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Water Stability of Soil Aggregates in a 50-Year-Old Soil Formation Experiment on Calcareous Glacial Till

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Abstract—Soil formation on the human time scale is immensely time consuming, although it can be significantly accelerated through the effects of vegetation. The content of water-stable aggregates (WSAs) is a useful indicator for determining both the soil development level and the soil quality. However, in severely degraded soils, especially in the Baltic pedoclimatic region, the effects of vegetation on the aggregate stability have been poorly studied. Therefore, to obtain more knowledge about the impact of vegetation on WSA, and thereby knowing how to improve it, this study was conducted on a long-term soil formation experiment in Estonia near Tartu. In 1964, the initial soil from an area of 20 × 8 m down to 100 cm depth was replaced with a sandy loam calcareous glacial till. The experiment started on April 26, 1965, when plants were sown on the plot. The topsoil (0–20 cm) samples were analyzed in 1966, 2000, 2007, and 2014. The study indicated that perennial grasses (meadow fescue and common meadow-grass) fertilized with $P_{40}K_{75}$, compared to $N_{150}P_{40}K_{75}$, decreased the WSA content, as well at the accumulation rate of soil organic carbon (SOC) and the total nitrogen content (N_{tot}). The hybrid alfalfa treatment resulted in the significantly highest SOC and N_{tot} accumulation, but not in the overall highest WSA content. Under barley, manure positively affected the WSA and SOC, though many other physical properties were not improved. Compared to the initial till under bare fallow, the SOC and N_{tot} contents were significantly higher under grown crops, but the WSA content remained the same. In addition, regardless of the grown crops, the WSA of larger (0.25–2 mm) aggregates was substantially higher than that of smaller (0.25–1 mm) aggregates. Also, as the relationship between WSA and SOC in the study was linear, the soil was far from C saturation and still in development. Overall, it can be concluded that the cultivation of perennial grasses and hybrid alfalfa on the severely eroded soil is the most rational option to improve the water stability of aggregates and increase the SOC and N_{tot} contents. However, because of the complexity of the aggregation process, further research is still needed.

Keywords: aggregate stability, calcareous glacial till, fertilization, soil organic carbon, vegetation treatments

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INTRODUCTION

Soil is an essential agricultural resource that largely determines the most appropriate land usage, as well as the type of crops and their yields. Unlike climate, soil can be directly manipulated by agricultural practices. Unsustainable management for a longer period of time will result in soil degradation. This, in most severe cases, with the combined effects of water and wind erosion could cause a complete loss of the developed topsoil until only parent material remains. Outside in agriculture and forestry, similarly degraded soils also occur due the consequence of open-pit mining [6]. Despite soil often being mentioned as a nonrenewable resource, soil formation is a naturally occurring process that could be classified as a renewable resource due to its immense time consumption, which is several magnitudes slower than degradation; it must be labeled more as a slowly renewable resource. According to Vasily Dokuchaev's modified soil formation concept by Hans Jenny [17], this process is mostly affected by (i) time, (ii) climate, (iii) biota, (iv) topography, (v) parent material and (iv) anthropogenic activity. Therefore, even on the human time scale, this process can be significantly accelerated by differences in soil management and plant vegetation. For instance, previous research with calcareous glacial till has shown that soil formation, in terms of C sequestration, is foremost accelerated if left under the permanent vegetation of perennial herbaceous grasses [33]. The state of soil recovery, as well as the soil organic carbon (SOC) content, can be evaluated by the improvements in soil physical properties, where changes in the stability of the soil structure are especially important. This is because soil structure, formed by aggregates, directly affects other physical, chemical and biological properties. While at the same time, water stability of soil aggregates is one of the most complex soil properties; due to the various interactions with other factors, it has a high short-term seasonal variability [1]. To see changes in the SOC due to soil management and crop selection, about 6–10 years are needed [37], while soil texture remains unaffected even after 100 years [23]. Although, in general, the positive correlation between the content of water-stable soil aggregates (WSAs) and SOC is a known fact, the persistent change of the WSA on the prolonged period is less known. Therefore, long-term field experiments are essential, because less perceivable changes accumulate over time and eventually become noticeable. Previous long-term studies have been predominantly focused on SOC dynamics in soils which were already well-developed [18]. However, since the past decade, emphasis on the WSA is steadily increasing as the importance of a stable soil structure on soil functionality is becoming more apparent. Many studies that were conducted on eroded soils used different shrubs and native pioneer species for vegetation, which therefore, greatly differ from those grown in large-scale agricultural production. In addition, those studies tend to be in slopes on mountainous areas [15] and in semiarid regions of different parent material [45]. However, research from those differ significantly from the Baltic pedo-climatic conditions and thus are not directly comparable. Therefore, to fill those missing knowledge gaps, this long-term study was conducted with the purpose to analyze how vegetation (perennial grasses, legumes and barley) with differences in fertilization, affects the soil aggregation, as well as other soil structure related physical-chemical properties, during the soil formation process in Estonia at calcareous glacial till.

