МАТИЦА СРПСКА
1826

МАТИЦА СРПСКА
ОДЕЉЕЊЕ ЗА ПРИРОДНЕ НАУКЕ
ЗБОРНИК
МАТИЦЕ СРПСКЕ ЗА ПРИРОДНЕ НАУКЕ

MATICA SRPSKA
DEPARTMENT OF NATURAL SCIENCES
MATICA SRPSKA J. NAT. SCI.

Покренут 1951 / First published in 1951.

Until volume 10, the journal was published under the title *Научни зборник Матице српске: Серија природних наука* (Scientific Proceedings of Matica Srpska: Natural Sciences Series) (1951–1955). Volume 11 was released under the title *Зборник Матице српске: Серија природних наука* (Matica Srpska Proceedings: Natural Sciences Series) (1956), volumes 12–65 under the title *Зборник за природне науке* (Proceedings for Natural Sciences) (1957–1983), and from volume 66 the journal was published under the title *Зборник Матице српске за природне науке* (Matica Srpska Proceedings for Natural Sciences) (1984–). From volume 84 (1993) the journal was published in English under the title *Matica Srpska Proceedings for Natural Sciences* (1993–2012), and since volume 125 under the title *Matica Srpska Journal for Natural Sciences* (2013–)

138

Articles are available in full-text at the web site of Matica Srpska and in the following data bases: Serbian Citation Index, EBSCO Academic Search Complet, abstract level at Agris (FAO), CAB Abstracts, CABI Full-Text and Thomson Reuters Master Journal List.

YU ISSN 0352-4906 UDK 5/6 (05)

MATICA SRPSKA
JOURNAL FOR
NATURAL SCIENCES

138

NOVI SAD
2020

CONTENTS / САДРЖАЈ

ARTICLES AND TREATISES / ЧЛАНЦИ И РАСПРАВЕ

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. Novi Sad, № 138, 9—18, 2020

UDC 631.417.4:504.7(497.11)
https://doi.org/10.2298/ZMSPN2038009V

Dragana D. VIDOJEVIĆ[1*], *Maja S. MANOJLOVIĆ*[2], *Aleksandar R. ĐORĐEVIĆ*[3], *Ljiljana M. NEŠIĆ*[2], *Tihomir M. PREDIĆ*[4]

[1] Ministry of Environmental Protection of the Republic of Serbia,
Environmental Protection Agency,
Ruže Jovanović 27a, Belgrade 11160, Serbia
[2] University of Novi Sad, Faculty of Agriculture, Department of Field and Vegetable Crops,
Trg Dositeja Obradović 8, Novi Sad 21000, Serbia
[3] University of Belgrade, Faculty of Agriculture, Department of Pedology and Geology,
Nemanjina 6, Zemun–Belgrade 11080, Serbia
[4] Agricultural Institute of Republic of Srpska, Department of Agroecology,
Knjaza Miloša 17, Banja Luka, Republic of Srpska

CORRELATIONS BETWEEN SOIL ORGANIC CARBON, LAND USE AND SOIL TYPE IN SERBIA

ABSTRACT: Correlation between soil organic carbon (SOC) and land use and soil type were investigated in the soils of the Republic of Serbia. The database included a total of 1,140 soil profiles. To establish the correlation between organic carbon content and soil type, a soil map of Serbia was adapted to the WRB classification and divided into 15,437 polygons (map units). The SOC stock values were calculated for each reference soil group based on mean values of SOC at 0–30 and 0–100 cm and their areas. The largest SOC stocks for the soil layers 0–30 cm were found in Cambisol 194.76 x 10^{12} g and Leptosol 186.43 x 10^{12} g and for the soil layers 0–100 cm in Cambisol 274.87 x 10^{12} g and Chernozem 230.43 x 10^{12} g. Using the Corine Land Cover (CLC) database, the major categories of land use were defined. Based on the obtained mean values of organic carbon content for the soil layers 0–30 and 0–100 cm and the areas indicated by Corine Land Cover categories of land use, the organic carbon stocks in agricultural soil, forest soil, semi-natural areas, and artificial areas were calculated. The correlation of organic carbon stocks and the different land use categories, soil reference group, and soil depth was studied for reference groups that occupy the major part of central Serbia, such as Cambisol (taking up 37.76% of the territory) and Leptosol (22.22% of the territory), and have a sufficient number of sites that were required for this type of analysis.

KEYWORDS: correlation, land use, organic carbon stocks, reference group

* Corresponding author. E-mail: dragana.vidojevic@sepa.gov.rs

INTRODUCTION

Soil is a natural resource with accumulated large organic carbon stocks (Lal, 2004; Manojlović, 2008). Appropriate land use aimed at increasing the level of organic carbon can increase the productivity and sustainability of agricultural ecosystems (Cole et al., 1997). Also, appropriate land use has a role in alleviating greenhouse gas effects, given that the soil is capable of either releasing or absorbing carbon. The total content of organic matter in the soil is higher in the conservation tillage as compared to plowing in winter wheat and sunflower production (Šeremešić et al., 2016). Statistical analysis has shown that there is a significant effect of the tillage system and the crop on the change of the total content of organic matter. Increasing organic matter in the soils is an important strategy of biological immobilization of carbon (Manojlović and Aćin, 2007; Manojlović et al., 2008). To ensure sustainable land use, the organic matter in the soils must be kept at a satisfactory level. The content of organic matter in specific layers of the soil is a basis for the calculation of organic matter up to a meter depth (Gobin et al., 2011; Vidojević and Manojlović, 2010). In this manner, organic carbon stocks can be assessed against the soil type and its specific use. Identifying parameters influencing the reduction of organic carbon stocks in the soil, as well as interconnectedness of these parameters lays the foundation for mapping the areas at risk of organic matter reduction. Organic carbon stocks in the soils of the Republic of Serbia have not been assessed consistently. The results of the assessment done so far have shown that a universal approach that would apply to a large area is not possible and that more detailed analyses will require more precise and reliable data at both regional and national levels.

MATERIAL AND METHODS

Soil database

In the period 2009–2011, a database was established which served as the basis for further research. Its objective was to collate all available data and to adapt them to fit the base. Presently, the database includes a total of 1,140 soil profiles which involve 4,335 horizons. The database comprises the data for analytical study collected in the period 1962–2010. The soil map of Serbia shows that the reference groups Histosol, Anthrosol, Calcisol, Podzol, Phaeozem, and Umbrisol are distributed over a limited area in the country, totaling 3.58%. The most extensive groups are Cambisols (27.99%), Chernozems (17.68%) and Leptosols (15.9%) (Vidojević et al., 2015).

To establish the relationship between organic carbon content and land use and soil type, a soil map of Serbia was adapted to the WRB classification and divided into 15,437 polygons (map units). The assessment was based on long-term research data and data from the Soil Information System of Environmental

Protection Agency (Vidojević and Manojlović, 2010). The assessment of organic carbon stocks in the soils of the Republic of Serbia was carried out in the period 2009–2013 and it was made in soil layers 0–30 cm and 0–100 cm and based on soil type and land use category.

Calculation of organic carbon stocks per land use categories

Using the Corine Land Cover (CLC) database for 2006, the areas of the major categories of land use were defined. Based on the obtained mean values of organic carbon content in soil layers 0–30 and 0–100 cm and the areas indicated by Corine Land Cover categories of land use, the organic carbon stocks in agricultural land, forest land, semi-natural areas, and artificial areas were calculated. The last category includes mostly the urban green areas and recreational areas. The database does not contain organic carbon data for other categories of land use.

Organic carbon stock for the soil layer 0–30 cm per land use category was calculated according to the following formula:

SOC 30 cm (t) = Σ {(ẋ) mean value of organic carbon content per category of land use for the soil layer 0–30 cm (t ha^{-1}) x area occupied by land use category (ha)}

Organic carbon stock for the soil layer 0–100 cm was calculated according to the following formula:

SOC 100 cm (t) = Σ {(ẋ) mean value of organic carbon content per category of land use for the soil layer 0–100 cm (t ha^{-1}) x area occupied by land use category (ha)}

Statistics

Statistical data analysis was used to identify and establish the correlation between the organic carbon stocks from the most represented soil reference group and the land use category.

The correlation of the organic carbon stocks and the different land use categories (agricultural land and forest land), soil reference group, and soil depth was studied for reference groups that occupy the major part of central Serbia, such as Cambisol (taking up 37.76% of the territory) and Leptosol (22.22% of the territory), and have a sufficient number of sites that were required for this type of analysis. The Cambisol reference group included 90 sites in agricultural land and 64 sites in forest land. The Leptosol reference group included 35 sites in agricultural land and 71 sites in forest land.

The analysis of soil organic carbon stocks was performed with a package IBM SPSS statistics 20. Indicators of descriptive statistics were obtained to identify a general trend in the variability of organic carbon stocks in different extreme conditions. The analysis of the variance during a three-factor experiment showed the importance of land use impacts, reference soil group, and soil depth for the organic carbon content – for 5% and 1% risk levels. Relative interdependence of characteristics was measured with the Pearson correlation coefficient, tested at the 5% and 1% level of significance.

RESULTS

Organic carbon stocks broken down by different land use categories were identified by examining the soils at 1,140 sites for assessment of soil organic carbon stocks in t/ha. The analysis of the share of land use category at investigated sites has shown that 6.5% of sites are classified as artificial areas for assessment of soil organic carbon stocks in t/ha, 50.6 % of sites are classified as agricultural land, and 42.9% of sites are classified as forests and semi-natural areas. Distribution of soil organic carbon was shown in relation to land use category as defined by the Corine Land Cover categories. In the Republic of Serbia, artificial areas, agricultural land, forests, and semi-natural areas, and wetlands and water surfaces cover 257,070 ha, 4,395,186 ha, 2,967,453 ha, and 127,691 ha, respectively. The respective percentages are 3.32%, 56.73%, 38.30%, and 1.65%.

The analysis of organic carbon content in agricultural land showed that in layer 0–30 cm the values ranged from 3.72 t ha^{-1} to 328.23 t ha^{-1} (Table 1). The mean value was 68.99 t ha^{-1}. In layer 0–100 cm the values ranged from 18.25 t ha^{-1} to 658.40 t ha^{-1}, with the mean value of 136.57 t ha^{-1}. The analysis of variation coefficients indicated that the mean values for this land use category were not sufficiently representative (CV > 50%) (Vidojević et al., 2015).

The analysis of organic carbon content in the category of forests and semi-natural areas showed that in the layer 0–30 cm the values ranged from 4.93 t ha^{-1} to 527.22 t ha^{-1}. The mean value was 116.35 t ha^{-1}. In layer 0–100 cm, the values ranged from 10.06 t ha^{-1} to 646.98 t ha^{-1}, with the mean value of 154.19 t ha^{-1}. The analysis of variation coefficients indicated that the mean values for this land use category were not sufficiently representative (CV > 50%).

The analysis of organic carbon content in the category of artificial areas showed that in layer 0–30 cm the values ranged from 30.71 t ha^{-1} to 133.51 t ha^{-1}. The mean value was 74.74 t ha^{-1}. In the layer 0–100 cm, the values ranged from 45.68 t ha^{-1} to 342.66 t ha^{-1}, with the mean value of 161.43 t ha^{-1}. The analysis of variation coefficients indicated that these mean values were representative (CV < 50%) for this land use category.

Based on the areas of the different land use categories, the values of organic carbon stocks for these categories were obtained. The results showed that the organic carbon stocks in the category of agricultural land were 303.22 x 10^{12} g (Tg) and 600.25 x 10^{12} g (Tg) for the soil layers 0–30 cm and 0–100 cm, respectively. In the category of forests and semi-natural areas, the organic carbon stocks were 345.26 x 10^{12} g (Tg) and 457.55 x 10^{12} g (Tg) for the layers 0–30 cm and 0–100 cm, respectively. In the category of artificial areas, which mainly included sites within urban green areas and recreational areas, the organic carbon stocks were 19.21 x 10^{12} g (Tg) and 41.50 x 10^{12} g (Tg) for the soil layers 0–30 cm and 0–100 cm, respectively. The category of wetlands was not investigated in this study.

Table 1. Soil organic carbon (SOC) content and SOC stocks by CLC categories in the Republic of Serbia

				0–30cm					0–100 cm				
				SOC content (t ha^{-1})					SOC content (t ha^{-1})				
	Area (ha)	Area (%)	n	Mean	Min	Max	SD	SOC stock (Tg)	Mean	Min	Max	SD	SOC stock (Tg)
Agricultural areas	4,395,186	56.73	577	68.99	3.72	328.23	36.68	303.22	136.57	18.25	658.40	72.86	600.25
Forestland and semi-natural areas	2,967,453	38.30	489	116.35	4.93	527.22	79.60	345.26	154.19	10.06	646.98	93.22	457.55
Artificial areas	257,070	3.32	74	74.74	30.71	133.51	22.61	19.21	161.43	45.68	342.66	65.71	41.50

n: Number of soil profiles in the database. **SD**: Standard deviation

The obtained data indicated that there existed a great variability in the content of organic carbon among the reference soil groups. The largest SOC stocks for the soil layers 0–30 cm were found in Cambisol 194.76 x 10^{12} g and Leptosol 186.43 x 10^{12} g and for the soil layers 0–100 cm in Cambisol 274.87 x 10^{12} g and Chernozem 230.43 x 10^{12} g.

Multi-regression and correlation analysis

Table 2 shows the results of descriptive statistics of the content of organic carbon in the soil as related to different land use categories and WRB groups, measured up to 30 cm and 100 cm depth. The lowest variability of the content of soil organic carbon, measured in two layers of the soil, was found in Cambisol in agricultural land (28.75% and 39.30% respectively), while Leptosol in agricultural land showed most variability (64.24% and 86.67% respectively). With increasing depth of agricultural land, the dispersion of the organic carbon stocks increases as well. When it comes to forest land, for both groups the variability of organic carbon content is almost regular. Generally speaking, the variability of soil organic carbon stocks is outstanding (Cv>30%), which means that the concentration of the observed element is impacted to a great extent by external factors.

Table 2. Descriptive statistics of the content of soil organic carbon for the soil layers 0–30 cm and 0–100 cm as related to different WRB groups and land use categories (t/ha)

Land use category	WRB group	n	$\bar{x} \pm S_{\bar{x}}$	**Xmax–Xmin**	**Cv (%)**
				0–30 cm	
Agricultural land	**Cambisol**	90	63.49 ± 1.94	134.68–20.44	28.75
	Leptosol	35	117.26 ± 12.73	328.23–18.25	64.24
Forestland	**Cambisol**	64	124.99 ± 9.18	347.62–40.50	58.27
	Leptosol	71	184.75 ± 12.75	471.70–32.48	58.05
				0–100 cm	
Agricultural land	**Cambisol**	90	102.72 ± 4.26	250.72–25.74	39.30
	Leptosol	35	162.10 ± 23.74	658.40–18.25	86.67
Forestland	**Cambisol**	64	154.73 ± 10.05	398.43–59.96	51.55
	Leptosol	71	217.43 ± 16.41	646.98– 32.48	63.60

n: Number of soil profiles

What is also worth noting is that organic carbon stocks for the Leptosol reference group are larger in comparison to the Cambisol reference group; the same applies to forest land when compared with agricultural land. Measured differences in the content of organic carbon stocks for different reference soil groups and land use categories for the soil layers 0–30 cm and 0–100 cm have been statistically tested (Table 3). The co-occurring influence of the three factors (reference soil group, land use category, and depth of soil layers) on the variability of organic carbon stocks has been examined. The variance analysis test has shown that individual factors have a very significant statistical impact on the change of organic carbon concentrations in the soil ($p<0.01$), while their interaction (on both levels) is not statistically significant ($p>0.05$).

Table 3. The analysis of variance (ANOVA) of tested factors and their influence on the variability of soil organic carbon

Test	**Land use (A)**	**Reference soil group (B)**	**Depth (C)**	**AxB**	**AxC**	**BxC**	**AxBxC**
F	52.212**	51.907**	20.063**	0.081^{NS}	0.438^{NS}	0.069^{NS}	0.007^{NS}
p-level	0.000	0.000	0.000	0.776	0.508	0.794	0.935
Partial eta-squared coef.	0.093	0.092	0.038	0.0001	0.0009	0.0001	0.0000

^{NS} >0,05 **<0,01

Apart from statistical importance, based on partial eta-squared coefficients, the effects of each factor were measured. The measurement has shown that land use category (agricultural or forest), as well as reference soil group (Cambisol and Leptosol) have nearly identical effects on the variability of organic carbon stocks: the land use category influences variability by 9.3%, and the reference group by 9.2%. The measured depth of soil layers modifies the values of SOC by 3.8%.

DISCUSSION

The map of organic carbon distribution depending on land use category showed that organic carbon stocks were higher in forests and semi-natural areas than in agricultural land, up to 40.71% and 11.43% for the soil layers 0–30 cm and 0–100 cm, respectively (Vidojević, 2016). Organic carbon content was found to be higher in artificial areas than in agricultural land, forestland, and semi-natural areas. The reasons for this are manifold, but the most probable explanation is that the samples for this category were taken from urban green areas and recreational areas which are intensively fertilized and the removal of organic carbon is reduced. This study showed that there occurred a great variability in results when categories of land use were analyzed. Only the sites belonging to the category of artificial areas, at 0-30 cm, produced sufficiently representative values of the mean content of organic carbon. It appears that organic carbon content depends more on other factors, such as soil type, climatic conditions, and altitude than on land use category.

The distribution of organic carbon stocks in the soil layer 0–30 cm has shown higher values in central Serbia, with larger areas of forested land in comparison to the Province of Vojvodina in the north of the country consisting of mostly agricultural fields (Vidojević et al., 2017). The distribution of stocks in the soil layer 0–100 cm has shown larger carbon content in the Province of Vojvodina in comparison to central Serbia. These are mostly the reference groups of Chernozem and Gleysol, occupying 76.03% of the area of the Province of Vojvodina, which has larger organic carbon stocks in comparison to the most prevalent referent group in central Serbia – Cambisol (Vidojević et al., 2016).

Analysis of the organic carbon content in the surface layer (0–20 cm) in the most prevalent soils of forest ecosystems in central Serbia: eutric Ranker, eutric Cambisol, and dystric Cambisol has shown that the mean value of organic carbon for all the tested soils amounts to 5.77 kg m^{-2} (Kadović et al., 2012).

The research done by Freibauer et al. (2004) has pointed out that average organic carbon stocks in European arable land are at the level of mean organic carbon stocks in European mineral soils (around 53 t/h) at a 0–30 cm depth when different soil types are taken into consideration. Consequently, it is likely that European soils vary to a great deal across climate regions and soil types.

Based on a research study of organic carbon stocks in agricultural land of Europe conducted using a CENTURY model (Lugato et al., 2014), a value of 17.63 Gt for a 0–30 cm layer was obtained. The model included the EU and non-EU countries (Serbia, Bosnia and Herzegovina, Croatia, Albania, Macedonia, and Norway). The results obtained by the model were tested against the European Environment and Observation Network (EIONET) results, as well as against approximately 20,000 soil samples from LUCAS 2009 study.

The organic carbon stocks in agricultural land were identified and measured based on the surface of agricultural land in the Republic of Serbia. The value shown in Gt is 0.35 Gt of organic carbon stocks at a 0–30 cm depth in arable land of the Republic of Serbia, or else 1.98% of total content for the agricultural soils of Europe tested with the CENTURY model (Vidojević et al., 2014).

CONCLUSION

The spatial distribution of organic carbon stocks and its variability in the Republic of Serbia is caused by various factors.

The co-occurring influence of reference soil group, land use category, and depth of soil layer on the variability of organic carbon stocks has been examined. The variance analysis test has shown that individual factors have a very significant statistical impact on the change of organic carbon concentrations in the soil, while their interaction (on both levels) is not statistically significant.

The research has shown a high variability of results for different categories of land use. The measurements have shown that land use category (agricultural or forest), as well as reference soil group Cambisol and Leptosol have nearly identical effects on the variability of organic carbon stocks.

The obtained results have shown that an inventory of the land cover provides essential information in a world that is quickly becoming aware of the environmental limitations and lack of natural resources. The compilation of data on organic carbon stocks and its distribution in the different soil reference groups and land use categories is the first step in the evaluation and monitoring of changes of organic carbon stocks in the soils of the Republic of Serbia.

ACKNOWLEDGMENT

This research was partly financed by the Norwegian Programme in Higher Education, Research and Development in the Western Balkans (HERD) within the project "Agricultural Adaptation to Climate Change – Networking, Education, Research and Extension in the West Balkans".

REFERENCES

Cole CV, Duxbury J, Freney J, Heinemeyer O, Minami K, Mosier A, Paustian K, Rosenburg N, Sampson N, Sauerbeck D, Zhao Q (1997): Global estimates of potential mitigation of greenhouse gas emissions by agriculture. *Nutr. Cycl. Agroecosyst.*, 49: 221–228.

Freibauer A, Rounsevell MDA, Smith P, Verhagen J (2004): Carbon sequestration in European agricultural soils. *Geoderma*, 122: 1–23.

Gobin, A., Campling, P., Janssen, L., Desmet, N., van Delden, H., Hurkens, J., Lavelle, P., Berman, S. (2011): *Soil organic matter management across the EU – best practices, constraints and trade-offs*. Final Report for the European Commission's DG Environment: 34.

Kadović R, Belanović S, Knežević M, Danilović M, Košanin O, Beloica J (2012): Organic carbon stock in some forest soils in Serbia. *Bull. Fac. Forest.*, 105: 81–98.

Lal R (2004): Soil carbon sequestration impacts on global climate change and food security. *Science* (Washington), 304: 1623–1627.

Lugato E, Panagos P, Bampa F, Jones A, Montanarella L (2014): A new baseline of organic carbon stock in European agricultural soils using a modelling approach. *Glob. Change Biol.*, 20: 313–326. (doi: 10.1111/gcb.12292).

Manojlović M (2008): Đubrenje i zaštita životne sredine. U monografiji: *Đubrenje u održivoj poljoprivredi*, urednik Maja Manojlović, Novi Sad: Poljoprivredni fakultet, 118–136.

Manojlović M, Aćin V (2007): Globalne promene klime i ciklus ugljenika u životnoj sredini. Letopis naučnih radova, Novi Sad: Poljoprivredni fakultet, 31: 187–195.

Manojlović M, Aćin V, Šeremešić S (2008): Long-term effects of agronomic practices on the soil organic carbon sequestration in Chernozem. *Arch. Agron. Soil Sci.*, 54: 353–367.

Šeremešić S, Ćirić V, Jaćimović G, Milošev D, Belić M, Vojnov B, Živanov M (2016): The influence of conventional and conservation tillage on content of total and labile soil organic matter. *Zemljiste i Biljka / Soil and Plant*, 65: 7–18.

Vidojević D, Manojlović M (2010): Procena sadržaja organske materije u zemljištima Srbije. XXIV savetovanje agronoma, veterinara i tehnologa. *Zbornik naučnih radova Instituta PKB Agroekonomik*, 16: 231–244.

Vidojević D, Manojlović M, Đorđević A, Dimić B (2014): Procena rezerve organskog ugljenika u poljoprivrednom zemljištu Republike Srbije. Zbornik naučnih radova Instituta PKB Agroekonomik, XXVIII Savetovanje agronoma, veterinara, tehnologa i agroekonomista. *Zbornik naučnih radova Instituta PKB Agroekonomik*, 20: 231–244.

Vidojević D, Manojlović M, Đorđević A, Nešić Lj, Dimić B (2015): Organic carbon stocks in the soils of Serbia. *Carpath. J. Earth Environ. Sci.*, 10: 75–83.

Vidojević D (2016): *Procena rezervi organske materije u zemljištima Srbije*. (Doctoral dissertation). Novi Sad: Univerzitet u Novom Sadu, Poljoprivredni fakultet.

Vidojević D, Manojlović M, Đorđević A, Nešić Lj, Dimić B (2016): *Organic carbon stocks in the chernozems of Serbia*. Eurosoil 2016, Istanbul, Abstract book, p. 280.

Vidojević D, Manojlović M, Đorđević A, Nešić Lj, Dimić B (2017): Spatial distribution of soil organic carbon stocks in Serbia. FAO 2017. *Proceedings of the Global Symposium on Soil Organic Carbon 2017*. Food and Agriculture Organization of the United Nations. Rome, Italy, 195–198.

КОРЕЛАЦИЈА ИЗМЕЂУ ОРГАНСКОГ УГЉЕНИКА У ЗЕМЉИ, КОРИШЋЕЊА ЗЕМЉИШТА И ВРСТЕ ОБРАДИВОГ ЗЕМЉИШТА У СРБИЈИ

Драгана Д. ВИДОЈЕВИЋ[1], Маја С. МАНОЈЛОВИЋ[2],
Александар Р. ЂОРЂЕВИЋ[3], Љиљана М. НЕШИЋ[2], Тихомир М. ПРЕДИЋ[4]

[1] Министарство заштите животне средине,
Агенција за заштиту животне средине,
Руже Јовановић 27а, Београд 11160, Република Србија
[2] Универзитет у Новом Саду, Пољопривредни факултет,
Департман за ратарство и повртарство,
Трг Доситеја Обрадовића 8, Нови Сад 21000, Република Србија
[3] Универзитет у Београду, Пољопривредни факултет,
Катедра за педологију и геологију
Немањина 6, Земун–Београд 11080, Република Србија
[4] Пољопривредни институт Републике Српске,
Завод за агроекологију,
Књаза Милоша 17, Бања Лука 78000, Република Српска

РЕЗИМЕ: У раду су представљени резултати истраживања које је имало за циљ утврђивање зависности садржаја органског угљеника у земљишту, начина коришћења земљишта и референтне групе земљишта у Републици Србији. База података је укључила укупно 1.140 профила земљишта. За потребе утврђивања зависности садржаја органског угљеника и типа земљишта педолошка карта Србије прилагођена је WRB класификацији и садржи 15.437 полигона. Урађена је калкулација за сваку референтну групу земљишта на основу резултата средњих вредности садржаја органског угљеника у слоју земљишта 0–30 cm и 0–100 cm за главне референтне групе и њихових површина. На основу средње вредности садржаја органског угљеника у земљишту и површине референтне групе утврђено је да највећу резерву органског угљеника у земљишту у слоју 0–30 cm има камбисол 194,76 x 1012 g и лептосол 186,43 x 1012 g, и у слоју 0–100 cm дубине камбисол 274,87 x 1012 g и чернозем 230,43 x 1012 g. На основу резултата средњих вредности садржаја органског угљеника у слоју 0–30 cm и 0–100 cm дубине и површине коју заузима Corine Land Cover категорија начина коришћења земљишта, израчуната је укупна вредност резерви органског угљеника за пољопривредна земљишта, шуме и полуприродна подручја и вештачке површине. Статистичка зависност садржаја органског угљеника у земљишту од начина коришћења земљишта, референтне групе земљишта и дубине слоја, рађена је за референтне групе које заузимају највећу површину посматрајући територију централне Србије и то за референтну групу камбисол (која заузима 37,76% територије), затим лептосол (која заузима 22,22% територије) и довољног броја локалитета који су били потребни за анализу.

КЉУЧНЕ РЕЧИ: резерве органског угљеника у земљишту, коришћење земљишта, зависност, референтна група

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. Novi Sad, № 138, 19—29, 2020

UDC 543.38:631.4(497.113)
https://doi.org/10.2298/ZMSPN2038019C

Vladimir I. ĆIRIĆ[*], *Maja S. MANOJLOVIĆ*, *Ljiljana M. NEŠIĆ*, *Milivoj Đ. BELIĆ*

University of Novi Sad, Faculty of Agriculture,
Trg Dositeja Obradovića 8, Novi Sad 21000, Serbia

DEPTH DISTRIBUTION OF ORGANIC MATTER CONCENTRATION AND STOCKS IN SOILS OF VOJVODINA

ABSTRACT: Despite the fact that soil organic matter (SOM) concentration is much lower compared to mineral portion, its importance is very valuable for soil fertility, agriculture, ecosystems and global environmental change. Soil organic carbon (SOC) is a key component of SOM. The amount of SOC varies greatly in surface layer of soil, but the vertical distribution of SOC is also very important. The purpose of the study was to investigate vertical distribution of SOC in soil profiles of the most common soil types in Vojvodina (Arenosols, Chernozems, Fluvisols, Vertisols, Solonetz) and in different land uses (arable land, meadow and forest). Soil samples were collected from 0–30, 30–60 and 60–100 cm depth. Dichromate wet oxidation method proposed by Tyurin's was performed to determine SOC concentration. Except arenosols, obtained results showed the decrease of SOC concentration with depth in all observed soil types and land uses. Vertisols had the highest SOC concentration in both surface (0–30 cm) and subsurface (30–60 cm) layers compared to the other soil types, while arenosols had the highest SOC concentration in the deepest layer (60–100 cm). Higher concentrations of SOC in surface layers were measured in forests and meadows in relation to arable land, while in the lower layers these differences were not detected. Differences in SOC concentration in observed soil types and land uses were more pronounced in surface than in deeper layers. Soil organic carbon concentrations in deeper layers were substantially different only between soil types.

