sciendo Agriculture (Poľnohospodárstvo), 65, 2019 (4): 136 – 146

Original paper

DOI: 10.2478/agri-2019-0014

ADAPTIVE ABILITIES OF BROAD BEAN (*VICIA FABA* L.) ACCESSION IN TERMS OF MAIN QUANTITATIVE TRAITS

NATALIA GEORGIEVA, VALENTIN KOSEV*

Institute of Forage Crops, Pleven, Bulgaria

GEORGIEVA, N. – KOSEV, V.: Adaptive abilities of broad bean (*Vicia faba* L.) accession in terms of main quantitative traits. Agriculture (Poľnohospodárstvo), vol. *65*, 2019, no. 4, pp. 136 – 146.

The experimental activity was conducted at the Institute of Forage Crops (Pleven) during the period 2016 – 2018. The adaptive ability of 10 broad bean accessions was determined with respect to main quantitative traits based on parametric and nonparametric analysis. The environment influences to the highest degree the traits of 1st pod height, pods number and seed weight per plant. The plant height and seeds number were strongly influenced by the genotype, and the mass of 100 seeds was determined by the genotype × environment interaction. The broad bean accessions can be distributed as follows: Fb 1929 has a high value of the 1st pod height (34 cm) and is characterized by high plasticity and stability; BGE 029055 and Fb 1896 are stable and form a large number of pods per plant (11 – 15); Fb 1896 and Fb 2486 are distinguished with good adaptability and stability, increased seed weight (28.01 and 30.28 g, respectively) and 100 seeds mass (105.48 g and 91.31 g). Accessions BGE 032012 and Fb 2481 represent a selection value in terms of plant height (61.36 and 65.83 cm); Fb 1929 – in 1st pod height (32.46 cm); and BGE 029055, Fb 1896 and Fb 2486 – in pods number (10.59, 9.67 and 11.89). Fb 1896, Fb 2486 and BGE 041470 can be used to develop a new genetic diversity in breeding aimed at increasing the mass of 100 seeds and seed productivity.

Key words: adaptability, selection value, trait, broad bean

Broad bean (*Vicia faba* L.) is a diploid species with 2n = 12 chromosomes. It belongs to the family of Fabaceae, subfamily of *Papilionoideae*, genus of *Vicia*. It is considered that broad bean is an important protein-rich food for animals and humans. It covers the requirements for a nutritional rate. Fortunately, the genetic variability of this species is large. Based on differences regarding seed weight, shape and size, most researchers focus on the following varieties: *V. faba paucijuga*, *V. faba major*, *V. faba equina* and *V. faba minor* (Fernandez *et al.* 1996; Ouji *et al.* 2011).

Such as other crops, the broad bean is exposed to biotic and abiotic stresses which decrease yield and affect stability. Abiotic stresses such as high temperature and drought mainly limit crop productivity (Pandey *et al.* 2017). The demand for genotypes with high yield and stability is a key aspect in breeding programs that aim to develop cultivars with high performances under different growing areas. The interaction of genotype × environment strongly affects genotype manifestations, making the creation of new cultivars difficult. One way to use this interaction is to identify genotypes with high productive capacity and phenotypic stability in various environments (Milioli *et al.* 2018).

Numerous methods for studying the stability and productivity of genotypes are described in the scientific literature. These methods differ in the estimated parameters and statistics. Investigations compar-

---

Valentin Kosev (*Corresponding author), Department of Breeding and Seed Production of Forage Crops, Institute of Forage Crops, Pleven, Bulgaria. Email: valkosev@hotmail.com

ing different methods have already been conducted in different crops such as wheat (Bornhofen *et al.* 2017), maize (Cargnelutti *et al.* 2009), common beans (Pereira *et al.* 2009), cowpea (Torres *et al.* 2016) and others. Among the basic methods applied to study the phenotypic stability of genotypes are univariate parametric models (Eberhart & Russell 1966), multivariate parametric models (Zobel *et al.* 1988; Yan 2001), nonparametric models (Lin & Binns 1988) and mixed models (Resende 2006).

The purpose of this study was to determine, by applying appropriate methods, broad bean accessions adapted to the local climatic conditions for the needs of combinative breeding.

MATERIAL AND METHODS

The experimental work was conducted at the Institute of Forage Crops (Pleven) during the period 2016 – 2018. The collection included 10 accessions of broad bean (*Vicia faba* L.), originating in Portugal (Fb 1896, Fb 1903, Fb 1929, Fb 2481, Fb 2486, Fb 3270) and Spain (BGE 002106, BGE 029055, BGE 032012, BGE 041470). It was used a randomized block method (Barov 1982). The sowing was done by hand, with a rate of 30 seeds/m^2, a plot size of 4 m^2 and three replications. Broad bean was grown under organic farming conditions without the use of fertilisers and pesticides. The biometric characteristics of the accessions included the following traits: plant height [cm], 1st pod height [cm], pods number per plant, pod length [cm], pod width [cm], seeds number per plant, seed weight per plant [g], 100 seeds mass [g].

The obtained data were processed by two-factor analysis of variance for each trait for determine of the influence of the factors of genotype (accessions) (G) and environment (E). For quantitative assessment of the stability and plasticity parameters was used the methodology of (Kilchevskiy & Hotyleva 1985a) and (Kilchevsky & Hotyleva 1985b) based on cultivar testing in different environments and allowing determination of: general (GAA) and specific adaptive ability (SAA), relative stability of the genotypes (Sgi), their interaction with environment (G×Egi) and reaction to the environment (Igi), as well as selective value of genotype (SVG) with an aim selecting high-productive and stable forms. The adaptability of the broad bean accessions was also determined by means of non-paramedical (rank) analysis (Nascimento *et al.* 2009), index of stability (IS) of the genotype (Nettevich 2001) and the stability parameter “bi” (Finlay & Wilkinson 1963)

All experimental data were processed statistically using the computer software GENES 2009.7.0 for Windows XP (Cruz 2006).

RESULTS

The results of the data on the variance of the productivity elements in the broad bean accessions were presented in Table 1. The environment (year), to the greatest degree, was a decisive factor for the performance of the traits of 1st pod height, pods number and seed weight per plant. The formation of plant height and seeds number was more strongly influenced by genotype than by the year. The mass of 100 seeds in the studied group of accessions was determined by the interaction genotype × environment, which occupied a major share in the total variation of this trait.

With regard to the trait of plant height (Figure 1), the average for the study period, accessions Fb 3270, Fb 1896 and BGE 032012 formed higher plants than others. Data on stability and adaptability showed that not all accessions whose plants were higher exhibited instability. According to the parameter „bi“ BGE 032012 was very stable (bi = 0.18), while Fb 1896 and Fb 1903 were unstable under unfavorable environmental conditions (Table 2).

The adaptability of genotype under specific environmental conditions, expressed by the values of SAA and Sgi, showed that the BGE 029055 and Fb 2486 were the least variable, but their plants were one of the lowest (65 – 68 cm). In terms of GAA, Fb 3270 was standing out, followed by Fb 1896 and Fb 1929. The higher the general adaptive ability, the better the genotype was adapted to different environmental conditions. The selective value of genotype was an aggregated indicator combining productivity and adaptability, more pronounced in BGE 032012 (61.36) and Fb 2481 (65.83).

The results of the biometric analysis showed a high-situated 1st pod (33 – 35 cm) in Fb 2481, Fb

1929 and Fb 3270, and respectively a low-located 1st pod (22 – 24 cm) in BGE 029055 and Fb 2486. The coefficient of regression (bi) gave similar information with the parameter Sgi regarding the trait variability under different environmental conditions. In terms of 1st pod height, no one of the accession was close to the "ideal" type (bi ≈ 1). Accessions BGE 002106, BGE 032012, Fb 2481, Fb 1903 and BGE 041470 had regression coefficients considerably exceeding one (bi > 1). Fb 2486 and Fb 3270 could be characterized as ecologically stable (bi < 1), but with a very low-situated 1st pod. Fb 1929 was manifested as stable both with respect to the regression coefficient and the values of the other parameters. The combination of good stability and high-situated 1st pod defined this accession as the most valuable one. According to the parameter SVG, Fb 2481 (31.18 cm) and BGE 032012 (30.59 cm) were also of interest.

Judging by the results regarding pods number per plant, accessions BGE 029055 and Fb 1896 were distinguished by the formation of more pods. For this trait, they were defined as stable, with coefficient „bi" < 0.3. BGE 002106 and BGE 041470 were also stable (bi = 0.34; bi = 0.26) but did not succeed to form more than 8 – 10 pods. Fb 2486 was unstable, but responsible and placed under favorable environmental conditions could form up to 10 pods per plant.

The accessions studied (with the exception of Fb 3270) were similar in GAA, and the values of this parameter were very close. Expression of the behavior of genotype in given environmental conditions is presented by the parameter of specific adaptive ability (SAA). More stable genotypes have low values for SAA. With such characteristics were Fb 2486 (0.04), BGE 032012 (0.19) and Fb 1896 (0.24). The ability of a genotype to maintain a certain level of the trait at a different background of the environment is represented by the parameter of relative stability (Sgi). This indicator as an interpretation is analogous to the coefficient of variation. According to the data obtained for Sgi, GAA, and SVG, selective valuable accessions were BGE 029055 (10.59), Fb 1896 (9.67) and Fb 2486 (11.89) (Table 2).

The accessions included in the study differed in the number of seeds formed (Figure 1) – from 22 (Fb 1929 and Fb 2481) to 41 (BGE 029055). BGE 029055 was one of the most stable (bi = 0.17) in this study. With a value for "bi" slightly above one was BGE 002106, which formed seeds above the average number for the group and can be referred to the plastic genotypes forming a sufficient number of seeds in both favorable and unfavorable environmental conditions. The accessions were characterized by different specific adaptive abilities, best shown regarding Fb 2481 (2.07), Fb 1929 (2.84) and Fb 1896 (6.71). With high values for SVG were Fb 2486 and BGE 041470 (26.19, 29.39).

When analyzing adaptability regarding 100 seeds mass, the broad bean accessions can be divided into

Table 1

Analysis of variance regarding the studied traits in broad bean accessions

Source of variation	*df*	Mean squares					
		plant height	1st pod height	pods number	seeds number	100 seeds mass	seed weight
Year	2	0.514	178.484^{++}	78.698^{++}	123.698^{++}	1.400^{++}	0.506^{++}
Replication ×Year	6	1.993	0.669	0.171	2.707	0.095	0.012^{++}
Accession	9	206.613^{++}	170.302^{++}	43.560^{++}	698.853^{++}	10.677^{++}	0.217^{++}
Accession × Year	18	174.118^{++}	68.676^{++}	37.603^{++}	182.961^{++}	12.209^{++}	0.091^{++}
Error	54	2.792	2.154	0.202	2.499	0.087	0.002
Corrected Total	89	–	–	–	–	–	–

$^{+}$ – significant at $P < 0.05$, $^{++}$ – significant at $P < 0.01$

several groups. Definitely stable (bi < 1) were BGE 041470 and Fb 1896 which had a high value for this trait, as well as BGE 032012, Fb 2486 and BGE 029055, which were with relatively lightweight seeds. Accessions Fb 1903, Fb 2481, Fb 3270 and BGE 002106 differed considerably in the performance of this trait, but they all showed very strong instability. However, an advantage can be given to Fb 3270, with a mass of 100 seeds over 102 g. Generally, in terms of general and specific adaptability, attention was paid to Fb 1896, which had the heaviest seeds. With higher requirements for specific environmental conditions were characterized accessions Fb 1903 (SAA = 17.31), BGE 041470 (SAA = 39.72) and Fb 2486 (SAA = 52.31). Fb 2486, BGE 041470 and Fb 1896 were distinguished by high values of the parameter SVG (24.89 – 30.97).

According to the shown stability level of seed weight, most of the low and medium-productive accessions (such as Fb 1929, Fb 3270 and BGE 032012) could be evaluated as stable, with the regression coefficient less than one (bi < 1) (especially

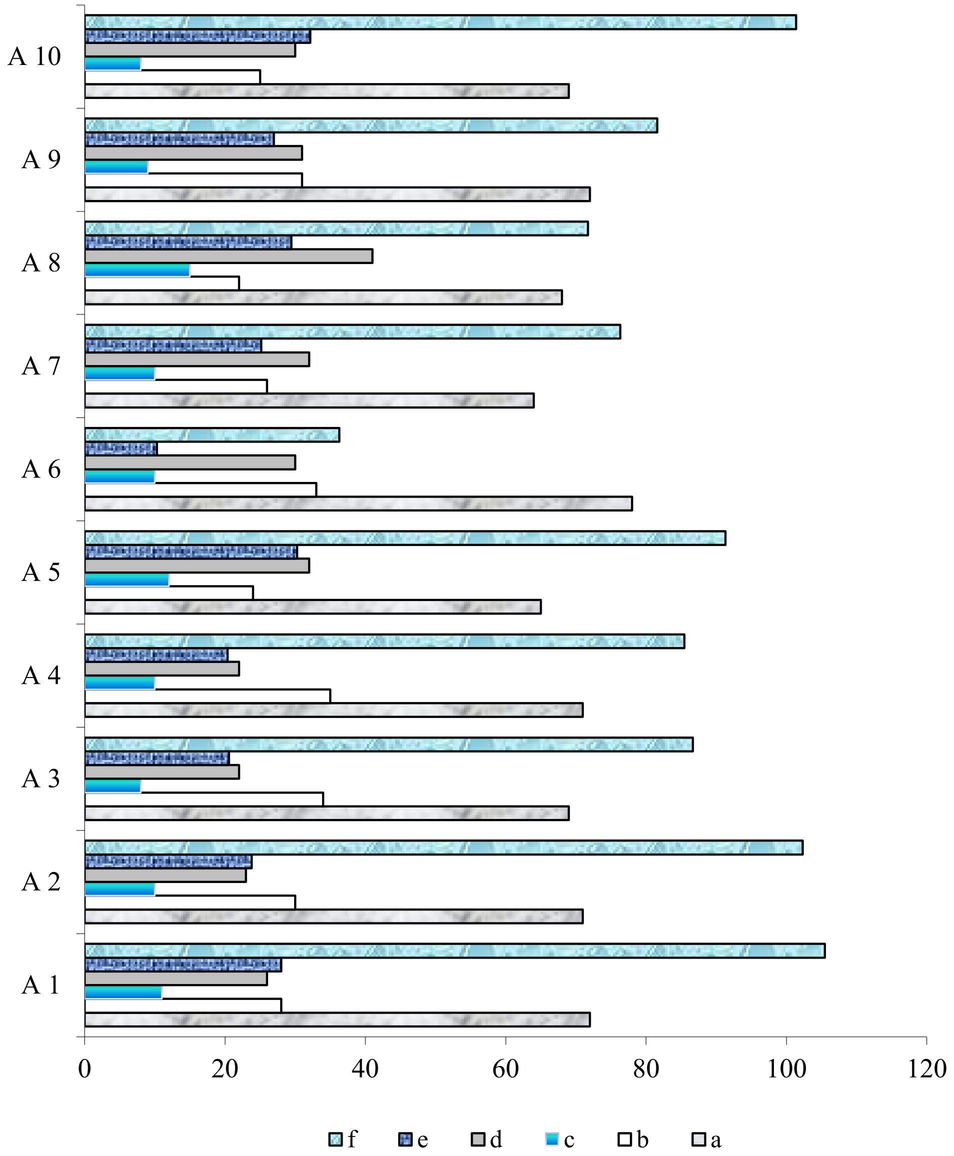

Figure 1. Quantitative traits in investigated accessions of broad bean

a – plant height; b – 1st pod height; c – pods number; d – seeds number; e – seed weight; f – 100 seeds mass
A1-Fb 1896, A2-Fb 1903, A3-Fb 1929, A4-Fb 2481, A5-Fb 2486, A6-Fb 3270, A7-BGE 002106,A8-BGE 029055, A9-BGE 032012, A10-BGE 041470

Table 2

Adaptive ability of quantitative traits in broad bean accessions

Accessions	b_i	G×Egi	SAA	GAA	Sgi [%]	Igi	SVG
Plant height							
Fb 1896	20.44	25.33	17.43	16.19	22.52	1.45	32.75
Fb 1903	5.42	64.04	23.28	4.29	6.01	2.75	61.03
Fb 1929	7.29	25.34	11.47	11.28	16.38	2.21	41.57
Fb 2481	0.34	29.39	10.77	2.03	2.87	2.73	65.83
Fb 2486	19.85	1.16	1.71	0.93	1.42	0.68	62.97
Fb 3270	6.64	240.31	116.96	116.86	149.12	2.05	−204.14
BGE 002106	31.29	21.92	14.04	0.58	0.91	1.56	62.59
BGE 029055	0.56	1.27	0.75	0.71	1.05	1.70	66.41
BGE 032012	0.18	6.67	4.68	4.42	6.14	1.42	61.36
BGE 041470	27.31	14.80	6.36	0.29	0.41	2.33	68.23
1st pod height							
Fb 1896	0.76	4.68	1.72	0.65	2.30	2.72	27.27
Fb 1903	1.58	7.52	3.54	3.48	11.59	2.13	24.5
Fb 1929	0.84	30.26	12.51	0.68	2.03	2.42	32.46
Fb 2481	1.74	29.07	21.41	2.32	6.67	1.36	31.18
Fb 2486	0.08	4.96	1.80	0.80	3.26	2.75	23.21
Fb 3270	0.19	80.89	42.13	42.08	127.97	1.92	−33.80
BGE 002106	2.44	20.75	21.18	10.47	40.06	0.98	9.54
BGE 029055	0.38	38.87	34.91	25.93	118.74	1.11	−19.25
BGE 032012	2.02	10.87	5.55	0.10	0.01	1.96	30.59
BGE 041470	1.24	3.63	1.36	0.73	2.91	2.68	23.81
Pods number							
Fb 1896	0.01	0.39	0.24	0.23	2.08	1.63	9.67
Fb 1903	2.17	5.87	4.17	0.39	4.05	1.41	6.96
Fb 1929	1.18	1.32	0.59	0.02	0.19	2.25	7.93
Fb 2481	0.64	2.03	1.17	0.02	0.18	1.73	9.39
Fb 2486	2.97	0.05	0.04	0.03	0.28	1.42	11.89
Fb 3270	0.93	14.97	6.30	6.06	61.35	2.38	−30.99
BGE 002106	0.34	0.31	0.35	0.19	1.87	0.89	8.71
BGE 029055	0.20	0.97	0.62	0.60	4.09	1.58	10.59
BGE 032012	1.32	0.37	0.19	0.01	0.01	1.91	9.44
BGE 041470	0.26	0.90	0.51	0.01	0.03	1.77	8.30
Seeds number							
Fb 1896	0.14	13.51	6.71	6.70	25.5	2.01	21.96
Fb 1903	3.17	34.39	18.05	0.02	0.09	1.90	22.91
Fb 1929	0.60	5.69	2.84	0.02	0.11	2.01	22.09
Fb 2481	0.07	4.88	2.07	0.21	0.94	2.36	21.89
Fb 2486	0.18	22.60	9.61	9.20	28.66	2.35	26.19
Fb 3270	3.20	269.46	143.29	142.81	468.48	1.88	−61.23
BGE 002106	1.26	58.53	51.49	39.16	121.49	1.14	7.08
BGE 029055	0.17	64.76	44.53	41.08	101.26	1.45	14.19
BGE 032012	2.85	50.90	17.14	8.22	26.58	2.97	25.66
BGE 041470	2.45	38.73	14.94	1.64	5.40	2.59	29.39