MATERIALS AND METHODS

Study site and treatments. The field experiment, established by Assoc. Prof. Arnold Sau (1928–1983), was conducted in Estonia, Tartu (N 58°22′04.09″, E 26°39′41.47″, elevation: 60 m above sea level). It was managed by the Chair of Crop Science and Plant Biology at the Estonian University of Life Sciences. In 1964, from an area of 20 × 8 m at a depth of 100 cm, the initial *Stagnic Luvisol* (WRB 2014) was dug out and replaced with reddish brown calcareous glacial till. This parent material was excavated near the field experiment in autumn of 1963 from a depth of 1.5–3.0 m and had the following initial specifications: dry bulk density (BD) 1.71 ± 0.02 g cm^{-3}, pH_{KCl} 7.0, cation-exchange capacity (CEC) 10.3 $cmol_c$/kg with 85% base saturation, $CaCO_3$ content 68 g kg^{-1}, total nitrogen (N_{tot}) 0.18 g kg^{-1} (0.018%), and soil organic carbon (SOC) 1.28 g kg^{-1} (0.128%). A more detailed description of the initial glacial tills chemical and physical properties were published earlier in the past [31, 35]. Furthermore, the initial WSA and SOC from 0.25–1 mm sized aggregates, which were both measured in 2014 from the earliest preserved soil material from year 1966, were 24.76% ± 0.68 SE (standard error) and 0.21% ± 0.026 SE, respectively. Based on another nearby located grass-herbaceous vegetation long-term soil formation experiment, which was established in 1963 on the same calcareous glacial till parent material; it can be derived that after 50 years the development of a *Calcaric Cambisol* [16] can be diagnosed [33]. Since the experiment establishment until 1979, there were 13 treatments. Individual plots (1 × 1.5 m), in total of 64, were separated from each other below the ground down to 100 cm depth with non-woven geotextile and above ground with a wooden framework made from pine. In 1980 the experiment was extended to 19 treatments; in regard to this, plot sizes remained unaltered, but the number of replications after this varied between two and four. The treatment replications were arranged in a completely randomized design, wherein the side by side occurrences of replications were avoided. The present study included those following eight vegetation treatments, which are commonly used in Estonia: (1) $\mathbf{0_T}$, control as bare fallow; (2) $\mathbf{B_T}$, spring barley (*Hordeum vulgare* L.) with $P_{40}K_{75}$ (phosphorous 40 kg ha^{-1} yr^{-1} and potassium 75 kg ha^{-1} yr^{-1}); (3) $\mathbf{B{+}M_T}$, spring barley with $P_{40}K_{75}$ and FYM (fermented cattle farmyard manure: 60 Mg ha^{-1} in 1995 and 25 Mg ha^{-1} in 2009); (4) $\mathbf{G{-}_T}$, perennial grasses: meadow fescue (*Festuca pratensis* Huds.) and common meadow-grass (*Poa pratensis* L.) without fertilization; (5) $\mathbf{G_T}$, perennial grasses with $P_{40}K_{75}$; (6) $\mathbf{G{+}N_T}$, perennial grasses with $N_{150}P_{40}K_{75}$ (nitrogen application: 2 × 75 kg N ha^{-1} yr^{-1}); (7) $\mathbf{WG_T}$, white clover (*Trifolium repens* L.) and perennial grass mixture with $P_{40}K_{75}$; (8) $\mathbf{HA_T}$, hybrid alfalfa (*Medicago sativa* subsp. *x varia* Martyn) with $P_{40}K_{75}$. Since the establishment of the experiment, no pesticides were used and until 2002, treatments with $P_{40}K_{75}$ and $N_{150}P_{40}K_{75}$ fertilization, received additionally Cu 6 kg ha^{-1} yr^{-1}, B 1 kg ha^{-1} yr^{-1}, Mo 1 kg ha^{-1} yr^{-1} micronutrients. Also, at the beginning of the experiment, plant residues and biomass yields from all vegetation treatments were removed. Barley treatments were sown annually in spring with a sowing rate of 400 kg ha^{-1} (60 g per plot) and were harvested from 15 cm height during late milk and early drought growth stages in the middle of July. Perennial grass treatments ($G{-}_T$, G_T, $G{+}N_T$) and legumes (WG_T, HA_T) were cut three and two times a year, respectively. The first cut was, depending on the situation, during the first or second week of June, the second cut was at the end of July, and the third cut was at the end of September. To conduct weed control, the study sampling years (2014, 2007, 2000) meant the last years before all perennial grass and legume treatments were renewed by tilling and re-seeding. For this, in spring, plants from all plots were removed, the soil was tilled with a shovel down to 20 cm depth and barley was sowed as cover crop. In autumn of the same year, barley as cover crop was removed; the soil was tilled once again down to 20 cm depth and in next spring the perennial vegetation was restored by re-seeding, whereupon the soil stayed untouched for the next six years. In addition, the control plots were tilled as well down to 20 cm depth, but nothing was sown in this treatment.

Soil sampling. The soil material for determining water-stable aggregate stability (WSA), particle size distribution (FAO classification) and plant unavailable water content (UWC) as well as for analyzing the contents of soil organic carbon (SOC) and total nitrogen (N_{tot}) was collected on the 16th October of 2014 from three different depths (0–5, 5–10 and 10–20 cm). Additionally, previously collected soil material from the autumn months of 2007 and 2000 from depths 0–5, 5–10 and 10–20 cm were used to determine the WSA and SOC, and for calculation of their relationship, additional soil material from 1966 in limited amounts were used. The soil material from 2014 were air-dried and sieved through 1 and 2 mm sieves; earlier (2007, 2000, 1966), the soil material had been sieved only through a 1 mm sieve, as this was a common standard that time. Also while in 2014 soil material from individual replications (two—0_T, B_T B+M_T; four—G—$_T$, G_T, G+N_T, WG_T, HA_T) were stored separately, in 2007 and 2000, soil material from all replications were mixed into one single paperboard box. The oldest preserved soil materials, date back to 1966 or one year after start of the experiment and were, therefore, considered to be unchanged from the initial glacial till. For measuring soil dry bulk density (BD), total porosity (TP), air-filled porosity (AFP) and plant available water content (AWC) the soil samples were collected also on 16th October of 2014 but only at 5–10 cm depth by stainless steel soil sampling cylinders (height: 40 mm, inner ∅: 53 mm) in four replications per plot.

Physical measurements. To ascertain the WSA from 0.25–1 mm (smaller) and 0.25–2 mm (larger) soil aggregates, air-dried soil was sifted through 1 and 2 mm sieves. Four replications of 4 g (varied between: 4000–4009 g) of soil were inserted into Eijkelkamp's *Wet Sieving Apparatus* (model: 08.13) with 0.25 mm sieves, which is based on the concept of Yoder [43] and modifications of Kemper and Koch [20] and Kemper and Rosenau [21]. To accelerate the dissolution of organic matter, those methods, however, was slightly modified—instead of the 0.2% NaOH solution, 0.4% was used. The WSA calculation steps were following:

$$Cup_{H_2O} = M_{H_2O} - Cup_{AL},$$

$$Cup_{NaOH} = M_{NaOH} - Cup_{316L} - 0.4g_{NaOH},$$

$$WSA = \frac{Cup_{NaOH}}{Cup_{H_2O} + Cup_{NaOH}} \times 100,$$

where Cup_{H_2O} is the weight (g) of dried soil which was earlier dissolved in purified water by 3 min of sieving; M_{H_2O} is Cup_{H_2O} including the weight (g) of the aluminum cup (Cup_{AL}); Cup_{NaOH} is the weight (g) of dried soil which was earlier dissolved by sieving in a 0.4% NaOH solution; M_{NaOH} is Cup_{NaOH} including the weights (g) of the stainless steel "316L" cup (Cup_{316L}) and dispersing solution ($0.4g_{NaOH}$). The liquids in both caps were evaporated firstly on a water bath at 95.5°C for ~10 h and then to ensure complete dryness placed into a convection oven at 105.5°C for 1 h.

Air-filled porosity (AFP) was determined on a sand barrel at –6 kPa (pF 1.8) water tension, where the soil samples stayed for 14 days. The exact AFP calculation process is the following:

$$AFP = TP - SM_{vol\%},$$

$$SM_{vol\%} = \frac{WS - DW}{DW} \times 100 \times BD,$$

where TP is total porosity (%); $SM_{vol\%}$ is soil moisture content in percentage by volume; WS is the weight (g) of soil at field moisture condition; DW is the weight (g) of dry soil and BD is soil dry bulk density (g cm^{-3}). The BD was determined by the commonly used oven drying method at 105.5°C for 24 h and was calculated by the following:

$$BD = \frac{DW}{V},$$

where DW is the weight (g) of absolute dry soil and V is the internal volume of soil sampling cylinders (cm^3). The TP was calculated between the BD and particle density by the formulas:

$$TP = \frac{PD \times BD}{PD} \times 100,$$

$$PD = 2.67 - 0.03 \times SOM,$$

where PD is soil particle density (g cm^{-3}), BD is soil dry bulk density (g cm^{-3}), 2.67 is the average mineral density of parent material (g cm^{-3}), 0.03 is coefficient and SOM is soil organic matter, which is obtained by assuming that SOM contains, average, 58% SOC.

The plant unavailable water content (UWC) was measured with Soil moisture Equipment Corp.'s *Pressure Extractor* (model: 1500F1) fitted with ceramic plates. Loose pre-wetted soil material in two replications were carefully inserted into small copper cylinders (height—5 mm, inner ∅—14 mm), saturated with purified water for 24 h and, afterwards, were placed on ceramic plates and pressurized at 1500 kPa (4.2 pF) for 30 days to simulate the permanent wilting point. It was later weighted, dried at 105.5°C for 24 h and was weighted again. The AWC was calculated by subtracting AFP and UWC from TP. The particle size distribution (FAO classification) of sand (>0.063 mm), silt (0.063–0.002 mm) and clay (<0.002 mm) fractions were determined by the pipette method.