KEYWORDS: soil organic matter, soil type, land use

INTRODUCTION

Soil organic matter (SOM) is considered as a highly valuable natural resource (Lal, 2004) that consists of both living and non-living matter which is created biologically and located in soil. The biggest part of the OM consists of non-living part, which is formed of dead organisms, soil flora and fauna, plant roots, decomposed or non-decomposed plant and animal residues and

[*] Corresponding author. E-mail: vladimir.ciric@polj.uns.ac.rs

OM – humus. Organic matter of soil is associated with mineral portion in greater or lesser extent. Human induced land use changes can greatly influence OM concentration.

In recent years, soil organic carbon (SOC) as main constituent of SOM occupies great place in soil science due to its significant impact on soil fertility, global carbon cycle, sustainability and stability of biosphere. Soil organic carbon loss is defined as one of the eight major threats to the soil in the EU Thematic Strategy for Soil Protection (Blum, 2008). Soil organic carbon is important intrinsic property of a particular soil type, which is formed and stabilized during the process of pedogenesis. Despite the fact that SOC concentration is much lower compared to mineral portion of soil, it is even more important for soil fertility, microbial, water-air and chemical properties of soil and therefore plant yield.

Understanding the effects of native vegetation conversion on different soils is essential for predicting and modelling future SOC concentration and sequestration potential. In the Serbian province of Vojvodina, main conversions of natural vegetation to arable land occurred about 200 years ago. Since then, Vojvodinian soils have lost most of their original SOC stocks. Considerable share of SOC variability depends on soil type (Robert, 2001) and thus all SOC estimation should be made on the basis of soil type. Despite the fact that SOM is a dynamic formation, at some point during pedogenesis its content stabilizes and forms an intrinsic characteristic of a particular soil type (Manojlovic et al., 2010).

The aim of this study was to determine the quantitative characteristics and depth distribution of SOC in the most common soil types in Vojvodina (Arenosols, Chernozems, Fluvisols, Vertisols and Solonetz) which are exposed to different land uses (forest, meadow, arable land).

MATERIALS AND METHODS

Field research was conducted in the Province of Vojvodina, Serbia, on five soil types (Arenosols, Chernozems, Fluvisols, Vertisols and Solonetz). Each soil type was examined at three locations and under three different land uses (arable land, meadow and forest) at every location (Figure 1, 2, 3 and 4). Soil samples were collected from 0–30, 30–60 and 60–100 cm depth, with three replicates approximately 10 m apart each other. For the determination of bulk density (BD), soil samples in a natural-undisturbed state were taken using Kopecky cylinders (V = 100 cm^3) in nine replicates. Dichromate wet oxidation method proposed by Tyurin (1934) was performed to determine SOC concentration. Soil organic carbon stock (t ha^{-1}) was calculated as follows

$$SOCstock = BD \times SOC \times D$$

where BD is soil bulk density (g cm^{-3}), SOC is the soil organic carbon concentration (%), D is the depth of the soil layer.

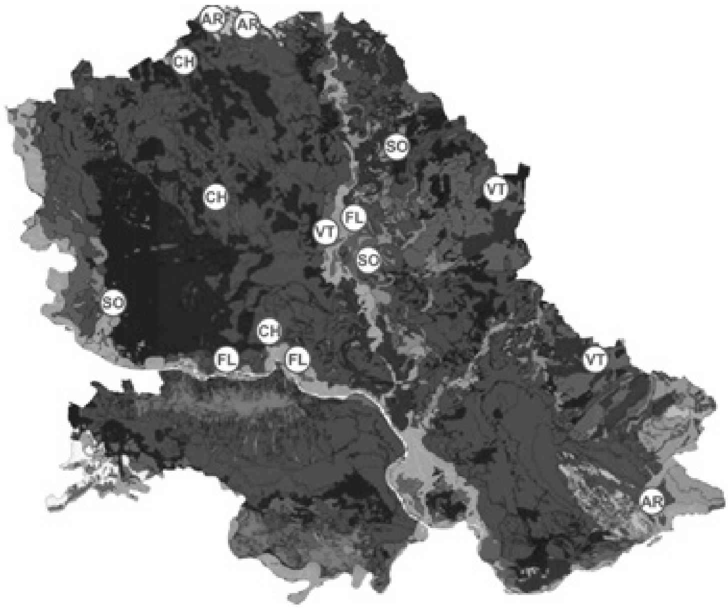


Figure 1. Locations of soil type sampling points included in the study

Figure 2. Different soil types included in the study

Figure 3. Different land uses included in the study

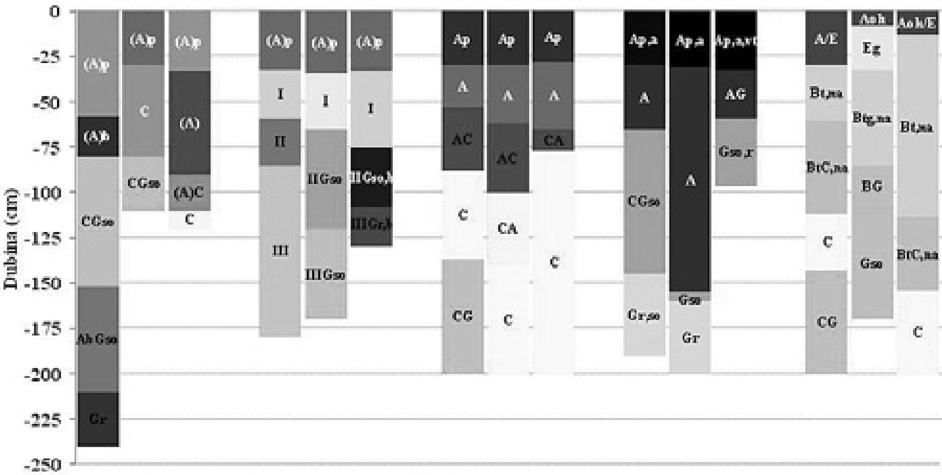


Figure 4. Soil profiles from the study (Arenosols 1–3; Fluvisols 4–6; Chernozems 7–9, Vertisols 10–12; Solonetz 13–15)

RESULTS

SOC concentration

The examined soil types contained different concentrations of SOC in the 0–30 cm layer: Vertisols (28.02 g kg^{-1}) > Chernozems (18.74 g kg^{-1}) > Solonetz (17.55 g kg^{-1}) > Fluvisols (15.38 g kg^{-1}) > Arenosols (11.50 g kg^{-1}) (Figure 5). Observed land uses also differed in SOC concentration in the layer of 0–30 cm: forests (19.57 g kg^{-1}) > meadows (19.18 g kg^{-1}) > arable land (15.95 g kg^{-1}).

Obtained results showed the decrease of SOC concentration with depth in all observed soil types and land uses except arenosols. Vertisols had the highest SOC concentration in both surface (0–30 cm) and subsurface (30–60 cm) layers compared to the other soil types, while arenosols had the highest SOC concentrations in the deepest layer (60–100 cm). Higher concentration of SOC in surface layer was measured in forests and meadows in relation to arable land, while in the lower layers these differences were not detected.

SOC stocks

Vertical distribution of SOC stocks showed decline with increasing soil depth for all soil types except arenosols, (Figure 6). Looking at the 0–30 cm layer, SOC stocks decline depending on the type of soil in the following order: Vertisols (99 t ha^{-1}), Chernozems (79 t ha^{-1}), Solonetz (71 t ha^{-1}), Fluvisols (58 t ha^{-1}), and Arenosols (47 t ha^{-1}).

Vertisols have the highest SOC stocks, compare to Chernozems and Solonetz. Arenosols are characterized with the lowest SOC stocks, while Fluvisols have larger reserves than Arenosols and smaller than Chernozem and Solonetz. In the 30–60 cm layer, Chernozems and Vertisols are characterized by the highest SOC content, with the statement that Vertisols have a significantly higher SOC stock than Chernozem, while the other soil types do not differ from each other and have lower stocks. In the 60–100 cm layer, Arenosols have higher SOC stocks compared to Chernozems, Vertisols and Solonetz, while fluvisols have the lowest stocks in this layer.

The total SOC stock 0–100 cm in different soil types showed the same order as in the layer 0–30 cm except that fluvisols had the lowest stocks: Vertisols (190 t ha^{-1}), Chernozems (150 t ha^{-1}), Solonetz (133 t ha^{-1}), Arenosols (118 t ha^{-1}), Fluvisols (111 t ha^{-1}). The topsoil (0–30 cm) accounts 40% of the SOC stock 0–100 cm depth in Arenosols, 52% in Chernozems, Fluvisols and Vertisols and 54% in Solonetz.

All analyzed land uses showed decline in SOC stocks with increasing soil depth. However, there were no differences in SOC stocks in topsoil and subsurface layers under different land uses. The differences in total SOC stock 0–100 cm between land uses were also not significant: forests (142 t ha^{-1}), meadows (141 t ha^{-1}), and arable land (137 t ha^{-1}). The topsoil SOC stock (0–30 cm) comprises 49% of SOC stock in 0–100 cm layer of arable land, 50% in forests and 53% in meadows.

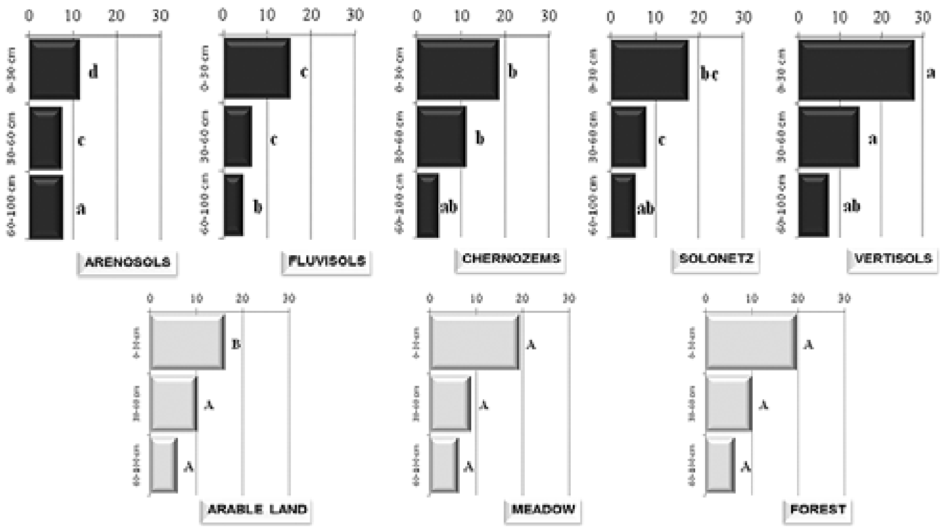


Figure 5. Vertical distribution of the SOC concentration (g kg^{-1}) in different soil types and land uses in 0–30, 30–60 and 60–100 cm layers

(The values marked with different lower case letters are significantly different within the soil type at the same depth ($p \leq 0.05$). The values marked with different capital letters are significantly different within the land uses of the same depth ($p \leq 0.05$).)

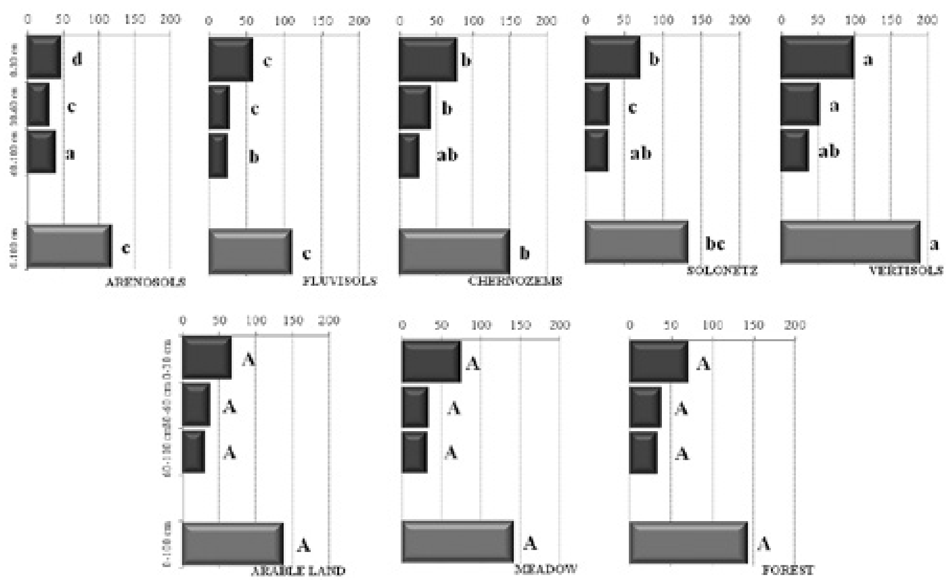


Figure 6. Vertical distribution of the SOC stocks (t ha^{-1}) in different soil types and land uses in 0–30, 30–60 and 60–100 cm layers.

(The values marked with different lower case letters are significantly different within the soil type at the same depth ($p \leq 0.05$). The values marked with different capital letters are significantly different within the land uses of the same depth ($p \leq 0.05$).)

Considering only the results of SOC stocks (without SOC concentration) can lead to wrong conclusion regarding the SOC stock loss on arable land compared to meadow and forest soils due to different values of bulk density in different land uses that are included in the calculation of SOC stocks. As a more reliable indicator of SOC loss, the SOC concentration has appeared. While differences between soil types in terms of SOC stocks and SOC concentration were minimal, in different land uses differences in SOC concentrations were significant, while there were no differences in SOC stocks. A significantly higher SOC concentration was found in forests (19.6 g kg^{-1}) and meadows (19.2 g kg^{-1}) than in arable land (15.9 g kg^{-1}), which represents a 19% reduction in relation to forest and 17% in relation to meadow land.

DISCUSSION

The various pedogenetic processes that dominate in particular soil types have a significant impact on ecosystem functioning and also on SOC stocks. Good assessment of SOC stocks and its vertical distribution in different systematic units of soil are needed for comparing SOC stocks and rates of attachment across horizons and depths (Civeira et al., 2012). The SOC content within the profile mainly varies depending on pedogenetic processes. Clay content and soil depth largely affect SOC stocks (Breulmann, 2011). Due to the still limited knowledge of specific features of soil types, global SOC stocks are difficult to assess (Sombroek et al., 1993; Batjes, 1996). Establishing a database as a basis for assessing the carbon content of the surface layer is critical in determining whether farmland is a source or reservoir for SOC (Freibauer et al., 2004). Most of SOC are stored in the surface layer, where they are likely to transform rapidly (Breulmann, 2011). Organic carbon from subsurface horizons is less exposed to oxidation and is important for the conservation of SOC land reserves.

According to the SOC stock in the 0-30 cm layer, soil types showed the following arrangement: rite blacks (99 t ha^{-1})> Chernozems (79 t ha^{-1})> Solonetz (71 t ha^{-1})> Fluvisols (58 t ha^{-1})> Arenosols (47 t ha^{-1}), as well as in the 0–100 cm layer: Vertisols (190 t ha^{-1})> Chernozems (150 t ha^{-1})> Solonets (133 t ha^{-1})> Arenosols (118 t ha^{-1})> Fluvisols (111 t ha^{-1}). This order highlights soils with humic-accumulative horizon in front of the initial lands.

The largest stocks of SOC in the 0–30 cm layer found in Vertisols are due to the accumulation of larger amounts of hydromorphic humus, which is formed under the influence of seasonal moisture, leading to reduced SOM oxidation and accelerate its accumulation in these soils. The second reason is to emphasize the vertic properties of these soils and increased clay content, which influences the stabilization and association of SOM with clay. In addition, the accumulation of humus in these soils may be increased by the deposition of silty particles by occasional flooding. Also SOC stock in the 0–100 cm layer, which is the largest in Vertisols, is the result of a powerful, deep humus horizon. The Vertisols contain hydromorphic humus and a humus horizon of 50–70 cm thickness (Ćirić, 1984).

Following Vertisols, large SOC stocks are determined in Chernozems, both in the 0–30 cm layer (which accounts 52% of the SOC stock up to a depth of 100 cm) and in the 0–100 cm layer. Chernozems as steppe black soils do not have the potential to accumulate SOC to the same extent as Vertisols due to the terrestrial pedogenesis. Rather good quality of humus caused by grassland-steppe vegetation and good structure, loamy mechanical composition with moderate clay and $CaCO_3$ content, which stabilize OM, as well as steppe pedo fauna and absence of eluvial-iluvial migration, significantly influence accumulation of SOC in Chernozems. High content of high quality OM in Chernozems has been described by many local (Živković et al., 1972; Ćirić, 1984; Škorić, 1986; Miljković, 1996) and foreign authors (Kononova, 1975; Mikhailova et al., 2000; Altermann et al., 2005). The Chernozems of Central and Eastern Europe contain 76–97 t ha^{-1} SOC in the 0–30 cm layer (Batjes, 2002). The average reserves in the Vojvodinian Chernozems on the loess terrace are 65 t ha^{-1} in the arable layer (0–30 cm) and range from 58–76 t ha^{-1} (Ćirić, 2008). In the 0–40 cm layer of Chernozems under the influence of different tillage systems SOC stocks ranged from 90.0–96.9 t ha^{-1} (Manojlović et al., 2008). Chernozems under different tillage systems contain average stocks of 60.2 t ha^{-1} at a depth of 0–30 cm (Šeremesić et al., 2011). Chernozems in Vojvodina contain average reserves of 72 t ha^{-1} SOC in the 0–30 cm layer and 165 t ha^{-1} in the 0–100 cm layer (Belić et al., 2013).

The specificity of Solonetz is reflected in a much higher amount of SOC in the surface layers than in the deeper ones (54%), which is a consequence of the most common grass vegetation on these lands, which leads to the accumulation of humus in a surface horizon of small thickness. Furthermore, SOM is unstable, which was also found in these studies, all because of the conditions of the alkaline reaction leading to an increase in the solubility of the humus and its eluviations. Vojvodinian Solonetz contains 55–89 t ha^{-1} of SOC (Belić et al., 2004).

The low degree of SOC accumulation in Fluvisols in the 0–30 cm layer and especially in the 0–100 cm layer, is due to the pedogenesis and character of the alluvial deposits on which this soil is formed. Recent alluvial deposition is formed in the event of occasional floods that precipitate suspended particles, so that the pedogenetic processes of humus accumulation and the formation of a stable organic-mineral complex are constantly interrupted and prevent the formation of a powerful humic-accumulative horizon and stabilization of SOC. To a large extent, SOC stocks also depend on the deposition properties, number of layers, fossil layers, mechanical and chemical composition.

Arenosols showed the lowest SOC stocks in the 0–30 cm layer that accounts for 40% of the SOC stocks up to 100 cm deep, but in 0–100 cm layer Arenosols have higher SOC stocks compared to Fluvisols, due to the larger stocks in the deeper layers resulting from the anthropogenisation. Arenosols are unable to accumulate larger SOC reserves due to their coarse mechanical composition, eolian erosion, and xerophytic vegetation on them. Arenosols had lower SOC stocks in earlier periods (Živković et al., 1972; Hadžić et al., 1997; Belić et al., 1999), but at present they are anthropogenized, relatively bound sands and peat-enriched.

The physicochemical characteristics inherent in each soil define the maximum degree of protection of the capacity of the SOC reserves, which limits its increase, i.e. soil binding even with increasing intake of organic residues (Six et al., 2002).

No differences in SOC stocks were found between different land uses in the 0–30 cm layer: nor in the 0–100 layer cm. Such results are opposite with the general view and expectations in this research that different uses differ in SOC stocks, i.e. that stocks are less on arable land than forests and meadows. There are significant differences in SOC between different types of vegetation, which could be attributed to differences in root and debris distribution above and below ground (Jobbagi and Jackson, 2000). Some studies report similar levels of SOC stocks among different uses (Smith et al., 2001) and point out that average SOC stocks in European arable land are at the level of average SOC stocks in mineral soils in Europe (about 53 t C ha^{-1}) at 0–30 cm depth when looking at different soil types. Therefore, it can be concluded that European soils vary widely among climate regions and land types (Freibauer et al., 2004).

However, the SOC socks calculation method includes SOC concentration and also bulk density that are negatively correlated with the SOC concentration. There are significant correlations between SOC, OM and volume mass (Périé and Ouimet, 2008). This can lead to the wrong conclusions when assessing the impact of land use on SOC stocks. It is known that after the converting natural vegetation to arable land, there is an increase in the bulk density of arable land and a decrease of SOC concentration due to the deterioration of structure and compaction. Simultaneous reductions in SOC concentration and a significant increase in bulk density lead to minimal differences in SOC stock results between arable soil and soil under natural vegetation, which can often lead to the conclusion that SOC changes were not affected by conversion. The established values of the share of the surface layer (0–30 cm) in the total SOC stocks of 0–100 cm are such that the surface layer accounts 49% of the SOC stocks up to a depth of 100 cm in arable land, 50% in forests and 53% in meadows, which agrees with the view that meadow vegetation with a shallow root system accumulates the most OM in the surface layer, forest slightly less, and arable land the least due to the decrease in SOC concentration caused by changes in land use and migration processes.

CONCLUSIONS

Different soil types and land uses contain different SOC concentrations in the 0–30 cm layer that are more pronounced than in deeper layers.

Different soil types contain different SOC stocks in the 0–30 cm layer but there were no differences between land uses.

Vertical distribution of SOC concentration and SOC stocks shows decreasing trend with depth in all observed soil types and land uses except Arenosols.

For the detecting SOC changes in the environment it is better to use SOC concentration than SOC stocks.

REFERENCES

Altermann M, Rinklebe J, Merbach I, Körschens M, Langer U, Hofmann B (2005): Chernozem – soil of the year 2005. *J. Plant Nutr. Soil Sci.*, 168: 725–740.

Batjes NH (2002): Carbon and nitrogen stocks in the soils of central and Eastern Europe. *Soil Use Manage.*, 18: 324–329.

Belić M, Hadžić V, Nešić Lj (2004): Characteristics of halomorphic soils in the Banat region and their possible for more intensive use. *Field Veg. Crop Res.*, 40: 73–89.

Belić M, Hadzić V, Ubavić M, Nesić L, Sekulić P, Gajić V (1999): Uticaj miliorativnih mera na kapacitet adsorbacije arenosola. *Zbornik radova Naučnog instituta za ratarstvo i povrtarstvo,* 32: 263–270.

Belić M, Manojlović M, Nešić Lj, Ćirić V, Vasin J, Benka P, Šeremešić S (2013): Pedoecological Significance of Soil Organic Carbon Stock in Southe-Eastern Panonnian Basin. *Carpathian. J. Earth Environ. Sci.*, 8: 171–178.

Blum WEH (2008): Characterisation of soil degradation risk: an overview. In: G. Tóth (ed.), *Threats to Soil Quality in Europe*. Luxemburg: JRC, Office for Official Publications of the European Communities, 5–10.

Breulmann M (2011): *Functional soil organic matter pools and soil organic carbon stocks in grasslands – An ecosystem perspective*. PhD thesis. Institut für Bodenkunde, Leibniz Universität Hannover.

Civeira G, Julieta I, Ileana RP (2012): Soil Organic Carbon in Pampean Agroecosystems: Horizontal and Vertical Distribution Determined by Soil Great Group. *Soil Horizons*, 53: 43–49.

Ćirić M (1984): *Pedologija*, Sarajevo: Svjetlost, 311 pag.

Ćirić V (2008): *Vodno-fizička svojstva černozema, kao činilac plodnosti u proizvodnji kukuruza* (magister thesis), Novi Sad: Poljoprivredni fakultet, 173 p.

Freibauer A, Rounsevellb MDA, Smith P, Verhagen J (2004): Carbon sequestration in the agricultural soils of Europe. *Geoderma*, 122: 1–23.

Hadžić V, Belić M, Ubavić M, Sekulić P, Nešić Lj (1997): Uticaj meliorativnih mera na promenu hemijskih svojstava arenosola. In: *Uređenje, korišćenje i očuvanje zemljišta*, Zbornik radova 9. kongresa za proučavanje zemljišta, 23–27 juni, Novi Sad, 248–258.

Jobbágy EG, Jackson RB (2000): The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol. Applic.*, 10: 423–436.

Kononova MM (1975): Humus of virgin and cultivated soils. In: *Soil components*, Berlin–Heidelberg: Springer, 475–526.

Lal R (2004): Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. *Science*, 304: 1623–1627.

Manojlovic M, Acin V, Seremesic S (2008): Long-term effects of agronomic practices on the soil organic carbon sequestration in Chernozem. *Arch. Agron. Soil Sci.*, 54: 353–367.

Manojlović M, Ćirić V, Nešić Lj, Belić M (2010a): The importance of soil organic carbon for soil, agriculture and global climate change. *Contemp. Agric.*, 59: 194–205.

Mikhailova EA, Bryant RB, Vassenev II, Schwager SJ, Post CJ (2000): Cultivation effects on soil carbon and nitrogen contents at depth in the Russian Chernozem. *Soil Sci. Soc. Amer. J.*, 64: 738–745.

Miljković N (1996): *Osnovi pedologije*. Novi Sad: Prirodno-matematički fakultet, Institut za geografiju.

Périé C, Ouimet R (2008): Organic carbon, organic matter and bulk density relationships in boreal forest soils. *Can. J. Soil Sci.*, 88: 315–325.

Robert M (2001): *Soil Carbon Sequestration for Improved Land Management*. World Soil Resources Report 96. Rome: Food and Agriculture Organization of the United Nations, 75 pag.

Six J, Conant RT, Paul EA, Paustian K (2002): Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant Soil*, 241: 155–176.

Smith P, Smith JU, Powlson DS (2001): Soil Organic Matter Network (SOMNET): 2001 Model and Experimental Metadata. GCTE Report 7, Second Edition, GCTE Focus 3, Wallingford, Oxon, 223 pag.

Sombroek WG, Nachtergaele FO, Hebel A (1993): Amounts, dynamics and sequestration of carbon in tropical and subtropical soils. *Ambio*, 22: 417–426.

Šeremešić S, Milošev D, Đalović I, Zeremski T, Ninkov J (2011): Management of soil organic carbon in maintaining soil productivity and yield stability of winter wheat. *Plant Soil Environ*, 57: 216–221.

Škorić A (1986): *Postanak, razvoj i sistematika tla*. Zagreb: Univerzitet u Zagrebu.

Tyurin IV (1934): New volumetric measuring method of humus using chromic acid. *Pochvovedenie*, 5–6: 36–47.

Živković B, Nejgebauer V, Tanasijević Đ, Miljković N, Stojković L, Drezgić P (1972): *Zemljišta Vojvodine*. Novi Sad: Institut za poljoprivredna istraživanja.