Table 2 continue

Seed weight							
Fb 1896	0.85	6.77	7.05	4.38	15.63	0.96	24.89
Fb 1903	2.11	51.04	17.31	6.77	28.45	2.95	18.99
Fb 1929	0.42	11.15	5.61	0.01	0.04	1.99	20.57
Fb 2481	1.07	4.74	2.34	0.03	0.16	2.03	20.35
Fb 2486	0.51	71.91	52.31	5.55	18.33	1.37	26.33
Fb 3270	0.30	107.68	63.71	62.22	604.6	1.69	–33.95
BGE 002106	1.01	1.33	0.61	0.27	1.06	2.19	24.99
BGE 029055	1.46	6.49	6.72	2.44	8.29	0.97	27.71
BGE 032012	0.10	27.83	9.52	5.81	21.55	2.92	22.83
BGE 041470	2.38	94.79	39.72	1.65	5.12	2.39	30.97
100 seeds mass							
Fb 1896	0.51	4.04	7.05	4.38	15.63	0.57	24.89
Fb 1903	6.11	48.31	17.31	6.77	28.45	2.79	18.99
Fb 1929	0.77	8.42	5.61	0.01	0.04	1.50	20.57
Fb 2481	6.22	2.01	2.34	0.03	0.16	0.86	20.35
Fb 2486	0.50	69.18	52.31	5.55	18.33	1.32	26.33
Fb 3270	3.74	104.95	63.71	62.22	604.6	1.65	–33.95
BGE 002106	3.72	–1.40	0.61	0.27	1.06	–2.30	24.99
BGE 029055	0.53	3.76	6.72	2.44	8.29	0.56	27.71
BGE 032012	0.11	25.1	9.52	5.81	21.55	2.64	22.83
BGE 041470	0.14	92.06	39.72	1.65	5.12	2.32	30.97

b_i – regression coefficient according to Finlay and Wilkinson (1963); (G×E)gi – criterion for estimation of the genotype ability to interact with environment; SAA – specific adaptive ability; GAA – general adaptive ability; Sgi – relative stability of the genotypes; Igi – reaction to the environment; SVG – selective value of genotype

Table 3

Adaptability determined by nonparametric analysis (Nascimento *et al.* 2009) in broad bean accessions

Accessions	Plant height	1st pod height	Pods number	Seeds number	100 seeds mass	Seed weight
Fb 1896	V	V	V	V	VI	V
Fb 1903	V	V	V	IV	VII	VII
Fb 1929	V	V	IV	IV	V	V
Fb 2481	V	VI	V	IV	V	V
Fb 2486	IV	IV	V	V	V	V
Fb 3270	II	II	III	III	IV	IV
BGE 002106	IV	V	V	V	V	V
BGE 029055	V	IV	VII	VI	V	VII
BGE 032012	V	V	V	V	V	V
BGE 041470	V	IV	IV	V	VII	VII

I: high general adaptability; II: adaptability under favorable conditions; III: adaptability under unfavorable conditions; IV: low adaptability; V: average general adaptability; VI: adaptability under average favorable conditions; VII: adaptability under average unfavorable conditions

BGE 032012 and Fb 3270). Fb 2486 (by the value of the trait) occupied the third position, with a seed weight of about 30 g. It had good stability (bi = 0.51) and, together with Fb 3270 and BGE 041470, interacted better with environmental conditions as compared to the rest of accessions.

On the basis of the parameters „bi“ and „G×Egi“, accession BGE 029055 reacted positively to changes in the environmental conditions and combined high productivity (29.45) and favorable general (2.44) and specific adaptive ability (6.72). Among the accession studied, Fb 1929, Fb 2481 and BGE 002106, were distinguished by lower values of the parameter Sgi. The values obtained regarding Igi for Fb 1896 and BGE 029055 assume that they will have a linear response to environmental changes. Accessions Fb 1896, Fb 2486, BGE 029055 and BGE 041470 were the compromise variant combining stability and high selection value of the genotype (24.89, 26.33, 27.71, 30.97).

Table 4

Index of stability (IS) (Nettevich 2001) of broad bean accessions

Accessions	Plant height	1st pod height	Pods number	Seeds number	Seed weight	100 seeds mass
Fb 1896	3.638	1.446	0.455	1.566	0.927	0.551
Fb 1903	2.636	1.246	0.280	0.551	0.747	0.529
Fb 1929	3.032	1.359	0.228	0.724	0.447	0.347
Fb 2481	2.672	1.250	0.325	0.687	0.703	0.344
Fb 2486	2.452	0.991	0.609	2.117	0.393	0.551
Fb 3270	1.931	0.824	0.474	0.786	0.404	0.238
BGE 002106	2.361	6.200	0.383	0.735	0.321	0.514
BGE 029055	2.608	2.702	0.681	1.154	0.576	0.633
BGE 032012	2.468	1.793	0.368	0.854	0.383	0.477
BGE 041470	2.512	1.179	0.273	0.983	0.547	0.635

Table 4

Correlation dependencies in broad bean accessions

Parameters	b_i	(G×E)gi	SAA	GAA	Sgi	Igi	SVG
(G×E)gi	0.143						
SAA	–0.064	0.945^{++}					
GAA	–0.332	0.640^{+}	0.698^{+}				
Sgi	–0.330	0.617	0.676^{+}	0.997^{++}			
Igi	0.203	0.154	–0.103	–0.104	–0.114		
SVG	0.381	–0.524	–0.579	$–0.974^{++}$	$–0.981^{++}$	0.048	
Seed weight per plant	–0.296	0.282	0.344	0.156	0.159	0.072	–0.119

$^{+}$ / $^{++}$ significant at 0.05/0.01

(G×E)gi – criterion for estimation of the genotype ability to interact with environment; SAA – specific adaptive ability; GAA – general adaptive ability; Sgi – relative stability of the genotypes; Igi – reaction to the environment; SVG – selective value of genotype

The results of the adaptability assessment obtained by applying the nonparametric method (Table 3) showed that following accessions had the worst adaptation: Fb 1929 – in terms of pods and seeds number, Fb 3270 – in 100 seeds mass and seed weight, Fb 2486 and BGE 002106 – in plant height. BGE 032012 can be defined as a genotype with a relatively good average general adaptability in terms of almost all traits. Similar behavior had Fb 1896, which (in contrast to BGE 032012) regarding 100 seeds mass was more responsive under environmental improving. On the other hand, adaptability under unfavorable conditions showed Fb 3270 (by pods and seeds number per plant), BGE 029055 (by pods number and seed weight), Fb 1903 and BGE 041470 (by 100 seeds mass and seed weight).

Based on the index of stability (IS) (Table 4), the accession assessment allows the selection of a suitable genotype with the highest value of the studied trait and adequate response to the environmental conditions. In plant height, Fb 1896 and Fb 1929 showed better stability, but in essence, they did not exceed considerably the remaining accessions, except for Fb 3270 (1.931).

In addition, Fb 1896 showed stability also in terms of seeds number, seed weight, and 100 seeds mass. Regarding 1st pod height, BGE 002106 stands out, followed by BGE 029055, which together with Fb 2486 showed stability in the number of pods and seeds per plant. Despite the fact that there is a certain discrepancy with the previous parameters, IS as a whole gives further information about the behavior of the broad bean accessions during the experimental period.

The information obtained from the dependencies between stability (adaptability) and productivity (seed weight) is important in the breeding process. The results of the correlation analysis (Table 5) showed that the seed weight was in a weak positive and insignificant correlation with G×Egi ($r = 0.282$), GAA ($r = 0.156$) and Igi ($r = 0.072$), and in mean correlation with SAA ($r = 0.344$).

A positive mean and high dependence was established regarding G×Egi with SAA ($r = 0.945$) and GAA ($r = 0.640$), and for GAA with Sgi ($r = 0.997$). A negative and statistically significant correlation coefficient was observed between SVG with GAA ($r = –0.974$) and Sgi ($r = –0.981$).

DISCUSSION

In the present study, the year factor had a strong influence on 1st pod height, pods number and seed weight per plant. Similar results were obtained by Mulusew *et al.* (2008), which reported that grain yield (productivity) was significantly affected by changes in the environment, followed by G × E interaction and genotype effects. The authors considered that G × E interaction minimizes the utility of genotypes by confounding their yield performances. Thus, it is very important to study in depth the yield levels, adaptation patterns and stability of faba bean genotypes in multiple environments.

Also, in our study, effects due to interactions between the genotype (accession) and the year for all the traits were significant ($P < 0.01$). This effect was particularly strong on 100 seeds mass as well as on plant height. The obtained results were in support of the studies of Temesgen *et al.* (2015). In a combined analysis of variance for grain yield of 16 faba bean genotypes, the authors established that the environment effect accounted for 89.27%, whereas genotype and G × E interaction effects presented 2.12% and 3.31% of the total variation, respectively. The results of the conducted study were in line with those obtained by Sharifi (2018) who reported that the largest influence factor for signs as grain productivity, pods per plant and pod length was the environmental factor, followed by the genotype factor.

Similar results were reported by Abrosimova and Fadeeva (2015). In collection accessions of peas, the trait of seed mass was strongly influenced by the genotype-environment interaction factor, and the genotype occupied the second position. It was established that regarding the parameter of general adaptation with priorities were the leaf and heterophilic pea forms (0.19 – 0.92). The authors found a high positive correlation of the general adaptive ability with the breeding value of the genotypes by this trait and negative (but nonsignificant) correlation with the relative stability of the genotype. This suggests that there are relatively stable high- and low-yielded cultivars in the studied group.

In determining the breeding value of broad bean genotypes, our research complements this one conducted by Bezuglova and Kazydub (2017), who have identified valuable genotypes for their breeding program. These genotypes were characterized with increased values of seeds number, 1,000 seeds mass and weight of seeds per plant. The authors indicated that the development of new broad bean varieties should be based primarily on the adaptability and stability of signs as number and weight of seeds per plant.

In earlier studies, Anohina and Mazuka (2006) found a specific variety response in estimation of bean genotypes in terms of adaptability and stability of main quantitative traits, which was confirmed in the present investigation.The authors used the general adaptive ability as a selection criterion and reported that cultivars with the highest average yields were also characterized by a high value of this parameter. According to their results for the number of seeds, the total adaptive capacity ranged from –38.94 to 27.26, and for the weight of seeds per plant – from 8.90 to –8.90. In regard to the parameter of breeding value of the genotype, the accessions showed the presence of polymorphism (–17.38 to 24.20).

In some other crops, such as wheat (Strizhkova 2003), the 1,000 seeds mass was found to be most consistent with the criterion of adaptability. This trait was an integrative and combining the final result of the interaction genotype-environment in the process of ontogenetic formation of productivity.

Singh *et al.* (2012) stated that plant breeding had the resource and could create new cultivars that adequately responded to changing climatic conditions. They considered that this ambitious task could be achieved by applying various methods, including from an initial selection of plants with desirable characteristics to more complicated classical or molecular techniques and approaches. With the development of modern biotechnology and its application in plant breeding, the creation of new adapted cultivars is a faster and more precise process.

According to some researchers as Potanin *et al.* (2014) in order to increase the efficiency of the breeding process in the development of new varieties with high productivity and environmental adaptability, it is necessary to apply non-traditional approaches for processing and analysis of the obtained information.

Nikiforova (2015) received data suggesting no significant relationship between plant productivity and relative stability of the genotype as a result of correlation analysis. This was due to the fact that in a group of accession (cultivars or populations) there may be both relatively stable and unstable, as well as high-productive and low-productive genotypes.

CONCLUSIONS

The analysis of variance regarding the productivity elements in the studied broad bean accessions showed that the environment influenced to the highest degree the traits of 1st pod height, pods number and seed weight per plant. The formation of plant height and seeds number was more strongly influenced by the genotype, and the mass of 100 seeds was determined by the genotype × environment interaction, which occupied a major share in the total variation of this trait.

According to the complex assessment of the adaptability and stability parameters, the broad bean accessions can be distributed as follows: BGE 032012 combines high stability (bi = 0.18), productivity and plant height (72 cm); Fb 1929 has a high value of the 1st pod height (34 cm) and is characterized by high plasticity and stability (bi = 0.84); BGE 029055 and Fb 1896 are stable (bi < 1) and form a large number of pods per plant (11 – 15); Fb 1896 and Fb 2486 are distinguished with good adaptability and stability, increased seed weight (28.01 and 30.28 g, respectively) and 100 seeds mass (105.48 g and 91.31 g, respectively).

Accessions BGE 032012 and Fb 2481 represent a selection value in terms of plant height (61.36 and 65.83 cm, respectively); Fb 1929 – in 1st pod height (32.46 cm); and BGE 029055, Fb 1896 and Fb 2486 – in pods number (10.59, 9.67 and 11.89). Fb 1896, Fb 2486 and BGE 041470 can be used to develop a new genetic diversity in breeding aimed at increasing the mass of 100 seeds and seed productivity.

Acknowledgement. The authors sincerely *acknowledge* the Agricultural Academy in Bulgaria for funding the study.

REFERENCES

ABROSIMOVA, T.N. – FADEEVA, A.N. 2015. Adaptive capacity and breeding value of vegetable varieties of peas collection. In *Scientific and Practical Per-reviewed Journal „Vegetable crops of Russia“*, vol. *1,* no. 26, pp. 27 – 30. УДК 635.656: 631.526.32

ANOHINA, V.S. – MAZUKA, E.S. 2006. Characteristics of inter-cultivar hybrids in relation to the adaptive potential of the original varieties. In *The Bulletin of BGU*, vol. *2*, no. 2, pp. 44 – 48.

BAROV, V. 1982. *Analysis and schemas of the field trial*. Sofia: Academic Press, pp. 668.

BEZUGLOVA, E.V. – KAZYDUB, N.G. 2017. Breeding value of source material of vegetable beans (*Vicia faba* L.) in the conditions of southern forest-steppe of Western Siberia. In *Bulletin of Omsk State Agrarian University*, vol. *1*, no. 25, pp. 11 – 16. УДК 635.651:631.53.01:631. 527(571.1)

BORNHOFEN, E. – BENIN, G. – STORCK, L. – WOYANN, L.G. – DUARTE, T. – STOCO, M.G. – MARCHIORO, S. 2017. Statistical methods to study adaptability and stability of wheat genotypes. In *Bragantia*, vol. *76*, no. 1, pp. 1 – 10. http://dx.DOI.org/10. 1590/1678-4499.557

CARGNELUTTI, F.A. – STORCK, L. – RIBOLDI, J. – GUADAGNIN, J.P. 2009. Association between adaptability and stability methods in corn. In *Ciência Rural,* vol. *39*, no. 2, pp. 340 – 347. http://dx.DOI.org/10.1590/S0103-84782008005000080

CRUZ, C.D. 2006. *Programa Genes: Biometria.* Version 7.0. Viçosa, Brazil: University of Federal Viçosa, pp. 382.

EBERHART, S. – RUSSELL, W.A. 1966. Stability parameters for comparing varieties. In *Crop Science,* vol. *6*, pp. 36 – 40. http://dx.DOI.org/10.2135/cropsci1966.0011183X0006000100 11x

FERNANDEZ, M. – LOPEZ-JURADO, M. – ARANDA, P. – URBANO, G. 1996. Nutritional assessment of raw and processed faba bean (*Vicia faba* L.) cultivar major in growing rats. In *Journal of Agriculture and Food Chemistry,* vol. *44*, pp. 2766 – 2772. https://pubs.acs.org/DOI/abs/ 10.1021/jf9505483

FINLAY, K.W. – WILKINSON, G.N. 1963. The analysis of adaptation in plant breeding programme. In *Australian Journal of Agricultural Research,* vol. *14*, pp. 742 – 754. http://dx.doi.org/10.1071/AR9630742

KILCHEVSKIY, A.V. – HOTYLEVA, L.V. 1985a. Methods of assessments of the adaptive abilities and stability of genotypes differentiating the abilities of environment. Messages 1. Justification of the method. In *Genetics*, vol. *21*, pp. 1481 – 1490.

KILCHEVSKY, A.V. – HOTYLEVA, L.V. 1985b. Methods of assessments of the adaptive abilities and stability of genotypes differentiating the abilities of environment. 2. Numerical example and discussion. In *Genetics,* vol. *21*, pp. 1491 – 1498.

LIN, C.S. – BINNS, M.R. 1988. A superiority measure of cultivar performance for cultivar × location data. In *Canadian Journal of Plant Science*, vol. *68*, pp. 193 – 198. http://dx.doi.org/10.4141/cjps88-018

MILIOLI, A.S. – ZDZIARSKI, A.D. – WOYANN, L.G. – SANTOS, R. – ROSA, A.C. – MADUREIRA, A. – BENIN, G. 2018. Yield stability and relationships among stability parameters in soybean genotypes across years. In *Chilean Journal of Agricultural Research,* vol. *78*, pp. 299 – 309. http://dx.doi.org/10.4067/S0718-58392018000200299

MULUSEW, F. – TADELE, T. – TESFAYE, L. 2008. Genotype–environment interaction and stability parameters for grain yield of faba bean (*Vicia faba* L.) genotypes grown in southeastern Ethiopia. In *International Journal of Sustainable Crop Production,* vol. *3*, pp. 80 – 87.

NASCIMENTO, M. – CRUZ, C.D. – CAMPANA, A.C.M. – TOMAZ, R.S. – SALGADO, C.C. – FERREIRA, R. 2009. Alteration of the centroid method to evaluate genotypic adaptability. In *Pesquisa Agropecuária Brasileira,* vol. *44*, pp. 263 – 269. DOI.Org/ 10.1590/ S0100-204x2009000300007

NETTEVICH, E.D. 2001. Potential productivity recommended for spring wheat and barley cultivation in the central district of the Russian federation and its realization in conditions of production. In *Reports of Russian Academy of Agrarian Sciences,* vol. *3*, pp. 50 – 55.

NIKIFOROVA, I.Y.U. 2015. Evaluation of adaptive potential of samples of common millet from various groups of ripeness on the statistical parameters calculated by sign “Productivity of grain”. In *Legumes and Cereals,* vol. *1*, no. 13, pp. 79 – 83. УДК 633.171:631.52

OUJI, A. – SUSO, M.J. – ROUAISSI, M. – ABDELLAOUI, R. – GAZZAH, M. 2011. Genetic diversity of nine faba bean (*Vicia faba* L.) populations revealed by isozyme markers. In *Genes & Genomics,* vol. *33*, no. 1, pp. 31 – 38. DOI 10.1007/s13258-010-0070-1

PANDEY, P. – IRULAPPAN, V. – BAGAVATHIANNAN, M. V. – KUMAR, M.S. 2017. Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. In *Fronties in Plant Science*, vol. *8,* pp. 1 – 15. DOI:10.3389/fpls.2017.00537

PEREIRA, H.S. – MELO, L.C. – PELOSO, M.J. – FARIA, L.C. – COSTA, J.G.C. – RAVA, C.A. – WENDLAND, M.J. 2009. Comparação de métodos de análise de adaptabilidade e estabilidade fenotípica em feijoeiro-comum. In *Pesquisa Agropecuária Brasileira,* vol. *44*, no. 4, pp. 374 – 383. DOI.org/10.1590/S0100-204X2009000400007

POTANIN, W.G. – ALEINIKOV, A.F. – STEPOCHKIN, P.I. 2014. A new approach to estimation of the ecological plasticity of plant varieties. In *Vavilov Journal of Genetics and Breeding*, vol. *18,* no. 3, pp. 548 – 552. УДК 633.111.1

RESENDE, M.D.V. 2006. O software selegen reml/blup. Embrapa gado de corte (embrapa gado de corte documentos), Campo Grande, Mato Grosso do Sul, Brasil. ISSN 1517-536X

SHARIFI, P. 2018. Biplot analysis of seed yield of faba bean genotypes at different planting dates. In *Agriculture & Forestry,* vol. *64*, no. 4, pp. 243 – 250. DOI: 10.17707/AgricultForest.64.4.24

SINGH, A.K. – BHATT, B.P. – UPADHYAYA, A. – KUMAR, S. – SUNDARAM, P.K. – SINGH, B.K. – CHANDR, N. – BHARATI, R.C. 2012. Improvement of faba bean (*Vicia faba* L.) yield and quality through biotechnological approach: A Review. In *African Journal of Biotechnology,* vol. *11*, pp. 15264 – 15271. DOI: 10.5897/AJB12.1926

STRIZHKOVA, F.M. 2003. Plasticity of spring wheat varieties. In *Agricultural Science*, vol. *4,* pp. 30 – 31.