Chemical analyses. In all soil samples the SOC was analyzed by Tuyrin's method [39, 41] and N_{tot} was measured with the Dumas method with an elemental analyzer *Vario MAX CNS analyzer* (ELEMENTAR, Germany).

Table 1. Mean air temperature (°C) and total precipitation (mm), with ± standard error, during the whole experiment period and separate decades

Period	Temperature, °C	Precipitation, mm
1965–1974	4.9^{A} (±0.3)	545^{A} (±29)
1975–1984	5.1^{A} (±0.3)	588^{AB} (±36)
1985–1994	5.5^{AB} (±0.4)	661^{AB} (±35)
1995–2004	6.0^{AB} (±0.2)	672^{AB} (±35)
2005–2014	6.4^{B} (±0.2)	690^{B} (±41)
1965–2014	**5.6 (±0.1)**	**631 (±17)**

Results of the one-way analysis of variance (ANOVA) in temperature and precipitation where $p = 0.007$ and $p = 0.027$, respectively. Means with different upper-case letters in same column indicate significant differences (Tukey's HSD test, $p < 0.05$) between decades.

Climate. Based on the Köppen–Geiger climate classification system, Estonia belongs to humid continental climate (Dfb) [28]. According to the Estonian Environment Agency weather station located in Tõravere (58°15′50.3″ N, 26°27′41.7″ E), in the period of January 1st 1965, until December 31st 2014, the average annual air temperature and precipitation were 5.6°C and 631 mm, respectively. However, if viewed in this region by decades, a steady increase in the air temperature and precipitation by the time was observed (Table 1). In the last decade (2005–2014), the air temperature was 1.5°C, or 30.1%, and the precipitation was 145 mm, or 26.6%, higher than in the first (1965–1974) decade. Based on data from the period of 1965–2014, following regression equations were generated for temperature: $T = -0.000181 \times \text{year}^2 + 0.761960 \times \text{year} - 792.613970$; $R^2 = 0.31$ and precipitation: $P = -0.075326 \times \text{year}^2 + 303.386318 \times \text{year} - 304791.132837$; $R^2 = 0.21$.

Data analysis. For testing the statistical significances of vegetation treatments, depths, years and their interactions on soil properties a full-factorial analysis of variance (ANOVA) was used. For *post hoc* analysis to see how those factors statistically differ between groups Tukey's HSD (honestly significant difference) test was used. Additional Pearson correlation coefficients were calculated in a pairwise configuration. Student's t-test with Bonferroni correction was used to calculate whether WSA values from 0–5, 5–10 and 10–20 cm depth in 2014, 2007 and 2000 at 0.25–1 mm sized aggregates significantly differ between glacial till samples from 1966. Weighted arithmetic mean was used to calculate the averages between depths 0–20 cm. The ANOVA, Tukey's HSD *post hoc* test and correlations were calculated by using Dell *Statistica*, version 13.2 (2016), while Student's t-test was performed by Microsoft Excel 2016 (v16.0). All tests results were considered to be significant if $p < 0.05$.

RESULTS

Analysis of variance. The analysis of variance showed that WSA was significantly ($p < 0.001$) affected by all factors, such as the vegetation treatment, soil depth, year and their combination(s) (Table 2). SOC and N_{tot}, were also both significantly affected ($p < 0.001$) by vegetation and depth and by their combination, although to a lesser extent. The findings also revealed that SOC was not significantly affected during the years of the study period as there were large variations. In the C : N ratio only depth was considered to be significant. In all particle fractions (sand, silt and clay), the impact of vegetation was near the threshold level ($p < 0.05$). The depth significantly influenced sand and silt fractions but had no effect on clay fractions.

Water stability of soil aggregates (WSA). In most cases, for both the control and barley treatments, the WSA values did not differ from the initial glacial till (Table 3). However, if manure was applied, the WSA increased significantly for the barley treatments in many years and depths. Nevertheless, in some vegetation treatments at certain depths, the WSA values were even lower than the initial ones. It was also found that perennial grass treatments that had been fertilized with $P_{40}K_{75}$, compared with without and $N_{150}P_{40}K_{75}$ fertilization, decreased the WSA. In most vegetation treatments, in 2014 and 2007, the WSA in 10–20 cm depths did not differ or was even lower than in the initial glacial till. However, generally speaking, in almost all vegetation treatments, there was a large variation of the WSA between different years during the study. It was additionally found in this study that the WSA values in larger (0.25–2 mm) aggregates compared with smaller (0.25–1 mm) aggregates were generally higher, and the decrease in stability caused by increasing the depth was less drastic. If the average values from the 2000–2014 period from all depths were taken, the WSA was the lowest in the control (24.11%) and without manure barley treatment (24.49%), while the WSA was highest on the $N_{150}P_{40}K_{75}$ fertilized grass treatment (37.60%), followed by non-fertilized perennial grass treatment (35.90%) and hybrid alfalfa (35.69%).

Soil organic carbon (SOC) and total nitrogen (N_{tot}). In 2014, the highest SOC and N_{tot} among the vegetation treatments were found under hybrid alfalfa, followed by $N_{150}P_{40}K_{75}$ fertilized perennial grass and white clover/perennial grass mixture treatment (Table 4). Compared to others, in those vegetation treatments that were enriched with nitrogen, the SOC was higher in all depths, while the N_{tot} was elevated only in 0–10 cm depths. In barley treatments, the use of a manure application increased both the SOC and the N_{tot} at all depths. By causing an average increase in the SOC by 40.0% and N_{tot} by 27.9% at 0–20 cm depth. However, those barley treatments still did not significantly differ from each other, as in the same *post hoc* test all other vegetation treatments were analyzed as well. The C : N

Table 2. Results of the full-factorial analysis of variance through which the effects of vegetation treatments, depth, year influence and their combined interactions were tested on different soil properties. Values considered statistically significant ($p < 0.05$) are presented in bold face

Parameter	WSA	SOC	C : N	N_{tot}	Sand	Silt	Clay
Vegetation (V)	$F_{7.216} = 141.41$ **$p < 0.001$**	$F_{7.54} = 15.78$ **$p < 0.001$**	$F_{7.54} = 1.72$ $p = 0.125$	$F_{7.54} = 16.32$ **$p < 0.001$**	$F_{7.54} = 2.29$ **$p = 0.041$**	$F_{7.54} = 1.93$ $p = 0.082$	$F_{7.54} = 2.17$ $p = 0.051$
Depth (D)	$F_{2.216} = 221.19$ **$p < 0.001$**	$F_{2.54} = 106.89$ **$p < 0.001$**	$F_{2.54} = 9.38$ **$p < 0.001$**	$F_{2.54} = 104.74$ **$p < 0.001$**	$F_{2.54} = 3.42$ **$p = 0.040$**	$F_{2.54} = 4.53$ **$p = 0.015$**	$F_{2.54} = 0.69$ $p = 0.506$
Year (Y)	$F_{2.216} = 174.64$ **$p < 0.001$**	$F_{2.54} = 0.97$ $p = 0.385$	–	–	–	–	–
V × D	$F_{14.216} = 17.75$ **$p < 0.001$**	$F_{14.54} = 2.89$ **$p = 0.003$**	$F_{14.54} = 1.43$ $p = 0.171$	$F_{14.54} = 2.06$ **$p = 0.029$**	$F_{14.54} = 0.44$ $p = 0.953$	$F_{14.54} = 1.60$ $p = 0.111$	$F_{14.54} = 0.75$ $p = 0.712$
V × Y	$F_{14.216} = 14.65$ **$p < 0.001$**	$F_{14.54} = 2.41$ **$p = 0.011$**	–	–	–	–	–
D × Y	$F_{4.216} = 31.77$ **$p < 0.001$**	$F_{4.54} = 8.01$ **$p = 0.001$**	–	–	–	–	–
V × D × Y	$F_{28.216} = 7.96$ **$p < 0.001$**	$F_{28.54} = 0.67$ $p = 0.876$	–	–	–	–	–