ВЕРТИКАЛНА ДИСТРИБУЦИЈА КОНЦЕНТРАЦИЈЕ И ЗАЛИХА ОРГАНСКЕ МАТЕРИЈЕ У ЗЕМЉИШТИМА ВОЈВОДИНЕ

Владимир И. ЋИРИЋ, Маја С. МАНОЈЛОВИЋ,
Љиљана М. НЕШИЋ, Миливој Ђ. БЕЛИЋ

Универзитет у Новом Саду, Пољопривредни факултет,
Трг Доситеја Обрадовића 8, Нови Сад 21000, Србија

РЕЗИМЕ: Упркос чињеници да је концентрација органске материје (ОМ) у земљишту знатно нижа у односу на минерални део, њен значај је веома важан за плодност земљишта, пољопривреду, екосистем, као и глобалне промене животне средине. Органски угљеник у земљишту (ОС) је кључна компонента ОМ. Количина ОС веома варира у површинском слоју земљишта, али је и вертикална дистрибуција ОС такође веома важна. Сврха истраживања била је да се испита вертикална дистрибуција ОС у профилима најзаступљенијих типова земљишта Војводине (ареносол, чернозем, флувисол, смоница, солоњец) који се користе на различите начине (обрадиво земљиште, ливада и шума). Узорци су прикупљени са дубине 0–30, 30–60 и 60–100 cm. Дихроматна метода мокре оксидације по Тјурину урађена је у циљу утврђивања концентрације ОС. Осим ареносола, добијени резултати показали су смањење концентрације ОС с дубином у свим посматраним типовима земљишта, као и у свим посматраним начинима коришћења. Смонице су имале највећу концентрацију ОС у површинском (0–30 cm) и потповршинском (30–60 cm) слоју у поређењу с осталим типовима земљишта, док ареносоли имају највећу концентрацију ОС у најдубљем слоју (60–100 cm). Веће концентрације ОС у површинским слојевима измерене су у шумама и ливадама у односу на обрадиво земљиште, док у нижим слојевима ове разлике нису биле значајне. Разлике у концентрацији ОС у посматраним типовима земљишта и начинима коришћења биле су израженије у површинским него у дубљим слојевима. Концентрације ОС у дубљим слојевима су биле битно различите само између типова земљишта.

КЉУЧНЕ РЕЧИ: органска материја земљишта, тип земљишта, начин коришћења

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. Novi Sad, № 138, 31—39, 2020

UDC 631.417.4:631.4
https://doi.org/10.2298/ZMSPN2038031S

Srđan I. ŠEREMEŠIĆ, Ljiljana M. NEŠIĆ[1], Vladimir I. ĆIRIĆ[1], Jovica R. VASIN[2], Ivica G. ĐALOVIĆ[2], Jelena B. MARINKOVIĆ[2], Bojan Đ. VOJNOV[1]*

[1] University of Novi Sad, Faculty of Agriculture Novi Sad,
Trg Dositeja Obradovića 8, Novi Sad 21000, Serbia
[2] Institute of Field and Vegetable Crops,
Maksima Gorkog 30, Novi Sad 21000, Serbia

SOIL ORGANIC CARBON FRACTIONS IN DIFFERENT LAND USE SYSTEMS OF CHERNOZEM SOIL

ABSTRACT: The relationship between soil carbon fractions in Chernozem soils was assessed in soil samples of three different environments: arable soil, grassland and oak forest. Grassland and oak forest had higher soil organic carbon (SOC), carbon soluble in hot water (HWC), particulate organic carbon (POC) and mineral-associated carbon (MOC) than the arable soil. The POC/MOC ratio was lowest in arable soil, indicating a smaller carbon pool for microbial turnover. POC increases with higher total SOC, indicating that the preservation of organic matter depends on the renewal of labile fractions. Our results showed that fertilization had active role in soil carbon stabilization, while crop rotation had less effect on a soil carbon turnover. Our result could contribute to the better understanding of SOC fractions composition and relevance in Chernozem soil, thus could help in selection of cropping management systems for SOC preservation.

KEYWORDS: land use systems, soil organic carbon, mineral-associated carbon, particulate organic carbon

INTRODUCTION

Different qualitative fractions of SOC derived from the formation pathways could be evaluated to better understand carbon cycling of the specific cropping systems. Soil organic carbon fractions are identified according to their role in the carbon turnover and relationship with the microbiological and soil properties (Cookson et al., 2005). In recent decades, increased number of physical and chemical fractionation approaches has been proposed for separation and

* Corresponding author. E-mail: srdjan.seremesic@polj.uns.ac.rs

the identification of the different labile and stable fractions as proxies for the SOC pool and their role in soil quality (Baldock and Nelson, 2000). Each of the fractions contains specific information relevant to estimate effects of land use on SOC pool turnover and stabilization. Changes in management practices in the long-term experiment over time could induce problems in quantification of SOC pool due to the large background amounts already present and the spatial variation (Haynes, 2000). Therefore, a current perspective in SOC interpretation based on total carbon pool lacks clarity and precision in explaining the relationship between SOC content and related soil properties. Moreover, soils can vary in the potential for organic matter mineralization (Najvirt et al., 2019). The turnover of the labile SOC fraction is relatively rapid and responds quickly to the land use changes and soil management, and therefore it could be anticipated as the early and sensitive indicator of the total SOC change (Hynes and Beare, 1996). Concentration and fluxes of the SOC fractions obtained with fractionation approaches results with the similar conceptual SOC pool but with the different perception on the factor causing their occurrence in soil. Decomposition studies by Gregorich et al. (2003) provided evidence that the hot water extractable carbon (HWC) fraction of soil C is highly labile and estimated that the HWC accounted for about 70% of the total water-soluble matter. Ghani et al. (2003) found that HWC reflected the changes in SOC caused by different soil management practices compared with stabile SOC pool. Particular organic carbon (POC) is considered as intermediary available SOC pool sensitive to the management practice and the grown crops (Cambardella and Elliott, 1993). Therefore, POC acts as a binding agent in soil, responsible in stabilization of the macro-aggregates and intra-aggregate structure (Six et al., 2002). Bayer et al. (2004) explained that organic matter in the POC is more sensitive to a management practices than C obtained in the total SOC pool. Martinez-Mena et al. (2012) emphasized the interaction between POC and mineral-associated carbon (MOC) to better understand the role of labile C in SOC dynamic. Due to pronounced decreasing trend in SOC, there is a need to explain how it reflects changes in the content of SOC fractions and to what extent it can affect soil productivity. The aim of this study was to determine the change in the SOC as a consequence of an interrelation of the different SOC fractions with different land use systems.

MATERIALS AND METHODS

The arable plots were selected from a long–term stationary experimental rotation-crop field “Plodoredi” (Šeremešić et al., 2011) of the Institute of Field and Vegetable Crops (N 45° 32’ 51”; W 19° 84’ 77”; 84 m above sea) in Novi Sad, Serbia and adjacent grassland and forest. The trial was set up on Calcic Chernozem (Aric, Loamic, Pachic) (abbreviated CH-cc-ai.lo.ph) (IUSS Working Group WRB, 2014) in 1946/1947, and the fertilized crop rotation systems were redesigned in 1969/70, while the unfertilized systems remained unchanged.

Experiment performance involved applications of organic and mineral fertilizers and plowing of the crop residue according to the following layout:

1. Maize monoculture (MO): mineral N – 120 kg/ha, P and K according to soil analyses, established in 1969/70.
2. Unfertilized 2-year rotation (maize-winter wheat) (N2): established in 1946/47 without any fertilizers and with plowing the crop residue since 1987.
3. Fertilized 2-year rotation (maize-winter wheat) (D2): mineral N – 120 kg ha^{-1}, P and K according to soil analyses, established in 1969/70.
4. Fertilized 3-year rotation (maize-soybean-winter wheat) (S3): mineral N – 120 kg ha^{-1}, 25 t ha^{-1} farmyard manure after winter wheat. P and K according to soil analyses, established 1969/70.
5. Grassland (UG): 45° 20,549', (E) 19° 51,492', at 81 m elevation
6. Oak forest (OF): 45° 20,591', (E) 19° 51,374', at 83 m elevation

Soil samples were taken at the beginning of maize growing period (5^{th} of May, 2012) and after maize harvest (5^{th} of October, 2012) with auger corer. The average soil sample consisted of 5 drillings. The disturbed soil samples (approximately 1 kg) were transferred to carton boxes and stored as air-dry samples at room temperature subsequent to analyses. The samples were ground in a mill and sieved (2 mm diameter). Soil organic carbon in the soil samples was determined with the Tyrin's titrimetric wet combustion method using dichromate ($K_2Cr_2O_7$) with external heating, followed by titration with ferrous ammonium sulphate (Mohr`s salt). In our study the 0–30 cm layer of soil was analyzed divided into 3 sub-layers (0–10 cm, 10–20 cm and 20–30 cm). Hot-water extractable C (HWC) was determined by adapting the procedures of Ghani et al. (2003). A previously published protocol (Cambardella and Elliott, 1993; Martinez-Mena et al., 2012) was used to obtain particulate organic matter (POC). The data on the SOC, HWC, POC and MOC were statistically assessed using the ANOVA on a significance level of $\alpha = 0.05$, and the LSD test was used for individual comparisons of the treatments' means. The data were statistically processed by using the program STATISTICA series 12.6.

RESULTS AND DISCUSSION

Comparison of different land use systems reveals that the highest content of SOC was in the grassland and the lowest in the unfertilized 2-year rotation. Significantly higher SOC content was obtained in samples taken in May (Table 1). Lowest SOC values obtained in the arable soils could be related to mineralization, which is faster on arable soils, whereas non-agricultural soil favors the accumulation of OM (McLauchan et al., 2006). Molnar et al. (2003) presented analyses from 1991 where 12.12 g kg^{-1} of SOC was found in N2, which suggests that continuous SOC decline, as a lack of the external inputs, is not sufficient to provide biomass for C stabilization. With the increasing soil depth, SOC decrease was more pronounced in grassland and oak forest where C accumulated in the top soil. The opposite distribution of SOC in the soil profile was found at the S3 where manure was applied. Many studies confirmed that the amount of crop residues and farmyard manure are responsible for SOC content change (Van Wesemael et al., 2010).

Table 1. Soil organic carbon content in different land use systems (g kg^{-1})

Sam-pling time	Depth (cm) **(De)**	Land use **(Lu)**						**Average De**	**Average St**
		MO	**N2**	**D2**	**S3**	**UG**	**OF**		
May	0–10	15.34±0.1	11.10±0.1	14.30±0.0	15.94±0.1	30.34±0.43	28.37±0.3	19.23	17.09[a]
	10–20	14.48±0.2	11.06±0.0	14.01±0.1	17.38±0.2	25.75±0.53	19.88±0.1	17.04	
	20–30	14.08±0.2	11.06±0.0	13.83±0.0	17.79±0.2	21.33±0.58	19.30±0.6	16.23	
Average Lu/St		**14.63**	**11.07**	**14.04**	**17.03**	**25.80**	**22.51**		
October	0–10	14.42±0.1	8.84±0.0	13.37±0.0	15.05±0.1	29.36±0.32	25.99±0.4	17.83	15.92[b]
	10–20	11.92±0.1	8.78±0.1	13.35±0.0	14.76±0.2	22.15±0.36	21.45±0.4	15.40	
	20–30	12.62±0.3	8.37±0.1	12.62±0.0	14.47±0.0	22.42±0.12	16.74±0.3	14.54	
Average Lu/St		**12.98**	**8.66**	**13.18**	**14.76**	**24.64**	**21.39**		
Average Lu/De		14.88	9.97	13.83	15.49	29.85	27.18	18.55[a]	
		13.20	9.92	13.68	16.07	23.95	20.66	16.27[b]	
		13.35	9.71	13.22	21.87	21.87	18.02	15.34[c]	
Average Lu		**13.80[D]**	**9.86[E]**	**13.57D**	**17.81[C]**	**25.22[A]**	**21.95[B]**		

Lu – land use; De – Depth (cm); St – Sampling time; [ABC] Data followed by the same capital letter within a row do not differ significantly at the $P \leq 0.05$ level; [abc] Data followed by the same capital letter within a collumn do not differ significantly at the $P \leq 0.05$ level.

The average HWC content for different land use systems ranged from 180 mg kg^{-1} to 1,454 mg kg^{-1} (Table 2). With the increased depth the content of HWC in all land use systems slightly decreased. Leinweber et al. (1995) found 430–650 mg kg^{-1} HWC in Bad Lauchstädt, while Sparling et al. (1998) determined HWC content which is comparative with our results. Chen et al. (2009) reported mean values of HWC in Cambisol, at a depth of 0–15 cm was 375 mg kg^{-1}, and at a depth of 15–30 cm it amounted to 243 mg kg^{-1}. The highest average content of HWC was found in samples taken from forest (860 mg kg^{-1}), while the lowest content was measured in N2 (231 mg kg^{-1}). We assumed that in the unfertilized maize rotation with lowest SOC small amount of labile organic SOC is produced and simultaneously stabilized with clay and $CaCO_3$ that prevent from further decline of SOC. Bouajila and Gallai (2008) also found less HWC content in soils with a higher content of $CaCO_3$. Significant variability was found among cropping systems and depth compared to sampling time. Leinweber et al. (1995) reported larger amounts HWC measured at the end of the growing season due to easily decomposable mucilage created by microorganisms living in the rhizosphere. The increase of labile HWC fractions in maize mono-culture compared with the arable land use systems is associated with more efficient utilization of nutrients from fertilizers and transformation of plant residues. Janzen et al. (1992) also found high content of labile SOC in continuous monoculture on Chernozem in Canada. Manure application has influenced the HWC content compared to maize fertilized and unfertilized 2-year rotation and by increased microbial activity. According to Šimon (2008) single organic manuring did not increase the HWC significantly as compared to the single NPK variant but increased the HWC content significantly in comparison with the control. These findings also support the fact that the manure application is more efficient with addition of NPK fertilizers (Blair et al., 2006). The highest distribution of HWC in total SOC was measured in the maize monoculture and it is attributed to the long-term production of large amounts of crop residues and presence of a large number of weeds (e.g. *Sorghum halepense*). Accordingly, increase or decrease in total biomass of the specific cropping systems, could contribute to soil carbon allocation through the soil profile.

Table 2. Hot water extractable carbon (HWC) in land use systems (mg kg^{-1})

Sampling time	Depth (cm) **(De)**	Land use **(Lu)**						**Average De**	**Average St**
		MO	**N2**	**D2**	**S3**	**UG**	**OF**		
May	0–10	528	289	406	497	1121	1454	716	598^{A}
	10–20	441	325	392	582	859	881	580	
	20–30	459	282	410	509	707	622	498	
Average Lu/St		**476**^{bc}	**299**^{c}	**402**^{bc}	**529**^{b}	**895**^{a}	**986**^{a}		
October	0–10	1256	180	360	451	968	1181	733	599^{A}
	10–20	1160	192	530	447	645	641	602	
	20–30	992	115	424	371	489	383	462	
Average Lu/St		**1136**^{a}	**162**^{d}	**438**^{c}	**423**^{c}	**701**^{b}	**735**^{b}		
Average Lu/De		892	234	383	474	1045	1317	724	
		800	258	461	515	752	761	**591**	
		725	199	417	440	598	503	**481**	
Average Lu		**806**^{A}	**231**^{C}	**420**^{B}	**477**^{B}	**800**^{A}	**860**^{A}		

Lu – land use; De – Depth (cm); St – Sampling time; $^{ABC/abc}$ Data followed by the same letter within a row or a column do not differ significantly at the $P \leq 0.05$.

Mineral-associated organic carbon (MOC) is considered an organic material difficult for microbial decomposition and represents the part of SOC which is commonly related to particle dimension composition (Table 3). Due to this, highest SOC in samples resulted with the highest MOC. Content of MOC is more stable C fraction than POC and positively correlated with clay content as clay minerals have a certain capacity to make complex with SOC (Mikutta et al., 2006). Comparing the different land use systems, non-agricultural soil samples were significantly higher in MOC content compared to arable land use (Table 3). Accordingly, changes in SOC contents induced by the land use are primarily caused by changes in the mineral associated SOC pool (John et al., 2005). Samples taken in May had a lower content of MOC compared to soil samples from October. This increase derived from non agricultural samples and can be attributed to soil organic matter chemical composition and straight of connection with the clay (Leifeld and Kögel-Knabner, 2005). Content of MOC had no clear pattern of change with the increasing soil depth. The differences are related to soil bulk density and content of the total SOC and tillage. The application of manure in S3 was significantly higher in MOC content than in 2-year rotations but not higher than MO, indicating that C from manure was mineralized and enriched POC fraction.

Table 3. Content of mineral-associated organic carbon (MOC) and particulate organic carbon (POC) in the soil of different land use systems (g kg^{-1})

C-fractions	Sampling time	Depth (cm) **De**	Land use systems (Lu)						**Average De**	**Average St**
			MO	**N2**	**D2**	**S3**	**UG**	**OF**		
MOC	May	0–10	16.70±0.3	15.81±0.3	13.71±0.1	20.21±0.8	24.31±0.1	25.41±0.2	19.36	18.69^{B}
		10–20	18.48±0.2	16.54±0.3	15.83±0.3	17.88±0.5	22.37±0.1	14.99±0.1	17.68	
		20–30	19.00±0.3	16.67±0.2	14.47±0.2	21.62±0.4	20.87±0.7	21.56±0.5	19.03	
	St/De		**18.06**	**16.34**	**14.67**	**19.91**	**22.51**	**20.65**		
	October	0–10	23.32±0.4	12.65±0.1	17.30±0.2	18.32±0.5	29.48±0.2	35.87±0.1	22.82	21.49^{A}
		10–20	17.46±0.3	15.45±0.2	16.46±0.2	19.74±0.2	33.48±0.1	26.91±0.3	21.58	
		20–30	19.26±0.2	14.67±0.2	15.21±0.4	18.33±0.3	27.94±0.1	24.89±0.3	20.05	
	St/De		**20.01**	**14.26**	**16.32**	**18.80**	**30.30**	**29.22**		
MOC (Average) Lu			**19.03^{b}**	**15.30^{c}**	**15.49^{c}**	**19.35^{b}**	**26.40^{a}**	**24.93^{a}**		
POC	May	0–10	2.55±0.7	1.29±0.1	2.37±0.2	2.47±0.5	9.82±1.4	8.91±0.7	4.57	**3.77^{A}**
		10–20	2.58±0.2	1.17±0.3	2.20±0.3	3.37±0.6	5.82±2.8	7.27±2.5	3.74	
		20–30	3.16±0.6	1.17±0.2	1.95±0.3	3.13±0.7	5.93±3.7	2.62±0.3	2.99	
	St/De		2.76	1.21	2.17	2.99	7.19	6.27		
	October	0–10	3.39±0.8	1.21±0.2	2.40±0.8	3.32±1.0	9.44±1.2	11.84±0.0	5.27	**3.93^{A}**
		10–20	2.40±0.6	1.23±0.1	2.49±0.6	2.54±0.1	6.73±0.8	7.76±1.1	3.86	
		20–30	2.36±0.1	1.31±0.2	2.37±0.3	2.83±0.2	3.50±0.1	3.51±0.3	2.65	
	St/De		**2.72**	**1.25**	**2.42**	**2.89**	**2.90**	**6.56**		
POC (Average) Lu			**2.74^{b}**	**2.23^{c}**	**2.29^{b}**	**2.94^{b}**	**5.04^{a}**	**6.41^{a}**		

Lu – land use; De – Depth (cm); St – Sampling time; $^{ABC/abc}$ Data followed by the same letter within a row or a column do not differ significantly at the $P \leq 0.05$

The highest content of POC was found in the non–agricultural land use systems, while the lowest contents were measured in N2 soil samples (Table 3). In our study statistical differences of the soil POC among two sampling periods were not observed. Crop rotation had no effect on POC, however fertilization significantly affected the content of POC. The plot where mineral N was ommited had the lowest content of POC which indicates a positive role of N addition to the formation of POC. The surface layer in grassland and oak forest were highest in POC due to the presence of fresh OM associated with soil particles and slower decomposition. According to Šeremešić et al. (2013) non-agricultural soils are composed of >30% macroaggregates compared to the <5% macroaggregates in arable soils. Given that the content of POC is related to the macroaggregates, we attributed highest POC content in forest and grassland samples to the better structural properties and better conditions for the soil aggregation. Likewise, the presence of hydrophobic substances in the non-agricultural soils coats the aggregates and slows the entry of water into solum and prevents their impairment (Blair et al., 2006). Changes in organic C by land use occurred mainly in the fraction of POM, however differences in MOC could be SOC background coupled with long-term soil tillage (De Figueiredo et al., 2010). The non-agricultural soil showed highest ratio of POC in SOC >20%, followed with the fertilized plots, and lower ratio was observed at unfertilized plots (6.58–7.66%). Besides, POC concentration was lower in arable soil compared with forest and grassland, reflecting the tillage activities that resulted with fast decomposition of easily available organic matter (Sandén et al., 2017).

CONCLUSION

The present study illustrates differences in SOC fractions concentration of the investigated land use systems. The highest values of carbon soluble in hot water were obtained on samples from non-agricultural soil and lowest level was found in the unfertilized soil. Arable soils had lower POC/MOC ration than grassland and forest indicating lower amount of labile carbon and potential for significant loss of OM in arable soils. This demonstrated substantial inflow and stabilization of the fresh OM in non-agricultural soils. Accordingly, management practices have a significant role in carbon transformation and fertilization has a significant role in maintenance and preservation of soil organic carbon.

ACKNOWLEDGMENT

This study is part of the TR031072 and TR031073 projects financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

REFERENCES

Baldock JA, Nelson PN (2000): Soil organic matter. In: Sumner ME (eds.), *Handbook of Soil Science*. Boca Raton: CRC Press

Bayer C, Matin-Neto L, Mielniczuk J, Pavinato A (2004): Carbon storage in labile fractions of soil organic matter in a tropical no-tillage Oxisol. *Pesqui. Agropecu. Bras*. 39: 677–683.

Blair N, Faulkner RD, Till AR, Körschens M, Schulz E (2006): Long-term management impacts on soil C, N and physical fertility. Part II. Bad Lauchstadt static and extreme FYM experiments. *Soil Tillage Res*. 91: 39–47.
Bouajila A, Gallali T (2008): Soil organic carbon fraction and aggregate stability in carbonated and no carbonated soil in Tunisia. *J. Agron*. 7: 127–137.
Cambardella CA, Elliott ET (1993): Methods for physical separation and characterization of soil organic matter fractions. *Geoderma* 56: 449–457.
Chen HQ, Marhan S, Billen N, Stahr K (2009): Soil organic carbon and total nitrogen stocks as affected by different land uses in Baden-Wurttemberg, Southwest Germany. *J. Plant Nutr. Soil Sci*. 172: 32–42.
Cookson WR, Abaye DA, Marschner P, Murphy DV, Stockdale EA, Goulding KWT (2005): The contribution of soil organic matter fractions to carbon and nitrogen mineralization and microbial community size and structure. *Soil Biol. Biochem*. 37: 1726–1737.
De Figueiredo CC, Resck,DVS, Carneiro MAC (2010): Labile and stable fractions of soil organic matter under management systems and native cerrado. *Rev. Bras. Cienc. Solo* 34: 907–916.
Ghani A, Dexter M, Perrott KW (2003): Hot–water carbon is an integrated indicator of soil quality. *Soil Biol. Biochem*. 35: 1231–1243.
Gregorich EG, Beare MH, Stoklas U, St-Georges P (2003): Biodegradability of soluble organic matter in maize cropped soils. *Geoderma* 113: 237–252.
Haynes RJ (2000): Interactions between soil organic matter status, cropping history, method of quantification and sample pre-treatment and their effects on measured aggregate stability. *Biol. Fert. Soils* 30: 270–275.
Haynes RJ, Beare MH (1996): Aggregation and organic matter storage in mesothermal, humid soils. In: Carter MR and Stewart BA (eds.), *Structure and organic matter storage in agricultural soils*, Boca Raton: CRC Press.
IUSS Working Group WRB (2014): World Reference Base for Soil Resources 2014. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports 106. FAO, Rome. Available to: http://www.fao.org/3/i3794en/I3794en.pdf.
Janzen HH, Campbell CA, Brandt SA, Lafond SA, Townley-Smith L (1992): Light-fraction organic matter in soils from long-term rotations. *Soil Sci. Soc. Am. J*. 56: 1799–1804.
John B, Yamashita T, Ludwig B, Flessa H (2005): Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use. *Geoderma* 128: 63–79.
Leifeld J, Kögel-Knabner I (2005): Soil organic matter fractions as early indicators for carbon stock changes under different land-use? *Geoderma* 124: 143–155.
Leinweber P, Schulten HR, Körschens M (1995): Hot water extracted organic matter: chemical composition and temporal variations in a long–term field experiment. *Biol. Fert. Soils* 20: 17–23.
Martinez-Mena M, Lopez J, Almagro M, Albaladejo J, Castillo V, Ortiz R, Boix-Fayos C (2012): Organic carbon enrichment in sediments: Effects of rainfall Characteristics under different land uses in Mediterranean area. *Catena* 94: 36–42.
McLauchan KK, Hobbie SE, Post WM (2006): Conversion from agriculture to grassland builds soil organic matter on decadal timescales. *Ecol. Appl*. 16: 143–153.
Mikutta R, Kleber M, Torn MS, Jahn R (2006): Stabilization of soil organic matter: association with minerals or chemical recalcitrance? *Biogeochemistry* 77: 25–56.
Molnar I (2003): Cropping systems in Eastern Europe: Past, Present and Future. *J. Crop Prod*. 9: 623–647.
Najvirt BD, Đurić SS, Hajnal-Jafari IT, Stamenov DS (2019): Characteristic of Agricultural organic matter degrading bacterial isolate from different type of soil. *Matica srpska J. Nat. Sci.* 163: 69–78.
Sandén T, Lair GJ, Van Leeuwen JP, Gísladóttir G, Bloem J, Ragnarsdóttir KV, Blum WE, (2017): Soil aggregation and soil organic matter in conventionally and organically farmed Austrian Chernozems. *Bodenkultur* 68: 41–55.
Seremesic S, Milosev D, Djalovic I, Zeremski T, Ninkov J (2011): Management of soil organic carbon in maintaining soil productivity and yield stability of winter wheat. *Plant Soil Environ*. 57: 216–221.
Six J, Conant RT, Paul EA, Paustian K (2002): Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant Soil* 241: 155–176.
Sparling G, Vojvodić-Vuković M, Schipper LA (1998): Hot-water-soluble C as a simple measure of labile soil organic matter: the relationship with microbial biomass C. *Soil Biol. Biochem*. 30: 1469–1472.

Šeremešić S, Milošev D, Đalović I, Belić M, Ćirić V, Đurđević B, Jaćimović G (2013): A comparative study of the winter wheat and maize effects on the changes in structural properties of Chernozem. *Proceedings of 2nd International Scientific Conference, Soil and Crop Management: Adaptation and Mitigation of Climate Change*, Osijek, Croatia, 155–165.
Šimon T (2008): The influence of Long-term Organic and Mineral Fertilization on Soil Organic Matter. *Soil Water Res*. 3: 41–51.
Van Wesemael B, Paustian K, Meersmans J, Goidts E, Barančíková G, Easter M (2010): Agricultural management explains historic changes in regional soil carbon stocks. *Proc. Natl. Acad. Sci. USA* (*PNAS*) 107: 14926–14930.

ФРАКЦИЈЕ ОРГАНСКОГ УГЉЕНИКА У РАЗЛИЧИТИМ НАЧИНИМА КОРИШЋЕЊА ЗЕМЉИШТА НА ЧЕРНОЗЕМУ

Срђан И. ШЕРЕМЕШИЋ[1], Љиљана М. НЕШИЋ[1],
Владимир И. ЋИРИЋ[1], Јовица Р. ВАСИН[2], Ивица Г. ЂАЛОВИЋ[2],
Јелена Б. МАРИНКОВИЋ[2], Бојан Ђ. ВОЈНОВ[1]

[1] Универзитет у Новом Саду, Пољопривредни факултет,
Трг Д. Обрадовића 8, Нови Сад 21000, Србија
[2] Институт за ратарство и повртарство,
Максима Горког 30, Нови Сад 21000, Србија

РЕЗИМЕ: Однос између фракција угљеника у земљишту испитиван је у узорцима земљишта пореклом са различитих начина коришћења: обрадиво земљиште, травњак и храстова шума. Травњак и храстова шума имали су већи садржај органског угљеника (SOC), угљеника растворљивог у топлој води (HWC), честичног органског угљеника (POC) у односу на ораницу. Однос POC/MOC је био најмањи у обрадивом земљишту, што указује на мању количину лабилног угљеника услед интензивијих процеса разградње. Вредности POC расту са већим укупним SOC, што указује да очување органске материје зависи од обнављања лабилних фракција. Наши резултати су показали да је ђубрење имало значајну улогу у стабилизацији угљеника у земљишту, док је плодоред имао мањи утицај. Добијени резултати могу допринети бољем разумевању улоге лабилне органске материје у чернозему, те на тај начин помоћи у одабиру система ратарења за управљање и очување SOC.