TEMESGEN, T. – KENEN, I.G. – SEFERA, T. – JARSO, M. 2015. Yield stability and relationships among stability parameters in faba bean (Vicia faba L.) genotypes. In *The Crop Journal,* vol. *3*, pp. 258 – 268. DOI.ORG/10.1016/J.CJ.2015.03.004

TORRES, F.E. – TEODORO, P.E. – FILHO, A.C. – SANTOS, A. – ROCHA, M.M. – SAGRILO, E. 2016. Adaptability and phenotypic stability of semi-prostate cowpea genotypes in Mato Grosso Do Sul. In *Bioscience Journal,* vol. *32,* no. 6, pp. 1435 – 1441. DOI: https://doi.org/10.14393/BJ-v32n6a2016-32813

YAN, W. 2001. GGE Biplot a windows application for graphical analysis of multienvironmental trial data and other types of two-way data. In *Agronomy Journal,* vol. *93,* no. 3, pp. 1111 − 1118. DOI:10.2134/agronj2001.9351111x

ZOBEL, R.W. – WRIGHT, M.J. – GAUCH, H.G. 1988. Statistical analysis of a yield trial. In *Agronomy Journal,* vol. *80*, pp. 388 − 393. DOI:10.2134/agronj1988.00021962008000030002x

Received: May 30, 2019
Accepted: November 11, 2019

Agriculture (Poľnohospodárstvo), 65, 2019 (4): 147− 154

sciendo

DOI: 10.2478/agri-2019-0015

Original paper

RHIZOSPHERE BACTERIOBIOME OF THE HUSK TOMATO GROWN IN THE OPEN FIELD IN WEST SIBERIA

NATALIA B. NAUMOVA[1]*, OLEG A. SAVENKOV[1], TATIANA Y. ALIKINA[2],
MARSEL R. KABILOV[2]

[1]Institute of Soil Science and Agrochemistry SB RAS, Novosibirsk, Russia
[2]Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia

NAUMOVA, N.B. – SAVENKOV, O.A. – ALIKINA, T.Y. – KABILOV, M.R.: Rhizosphere bacteriobiome of the husk tomato grown in the open field in West Siberia. Agriculture (Poľnohospodárstvo), vol. *65*, 2019, no. 4, pp. 147 – 154.

The composition and structure of rhizosphere bacteriobiome of the husk tomato (*Physalis philadelphica* Lam.) plants grown on Phaeozem in the open field in West Siberia, Russia (55°15’ NL, 83°31’ EL) were studied using Illumina MiSeq sequencing of the V3-V4 hypervariable region of 16S rRNA genes. In total 5898 OTUs (Operational Taxonomic Units) were found in the study, representing 20 phyla and 53 identified and 15 non-identified (below the phylum level) classes. The most OTU-rich phyla were *Proteobacteria*, *Acidobacteria* and *Actinobacteria*, their relative abundance in the total number of sequence reads being 26, 22 and 19%, respectively. *Bacteroidetes*, *Gemmatimonadetes* and *Verrucomicrobia* phyla each accounted for 2 – 4%. The rest 14 of the identified phyla were quite negligible, contributing less than 0.5% each. At the OTUs level, the structure was very even and equitable, as only 7 OTUs had relative abundance ranging from 0.5 to 1.1%. The main dominant OTU represented *Bradyrhizobiaceae* family, implying the importance of nitrogen-fixing bacteria for plant growth and development without any mineral fertilisation. The dominance biodiversity index was very low (0.001), while Shannon index was rather high (7.5). We believe the presented husk tomato rhizosphere bacteriobiome, as the first study using new generation sequencing platform for this species, will help get a better picture of *Solanaceae* microbiomes in different environments, thus contributing to a more comprehensive understanding of shaping microbial communities by plant roots.

Key words: *Physalis philadelphica,* rhizosphere soil, 16S rDNA sequencing, bacterial diversity

Sustainable crop production is of utmost importance for providing food, bioenergy, timber and other plant materials for the ever-growing human population. Except for carbon, plants derive macro- and micronutrients from soil, affecting the latter by their rhizodeposition and aboveground litter. Rhizosphere is the primary interface between plant and soil. The rhizosphere microbiota influences the plant fitness, which, in its turn, determines the quantity and quality of phytomass production. Thus understanding plant-microbe interactions is indispensable for maintaining, increasing or restoring plant health (Berendsen *et al.* 2012; Wallenstein 2017) and agricultural ecosystem productivity (Ahkami *et al.* 2017). Ideally, rhizosphere microbiota should be considered while breeding new cultivars (Saleem *et al.* 2018), developing microbial preparations for plant protection from pathogens, for growth promotion.

Natalia B. Naumova (*Corresponding author), Oleg A. Savenkov, Institute of Soil Science and Agrochemistry SB RAS, Lavrentieva 8/2, Novosibirsk 630090, Russia. E-mail: naumova@issa-siberia.ru
Tatiana Y. Alikina, Marsel R. Kabilov, Institute of Chemical Biology and Fundamental Medicine SB RAS, Lavrentieva 8, Novosibirsk 630090, Russia. E-mail: alikina@niboch.nsc.ru; kabilov@niboch.nsc.ru

In the family *Solanaceae Physalis* L. is one of the most important genera with *ca.* 100 species, originating and distributed in tropical and temperate regions of America. Husk tomato (*Physalis philadelphica* Lam.), being native for Mexico and Guatemala (Zamora-Tavares *et al.* 2015; Martinez *et al.* 2017) and one of the most abundant *Physalis* species there (Zamora-Tavares *et al.* 2015), recently enjoys growing popularity in other regions of the world, attracting attention for breeding and selection as a crop of nutritional quality (Valdivia-Mares *et al.* 2016). Its fruits, rich in vitamins, minerals and antioxidants, recently were proved to have several beneficial properties on human health as a source of bioactive compounds (Khan *et al.* 2018) with anticarcinogenic (Zheng *et al.* 2019) and antimicrobial (Khan *et al.* 2016) properties. Even the waste of the crop has been attracting attention as an important source of pectin with highly distinctive physicochemical characteristics (Morales-Contreras *et al.* 2017).

The forecast of further climate warming in the Asian part of northern Eurasia is promoting the cultivation of new unconventional crops in the region, including husk tomato, albeit so far on a small scale. This actualizes studies of husk tomato growth and development in the open field of this region (Naumova *et al.* 2019). As there is no information about husk tomato rhizosphere microbiome, its composition and structure, the aim of our study was to profile 16S rRNA gene diversity in the rhizosphere of the husk tomato grown in the open field in West Siberia on Phaeozem, a widely spread soil type in agroecosystems of the region.

MATERIAL AND METHODS

Experimental setup

A micro plot field experiment was carried out during 2016 growing season in the open field in the forest-steppe zone in West Siberia (55°15' NL, 83°31' EL) as part of the larger experiment carried on simultaneously at several experimental stations to study husk tomato yield and fruit properties (Naumova *et al.* 2019). The climate of the region is sharply continental with average (June, July and August) maximal temperatures in summer ranging 22 – 26ºC and average precipitation ranging 40 – 65 mm per month, with 119 days of the frost-free period. This type of climate is in stark contrast with the climate in Mexico, i.e. the country of the species origin, where *Physalis sp.* grows in the areas with tropical wet and dry climate with yearly temperatures varying from 20 to 24°C and up to 2,000 mm precipitation. The temperatures recorded over the growing period (103 days) by the data loggers placed 2 m above soil surface and in the soil at 1, 10 and 20 cm depths allowed to estimate active (>0ºC) temperature sums as 1,400ºC·day for the air and 1,544, 1,387 and 1,458ºC·day for the respective soil layers. The experiment was conducted on loamy agricultural soil, common for the region, classified as Luvic Greyzemic Phaeozem (Siltic,

Table 1

Some yield (fresh mass) and phytomass properties of the husk tomato grown in the open field in West Siberia

Property	Median	Min	Max
Number of fruits per 1 plant [pcs.]	74	43	110
Fruit yield per 1 plant [g] (F)	1,942	1,117	4,240
Maximal fruit mass [g]	44.6	31.5	65.7
Mean fruit mass [g]	26.5	18.0	38.5
Aboveground phytomass [g] (AG)	790	315	1800
Belowground phytomass [g] (R)	80	35	130
Ratio AG/R	11.5	7.9	21.2
Ratio AG/F	0.5	0.3	0.6

Aric) according to the World Reference Base for Soil Resources (IUSS Working Group 2014). The soil had rather high soil organic carbon content of 6.7%, a slightly acidic pH of 5.77, being favourable for plant growth and development. Interestingly, in Mexico Phaozems are the main agricultural soils for vegetable production (Marquez-Santacruz *et al.* 2010).

In the study, we used *Physalis philadelphica* Lam. (*Solanaceae*) of the 'Konditer' (Confectioner) cultivar which has been listed in the Russian State Crop Register since 1990 and recommended for most of the regions of the country. The cultivar is characterized by rounded/flat-rounded shaped fruits that are light green during the unripe stage and light yellow when ripe (Naumova *et al.* 2019). The seeds for the study were provided by the seed bank of the Central Siberian Botanical Garden SB RAS (Novosibirsk, Russia). No treatment was applied to the seeds prior to sowing into the plastic cassettes; the emerged plants were grown in greenhouse conditions for 50 days. Then on June 10, 2016 the young plants were planted out into the open field micro plot 2 m × 4 m at the density of 1 plant per 0.25 m^2 with 0.5 m interrow spacing. No fertilisers were applied. The weeds were manually removed throughout the season. The plant grew and developed very well (Table 1), producing similar or higher yields as compared to its native environment (Smith *et al.* 1999; Ramirez-Godina *et al.* 2013).

Methods

Soil sampling

At the end of the growing season, rhizosphere soil was collected from four individual plants, grown on one of the experimental sites described earlier (Naumova *et al.* 2019), as the soil strongly adhering to 1 – 3 mm thick roots after gentle shaking (Zhao *et al.* 2010). The sampled soil was brought into the laboratory, thoroughly mixed, and the resulting soil samples were stored at –20°C prior to DNA extraction.

Extraction of total nucleic acid from the soil

Total DNA was extracted from the four soil samples using the DNA isolation Kit (DNeasy PowerSoil Kit, Qiagen, Germany) as per manufacturer's instructions. The bead-beating was performed using TissueLyser II (Qiagen, Germany) 10 min at 30 Hz. The quality of the extracted DNA was assessed by the spectrophotomer NanoDrop ND-1000 (Thermo Fisher, USA), by agarose gel electrophoresis and pilot PCR. No further purification of the DNA was needed.

16S rRNA gene metagenomic sequencing

The 16S DNA region was amplified with the primer pair V3-V4 combined with Illumina adapter sequences (Fadrosh *et al.* 2014). PCR amplification was performed as described early (Igolkina *et al.* 2018). A total of 200 ng PCR product from each sample was pooled together and purified through MinElute Gel Extraction Kit (Qiagen, Germany). The obtained libraries were sequenced with 2 × 300 bp paired-ends reagents on MiSeq (Illumina, USA) in SB RAS Genomics Core Facility (ICBFM SB RAS, Novosibirsk, Russia). The read data reported in this study were submitted to the GenBank under the study accession PRJNA556565 and the sequence read archive (SRA) accession number SRP216321.

Bioinformatics and statistical analysis

Raw sequences were analyzed with UPARSE pipeline (Edgar 2013) using Usearch v10.0. The UPARSE pipeline included the merging of paired reads; read quality filtering; length trimming; merging of identical reads (dereplication); discarding singleton reads; removing chimeras and operational taxonomic unit (OTU) clustering using the UNOISE-OTU algorithm. The OTU sequences were assigned taxonomically using the SINTAX (Edgar 2016) and 16S RDP training set v.16 (Wang *et al.* 2007). Taxonomic structure of thus obtained sequence assemblages, i.e. a collection of different species at one site at one time (Fauth *et al.* 1996), was estimated by the ratio of the number of taxon-specific sequences reads to the total number of sequence reads, i.e. by the relative abundance of taxa, expressed as a percentage. The data were represented as a median. Biodiversity indices were calculated with the help of the PAST 3.19 software (Hammer *et al.* 2001).

RESULTS

The taxonomic richness of the husk tomato rhizosphere bacteriobiome

After quality filtering and chimera removal 5898 OTUs were found in the husk tomato rhizosphere soil samples. *Archaea* were represented by 17 OTUs, with 9 and 8 OTUs representing *Euryarchaeota* and *Thaumarchaeota* phyla, respectively. The 16S amplicon sequence reads datasets were analyzed by individual rarefaction (Figure 1): the number of OTUs detected, reaching a plateau with increasing number of sequences, showed that the sampling effort was close to saturation for all samples, thus being enough to compare diversity (Hughes & Hellmann 2005).

The number of OTUs per sample averaged 4,335, ranging from 4,100 – 4,500. In total, 20 bacterial phyla and 53 identified and 15 non-identified (below the phylum level) classes were found. Quite a lot of sequences (16.6%) could not be classified below the domain level, most likely signifying their absence in the database. The most OTU-rich phylum was *Proteobacteria* with 2,552 OTUs, followed by *Acidobacteria* with 1,724 OTUs and *Actinobacteria*, represented by 1,384 OTUs.

Taxonomic structure of the husk tomato rhizosphere bacteriobiome

As for the relative abundance of taxa, assessed by the ratio of a taxon-specific sequences to the total number of sequence reads, the bacteriobiome of the husk tomato rhizosphere was dominated by *Proteobacteria*, *Acidobacteria* and *Actinobacteria* phyla (Figure 2), with other phyla, such as *Bacteroidetes*, *Gemmatimonadetes* and *Verrucomicrobia* each accounting for 2 – 4%. The rest of the identified phyla were quite negligible, contributing less than 0.5% each. At the class level *Actinobacteria* were the most abundant (Figure 3), followed by *Alphaproteobacteria* and *Acidobacteria group 6*. *Betaproteobacteria* and *Deltaproteobacteria* contributed slightly over 2% each into the total bacteriobiome. Other *Acidobacteria* classes, i.e. groups 16, 3 and 4, accounted for 2 – 4%. At the order level, some unclassified *Acidobacteria group 6* were the most abundant, followed by *Rhizobiales, Actinomycetales, Gaiellales* and unclassified *Actinobactria* (Figure 4).

At the OTUs level, the structure was very even and equitable: only 7 OTUs had relative abundance ranging from 0.5 to 1.1%. The main dominant OTU represented *Bradyrhizobiaceae* family and accounted for just 1.1%. Other dominants represented *Spartobacteria* class (one OTU with 0.8%), *Acidobacteria group 16* (one OTU with 0.7%), *Sphagnomonas* genus of the *Alphaproteobacteria* (two OTUs with 0.5 – 0.7%) and *Actinobacteriales* and *Gaiellales* orders of *Actinobacteria* class (also two OTUs with 0.5 – 0.7%). Overall such low relative abundance for an OTU together with a very high number of OTUs detected resulted in very high diversity indices and extremely low dominance indices (Table 2).

DISCUSSION

In our study, we found very diverse bacteriobiome in the rhizosphere of the husk tomato grown on naturally developed Phaeozem in the open field. Among several thousands of detected OTUs only two OTUs had relative abundance of sequence reads

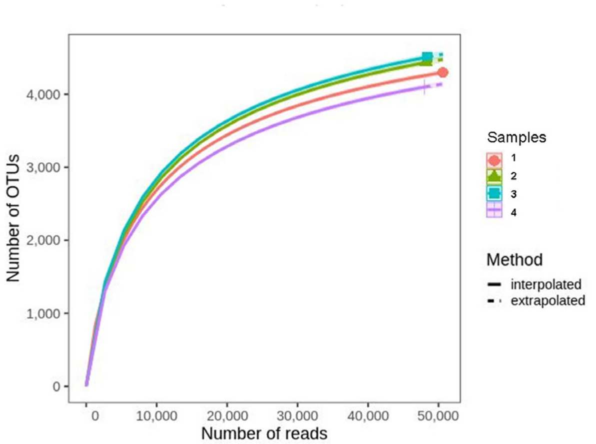

Figure 1. The rarefaction curves for the husk tomato rhizosphere soil samples. OTUs – operational taxonomic units

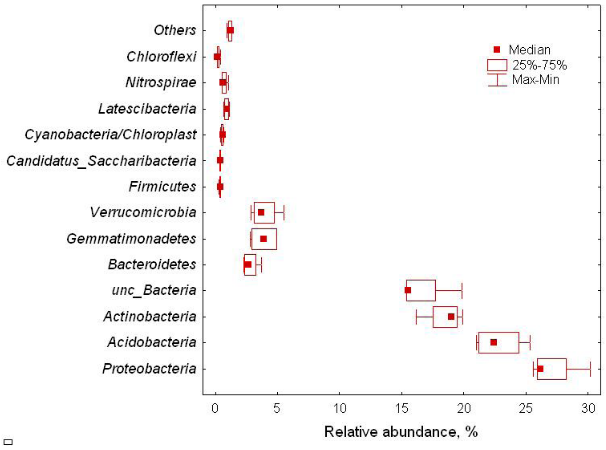

Figure 2. The relative abundance of phylum-specific sequences in the rhizosphere soil bacteriobiome of the husk tomato grown on Luvic Phaeozem in the open field in West Siberia

exceeding 1%, one being an unclassified representative of *Bradyrhizobiaceae* in all for samples, which may imply the importance of nitrogen fixation for plant growth and development; the other being an unclassified *Spartobacteria* detected at the level of ≥1.0% only in one sample.