WSA, water-stable soil aggregates, %, 0.25–1 mm fraction; SOC, soil organic carbon, %; C : N, ratio between soil organic carbon and total nitrogen; N_{tot}, total nitrogen, %; Sand, >0.063 mm soil particles; Silt, 0.063–0.002 mm soil particles; Clay, <0.002 mm soil particles.

ratio between vegetation treatments significantly differed only in the depths of 5–10 cm, while being significantly lowest in $N_{150}P_{40}K_{75}$ and $P_{40}K_{75}$ fertilized perennial grass treatments (13.3 : 1 and 13.5 : 1, respectively) and highest in the control (26.6 : 1). With the exception in manure-applied barley, the C : N ratio increased by depth in all vegetation treatments, however only in white clover/perennial grass mixture this occurred significantly.

Nevertheless, after a 50-year period, there were several unusual SOC and N_{tot} associated findings. For instance, SOC in the control at all depths and N_{tot} at 0–5 cm depth had values higher than those of the initial glacial till, which were 0.21% and 0.018%, respectively. In addition, despite periodic soil disturbance at 20 cm depth due to the renewal of vegetation in the treatments, both SOC and N_{tot} still decreased with depth, wherein most cases, it did so significantly. It was also found that $P_{40}K_{75}$ fertilized perennial grasses compared to non-fertilized grass treatments, in almost all cases had lower SOC by 9.3, 20.5, 9.5% at 0–5, 10–20 and 0–20 cm depths, respectively.

Soil particle-size distribution. Among vegetation treatments there was a significant difference in the content of clay particles (<0.002 mm) at 0–5 cm depth; particularly, under herbaceous vegetation (especially with legumes), the clay content was higher than in barley treatments and in the control (Table 5). Under barley treatments, the clay content increased with the depth. In silt particles (0.063–0.002 mm), both herbaceous vegetation treatments and the control had the lowest content in 5–10 cm depth, of which only non-fertilized perennial grass treatment differed significantly. Furthermore, under herbaceous vegetation, the silt content slightly increased by depth, but in the barley treatments and in the control, the silt content decreased. In sand particles (>0.063 mm), the average of all depths (0–20 cm) showed, with the lowest (60.69%) in sand content in non-fertilized perennial grass and the highest (63.17%) in $N_{150}P_{40}K_{75}$ fertilized perennial grass treatments. However, those differences were not significant. In addition, in all vegetation treatments (except in the control), the sand content was lowest at 10–20 cm depths, while with the control, the lowest occurred in 0–5 cm depths; in both cases, those differences were not significant. In overall the average of all treatments and depths in 2014 showed the following soil particle fraction distribution of 62.2% sand (>0.063 mm), 23.8% silt (0.063–0.002 mm) and 14.0% clay (<0.002), which means a sandy loam texture in FAO classification.

Soil physical properties: porosity, density, and water content. All soil physical properties were significantly affected by vegetation (Table 6). The total porosity (TP) was lowest in the control (35.72%) and the highest without manure barley treatment (43.67%); at the same time, the TP in barley treatment with manure application was the second lowest (38.80%). The air-filled porosity (AFP) had a similar sequence between vegetation types as those in TP, although the significances between vegetation treatments were more homogeneous. The soil dry bulk density (BD) was inversely related to both TP and AFP. The plant available water content (AWC) was significantly higher

Table 3. The average soil water-stable aggregate stability (%) (with ± standard error) by vegetation treatment, depth, year and aggregate size

	Depth	Vegetation treatments							
		0_T	B_T	$B+M_T$	$G\text{—}_T$	G_T	$G+N_T$	WG_T	HA_T
		0.25–2 mm sized aggregates							
Year: 2014	0–5	32.69 ± 1.72	37.46 ± 1.68	43.63 ± 1.26	47.44 ± 0.95	46.34 ± 1.36	49.90 ± 0.66	49.81 ± 0.66	49.20 ± 1.82
	5–10	25.19 ± 1.70	34.02 ± 0.33	35.12 ± 1.42	42.97 ± 0.97	39.12 ± 0.40	39.91 ± 0.91	41.65 ± 1.60	47.61 ± 0.89
	10–20	19.18 ± 0.71	26.86 ± 0.86	22.66 ± 2.95	28.76 ± 0.46	32.37 ± 2.27	32.44 ± 0.59	30.78 ± 0.64	33.89 ± 1.54
	0–20	24.06	31.30	31.02	36.98	37.55	38.67	38.25	41.15
		0.25–1 mm sized aggregates							
Year: 2014	0–5	28.16^{ABa}● ± 1.21	23.83^{Aa}● ± 1.51	39.44^{CDa} ± 2.97	38.40^{CDa} ± 0.61	34.31^{BCa} ± 0.16	44.54^{D} ± 1.18	36.70^{Ca} ± 1.04	41.15^{CDa} ± 1.29
	5–10	19.85^{Aa}● ± 1.73	23.99^{ABa}● ± 0.71	33.49^{D} ± 0.64	33.54^{Da} ± 0.55	26.04^{BCa}● ± 1.95	35.17^{Da} ± 0.92	31.23^{CDa} ± 0.66	35.03^{Da} ± 0.95
	10–20	21.50^{CD}● ± 0.53	10.72^{A} ± 1.11	29.20^{F}● ± 0.64	26.51^{EF}● ± 1.15	16.84^{B} ± 1.17	25.71^{DEF}● ± 0.87	18.09^{BC} ± 0.84	24.29^{DE}● ± 0.76
	0–20	22.75	17.32	32.83	31.24	23.51	32.78	26.02	31.19
Year: 2007	0–5	24.87^{Aa}● ± 1.65	30.03^{Ab}● ± 0.49	29.39^{Ab}● ± 1.54	42.75^{Bb} ± 1.02	41.55^{Bb} ± 2.12	42.14^{B} ± 0.59	41.77^{Bb} ± 0.99	45.36^{Ba} ± 1.73
	5–10	21.86^{Aa}● ± 0.58	35.19^{Bb} ± 2.28	35.87^{B} ± 0.90	35.40^{Bab} ± 1.25	39.52^{Bb} ± 1.23	41.17^{Bb} ± 1.70	36.48^{Ba} ± 1.47	40.05^{Ba} ± 2.26
	10–20	26.67^{A}● ± 2.34	23.87^{A}● ± 1.00	36.64^{C} ± 0.34	28.18^{AB}● ± 2.30	27.15^{A}● ± 2.31	35.67^{BC} ± 1.51	26.11^{A}● ± 1.36	24.84^{A}● ± 2.05
	0–20	25.02	28.24	34.63	33.63	33.84	38.66	32.61	33.77
Year: 2000	0–5	13.19^{Ab} ± 1.70	29.54^{Bb}● ± 1.14	35.82^{BCab} ± 2.24	41.56^{CDab} ± 0.85	34.35^{BCa} ± 1.69	46.64^{DE} ± 1.88	48.36^{DEc} ± 1.02	52.84^{Eb} ± 1.35
	5–10	31.02^{Bb}● ± 1.74	23.03^{Aa}● ± 0.60	34.83^{BC} ± 0.47	38.74^{CDb} ± 0.98	37.65^{CDb} ± 1.24	45.42^{Eb} ± 0.98	43.58^{DEb} ± 2.28	47.34^{Eb} ± 1.15
	10–20	27.04^{A}● ± 1.57	29.52^{AB}● ± 0.88	31.15^{AB}● ± 2.69	45.54^{D} ± 2.13	35.75^{BC} ± 1.06	36.65^{BC} ± 1.36	39.65^{CD} ± 1.44	34.10^{ABC} ± 1.92
	0–20	24.57	27.90	33.24	42.85	35.88	41.34	42.81	42.10