КЉУЧНЕ РЕЧИ: системи коришћења земљишта, органски угљеник у земљишту, угљеник везан у минералима, честични органски угљеник

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. Novi Sad, № 138, 41—49, 2020

UDC 633.34:579.8
https://doi.org/10.2298/ZMSPN2038041M

Jelena B. MARINKOVIĆ[*], *Dragana Đ. BJELIĆ*, *Branislava B. TINTOR*, *Vuk B. ĐORĐEVIĆ*, *Svetlana N. BALEŠEVIĆ-TUBIĆ*, *Vojin H. ĐUKIĆ*, *Marina R. ĆERAN*

Institute of Field and Vegetable Crops,
Maksima Gorkog 30, Novi Sad 21000, Serbia

SOIL MICROBIAL PROPERTIES UNDER DIFFERENT MANAGEMENT SYSTEMS IN SOYBEAN PRODUCTION

ABSTRACT: The aim of the study was to examine the effects of management practice on microbial properties of soil under soybean production. The study included 180 samples of soil under certified organic soybean production and 80 samples in conventional production system. An abundance of the examined microbial groups was assessed using the indirect dilution method, followed by plating of soil suspension on different selective media, while dehydrogenase and β-glucosidase activity was measured spectrophotometrically. Our data indicated that the management practice affected the structure and activity of microbial communities. A significant positive effect of organic farming on *Azotobacter* spp., free N-fixers and abundance of actinomycetes was identified. The influence of management system for the total number of bacteria, ammonifiers and fungi was not observed. Significantly higher dehydrogenase and β-glucosidase activity was recorded in the soils under organic farming compared to the conventional farming. The obtained results showed an increase in organic matter content, associated with organic soil management, and its positive correlation with soil microbial properties.

KEYWORDS: dehydrogenase, β-glucosidase, microbial abundance, organic and conventional management, soybean

INTRODUCTION

The presence and activity of microbial communities have a crucial role in numerous biochemical cycles important in the functioning of soil ecosystem. Soil microorganisms play the key role in organic matter decomposition, transformation, mineralization and release of carbon, nitrogen, phosphorus, sulfur and other nutrients important for plant nutrition. Soil microbes are also involved,

[*] Corresponding author. E-mail: jelena.marinkovic@nsseme.com

either directly or indirectly, in many other processes which provide stability to the soil ecosystem, such as control of erosion through the formation of stable soil aggregates and soil structure, pesticide degradation, pest and disease regulation and bioremediation (Nannipieri et al., 2003).

Microbial abundance, diversity and activity are important indicators of soil quality. Relationships between the stability and functioning of the soil ecosystem and the changes in microbial community structure are very complex (Stagnari et al., 2014). Intensification of agriculture, use of cultivation techniques in conventional production and intensive anthropogenic activities cause serious soil degradation. Microbial community structure is mostly determined by soil chemical and physical characteristics, but soil management practices have the potential to modify the biomass, diversity and physiology of microbes in agricultural fields. Agricultural practice has a complex and diverse influence on soil microorganisms and composition of their communities (Wang et al., 2016). Conventional agriculture relies on the use of synthetic mineral fertilizers and pesticides, which cause adverse impacts on soil health and productivity. However, they are also regarded as the primary factors which induce negative changes in soil microbial population, while shaping their size and structure. Organic production minimizes the impact of agricultural practice on soil quality and the environment, and represents the best alternative to conventional production. Approximately 1% of the world's arable land is under organic soil management (Lori et al., 2017) with a 14% annual increase in surface area (Wang et al., 2016). Organic farming improves soil physical and chemical properties, while the application of different organic fertilizers enriches soil by providing it with organic matter, and thus enhances soil fertility. Organic farming system aims to close nutrition cycles and this concept relies heavily on microbial activity. Soils managed organically exhibit higher microbial diversity, biomass and activity. However, complex and diverse overall response of soil microbial populations to soil management type is still insufficiently understood (Stagnari et al., 2014).

The impact of agricultural management on soil microbial abundance, diversity and activity is very complex and diverse. For this reason, the aim of the study was to identify and compare microbial community abundance and enzymatic activity in soils under organic or conventional farming systems.

MATERIALS AND METHODS

Soil samples were collected from several fields under organic soybean production at the location Čurug–Gospođinci, AP Vojvodina. The largest areas under organic soybean production in AP Vojvodina were registered under Global Seed d.o.o. (Čurug), at the total area of 390 ha (soybean variety Rubin on 170 ha, NS Maximus on 110 ha, Fortuna on 40 ha, varieties Galina and Valjevka on 35 ha, each). Organic management practice had been applied for at least three consecutive years prior to sampling all the tested plots.

Samples of soil under conventional soybean production were collected at several fields at the location Rimski Šančevi – Čenej (AP Vojvodina). A total of 180 samples of soil under certified organic production and 80 samples of soil under conventional production were collected. Soil samples were collected randomly form soybean rizosphere, at 0–20 cm, at the beginning of June, in the period of soybean full bloom (R3), while the number of samples depended on the size of the plot. Soil samples were stored for microbial analysis at 4 °C, and maintained until laboratory analysis.

Soil chemical analyses were conducted on air-dried samples, as previously described (Marinković et al., 2018).

The abundance of the total and specific bacterial and fungal communities was assessed by an indirect dilution spread-plating method on an appropriate nutritive media. The total number of bacteria was determined on a soil agar, ammonifiers on a nutrient agar (NA), *Azotobacter* spp. and free N-fixing bacteria on a nitrogen-free medium (Fyodorov's medium), fungi on a Czapek-Dox agar, and actinomycetes on a synthetic medium (Krasilnikov's agar). Plates were incubated at the temperature of 28 °C, while incubation time depended on the tested microbial group. The number of *Azotobacter* spp., ammonifiers and actinomycetes was detected after 2, 3 and 7 days respectively, while the total number of bacteria, free N-fixing bacteria and fungi was recorded after 5 days. After incubation, the average number of colony forming units (CFU) was calculated at 1.0 g of absolutely dry soil (Briones and Reichardt, 1999). Dehydrogenase activity (DHA) (EC 1.1.1.) was done by measuring the extinction of colored triphenylformazan (TPF) formed by reducing a colorless triphenyl-tetrazolium chloride (TTC) (Casida et al., 1964). TPF concentration was measured at 485 nm, and the results were expressed as μg TPF g^{-1} dry soil. Activity of β-glucosidase (β-glc) (EC 3.2.1.21) was determined by measuring the extinction of colored p-nitrophenol, formed by reducing a colorless p-nitrophenyl β-d-glucoside (PNG) (Hayano, 1973). The intensity of the yellow colored p-nitrophenol was measured at 400 nm and the average β-glc was expressed as an enzyme unit. One unit of enzyme was defined as 1 μmol of p-nitrophenol released per min at 30 °C. All microbiological analyses were performed in three replications.

The variables were analyzed using two-way analysis of variance (ANOVA), followed by mean separation according to Tukey's test at the $P < 0.05$ level of probability.

RESULTS AND DISCUSIONS

Chemical characteristics of soils under organic and conventional agriculture production are presented in Table 1. The results showed that soil samples under both organic and conventional production were slightly alkaline (pH 7.26–7.42), and only one sample was acidic (pH 5.43). Soils under organic farming belong to the class of humic soils (> 3%), while soils samples in conventional fields were characterized by the lower humus content (< 3%) (Table 1).

Table 1. Chemical soil properties of the examined fields

Sample no.	Agricultural management	pH		$CaCO_3$ %	Humus %	Total N %	AL-P_2O_5 (mg 100 g^{-1})	AL-K_2O (mg 100 g^{-1})
		in KCl	in H_2O					
1	**Organic**	7.33	8.10	6.76	3.57	0.245	22.9	24.00
2	**Organic**	7.26	8.06	4.22	3.52	0.241	16.2	22.00
3	**Organic**	7.27	8.07	5.07	3.45	0.235	12.10	21.00
4	**Organic**	7.27	8.03	8.44	3.52	0.241	12.20	25.00
5	**Organic**	5.43	6.78	0.76	3.47	0.238	4.50	19.00
6	**Conventional**	7.24	8.29	7.16	2.19	0.163	27.76	29.23
7	**Conventional**	7.38	8.22	3.38	2.58	0.192	27.90	25.00
8	**Conventional**	7.42	8.26	4.64	2.62	0.195	26.90	23.91
9	**Conventional**	7.38	8.21	3.80	2.65	0.197	27.40	39.81
10	**Conventional**	7.37	8.16	4.64	2.38	0.177	25.50	32.37

Organically managed soils enhance microbial community structure, abundance, diversity and activity.

Table 2. Microbial abundance and activity depending on the management system

Sample no.	Agricultural management	Soybean variety	*Azotobacter* spp.	AMN ×10^7	TNB ×10^7	FNB ×10^6	FNG ×10^4	ACT ×10^4	DHA (µg TPF g^{-1} soil)	β-glc (mU g^{-1} soil)
			CFU g^{-1} soil							
1	**Organic**	Rubin	1310 a	173 ab	162 a	230 a	19 a	17 a	318 a	74.6 a
2	**Organic**	Maximus	1150 a	211 a	148 ab	231 a	20 a	20 a	217 ab	75.7 a
3	**Organic**	Galina	690 b	206 a	160 a	194 ab	17 a	20 a	309 a	82.5 a
4	**Organic**	Valjevka	740 b	192 a	193 a	243 a	14 a	19 a	314 a	79.0 a
5	**Organic**	Fortuna	2 c	135 b	121 ab	77 c	28 a	3 b	35 c	71.4 a
	Organic	**Average**	**778 A**	**183 A**	**157 A**	**195 A**	**20 A**	**16 A**	**239 A**	**76.6 A**
6	**Conventional**	Rubin	0 c	204 a	157 a	190 ab	25 a	7 ab	60 bc	47.2 b
7	**Conventional**	Maximus	2 c	147 b	143 ab	105 b	17 a	6 ab	73 bc	34.7 b
8	**Conventional**	Galina	0 c	191 a	192 a	122 b	18 a	12 ab	110 b	35.6 b
9	**Conventional**	Valjevka	1 c	213 a	163 a	138 b	28 a	8 ab	83 bc	39.8 b
10	**Conventional**	Fortuna	1 c	197 a	136 ab	125 b	16 a	11 ab	63 bc	50.4 b
	Conventional	**Average**	**1 B**	**190 A**	**158 A**	**136 B**	**21 A**	**9 B**	**78 B**	**41.5 B**

AMN – ammonifiers; TNB – total number of bacteria; NFB – nitrogen-fixing bacteria; FNG – fungi; ACT – actinomycetes; DHA – dehydrogenase; β-glc – β-glucosidase.

* The different letters indicate a significant difference at $P < 0.05$

The results of the study showed significant increase in the abundance of *Azotobacter* spp., free N-fixing bacteria, actinomycetes and dehydrogenase and β-glucosidase activity in soils under organic management. Influence of soil management system was not significant for the total number of bacteria, ammonifiers and fungi (Table 2). The two different soil management systems did not significantly affect the total bacterial population and fungal abundance in the previous studies (Scullion et al., 1998; Stagnari et al., 2014; Marinkovic et al., 2018), which is in accordance with our results. The total number of bacteria and fungi were similar in both systems due to their diversity and ability to adapt and grow under various environmental conditions (Anand et al., 2006).

Azotobacter spp. is among the most important free-living, heterotrophic, aerobic soil bacteria, capable of fixing an average of 20 kg N ha^{-1} per year (Jnawali et al., 2015). *Azotobacter* strains are very sensitive to adverse environmental conditions, and their presence is therefore considered an indicator of soil quality, health and fertility (Kizilkaya, 2009). Favorable conditions in the soils under organic production – slightly alkaline pH and organic matter content above 3%, resulted in an abundance of *Azotobacter* spp. (690–1310 CFU g^{-1} soil) and free N-fixers (194–243×10^6 CFU g^{-1} soil) (Table 2). A significant decrease in the presence of *Azotobacter* spp. (2 CFU g^{-1} soil), free N-fixers (77×10^6 CFU g^{-1} soil) and low phosphorus content were noted in sample no. 5 with pH < 5.43. *Azotobacter* strains are sensitive to acidic pHand, for this reason, its population is the most abundant in neutral and slightly alkaline soils (Barnes et al., 2007). Samples of soil under conventional production also had slightly alkaline pH, so *Azotobacter* spp. strains were recorded in extremely low number or not detected at all (Table 2). The average abundance of free N-fixers (136×10^6 CFU g^{-1} soil) was also significantly lower compared with their presence in fields under organic production (Table 2). Previous studies showed that populations of different bacterial N-fixing genera were more abundant under organic soil management (Jangid et al., 2008; Orr et al., 2012; Stagnari et al., 2014). Extensive use of mineral fertilizers and pesticides may reduce the number of *Azotobacter* spp. and other nitrogen fixing bacteria in the soil (Khudhur and Askar, 2013; Jnawali et al., 2015; Shaid et al., 2019). Considering that soil chemical analysis conducted in our research did not reveal high concentrations of P and K in fields under conventional management, excessive pesticide application is assumed to have an extreme negative effect on *Azotobacter* spp. population. Nitrogen fixing bacteria are known for their sensitivity to pesticides (Moreno et al., 2009; Orr et al., 2012). Some pesticides may therefore cause deleterious impact to *Azotobacter* populations (Shahid et al., 2019) and nitrogen fixers (Walvekar et al., 2017).

Actinomycetes are involved in organic matter turnover, nutrient recycling, humus synthesis, but they are also known for their ability to produce antimicrobial compounds (Aislabie and Deslippe, 2013). Through the production of extracellular enzymes, these microbes are able to degrade complex organic compounds, including lignin, cellulose, chitin and pectin, into less recalcitrant molecules (Eilers et al., 2010; Li et al., 2012). Actinomycetes have a strong negative relationship with soil pH, thus being more abundant in neutral and slightly alkaline soils (Sreevidya et al., 2016). The significantly lowest number of actinomycetes (3×10^4 CFU g^{-1} soil) (Table 2) was recorded in the acidic soil sample. Soil management significantly affected the number of actinomycetes, while organic farming showed higher average values (16×10^4 CFU g^{-1} soil) than the conventional (9×10^4 CFU g^{-1} soil) (Table 2). Increase in organic carbon sources, associated with organic fertility management, had a positive correlation with the population of actinomycetes (Chen et al., 2018). Recent studies confirmed a significant influence of soil management system and an enhanced population of actinomycetes in soils under organic farming (Stagnari et al., 2014; Chen et al., 2018).

Enzymatic activity has been proposed as an integrative determinant of soil quality, because of the key role in numerous biochemical and nutrient-cycling processes in soils (Stott et al., 2010). Dehydrogenase is widely used as a reliable indicator of the total oxidative activity of soil microorganisms and overall soil metabolic activity (Nannipieri et al., 2003). Unfavorable environmental conditions, such as low/high temperature, low/high pH, lower content of organic matter, and presence of heavy metals, fertilizers and pesticides, could have an adverse impact on dehydrogenase activity. The lowest DHA (35 μg TPF g^{-1} soil) (Table 2) was recorded in the acidic soil sample, confirming significant relation between dehydrogenase activity and soil pH (Fernandez-Calvino et al., 2010). Microbial communities in soils under organic production showed DHA values up to three times higher than those in soils under conventional production. Significantly higher average DHA was recorded in organic (239 μg TPF g^{-1} soil) than in conventional systems (78 μg TPF g^{-1} soil) (Table 2). Significant differences in DHA between production systems could be due to lower soil organic matter content in conventional fields. Previous research emphasized a positive correlation between soil DHA and organic matter content (Zhao et al., 2010; Yuan and Yue, 2012). The β-glucosidase enzyme catalyzes the final step of cellulose hydrolysis, and plays a major role in degradation of plant residues (Stott et al., 2010). The activity of β-glucosidase is sensitive to changes in crop residue management, but could also be an early indicator of changes in soil organic matter content (Stott et al., 2010). The average β-glucosidase activity in the fields under organic management was significantly higher (76.6 mU g^{-1} soil) than under conventional management (41.5 mU g^{-1} soil) (Table 2). The results confirm that β-glucosidase activity is sensitive to changes caused by differences in soil management systems. Application of organic materials in organic production results in increased β-glucosidase activity. Elevated levels of β-glucosidase activity are confirmed in the previous research (De la Hora et al., 2003; Acosta-Martínez et al., 2007; Stott et al., 2010).

Previous studies showed that soil pH is one of the most important factors affecting abundance and diversity of bacterial populations, with the optimum pH close to neutral (Lauber et al., 2009). Our study confirmed significantly the lowest number of azotobacter, free N-fixers, actinomycetes, and dehydrogenase activity in the acidic soil sample. Soil organic matter substrate to soil microorganisms and it is an essential plant nutrient source through mineralization (Haynes, 2005). Soils in AP Vojvodina naturally have a high content of organic matter, but inadequate cultivation practices applied during a longer period caused significant reduction in soil organic matter content (Vasin et al., 2013). Organic matter declines significantly after an extended time of agricultural production (Powlson et al., 2012), and it is lower in some agricultural soils than in abandoned soils of the same type (Marinković et al., 2018). Organic management has a positive effect on soil organic matter content through incorporation of manure, compost, green manure, and plowing crop residues (Wang et al., 2016). Increased organic matter content in organic farming positively influences microbial growth, biomass and enzymatic activity (Lori et al., 2017). Significantly higher average number of azotobacter, free N-fixers and actynomicetes, as well as dehydrogenase and β-glucosidase activityfound in our study, confirm the positive correlation with soil organic matter content.

CONCLUSIONS

Organic soil management practices improved soil nutrient status and impacted positively most of the investigated microbial indicators. Humus content was higher in all organic fields than on conventional plots. The abundance of *Azotobacter* spp., free N-fixing bacteria and actinomycetes was significantly enhanced in fields under organic farming. There were no significant changes in the total number of microorganisms, ammonifiers and fungi under specific agricultural management practices. The average dehydrogenase and β-glucosidase activity was significantly higher in soils under organic management than in conventional fields. These results allow better understanding of linkage between soil management and soil nutritional status, as well as structure and activity of soil microbial populations.

ACKNOWLEDGEMENT

The research was supported by the Provincial Secretariat for Higher Education and Scientific Research, Vojvodina, Serbia, Project No. 114-451-2739/2016: "Sustainable production of plant proteins: soybean, microorganisms, response to climate changes", and Ministry of Education, Science and Technological Development, Republic of Serbia (Project No. TR 30122)

REFERENCES

Acosta-Martínez V, Mikha MM, Vigil MF (2007): Microbial communities and enzyme activities in soils under alternative crop rotations compared to wheat–fallow for the central Great Plains. *Appl. Soil Ecol.* 37: 41–52.

Aislabie J, Deslippe JR (2013): Soil microbes and their contribution to soil services. In: *Dymond JR ed. Ecosystem services in New Zealand – conditions and trends*. Manaaki Whenua Press, Lincoln, New Zealand.

Anand P, Isar J, Saran S, Saxena RK (2006): Bioaccumulation of copper by *Trichoderma viride*. *Biores. Technol.* 97: 1018–1025.

Askar KA, Khudhur AM (2013): Effect of some pesticides on growth, nitrogen fixation and nif genes in *Azotobacter chroococcum* and *Azotobacter vinelandii* isolated from soil. *J. Toxicol. Environ. Health Sci.* 5: 166–171.

Barnes RJ, Baxter SJ, Lark RM (2007): Spatial covariation of *Azotobacter* abundance and soil properties: A case study using the wavelet transform. *Soil Biol. Biochem*. 39: 295–310.
Bhatia R, Ruppel S, Narula N (2009): Diversity studies of *Azotobacter* spp. from cotton-wheat cropping systems of India. *J. Basic Microbiol*. 48: 455–463.
Briones AM, Reichardt W (1999): Estimating microbial population counts by 'most probable number' using Microsoft Excel. *J. Microbiol. Methods* 35: 157–161.
Casida LEJ, Klein DA, Santoro T (1964): Soil dehydrogenase activity. *Soil Sci*. 98: 371–376.
Chen H, Xia Q, Yang T, Shi W (2018): Eighteen-Year Farming Management Moderately Shapes the Soil Microbial Community Structure but Promotes Habitat-Specific Taxa. *Front Microbiol*. 9: 1776.
De la Horra AM, Conti ME, Palma RM (2003): β-Glucosidase and proteases activities as aff ected by long-term management practices in a Typic Argiudoll soil. Commun. *Soil Sci. Plant Anal*. 34: 2395–2404.
Eilers KG, Lauber CL, Knight R, Fierer N (2010): Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. *Soil Biol. Biochem*. 42: 896–903.
Fernandez-Calvino D, Soler-Rovira P, Polo A, Diaz-Raviña M, Arias-Estevez M, Plaza C (2010): Enzyme activities in vineyard soils long-term treated with copper-based fungicides. *Soil Biol. Biochem*. 42: 2119–2127.
Hayano K (1973): A method for the determination of ß-glucosidase activity in soil. *Soil Sci. Plant Nutr*. 19: 103–108.
Haynes RJ (2005): Labile organic matter fractions as central components of the quality of agricultural soils: an overview. *Adv. Agron*. 85: 221–268.
Jangid K, Williams MA, Franzluebbers AJ, Sanderlin JS, Reeves JH (2008): Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. *Soil Biol. Biochem*. 40: 2843–2853.
Jnawali AD, Ojha RB, Marahatta M (2015): Role of *Azotobacter* in Soil Fertility and Sustainability–A Review. *Adv. Plants Agric. Res*. 2: 250–253.
Kizilkaya R (2009): Nitrogen fixation capacity of *Azotobacter* spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. *J. Environ. Biol*. 30: 73–82.
Lauber C, Hamady M, Knight R, Fierer N (2009): Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl. Environ. Microbiol*. 75: 5111–5120.
Li R, Khafipour E, Krause DO, Entz MH, de Kievit TR, Fernando WGD (2012): Pyrosequencing reveals the influence of organic and conventional farming systems on bacterial communities. *PLOS One* 7: e51897.
Lori M, Symnaczik S, Mader P, De Deyn G, Gattinger A (2017): Organic farming enhances soil microbial abundance and activity—A metaanalysis and meta-regression. *PLOS One* 12: e0180442.
Marinković J, Bjelić D, Ninkov J, Vasin J, Tintor B, Živanov M (2018): Effect of different soil usage on microbial properties in soils of Central Serbia. *Ratar. Povrt*. 55: 58–64.
Moreno B, Garcia-Rodriguez S, Cañizares R, Castro J, Benıtez E (2009): Rainfed olive farming in south-eastern Spain: Long-term effect of soil management on biological indicators of soil quality. *Agri. Ecosys. Environ*. 131: 333–339.
Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003): Microbial diversity and soil functions. *Eur. J. Soil Sci*. 54: 655–670.
Orr CH, Leifert C, Cummings SP, Cooper JM (2012): Impacts of organic and conventional crop management on diversity and activity of free-living nitrogen fixing bacteria and total bacteria are subsidiary to temporal effects. *PLOS One* 7: e52891.
Powlson DS, Bhogal A, Chambers BJ, Coleman K, Macdonald AJ, Goulding KWT, Whitmore AP (2012): The potential to increase soil carbon stocks through reduced tillage or organic material additions in England and Wales: A case study. *Agric. Ecosyst. Environ*. 146: 23–33.
Scullion J, Eason WR, Scott EP (1998): The effectivity of arbuscular mycorrhizal fungi from high input conventional and organic grassland and grassarable rotations. *Plant Soil* 204: 243–254.
Shahida M, Zaidia A, Ehtramb A, Khana MS (2019): In vitro investigation to explore the toxicity of different groups of pesticides for an agronomically important rhizosphere isolate *Azotobacter vinelandii*. *Pestic. Biochem. Physiol*. 157: 33–44.
Sreevidya M, Gopalakrishnan S, Kudapa H, Varshney RK (2016): Exploring plant growth-promotion actinomycetes from vermicompost and rhizosphere soil for yield enhancement in chickpea. *Braz. J. Microbiol*. 47: 85–95.

Stagnari F, Perpetuini G, Tofalo R, Campanelli G, Leteo F, Della Vella U, Schirone M, Suzzi G, Pisante M (2014): Long-term impact of farm management and crops on soil microorganisms assessed by combined DGGE and PLFA analyses. *Front. Microbiol*. 5: 644.
Stott DE, Andrews SS, Liebig MA, Wienhold BJ, Karlen DL (2010): Evaluation of β-Glucosidase Activity as a Soil Quality Indicator for the Soil Management Assessment Framework. *Soil Sci. Soc. Am. J.* 74: 107–119.
Vasin J, Milić S, Zeremski T, Ninkov J, Marinković J, Sekulić P (2013): *Potencijali Republike Srbije u pogledu kvaliteta zemljišta za organsku poljoprivrednu proizvodnju*. Novi Sad: Institut za ratarstvo i povrtarstvo.
Walvekar VA, Bajaj S, Singh DK, Sharma S (2017): Ecotoxicological assessment of pesticides and their combination on rhizosphere microbial community structure and function of Vigna radiata. *Environ. Sci. Pollut. Res*. 24: 17175–17186.
Wang W, Wang H, Feng Y, Wang L, Xiao X, Xi Y, Luo X, Sun R, Ye X, Huang Y, Zhang Z, Cui Z (2016): Consistent responses of the microbial community structure to organic farming along the middle and lower reaches of the Yangtze River. *Sci. Rep.* 6: 35046.
Yuan B, Yue D (2012): Soil microbial and enzymatic activities across a chronosequence of chinese pine plantation development on the loess plateau of China. *Pedosphere* 22: 1–12.
Zhao B, Chen J, Zhang J, Qin S (2010): Soil microbial biomass and activity response to repeated drying-rewetting cycles along a soil fertility gradient modified by long-term fertilization management practices. *Geoderma* 160: 218–224.

МИКРОБИОЛОШКЕ КАРАКТЕРИСТИКЕ ЗЕМЉИШТА У РАЗЛИЧИТИМ СИСТЕМИМА ГАЈЕЊА СОЈЕ

Јелена Б. МАРИНКОВИЋ, Драгана Ђ. БЈЕЛИЋ, Бранислава Б. ТИНТОР, Вук Б. ЂОРЂЕВИЋ, Светлана Н. БАЛЕШЕВИЋ-ТУБИЋ, Војин Х. ЂУКИЋ, Марина Р. ЋЕРАН

Институт за ратарство и повртарство
Максима Горког 30, Нови Сад 21000, Србија

РЕЗИМЕ: Циљ рада био је да се испита утицај система гајења на микробиолошке карактеристике земљишта које је засејано сојом. Истраживање је обухватило 180 узорака земљишта под сертификованом органском производњом соје и 80 узорака у конвенционалној производњи. Бројност испитиваних група микроорганизама одређена је индиректном методом разређења, засејавањем земљишне суспензије на одговарајуће селективне подлоге, док је активност дехидрогеназе и β-глукозидазе утврђена спектрофотометријски. Добијени резултати указују да је систем гајења утицао на структуру и активност микробних заједница. Значајни позитивни ефекти органске производње одразили су се на бројност *Azotobacter* spp., слободних азотофиксатора и актиномицета. Утицај система гајења није запажен у промени укупног броја бактерија, амонификатора и гљива. Значајно виша активност дехидрогеназе и β-глукозидазе забележена је под органском производњом у поређењу са конвенционалном. Добијени резултати указују да је повећање у садржају органске материје, које је повезано са органским системом ђубрења, у позитивној корелацији са микробиолошким особинама земљишта.

КЉУЧНЕ РЕЧИ: бројност микроорганизама, дехидрогеназа, β-глукозидаза, органска и конвенционална производња, соја

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. Novi Sad, № 138, 51—59, 2020

UDC 631.46:633.11
https://doi.org/10.2298/ZMSPN2038051B

*Dragana Đ. BJELIĆ**, *Jelena B. MARINKOVIĆ*, *Branislava B. TINTOR*, *Jordana M. NINKOV*, *Jovica R. VASIN*, *Milorad S. ŽIVANOV*, *Snežana P. JAKŠIĆ*

Institute of Field and Vegetable Crops,
Maksima Gorkog 30, Novi Sad 21000, Serbia

POSSIBILITY OF USING *Bacillus* AND *Trichoderma* STRAINS FOR DECOMPOSITION OF CROP RESIDUES

ABSTRACT: The objective of this study was to investigate the possibility of using microbial strains as residue decomposers and to determine the effect of these strains on chemical and microbial properties in the residue-amended soil. Greenhouse experiment consisted of eight *Bacillus* treatments, three *Trichoderma* treatments, and their combination, all applied to non-sterile chernozem soil amended with wheat straw. Incorporation of wheat straw improved soil chemical and microbial properties, while the extent of residue decomposition under microbial strains was intensified. Microbial treatments significantly affected the soil pH, the content of carbonate, total carbon, soil organic carbon, humus, and available phosphorus and potassium. Bacterial and fungal treatments also significantly influenced the total microbial number, ammonifiers, N_2-fixers, fungi, actinomycetes, oligotrophs, copiotrophs, and cellulolytic microorganisms. The effect of microbial treatments varied depending on the applied strains and examined properties, with *Bacillus* strains being more promising residue decomposers compared to *Trichoderma* strains. The most effective microbial strains could be used as potential decomposers of crop residues.