Our data about the rhizosphere microbiome of the husk tomato were obtained by investigating rhizosphere soil samples collected from the plants grown in the open field in West Siberia, i.e. very far from the species native environment of this species in Mexico. Anyway, we compared our data with the respective data obtained in the crop's native environment. We were able to find just one paper with the information about *Physalis ixocarpa* Brot. rhizosphere microbiome diversity, published in 2010 (Marquez-Santacruz *et al.* 2010) and since then apparently remaining the main publication on the subject. Although the authors did not specify soil type in their article, most likely it was the same as in our study, as Phaeozems are the main agricultural soils cropped for vegetables in Guanajuato, Mexico, where husk tomato plants were sampled for that study. By cloning PCR V1-V9 amplicons and sequencing clone libraries the authors detected "only common soil bacteria", including *Stenotrophomonas*, *Burkholderia*, *Bacillus*, and *Pseudomonas,* in a 16S rDNA gene library of the rhizosphere. We also detected members of these genera, but in our study, they were extremely rare ones, represented by 7, 6, 29 and 37 OTUs, respectively, each of those OTUs contributing much less than 0.01% into the total number of sequence reads. Such a drastic difference in husk tomato rhizosphere bacterial diversity is due to the differences between the metagenomic DNA analyses, e.g. between sequencing clone libraries and direct sequencing, as well as differences in bioinformatic tools used. Also, partially the difference may be attributed to some differences in soil prop-

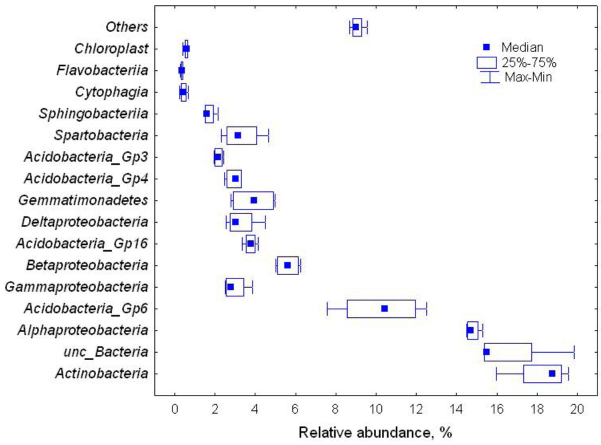

Figure 3. The relative abundance of class-specific sequences in the rhizosphere soil of the husk tomato grown on Luvic Phaeozem in the open field in West Siberia

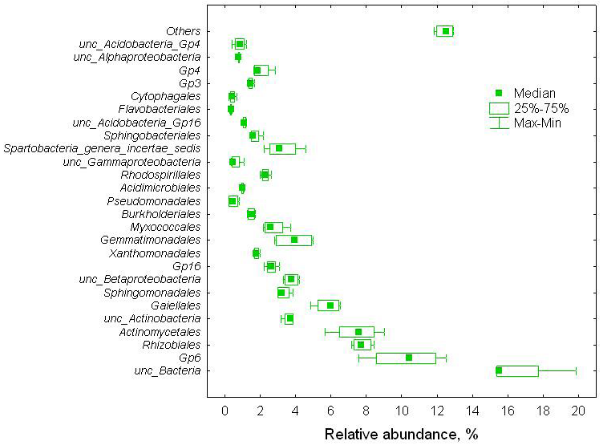

Figure 4. The relative abundance of order-specific sequences in the rhizosphere soil of the husk tomato grown on Luvic Phaeozem in the open field in West Siberia

Table 2

Alpha biodiversity indices of the rhizosphere bacteriobiome of the husk tomato grown on Phaeozem in the open field in West Siberia

Index	Median	Max	Min
Total number of identified OTUs (richness)	4,367	4,508	4,098
Dominance (D)	0.0013	0.0015	0.00112
Simpson (1-D)	0.9987	0.9988	0.9985
Shannon	7.50	7.60	7.40
Evenness	0.41	0.43	0.38
Brillouin	7.30	7.40	7.20
Menhinick	19.70	20.50	18.70
Margalef	404	418	380
Equitability	0.89	0.90	0.88
Fisher-alpha	0.00	0.00	0.00
Berger-Parker	0.011	0.012	0.010
Chao-1	5,000	5,235	4,674
Jost	1,137	1,267	990
Robbins	0.20	0.20	0.19

erties (Xue *et al.* 2018), for instance, to the much higher pH in the Mexican Phaeozem (Marquez-Santacruz *et al.* 2010).

Despite the wide use in Latin American cuisine and beneficial properties for human health, currently *Physalis* plants in general and husk tomato, in particular, are not the most economically important *Solanaceae* crops; therefore the lack of information about their microbiome does not seem surprising. Understandably, for such a major *Solanaceae* crop as tomato, there is more information available about its rhizosphere microbiome. So, being aware of plant species-associated differences in the rhizosphere microbiome, we tried to relate our results to some earlier ones reported on the tomato rhizosphere. One of the most complete characterization of the microorganism diversity associated with the rhizosphere of tomato *Solanum lycopersicum* L. cultivated in the open field was done with plants grown in Mexico on a Fluvisol, also by using cloning of PCR products and sequencing clone libraries: the phylum *Firmicutes* was found to be the most abundant (45%), followed by *Proteobacteria* (15%) and *Gemmatimonadetes* (13%) (Cordero-Ramirez *et al.* 2012). So even at the dominant phyla level, there was no semblance between rhizosphere bacteriobiome phylum compositions of husk tomato grown in West Siberia and tomato grown in Mexico. In a study, performed by employing the Illumina MiSeq sequencing platform, albeit different bioinformatic tools, the rhizosphere of tomato plants, grown in greenhouses in Korea, was found to be dominated by *Proteobacteria*, followed by *Actinobacteria* and *Bacteroidetes* (Lee *et al.* 2016). *Acidobacteria* representatives were also found among moderate (3.5%) dominants in the study, which is much less as compared with our study (*ca.*23%). Interestingly, in this Korean study, only 1.3% of all the MiSeq reads could not be classified at the phylum level, whereas in our study the number of such reads was substantially higher (*ca.*17%). We found two *Sphingomonas* sp. OTUs among the few most prevailing ones, albeit with just 0.5% of relative abundance, as compared to the very pronounced dominance of the genus in the tomato rhizosphere, where together with *Sphignobium* it accounted for 53%. Another marked difference was the dominance of *Bradyrhizobiaceae* in the husk tomato rhizosphere. The latter can be most likely attributed to the fact that in our study no fertilisation was applied, thus possibly recruiting and/or promoting nitrogen-fixing bacteria from the bulk soil. Although it was not explicitly stated (Lee *et al.* 2016), one can assume that tomatoes grown in the greenhouses received some mineral fertilisation, which did not favour nitrogen-fixers growth in the rhizosphere. We also detected higher numbers of taxa at different taxonomical levels. Overall, the differences between the rhizosphere bacteriobiomes of the husk tomato in the open field on naturally-developed Phaeozem in Siberia and tomato grew in the greenhouse in Korea can be attributed mostly to the differences between the soil/soil substrate and then to the differences between the crops. We believe, however, that at the high taxonomic level of phyla and, the more so, at the dominant phyla, more semblance between rhizosphere bacteriobiome profiles of the same or closely related plant species could have been revealed despite different methodology. Thus the differences may most likely be resultant mainly from the differences in soil properties and indigenous soil microbiota pool, from which plants by root exudates and litter draw certain microbial species to compose their rhizosphere assemblage.

In contrast to our results, a study of the rhizosphere bacteriobiome of tomatoes grown in the open field in Maryland (USA) on a silt loam soil reported the dominance of *Firmicutes* (*Bacilli*) and *Gammaproteobacteria* (Allard *et al.* 2016). In our view, the *Firmicutes* dominance in the tomato rhizosphere is somewhat surprising and might be related to the fertilisation history, i.e. application of cattle manure at the experimental site (Wang *et al.* 2018), which was not specified in the article.

A recent study from China (Li *et al.* 2019) reported that the main dominant phyla in soil under tomato plants grown with groundwater irrigation were *Proteobacteria*, *Acidobacteria* and *Actinobacteria,* i.e. similar to the ones in our study with husk tomato. However, the alpha-diversity indices of the husk tomato rhizosphere bacteriobiome are much higher, Shannon and Chao-1 being 7.5 and 5,000 *vs*. 1.9 and 21 (Li *et al.* 2019), respectively. Yet in an earlier study in greenhouse pot experiment the estimated alpha-diversity indices for tomato rhizosphere bacteriobiome (Cai *et al.* 2017) were 5.4 and 2,400, i.e. closer to the indices we present in this article. Another study from China (Wang *et al.* 2018) recently reported for the bulk soil under tomato very high Shannon indices of 9.4 – 9.9 and Chao-1, somewhat lower than reported in this study.

Since no fertilisation was used in our study, the husk tomato rhizosphere bacteriobiome, presented here, was shaped by the crop's nutrient preferences (Cai *et al.* 2017), alongside with its root exudates and litter, as well as edaphic and other environmental factors.

CONCLUSIONS

Our exploratory study provides a glimpse into the composition and structure of the husk tomato rhizosphere microbiome: it was interesting to find highly diverse and equitable bacterial assemblage was at the interface between a non-conventional crop, originated in America, and a naturally developed soil in North Eurasia. The presented husk tomato rhizosphere bacteriobiome is the first one obtained by deep amplicon sequencing on Illumina MiSeq platform and may serve as a reference one for further research to get a better picture of specifics of *Solanaceae* microbiome in different environments, thus contributing to a more comprehensive understanding of shaping microbial communities by roots of these globally important crops.

Acknowledgements: The study was supported by Russian State funded budget project AAAA-A17-117020210021-7. The Ministry of Science and Higher Education of the Russian Federation funded the research carried out by the Institute of Soil Science and Agrochemistry (Novosibirsk, Russia).

The authors thank Dr. Yury V. Fotev (Central Siberian Botanical Garden, SB RAS, Novosibirsk, Russia) for supplying the husk tomato seedlings for the study.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

AHKAMI, A.H. – WHITE, R.A. – HANDAKUMBURA, P.P. – JANSSON, C. 2017. Rhizosphere engineering: Enhancing sustainable plant ecosystem productivity. In *Rhizosphere,* vol. *3,* no. 2, pp. 233 – 243. DOI: 10.1016/j.rhisph.2017.04.012

ALLARD, S.M. – WALSH, C.S. – WALLIS, A.E. – OTTESEN, A.R. – BROWN, E.W. – MICALLEF, S.A. 2016. *Solanum lycopersicum* (tomato) hosts robust phyllosphere and rhizosphere bacterial communities when grown in soil amended with various organic and synthetic fertilizers. In *Science of the Total Environment*, vol. *573*, pp. 555 – 563. DOI: 10.1016/j.scitotenv.2016.08.157

BERENDSEN, R.L. – PIETERSE, C.M.J. – BAKKER, P.A.H.M. 2012. The rhizosphere microbiome and plant health. In *Trends in Plant Science*, vol. *17*, no. 8, pp. 478 – 486. DOI: 10.1016/j.tplants.2012.04.001

CAI, F. – PANG, G. – MIAO, Y. – LI, R. – LI, R. – SHEN, Q. – CHEN, W. 2017. The nutrient preference of plants influences their rhizosphere microbiome. In *Applied Soil Ecology*, vol. *110*, pp. 146 – 150. DOI: 10.1016/j.apsoil.2016.11.006

CORDERO-RAMÍREZ, J.D. – LÓPEZ-RIVERA, R. – CALDERÓN-VÁZQUEZ, C.L .– FIGUEROA-LÓPEZ, A.MI. – MARTÍNEZ-ÁLVAREZ, J.C. – LEYVA-MADRIGAL, K.Y. – CERVANTES-GÁMEZ, R.G. – MALDONADO-MENDOZA, I.E. 2012. Microorganismos asociados a la rizosfera de jitomate en un agroecosistema del valle de Guasave, Sinaloa, México. In *Revista Mexicana de Biodiversidad*, vol. *83,* no. 3, pp. 712 – 730. DOI: 10.7550/rmb.17897

EDGAR, R.C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. In *Nature Methods*, vol. *10,* pp. 996 – 998. DOI: 10.1038/nmeth.2604

EDGAR, R.C. 2016. SINTAX, a Simple Non-Bayesian Taxonomy Classifier for 16S and ITS Sequences. bioRxiv., DOI: 10.1101/074161

FADROSH, D.W. – MA, B. – GAJER, P .– GAJER, P. – SENGAMALAY, N. – OTT, S. – BROTMAN, R.M. – RAVEL, J. 2014. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. In *Microbiome*, vol. *2,* no. 1, pp. 6. DOI: 10.1186/2049-2618-2-6

FAUTH, E. – BERNARDO, J. – CAMARA, M. – RESETARITS, W.J. – VAN BUSKIRK, J. – MCCOLLUM, S.A. 1996. Simplifying the Jargon of community ecology: A conceptual approach. In *The American Naturalist*, vol. *147*, pp. 282 – 286.

HAMMER, Ø. – HARPER, D.A.T. – RYAN, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. In *Palaeontologia Electronica*, vol. *4*, pp. 1 – 9.

HUGHES, J.B. – HELLMANN, J.J. 2005. The application of rarefaction techniques to molecular inventories of microbial diversity. In *Methods in Enzymology,* vol. *397*, pp. 292 – 308. https://doi.org/10.1016/S0076-6879(05)97017-1

IGOLKINA, A.A. – GREKHOV, G.A. – PERSHINA, E.V. – SAMOSOROVA, G.G. – LEUNOVA, V.M. – SEMENOVA, A.N. – BATURINA, O.A. – KABILOV, M.R. – ANDRONOV, E.E. 2018. Identifying components of mixed and contaminated soil samples by detecting specific signatures of control 16S rRNA libraries. In *Ecoogical Indicators*, vol. *94,* no.1, pp. 446 – 453. DOI: 10.1016/j.ecolind.2018.06.060

IUSS WORKING GROUP. 2014. *WRB, World Reference Base for Soil Resources*. *International soil classification system for naming soils and creating legends for soil maps*. Rome: FAO.

KHAN, W. – BAKHT, J. – NAIR, M.G. – UDDIN, M.N. – SHAFI, M. 2018. Extraction and isolation of important bioactive compounds from the fruit of *Physalis ixocarpa*. In *Pakistan Journal of Pharmaceutical Sciences*, vol. *31*, no. 6, pp. 2463 – 2469.

KHAN, W.– BAKHT, J.– SHAFI, M. 2016. Antimicrobial potentials of different solvent extracted samples from Physalis ixocarpa. In *Pakistan Journal of Pharmaceutical Sciences*, vol. *29*, no. 2, pp. 467 – 475.

LEE, S.A. – PARK, J. – CHU, B. – KIM, J.M. – JOA, J.H. – SANG, M.K. – SONG, J. – WEON, H.Y. 2016. Comparative analysis of bacterial diversity in the rhizosphere of tomato by culture-dependent and -independent approaches. In *Journal of Microbioogy*, vol. *54,* no. 12, pp. 823 – 831. DOI: 10.1007/s12275-016-6410-3

LI, B. – CAO, Y. – GUAN, X. – LI, Y. – HAO, ZH. – HU, W. – CHEN, L. 2019. Microbial assessments of soil with a 40-year history of reclaimed wastewater irrigation. In *Science of the Total Environment*, vol. *651*, no. 1, pp. 696 – 705. DOI: 10.1016/j.scitotenv.2018.09.193

MARQUEZ-SANTACRUZ, H.A. – HERNANDEZ-LEON, R. – OROZCO-MOSQUEDA, M.C. – VELAZQUEZ-SEPULVEDA, I. – SANTOYO, G. 2010. Diversity of bacterial endophytes in roots of Mexican husk tomato plants (*Physalis ixocarpa*) and their detection in the rhizosphere. In *Genetic and Molecular Research,* vol. *9,* no. 4, pp. 2372 – 2380. DOI: 10.4238/vol9-4gmr921

MARTINES, M. – VARGAS-PONCE, O. – RODRIGUEZ, A. – CHIANG, F. – OCEGUEDA, S. 2017. Solanaceae family in Mexico. In *Botanical Sciences*, vol. *95*, no. 1, pp. 1–15. DOI: 10.17129/botsci.658

MORALES-CONTRERAS, B.E. – CONTRERAS-ESQUIVEL, J.C. – WICKER, L. – OCHOA-MARTÍNEZ, L.A. – MORALES-CASTRO, J. 2017. Husk Tomato (Physalis ixocarpa Brot.) Waste as a Promising Source of Pectin: Extraction and Physicochemical Characterization*.* In *Journal of Food Science*, vol. *82,* no. 7, pp. 1594 – 1601. DOI: 10.1111/1750-3841.13768

NAUMOVA, N.B. – SAVENKOV, O.A. – NECHAEVA, T.V. – FOTEV, Y.V. 2019. Yield and fruit properties of husk tomato (*Physalis phyladelphica*) cultivars grown in the open field in the South of West Siberia. In *Horticulturae*, vol. *5,* no. 1, pp. 19. DOI: 10.3390/horticulturae5010019

RAMÍREZ-GODINA, F. – ROBLEDO-TORRES, V. – FOROUGHBAKHCH-POURNABAV, R. – BENAVIDES-MENDOZA, A. – HERNÁNDEZ-PIÑERO, J.L. – REYES-VALDES, M.H. – ALVARADO-VÁZQUEZ, M.A. 2013. Yield and fruit quality evaluation in husk tomato autotetraploids (*Physalis ixocarpa*) and diploids. In *Australian Journal of Crop Science*, vol. *933*, pp. 933 – 940.

SALEEM, M. – LAW, A.D. – SAHIB, M.R. – PERVAIZ, Z.H. – ZHANG, Q. 2018. Impact of root system architecture on rhizosphere and root microbiome. In *Rhizosphere*, vol. *6*, no. 1, pp. 47 – 51. DOI: 10.1016/j.rhisph.2018.02.003

SMITH, R. – JIMENEZ, M.– CANTWELL, M. 1999. *Tomatillo production in California.* Available at http://anrcatalog.ucanr.edu/pdf/7246.pdf (accessed July 23, 2019)

VALDIVIA-MARES, L.E. – RODRÍGUEZ ZARAGOZA, F.A. – SÁNCHEZ GONZÁLEZ, J.J. – VARGAS-PONCE, O. 2016. Phenology, agronomic and nutritional potential of three wild husk tomato species (*Physalis, Solanaceae*) from Mexico. In *Scientia Horticulturae*, vol. *200*, pp. 83 – 94. DOI: 10.1016/j.scienta.2016.01.005

WALLENSTEIN, M.D. 2017. Managing and manipulating the rhizosphere microbiome for plant health: A systems approach. In *Rhizosphere*, vol. *3*, no. 2, pp. 230 – 232. DOI: 10.1016/j.rhisph.2017.04.004

WANG, Q. – GARRITY, G.M. – TIEDJE, J.M. – COLE, J.R. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. In *Applied and Environmental Microbiology*, vol. *73*, pp. 5261 – 5267. DOI: 10.1128/AEM.00062-07

WANG, M. – CHEN, L. – LI, Y. – CHEN, L. – LIU, ZH. – WANG, X. – YAN, P. – QIN, S. 2018. Responses of soil microbial communities to a short-term application of seaweed fertilizer revealed by deep amplicon sequencing. In *Applied Soil Ecology*, vol. *125,* pp. 288 – 296. DOI: 10.1016/j.apsoil.2018.02.013

XUE, D. – CHRISTENSON, R. – GENGER, R. – GEVENS, A. – LANKAU, R.A. 2018. Soil microbial communities reflect both inherent soil properties and management practices in Wisconsin potato fields. In *American Journal of Potato Research*, vol. *95*, pp. 696. DOI: 10.1007/s12230-018-9677-6

ZAMORA-TAVARES, P. – VARGAS-PONCE, O. – SANCHEZ-MARTINEZ, J. – CABRERA-TOLEDO, D. 2015. Diversity and genetic structure of the husk tomato (*Physalis philadelphica* Lam.) in Western Mexico. In *Genetic Resources and Crop Evolution*, vol. *62*, pp. 141 – 153. DOI: 10.1007/s10722-014-0163-9

ZHAO, Q. – ZENG, D.H. – FAN, Z.-P. 2010. Nitrogen and phosphorus transformations in the rhizospheres of three tree species in a nutrient-poor sandy soil. In *Applied Soil Ecology*, vol. *46*, pp. 341 – 346. DOI: 10.1016/j.apsoil.2010.10.007

ZHENG, M. – GUO, J. – XU, J. – YANG, K. – TANG, R. – GU, X. – LI, H. – CHEN, L. 2019. Ixocarpalactone A from dietary tomatillo inhibits pancreatic cancer growth by targeting PHGDH. In *Food & Function*, vol. *10,* no. 6, pp. 3386 – 3395. DOI: 10.1039/c9fo00394k

Received: August 8, 2019
Accepted: November 11, 2019

Agriculture (Poľnohospodárstvo), 65, 2019 (4): 155− 163

sciendo

DOI: 10.2478/agri-2019-0016

Original paper

ABOVE AND BELOW GROUND BIOMASS AND CARBON STOCK IN PERMANENT GRASSLANDS OF SLOVAKIA

MIRIAM KIZEKOVÁ[1]*, RADOSLAVA KANIANSKA[2], ĽUBICA JANČOVÁ[1],
JOZEF ČUNDERLÍK[1], ZUZANA DUGÁTOVÁ[1], JARMILA MAKOVNÍKOVÁ[3]

[1]National Agricultural and Food Centre – Grassland and Mountain Agriculture Research Institute, Banská Bystrica, Slovak Republic
[2]Matej Bel University in Banská Bystrica, Slovak Republic
[3]National Agricultural and Food Centre – Soil Science and Conservation Research Institute, Slovak Republic

KIZEKOVÁ, M. – KANIANSKA, R. – JANČOVÁ, Ľ. – ČUNDERLÍK, J. – DUGÁTOVÁ, Z. – MAKOVNÍKOVÁ, J.: Above and below ground biomass and carbon stock in permanent grasslands of Slovakia. Agriculture (Poľnohospodárstvo), vol. *65*, 2019, no. 4, pp. 155 – 163.