0_T—bare fallow as control; B_T—barley with $P_{40}K_{75}$; $B+M_T$—barley with $P_{40}K_{75}$ and manure; $G\text{—}_T$—non-fertilized perennial grasses; G_T—perennial grasses with $P_{40}K_{75}$; $G + N_T$—perennial grasses with $N_{150}P_{40}K_{75}$; WG_T—white clover and perennial grass mixture with $P_{40}K_{75}$; HA_T—hybrid alfalfa with $P_{40}K_{75}$. Mean values with different upper-case letters in same row indicate significant differences (Tukey's HSD test, $p < 0.05$) between vegetation treatments on same depth, while means with lower-case letters indicate differences in different years on same depth at the same vegetation treatment. Mean values from 0.25–2 mm sized aggregates, as well as weighed averages from 0–20 cm depth in all years were excluded. Symbol "●" indicates in 0.25–1 mm aggregates (0–20 cm depths and 0.25–2 mm aggregates excluded) non-significant differences between 1966 year mean samples.

under herbaceous vegetation than in the control and without manure barley treatment. The plant unavailable water content (UWC) was significantly highest in hybrid alfalfa (11.13%) and significantly lowest in barley without manure treatment (8.94%).

WSA–SOC relationship. This study showed a linear relation between WSA and SOC, with the following regression equation: WSA = 17.9522 × SOC + 21.1257 ($R^2 = 0.44$, $p < 0.001$); which was based from values from all years and depths (Fig. 1). Therefore, based on measurements, the soil was far from any C saturation. It was also noticeable that the WSA and SOC relationship was not consistent and had a high periodic variability. The WSA in some years, especially in 2014 at 10–20 cm depth, were lower than in the initial glacial till from 1966. While WSA in 2014 had high variability, in 1966, the results were more densely clustered. The correlations found between the WSA and the SOC were the following: 1966 ($r = -0.33$), 2000 ($r = +0.68$), 2007 ($r = +0.56$), and 2014 ($r = +0.82$); in all years but 1966, correlations were significant ($p < 0.05$).

Table 4. Average content of soil organic carbon (SOC) (%), total nitrogen (N_{tot}) (%) and the ratio between SOC and N_{tot} (C : N), with ± standard error, between different vegetation treatments and soil depths in year 2014

	Depth	Vegetation treatment							
		0_T	B_T	$B + M_T$	$G{—}_T$	G_T	$G + N_T$	WG_T	HA_T
SOC	0–5	0.57^{A} ± 0.118	0.84^{AB} ± 0.163	1.00^{ABb} ± 0.057	1.09^{Bc} ± 0.071	0.99^{ABb} ± 0.088	1.18^{Bb} ± 0.091	1.32^{BCc} ± 0.062	1.64^{Cc} ± 0.103
	5–10	0.31^{A} ± 0.035	0.55^{AB} ± 0.080	0.84^{BCb} ± 0.114	0.55^{ABb} ± 0.051	0.56^{ABa} ± 0.113	0.71^{ABCa} ± 0.107	0.83^{BCb} ± 0.042	1.07^{Cb} ± 0.044
	10–20	0.16^{A} ± 0.013	0.27^{AB} ± 0.069	0.32^{ABCa} ± 0.041	0.32^{ABa} ± 0.007	0.25^{ABa} ± 0.035	0.39^{BCa} ± 0.060	0.48^{Ca} ± 0.022	0.51^{Ca} ± 0.014
	0–20	0.30^{A} ± 0.045	0.48^{AB} ± 0.095	0.62^{ABC} ± 0.063	0.57^{ABC} ± 0.029	0.51^{AB} ± 0.067	0.67^{BC} ± 0.069	0.78^{CD} ± 0.018	0.93^{D} ± 0.037
N_{tot}	0–5	0.045^{A} ± 0.019	0.047^{Ab} ± 0.008	0.055^{Ab} ± 0.004	0.065^{Ab} ± 0.006	0.06^{Ab} ± 0.013	0.086^{ABc} ± 0.007	0.089^{ABc} ± 0.006	0.122^{Bc} ± 0.008
	5–10	0.013^{A} ± 0.006	0.028^{ABab} ± 0.003	0.040^{ABb} ± 0.004	0.030^{ABa} ± 0.002	0.041^{ABab} ± 0.005	0.053^{BCb} ± 0.003	0.048^{Bb} ± 0.002	0.076^{Cb} ± 0.011
	10–20	0.006 ± 0.001	0.011^{a} ± 0.000	0.021^{a} ± 0.000	0.017^{a} ± 0.002	0.016^{a} ± 0.004	0.019^{a} ± 0.007	0.017^{a} ± 0.004	0.031^{a} ± 0.007
	0–20	0.017^{A} ± 0.006	0.025^{A} ± 0.001	0.034^{A} ± 0.002	0.032^{A} ± 0.002	0.034^{A} ± 0.005	0.044^{AB} ± 0.006	0.043^{AB} ± 0.002	0.065^{B} ± 0.007
C : N	0–5	14.41 ± 3.64	17.83 ± 0.36	18.12 ± 0.22	16.84 ± 0.50	16.17 ± 1.96	13.79 ± 1.14	14.92^{b} ± 0.62	13.53 ± 0.70
	5–10	26.57^{B} ± 8.60	19.78^{AB} ± 4.80	20.54^{AB} ± 0.97	18.37^{AB} ± 1.50	13.47^{A} ± 1.46	13.33^{A} ± 1.53	17.40^{ABb} ± 0.21	14.70^{AB} ± 1.62
	10–20	29.17 ± 6.08	23.85 ± 5.43	15.33 ± 1.89	19.86 ± 2.12	20.47 ± 5.01	26.28 ± 6.62	32.05^{a} ± 5.78	18.04 ± 2.96
	0–20	24.83 ± 0.02	21.33 ± 4.00	17.33 ± 1.13	18.73 ± 1.20	17.65 ± 2.64	19.92 ± 3.23	24.10 ± 2.85	16.08 ± 1.67

0_T—bare fallow as control; B_T—barley with $P_{40}K_{75}$; $B+M_T$—barley with $P_{40}K_{75}$ and manure; $G{—}_T$—non-fertilized perennial grasses; G_T—perennial grasses with $P_{40}K_{75}$; $G + N_T$—perennial grasses with $N_{150}P_{40}K_{75}$; WG_T—white clover and perennial grass mixture with $P_{40}K_{75}$; HA_T—hybrid alfalfa with $P_{40}K_{75}$. Means with different upper-case letters in same row indicate significant differences (Tukey's HSD test, $p < 0.05$) between vegetation treatments, while means with lower-case letters indicate differences in depths of each soil property.