KEYWORDS: *Bacillus*, soil microorganisms, soil organic matter, *Trichoderma*, wheat straw

INTRODUCTION

A sustainable agroecosystem relies upon an adequate amount of soil organic matter (SOM). Organic matter plays an important and multiple roles in soil, affecting physical, chemical, and biological soil properties, such as soil structure, cation exchange capacity, soil pH, and nutrient and energy supply for microbial biomass and higher plants (Walsh and McDonnell, 2012). The amount of organic matter in soil is largely influenced by cropping (Liu et al., 2005). Management practices such as repetitive tillage, removal or burning of crop residues, and

* Corresponding author. E-mail: dragana.bjelic@ifvens.ns.ac.rs

inadequate fertilization result in a decrease of SOM content (Ghimire et al., 2017). Conversely, increasing or preserving the SOM content requires a sustained effort that includes reduced or no-tillage, crop residue amendment, integration of organic and chemical fertilizers, and crop rotations (Shrestha et al., 2013).

Incorporation of crop residues or other forms of organic material may reduce the application of chemical fertilizers while maintaining SOM levels and improving fertility and productivity of agricultural soils (Singh and Rengel, 2007). A great proportion of nutrient input during cultivation i.e. 30–35% of applied nitrogen (N) and phosphorus (P), and 70–80% of applied potassium (K), remains in crop residues (FAO, 2006). For instance, incorporation of wheat straw saves 50% of the recommended fertilizer quantity, followed by increased productivity of crops (Rajput, 1995). Additionally, crop residues are the main contributor to soil organic carbon (SOC) pool, which is a key index for soil fertility and a major carbon (C) reservoir in an agroecosystem (Manlay et al., 2007). Soil organic matter dynamics depends on the balance between C inputs via return of crop residues or organic amendments and C outputs primarily through SOC decomposition (Wang et al., 2015).

Crop residues are composed of lignin, cellulose, hemicellulose, and nutrients. The decomposition of crop residues is primarily determined by soil microorganisms (Schneider et al., 2012). Burning or removal of residues can decrease microbial biomass and microbial activity compared with soils where crop residues are returned to the soil (Chowdhury et al., 2015). Since soil microbial communities are the main regulators of nutrient cycling and soil carbon processes, differences in their composition have the potential to influence the provisioning of crop nutrients and the retention of C in residue-amended soils (Bending et al., 2002). The intensification of microbial and chemical processes during decomposition can be achieved using microbial strains as potential residue decomposers (Miki et al., 2010).

Species of *Bacillus* and *Trichoderma* are widely distributed in soil and well known for their beneficial effects on crop productivity (Bhattacharyya et al., 2016). They are among the most investigated biocontrol and plant growth-promoting agents that contribute to the suppression of plant pathogens and enhancement of plant growth (Abhilash et al., 2016). However, little is known about the impact of these microbial strains on residue decomposition and their relation to the soil microbiome during this process. Therefore, the objective of this study was to investigate the possibility of using *Bacillus* and *Trichoderma* strains as residue decomposers and to determine the effect of microbial treatments on chemical and microbial properties in the residue-amended soil.

MATERIALS AND METHODS

Microbial strains

Bacillus strains used in this study were obtained from the collection of the Department of Microbiological Preparations, Institute of Field and Vegetable

Crops, Novi Sad, Serbia. The strains were originally isolated from the soil samples, which included the rhizosphere of plants, agricultural and non-agricultural soils from different locations in the Province of Vojvodina. The bacteria were cultured for 48 h in nutrient broth (NB) at 30 °C to obtain the 10^9 cells ml^{-1} inoculum density. *Trichoderma* strains originated from the soil samples collected at Rimski šančevi experimental field of the Institute of Field and Vegetable Crops. The fungi were grown on potato dextrose agar (PDA) for seven days at 27 °C. After incubation, a sample of each fungus was taken and suspended in sterile distilled water to prepare the 10^9 spores ml^{-1} inoculum density.

Greenhouse experiment

The experiment was conducted in plastic boxes (36 × 28 × 15 cm) in a greenhouse under non-sterile, ambient temperature conditions. Each box contained 4 kg of non-sterile chernozem soil, 50 g of wheat straw, and 750 ml of microbial inoculum. Experiment included eight bacterial treatments: *Bacillus safensis* (BS), *Lysinibacillus fusiformis* (LF), *Bacillus megaterium* (BM), *Bacillus thuringiensis* (BT), *Lysinibacillus sphaericus* (LS), *Bacillus pumilus* (BP), *Bacillus cereus* (BC), and *Bacillus* mixture (Bmix); three fungal treatments: *Trichoderma harzianum* (TH), *Trichoderma asperellum* (TA), and *Trichoderma* mixture (Tmix); and a combination of bacterial and fungal mixture (BTmix). Mixtures consisted of bacterial and/or fungal strains mixed in an equal ratio. The effect of applied microbial treatments was compared to soil and soil with wheat straw, both with 750 ml of water (control). There were three replications of each treatment. The experiment was conducted for six months and watered weekly to maintain optimal conditions for microbial activity.

Soil chemical analysis

Soil samples for chemical analysis were collected at the end of the experiment (after 180 days). The samples were dried at the room temperature, milled and sieved to a particle size of < 2 mm. Chemical properties of examined soil were determined at the Laboratory for Soil and Agroecology of the Institute of Field and Vegetable Crops, using standard methods. The pH value in 1:5 (v/v) of soil suspension in 1 mol l^{-1} KCl was determined potentiometrically. Carbonate content ($CaCO_3$) (%) was measured with a Scheibler calcimeter. Contents of total nitrogen (N) (%), total carbon (C) (%), and soil organic carbon (SOC) (g/kg) were obtained via CHNS elemental analysis (Vario EL III, Elementar). The humus content (%) was assessed by oxidation of organic matter by the method of Tyurin. Available K_2O and P_2O_5 (mg/100 g) were determined by extraction with ammonium lactate according to Egner-Riehm. Potassium content (K) was determined using the flame photometer (Evans Electroselenium Ltd.). Phosphorus (P) content was analyzed using the blue method in a spectrophotometer (Agilent Cary 60, Agilent Technologies). All chemical analyses were performed in four replicates.

Soil microbial analysis

Soil samples for microbial analysis were collected four times during the experiment (after 45, 90, 130, and 180 days). The microbial number was analyzed at the Department of Microbiological Preparations of the Institute of Field and Vegetable Crops, using indirect dilution method followed by plating of soil suspension on selective nutritive media. The total number of microorganisms (TNM) was determined on an agarized soil extract, ammonifiers (AMN) on a meat-peptone agar (MPA), and free N_2-fixers (NFB) on a nitrogen-free agar. The number of fungi (FNG) was determined on Czapek-Dox agar, actinomycetes (ACT) on synthetic agar, oligotrophic (OB) and copiotrophic (CB) bacteria on (C)- poor and (C)- rich medium, and cellulolytic microorganisms (CEL) on Waksman-Carey medium. The incubation temperature was 28 °C, while the incubation time depended on the tested group of microorganisms (Jarak and Đurić, 2006). The average number of colony-forming units (CFU) was calculated per 1.0 g of absolute dry soil. All microbial analyses were performed in four replicates.

Statistical analysis

Data were subjected to analysis of variance (ANOVA). Means were compared using Tukey's honest significant difference (HSD) test at the $P < 0.05$ level. All analyses were performed in STATISTICA 12.6 (StatSoft Inc., USA).

RESULTS AND DISCUSSION

Microbial strains used in this study were selected according to a previously performed screening of bacteria and fungi for potential cellulolytic activity using enzymatic and dry fermentation assays (data not shown). Chemical analysis showed that experimental soil was slightly alkaline and slightly humic, with a high content of easily accessible phosphorus and optimal supply of easily accessible potassium. Significant differences between soil and soil amended with wheat straw were observed for pH, SOC, and K (Table 1). Applied microbial treatments significantly affected all examined soil chemical properties except total N (Table 1). All *Bacillus* treatments increased the pH values, while *Trichoderma* treatments had a lower pH compared to control. A significant increase in pH was recorded with *Bacillus safensis*, *Bacillus cereus,* and *Bacillus* mixture. *Trichoderma harzianum* significantly increased the content of $CaCO_3$, while other treatments had a negligible impact on the carbonates. Similarly, significant differences in N content between experimental treatments were not recorded. Bacterial treatments positively affected the total C and SOC, while a significant increase was observed in all *Bacillus* treatments, except *Bacillus cereus*. Conversely, fungal treatments slightly decreased the content of total C and significantly decreased the content of SOC. All *Bacillus* individual treatments, as well as *Trichoderma harzianum* and *Bacillus-Trichoderma* mixture treatments significantly affected the humus content. A significant increase in P and K content was recorded with *Bacillus* individual treatments and both *Bacillus* mixtures. According to Ogbodo [2011], organic matter from residue improved the soil pH status by increasing the soil buffer capacity. An increase in soil pH followed by an increase in nutrient content in residue–amended soil is frequently reported [Butterly et al., 2013]. The organic matter acts as a storage from which basic cations are released into the soil solution, while the improved soil nutrient status leads to better soil quality and crop productivity [Manlay et al., 2007].

Table 1. Chemical properties of residue-amended soil depending on examined microbial treatments

Treatment	pH	$CaCO_3$ (%)	Total N (%)	Total C (%)	SOC (g kg^{-1})	Humus (%)	P (mg)*	K (mg)*
Soil	7.35 f	0.93 bc	0.184 a	1.95 d	17.03 h	2.48 h	26.1 d	22.7 c
Control	7.56 cde	1.17 bc	0.189 a	2.06 cd	18.69 f	2.55 gh	25.2 d	30.5 b
BS	7.70 ab	1.17 bc	0.208 a	2.24 ab	19.74 b	2.80 ab	39.3 a	38.2 a
LF	7.68 abc	0.93 bc	0.203 a	2.22 ab	19.10 c	2.73 bcd	35.0 bc	39.1 a
BM	7.64 bcd	1.17 bc	0.200 a	2.27 a	20.31 a	2.69 cdef	34.0 bc	38.6 a
BT	7.65 abcd	0.93 bc	0.205 a	2.23 ab	19.59 b	2.76 abc	32.3 c	40.0 a
LS	7.68 abc	1.17 bc	0.205 a	2.18 ab	19.08 cd	2.76 abc	34.3 bc	40.0 a
BP	7.68 abc	1.17 bc	0.210 a	2.18 ab	18.89 e	2.83 a	32.5 c	38.6 a
BC	7.71 ab	0.93 bc	0.207 a	2.15 bc	18.68 f	2.78 abc	32.6 c	38.6 a
BMix	7.77 a	1.35 b	0.195 a	2.22 ab	18.93 de	2.63 efg	32.7 c	40.9 a
TH	7.51 e	4.20 a	0.197 a	1.96 d	18.31 g	2.65 def	24.0 d	29.1 b
TA	7.54 de	1.35 b	0.193 a	1.99 d	18.38 g	2.60 fg	26.6 d	30.5 b
TMix	7.48 e	1.17 bc	0.194 a	1.99 d	18.36 g	2.61 fg	26.0 d	30.0 b
BTmix	7.68 abc	0.75 c	0.201 a	2.17 abd	18.54 f	2.71 bcde	35.8 b	38.2 a
P	0.000	0.000	0.091	0.000	0.000	0.000	0.000	0.000

Values are the means of 4 replicates. Values in a column with different letters are statistically different ($P < 0.05$), according to Tukey's HSD test. *In a 100 g sample of soil. BS – *Bacillus safensis*; LF – *Lysinibacillus fusiformis*; BM – *Bacillus megaterium*; BT – *Bacillus thuringiensis*; LS – *Lysinibacillus sphaericus*; BP – *Bacillus pumilus*; BC – *Bacillus cereus*; Bmix – *Bacillus* mixture; TH – *Trichoderma harzianum*; TA – *Trichoderma asperellum*; Tmix – *Trichoderma* mixture; BTmix – *Bacillus* and *Trichoderma* mixture.

Different cropping systems can have a positive or negative effect on microbial number and activity, which directly reflect the fertility of the soil. Our research revealed that wheat straw amendment increased the presence of all microbial communities, and led to a significant change in the total microbial number, number of ammonifiers, N_2-fixers, fungi, and copiotrophs compared to soil without crop residues. Microbial treatments significantly affected the number of all examined microbial groups (Table 2). All microbial treatments, except *Trichoderma harzianum*, increased the total microbial number. A significant increase in the total microbial number was achieved in all treatments apart from *Bacillus safensis*. The number of ammonifiers and N_2-fixers was increased in applied treatments, while the significant effect was observed for all treatments but *Trichoderma harzianum*. The number of fungi was significantly increased with *Trichoderma harzianum*, while an increase in the number of this microbial group was also recorded with *Bacillus safensis*, *Bacillus cereus*, *Trihoderma asperellum,* and both *Trichoderma* mixtures. Interestingly, treatments with *Lysinibacillus fusiformis*, *Bacillus megaterium*, and *Bacillus thuringiensis* led to a significant decrease in the number of fungi. The number of actinomycetes was significantly higher in *Lysinibacillus fusiformis, Bacillus cereus*, and *Trichoderma* mixture treatments, while their population was not considerably altered in other experimental treatments. All microbial treatments, except *Trihoderma harzianum*, positively affected the number of oligotrophs, while a significant increase was obtained with *Bacillus safensis*, *Bacillus cereus*, and *Trichoderma* mixture. The number of copiotrophs was higher in all microbial treatments when compared to control, while the significant effect on this microbial group was recorded in *Bacillus* individual treatments, *Bacillus* mixture, and *Trichoderma asperellum* treatments. Cellulolytic microorganisms were significantly increased with *Lysinibacillus sphaericus, Bacillus mixture, Trichoderma asperellum*, and *Trichoderma* mixture, while a positive effect was obtained in most other treatments.

Examined microbial parameters in this study are important indicators of nutrient cycling and carbon processes, as well as the soil perturbations during residue decomposition. Ammonifiers degrade organic nitrogen compounds, while nitrogen-fixing bacteria reduce atmospheric nitrogen (Isobe et al., 2014). Actinomycetes and fungi are effective at decomposing complex organic compounds including lignin and cellulose (Bai et al., 2016). Copiotrophs and oligotrophs indicate the availability of carbon source, while cellulolytic microorganisms are the main decomposers of plant biomass (Ho et al., 2017). In this study, higher microbial abundance was accompanied by higher enzyme activities (data not shown). The higher enzyme activities may coincide with greater capacity to produce enzymes by the larger microbial biomass or might be partially attributed to higher substrate quantity and complexity (Yang et al., 2011). Previous studies reported the importance of crop residue returns for improving microbial community structure and maintaining soil fertility (Pascault et al., 2010; Arcand et al., 2016), while this research emphasized its significance through the application of adequate microbial strains.

Table 2. Microbial properties of residue-amended soil depending on examined treatments

Treatment	TNM (CFU×10^6^)	AMN (CFU×10^6^)	NFB (CFU×10^5^)	FNG (CFU×10^3^)	ACT (CFU×10^3^)	OB (CFU×10^6^)	CB (CFU×10^6^)	CEL (CFU×10^5^)
	g^{-1} soil							
Soil	107 h	67 i	60 f	17 efg	0 d	191 g	251 g	4 d
Control	344 f	166 h	197 e	24 bcde	2 cd	310 defg	529 f	21 cd
BS	399 ef	394 def	269 d	28 abcd	2 cd	461 abc	649 de	27 cd
LF	445 de	397 def	361 bc	12 gh	11 a	501 ab	677 de	41 bc
BM	446 de	420 cde	389 b	15 fgh	3 cd	384 bcde	676 de	40 bc
BT	509 bc	404 def	372 bc	9 h	3 cd	337 cdef	655 de	39 bc
LS	510 bc	609 a	603 a	21 def	2 cd	371 cdef	841 ab	58 ab
BP	597 a	493 b	397 b	18 efg	0 d	349 cdef	928 a	20 cd
BC	562 ab	370 ef	329 c	30 abc	6 b	266 efg	818 bc	26 cd
BMix	564 ab	477 bc	358 bc	22 cdef	1 cd	428 abcd	734 cd	54 ab
TH	245 g	216 gh	205 e	36 a	2 cd	249 fg	589 ef	19 cd
TA	489 cd	244 g	407 b	31 ab	1 cd	352 cdef	700 d	60 ab
TMix	519 bc	347 f	380 bc	28 abcd	4 c	541 a	538 f	79 a
BTmix	491 cd	457 bcd	259 d	25 bcde	1 cd	359 cdef	589 ef	20 cd
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Values are the means of 4 replicates. Values in a column with different letters are statistically different ($P < 0.05$), according to Tukey's HSD test. TNM: total microbial number, AMN: ammonifiers, NFB: N_2-fixers, FNG: fungi, ACT: actinomycetes, OB: oligotrophs, CB: copiotrophs, CEL: cellulolytic microorganisms. BS – *Bacillus safensis*; LF – *Lysinibacillus fusiformis*; BM – *Bacillus megaterium*; BT – *Bacillus thuringiensis*; LS – *Lysinibacillus sphaericus*; BP – *Bacillus pumilus*; BC – *Bacillus cereus*; Bmix – *Bacillus* mixture; TH – *Trichoderma harzianum*; TA – *Trichoderma asperellum*; Tmix – *Trichoderma* mixture; BTmix – *Bacillus* and *Trichoderma* mixture.

CONCLUSIONS

Our study confirmed the importance of residue incorporation and microbial decomposition in agricultural soil. Incorporation of wheat straw had a positive effect on examined soil properties, while microbial treatments improved chemical properties and increased microbial number. Overall, *Bacillus* treatments had a better effect on examined soil properties compared to *Trichoderma* treatments. Individual *Bacillus* strains mostly had an advantage over their combined application, while *Trichoderma* strains had the best effect in the mixtures. The most effective microbial strains could be used for decomposition of wheat straw in soils with similar properties. A further selection of microbial strains through greenhouse and field trials will be necessary to establish their efficiency as individual and combined decomposers of various crop residues in different soil environments.

ACKNOWLEDGMENTS

This study is part of the TR31072 project financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

REFERENCES

Abhilash PC, Dubey RK, Tripathi V, Gupta VK, Singh HK (2016): Plant growth promoting microorganisms for environmental sustainability. *Trend. Biotechnol.* 34: 847–850.

Arcand MM, Helgason BL, Lemke RL (2016): Microbial crop residue decomposition dynamics in organic and conventionally managed soils. *Appl. Soil Ecol.* 107: 347–359.

Bai Z, Liang C, Bodé S, Huygens D, Boeckx P (2016): Phospholipid ^{13}C stable isotopic probing during decomposition of wheat residues. *Appl. Soil Ecol.* 98: 65–74.

Bending GD, Turner MK, Jones JE (2002): Interactions between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. *Soil Biol. Biochem.* 34: 1073–1082.

Bhattacharyya PN, Goswami MP, Bhattacharyya LH (2016): Perspective of beneficial microbes in agriculture under changing climatic scenario: a review. *J. Phytol.* 8: 26–41.

Butterly CR, Baldock JA, Tang CX (2013): The contribution of crop residues to changes in soil pH under field conditions. *Plant Soil* 366:185–198.

Chowdhury S, Farrell M, Butler G, Bolan N (2015): Assessing the effect of crop residue removal on soil organic carbon storage and microbial activity in a no-till cropping system. *Soil Use Manag.* 31: 450–460.

Food and Agricultural Organization (2006): Plant Nutrition for Food Security: A Guide for Integrated Nutrient Management, *Fert. Plant Nutr. Bull.* 16. FAO.

Ghimire R, Lamichhane S, Acharya BS, Bista P, Sainju UM (2017): Tillage, crop residue, and nutrient management effects on soil organic carbon in rice-based cropping systems: A review. *J. Integr. Agric.* 16: 1–5.

Ho A, Di Lonardo DP, Bodelier PL (2017): Revisiting life strategy concepts in environmental microbial ecology. *FEMS Microbiol. Ecol.* 93: fix006.

Isobe K, Ohte N (2014): Ecological perspectives on microbes involved in N-cycling. *Microbes Environ.* 29: 4–16.

Jarak M, Đurić S (2006): *Praktikum iz mikrobiologije*. Univerzitet u Novom Sadu: Poljoprivredni fakultet.

Liu X, Herbert SJ, Hashemi AM, Zhang X, Ding G (2005): Effects of agricultural management on soil organic matter and carbon transformation – a review. *Plant Soil Environ.* 56: 531–543.

Manlay R, Feller C, Swift M (2007): Historical evolution of soil organic matter concepts and their relationships with the fertility and sustainability of cropping systems. *Agric. Ecosyst. Environ.* 119: 217–233.

Miki T, Ushio M, Fukui S, Kondoh M (2010): Functional diversity of microbial decomposers facilitates plant coexistence in a plant-microbe-soil feedback model. *Proc. Natl. Acad. Sci. USA* 107: 14251–14256.

Ogbodo EN (2011): Effect of Crop Residue on Soil Chemical Properties and Rice Yield on an Ultisol at Abakaliki, Southeastern Nigeria. *World J. Agric. Sci.* 7: 13–18.

Pascault N, Cécillon L, Mathieu O, Hénault C, Sarr A, Lévêque J, Farcy P, Ranjard L, Maron P-A (2010): *In situ* dynamics of microbial communities during decomposition of wheat, rape, and alfalfa residues. *Microb. Ecol.* 60: 816–828.

Rajput AL (1995): Effect of fertilizer and organic manure on rice (*Oryza sativa*) and their residual effect on wheat (*Triticum aestivum*). *Indian J. Agron.* 40: 292–294.

Schneider T, Keiblinger KM, Schmid E, Sterflinger-Gleixner K, Ellersdorfer G, Roschitzki B, Richter A, Eberl L, Zechmeister-Boltenstern S, Riedel K (2012): Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. *ISME J.* 6: 1749–1762.

Shrestha BM, McConkey BG, Smith WN, Desjardins RL, Campbell CA, Grant BB, Miller PR (2013): Effects of crop rotation, crop type and tillage on soil organic carbon in a semiarid climate. *Can. J. Soil Sci.* 93: 137–146.
Singh B, Rengel Z (2007): The role of crop residues in improving soil fertility. In: P. Marschner, Z. Rengel Z. (eds), *Nutrient Cycling in Terrestrial Ecosystems*, Berlin: Springer Verlag, 183–214.
Walsh E, McDonnell KP (2012): The influence of added organic matter on soil physical, chemical, and biological properties: a small-scale and short-time experiment using straw. *Arch. Agron. Soil Sci.* 58: S201–S205.
Wang J, Wang X, Xu M, Feng G, Zhang W, Yang X, Huang S (2015): Contributions of wheat and maize residues to soil organic carbon under long-term rotation in north China. *Scientific Reports* 5: 11409.
Yang B, Dai Y, Ding S-Y, Wyman CE (2011): Enyzmatic hzdrolzsis of cellulosic biomass. *Biofuels* 2: 421–450.

МОГУЋНОСТ КОРИШЋЕЊА *Bacillus* И *Trichoderma* СОЈЕВА ЗА РАЗЛАГАЊЕ ЖЕТВЕНИХ ОСТАТАКА

Драгана Ђ. БЈЕЛИЋ, Јелена Б. МАРИНКОВИЋ, Бранислава Б. ТИНТОР, Јордана М. НИНКОВ, Јовица Р. ВАСИН, Милорад С. ЖИВАНОВ, Снежана П. ЈАКШИЋ

Институт за ратарство и повртарство,
Максима Горког 30, Нови Сад 21000, Република Србија

РЕЗИМЕ: Циљ рада био је да се утврди могућност коришћења микробиолошких сојева у разлагању жетвених остатака, као и ефекат ових сојева на хемијска и микробиолошка својства у земљишту са додатком жетвених остатака. Оглед у стаклари обухватио је осам *Bacillus* третмана, три *Trichoderma* третмана и њихову смешу, који су примењени у нестерилном земљишту типа чернозем са пшеничном сламом. Уношење сламе побољшало је хемијска и микробиолошка својства земљишта, док је примена микробиолошких сојева интензивирала разградњу жетвених остатака. Микробиолошки третмани значајно су утицали на pH земљишта, садржај карбоната, укупног угљеника, органског угљеника, хумуса, приступачног фосфора и калијума. Третмани са бактеријама и гљивама такође су значајно утицали на укупан број микроорганизама, бројност амонификсатора, азотофиксатора, гљива, актиномицета, олиготрофа, копиотрофа и целулолитичких микроорганизама. Ефекат примењених третмана варирао је у зависности од примењеног соја и испитиваних својстава, при чему су сојеви *Bacillus*-а имали бољи ефекат у разградњи жетвених остатака у поређењу са *Trichoderma* сојевима. Најефикаснији микробиолошки сојеви могу се користити као потенцијални разлагачи жетвених остатака.

КЉУЧНЕ РЕЧИ: *Bacillus*, земљишни микроорганизми, органска материја земљишта, *Trichoderma*, пшенична слама

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. Novi Sad, № 138, 61—69, 2020

UDC 631.878(497.113)
UDC 631.452(497.113)
https://doi.org/10.2298/ZMSPN2038061Z

Milorad S. ŽIVANOV[1*], *Srđan I. ŠEREMEŠIĆ*[2], *Dragana Đ. BJELIĆ*[1], *Jelena B. MARINKOVIĆ*[1], *Jovica R. VASIN*[1], *Jordana M. NINKOV*[1], *Stanko B. MILIĆ*[1]

[1] Institute of Field and Vegetable Crops,
Maksima Gorkog 30, Novi Sad 21000, Serbia
[2] University of Novi Sad, Faculty of Agriculture,
Trg Dositeja Obradovića 8, Novi Sad 21000, Serbia

RESPONSE OF CHEMICAL AND MICROBIAL PROPERTIES TO SHORT-TERM BIOCHAR AMENDMENT IN DIFFERENT AGRICULTURAL SOILS

ABSTRACT: The objective of this study was to assess the effect of biochar soil amendment (BSA) on chemical and microbial properties in different agricultural soils in Vojvodina Province. Short-term pot experiment consisted of five biochar application doses (0, 0.5, 1, 2, and 3%) and five contrasting soil types (Mollic Gleysol, Eutric Cambisol, Calcaric Fluvisol, Gleyic Chernozem, and Haplic Chernozem), planted with sunflower (*Helianthus annuus* L.) and winter wheat (*Triticum aestivum* L.). The examined chemical and microbial properties were significantly influenced by soil type and interaction of experimental factors. Significant influence of biochar on the contents of calcium carbonate ($CaCO_3$), total nitrogen (N), total carbon (C), soil organic carbon (SOC), humus and potassium (K) of the tested soils was observed. Biochar also significantly affected the number of azotobacters (AZB), fungi (FNG), actinomycetes (ACT) and copiotrophic bacteria (CB). The effect of BSA varied depending on the applied dose, with higher values of the examined chemical and microbial parameters at higher doses of application. Further studies on using biochar in soils with low fertility will be necessary to establish its efficiency as an enhancer for agricultural production in Serbia.
KEYWORDS: biochar, carbon, humus, nitrogen, microbial number, soil type

INTRODUCTION

Biochar is a stable C-rich material produced by thermal degradation of plant-derived biomass under oxygen-free to oxygen-deficient conditions (Sohi et al., 2009). Application of biochar into agricultural soils can improve soil fertility, increase carbon sequestration and mitigate greenhouse effects (Brassard

[*] Corresponding author. E-mail: milorad.zivanov@nsseme.com

et al., 2016). Numerous studies confirmed that biochar soil amendment (BSA) changes physical and chemical soil properties (water holding capacity, soil aeration and pH, soil structure, nutrient retention and availability, release of soluble C and availability of micronutrients), decreases fertilizer requirements, and increases sorption of toxic compounds (Lehmann and Joseph, 2009). Positive priming effects of biochar on crop biomass and yield have also been reported (Castaldi et al., 2011).