This paper aimed to monitor the dry matter biomass production and carbon stocks of above-and below-ground biomass in five types of grasslands in Slovakia: i) lowland oversowed pasture ii) lowland hay meadows, iii) mesophilous pasture, iv) mountain hay meadows, v) abandoned grassland. Averaged over two cropping seasons the total above-and below-ground biomass differed significantly across the monitored grasslands. It ranged respectively from 2.18 to 7.86 t/ha and from 9.64 to 22.67 t/ha dry matter depending on the pedoclimatic condition and the botanical composition of each grassland type. Consequently, this resulted also in the carbon stocks in above-and below-ground biomass. Generally, the mean carbon stocks were 1.56 t/ha for above-ground biomass (24%) and 4.83 t/ha for below-ground biomass (76%). The botanical composition for all the grassland types was also described. The highest number of plant species (55) was observed in lowland hay meadow located in Slovak Karst, the lowest one (23) for the oversowed grassland located in Eastern Slovak Upland. This monitoring paper showed that semi-natural grassland habitats and improved grasslands as well are an important carbon sink, and they can play a key role in global climate change mitigation.

Key words: ecosystem services environmental indicators, botanical composition, grasslands

In the past a large area of the EU land was covered by grasslands (Hejcman *et al*. 2013). Nowadays their role has weakened since land use has been intensified and several agricultural areas have been converted to other uses or even abandoned (FAOSTAT 2011). This reduction of grassland areas is due to several factors i.e. urbanisation, conversion to arable land and afforestation. In arable land, the decline of temporary grasslands was mostly in favour of the production of annual crops and fodder maize. Modern agricultural practices, including monoculture, have disrupted the carbon cycle. They have removed large amounts of carbon from grasslands and transferred great levels of it to air and water providing serious consequences to the environment (Loges *et al*. 2018; Tobiášová *et al*. 2013). A strong conversion to modern agricultural practices is present also in Slovakia. Grassland areas are abandoned both in lowlands and mountain areas and are preserved only in regions where it is not cultivat-

---

Ing. Miriam Kizeková, PhD. (*Corresponding author), National Agricultural and Food Centre – Grassland and Mountain Agriculture Research Institute, Mládežnícka 36, 974 21 Banská Bystrica, Slovak Republic. E-mail: miriam.kizekova@nppc.sk

ed intensively or in marginal areas where livestock production persists traditional. In 2017 permanent grasslands area in Slovakia was equal to 853,756 ha of which only 517,679 was used (MARD SR 2018). Grasslands conserve their traditional main function i.e. to provide a number of products including food crops and meat but they have many other functions that are the so-called ecosystem services (Huyghe *et al*. 2014; Sarzeaud *et al.* 2008). These include provisioning, regulating, and cultural services that directly affect people, as well as supporting services needed to maintain the other services (MEA 2005). Overall, farmers need to supply more milk and meat to satisfy the market requirements (Corazzin *et al*. 2019), so very high yielding dairy cows and sheep are fed mainly with maize and concentrates instead of grass. Nevertheless, grasslands have still an important role and farmers and stakeholders increase the reliance on grasslands for competitive and sustainable ruminant production systems. Besides feed for animals, grasslands are judged important for many other reasons i.e. for the conservation of habitats, accumulation of greenhouse gas emissions and mitigation of climate change impacts. Grasslands act as carbon sink, improving the organic carbon and might mitigate soil pollution and lower erosion. These benefits are lost when soils covered by grasslands are tilled (Lal *et al.* 2007). The EU has acknowledged the ecological functions of grassland and introduced measures within the Common Agricultural Policy for its protection against conversion into arable land as well as for preserving or enhancing its ecological quality. In addition activities at national or regional levels may regulate the maintenance of grassland. In grasslands, there are different storages of carbon i.e. the above-and below-ground biomass of grasses, litter on the grassland floor, dead grass and soils. It is well known that the most soil carbon is derived from recent photosynthesis that takes carbon from the above-ground biomass into root structure and further into below-ground storage via exudates (Kell 2012; Jones & Donnelly 2004). Above-ground biomass carbon is relatively short-lived due to grazing, cutting, senescence. In contrast, below-ground biomass is characterized by a large fibrous root system that often stocks up 60–80% of the carbon (Janowiak *et al*. 2017).

Semi-natural grasslands have a rich flora while intensively managed grasslands are characterized by the use of highly efficient grass and legume species. Hungate *et al.* (2017) reported that carbon storage is one of the many features of a species value. However, currently, there is little known how plant diversity influences carbon storage in ecosystems including above- and below-ground biomass as well (Catovsky *et al.* 2002). Moreover, only some studies determinate carbon storage in root biomass. In this paper, we focus on botanical composition and above-and below-ground biomass carbon stocks as good ecosystem indicators to predict the efficiency of carbon sink capacity of grasslands.

MATERIAL AND METHODS

The monitoring was conducted during 2014 − 2015 on five permanent grasslands in different environmental conditions and used by farmers with common agricultural practices (Table 1, Figure 1): i) in Nacina Ves (NV) was chosen a permanent pasture for cattle oversown by a grass/legume mixture (*Lolium multiflorum* L. + *Trifolium pretense* L.) in 2014; ii) in Kečovo (KE) a lowland hay meadow – alliance *Arrhenatherion elatioris* (Natura 2000 code 6510) used by mowing in spring and by extensive cattle grazing in autumn; iii) in Tajov (TA) a mesophilous pasture (habitat of national importance) season-long continuously grazed by sheep and in Liptovská Teplička (LT) iv) a mountain hay meadow (Natura 2000 code 6520) mowed in spring and extensive sheep grazing in autumn and v) abandoned grassland (LT2) which was not utilized for about 5 years on the edge of a small wood.

Botanical composition

The botanical composition was estimated in percentage on four replications of 16 m^2 large plots. In the relevés, the cover value of each species was visually determined. In this paper, we report only the five most dominant species per each site what is considered possible to characterize quite well the plant associations in this way. This is also of great agronomic interest as argued by Sarno *et al.* (1989) who monitored grasslands of 16 sites in Italy.

Above-and below-ground biomass sampling and analysis

At each site, four quadrates (each with an area of 0.5 × 0.5 m^2 within the 16 m^2 quadrats chosen for botanical relevés) were sampled for above-ground biomass (AGB), for soil samples and for root biomass samples determination. Below-ground biomass (BGB) was taken at the identical place as AGB immediately before cutting or grazing. The distance between the quadrates was 10 m in the field and they were organized as a letter Z as described by Hrivňáková *et al*. (2011). AGB was clipped twice a year in May and September. The content of dry matter (DM) yield was determinate by overnight drying at 60°C. Four soil cores (5 cm in diameters, 15 cm depth) were collected in May. Collected samples were washed on sieves of 0.5 mm mesh size, dried at 60°C and weighted the roots as BGB. To calculate both contents of carbon in AGB and BGB we used the conversion factor 0.475 (Schlesinger 1991).

Data analyses

Statistical analyses were computed by using the statistical program package PASW Statistics. Assessment of the normality of all data was made by testing procedures available in this package.

Consequently, AGB and BGB parameters were

Table 1

Geographic characteristics, soil properties and land management of the five study sites

Study site	Nacina Ves	Kečovo	Tajov	Liptovská Teplička	Liptovská Teplička Abandon
Geographical location	Eastern Slovak Upland	Slovak Karst	Kremnické vrchy Mts	Low Tatras Mts	Low Tatras Mts.
Altitude [m]	121	344	597	931	950
Mean annual rainfall [mm]	559	620	795	950	950
Mean annual temperature [°C]	8.9	8.6	8.1	6.2	6.2
Soil type	Humic Regosol (Fluvisol)	Eutric Cambisol	Dystric Cambisol	Regosol (Rendzina)	Regosol (Rendzina)
Geological substrate	Fluvial sediments	Limestones and dolomites	Volcanic	Limestones and dolomites	Limestones and dolomites
Grassland management	Cattle pasture	Cut / pasture	Sheep pasture	Meadow	Abandon

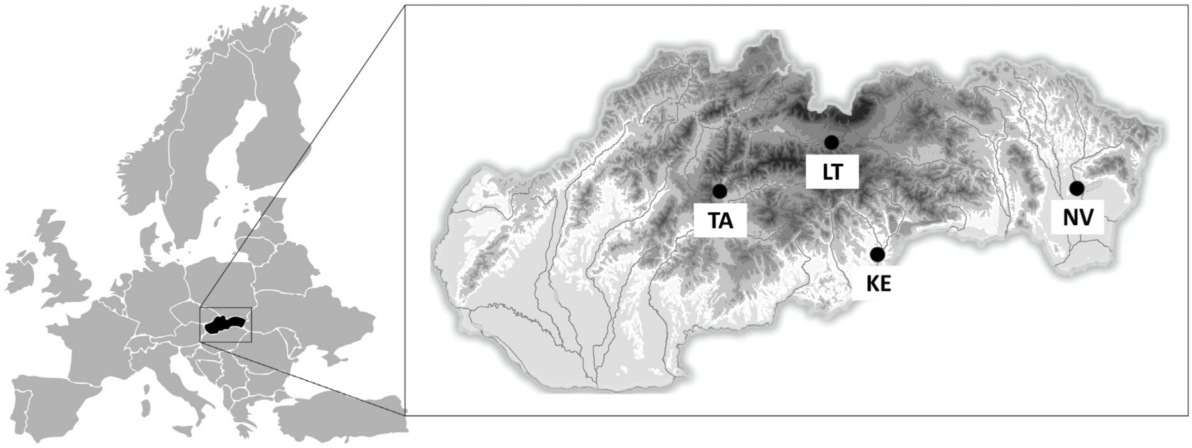

Figure 1. Map of the study sites in Slovakia
NV – Nacina Ves; KE – Kečovo; TA – Tajov; LT – Liptovská Teplička

analysed using one-way ANOVA analysis of variance followed by post hoc comparison using the Tukey HSD test based on the studentized range distribution to identify differences among study sites with different environmental conditions and grassland management.

RESULTS

Botanical composition

On average in the five monitored grasslands have been identified and classified 33 species, with a minimum number (23) for the natural grassland

Table 2

Botanical survey on the five study sites

Study site	NV	KE	TA	LT1	LT2
Agrobotanical group/species	Coverage [%]				
Grasses	68.5	40.6	61.4	53.6	41.5
Legumes	9.8	11.3	29.3	11.7	14.5
Forbs	21.3	48.1	18.3	33.7	32.5
Bare grounds	0.4	–	–	1.0	8.4
Trees	–	–	–	–	3.1
Lolium multiflorum L.	30.2	–	–	–	–
Festuca pratensis L.	17.2	–	–	–	–
Agropyron repens Beauv.	13.0	–	–	–	–
Trifolium pratense L.	5.5	–	–	–	–
Poa pratensis L.	4.0	–	–	–	–
Festuca ovina L.	–	11.7	–	–	–
Dactylis glomerata L.	–	5.5	–	10.6	7.2
Trifolium repens L.	–	5.4	10.5	–	–
Festuca arundinacea Schreb.	–	5.2	–	–	6.1
Avenula pubescens Huds.	–	4.4	–	9.2	5.8
Bromus erectus Huds.	–	–	24.0	–	–
Festuca rubra L.	–	–	14.4	–	8.1
Festuca rupicola Heuffel	–	–	8.7	–	–
Agrostis capillaris L.	–	–	7.0	–	–
Arrhenatherum elatius L.	–	–	–	7.0	8.7
Anthoxantum odoratum L.	–	–	–	6.5	–
Alopecurus pratensis L.	–	–	–	5.0	–
Number of species					
Grasses	6	10	7	10	7
Legumes	3	6	6	6	8
Forbs	14	39	17	20	18
Trees	–	–	–	–	1

NV – Nacina Ves; KE – Kečovo; TA – Tajov; LT1 – Liptovská Teplička; LT2 – Liptovská Teplička abandon of the NV site which was owersown with a productive mixture (Table 2). The maximum number of species (55) was observed for the site KE with a limestone and dolomite geological substrate. The grasslands of LT1, LT2 and TA sites have a presence of respectively 36, 33 and 30 species. Per each of these sites are reported also the five most covering species (Table 2). Grasses are the most representative (53.12%) followed by forbs (30.87%) and legumes (15.32%). Only in the site KE, forbs are the most representative (48.1%) while the highest percentage of legumes (29.3%) is present in TA.

Above- and below-ground biomass

On average the AGB yield (DM) was 4.58 t/ha and there was no significant difference between the two years of monitoring (Table 3). To contrary, there were significant differences between the intensive pasture in NV (7.86 t/ha DM) and lowland hay meadow in KE (2.18 t/ha DM) and mesophilous pasture in TA (2.82 t/ha DM), as reported in Table 3. In the same Table 3 may be found that there are no significant differences between NV and LT2 (7.86 and 6.27 t/ha DM) and between TA and KE (2.82 and 2.18 t/ha DM). The BGB yield (DM) fluctuated from 9.64 t/ha in the mountain meadow LT1 to 22.67 t/ha in the mountain sheep pasture TA. Similarly, to AGB, in this case, there was no significant difference between the two years of monitoring. Significant differences were not verifiable between TA and LT2 (22.67 and 17.91 t/ha DM) and between NV, KE, LT1 (11.01, 10.93, 9.64 t/ha DM) for this parameter.

The BGB:AGB ratio varied in a broad range. The BGB DM yield was greater by a factor 8.04 than the AGB DM yield in TA whereas the lowest BGB:AGB ratio was found in NV (1.41). KE, LT1, LT2 showed ratios respectively of 5.01, 2.54, 2.85. The total average yield (AGB+BGB) for the five sites was of 19.02 DM t/ha while the highest yields were registered in TA and LT2 (25.49 and 24.18 DM t/ha) and the lowest in NV, LT and KE (18.87, 13.43 and 13.11 DM t/ha). Nevertheless, in this case, the differences were not significant.

Above and below-ground carbon content

On average the above-ground biomass carbon content (AGBC) for the five sites was of 1.56 t/ha while the highest AGBC was registered in NV and LT2 (2.56 and 2.17 t/ha). The lowest values were observed in LT1, TA and KE (1.9, 1.06 and 0.93 t/ha). Concerning the average below-ground biomass carbon content (BGBC) the values observed were of 4.83 t/ha (Table 3). Unlike what

Table 3

Above-ground dry matter yield, below-ground dry matter yield, total biomass yield, content of carbon in above-ground plant biomass, content of carbon in below-ground plant biomass (roots), content of total carbon in plant biomass ($P < 0.01$)

Factor		AGB DMY [t/ha]	BGB DMY [t/ha]	Total DMY [t/ha]	AGBC [t/ha]	BGBC [t/ha]	TBC [t/ha]
Study site	NV	7.86^{a}	11.01^{bc}	$18.87^{n.s.}$	2.56^{a}	3.86^{bc}	6.42^{ab}
	KE	2.18^{c}	10.93^{bc}	$13.11^{n.s.}$	0.93^{b}	2.89^{c}	3.82^{b}
	TA	2.82^{c}	22.67^{a}	$25.49^{n.s.}$	1.06^{b}	7.27^{a}	8.33^{a}
	LT1	3.79^{bc}	9.64^{c}	$13.43^{n.s.}$	1.09^{b}	3.63^{bc}	4.72^{b}
	LT2	6.27^{ab}	17.91^{ab}	$24.18^{n.s.}$	2.17^{ab}	6.48^{ab}	8.65^{a}
$HSD_{0.01}$		0.63	1.31	1.67	0.28	0.57	0.62
Year	2014	$4.23^{n.s.}$	$11.65^{n.s.}$	$15.87^{n.s.}$	2.07^{a}	$4.43^{n.s.}$	$6.50^{n.s.}$
	2015	$4.94^{n.s.}$	$17.22^{n.s.}$	$22.17^{n.s.}$	1.06^{b}	$5.22^{n.s.}$	$6.28^{n.s.}$
$HSD_{0.01}$		0.39	1.02	1.06	0.18	0.36	0.39

NV – Nacina Ves; KE – Kečovo; TA – Tajov; LT1 – Liptovská Teplička; LT2 – Liptovská Teplička abandon; AGB – above-ground biomass; BGB – below-ground biomass; AGBC – above-ground biomass carbon content; BGBC – below-ground biomass carbon content; TBC – total biomass carbon content; DMY – dry matter yield previously noted, the highest values were registered for the site TA (7.24 t/ha) and again for site LT2 (6.48 t/ha). In sites NV, LT1 and KE were accumulated in the below-ground similar quantities of carbon i.e. respectively 3.86, 3.63 and 2.89 t/ha. Total C (AGBC + BGBC) stored on average by our five grassland types was 6.39 t/ha. The highest stored C quantities were observed for LT2 (8.65 t/ha) and TA (8.30 t/ha). NV, LT1 and KE stored in descending order 6.31, 4.72 and 3.82 t/ha of DM.