Table 5. Average percentage composition of sand (>0.063 mm), silt (0.063–0.002 mm) and clay (<0.002 mm) fractions (±standard error) between different vegetation treatments and soil depths in year 2014

Frac-tion	Depth	Vegetation treatments							
		0_T	B_T	$B + M_T$	$G{—}_T$	G_T	$G+N_T$	WG_T	HA_T
Sand	0–5	60.99 ± 1.97	61.71 ± 2.50	63.28 ± 1.13	60.92 ± 0.42	63.04 ± 0.78	63.13 ± 0.42	61.64 ± 0.76	62.41 ± 0.54
	5–10	64.60 ± 0.48	62.16 ± 0.66	62.92 ± 2.08	61.67 ± 0.72	63.43 ± 0.65	63.31 ± 0.51	62.39 ± 0.39	63.07 ± 0.61
	10–20	62.62 ± 0.65	61.45 ± 0.26	62.49 ± 1.26	60.08 ± 0.92	61.23 ± 1.28	63.11 ± 1.28	60.29 ± 0.59	61.83 ± 1.47
	0–20	62.71 ± 0.70	61.69 ± 0.92	62.79 ± 1.44	60.69 ± 0.68	62.23 ± 0.89	63.17 ± 0.55	61.15 ± 0.51	62.28 ± 0.90
Silt	0–5	26.63 ± 2.42	26.31 ± 3.33	24.14 ± 0.42	24.77 ± 0.39	23.04^{b} ± 0.71	22.91 ± 0.50	23.71 ± 0.57	22.82 ± 0.53
	5–10	21.23 ±1.02	25.00 ± 1.64	24.24 ± 1.52	23.91 ± 0.31	22.33^{b} ± 0.41	22.74 ± 0.70	23.16 ± 0.30	22.56 ± 0.60
	10–20	23.79 ± 0.04	24.20 ± 0.49	23.72 ± 0.68	25.06 ± 0.80	26.11^{a} ± 1.02	23.26 ± 0.92	24.79 ± 0.55	24.02 ± 1.14
	0–20	23.86 ± 0.33	24.93 ± 1.49	23.95 ± 0.82	24.70 ± 0.54	24.40 ± 0.42	23.04 ± 0.34	24.11 ± 0.44	23.36 ± 0.71
Clay	0–5	12.38^{AB} ± 0.45	11.98^{A} ± 0.83	12.58^{ABC} ± 0.71	14.31^{CD} ± 0.29	13.93^{BCD} ± 0.29	13.95^{BCD} ± 0.33	14.65^{D} ± 0.28	14.77^{D} ± 0.17
	5–10	14.17 ± 0.54	12.84 ± 0.98	12.84 ± 0.56	14.42 ± 0.61	14.24 ± 0.38	13.95 ± 0.24	14.46 ± 0.17	14.37 ± 0.24
	10–20	13.59 ± 0.69	14.35 ± 0.23	13.80 ± 0.59	14.86 ± 0.72	12.66 ± 2.25	13.63 ± 0.43	14.92 ± 0.24	14.15 ± 0.40
	0–20	13.43 ± 0.37	13.38 ± 0.57	13.26 ± 0.61	14.61 ± 0.58	13.37 ± 1.15	13.79 ± 0.35	14.74 ± 0.20	14.36 ± 0.28

0_T—bare fallow as control; B_T—barley with $P_{40}K_{75}$; $B+M_T$—barley with $P_{40}K_{75}$ and manure; $G{—}_T$—non-fertilized perennial grasses; G_T—perennial grasses with $P_{40}K_{75}$; $G + N_T$—perennial grasses with $N_{150}P_{40}K_{75}$; WG_T—white clover and perennial grass mixture with $P_{40}K_{75}$; HA_T—hybrid alfalfa with $P_{40}K_{75}$. Means with different upper-case capital letters in same row indicate significant differences (Tukey's HSD test, $p < 0.05$) between vegetation treatments.

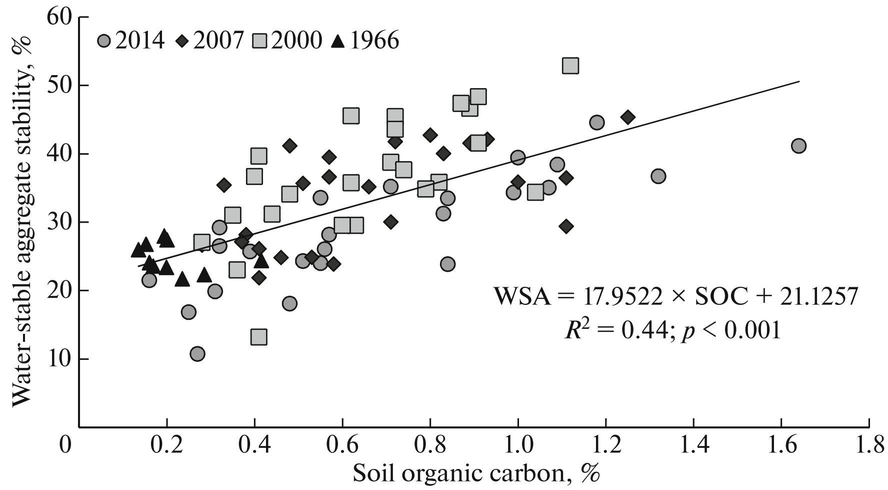

Fig. 1. Shatter plot of the relationship between soil water-stable aggregate stability (%) and soil organic carbon content (%) from all vegetation treatments, depths and years. Different years are highlighted separately.

DISCUSSION

The WSA differences among vegetation treatments were most likely caused by variations in root biomass and morphology, which means differences in root exudates; those, in turn, are effective binding agents for soil aggregates. Fertilization, which was part of the treatments, is also known to affect the root system. For instance, N fertilization is known to increase the aboveground biomass [34], as well as the root biomass, but this to a much lesser extent [8]. While on the other hand P might increases the root biomass. Nevertheless, this is more complicated, as it depends on the fertilizer's nutrient composition as well as the quantity. Differences in soil management by the annual tillage operations in barley treatments are known to cause soil disturbances and a disruption of aggregates; therefore, the mineralization of soil organic matter (SOM) increases. Despite this, the use of manure in barley greatly reduced the consequences of tillage. The year's influence on the WSA was more complicated. According to Zhang et al. [44], the fluctuating content of binding agents in form of glomalin-related soil proteins and microbial biomass determines 79% of the WSA variability. Furthermore, the high sand content and low SOC, as found in the present study, are widely

Table 6. The average mean and standard error of total porosity (TP) (%), air-filled porosity (AFP) (%), dry bulk density (BD) (g cm^{-3}), available water content (AWC) (weight %) and unavailable water content (UWC) (vol %), depending on vegetation treatment in year 2014 from 5–10 cm depth