Studies of BSA have mainly been conducted in regions with tropical and humid climate, on soils which are more degraded, while the possibility of biochar application on soils in regions with temperate climate remains unexplored (Jeffery et al., 2011; Liao et al., 2016). There is no experimental confirmation on using biochar and its effects on soil properties in the environmental conditions of Vojvodina Province (South Pannonian Plain), Serbia. The soils in Vojvodina are potentially fertile. However, a downward trend in the content of humus and soil organic carbon (SOC) has been observed over the past few decades as a result of tillage, insufficient fertilization, removal and burning of crop residues (Šeremešić et al., 2013).

For the long-term preservation of soil fertility and protection of agroecological systems, it is necessary to enable intensification of microbiological activity by applying appropriate measures, such as BSA. Soil microorganisms can be affected by BSA because of their role in mineralization of organic matter and involvement in nutrient cycles (Ferrell et al., 2013). Improved understanding of the dynamics and interrelationship between chemical and microbial properties in response to biochar amendment can provide valuable information on soil fertility potential and assessment of strategic biochar application to agricultural soils (Quilliam et al., 2013). Therefore, the objective of this study was to assess the effect of biochar amendment on chemical and microbial properties in different agricultural soils in Vojvodina Province (Serbia).

MATERIAL AND METHODS

Soil description

Soil types were selected based on the most common use by farmers in the study area (Vojvodina Province): Mollic Gleysol (clayic): GL-mo-ce (Novi Bečej, 45° 58’ N; 20° 08’ E; 70 m), Calcaric Fluvisol (arenic): FL-ca-ar (Šangaj, 45° 29’ N; 19° 87’ E; 75 m MASL), Eutric Cambisol (clayic): CM-eu-ce (Bukovac, 45° 20’ N, 19° 90’ E, 198 m MASL), Gleyic Chernozem (arenic): CH-gl-ar (Futog, 45° 24’ N; 19°70’ E; 78 m MASL) and Haplic Chernozem (loamic): CH-ha-lo (Čenej, 45° 35’ N; 19° 79’ E; 79 m MASL). Soils were classified according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2014). Vojvodina Province extends in the Pannonian Plain, characterized by typically temperate, continental climate.

Biochar

Biochar was purchased from a local company in Serbia. Chemical properties of biochar were analyzed according to the methods applied in soil analysis. Ash content of biochar was < 4 %, with the density of 0.402 g cm^{-3}, and moisture of 4–10%. Biochar contained 74.51% carbon (C), 95.03% organic carbon (C_{org}), and 0.547% nitrogen (N). Available phosphorus (P) content was 53.8 mg / 100 g, available potassium (K) content was 291 mg / 100 g, whereas pH of biochar was 7.54 (in KCl) and 8.24 (in H_2O). Before application into the soil, biochar was milled and passed through a brass sieve with a 2 mm aperture size and weighed in bags with the planned amount for each pot.

Pot experiment

The experiment was conducted in a greenhouse under semi-controlled conditions, characterized by the complete control of soil moisture, partial control of light and protection from adverse mechanical influences, without controlling the air temperature. Soils and biochar were simultaneously distributed in pots (v/w 10 l) in the amount of 5 kg per pot. The experiment consisted of five biochar application rates: 0 (0%), 25 (0.5%), 50 (1%), 100 (2%) and 150 (3%) g pot^{-1} (w/w). Treatments were arranged in a randomized block design with four replications. The experimental crops were winter wheat (*Triticum aestivum* L.) and sunflower (*Helianthus annuus* L.). Sunflower was sown in early April and matured in late August. Winter wheat was sown in early October and harvested in early June of the following year.

Soil sampling

After harvesting winter wheat at the end of the experiment, the samples were collected from the experimental pots assembled for each soil type and biochar dose in order to examine the effect of biochar application. After removing approximately 3 cm of the soil surface, about 0.5–1 kg of soil was taken from each pot. The samples were placed into polyethylene bags and transported to the laboratory. The samples were sieved at < 2 mm and stored at room temperature for chemical analysis. An aliquot of each soil sample was stored in refrigerator at 4 °C before microbiological analysis. All chemical and microbial analysis were performed in four replicates.

Soil chemical analysis

The main chemical properties of examined soils were determined using standard methods. pH in soil suspension with water or 1M KCl was analyzed potentiometrically (Mettler Toledo SevenCompact pH/ion). The content of calcium carbonate ($CaCO_3$) (%) was determined with a Scheibler calcimeter. Humus content (%) was determined by oxidation of organic matter by the method of Tyurin. Contents of total nitrogen (N) and total and organic carbon (C and C_{org}) (%) were analyzed on elemental CHNS analyzer (Vario EL III, Elementar). C_{org} (%) content was expressed in the SI unit as g C/kg soil (SOC g/kg). Available K_2O and P_2O_5 (mg/100 g) were analyzed by AL-method according to Egner-Riehm, by extraction with ammonium lactate. Potassium content (K) was determined by the flame photometer (Evans Electroselenium Ltd.) and phosphorus (P) content was assessed using the blue method in a spectrophotometer (Agilent Cary 60, Agilent Tehnologies).

Soil microbial analysis

Total cultivable bacterial and fungal colony forming units (CFU) were measured by the dilution plate method on the appropriate nutritive media. The total number of microorganisms (TNM) was determined on a soil agar (5 days, at 28 °C), the number of *Azotobacter* sp. (AZB) and free N_2-fixers (NFB) on nitrogen-free medium (Fyodorov's medium) (48 h and 5 days, respectively, at 28 °C). The number of ammonifiers (AMN) was determined on a meat peptone agar (3 days, at 28 °C), actinomycetes (ACT) on Krasilnikov's agar (7 days, at 28 °C), fungi (FNG) on Czapek-Dox agar (5 days, at 28 °C), copiotrophic (CB) and oligotrophic (OB) bacteria on high and low C content medium (7 and 14 days, at 28 °C). The average number of colony forming units (CFU) was calculated per 1.0 g of soil dry weight.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using software STATISTICA 12.6 (Statsoft, Tulsa, Oklahoma, USA). Means were separated using Tukey's HSD (honest significant difference) test at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Chemical properties of the examined agricultural soils varied significantly depending on the type of soil (Table 1). Investigated soil types had a slightly alkaline pH reaction, which is a favorable environment for the growth and development of most plants and microorganisms. Variability of the other tested chemical properties was very apparent because of differences between the studied soil types. The highest contents of total N, total C, SOC, and humus were observed in Haplic Chernozem, followed by Mollic Gleysol. The lowest values of these parameters were detected in Gleyic Chernozem, where the highest contents of available P and K were found. Lower contents of humus, N and SOC were also determined in Eutric Cambisol and Calcaric Fluvisol (Table 1).

Biochar had significant influence on the content of $CaCO_3$, total N, total C, SOC, humus and K. Interaction of soil type and biochar doses significantly affected the pH, total N, total C, SOC humus and K. Except pH and $CaCO_3$, concentration of all the examined chemical properties showed an upward trend after applying an increased dose of biochar (Table 1). The highest increase of examined chemical properties was recorded at 2% and 3% biochar treatments. The results are in accordance with those presented by Ippolito et al. (2014), who reported positive effects of BSA on chemical properties in calcareous soils. A significant increase in humus content along with increased doses of BSA could be explained by chemical content of BSA and high content of total C (74.51%). The positive effects of BSA on C and humus content suggested that application of biochar may promote the formation of stable soil organic matter (SOM) (Muhammad et al., 2014; Prayogo et al., 2014).

Table 1. Chemical properties depending on examined soil types and biochar doses

Factor/Variable		pH	$CaCO_3$ (%)	Total N (%)	Total C (%)	SOC (g kg^{-1})	Humus (%)	P (mg)*	K (mg)*
Soil (S)	GL-mo	7.23 e	0.68 d	0.25 b	4.34 a	35.5 a	3.74 b	14.42 c	25.78 b
	CM-eu	7.42 d	0.67 d	0.15 d	2.01 b	14.2 c	1.82 d	9.16 d	16.13 d
	FL-ca	7.75 a	21.66 a	0.17 c	4.35 a	17.8 b	2.21 c	15.07 c	7.41 e
	CH-gl	7.61 b	5.40 c	0.14 e	1.79 b	13.2 c	1.71 d	40.95 a	39.67 a
	CH-ha	7.49 c	7.35 b	0.30 a	4.68 a	36.3 a	4.75 a	23.45 b	19.03 c
Bio-char (B)	0%	7.51 a	7.57 a	0.18 d	2.60 b	17.3 c	2.38 d	20.30 b	20.61 b
	0.5%	7.50 a	7.18 ab	0.19 c	2.96 b	19.3 c	2.56 c	20.48 ab	21.26 b
	1%	7.52 a	7.09 ab	0.20 b	3.13 b	22.8 b	2.81 b	20.57 ab	21.56 b
	2%	7.47 a	6.83 ab	0.22 a	4.12 a	28.4 a	3.16 a	20.62 ab	21.14 b
	3%	7.49 a	7.08 b	0.23 a	4.36 a	29.2 a	3.32 a	21.08 a	23.45 a
P	S	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	B	0.069	0.008	0.000	0.000	0.000	0.000	0.076	0.000
	S × B	0.024	0.085	0.000	0.012	0.000	0.000	0.115	0.007

Values are the means of 4 replicates. Values in a row with different letters are statistically different ($P < 0.05$), according to Tukey's HSD test. GL-mo: Mollic Gleysol, CM-eu: Eutric Cambisol, FL-ca: Calcaric Fluvisol, CH-gl: Gleyic Chernozem, CH-ha: Haplic Chernozem.
* In a 100 g sample of soil.

Soil properties have a strong impact on a range of processes influencing crop yield, including microbial diversity (Coleman, 2011). The abundance and activity of certain microbial groups are positively or negatively correlated with soil chemical properties (Falkowski et al., 2008; Liang and Balser, 2011). Soil microorganisms can be affected by BSA because of their role in maintaining crop productivity through mineralization of complex organic compounds in soil, as well as their sensivity to environmental change (Ferrell et al., 2013; Muhammad et al., 2014). The analysis of variance revealed that the soil type significantly influenced the number of all investigated microbial groups (Table 2). The TNM, NFB, AMN, CB and OB were significantly higher in Mollic Gleysol compared with other soil types. AZB and ACT were most abundant in Chernozem soils, while the number of FNG was the highest in Calcaric Fluvisol (Table 2).

BSA had significant influence on the number of AZB, FNG, ACT and CB. Interaction of experimental factors significantly affected the number of all the examined microbial groups (Table 2). The effect of BSA depended on microbial group and the applied dose. Better effects on the tested microbial parameters were obtained with higher doses of biochar (1–3%). The treatments negatively affected the number of AMN and FNG.

Table 2. Microbial properties depending on examined soil types and biochar doses (g^{-1} soil)

Factor/ Variable		AZB ($CFU \times 10^2$)	TNM ($CFU \times 10^6$)	AMN ($CFU \times 10^6$)	NFB ($CFU \times 10^5$)	FNG ($CFU \times 10^3$)	ACT ($CFU \times 10^3$)	OB ($CFU \times 10^6$)	CB ($CFU \times 10^6$)
Soil (S)	GL-mo	46 ab	467 a	242 a	637 a	67 b	2 d	371 a	371 a
	CM-eu	12 c	255 b	111 b	402 bc	62 b	4 cd	294 b	224 c
	FL-ca	40 b	325 b	153 b	500 b	85 a	7 bc	389 a	283 bc
	CH-gl	60 a	149 c	90 b	327 c	29 c	11 b	156 c	302 ab
	CH-ha	54 ab	137 c	117 b	361 c	35 c	19 a	160 c	256 bc
Bio-char (B)	0%	38 b	270 ab	172 a	454 a	75 a	6 b	270 a	250 b
	0.5%	36 b	271 ab	162 a	430 a	45 b	12 a	255 a	247 b
	1%	42 ab	254 ab	125 a	428 a	50 b	12 a	295 a	373 a
	2%	39 ab	313 a	152 a	475 a	52 b	6 b	304 a	336 a
	3%	57 a	225 b	100 a	439 a	56 b	7 b	247 a	229 b
P	S	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	B	0.019	0.052	0.076	0.689	0.000	0.000	0.124	0.000
	S × B	0.012	0.000	0.008	0.004	0.000	0.011	0.000	0.000

Values are the means of 4 replicates. Values in a row with different letters are statistically different ($P < 0.05$), according to Tukey's HSD test. GL-mo: Mollic Gleysol, CM-eu: Eutric Cambisol, FL-ca: Calcaric Fluvisol, CH-gl: Gleyic Chernozem, CH-ha: Haplic Chernozem. AZB: azotobacters, TNM: total microbial number, AMN: ammonifiers, NFB: N_2-fixers, FNG: fungi, ACT: actinomycetes, OB: oligotrophs, CB: copiotrophs.

Our results are similar to those reported by Hu et al. (2014) who found 12%, 30%, 37% higher bacterial diversity, and 17%, 40%, 23% lower fungal diversity in biochar amended soil. Similarly, biochar application to a calcareous soil caused an increase in soil C content, soil respiration rates and bacterial populations (Ippolito et al., 2014). Prayogo et al. (2014) observed that the amount of bacterial biomass was increased by BSA, providing evidence of stimulated abundance of Gram-negative bacteria and actinobacteria. Anderson et al. (2011) discovered that BSA had a positive influence on the abundance of bacterial families involved in nitrate denitrification, while organisms involved in nitrification were less abundant.

AMN participate in the processes of decomposition and transformation of organic nitrogen compounds in the soil, while NFB have the ability to reduce atmospheric nitrogen, transform it into plant-available forms, and thus enrich the soil with this important element. CB and OB were selected to determine bacterial response to alteration in C availability. As active decomposers of organic matter, ACT and FNG are included in the cycle of C, N, P, and other nutrients. Positive effects on the analyzed chemical and microbial properties revealed that BSA could potentially affect soil C and N cycling in the examined agricultural soils. Although it is accepted that most of the biochar-C is largely unavailable to microbes (Thies and Rillig, 2009; Farrell et al., 2013), it is clear that BSA can have positive influence on microbial community structure and soil fertility (Sun et al., 2013; Gul et al., 2015), which was confirmed by this research.

CONCLUSION

The examined soil properties were significantly influenced by soil type and interaction of experimental factors. Biochar amendment significantly affected the contents of calcium carbonate, total nitrogen, total and soil organic carbon, humus, potassium, as well as the number of azotobacters, fungi, actinomycetes and copiotrophic bacteria of tested soils. This was the first experiment examining biochar implementation in Serbia, in the continental ecological conditions of Vojvodina Province, and presents the preliminary results of its effect on the chemical and microbial properties of agricultural soils. Further research will include testing the effect of biochar application in field conditions, on a range of crop species and soil types. In addition to soil properties, it will be necessary to establish the effect of biochar application on yield components and quality of agricultural products.

ACKNOWLEDGEMENTS

This study is a part of the TR31072 project supported by the Ministry of Education, Science and Technological Development of Republic of Serbia.

REFERENCES

Anderson CR, Condron LM, Clough TJ, Fiers M, Stewart A, Hill RH, Sherlock RR (2011): Biochar induced soil microbial community change: Implications for biogeochemical cycling of carbon, nitrogen and phosphorus. *Pedobiologia* 54: 309–320.

Brassard P, Godbouat S, Raghavan V (2016): Soil biochar amendment as a climate change mitigation tool: Key parameters and mechanisms involved. *J. Environ. Manage.* 181: 484–497.

Castaldi S, Riondino M, Baronti S, Esposito FR, Marzaioli R, Rutigliano FA, Vaccari FP, Miglieta F (2011): Impact of biochar application to a Mediterranean wheat crop on soil microbial activity and greenhouse gas fluxes. *Chemosphere* 85: 1464–1471.

Chen J, Liu X, Zheng J, Zhang B, Lu H, Chi Z, Pan G, Li L, Zheng J, Zhang X, Wang J, Yu X (2013): Biochar soil amendment increased bacterial but decreased fungal gene abundance with shifts in community structure in a slightly acid rice paddy from Southwest China. *Appl. Soil Ecol.* 71: 33–44.

Coleman DC (2011): Understanding soil processes: one of the last frontiers in biological and ecological research. *Australas. Plant Path*. 40: 207–214.

Falkowski PG, Fenchel T, Delong EF (2008): The microbial engines that drive Earth's biogeochemical cycles. *Science* 320: 1034–1039.

Farrell M, Kuhn TK, Macdonald LM, Maddern TM, Murphy DV, Hall PA, Singh BP, Baumann K, Krull ES, Baldock JA (2013): Microbial utilisation of biochar derived carbon. *Sci. Total Environ*. 465: 288–297.

Gul S, Whalen JK, Thomas BW, Sachdeva V, Deng H (2015): Physico-chemical properties and microbial responses in biochar-amended soils: Mechanisms and future directions. *Agric. Ecosys. Environ*. 206: 46–59.

Hu L, Cao L, Zhang R (2014): Bacterial and fungal taxon changes in soil microbial community composition induced by short-term biochar amendment in red oxidized loam soil. *World J. Microbiol. Biotechnol*. 30: 1085–1092.

Ippolito JA, Stromberger ME, Lentz RD, Dungan RS (2014): Hardwood biochar influences calcareous soil physicochemical and microbiological status. *J. Environ. Qual*. 43: 681–689.

IUSS Working Group WRB. (2014): *World Reference Base for Soil Resources 2014*. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. World Soil Resources Reports No. 106. Rome: FAO.

Jeffery S, Verheijen F, Van Der Velde M, Bastos A (2011): A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agric. Ecosys. Environ*. 144: 175–187.

Lehmann J, Joseph S (2009): Biochar for environmental management: an introduction. In: Lehmann J, Joseph S (eds.), *Biochar for environmental management, science and technology*, London: Earthscan, 1–12.

Liang C, Balser TC (2011): Microbial production of recalcitrant organic matter in global soils: implications for productivity and climate policy. *Nat. Rev. Microbiol*. 9: 75.

Liao N, Li Q, Zhang W, Zhou G, Ma L, Min W, Ye J, Hou Z (2016): Effects of biochar on soil microbial community composition and activity in drip-irrigated desert soil. *Eur. J. Soil Biol.* 72: 27–34.

Muhammad N, Dai Z, Xiao K, Meng J, Brookes PC, Liu X, Wang H, Wu J, Xu J (2014): Changes in microbial community structure due to biochars generated from different feedstocks and their relationships with soil chemical properties. *Geoderma* 226: 270–278.

Prayogo C, Jones JE, Baeyens J, Bending GD (2014): Impact of biochar on mineralisation of C and N from soil and willow litter and its relationship with microbial community biomass and structure. *Biol. Fert. Soils* 50: 695–702.

Quilliam RS, Glanville HC, Wade SC, Jones DL (2013): Life in the 'charosphere' – does biochar in agricultural soil provide a significant habitat for microorganisms? *Soil Biol. Biochem*. 65: 287–293.

Sohi SP, Krull E, Lopez-Capel E, Bol R (2009): A review of biochar and its use and function in soil. *Adv. Agron*. 105: 47–82.

Sun D, Meng J, Chen W (2013): Effects of abiotic components induced by biochar on microbial communities. *Acta Agric. Scan.* Sec. B 63: 633–641.

Šeremešić S, Đalović I, Milošev D, Jocković Đ, Pejić B (2013): Maize (*Zea mays* L.) yield stability dependence on crop rotation, fertilization and climatic conditions in a long-term experiment on Haplic Chernozem. *Zemdirbyste* 100: 137–142.

Vedove G, De Neve S (2014): C mineralization and microbial activity in four biochar field experiments several years after incorporation. *Soil Biol. Biochem*. 78: 195–203.

ПРОМЕНЕ ХЕМИЈСКИХ И МИКРОБИОЛОШКИХ СВОЈСТАВА НАКОН ПРИМЕНЕ БИОУГЉА НА РАЗЛИЧИТЕ ТИПОВЕ ОБРАДИВОГ ЗЕМЉИШТА

Милорад С. ЖИВАНОВ[1], Срђан И. ШЕРЕМЕШИЋ[2],
Драгана Ђ. БЈЕЛИЋ[1], Јелена Б. МАРИНКОВИЋ[1], Јовица Р. ВАСИН[1],
Јордана М. НИНКОВ[1], Станко Б. МИЛИЋ[1]

[1] Институт за ратарство и повртарство,
Максима Горког 30, Нови Сад 21000, Србија
[2] Универзитет у Новом Саду, Пољопривредни факултет,
Трг Доситеја Обрадовића 8, Нови Сад 21000, Србија

РЕЗИМЕ: Циљ ових истраживања био је да се процени утицај биоугља, као оплемењивача земљишта на хемијска и микробиолошка својства на различитим типовима пољопривредног земљишта у Војводини. Краткорочни експеримент у судовима састојао се од пет доза примене биоугља (0, 0.5, 1, 2 и 3%) и пет различитих типова земљишта (ритска црница, еутрични камбисол, алувијално земљиште, чернозем на алувијалном наносу и чернозем на лесној тераси), где су у две производне године гајени озима пшеница и сунцокрет. На испитивана хемијска и микробиолошка својства значајно су утицали тип земљишта и интеракција експерименталних фактора. Уочен је значајан утицај биоугља на садржај калцијум-карбоната ($CaCO_3$), укупног азота (N), укупног угљеника (C), органског угљеника (SOC), хумуса и калијума (K) у испитиваном земљишту. Биоугаљ је такође значајно утицао на број азотобактера (AZB), гљива (FNG), актиномицета (ACT) и копиотрофних бактерија (CB). Ефекат биоугља варирао је у зависности од примењене дозе, са вишим вредностима испитиваних хемијских и микробиолошких параметара при вишим дозама примене. Да би се утврдила његова ефикасност, као оплемењивача земљишта и унапређења пољопривредне производње у Србији, потребно је урадити још истраживања са коришћењем биоугља на земљиштима лошијих производних својстава.

КЉУЧНЕ РЕЧИ: азот, биоугаљ, бројност микроорганизама, тип земљишта, угљеник, хумус

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. Novi Sad, № 138, 71—82, 2020

UDC 633.31:631.526
https://doi.org/10.2298/ZMSPN2038071U

Vladan M. UGRENOVIĆ[1*], *Vladimir M. FILIPOVIĆ*[2], *Dušica I. DELIĆ*[1], *Vera M. POPOVIĆ*[3], *Olivera S. STAJKOVIĆ-SRBINOVIĆ*[1], *Aneta V. BUNTIĆ*[1], *Gordana K. DOZET*[4]

[1] Institute of Soil Science,
Teodora Drajzera 7, Belgrade 11000, Serbia
[2] Institute for Medicinal Plants Research "Dr Josif Pančić",
Tadeuša Košćuška 1, Belgrade 11000, Serbia
[3] Institute of Field and Vegetable Crops,
Maksima Gorkog 30, Novi Sad 21000, Serbia
[4] University of Megatrend, Faculty of Biofarming,
Maršala Tita 39, Bačka Topola 24300, Serbia

MAINTENANCE OF SOIL FERTILITY ON ORGANIC FARM BY MODELING OF CROP ROTATION WITH PARTICIPATION ALFALFA

ABSTRACT: The aim of this paper is to maintain soil fertility on an organic farm without livestock production by using alfalfa green biomass. The research was carried out on the farm of Mokrin PP company, by modeling and sizing of crop rotation with alfalfa (*Medicago sativa* L.) on the non-carbonate humoglay. To ensure a cost-effective technical solution, alfalfa seed production was organized. In the autumn of 2015 alfalfa sowing was carried out in a field of 5 ha. Green biomass of the first and third cuttings, as well as crop residue after harvesting of seeds in the second cutting, were mowed and chopped by harvester for low silage and stored in the silage-pit. After nine months, a mature alfalfa compost was obtained with optimum values of total nitrogen (5.04%), organic matter (42.56%), C/N, pH, humidity, and EC.

Two-year alfalfa utilization is the recommended time in this research because to the following benefits: in crop rotation, alfalfa field is provided with nitrogen by symbiotic nitrogen fixation and the alfalfa is cultivated every five years in the same field, while in the middle of that period the field is fertilized with compost produced on the farm. The amount of compost obtained by crop rotation (2016 – 48.80 t; 2017 – 62.30 t) is enough for about 20% of the arable area per year. Thus, the fields are fertilized every fourth year with 10 t ha^{-1} of compost. Thanks to alfalfa biomass and seed and also nitrogen fixation, maintaining soil fertility is resolved in a sustainable and natural way.

KEYWORDS: alfalfa, composting, fertility, organic production

[*] Corresponding author. E-mail: vladan.ugrenovic@gmail.com

INTRODUCTION

Organic production of food is based on ecological practice, a high degree of biodiversity, and the conservation of natural resources. Council Regulation (EC) no. 834/2007 from 28 June 2007, especially points out the rational use of soil, water, and soil organic matter, by application of procedures that are not harmful to them. The plant nutrition should be provided through the ecosystem of the soil, and not by using soluble fertilizers that would be added to it. The fertility and biological activity of the soil is maintained and increased by the practice of wider crop rotation, with a greater share of legumes and the introduction of cover crops (Ugrenović and Filipović, 2017), using composted organic fertilizers originating from livestock production or farm waste. Particular emphasis is placed on the importance of livestock production for organic agricultural holdings since they provide them with the necessary organic matter for cultivated land. However, there are only forty producers in organic livestock production in Serbia (DNRL 2016). Bearing in mind the ban on the use of synthetic mineral fertilizers, and the application of commercial certified fertilizers for organic production is often not economically justified, a small volume of organic livestock production undermines the sustainability of total organic production, especially when it comes to maintaining of soil fertility. An important principle of organic production is the preservation and improvement of biodiversity. The intensification of agriculture in the last decades has severely affected biodiversity (Ugrenović et al., 2012), and the inappropriate use of synthetic agrochemicals has also caused land biodiversity loss.

A large number of researchers have considered the maintenance of soil fertility and crop-rotation in organic production in the world (Watson et al., 2008; Mohler & Johnson, 2009; Altieri, 2015; Jat et al., 2015; Wande, 2015). The obtained results can only be partially applied in the organic production of Serbia, since the agro-ecological, technical, technological, and social conditions are significantly different. Attention has not been paid yet to the maintaining of the soil fertility on organic manors without animal husbandry by modelling crop relation with the use lucerne (*Medicago sativa* L.). Compost from biomass of alfalfa has not been produced so far. Research of Ćupina et al. (2017) deals with the topic of annual cover crops and the nitrogen budget. Several transparent works are available (Ćupina et al., 2004, 2004a, Čuvardić, 2006, Ugrenović and Filipović, 2017) and additional researches with various commercial, certified organic fertilizers and soil cultivators (Filipović et al., 2012; Bogdanović et al., 2013; Popović et al., 2014; 2019; Dozet et al., 2017).