DISCUSSION

The five grassland types are chosen prove the richness of the Slovak flora which is considered of high biodiversity. It is important to underline that the highest number of species (55) is present in the site KE where the soil is a cambisol with limestones and dolomites geological substrate (Poschlod & Wallis De Vries 2002; Dengler *et al*. 2014). To contrary permanent grasslands used for intensive ruminant husbandry have usually been improved by overseeding productive grass/clover mixtures and/or high mineral fertilisation resulted very often in decreasing of plant diversity while moderate fertilisation may preserve biodiversity even with economic benefits (Plantereux *et al*. 2005; Jaurema *et al.* 2016; Bryan 1985; Willems *et al*. 1996; Kizekova *et al.* 2017; Samuil *et al*. 2013). Differences in the number of plant species in semi-natural grasslands in TA and LT1 sites may be attributed to different grassland management. Due to selective biting, sheep affected plant diversity at the continuously grazed pasture in TA as reported by Rook *et al*. (2004) and Metera *et al.* (2010).

The highest AGB yield of the NV grassland may be attributed to the presence of productive species i.e. *Lolium multiflorum* L., *Festuca pratensis* L., *Agropyron repens* Beauv., *Trifolium pratense* L. and *Poa pratensis* L. These findings are within the range reported by other studies for oversown grasslands on fertile soils (Hofmann & Isselstein 2005; Michaud *et al.* 2011). For the LT2 grassland we have to register a high AGB yield to be attributed to the presence of tall species like *Dactylis glomerata* L., *Festuca arundinacea* Schreb., *Avenula pubescens* Huds., *Festuca rubra* L. and *Arrhenatherum elatius* L. All these species prove that this grassland was more anthropized than the less productive grasslands. Similarly, an increase in the cover of tall species like *Festuca rubra* L. in abandoned mountain grasslands has been found by several authors (Pavlů *et al.* 2012; Pavlů *et al*. 2013; Pavlů *et al*. 2016). Mašková *et al.* (2009) also reported higher AGB yield in unmanaged fallow treatment compared to a mown mountain meadow. In general, ABG DM yield from mesophilous semi-natural grasslands is low, especially when plant diversity is high (Hopkins 2004) and can range from less than 2.00 t/ha to 8.00 t/ha (Tallowin & Jefferson 1999). LT1 mountain meadow was the third most productive grassland. In this situation the significative presence of *Dactylis glomerata* L., *Avenula pubescens* Huds. and *Arrhenaterum elatius* L. determines more the yield comparing to KE and TA where the yield was of 2.18 t/ha and 2.82 t/ha respectively. In addition of high occurrence of short grass *Festuca ovina* L. in KE lowland meadow, the lowest herbage yield detected may also have been affected by the warm and dry climatic conditions in the region.

It is more difficult to attribute the differences between BGB DM yields to the different botanical composition of the grasslands. The same species have a different roots structure, growth and biomass if they grow in different environmental conditions i.e. temperature, altitude, light, air, water, soil, minerals, management, etc. (Kutschera 1960). The BGB is very often higher where the AGB is lower so that there is no significant difference between the total DM yields for the five grassland types. BGB DM yield ranged from a minimum of 9.64 t/ha in LT to a maximum of 22.67 t/ha in TA. In this grassland we may notice that there is a significative presence (24%) of the species *Bromus erectus* Huds.. This grass is characteristic of poor meadows and has a slow root turnover in comparison with *Dactylis glomerata* L. and *Arrhenatherum elatius* L. (Schläpfer & Ryser 1996). We have to notice that in the TA grassland we observed the highest BGBC content (7.24 t/ha) and the highest BGB DM (22.67 t/ha). This pasture was season-long continuously grazed by sheep and the BGB:AGB was greater by a factor 8.04. Very few studies have directly measured the response of grassland root production to grazing. One paper shows that grazers in nine variable sites in Yellowstone Park stimulated AGB, BGB and whole-grassland productivity. Root production was stimulated seven times more than shoot production, indicating that the major effect of grazing was positive feedback on root growth (Frank *et al*. 2002). Hui and Jackson (2006) reported that the pattern of below-ground biomass production could also be the result of plant species response to environmental conditions. A large fluctuation in BGB DM yield in grasslands is in line with the study of Fiala *et al.* (2014) and Jančovič *et al*. (2002) who reported variation in BGB DM yield according to geographical location and grassland management. Several studies showed that BGB DM yield was significantly higher in grazed grasslands comparing to mown meadows (Garcia-Parsas *et al*. 2011; López-Marsico *et al*. 2015). In general, the BGB was greater by a factor of 3.15. In many grasslands, BGB plant biomass is greater than AGB biomass, in some cases even by a factor of five or more (Tomaškin *et al*. 2013) and this is even typically greater in grasslands with high water and nutrient limitations like in our monitored grasslands (Blair *et al.* 2014). The total DMY (Table 3) shows no significant differences between the five sites. This can be explained by the high variability of monitored grassland types and years. As for the carbon content in the biomasses, it is clear that the BGB content is also more than three times greater than in AGB. Similar to our results Loges *et al*. (2018) reported that grassland BGB fraction of the total DMY was higher up to 35% and absolute C input reached 5.3 t/ha. The carbon within AGB is small proportion of the total carbon pool in the ecosystem, and it is equally clear that this AGB carbon has a short life. BGB provides abundant carbon to soils and this soil carbon makes up just about 81% of the total ecosystem carbon found in grasslands (Adams *et al.* 1990). It must finally be remarked that in experiments where there were added species in the grassland composition the carbon storage increased in the plant, soil and ecosystem C pools over 50 years (Hungate *et al*. 2017). Therefore, a preservation policy of grasslands is urgent because even a return to previous management would not re-establish the complex structure of these habitats (Ruda *et al*. 2011).

CONCLUSIONS

Our main goal in this paper was to monitor the capacity of five permanent grasslands to produce AGB and BGB as well as to measure their carbon content. The AGB DM yields were the highest for intensive oversown and abandoned grassland while the BGB DM yield was significantly highest in a mesophilous pasture season-long continuously grazed by sheep. All grassland types showed not significant differences in the AGB + BGB DM yield because of high differences in the AGB:BGB ratios. AGB and BGB DM yields have obviously also affected their carbon content and similar conclusions can be made for AGBC and BGBC. For the contents of AGBC + BGBC, the intensive oversown, abandoned and mesophilous grasslands showed significantly the highest carbon storage capacities. It must be reminded that grasslands have a very high ecological value and in addition to providing food and feed, they fulfil many valuable ecosystem functions, such as product quality, biodiversity, landscape effect, water quality, erosion prevention, and, as demonstrated in this paper, carbon sequestration. Our results highlight that grasslands are an important carbon sink. They have the capacity to trap large amounts of C which would otherwise escape into the atmosphere as CO_2 which is one the worst greenhouse gases. This function is one of the most important between all multifunctional roles which grasslands have.

Acknowledgements. The authors acknowledge the Slovak Research and Development Agency for financial support via Project no. APVV-0098-12 “Analysis, modeling and evaluation of agroecosystem services” and the Ministry of Agriculture and Rural Development of the Slovak Republic via the Contract no. 318/2018/MPRV SR-300.

REFERENCES

ADAMS, J.M. – FAURE, H. – FAURE-DENARD, L. – MCGLAVE, J.M. – WOODWARD, F.I. 1990. Increases in terrestrial carbon storage from the last glacial maximum to the present. In *Nature*, vol. *348,* pp. 711 – 714.

BLAIR, J. – NIPPERT, J. – BRIGGS, J. 2014. Grassland ecology. In MONSON, R.K. (Ed.) *Ecology and the Environmental*. The Plant Science 8. New York : Springer Science, pp. 389 – 424. DOI: 10.1007/978-1-4614-7501-9_14

BRYAN, W.B. 1985. Effects of sod-seeding legumes on hill land pasture productivity and composition. In *Agronomy Journal*, vol. *77*, pp. 901 – 905.

CATOVSKY, S. – BRADFORD, M.A. – HECTOR, A. 2002. Biodiversity and ecosystem productivity: implications for carbon storage. In *Oikos*, vol. *97*, no. 3, pp. 443 – 448.

CORAZZIN, M. – ROMANZIN, A. – SEPULCRI, A. – PINOSA, M. – PIASENTIER, E. – BOVOLENTA, S. 2019. Fatty acid profiles of cow's milk and cheese as affected by mountain pasture type and concentrate supplementation. In *Animals* (Basel), vol. *9*, no. 2, pp. 68. DOI: 10.3390/ani9020068

DENGLER, J. – JANIŠOVÁ, M. – TÖRÖK, P. – WELLSTEIN, C. 2014. Biodiversity of Palaearctic grasslands: a synthesis. In *Agriculture, Ecosystems and Environment*, vol. *182*, pp. 1 – 14. DOI: 10.1016/j.agee.2013.12.015

FAOSTAT 2011. http://faostat.fao.org/. Food and Agriculture Organization of the United Nations, Rome, Italy.

FRANK, D.A. – KUNS, M.M. – GUIDO, D.R. 2002. Consumer control of grassland plant production. In *Ecology*, vol. *83*, no. 3, pp. 602 – 606. DOI: 10.1890/0012

FIALA, K. – TŮMA, I. – HOLUB, P. 2014. Proportions of root production in several temperate grasslands of Central Europe. In *Ekológia (Bratislava)*, vol. *33*, no. 3, pp. 232 – 241. DOI: 10.2478/eko-2014-0022

GARCIA-PAUSAS, J. – CASALS, P. – ROMANYÀ, J. – VALLECILLO, S. – SEBASTIÀ, M.T. 2011. Seasonal patterns of belowground biomass and productivity in mountain grasslands in the Pyrenees. In *Plant and Soil*, vol. *340*, pp. 315 – 326. DOI: 10.1007/s11104-010-0601-1

HRIVŇÁKOVÁ, K. et al. 2011. *Jednotné pracovné postupy rozborov pôd* [*Uniform working methods for soil analysis*]. Bratislava: SSCRI, 145 pp. ISBN 978-80-89128-89-1

HEJCMAN, M. – HEJCMANOVA, P. – PAVLŮ, V. – BENEŠ, J. 2013. Origin and history of grasslands in Central Europe – a review. In *Grass and Forage Science*, vol. *68*, no. 3, pp. 345 – 364. DOI: 10.1111/gfs.12066

HOFMANN, M. – ISSELSTEIN, J. 2005. Species enrichment in an agriculturally improved grassland and its effects on botanical composition, yield and forage quality. In *Grass and Forage Science*, vol. *60*, pp. 136 – 145. DOI: /10.1111/j.1365-2494.2005.00460.x

HOPKINS, A. 2004. Relevance and functionality of semi-natural grassland in Europe – status quo and future prospective. In I*nternational Workshop of the SALVERE Project,* p. 9 – 14.

HUNGATE, B.A. – BARBIER, E.B. – ANDO, A.W. – MARKS, S.P. – REICH, P.B. – VAN GESTEL, N. – TILMAN, D. – KNOPS, J.M.H. – HOOPER, D.U. – BUTTERFIELD, B.J. – CARDINAL, B.J. 2017. The economic value of grassland species for carbon storage. In *Science Advances*, vol. *3*, no. 4, e1601880. DOI: 10.1126/sciadv.1601880

HUI, D. – JACKSON, R.B. 2006. Geographical and interannual variability in biomass partitioning in grassland ecosystems: a synthesis of field data. In *New Phytologist,* vol. *169,* pp. 85 – 93. DOI: 10.1111/j.1469-8137.2005.01569.x

HUYGHE, C. – DE VLIEGHER, A. – VAN GILS, B. – PEETERS, A. 2014. *Grasslands and herbivore production in Europe and effects of common policies*. Versailles: Editions Quae, 287 pp. ISBN 978-2-7592-2156-1

JANČOVIČ, J. – SLAMKA, P. – VORÁR, Ľ. – KOVÁČIK, P. 2002. Changes of root system of various types of grasslands influenced by mineral nutrition. In *Ekológia (Bratislava)*, vol. *21,* no. 4, pp. 356 – 362.

JAUREMA, M.A. – LEZEMA, F. – SALVO, L. – CARDOZO, G.A. – AYALA, W. – TERRA, J. – NABINGER, C. 2016. The dilemma of improving native grasslands by overseeding legumes: Production intensification on diversity conservation. In *Rangeland Ecology and Management*, vol. *69,* no. 1, pp. 35 – 42.

JONES, M.B. – DONNELLY, A. 2004. Carbon sequestration in temperate grassland ecosystem and the influence of managing, climate and elevated CO_2. In *New Phytologist*, vol. *164,* no. 3, p. 423 – 439. DOI: 10.1111/j.1469-8137.2004.01201.x

KELL, D.B. 2012. Large-scale sequestration of atmospheric carbon via plant roots in natural and agricultural ecosystems: why and how. In *Philosophical Transactions of the Royal Society B*, vol. *367,* pp. 1589 – 1597. DOI: 10.1098/rstb.2011.0244

KIZEKOVA, M. – FEOLI, E. – PARENTE, G. – KANIANSKA, R. 2017. Analysis of the effects of mineral fertilisation on species diversity and yield of permanent grasslands: Revisited data to mediate economic and environmental needs. In *Community Ecology*, vol. *18,* no. 3, pp. 295 – 304. DOI: 10.1556/168.2017.18.3.8

KUTSCHERA, L. 1960. *Wurzelatlas, mitteleuropaeischer Ackerunkrauter und Kulturpplanzen*. DLG-VERLAGS-GMBH. Frankfurt am Main, 374 pp.

LAL, R. – REICOSKY, D.L. – HANSON, J.D. 2007. Evolution of the plow over 10,000 years and the rationale for no-till farming. In *Soil and Tillage Research*, vol. *93,* no. 1, pp. 1 – 12. DOI: 10.1016/j.still.2006.11.004

LOGES, R. – BUNNE, I. – REINSCH, T. – MALISCH, C. – KLUSS, C. – HERMANN, A. – TAUBE, F. 2018. Forage production in rotational systems generates similar yields compares to maize monocultures but improves soil carbon stock. In *Agriculture, Ecosystems and Environment*, vol. *97,* pp. 11 – 19. DOI: 10.1016/j.eja.2018.04.010

LÓPEZ-MÁRSICO, L. – ALTESOR, A. – OYARZABAL, M. – BALDASSINI, P. – PARUELO, J.M. 2015. Grazing increases below-ground biomass and net primary production in a temperate grassland. In *Plant and Soil,* vol. *392,* no. 1 – 2, pp. 155 – 162. DOI: 10.1007/s11104-015-2452-2

MAŠKOVÁ, Z. – DOLEŽAL, J. – KVĚT, J. – ZEMEK, F. 2009. Long-term functioning of a species-rich mountain meadow under different management regimes. In *Agriculture, Ecosystems and Environment*, vol. *132,* pp. 192 – 202. DOI: 10.1016/j.agee.2009.04.002

MEA, Millennium Ecosystem Assessment, 2005. *Ecosystems and human well-being*. Washington DC: Island Press, 155 pp. ISBN 1-59726-040-1

METERA, E. – SAKOWSKIN, T. – SŁONIEWSKI, K. – ROMANOWICZ, B. 2010. Grazing as a toll to maintain biodiversity of grassland – a review. In *Animal Science Papers and Reports*, vol. *28,* no. 4, pp. 315 – 334.

MICHAUD, A. – ANDUEZA, D. – PICARD, F. – PLANTUREUX, S. – BAUMONT, R. 2011. Seasonal dynamics of biomass production and herbage quality of three grasslands with contrasting functional compositions. In *Grass and Forage Science*, vol. *67,* pp. 64 – 76. DOI: 10.1111/j.1365-2494.2011.00821.x

Ministry of Agriculture and Rural Development of the Slovak Republic (MARD SR), 2018. *Report on Agriculture and Food Sector in the Slovak Republic 2017* (Green Report), 76 p. ISBN 978-80-8058-626-3

JANOWIAK, M. – CONNELLY, W.J. – DANTE-WOOD, K. – DOMKE, G.M. – GIARDINA, C. – KAYLER, Z. – MARCINKOOWSKI, K. – ONTL, T. – RODRIGUEZ-FRANCO, C. – SWANSTON, C. – WOODALL, C.W. – BUFORD, M. 2017. *Considering forest and grassland carbon in land management*. Technical Report WO-95. Washington, D.C.: United States Department of Agriculture, Forest Service. 68 p.

PAVLŮ, V. – GAISLER, J. – PAVLŮ, L. – HEJCMAN, M. –

LUDVIKOVÁ, V. 2012. Effect of fertilizer application and abandonment on plant species composition of *Festuca rubra* grassland. In *Acta Oecologica*, vol. *45,* pp. 42 – 49. DOI: 10.1016/j.actao.2012.08.007

PAVLŮ, L. – PAVLŮ, V. – GAISLER, J. – HEJCMAN, M. 2013. Relationship between soil and biomass chemical properties, herbage yield and sward height in cut and unmanaged mountain hay meadow (Polygono-Trisetion). In *Flora*, vol. *208,* pp. 599 – 608. DOI: 10.1016/j.flora.2013.09.003

PAVLŮ, L. – GAISLER, J. – HEJCMAN, M. – PAVLŮ, V. 2016. What is the effect of long-term mulching and traditional cutting regimes on soil and biomass chemical properties, species richness and herbage production in *Dactylis glomerata* grassland? In *Agriculture, Ecosystems and Environment*, vol. *217,* pp. 13 – 21. DOI: 10.1016/j.agee.2015.10.026

POSCHLOD, P. – WALLIS DE VRIES, M.F. 2002. The historical and socioeconomic perspective of calcare*ous grasslands: lessons from distant and recent past.* In *Biological Conservation,* vol. *104,* no. 3, p. 361 – 376. DOI: 10.1016/S0006-3207(01)00201-4

PLANTEREUX, S. – PEETERS, A. – MCKRACKEN, D. 2005. Biodiversity in intensive grassland: Effect of management, improvement and challenges. In *Agronomy Research*, vol. *3,* no. 2, pp. 153 – 164.

ROOK. A.J. – DUMONT, B. – ISSELSTEIN, J. – OSORO, K. – WALLIS DE WRIES, M.F. – PARENTE, G. – MILLS, J. 2004. Matching type of livestock to desired biodiversity outcomes in pastures: a review. In *Biological Conservation,* vol. *119,* pp. 137 – 150. DOI: 10.1016/j.biocon.2003.11.010

RUDA, P. – SALIS, M. – CARRONI, A.M. 2011. Effectiveness of the GAEC cross compliance standard protection of permanent pasture into arable crops in avoiding habitat deterioration. In *Italian Journal of Agronomy*, vol. *6,* no. 1, p. 87 – 91. DOI: 10.4081/ija.2011.6. s1.e12

SAMUIL, C. – VINTU, V. – SIRBU, C. – STAVANCHE, M. 2013. Influence of fertilizers on the biodiversity of seminatural grasslands in the Eastern Carpathins. In *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, vol. *41,* pp. 195 – 200. DOI: 10.15835/nbha4118363

SARNO, R. – TALAMUCCI, P – CAVALLERO, A. – STRINGI, L. 1989. *Distribuzione della produzione ei pascoli in ambienti marginali italiani*. Guida alla valutazione della produttivita. Palermo: Progetto finalizzato CNR-IPRA Aree marginali, pp. 175.

SARZEAUD, P. – PFLIMLIN, A. – PERROT, C. – BECHEREL, F. 2008. Diversity of beef farming systems and grassland use in Europe. In *Grassland Science in Europe*, vol. *13,* pp. 693 – 705.

SCHLÄPFER, B. – RYSER, P. 1996. Leaf and root turnover of three ecologically contrasting grass species in relation to their performance along a productivity gradient. In *Oikos*, vol. *75,* no. 3, pp. 398 – 406.