Soil properties	Vegetation treatments							
	0_T	B_T	$B+M_T$	$G-_T$	G_T	$G+N_T$	WG_T	HA_T
TP $p < 0.001$	35.72^A ± 1.20	43.67^C ± 1.40	38.80^{AB} ± 1.02	41.11^{BC} ± 0.43	41.53^{BC} ± 0.49	42.44^C ± 0.56	40.79^{BC} ± 0.65	40.83^{BC} ± 0.71
AFP $p = 0.001$	11.48^A ± 1.47	19.78^B ± 2.17	12.26^A ± 1.13	14.01^A ± 0.88	12.69^A ± 1.15	16.17^{AB} ± 1.02	13.59^A ± 1.03	12.74^A ± 1.11
BD $p < 0.001$	1.71^C ± 0.03	1.49^A ± 0.04	1.61^{BC} ± 0.03	1.56^{AB} ± 0.01	1.54^{AB} ± 0.01	1.52^{AB} ± 0.02	1.56^{AB} ± 0.02	1.55^{AB} ± 0.02
AWC $p < 0.001$	13.93^A ± 0.18	14.95^{AB} ± 0.20	15.61^{ABC} ± 0.96	17.01^{BC} ± 0.34	18.38^C ± 0.65	16.59^{ABC} ± 0.64	16.89^{BC} ± 0.47	16.96^{BC} ± 0.51
UWC $p < 0.001$	10.32^{ABC} ± 0.54	8.94^A ± 0.56	10.93^{BC} ± 0.63	10.09^{ABC} ± 0.15	10.46^{ABC} ± 0.36	9.69^{AB} ± 0.12	10.32^{ABC} ± 0.26	11.13^C ± 0.28

0_T—bare fallow as control; B_T—barley with $P_{40}K_{75}$; $B+M_T$—barley with $P_{40}K_{75}$ and manure; $G-_T$—non-fertilized perennial grasses; G_T—perennial grasses with $P_{40}K_{75}$; G + N_T—perennial grasses with $N_{150}P_{40}K_{75}$; WG_T—white clover and perennial grass mixture with $P_{40}K_{75}$; HA_T—hybrid alfalfa with $P_{40}K_{75}$. Means with different upper-case capital letters in same row indicate significant differences (Tukey's HSD test, $p < 0.05$) between vegetation treatments.

known to have a negative impact on WSA and simultaneously cause the soil structure to become more prone to high seasonal variability [11]. Additionally, according to [5], in soils with a low SOC, much of its organic matter, which is responsible for forming stable aggregates, has a microbial origin, and their effects are transient. In addition, the high soil porosity could enhance mineralization of organic matter, which is further contributed to by the mineralization favorable C : N ratio, which in all treatments was below 25 : 1. Additionally, the year's influence on WSA was also the combination of abiotic factors, such as wetting-drying [9] and freeze-thaw cycles [22], which are all affected by changes and patterns of temperature, precipitation and solar radiation. The reason why WSA decreased with depth under herbaceous vegetation could be explained by the rapid decrease of SOC and N_{tot} below 10 cm depth, as according to Gill et al. [13] under perennial grasses 75% of root mass is located within 0–15 cm depth. However, the reasons why the SOC and N_{tot} both decreased by depth in barley treatments, despite each year evenly disturbing the soil down to 20 cm depth by digging, could not be explained.

The primary reason why larger (0.25–2 mm) soil aggregates in this study had a higher WSA could be that smaller (0.25–1 mm) aggregates had a higher content of mineral particles, especially sand fractions, and less all type of organic matter than in larger aggregates. This assumption is supported by Oades [27] if clay content is <15%, the soil structure is predominantly maintained with organic matter, not with abiotic factors such as clay. In addition, a majority of organic matter (including root residues) could be agglomerated into larger clods and therefore be located with a higher content in >1 mm soil aggregates [14, 25]. This could also explain why in 2014, the SOC and N_{tot} had a higher correlation between WSA in larger (0.25–2 mm) aggregates ($r = +0.64$ and $r = +0.65$, respectively) than in smaller (0.25–1 mm) aggregates ($r = +0.54$ and $r = +0.47$), respectively. Although according to the aggregate hierarchy theory [38], smaller aggregates are more stable than larger ones due to stronger internal forces. In this study, both sized soil aggregates were classified as macro-aggregates (>250 μm); therefore, both are susceptible to the same aggregate formation, degradation and stabilization processes [3].

Compared with the initial glacial till, the SOC and N_{tot} increase in bare fallow treatment, which acted as control, could be caused by various reasons. First, due to the experiment fields' small scale, contamination with organic matter from the surrounding environment in the form of wind-blown tree leaves and grass hey pieces could occur. In addition, it cannot be excluded the possibility of contamination due to dissolved organic matter, which could be transported by water from areas nearby the plots. Second, there is a possibility that this was caused by the life activity of soil bacteria and fungi. According to Rashid et al. [29], those microorganisms are omnipresent in soil, and their exudates and dead cells contribute to an increase in both SOC and WSA [10]. In addition, bacteria, with the interaction of fungi, helps N fixation even in bulk soil [29]. This could explain why the N_{tot} content in the control at 0–5 cm depth was confusingly similar to those in barley treatment without manure and did not significantly differ from manure-applied barley along with non-fertilized and $P_{40}K_{75}$ fertilized perennial grass treatments. Furthermore, these microorganisms that caused effects in the present study could be enhanced in this study due to the absence of pesticides after the experiments were established. Besides this, the increase in N_{tot} can be additionally linked to the natural nitrogen enrichment by precipitation. According to the chemical analyses of atmospheric precipitation, which were conducted by the Estonian Environmental Research Center, near Tartu during the period 2011–2016, the mean inorganic nitrogen (NH_4^+–N + NO_3^-–N) enrichment with precipitation was approximately 5.5 kg ha^{-1} yr^{-1} ± 0.8 SE.