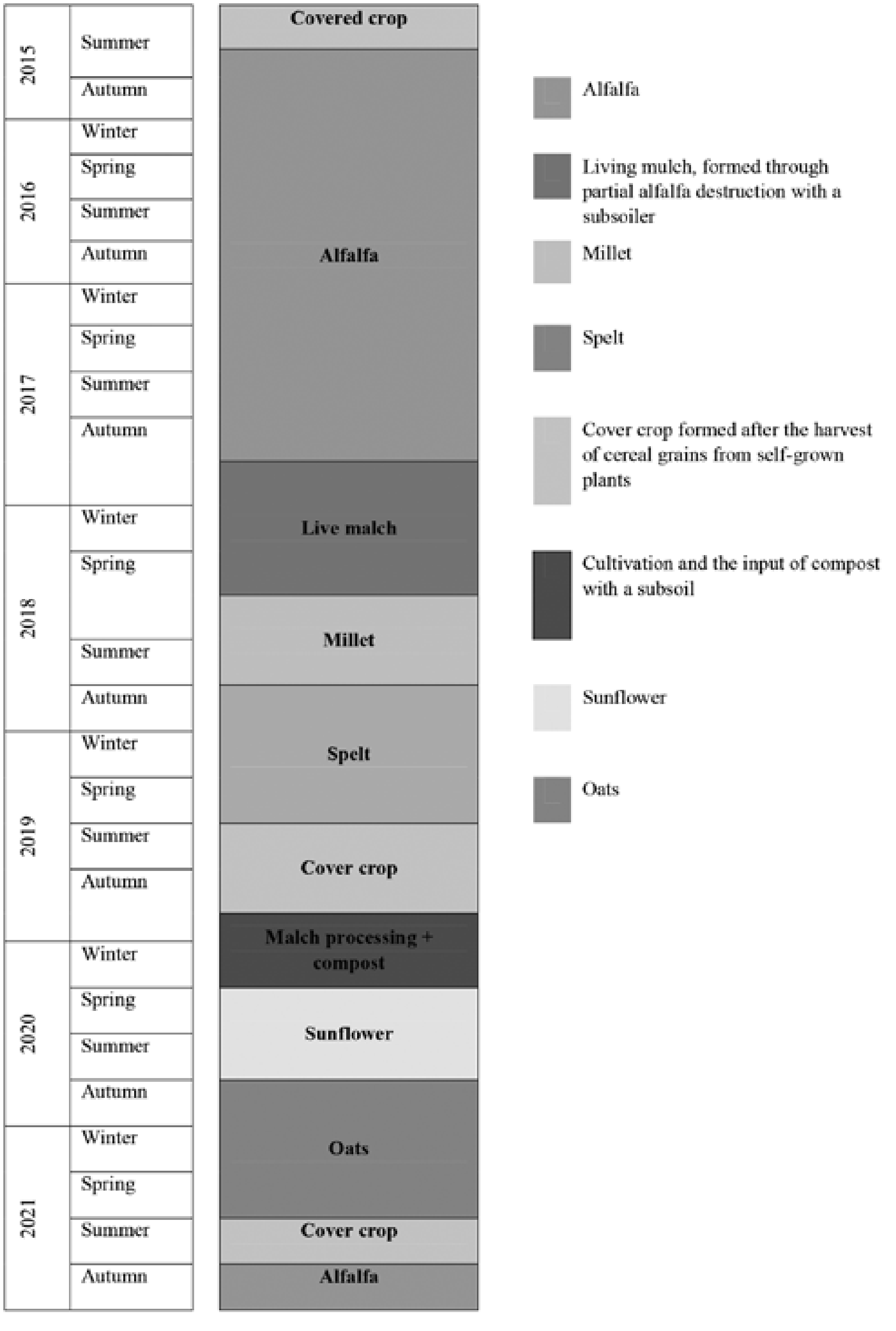
Organic production is controlled, and the Law on Organic Production (*Official Gazette of the Republic of Serbia*, 30/2010) for the maintenance of soil fertility, application of wider crop rotation and preservation of biodiversity, particularly defines the criteria that the organic producer must fulfill in the process of control and certification. In this sense, the proposed technical solution aims to address the issue of soil fertility maintenance on organic farms that do not have livestock production, modeling an optimally sized crop rotation, with the participation of alfalfa (*Medicago sativa* L.), which should: 1) provide biomass for composting to produce sufficient quantity of quality compost on the farm, 2) provide symbiotic nitrogen fixation of soil with nitrogen for future crops in crop rotation, 3) protect and improve soil biodiversity by increasing the number of useful microorganisms, 4) obtain the primary product, interesting for the processing industry and the market. All this should be modeled in a way that does not increase production costs.

MATERIAL AND METHOD

The proposed technical solution was studied on 25 ha of Agricultural enterprise Mokrin doo production areas (N 45° 57' 1213", E 20° 22' 3830") in the system of organic production, on the non-carbonate humoglay soil. To solve the soil fertility issue on organic farms with no livestock production, there was modeled and sized a five-field crop rotation, with a share of alfalfa (20%), millet (*Panicum miliaceum* L.) 20%, spelt (*Triticum spelta* L.) 20%, sunflower (*Helianthus annuus* L.) 20%, and oats (*Avena sativa* L.) 20% (Scheme 1). The goal of using alfalfa was to provide biomass for the production of compost in crop rotation for the maintenance of soil fertility. To ensure economic viability in such circumstances, the production of alfalfa seed is organized. At the end of 2015, on the surface of 5 ha, on which the pre-sowing crop was wheat, conservation treatment of the soil by both full-scale shallow cultivation and deep cultivation in one single pass was performed by Vaderstad-Top Down 300S (30 cm depth). Then, on 8 September, alfalfa (Banat VS variety) was sown by Vaderstad-Rapid 400 C sower, at a row distance of 12.5 cm, to a depth of 2–2.5 cm, with 16 kg ha^{-1} of seed. For the next two years (2016, 2017) when the crop of the first and third branches was in the phase of forming the flower buds until the beginning of flowering, the over-ground biomass was harvested with silorator for low silage, cut and stored in the silage holes on the farm in Mokrin. In the second cutting in both years, when most of the pods in alfalfa crop received a dark color, two-phase harvesting of seeds was done. Herbal residues after threshing were also utilized for composting. In the autumn of 2017, the crop was partially destroyed by soil cultivation. During the vegetation period of alfalfa, phenological observations were carried out: sprouting, intense growth, flowering, butonization, and maturation. During transportation, measurements of the amount of green biomass and recalculation of yield (t ha^{-1}) were carried out.

Process of preparation of alfalfa compost – Produced green biomass of the first and third cutting of alfalfa and dried mass of plant residues after alfalfa seed harvesting was used for composting. This mass is stored in the Power Plant PP Mokrin. To accelerate the degradation process, the microbial preparation Ecovital (2.0 l / 10 t^{-1}) was used. During the production, care measures were carried out permanently: mixing, watering, and covering of the compost pile, and for their timely implementation, measurements of temperature and humidity preservation.

Scheme 1. The model of crop rotation with the participation of alfalfa on an organic farm



The mixing was done on several occasions with loader, depending on the state of the humidity of the compost pile, to ensure the access of a sufficient amount of oxygen. Water was added as needed to maintain the humidity of the compost heap at about 65%. After nine months, a mature compost was obtained, which was measured during transport to calculate the yield (t ha^{-1}).

Methods of performed analyzes – After the plowing of alfalfa in autumn 2017, sampling was carried out in the spring next year to determine the basic fertility parameters of the soil: total nitrogen and carbon (CNS Elemental Analyzer Varian EL III), humus (calculation from organic carbon – CNS Analyzer), pH of the soil values (in H_2O and KCl, potentiometric), and readily available-accessed potassium (K_2O) and phosphorus (P_2O_5) by the Egner and Riehm Al methods (Egner et al., 1960). Microelements in the compost are determined by extraction using aqua regia. Also, sampling and analyses were done to determine the microbiological activity of the soil and compost.

The total number of microorganisms was determined by the plate count method on the agarized soil extract, the number of fungi on the Chapek medium, *Actinomycetes* on the medium according to Krasiljnikov, and oligonitrophiles on the Fyodorov medium. The other two groups of microorganisms Ammonifiers and *Azotobacter* spp. were determined by the most probable number (MPN) method in the liquid medium with asparagines or mannitol, respectively (Sarić, 1989; SRPS ISO 11465:2 002).

Soil respiration was determined by laboratory incubation with constant temperature and moisture. The respired carbon dioxide was trapped in the NaOH, and the remaining amount of OH^- ions was back-titrated with the solution of HCl. The amount of released CO_2 during the incubation period was calculated (Horwath et al., 1996). The presence and the most probable number (MPN) of bacterial species *S. meliloti* in the soil were determined by the indirect method „plant infection count“ (Vincent, 1970). The results were statistically analyzed by the variance analysis method (ANOVA), and the level of difference significance was tested with the LSD test, at the level $P < 0.05$ (COSTAT program). To perform the analysis, benefits, costs, and variable costs were monitored.

RESULTS AND DISCUSSION

Production of biomass for composting, to produce sufficient quantity of quality compost on the farm – Total yield of alfalfa green biomass in 2016 was 16.40 t ha^{-1} (Table 1), the weight of plant residues after threshing alfalfa seeds was 2.40 t ha^{-1}, and the amount of compost obtained was 9.76 t ha^{-1}. In the second year of production (2017), the total yield of green biomass was 25.07 t ha^{-1}, the mass of plant residues after threshing of seed was 3.11 t ha^{-1}, and 12.46 t ha^{-1} of compost was obtained. The total quantity of compost produced in 2016 was 48.80 t, and in 2017 it was 62.30 t.

Table 1. The yield of the biomass of alfalfa for composting and the amount of obtained compost

	The first and third cutting	Second cutting	
	Green biomass ($t\ ha^{-1}$)	Biomass of residuals after threshing seed ($t\ ha^{-1}$)	Amount of compost ($t\ ha^{-1}$) / total (t)
2016	16.4	2.40	9.76 / 48.80
2017	25.0	3.11	12.46 / 62.30

The average value of the C/N ratio of the produced alfalfa compost is 8.51 (Table 2). The ratio of C/N at the formation of the compost heap and start of the aerobic process should be 40:1 optimally, and finally after ripening 10 (12): 1, indicating somewhat lower efficiency of the produced compost in fertilizing, but its excellent characteristics in terms of physical properties. Values: humidity, pH, and EC are optimal. According to the measured content of total nitrogen (5.04%), alfalfa compost is well supplied with nitrogen, and the content of organic matter is 42.56%.

Table 2. Agrochemical properties of alfalfa compost

C/N	Moisture %	EC mS/cm^2 g/l	pH		Organic matter			Content of readily – available				
			in KCL	in H_2O	C%	N%	(Org.C) %	P_2O_5 mg/100 g	K_2O mg/100 g	NH_4 mg/kg	NO_3 mg/kg	NH_4+NO_3 mg/kg
8.51	61.13	7.97 3.99	6.61	7.33	38.04	5.04	42.56	1.40	4.11	46.7	1437.3	1484

The total microflora in the alfalfa compost was represented in a significant number ($65.22x10^6$ microorganisms per gram of dry compost) (Table 3) indicating intense microbiological activity. The determined number of fungus (98.44x104) indicates a high content of organic matter and optimal substrate moisture, which confirms the good quality of the compost obtained. Also, the number of *Actinomycetes* ($2.43x10^4$) was significant. The presence of *Azotobacter* as a fertility indicator confirms the good water-air properties of compost, and the presence of amonifiers ($7.97x10^5$) the significant presence of nitrogenous organic compounds. The content of heavy metals was within the boundaries of the MAC (Official Gazette of RS, No. 23/94) (Table 4).

Table 3. Number of microorganisms in alfalfa compost

Total number of microorganisms ($x10^6$)	Number of fungi ($x10^4$)	Number of *Actinomycetes* ($x10^4$)	Number of *Azotobacter* (MPN)*	Number of amonifiers ($x10^5$)
Number of microorganisms/g in dry compost				
5.22	98.44	2.44	25.00	7.97

* MPN – the most likely number from Mec Credy table.

Table 4. Content of microelements in alfalfa compost

As	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn	Fe
				mg/kg					%
1.48	0.22	2.29	17.42	31.89	228.5	12.75	3.7	77.63	4.70

According to the recommendation of the Bundesgütegemeinschaft Kompost e.V. (2004), for the production of most arable crops, 10 to 15 t ha^{-1} of compost is applied during autumn fertilization. The amount of compost obtained by modeling the crop rotation on the Mokrin estate (Table 1) is sufficient for about 20% of the area of the field, on an annual basis. Thus, every fourth year, they were fertilized with about 10 t ha^{-1} of compost, excluding the predicted 20% of the area for establishing the alfalfa, so that the fertility of the soil is maintained in a natural way.

Providing nitrogen for future crops in the crop rotation – Total N in the soil after two years of planting alfalfa was 0.25% (Table 5), which is higher than the control plot (0.22%). This clearly demonstrated the significant influence of alfalfa on the provision of nitrogen in the soil. The obtained results confirm a large number of *S. meliloti* (1.78x104 g^{-1} absolutely dry soil) in the soil after 2 years of growing alfalfa, which is significantly higher than in the control soil ($2.00x10^2$ g^{-1} absolutely dry soil). This confirms the well-known fact that alfalfa as a host plant positively influences the number of *S. meliloti* (Delić et al., 2016), and thus the establishment of an ecological and economically significant process of symbiotic nitrogen fixation (Delić, 2014).

Table 5. Chemical properties of soil after two years of cultivating alfalfa, on non-carbonate, marsh dark soil

Parameters	pH		Humus	Min. N	Total	P_2O_5	K_2O
Treatment	in KCL	in H_2O	%	mg/kg	N %	mg/100 g	mg/100 g
Alfalfa field	6.0	6.7	4.36 a	22.16 a	0.25 a	13.76 a	19.07 a
Control	6.1	7.3	3.59 b	11.08 a	0.22 b	15.16 a	20.66 a
LSD 0.05			0.730	14.26 ns	0.023	4.13 ns	2.23 ns

The mean values indicated by the same letter within one column do not differ significantly ($p < 0.05$); ns – not statistically significant

Protecting and improving biodiversity by increasing the number of useful microorganisms in the soil – After two years of alfalfa cultivation, the total number of microorganisms ($18.22x10^6$ g^{-1} absolutely dry soil) was higher than the control plot ($16.22x10^6$ g^{-1} absolutely dry soil) (Table 6). These results are in correlation with the intensity of respiration, which was twice as high in the soil on which alfalfa was grown. The number of fungi was also significantly higher after the production of alfalfa ($22.00x10^4$ g^{-1}) compared to the control ($12.22x10^4$ g^{-1}), and their role in the synthesis of humus was confirmed, since there was significantly more humus, after plowing the alfalfa field.

Although the number of ammonifiers did not change significantly, the role of alfalfa in increasing fertility of the soil indicates a significant number of *Azotobacter*, which was eight times higher than in the control land. Alfalfa also significantly influenced the increase in the number of bacteria *S. meliloti* ($178x10^2$ g^{-1}) compared to the control ($2x10^2$ g^{-1}). Results of the total number of microflora, ammonifiers, fungi, and *Azotobacter* sp. indicate a positive effect of alfalfa on biodiversity and the activity of microorganisms in the soil.

Table 6. Microbiological properties of soil after two years of cultivating alfalfa on non--carbonate marsh dark soil

Parameters / Treatment	Total microflora ($x10^6$/g)	Fungi ($x10^4$/g)	*Azotobacter* number /g MPN	Ammonifiers ($x10^5$) MPN*	*Sinorhizobium meliloti* ($x10^2$) MPN	Respiration (mg CO_2/kg of soil /7 days)
Alfalfa field	18.22 a	22.00 a	316.66 a	3.16 a	178 a	733.35 a
Control	16.22 a	12.22 b	41.33 b	3.83 a	2b	476.16 b
LSD 0.05	18.78 ns	9.41	200.24	2.62 ns	195	256.98

The mean values indicated by the same letter within one column do not differ significantly ($p < 0.05$); ns – not statistically significant; * MPN – the most likely number

Alfalfa seed – a product, interesting for the processing industry and the market – Average annual level of alfalfa production in Serbia is 104,584.7 hectares (Stat. Year Book of Serbia, 2015), and as its production requires the use of quality declared seed, the need for it on the market is large. On the other hand, by cultivating alfalfa seed in primary production conditions, it is possible to generate higher income per unit area (Karagić et al., 2006). In the first year the total yield of produced natural seed was 1.95 t (0.39 t ha^{-1}), and in the second 3.15 t (0.63 t ha^{-1}). In the analyzed two-year period (2016 and 2017) in the production of alfalfa seed together with compost production, a favorable gross financial result was achieved (64,050.00 RSD ha^{-1} in 2016 and 112,020.00 RSD ha^{-1} in 2017) (Table 7). In other words, sufficient resources have been made to cover fixed costs, and the achieved profitability ensures the sustainability of the proposed technology.

The essence of the technical solution is in solving the issue of maintaining the fertility of the soil on an organic farm without livestock production, modeling and dimensioning of rotating the crops on five fields with the participation of alfalfa. The yields of alfalfa can reach 52 t ha^{-1} of green biomass, i.e. 12.6 t ha^{-1} of dry matter per year (Katić et al., 2011). By implementing the proposed technical solution a part of this biomass was used for the production of compost, to maintain the fertility of the soil on the farm, and this is precisely the innovation because alfalfa compost has not been produced in Serbia so far. To ensure economic sustainability, the production of seeds of alfalfa was realized, with very good economic indicators (Pajić and Marković, 2016). In this sense, the first and third cuttings were used to provide biomass for the production of compost, and the second for the production of seeds.

Table 7. Analytical calculation based on variable costs in the production of alfalfa seed and alfalfa compost

Description	Year	
	2016.	2017.
Production value (RSD/ha^{-1})*	**135,160.00**	**167,560.00**
Quantity of natural seed (kg ha^{-1})	390.00	630.00
Price of natural seed (RSD ha^{-1})	324.00	252.00
Compost quantity (t ha^{-1})**	9.76	12.46
Incentives (RSD ha^{-1})	8,800.00	8,800.00
Variable production costs (RSD ha^{-1})	**71,110.00**	**55,540.00**
Basic material:	*8,800.00*	*1,800.00*
Seed	7,200.00	–
Microbiological preparation	1,500.00	1,800.00
*Costs of work utilizing mechanization and operator***:*	*54,310.00*	*45,740.00*
Plowing	7,950.00	–
Seedbed tilling	2,570.00	–
Sowing	2,460.00	–
Smashing biomass with combines (x2)	13,140.00	13,140.00
Threshing of seed	10,360.00	10,360.00
Transport	8,330.00	12,740.00
Compost mixing with loader (x2)	9,500.00	9,500.00
Certification costs	*8,000.00*	*8,000.00*
Certification costs	8,000.00	8,000.00
Gross financial result (RSD ha^{-1})	**64,050.00**	**112,020.00**

* Gross financial result (RSD ha^{-1}). ** The compost value was not assessed. *** Based on the price list of the Cooperative Association of Vojvodina (2017)

According to this new technology, it is recommended that the exploitation time of the alfalfa crop should be two years. This is an advantage for farmers because, thanks to symbiotic nitrogen fixation, the entire surface in a crop rotation can be provided with nitrogen more efficiently since the accumulation of nitrogen by this process in the soil in later years of alfalfa crop decreases. In this way, in crop rotation, alfalfa returns to the same field every fifth year and in the middle of the period, organic fertilizer produced on the farm is applied. Thanks to the production of alfalfa seed economic viability is assured, in addition to the maintenance of soil fertility in a sustainable and natural way.

CONCLUSION

The proposed new technology recommends the use of alfalfa for two years. In that way, the cultivated soil could be sufficiently provided with nitrogen under proposed crop rotation, by symbiotic nitrogen fixation. In the five-year crop rotation, alfalfa returns to the same field every five years, while in between the soil receives nutrients from composted alfalfa. This technology is precisely the innovation because such compost has not been produced in Serbia so far. The quantity of alfalfa compost obtained by designing the crop rotation with alfalfa on an organic farm in Mokrin is enough for about 20% of the arable land, on an annual level, and with which every fourth year they fertilize it with about 10 t ha^{-1} of compost. Results of the total number of microflora, ammonifiers, fungus, and *Azotobacter* sp. indicate a positive effect of alfalfa on biodiversity and the activity of microorganisms in the soil.

Thanks to the production of alfalfa seed that provides economic sustainability, the proposed technology for maintaining soil fertility is done in a sustainable and natural way.

ACKNOWLEDGMENT

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Projects: III 46005 and III 46006).

REFERENCES

Altieri MA (2015): *Agroecology, the Science of Sustainable Agriculture*, 2nd Edition, Boca Raton: CRC Press. Available to: https://www.taylorfrancis.com/books/e/9780429964015.

Bogdanović D, Latković D, Berenji J, Manojlović M, Barač R (2013): Zavisnost prinosa hibrida kukuruza od primene organskog đubriva guana i biofertilizatora, *Letopis naučnih radova Poljoprivrednog fakulteta*, Univerzitet u Novom Sadu, 37: 110–119.

Bundesgütegemeinschaft Kompost e.V. (2004): "5 – jährige Ergebnisse zur Kompostdüngung in Landwirtschaft und Gemüsebau" Available to: http://www.kompost.de/fileadmin/docs/Archiv/Anwendung/5-jaehrige_Ergebnisse_zur_Kompostduengung_in_Landwirtschaft_und_Gemuesebau.

Ćupina B, Erić P, Krstić Đ, Vučković S (2004): Forage catch crops in sustainable agriculture and organic farming. *Acta Agric. Serb.* 9: 451–459.

Ćupina B, Erić P, Mihailović VM, Mikić AM (2004a): Značaj i uloga međuuseva u održivoj poljoprivredi. *Zbornik radova: Poljoprivredni fakultet, Institut za ratarstvo i povrtarstvo*, 40: 419–430.

Ćupina B, Vujic S, Krstic Dj, Radanovic Z, Cabilovski R, Manojlovic M, Latkovic D (2017): Winter cover crops as a green manure in a temperate region: the effect on nitrogen budget and yield of silage maize. *Crop Pasture Sci.* 68: 1060–1069.

Čuvardić M (2006): Primena đubriva u organskoj poljoprivredi. *Zbornik radova: Poljoprivredni fakultet, Institut za ratarstvo i povrtarstvo*, 42: 369–376.

Delić D (2014): *Rizobijalne bakterije u poljoprivrednoj proizvodnji leguminoza*. Beograd: Zadužbina Andrejević.

Delić D, Stajković-Srbinović O, Knežević-Vukčević J (2016): Alfalfa (*Medicago sativa* L.) and Sinorhizobium meliloti: prospects of using rhizobial inoculants in Serbia. *Bot. Serb.* 40: 13–19.

DNRL (2016): List of organic product manufacturers for 2016. Directorate for National Reference Laboratory, Ministry of Agriculture, Agriculture, Forestry and Water management). Available to: http://www.dnrl.minpolj.gov.rs/o_nama/organska/proizvodjaci_organskih_proizvoda.html.

Dozet G, Cvijanović G, Milenković S, Ninkov N, Kostov Lj, Kaluđerović D (2017): Komponente prinosa pasulja u zavisnosti od primene guanita i mikrobioloških đubriva. *XXII savetovanje o biotehnologiji. Čačak 2017. Zbornik radova*, 1: 69–74. Available to: https://www.afc.kg.ac.rs/files/data/sb/zbornik/Zbornik%20radova%20Knjiga%201-SB%202017.pdf.
Egner H, Riehm H, Domingo WR (1960): Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nahrstoffzustandes der Boden, II: Chemische Extractions-metoden zu Phosphorund Kaliumbestimmung. *Kungliga Lantbrukshügskolans Annaler*, 26: 199–215.
Filipović V, Radivojević S, Ugrenović V, Jaćimović G, Kuzevski J, Subić J, Grbić J (2012): Effects of a certified organic fertilizer on yield and market quality of root parsley (*Petroselinum crispum* (Mill) Nym. Ex A.W. Hill ssp. Tuberosum (Bernh.) Crov.). *Afr. J. Biotechnol*., 11: 9182–9188.
Horwath WR, Paul EA, Harris D, Norton J, Jagger L, Hortont KA (1996): Defining a realistic control for the chloroform fumigation-incubation method using microscopic 10 counting and l4C'substrates. *Can. J. Soil Sci*., 76: 459–467.
Jat LK, Singh YV, Meena SK, Meena SK, Parihar M, Jatav HS, Meena RK, Meena VS (2015): Does Integrated Nutrient Management, Enhance Agricultural Productivity? *J. Pure Appl. Microbiol.* 9: 1211–1221.
Karagić Đ, Katić S, Mihailović V, Erić P, Milić D (2006): Proizvodnja semena lucerke u Vojvodini. *Zbornik radova: Poljoprivredni fakultet, Institut za ratarstvo i povrtarstvo*, 42: 115–130.
Katić S, Milić D, Katanski S, Karagić Đ, Vasiljević S (2011): Genetički doprinos oplemenjivanju lucerke: prinos eksperimentalnih populacija u odnosu na priznate sorte. *Ratarstvo i povrtarstvo*, 48: 91–98.
Mohler LC, Johnson SE (2009): Crop Rotation on Organic Farms: A Planning Manual. Natural Resource, Agriculture, and Engineering Service (NRAES) Cooperative Extension PO Box 4557 Ithaca, NY, USA 14852–4557. Available to: https://www.organicconsumers.org/sites/default/files/crop_rotation_on_organic_farms_planning_manuel.pdf.
Pajić N, Marković T (2016): Economic results in the production of alfalfa seed. *Plant Breed.* 53: 111–115.
Popović V, Sikora V, Simić D, Živanović Lj, Ugrenović V, Filipović V, Zejak D (2014): Efekat folijarne prihrane na produktivnost heljde (*Fagopyrum esculentum* Moench) u organskom sistemu gajenja. *Radovi sa XXVIII savetovanja agronoma, veterinara, tehnologa i agroekonomista*, 20: 83–92. Available to: https://www.pkbae.rs/index.php?action=page&id=zbornici.
Popović V, Maksimović L, Adamović D, Sikora V, Ugrenović V, Filipović V, Mačkić M (2019): Yield of biomass and essential oil of dill (*Anethum graveolens* L.) grown under irrigation. *Ratarstvo i povrtarstvo* 56: 1–10.
Regulation of allowed quantities dangerous and harmful substances in soil and water for irrigation and methods of their testing (1994): *Off. Jour. of Republic of Serbia*, 23/94. Available to: http://www.pravno-informacionissistem.rs/SlGlasnikPortal/eli/rep/sgrs/ministarstva/pravilnik/1994/23/1/reg.
Sarić Z (1989): *Praktikum iz mikrobiologije*. Beograd: Naučna knjiga.
SRPS ISO 11465:2002 (2002): Determination of dry matter and water content on a mass basis – Gravimetric method.
Ugrenovic V, Filipovic V (2017): Cover Crops: Achievement of Sustainability in the Ecological Systems of Agriculture. In: A. Jean-Vasile & D. Nicolò (Eds.), *Sustainable Entrepreneurship and Investments in the Green Economy*, IGI Global, USA, 255–278.
Ugrenović V, Filipović V, Glamočlija Đ, Subić J, Kostić M, Jevđović R (2012). Benefits of Using Fennel as Isolation Buffer in Organic Agriculture. *Ratarstvo i povrtarstvo*, 49: 126–131.
Vincent JM (1970): *A manual for the practical study of rootnodule bacteria*. IBP Handbook 15, Blackwell Scientific Publications.
Wande M (2015). Soil Fertility in Organic Farming Systems: Much More than Plant Nutrition. Available to: https://articles.extension.org/pages/18636/soil-fertility-in-organic-farming-systems:-much--more-than-plant-nutrition.
Watson CA, Stockdale EA, Rees RM (2008): Assessment and maintenance of soil fertility in temperate organic farming. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, 3: 1–11.

ОДРЖАВАЊЕ ПЛОДНОСТИ ЗЕМЉИШТА НА ОРГАНСКИМ ФАРМАМА МОДЕЛИРАЊЕМ ПЛОДОРЕДА КОРИШЋЕЊЕМ ЛУЦЕРКЕ

Владан М. УГРЕНОВИЋ[1], Владимир М. ФИЛИПОВИЋ[2], Душица И. ДЕЛИЋ[1], Вера М. ПОПОВИЋ[3], Оливера С. СТАЈКОВИЋ-СРБИНОВИЋ[1], Анета В. БУНТИЋ[1], Гордана К. ДОЗЕТ[4]

[1] Институт за земљиште,
Теодора Драјзера 7, Београд 11000, Србија
[2] Институт за проучавање лековитог биља „Др Јосиф Панчић",
Тадеуша Кошћушка 1,
Београд 11000, Србија
[3] Институт за ратарство и повртарство,
Максима Горког 30, Нови Сад 21000, Србија
[4] Мегатренд универзитет, Факултет за биофарминг,
Маршала Тита 39, Бачка Топола 24300, Србија

РЕЗИМЕ: У органској производњи се истиче значај сточарске производње, јер она обезбеђује неопходне органске материје за обрађено земљиште. Међутим у Србији се органска сточарска производња одвија код свега четрдесет произвођача што доводи у питање одрживост овог система пољопривредне производње. Циљ техничког решења је одржавање плодности земљишта на органском газдинству које нема сточарску производњу. Истраживање је спроведено на имању ПП „Мокрин", на површини од 25 ha, моделирањем и димензионирањем плодореда са учешћем луцерке, на бескарбонатној ритској црници. Како би се обезбедила економска одрживост организована је производња семена луцерке, а сетва је обављена у јесен 2015. године на 5 ha. Зелена биомаса првог и трећег откоса, као и просушена биомаса после бербе семена, кошене су и сецкане комбајном за ниску силажу и складиштене у сило јаме. После девет месеци добијен је зрео луцеркин компост, добро обезбеђен азотом (укупан азот 5,04%), органском материјом (42,56%) и оптималних вредности: C/N, pH, влажности и EC.