SCHLESINGER, W.H. 1991. *Biochemistry: An Analysis of Global Change*. San Diego: Academic press, 432 pp. ISBN 978-0-12-625157-9

Statistical Office of the Slovak Republic (SO SR), 2019. Available at: http://www.slovakstatistics.sk.

TALLOWIN, J.R.B. – JEFFERSON, R.G. 1999. Hay production from lowland semi-natural grasslands: a review of implications for ruminant livestock systems. In *Grass and Forage Science*, vol. *54,* no. 2, pp. 99 – 115. DOI: 10.1046/j.1365-2494.1999.00171.x

TOBIÁŠOVÁ, E. – ŠIMANSKY, V. – DĘBSKA, B. – BANACH-SZOTT, M. 2013. Soil structure and soil organic matter of selected soil types in different ecosystems. In *Agriculture (Poľnohospodárstvo)*, vol. *59,* no. 1, pp. 1 – 8. DOI: 10.2478/agri-2013-0001

TOMAŠKIN, J. – JANČOVIČ, J. – VOZÁR, Ľ. – TOMAŠKINOVÁ, J. 2013. The effect of mineral fertilization on belowground plant biomass of grassland ecosystems. In *Acta Universitatis Agriculturae Silviculturae Mendelianae Brunensis*, vol. *61,* no. 5, pp. 1461 – 1440. DOI: 10.11118/actaun201361051431

WILLEMS, J.H. – VAN NIEUWSTADT, M.G.L. 1996. Long-term effects of fertilization on above-ground phytomass and species diversity in calcareous grassland. In *Journal of Vegetation Science*, vol. *7,* pp. 177 – 184. DOI: 10.2307/3236317

Received: June 4, 2019
Accepted: November 11, 2019

sciendo

Agriculture (Poľnohospodárstvo), 65, 2019 (4): 164 − 171

Original paper

DOI: 10.2478/agri-2019-0017

SORPTION CAPACITY OF SANDY SOIL UNDER LONG-TERM FERTILISATION

VLADIMÍR ŠIMANSKÝ [1]*, JERZY JONCZAK[2]

[1]Slovak University of Agriculture in Nitra
[2]Warsaw University of Life Sciences

ŠIMANSKÝ, V. – JONCZAK, J.: Sorption capacity of sandy soil under long-term fertilisation. Agriculture (Poľnohospodárstvo), vol. *65*, 2019, no. 4, pp. 164 – 171.

In this paper, the results of an investigation of the effects of particle-size distribution, soil organic matter content and its parameters on soil sorption capacity are presented and their mutual relationships in sandy soils under long-term fertilisation experiments are determined. Soil samples were taken at the experimental station of Warsaw University of Life Sciences located in Skierniewice, (Poland) in spring 2017. The study included 94- and 41-year-old experiments with mineral fertilisation (no fertilisation, NPK, CaNPK) and 25-year-old experiment with mineral fertilisation + farmyard manure (FYM) in 4-year cycle: FYM, FYM+NPK and FYM+CaNPK. The results show that in the 94-year-old experiment in NPK and CaNPK treatments, hydrolytic acidity (Ha) decreased in comparison with the control by 30% and 88%, respectively, while in 25- and 41-year-old experiments only the application of NPK significantly increased Ha values. The sum of basic cations increased by a factor of 10 at the most in the CaNPK treatment in the 94-year-old experiment. The same effect was also observed in the 25-year-old experiment. On the one hand, the sorption complex gradually became fully saturated as a result of fertilisation in the 94-year-old experiment. On the other hand, in the 25- and 41-year-old experiments, base saturation was substantially reduced. A higher humus stability was an important agent for improving soil sorption capacity in 41- and 94-year old experiments.

Key words: base saturation, cation exchange capacity, hydrolytic acidity, long-term fertilisation, sandy soil

Soil is a heterogeneous body consisting of inorganic and organic solid particles, air, water and living organisms. All aforementioned phases or components affect each other. Reactions of the solids affect air and water quality, air and water are key-factors of mineral substrate weathering, microorganisms catalyze numerous reactions and control biogeochemical cycling of elements and various substances, influencing soil quality and productivity. Humankind is dependent on soils – and to a certain extent good soils are dependent upon man and the use he makes of them (Foth 1990).

Soil sorption capacity is considered as one of the most significant factors affecting soil fertility. The term soil sorption capacity expresses its potential to sequester – or sorb – ions or molecules of different substances from the soil solution. This very important soil feature is associated with the soil colloidal complex that includes mineral and organic components. Thanks to this soil property, plant root system is supported by a supply of biogenic elements from the soil; meaning that farmers do not need to add these elements on a daily basis (Foth 1990; Lorandi 2012; Šimanský & Polláková 2014; Šimanský *et al.* 2018). Soil organic matter, especially the decomposed and humified variety, together with soil clay content significantly influence soil sorption capacity (Foth 1990, Manjaiah *et al.* 2019). In addition,

doc. Ing. Vladimír Šimanský, PhD. (* Corresponding author), Department of Soil Science, FAFR – SUA Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic. E-mail: vladimir.simansky@uniag.sk
Dr. hab. Jerzy Jonczak, Department of Soil Science, Warsaw University of Life Sciences – SGGW, Nowoursynowska Str. 159, 02-776 Warszawa, Poland. E-mail: jerzy_jonczak@sggw.pl

the cation exchange capacity (CEC) is one of the most important parameters of soil sorption capacity and rages in sandy, loamy, clay and organic soils between 20 − 100, 200 − 300, 400 − 500 and more than 1,500 mmol (p^+)/kg of soil, respectively (Hanes 1999).

In arable soils, the soil sorption capacity can be altered by different soil management practices (Lorandi 2012; Šimanský & Polláková 2014). Fertilisers are an important factor of crop production, offer intensifying modern agriculture. In addition to the positive effects on crop yields, they also affect soil properties (Banerjee *et al.* 2019). Fertilisation improves crop supporting properties of sandy soils in particular, due to their coarse texture, high permeability and low water and nutrient storage capacity. In addition to the increase in yields, the use of fertilisers in the soil can increase the biomass of plants and roots, eventually resulting in SOM increase (Tian *et al.* 2015). Murawska *et al.* (2017) have shown that after 36 years of fertilising the sandy soil by NPK without or with farmyard manure, the content of SOM increased, and at the same time the quality of humus improved in this soil. Higher content of SOM and humus quality are considered to be major factors influencing soil sorption complex.

In this work, the effects of long-term fertilisation on changes in soil sorption capacity are focused. The objectives of this study are (i) to quantify the effects of long-term fertilisation on the soil sorption parameters of sandy soils and (ii) to identify feedbacks between soil sorption parameters and soil organic matter and particle-size distribution in long-term experiments with fertilisers and manure application. The following hypotheses were tested: (H1) application of mineral fertilisers and farmyard manure improves soil sorption capacity, mainly by increasing the SOM content, and (H2) the length of fertilisation significantly affects the relationship between soil sorption parameters, and SOM or soil clay content.

MATERIAL AND METHODS

The experimental field plot was located in Skierniewice (experimental station of Warsaw University of Life Sciences – SGGW; 51°57’54.3”N 20°09’31.8”E). The annual average temperature and precipitation are 8°C and 530 mm respectively, measured at a meteorological station in Skierniewice. The soil was classified as Arenic Planosol (IUSS Working Group WBR 2015). The soil were characterized by loamy sand or sandy loam texture in A-horizons and contained 76.0 − 88.2% of sand, 7.1 − 16.7% silt and 4.0 − 7.7% clay. The soils were relatively poor in organic carbon and their pH varied from strongly acidic to slightly acidic, depending on experiment option.

Prior to the experiment, the soil at the experimental site were cultivated using conventional agriculture techniques adequate of actual period. The oldest experiment was established in 1923 (94-year-old experiment), the second experiment in 1975 (41-year-old experiment) and the third in 1992 (25-year-old experiment). The experimental design is shown in Figure 1. In all experiments, each treatment had three replications. The investigated fertilisation treatments and planted crops are presented in Table 1. The study was carried out on 27 plots of 36 m^2 (4 m × 9 m) each. The spacing between the neighbouring replications was 2 m. In all long-term experiments, nitrogen was applied as ammonium sulphate (30 kg/ha of N every year from 1921 to 1976 and 90 kg/ha of N from 1976), phosphorus as superphosphate (every year at rate of 30 kg/ha of P_2O_5 from 1921 to 1976 and 26 kg/ha of P_2O_5 from 1976) and potassium as potassium chloride (30 kg/ha of K_2O every year from 1921 to 1976 and 91 kg/ha of K_2O from 1975). Calcium was introduced as 1.6 t/ha of CaO every 4 years. In 25-year-old experiment the used doses of farmyard manure at the plots were 25 t/ha every 4 years beginning from 1992.

The soils were sampled at a depth of 0 − 20 cm in autumn 2017. Three randomly distributed soil sub-samples per each plot were collected and mixed into one average sample. Soil samples were then air-dried, crushed and sieved (2 mm) for analyses. Standard procedures of soil analyses were used to determine soil characteristics. Particle-size distribution was analysed by the pipette method described by Hrivňaková *et al.* (2011). Soil pH was measured potentiometrically in distilled water (at ratio 1:2.5, soil: distilled water). Soil organic carbon content (SOC) was estimated by the Tyurin wet oxidation method (Dziadowiec & Gonet 1999). Fractional composition of humus was analysed using the Tyurin method as modified by Ponomareva and Plotnikova (Dziadowiec & Gonet 1999). Optical characteristics of humus quality were assessed as absorbance of humic substances and humic acids measured at wavelength of 465 and 650 nm using a Jenway Model 6400 spectrophotometer. Labile carbon content (C_L) was determined according to Łoginow *et al.* (1987). Soil sorption parameters such as: hydrolytic acidity (Ha) and sum of basic cations (SBC) were determined by the Kappen method (Hrivňáková *et al.* 2011) and based on values of Ha and SBC cation exchange capacity (CEC), and base saturation (Bs) were calculated according to Equations 1 – 2.

Table 1

Experiment design

Experiment name	FYM	Mineral fertilisation	Crop rotation
94-year-old experiment with mineral fertilisation	No	No	Cereals from 1923
	No	NPK	
	No	CaNPK	
41-year-old experiment with mineral fertilisation	No	No	Cereals from 1923 to 1975, blueberry from 1976, no tillage from 1976
	No	NPK	
	No	CaNPK	
25-year-old experiment with mineral fertilisation + FYM in 4-year cycle	Yes	No	Cereals from 1923, farmyard manure from 1992
	Yes	NPK	
	Yes	CaNPK	

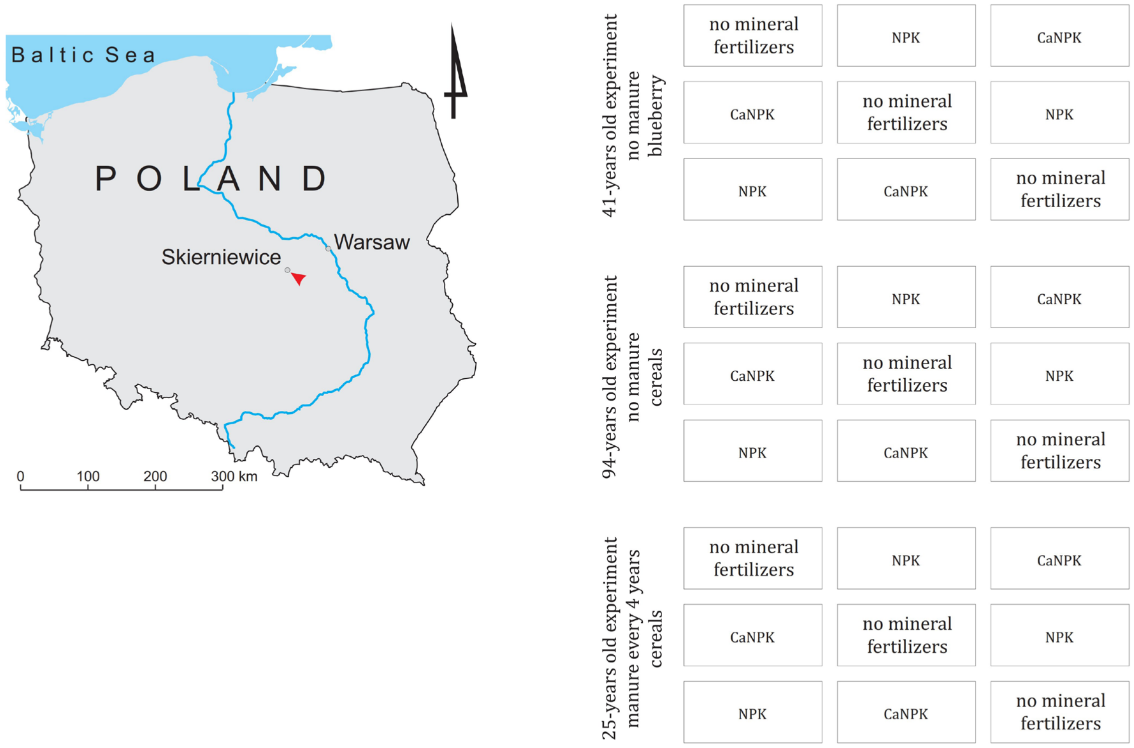

Figure 1. Study site location and schematic layout of the experimental field

$$CEC = Ha + SBC \quad (1)$$

$$Bs = SBC/CEC \times 100 \quad (2)$$

All data was analysed using the Statgraphics Centurion XV.I programme (Statpoint Technologies, Inc., USA). The data was analysed using one-way ANOVA and the means were compared with *LSD* test at $P < 0.05$. The link between the sorption parameters and SOM and particle-size distribution were assessed using a correlation matrix.

RESULTS AND DISCUSSION

Each experiment has been analysed separately and all experiments were based on the same soil type (Arenic Planosol) with comparable textures (clay content ranged from 4.40% to 5.77% in fertilisation experiments). We also compared soil parameters describing experiments of different duration. Fertilisation can affect soil properties in different ways as shown in our results (Figure 2). We found significant influence on soil pH in all long-term experiments, but the strength of the effect was modified by the duration of the experiment. Compared with the control plot, the NPK ($P > 0.05$) and CaNPK ($P < 0.05$) treatments increased soil pH by 25% and 86%, respectively in 94-year-old experiment, whereas in 41- and 25-year-old experiments the NPK treatment had only shown a significant decrease in the soil pH, which decreased by 24% and 16%, respectively. The pH was associated with SOC. Between SOC and soil pH exist negative linear relationship – the higher SOC content is the reason for a lower soil pH (Jagadamma *et al.* 2008). As Nardi *et al.* (2004) presented, soil acidification can be related to the SOM mineralisation process, which produces nutrients (in particular NH_3), whose oxidation may contribute to H^+ production (Tan 1998). This reaction may also be affected by mineral fertilisers, especially NH^+_4 sources (Havlin *et al.* 1999), an effect apparent also in our study (N applied as ammonium sulphate). In addition, application of CaO every 4 years significantly reduced soil acidification in all long-term experiments. Positive effect of liming on soil pH have been described previously by many authors (e.g. Merha *et al.* 2019). Soil pH negatively correlated with values of hydrolytic acidity (Ha) in all treatments and all long-term experiments. Higher values of soil pH resulted in

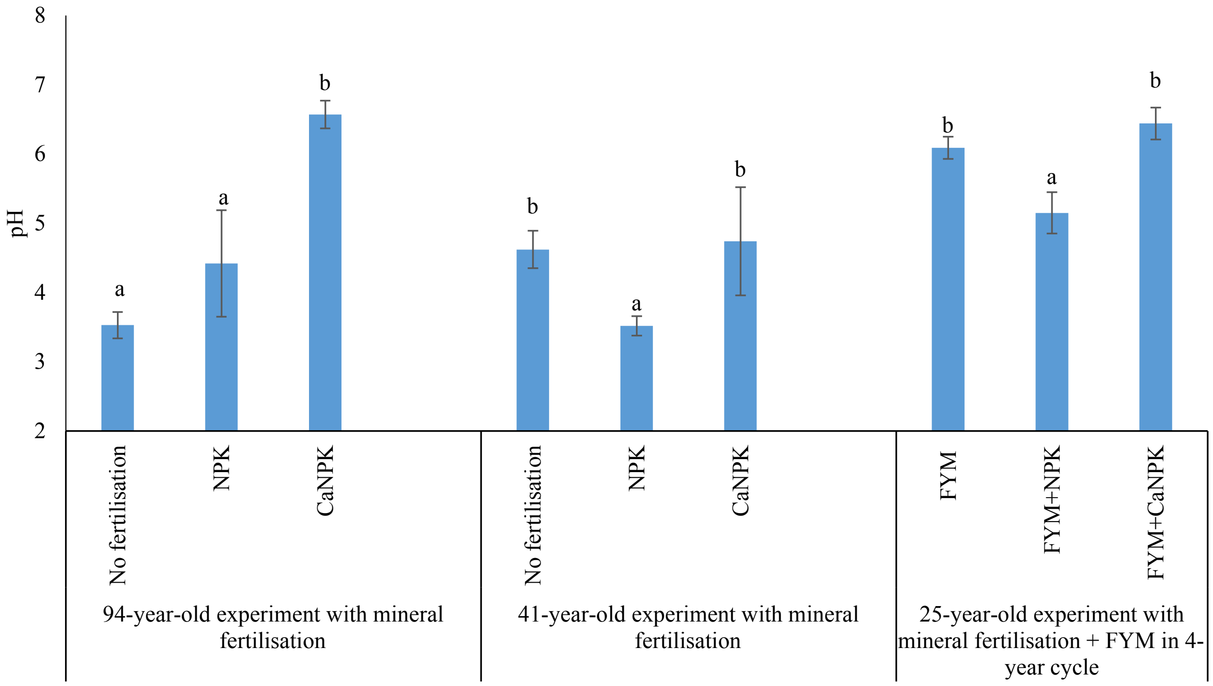

Figure 2. Effect of fertilisation on soil pH in long-term experiments

Different letters (a, b) between columns indicate that treatment means are significantly different at $P < 0.05$ according to *LSD* test.

lower Ha (in 94-year-old: $r = –0.986$, $P < 0.001$; in 41-year-old: $r = –0.887$, $P < 0.01$; in 25-year-old: $r = –0.896$, $P < 0.01$). Values of Ha were affected by long-term experiments. In 94-year-old experiment added NPK (NPK) and Ca in NPK treatment (CaNPK) decreased Ha by 30% and 88%, respectively compared with control treatment, while in 25- and 41-year-old experiment only application of NPK significantly increased Ha values. If all experiments were compared, the lowest values of Ha in control treatments were observed in 25-year-old experiment. Probably applied FYM every 4-year in this experiment is responsible for better values of Ha in comparison to other both experiments. Applied FYM to the soil can affect buffering capacity of soil (Hanes 1999).