Regardless of the increase of SOC and N_{tot} in the control treatment, compared with initial glacial till, it seemingly had no effect on WSA in smaller aggregates (0.25–1 mm) and a limited effect in larger aggregates (0.25–2 mm) at 0–5 cm depths. Such finding also occurred in all other vegetation treatments in 2014 and 2007 at 10–20 cm depths at smaller aggregates. One explanation for this could be that, at such low level of SOC, the WSA cannot be persistently maintained, as the SOC is known to be the major binding agent of aggregates [38]. The second possible explanation, even though it was not analyzed in the present study, could have to do with the differences in SOC composition, as a higher content of labile soil organic carbon affects the WSA less or even negatively [40] than compared to the content of stabile soil organic carbon fractions. In barley treatment without manure, in smaller aggregates the WSA was similar to those of control, despite having higher SOC, which could be explained by the annual soil disturbance caused by sowing, which disrupted aggregates. The SOC and N_{tot} decline by depth, foremost in the control, and could be due to the decreased soil air exchange rate, which could inhibit microbial life activity. Despite P and K being known to increase soil above- and belowground biomass and thereby contribute to C sequestration, especially in nutrient poor soils. In the present study, the SOC was lower in $P_{40}K_{75}$ fertilized grass treatment compared with non-fertilized grass treatment, which could be because $P_{40}K_{75}$ fertilization increased the life activity of soil microorganisms, which contributed to mineralization [24] at a greater extent than in the non-fertilized treatment. For this assumption, evidence can be found in the C : N ratio, which was much narrower in $P_{40}K_{75}$ fertilized treatment than in the unfertilized treatment. The above-ground biomass yields of dry matter in 2014 from every vegetation treatment were: B_T (1.76 Mg ha^{-1}), $B+M_T$ (2.35 Mg ha^{-1}), $G-_T$ (1.86 Mg ha^{-1}), G_T (2.28 Mg ha^{-1}), $G+N_T$ (6.11 Mg ha^{-1}), WG_T (2.08 Mg ha^{-1}) and HA_T (9.09 Mg ha^{-1}). It can be assumed, with similar fertilization rates based on grassland research by Kauer et al. [19], if the aboveground biomass after each cut were returned, the yields in $P_{40}K_{75}$ and $N_{150}P_{40}K_{75}$ fertilized grassland treatments would eventually increase ~7 and ~18%, respectively. This, in turn, would improve both the SOC and WSA and other soil physical properties. The exact reasons, why the average WSA values from 2000–2014 period where highest under perennial grasses and hybrid alfalfa could be associated with root development, which were part of the SOC and N_{tot} accumulation, while the lowest WSA values are mostly the consequence of lack of vegetation in control and due to tillage in without manure barley treatment. The high similarity in N_{tot} content between $N_{150}P_{40}K_{75}$ fertilized grassland and white clover grass mixture treatment in all layers indicates that the root nodule nitrogen-fixing ability in the white clover grass mixture was equivalent to 150 kg ha^{-1} yr^{-1} N fertilization. Nevertheless, according to a large-scale meta-analysis conducted by Carlsson, Huss-Danell [7], the N fixation in similar climatic conditions has a large annual variation as well as according to meta-analysis by Divito, Sadras [12] the root nodule activity is also highly sensitive to P, K and S deficiency. The high positive correlation between N_{tot} and WSA could most likely be explained by the increased root mass due to additional fertilization in the nutrient poor soil, which, in turn, released a greater amount of aggregate binding agents into the soil [38] and clearly indicates the importance of inputs in the form of organic material and fertilizers that contain N for soil development. Nevertheless, for instance, in well developed soils, the extensive N fertilization was shown to have rather negative effects on WSA [4].

The linear relation between WSA and SOC found in this study clearly indicates that there is no sign of C saturation and therefore the soil is still steadily developing. Due to the strong significant correlation between WSA and SOC, the fluctuation in either affects the other in the same direction; as SOC is the major binding agent for aggregates, while at the same time, inside of aggregates, the SOC is physically protected from fast decomposition [38]. Because of the coarse soil texture in the present study, aggregates are more susceptible to tillage-caused degradation and rainfall impact than in finer texture [2]. This could be one of the reasons why WSA in 2014, under many vegetation treatments, was lower than in years 2007 and 2000. In addition to this, the temperature and precipitation increased due to climate change, which could accelerate microbial activity and therefore a faster soil organic matter decomposition rate [42] by increasing further fluctuations in WSA. Despite this, the effects of climate change could have controversial effects on WSA. On the one hand, in those particular conditions, soil formation from a WSA point of view could be increased by enhanced weathering of parent material, as according to the Arrhenius equation, the speed of chemical and biological reactions often double by an increase of each 10°C in temperature. On the other hand, it could cause extensive leaching, but also increase the number of wetting-drying and freeze-thaw cycles, which in turn could increase mineralization rate of soil organic matter [36].

The significantly lowest BD in the barley treatment without manure application was most likely caused by annual sowing, through which soil disturbance left the soil relatively loose. This assumption was also supported by the results of TP and AFP, which were significantly highest among all treatments. With the manure-applied barley treatment, the opposite results were most likely caused due by slight compaction, which most likely occurred during annual sowing. Nevertheless, despite this, the WSA, especially in smaller aggregates (0.25–1 mm), was significantly higher compared to barley treatment without manure. Considering that the SOC was higher in the manure-applied barley treatment, this clearly indicates the importance of SOC for maintaining soil structure at a far greater extent than TP and AFP, in such less developed coarse textured soil. In the control treatment, the significantly highest BD and lowest AWC, TP and AFP values were the consequence of having the lowest WSA and SOC among all other treatments, which were caused by the lack of vegetation. This also meant that the soil in this treatment was also directly exposed to rainfall impact, which is known to be highly destructive to aggregates. Under herbaceous vegetation, despite AFP and WSA were weakly and not significantly correlated, it could be still assumed that with a higher WSA, the collapse of soil structure under wetting stress will be less severe; therefore, the air-filled pores were likely to become clogged and sealed with soil particles during the aggregate degradation under rainfall. Despite the AWC being highest in herbaceous vegetation, there was no clear correlation between of either WSA or SOC with AWC and/or UWC. Therefore, those findings match the results of a resent large-scale meta-analysis [26], in which the increase of SOC has only a very small positive effect on AWC, despite some studies [2] showing much greater importance of SOC on AWC. However the reason why barley without manure had the significantly lowest UWC could be associated with the soil initial volumetric water content, as AWC and UWC, where both significantly correlated with the soil initial volumetric water content, $r = 0.67$; $p < 0.001$ and $r = 0.33$; $p = 0.001$, respectively. Furthermore, the soil initial volumetric water content was also positively correlated with SOC ($r = 0.22$; $p = 0.027$). Thus, treatments with higher SOC contained simply more volumetric water and therefore both more AWC and UWC. The reason clay content on herbaceous vegetation significantly differed from barley treatments at 0–5 cm depth was mostly due to the absence of annual soil disturbances within 20 cm depth, but it is also due to the possibility that greater amounts of clay particles were enmeshed into soil organic matter, as those treatments had higher WSA and SOC than that in barley treatments. The decreased silt content in all herbaceous vegetation treatments and in the control at 5–10 cm depth indicate the presence of a slight leaching process, as those findings on leaching are similar to those of another long-term soil formation experiment based on the same parent material located nearby [33], and according to this study, it can be concluded that the leaching was, to a larger extent, caused by translocation rather than transformation of particle fractions by weathering. In the present study, the possible reason for the lowest sand content in the control at 0–5 cm depth could be an indication of the signs of weathering, as similar conclusions were reached by Reintam [33], although in present study the difference was not significant. Furthermore, a long-term study by Li and Shao [23] found that, compared with other properties, soil texture is a stable property, while it is the particle fraction distribution that changes in a small amount [32]. According to the long-term climate data (Table 1), due to climate warming, it is expected that the weathering and leaching intensifies in future. This, under the influence of several fungi and bacteria species, could increase the phosphorus and foremost K solubilization [30], as the control treatment showed a possible bacteria-fungi influence, which was mentioned earlier in the discussion, and the increase of plant nutrient uptake could, in turn, somewhat accelerate the SOC formation, which could, in some way, affect the WSA.

CONCLUSIONS

Based on this study, it can be concluded that the most rational way to improve the water stability of soil aggregates (WSA), as well as increase the soil organic carbon (SOC) and total nitrogen (N_{tot}) contents were archived if the calcareous glacial till was left under vegetation of perennial grasses and hybrid alfalfa. Fertilization also had a positive effect on the soil properties; however, on perennial grasses rather in a complicated manner, as the WSA, SOC and air-filled porosity in $P_{40}K_{75}$ fertilization treatments were lower than those in $N_{150}P_{40}K_{75}$ or non-fertilized treatments. Still, the aggregation process was highly complicated. In addition, it is important to notice that the findings of this study are experiment specific. Therefore, differences in the fertilization rates and residue retention management could cause significant differences in the WSA among those vegetation treatments.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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