aggregates $WSA_{F1\text{-}3}$ in fraction 1-3 mm:

$$WSA_{F1\text{-}3} = -372.7^{+} + 0.122^{+} \times Cmic - 112.4^{+++} \times Ch + + 46.0^{+} \times pH \quad (5)$$

R^2 = 97.8%, R^2 adj for d.f. = 95.6%, SEE = 3.5%

The average values of the predictors (eq. 5) in the formed three groups of water stability are presented in Table 6.

Discussion

The water stable aggregates (WSA> 0.25 mm) in the surface layer of non-cultivated soil under forest (V1) is 44%, which is half of the amount of WSA (80-90%) reported by Dilkova (2014) using the same method for Kastanozems with similar texture and humus content (about 3%), but under grassland in plain conditions. The forest vegetation creates conditions for the predominant formation of fulvic acids, which are more mobile and for spread of microbiological activity to a greater depth and in general to a lower content of organic carbon in the surface layer (Filcheva et al., 2021). Grass vegetation is known as a factor for the formation of water stable macroaggregates (Jensen et al., 2020).

In two of cultivated variants (V2) and (V3), the reduction of the water stability of soil aggregates by 40% compared to the non-cultivated (V1) can be directly related to the reduction of the content of organic matter or its components. In the upper part of the slope with inclination of 7.5° (V3), this is due to soil erosion, while in the newly plowed area after forest (V2) – to intensive mineralization due to tillage. The drastic reduction of the water resistance of the aggregates

Table 6. Average values of some soil parameters grouped by water resistance of the aggregates

Group	VariVariants	$WSA_{F1\text{-}3}$,%	Cmic, mg/kg	Ch, %	pH
I	(V1)	41.9	738	0.30	7.74
II	(V2), (V3)	25.3±0.8	265±16	0.11±0.02	8.24±0.06
III	(V4), (V5), (V6), (V7)	1.7±1.7	322±130	0.24±0.08	7.86±0.15

in the other cultivated variants cannot be explained by the organic matter, as there is an increase in the organic matter due to deposition of eroded material (V4, V5, V7) or due to manure application (V6). The obtained in previous study (Kuncheva, 2019) data on the content of organic matter in eroded soil, showed that the sediment enrichment ratio is on average between 0.95 and 1.11. The indirect confirmation of the source of increase of the organic matter in the present study is the increase of il (< 1 μm) (Figure 1) and the high correlation coefficients of this textural fraction with the content of Cextr (Table 5). The lack of a correlation between the water stability of macroaggregates (> 0.250 mm) and organic matter in semi-arid regions has been noted also by other authors (Caravaca et al., 2004).

Jensen et al. (2019) indicate that the bonding effect of organic matter is more pronounced in microaggregates (< 0.250 mm), while the mechanisms for the formation of macroaggregates are more difficult to determine, but definitely depend on land use.

The results of our study show the highest correlations between the water stability of the aggregates in the fraction 1-3 mm and the content of microbial carbon. It, in turn, is determined to the greatest extent by the content of actinomycetes and fungi (eq. 3). Macroaggregates are generally considered to be dominated by fungal microorganisms (Frey et al., 2005), but most studies on the microbial composition in macroaggregates are focused on bacteria and little is known about fungal communities (Gupta & Germida, 2015).

The search for other factors explaining the water instability of soil macroaggregates in the considered variants leads to the addition of two more variables (eq. 5) – negative connection with humic acids and positive with pH. These relationships are surprising because they contradict other studies showing an increase in water stability with decreasing pH and increasing humic acids (Regelink et al, 2015). The result obtained can be interpreted by the specific processes occurring in slope erosion, namely the removal of microaggregates or primary soil particles rich in organic carbon on the slope and their appearance in the middle (V4, V6 and V7) and at the foot of the slope (V5). These eroded microparticles cannot form stable macroaggregates due to the applied tillage, low soil moisture during the hot summer with southeastern exposure of the slope. The data on the aggregate composition of the studied variants show that their binding in larger aggregates depends on the silt content and the activity of the ammonifying bacteria, as shown by Equation 4. The role of carbonates is not evident as a binding factor in the topsoil of the studied variants. The instability of the soil aggregates is due to the lack of binding agents, such as fungi and roots. In addition to these factors the high content of potassium from the soil-forming material, also prevents strong bonding of the organic matter with the mineral part.

The instability of the aggregates formed by eroded particles has an impact on the lower available water capacity of all variants compared to the forest (Table 4), the most significant is the reduction in sediment – by nearly 11% and at lowest in the variant with mulching with manure – by 4%.

Conclusion

The study presents data and relationships for the main factors influencing the structural stability of Kastanozems, subject to water erosion on sloping land under different position, land cover and tillage No correlation has been established between the water stability of soil aggregates and the humic and chemical soil parameters. The contents of organic matter, fulvic and humic acids, and the insoluble carbon show a correlation with the water retention properties. Quantitative assessments of the influence of microbiological indicators in the formation of water stable structural aggregates have been obtained.

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