In sandy soils, even a small increase in fine-grain fraction or SOM can significantly affect sorption capacity. Our results show that ninety-four years of continuous inputs of mineral fertilisers (NPK) increased the SOC content, while the application of CaNPK caused the SOC content to increase from 4.07 to 5.99 g/kg. The same effect was observed in the shorter experiments – in 41- and 25-years-old (Figure 3). More detailed results with respect to SOM parameters and humus quality in these experiments are presented in Šimanský *et al.* (2019). Up to 10 times, sum of basic cations (SBC) increased in CaNPK treatment at 94-year-old experiment. The same effect was observed in 25-year-old experiment, but on the other hand in 41-year-old experiment any significant effect on SBC values due to fertilisation was determined. This means that the SBC values were affected in all except the long-term fertilisation also by a combination of mineral and organic fertilisation. CEC increased significantly in the 94- and 25-year experiments due to the addition of Ca to NPK treatment, while in the 41-year experiment it has been significantly increased only in NPK. In this case it is associated with the extreme increase Ha (in this treatment). The results of Reeves (1997) also showed that the CEC increased after fertilisation with organic fertilisers and NPK fertiliser. The sorption complex gradually fully saturated as a result of fertilisation in the 94-year-old experiment, on the other hand, in 25- and 41-year-old experiments the base saturation substantially reduced (Table 2).

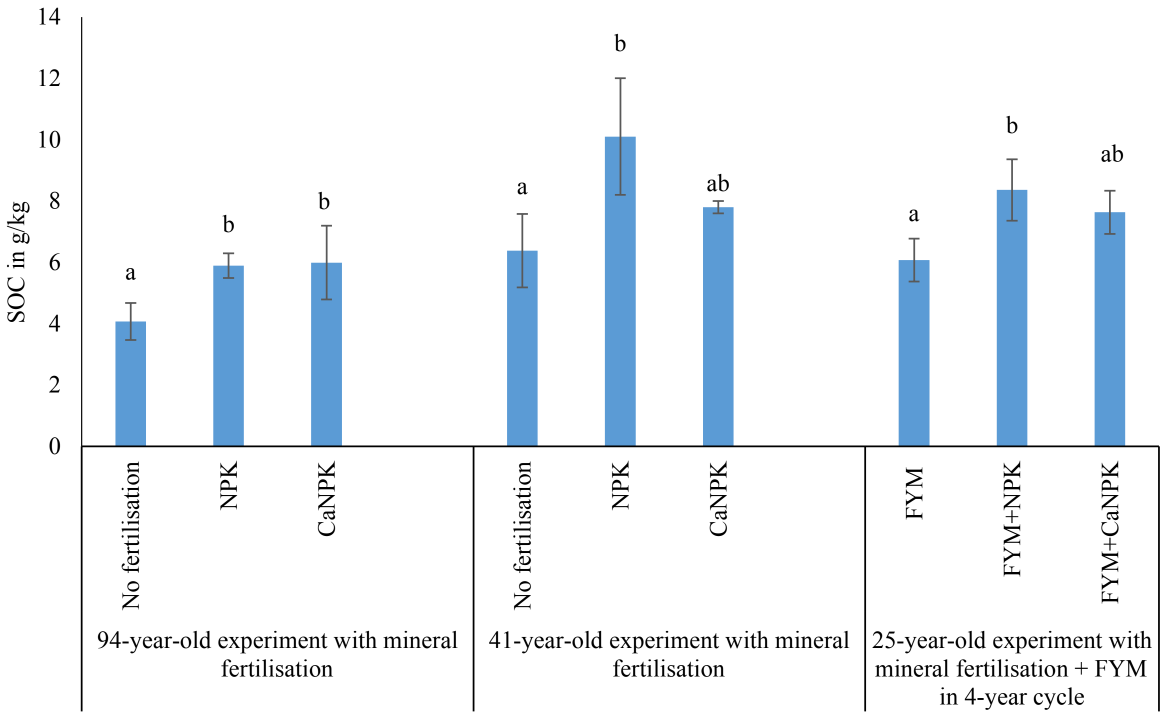

Figure 3. Effect of fertilisation on SOC in long-term experiments

Different letters (a, b) between columns indicate that treatment means are significantly different at $P < 0.05$ according to *LSD* test.

Correlation coefficients between sorption parameters, particle-size distribution and SOM parameters are shown in the Table 3. It is likely that the fertilisation period might be responsible for stronger correlation between the soil sorption parameters and SOM as well as clay content, however, our data cannot confirm this conclusively. The highest number of relationships was determined in the 41-year-old experiment, followed by the 94- > 25-year-old experiment. In the 94-year-old experiment, only Bs values positively correlated with C_L on the one hand, and negative with HS, HA, Q_{HS} and Q_{HA} on the other. This indicates that the labile fraction of SOM, but also humus stability, are responsible for observed saturation of the sorption complex. Overall, all sorption parameters were positively affected through higher stability of humic substances. Humus stability has positive effects on soil properties (Stevenson 1994; Lorandi 2012). In the 41-year-old experiment, higher sand content decreased Ha and CEC, but higher silt and SOC content increased the values of Ha and CEC. Grain size, together with SOM, are the key factors of soil sorption capacity (Stevenson 1994; Šimanský & Polláková 2014). A higher content of HS resulted in a decrease of Ha and an increase of SBC. Higher humus quality in the soil caused higher decreases in the hydrolytic acidity and improves sorption parameters at the same time. In addition, soil sorption complex was fully saturated due to better humus quality. The lower the values of colour quotient of humic substances and humic acids, the more improved soil sorption parameters in the 41-year-old experiment. These results support the fact that the application of mineral fertilisers can increase SOM quality (Fröberg *et al.* 2013). SOM quality is associated with higher soil sorption capacity (Foth 1990; Šimanský & Polláková 2014). In the shortest experiment, the sand content negatively correlated with SBC and CEC, however the silt content positively correlated with values of SBC and CEC. Clay content did not show statistically significant effects on sorption parameters (Table 3), which was surprising based on literature data (e.g. Ding *et al.* 2014). Negative effects of higher con-

Table 2

Statistical evaluation of soil sorptive parameters

Treatments	Ha	SBC	CEC	Bs
94-year-old experiment with mineral fertilisation				
No fertilisation	27.40 ± 0.52^{b}	7.31 ± 1.51^{a}	34.7 ± 1.48^{a}	20.9 ± 3.47^{a}
NPK	19.10 ± 7.85^{b}	20.50 ± 7.56^{a}	39.6 ± 2.41^{a}	51.9 ± 18.6^{b}
CaNPK	3.15 ± 1.11^{a}	77.60 ± 19.10^{b}	80.8 ± 18.5^{b}	95.9 ± 2.15^{c}
P-value	*0.0018*	*0.0008*	*0.0037*	*0.0005*
41-year-old experiment with mineral fertilisation				
No fertilisation	20.2 ± 2.65^{a}	25.5 ± 4.00^{a}	45.7 ± 1.90^{a}	55.6 ± 6.94^{b}
NPK	66.5 ± 5.26^{b}	11.3 ± 4.47^{a}	76.8 ± 6.73^{b}	14.4 ± 4.98^{a}
CaNPK	34.9 ± 15.95^{a}	22.2 ± 12.5^{a}	57.0 ± 7.46^{a}	39.8 ± 25.5^{ab}
P-value	*0.0032*	*0.1535*	*0.0019*	*0.0459*
25-year-old experiment with mineral fertilisation + FYM in 4-year cycle				
FYM	6.18 ± 1.53^{a}	31.7 ± 4.54^{a}	37.9 ± 3.56^{a}	83.5 ± 5.00^{b}
FYM+NPK	25.4 ± 4.20^{b}	18.2 ± 5.80^{a}	43.6 ± 3.65^{a}	41.4 ± 10.9^{a}
FYM+CaNPK	10.4 ± 2.38^{a}	73.6 ± 19.5^{b}	84.0 ± 17.7^{b}	86.9 ± 5.62^{b}
P-value	*0.0005*	*0.0032*	*0.0034*	*0.0006*

Ha – hydrolytic acidity; SBC – sum of basic cations; CEC – cation exchange capacity; Bs – base saturation Different letters (a, b, c) between lines indicate that treatment means are significantly different at $P < 0.05$ according to *LSD* test tents of extracted humic substances mainly fulvic acids indicate the correlations between HS and Ha ($r = 0.776$, $P < 0.01$) also between FA and Ha ($r = 0.808$, $P < 0.01$) and between HS and Bs ($r = -0.752$, $P < 0.01$) as well as between FA and Bs ($r = -0.690$, $P < 0.05$). In the soil, fulvic acids are more aggressive and it had a higher mobility in the soil in comparison to humic acids (Tate 1987; Stevenson 1994). Organic matter after FYM application is decomposed – except mineralization through humification process. In soils with low biological activity such as sandy soils, FA are accumulated as a result of the humification process (Tate 1987), with negative effects on the sorption parameters (Table 3). Low humus quality due to long-term application of mineral fertilisation but as well as its combination with FYM in these experiments was also observed (Šimanský *et al.* 2019).

CONCLUSIONS

The results of this study showed that higher humus stability is the important agent for improving soil sorption capacity in the 41- and 94-year old experiments. In the 25-year-old experiment, this relationship was not observed. The strongest relationships between grain size distribution, SOM parameters and sorption parameters were determined in the 41-year-old

Table 3

Correlation coefficients between particle-size distribution, SOM and humus parameters and soil sorption parameters with depend on fertilisation

	Clay	Sand	Silt	SOC	C_L	C_{HS}	C_{HA}	C_{FA}	C_{HA}:C_{FA}	Q_{HS}	Q_{HA}
94-year-old experiment with mineral fertilisation											
pH	n.s.	n.s.	n.s.	n.s.	n.s.	-0.774^{+}	-0.666^{+}	n.s.	n.s.	-0.772^{+}	-0.737^{+}
Ha	n.s.	n.s.	n.s.	n.s.	n.s.	0.734^{+}	n.s.	n.s.	n.s.	0.701^{+}	0.744^{+}
SBC	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.827^{++}	n.s.
CEC	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.844^{++}	n.s.
Bs	n.s.	n.s.	n.s.	n.s.	0.692^{+}	-0.778^{+}	-0.693^{+}	n.s.	n.s.	-0.683^{+}	-0.707^{+}
41-year-old experiment with mineral fertilisation											
pH	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.727^{+}	-0.734^{+}	n.s.
Ha	n.s.	-0.674^{+}	0.799^{++}	0.811^{++}	n.s.	n.s.	-0.733^{+}	n.s.	-0.895^{++}	0.865^{++}	0.838^{++}
SBC	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.735^{+}	n.s.	0.819^{++}	-0.713^{+}	n.s.
CEC	n.s.	-0.841^{++}	0.919^{+++}	0.825^{++}	n.s.	n.s.	n.s.	n.s.	0.807^{++}	-0.825^{++}	-0.889^{++}
Bs	n.s.	n.s.	n.s.	-0.687^{+}	n.s.	n.s.	0.728^{+}	n.s.	0.903^{+++}	-0.773^{+}	-0.714^{+}
25-year-old experiment with mineral fertilisation + FYM in 4-year cycle											
pH	n.s.	n.s.	n.s.	n.s.	n.s.	-0.686^{+}	n.s.	n.s.	n.s.	n.s.	n.s.
Ha	n.s.	n.s.	n.s.	n.s.	n.s.	0.776^{++}	n.s.	0.808^{++}	n.s.	n.s.	n.s.
SBC	n.s.	-0.839^{++}	0.830^{++}	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CEC	n.s.	-0.891^{++}	0.891^{++}	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Bs	n.s.	n.s.	n.s.	n.s.	n.s.	-0.752^{++}	n.s.	-0.690^{+}	n.s.	n.s.	n.s.

SOC – soil organic carbon; C_L – labile carbon; C_{HS} – content of humic substances carbon; C_{HA} – content of humic acids carbon; C_{FA} – content of fulvic acids carbon; C_{HA}:C_{FA} – humic acids carbon to fulvic acids carbon ratio; Q_{HS} – colour quotient of humic substances; Q_{HA} – colour quotient of humic acids; Ha – hydrolytic acidity; SBC – sum of basic cations; CEC – cation exchange capacity; Bs – base saturation.
n.s. $P > 0.05$; $^{+}P < 0.05$; $^{++}P < 0.01$; $^{+++}P < 0.001$

experiment, followed by the 94- > 25-year-old experiment.

This research provides information for farmers on optimizing soil management practices in sandy soils and on improving soil ecological functions and stability to avoid environmental degradation due to application of mineral fertilisers to the soil. The results of this study emphasize the importance of organic matter quantity and quality in relation to sorption capacity, especially in sandy soils under long-term mineral fertilisation.

REFERENCES

BANERJEE, H. – KRISHNENDU, R. – DUTTA, S. KR. 2019. Fertilizer best management practices: Concepts and recent advances. In RAKSHIT, A. – SARKAR, B. – PURUSHOTHAMAN, CH. A. (Eds.) *Soil amendments for sustainability. Challenges and perspectives*. Boca Raton, Fl : CRC Press Taylor & Francis Group, pp. 33 – 46. ISBN 978-0-8153-7077-3

DING, F. – HUANG, Y. – SUN, W. – JIANG, G. – CHEN, Y. 2014. Decomposition of organic carbon in fine soil particles is likely more sensitive to warming than in coarse particles: an incubation study with temperate grassland and forest soils in Northern China. In *PLos One*, vol. *9*, pp. e95348. DOI: 10.1371/journal.pone.0095348

DZIADOWIEC, H. – GONET, S.S. 1999. *Przewodnik metodyczny do badań materii organicznej gleb* [Methodical guide-book for soil organic matter studies]. Prace Komisji Naukowych Polskiego Towarzystwa Gleboznawczego, N. 120, Komisja chemii gleb, Zespół Materii Organicznej Gleb, N II/16, 65 p.

FRÖBERG, M. – GRIP, H. – TIPPING, E. – SVENSSON, M. – STRÖMGREN, M. – KLEJA, D.B. 2013. Long-term effects of experimental fertilization and soil warming on dissolved organic matter leaching from a spruce forest in Northern Sweden. In *Geoderma* vol. *200–201,* pp. 172 – 179. DOI: 10.1016/j.geoderma.2013.02.002

FOTH, H.D. 1990. *Fundamentals of soil science.* New York : John Wiley and Sons, pp. 360.

HANES, J. 1999. *Analýza sorpčných vlastností pôd* [Analyzes of sorptive characteristics]. Bratislava : SSCRI, pp. 138.

HAVLIN, J.L. – BEATON, J.D. – TISDALE, S.L. – NELSON, W.L. 1999. *Soil fertility and fertilizers.* An Introduction to Nutrient Management. NJ : Prentice Hall, Upper Saddle, pp. 485.

HRIVŇÁKOVÁ, K. – MAKOVNÍKOVÁ, J. – BARANČÍKOVÁ, G. – BEZÁK, P. – BEZÁKOVÁ, Z. – DODOK, R. – GREČO, V. – CHLPÍK, J. – KOBZA, J. – LIŠTJAK, M. – MALIŠ, J. – PÍŠ, V. – SCHLOSSEROVÁ, J. – SLÁVIK, O. – STYK, J. – ŠIRÁŇ, M. 2011. *Jednotné pracovné postupy rozborov pôd* [Uniform methods of soil analyses]. Bratislava : VÚPOP, pp 136.

IUSS Working Group WRB. 2015. *World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps. Update 2015.* World Soil Resources Reports No. 106, Rome: FAO, pp. 192.

JAGADAMMA, S. – LAL, R. – HOEFT, R.G. – NAFZIGER, E.D. – ADEE, E.A. 2008. Nitrogen fertilization and cropping system impacts on soil properties and their relationship to crop yield in the central Corn Belt, USA. In *Soil and Tillage Research*, vol. *98,* pp. 120 – 129.

ŁOGINOW, W. – WISNIEWSKI, W. – GONET, S.S. – CIESCINSKA, B. 1987. Fractionation of organic carbon based on susceptibility to oxidation. In *Polish Journal of Soil Science*, vol. *20,* pp. 47 – 52.

LORANDI, R. 2012. Evaluation of Cation Exchange Capacity (CEC) in Tropical Soils Using Four Different Analytical Methods. In *Journal of Agricultural Science*, vol. *4,* pp. 278 – 289. DOI: 10.5539/jas.v4n6p278

MANJAIAH, K.M. – MUKHOPADHYAY, R. – NARAYANAN, N. 2019. Clay amendments for environmental clean-up. In RAKSHIT, A. – SARKAR, B. – PURUSHOTHAMAN, CH. A. (Eds.) *Soil amendments for sustainability. Challenges and perspectives*. Boca Raton, Fl : CRC Press Taylor & Francis Group, pp. 19 – 32. ISBN 978-0-8153-7077-3

MEHRA, P. – SARKAR, S. – BOLAN, N. – CHOWDHURY, S. – DESBIOLLES, J. 2019. Impact of carbonates on the mineralisation of surface soil organic carbon in response to shift in tillage practice. In *Geoderma*, vol. *339,* pp. 94 – 105. DOI: 10.1016/j.geoderma.2018.12.039

MURAWSKA, B. – KONDRATOWICZ-MACIEJEWSKA, K. – SPYCHAJ-FABISIAK, E. – RÓZAŃSKI, S. – KNAPOWSKI, T. – RUTKOWSKA, B. 2017. The impact of long-term application of inorganic nitrogen fertilizers and manure on changes of selected properties of organic matter in sandy loam soil. In *Journal of Central European Agriculture*, vol. *18,* pp. 542 – 553. DOI: 10.5513/JCEA01/18.3.1928

NARDI, S. – MORARI, F. – BERTI, A. – TOSONI, M. – GIARDINI, L. 2004. Soil organic matter properties after 40 years of different use of organic and mineral fertilisers. In *European Journal of Agronomy*, vol. *21,* pp. 357 – 367. DOI: 10.1016/j.eja.2003.10.006

REEVES, D.W. 1997. The role of soil organic matter in maintaining soil quality in continuous cropping system. In *Soil and Tillage Research*, vol. *43,* pp. 131 – 167. DOI: 10.1016/S0167-1987(97)00038-X

STEVENSON, J.F. 1994. *Humus chemistry.* New York : John Wiley & Sons, pp. 512.

ŠIMANSKÝ, V. – HORÁK, J. – IGAZ, D. – BALASHOV, E. – JONCZAK J. 2018. Biochar and biochar with N fertilizer as a potential tool for improving soil sorption of nutrients. In *Journal of Soils and Sediments*, vol. *18,* pp. 1432 – 1440. DOI: 10.1007/s11368-017-1886-y

ŠIMANSKÝ, V. – JURIGA, M. – JONCZAK, J. – UZAROWICZ, L. – STAPIEŃ, W. 2019. How relationships between soil organic matter parameters and soil structure characteristics are affected by the long-term fertilization of a sandy soil. In *Geoderma*, vol. *342,* pp. 75 – 84. DOI: 10.1016/j.geoderma.2019.02.020

ŠIMANSKÝ, V. – POLLÁKOVÁ, N. 2014. Soil organic matter and sorption capacity under different soil management practices in a productive vineyard. In *Archives of Agronomy and Soil Science*, vol. *60,* pp. 1145 – 1154. DOI: 10.1080/03650340.2013.865837

TAN, K.W. 1998. *Principles of soil chemistry.* 3rd ed. New York : Marcel Dekker, pp. 556.

TATE, L.R. 1987. *Soil organic matter: Biological and ecological effects.* New York : John Willey & Sons, pp. 340.

TIAN, K. – ZHAO, Y. – XU, X. – HAI, N. – HUANG, B. – DENG, W. 2015. Effects of long-term fertilization and residue management on soil organic carbon changes in paddy soils of China: a meta-analysis. In *Agriculture Ecosystem & Environment*, vol. *204,* pp. 40 – 50.

Received: May 6, 2019
Accepted: November 11, 2019