Искоришћавање луцерке од две године је препоручено време у овом истраживању. Тиме се цела површина у плодосмени симбиотском азотофиксацијом брже обезбеђује азотом, луцерка се на исту њиву враћа сваке пете године, а на половини тог периода њива се ђубри компостом произведеним на имању. Количина компоста добијена моделирањем плодореда у Мокрину (2016. год. 48,80 t, а 2017. год. 62,30 t), довољна је за око 20% површина њива, на годишњем нивоу. Тако се оне у плодосмени, сваке 4 године ђубри са 10 t ha^{-1} компоста. Захваљујући производњи семена луцерке, која обезбеђује економску одрживост, предложеном технологијом одржавање плодности земљишта решава се на одржив и природан начин.

КЉУЧНЕ РЕЧИ: луцерка, компостирање, органска производња, плодност

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. Novi Sad, № 138, 83—95, 2020

UDC 630*4:631.81.031(497.11)
https://doi.org/10.2298/ZMSPN2038083K

Olga A. KOSTIĆ[*], *Miroslava M. MITROVIĆ*, *Pavle Ž. PAVLOVIĆ*

University of Belgrade, Institute for Biological Research "Siniša Stanković", National Institute of the Republic of Serbia, Department of Ecology, Bulevar Despota Stefana 142, Belgrade 11060, Serbia

DOUGLAS FIR IMPACT ON THE DYNAMICS AND COMPOSITION OF HUMUS IN THE SOIL OF INDIGENOUS BEECH FOREST IN WESTERN SERBIA

ABSTRACT: This study investigates the impact of organic matter from Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) on the amount and composition of humus in acid brown soil in a climatoregional beech forest (*Fagetum moesiacae montanum* B. Jov. 1967 s.l.) on Mt. Maljen. To accomplish this objective, we performed a one-year litterbag decomposition experiment with litterfall from *Fagus moesiaca* and *Pseudotsuga menziesii.* The quantitative and qualitative content of humus and the intensity of the decomposition process of organic matter from beech and Douglas fir were analysed. Less humus was found during the experiment under Douglas fir than under autochthonous beech at the control site, as well as a decreasing trend for humus levels and quality (unfavourable chemical composition). It was concluded that these changes, caused by the effects of the clearcutting of beech and, in the future, of Douglas fir, and the slower decomposition of organic matter from Douglas fir will contribute to further degradation of the beech habitat on Mt. Maljen in terms of productivity.

KEYWORDS: soil organic matter, *Fagus moesiaca*, *Pseudotsuga menziesii* plantation, silviculture, humus content, humic and fulvic acids, litter decomposition, litterbag experiment

INTRODUCTION

Organic matter is of primary importance to the sustainability of long-term site productivity in forest ecosystems (Prescott et al., 2000). However, it influences several critical soil functions and is affected by land management practices. Soil organic matter content is a function of organic matter inputs (residues and roots – plant litter) and litter decomposition (Bot and Benites, 2005). Even though these processes are related to moisture, temperature, aeration and the physical and chemical properties of the soils, as well as soil macrofauna activity,

[*] Corresponding author. E-mail: olgak@ibiss.bg.ac.rs

leaching by water and humus stabilisation (organomineral complexes and aggregates), the primary factor in these processes is the amount and quality of litter from woody species. For this reason, tree species substitution affects the quality and quantity of annual organic matter stock, as well as the decomposition rate, and can thus have a considerable impact on the modification of the physical and chemical characteristics of soil and on the processes in the soil (Pavlović et al., 1998; Albers et al., 2004; Moukoumi et al., 2006; Kostić et al., 2012, 2016). Hence, the amount of organic matter and its dynamics have become a focus of research (Augusto et al., 2002; Moukoumi et al., 2006; Bonifacio et al., 2008; Kostić et al., 2012, 2016).

The decomposition of organic matter is essential for the short-term availability of nutrients for tree growth and long-term site fertility due to humus formation (Prescott et al., 2000). Vegetation type is considered to be one of the main parameters affecting differences in the decomposition process. It has been established that litter from broadleaf forest species decomposes more quickly and leads to mull humus formation, while coniferous species decompose more slowly and form mor or moder humus (Binkley, 1995; Rovira and Vallejo, 2002; Pavlović et al., 1998a, b). This slower decomposition in coniferous forests results in the accumulation of forest litter, and thus of nutrients in insoluble forms (Vogt et al., 1986; Raulund-Rasmussen and Vejre, 1995). Over time, this can reduce the supply of available nutrients to plants, having a negative impact on habitat productivity thereby.

Humic acids are the most significant part of humus. They affect soil structure, participate in the transfer of micronutrients from soil to plants, improve water retention, increase seed germination and stimulate the development of microflora populations in soil, respiration and photosynthesis (Nardi et al., 2002; Chen et al., 2004; Velasco et al., 2004; Pena-Mendez et al., 2005). Humic acids also decelerate the evaporation of water from soil. This is of particular importance in soils containing only a low proportion of clay and sandy soils, which do not have the capacity to retain water. Fulvic acids, which behave like strong organic acids due to their acidity, act destructively on soil minerals. In addition, these acids can cause soil podzolisation as they form complex compounds with sesquioxides, which translocate from the surface layers of soil and accumulate in the B horizon (Riise et al., 2000). For example, planting spruce trees on acid brown soil intensifies the processes of podzolisation (Nielsen et al., 1999).

Due to the ever-increasing needs of society for wood, large areas of European broadleaf forests are being replaced by fast-growing coniferous cultures. This is also true for Serbia, where the high number of coppice and degraded forests meant the production potential of the habitat could not be exploited, resulting in the mass introduction of coniferous species, especially in low and degraded beech forests. Either translocated autochthonous species like Norway spruce were used or allochthonous species like Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) (Kostić et al., 2012, 2016) and *Pinus strobus* L. (Pavlović et al., 1997; Pavlović, 1992). Research has shown the impact of anthropogenic activities in forest ecosystems on the quantitative and qualitative dynamics of humus (particularly humins, humic and fulvic acids) and the dynamics of organic matter (Wollum Schubert, 1975; Piene and VanClave, 1978; Vesterdal et al., 1995; Pavlović et al., 1997; Ussiri and Johnson, 2001; Kawahigashi and Sumida, 2006).

This study was part of wider ecological research aimed at investigating the impact of conifer cultures on pedogenesis and soil properties in a climatoregional zone of autochthonous montane beech forests. Alongside an experiment designed to determine the decomposition process model of organic matter from beech (*Fagus moesiaca* [Domin, Maly] Czeczott) and Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco), the spatial and temporal dynamics of humus were observed with the aim of establishing whether introducing allochthonous coniferous species disturbed the balance between the processes of organic matter humification and mineralisation. Furthermore, on the basis of humus composition and by monitoring the intensity of organic matter decomposition, the effect of the stated anthropogenic activities on habitat productivity in a zone of beech forest was analysed.

MATERIALS AND METHODS

Research was undertaken in the climatoregional belt of montane beech forests (*Fagetum moesiacae montanum* B. Jov. 1967 s.l.) on Mt. Maljen (lat. 44° 10' N; long. 20° 5' E), in the locality of Kaona (880 MASL). There were silviculture activities undertaken here, whereas, after the clearcutting of beech trees in 20 m-wide strips, plantations of Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) were established, with these strips alternating with the strips of coppice forests of beech. The climatic conditions of this region are moderate continental, which is characteristic for the mountainous zone of Central European broadleaf forests (the mean annual temperature is 9.16 °C and mean annual precipitation is 890 mm). The study area lying within the forty-year-old silvicultural system of Douglas fir and the control area within the autochthonous beech forest are characterised by the same ecological conditions, i.e. western exposure, a 2–5 ° slope, and the same soil types, formed on the same geological substrate (diabase), (WRB, Cambisol, dystric 2006; Kostić et al., 2016; Pavlović et al., 2017).

To determine the decomposition intensity of leaf litter from beech and Douglas fir (6 and 12 months after the experiment started), a 'litterbag approach' was used. Fresh leaf litter (20 g), dried at 65 °C to a constant weight, was enclosed in bags (20x20 cm) made of 1mm nylon mesh. In October, ten bags were randomly placed on the surface of the forest floor for each species. Five of the bags (n=5) were collected from both areas in April and the remaining five in October. In order to determine the weight of the organic matter after decomposition, each bag was cleaned of soil and roots and dried to a constant weight (at 65 °C). Samples of soil (n=5) were taken from under each of the litterbags, up to a depth of 5 cm, so as to determine the quantity of humus using the Simakov modification of the Tyurin method (Beljčikova, 1975).

A qualitative analysis of humus, i.e. an analysis of the humic fractions of the leaf litter after extraction of bitumen (Ponomareva and Plotnikova, 1975a) and soil (Ponomareva and Plotnikova, 1975b), was conducted in soil profiles opened at both study sites. The content of Fe_2O_3 was determined using a complexometric method (Škorić and Sertić, 1963), while the content of R_2O_3 was established using gravimetry. The Al_2O_3 content, the R_2O_3:FK ratio and the Al_2O_3:Fe_2O3 ratio were calculated. Soil acidity in H_2O was determined using a potentiometer.

A prognosis for the decomposition of organic matter from beech and Douglas fir was given based on Olson's decomposition model and Olson's constant loss rate (Olson, 1963), using the formula:

$$M_t/M_0=e^{-kt}$$

where M_0 is the initial mass of organic matter, M_t is the mass of the organic matter after (t) years of decomposition, and k is Olson's constant loss rate after 12 months of decomposition. According to this decomposition model, half the decomposition time is $t_{1/2}$=0.639k, and the time constant k=0.368 of 1/e. To calculate the decomposition prognosis for 95% and 99% of the organic matter, the coefficients 3/k and 5/k were used.

The quantitative distribution of humus, i.e. its spatial (between the study sites) and temporal (during the experiment at each of the sites) distribution in the surface layer of soil and the coefficients of loss, as well as the prognosis for the decomposition of organic matter at the study sites were analysed using one-way analyses of variance (ANOVA), and significant differences were confirmed using a t-test.

RESULTS AND DISCUSSION

A comparison of the amount of humus in the layer of topsoil at the study sites at the beginning of the experiment revealed that the soil under the control beech stand was richer in humus (16.2%) than that under the Douglas fir stand (9.89%). This spatial trend was also true 6 (18.96% : 9.21%) and 12 months (17.40% : 9.89%) after the experiment started (Figure 1). The lower levels of humus in the topsoil from the Douglas fir stand are a result of the negative effects of clearcutting as a land management practice in this area. Specifically, the long period where the habitat was left open before the establishment of the Douglas fir stand led to the mineralisation process of humus intensifying and it being washed away (Pavlović et al., 1997; Ranger et al., 2008).

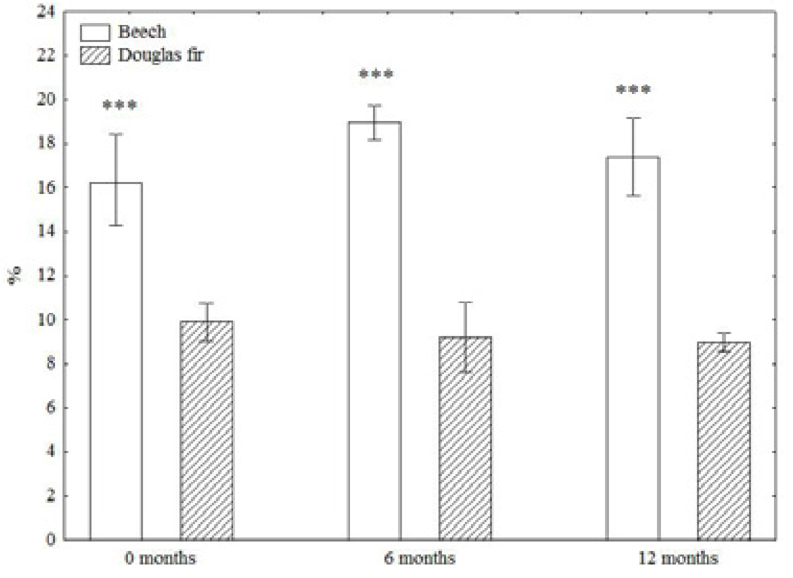


Figure 1. Spatial analysis of the amount of humus in topsoil (0–5 cm) (ANOVA, n=5; ***p<0.001)

In terms of time, the only significant increase in the amount of humus was determined in topsoil at the beech stand 6 months into the experiment (Figure 2).

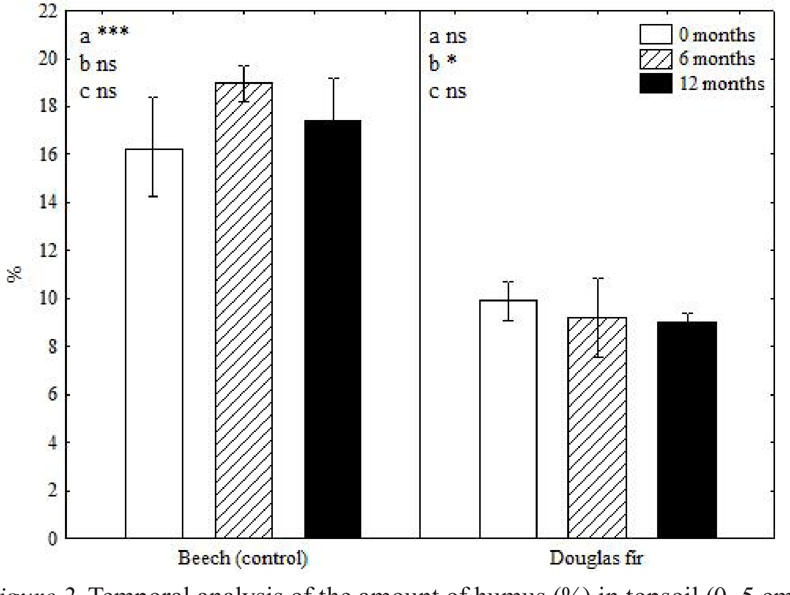


Figure 2. Temporal analysis of the amount of humus (%) in topsoil (0–5 cm) (ANOVA, n=5; ***p<0.001, *p<0.05, ns – not significant; a – 0 months/6 months; b – 0 months/12 months; c – 6 months/12 months)

During the decomposition of organic matter in soil, two processes occur simultaneously – the processes of humification and mineralisation of organic matter (Guggenberger, 2005; Ćirić, 1984). In the early stages of organic matter decomposition, humification is the dominant process, which explains the increase in the amount of humus 6 months into the experiment at the beech stand. In the later stages, the process of humification slowed down, i.e. the domination of organic matter mineralisation, which occurred during the second phase of the experiment from the 6th to 12th month, resulted in a decrease in the amount of humus in the topsoil (at a depth of 0–5 cm) at the beech stand. Even so, the lack of any difference in the amount of humus in the soil at the beech stand at the beginning and end of the experiment shows that the processes of organic matter humification and mineralisation are in dynamic equilibrium, which is characteristic of stable ecosystems (Ćirić,1984; Swift et al., 1979; Pavlović et al., 1998). The lower humus content in the topsoil under the Douglas fir stand after 12 months (Figure 2) indicates a continuing trend of depletion due to the clearcutting of already partially degraded habitats of beech subject to silviculture activities. Previous research showed that Douglas fir, as a fast-growing species, leads to the depletion of nutrients in soil, as indicated by the negative input-output budget of elements such as C, N, P, S, K, Ca and Mg (Marques et al., 1997; Ranger et al., 1997, 2002; Martinik, 2003; Kostić et al., 2016). These differences are more noticeable in young stands, particularly during periods of dormancy and are more pronounced as the time period between two rotations is shortened. Bearing in mind the fact that maintaining nutrient reserves at a level characteristic for each, individual ecosystem is a fundamental prerequisite for the stability of the ecosystem in question, disturbing the balance in their dynamics through reducing the amount of humus can have a negative impact on habitat productivity (Pavlović et al., 1997; Ranger et al., 1997; Kostić et al., 2016).

A morphological analysis of the soil profile and a qualitative analysis of the humus at the control site in the beech stand revealed the formation of mulmoder type humus, while the presence of an Oh horizon at the Douglas fir stand indicated that the planting of conifers at the beech habitat has resulted in the inhibition of the coupling of organic and mineral components in soil, i.e. the occurrence of moder-type humus (Table 1). Qualitative changes in humus were detected in terms of lower pH values in the soil under the Douglas fir stand (Table 2). The acidification of soil under conifer cultures, most marked in the surface layer, is the result of the impact of litter quality and the transformation of organic matter from conifers, which evolves in the direction of acidic humic substances (Kostić et al., 2012; Podrázský et al., 2009; Kostić et al., 2016). Although humus composition in both areas revealed a trend characteristic of acid brown soils, i.e. a ratio of humic (Ch) to fulvic (Cf) acids of less than one, which narrows with depth in the soil profile (Avdalović, 1971), the values of this ratio were higher at the control site under beech than within the Douglas fir stand. In terms of humic acids, fraction 1 was most present, with its highest content in the humus-accumulative horizon (18.32–20.14%) at both study communities, and the dominant proportion at the beech stand (Table 1). As for fulvic acids (Cf), fraction 1, which is bound with mobile R_2O_3 and humic acids of fraction 1, was

Table 1. Group and fractional composition of humus in soil and litter under beech and Douglas fir

Plot	Hor.	Depth cm	Total C	Humic acid			Fulvic acid				Total acids sum	Humin	Ch : Cf
				1	2	sum	1a	1	2	sum			
Beech (control)	O_{LF}	0–4 cm	37.54	4.44	0.15	4.59	0.48	4.90	0.35	5.73	10.32	27.22	0.80
			100.00	11.83	0.40	12.23	1.28	13.05	0.93	15.27	27.49	72.51	
	A	4–9 cm	10.40	2.02	0.00	2.02	0.44	2.05	0.17	2.66	4.68	5.72	0.76
			100.00	19.42	0.00	19.42	4.23	19.71	1.60	25.54	44.96	55.04	
	A	4–13/19 cm	7.30	1.47	0.00	1.47	0.36	1.53	0.07	1.96	3.43	3.87	0.75
			100.00	20.14	0.00	20.14	4.93	20.96	0.96	26.85	46.99	53.01	
	(B)	13/19–50 cm	2.02	0.34	0.00	0.34	0.20	0.29	0.10	0.59	0.93	1.09	0.58
			100.00	16.83	0.00	16.83	9.90	14.36	4.95	29.21	46.04	53.96	
	(B)	50–70 cm	0.81	0.09	0.00	0.09	0.08	0.09	0.07	0.24	0.33	0.48	0.38
			100.00	11.11	0.00	11.11	9.88	11.11	8.64	29.63	40.74	59.26	
Douglas fir	O_{LF}	0–1/2 cm	36.17	3.27	0.00	3.27	0.50	4.67	0.00	5.17	8.44	27.73	0.63
			100.00	9.04	0.00	9.04	1.38	12.91	0.00	14.29	23.33	76.67	
	O_H	1/2–2 cm	14.53	2.18	0.00	2.18	0.38	3.14	0.00	3.52	5.70	8.83	0.62
			100.00	15.00	0.00	15.00	2.62	21.61	0.00	24.23	39.23	60.77	
	A	2–8 cm	5.35	0.98	0.00	0.98	0.39	1.15	0.15	1.69	2.67	2.68	0.58
			100.00	18.32	0.00	18.32	7.29	21.50	2.80	31.59	49.91	50.09	
	(B)	8–50 cm	0.90	0.15	0.00	0.15	0.12	0.20	0.00	0.32	0.47	0.43	0.47
			100.00	16.67	0.00	16.67	13.33	22.22	0.00	35.56	52.23	47.77	
	(B)	50–103 cm	0.39	0.06	0.00	0.06	0.05	0.08	0.00	0.13	0.19	0.20	0.46
			100.00	15.38	0.00	15.38	12.82	20.51	0.00	33.33	48.71	51.29	

Table 2. Composition of 0.1 M H_2SO_4 extract from samples of soil under beech and Douglas fir

Plot	Horiz.	Depth cm	pH H_2O	Fulvic acid 1a (Cx2)	R_2O_3 %	Fe_2O_3 %	Al_2O_3 %	R_2O_3 : FK	Al_2O_3 : Fe_2O_3	Fe (B)hor : Fe A hor
Beech (control)	O_{LF}	0–4 cm	5.06	1.02	0.20	0.15	0.05	0.20	0.33	
	A	4–9 cm	4.86	0.88	1.30	0.42	0.88	1.48	2.10	
	A	4–13/19 cm	4.79	0.72	1.29	0.47	0.82	1.79	1.74	
	(B)	13/19–50 cm	5.27	0.40	1.19	0.37	0.82	2.98	2.22	0.79
	(B)	50–70 cm	5.54	0.16	0.86	0.28	0.58	5.38	2.07	0.60
Douglas fir	O_{LF}	0–1/2 cm	4.95	1.00	0.48	0.28	0.20	0.48	0.70	
	O_H	1/2–2 cm	4.60	0.76	1.58	0.48	1.10	2.07	2.28	
	A	2–8 cm	4.55	0.78	2.17	0.40	1.77	2.78	4.43	
	(B)	8–50 cm	5.15	0.24	1.44	0.39	1.05	6.00	2.69	0.98
	(B)	50–103 cm	5.36	0.10	1.34	0.33	1.01	13.40	3.06	0.83

the most present. There was no regularity in its increase or decrease through the profile, as already concluded by several authors (Avdalović, 1971; Pavlović, 1992, 1998; Pavlović et al., 1997; Kostić, 2007). The proportion of fraction 1 of the fulvic acids was higher at the Douglas fir stand through the whole depth of the profile, apart from in the litter horizon. This difference was most pronounced in the cambic horizon, where it amounted to 20.51–22.22 % at the Douglas fir stand, while at the control area of beech it was 11.11–14.36% (Table 1). This fraction was higher than the content of the aggressive fraction 1a throughout almost the whole profile, while its increase with depth was more marked in the soil under Douglas fir than under beech (13.33% : 9.90%; 12.82 : 9.88 in the cambic horizon, Table 1). As expected, the highest content of insoluble residue, due to the highest content of resistant material of lignin, was found in the litter horizon at both study sites. The proportion of humins in the humus was higher under Douglas fir than under the control beech (76.67% : 72.51%; Table 1). On the basis of these results, it can be seen that the humus matter formed in the soil under Douglas fir has a less favourable chemical composition than that at the control site in the autochthonous beech stand, which is reflected in the greater proportion of insoluble residue in humus, a lower proportion of humic acids, a higher proportion of fulvic acids and fraction 1a in the soil horizons, and a narrower Ch:Cf ratio at the Douglas fir stand. Specifically, soils which develop under coniferous vegetation are richer in fulvic acids than those formed under broadleaf vegetation (McKeague et al., 1986; Stefanović and Pavlović, 1991; Pavlović et al., 1997). An analysis of the parameters of the composition of soil extract in 0.1M H_2SO_4 revealed a dynamic that is characteristic of Dystric Cambisol (Table 2), i.e. there was not found a translocation of fulvic acid 1a and the organomineral complex of iron from the surface layers into the lower parts of the profile (Avdalović, 1971). This can be explained by the fact that the level of sesquioxides was enough to bind the aggressive fraction of fulvic acid 1a to the organomineral complex of iron and accumulate it from the very surface of the profile. This was supported by both the ratio of R_2O_3 to fulvic acid 1a, which was always higher than one, and also the Fe (B) hor./FeAhor. ratio, which was less than one. As far as aluminium is concerned, its migration was noted only in the area of the Douglas fir stand, and only within the humus-accumulative horizon.

Through an analysis of litter decomposition intensity, it was determined that beech organic matter decomposed more quickly than that from Douglas fir during the whole experiment (Figures 3 and 4). The greatest difference in decomposition intensity was found 6 months after the start of the experiment ($p < 0.001$) (Figure 3).

At this point, beech organic matter was 18.2% decomposed, while that from Douglas fir was 9.48% decomposed; however, after a year, the ratio was 32.76% : 24.98%. The slower decomposition of conifer litter is a result of the lower concentrations of nutrients and higher levels of lignin and polyphenols in coniferous organic matter, and of the specific microclimate (lower light intensity and lower moisture content) characteristic for coniferous ecosystems (Millar, 1974; Flanagan and VanCleve, 1983; Pavlović, 1992; Mudrik et al., 1994; Cornelissen, 1996; Pavlović, 1998; Prescott et al., 2000, 2004; Kostić et al., 2003; Pavlović et al., 2003; Kostić, 2007; Kostić et al., 2016).

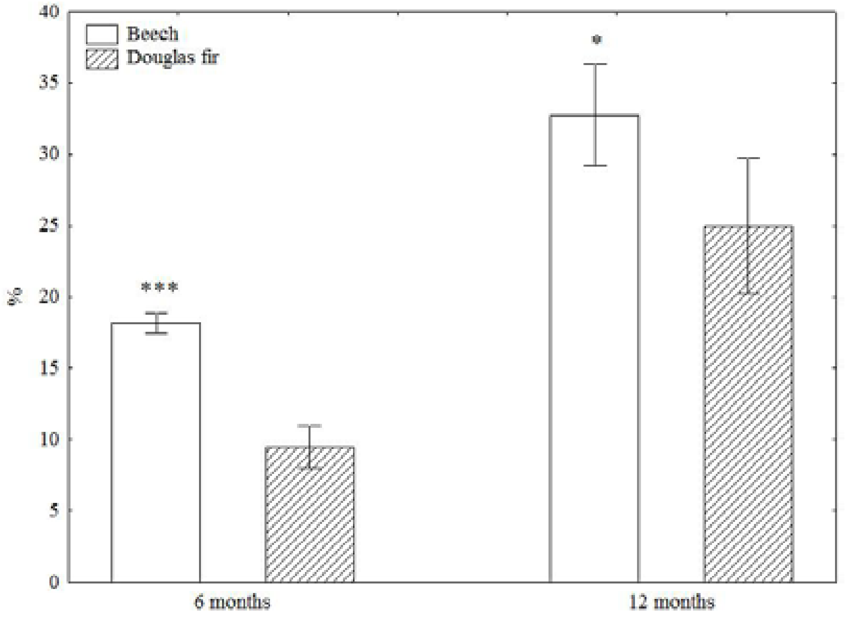


Figure 3. Decomposition intensity of organic matter from beech and Douglas fir (ANOVA, n=5; ***p<0 .001, * p<0.05) (Kostić et al., 2016).

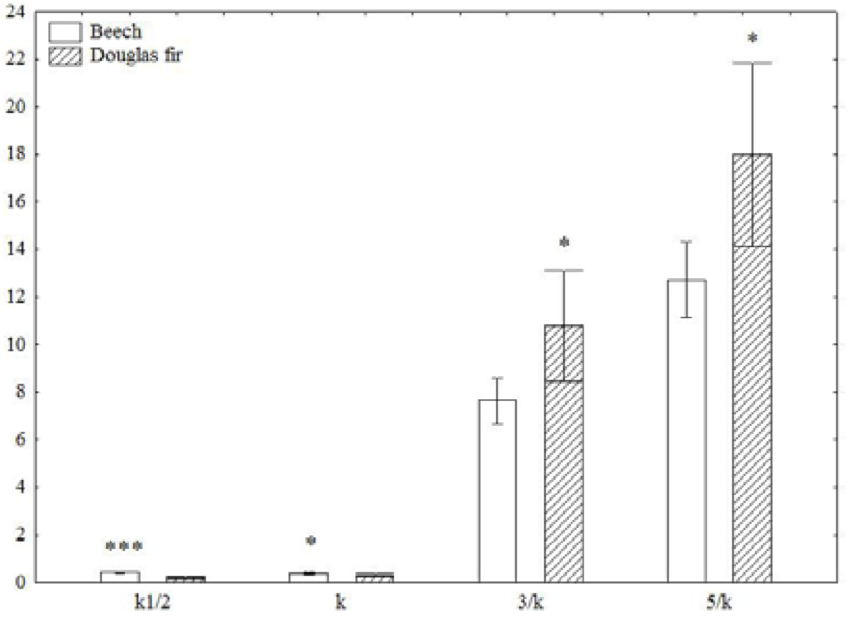


Figure 4. Analysis of the decomposition constants (k1/2 and k) and the prognosis constants (3/k and 5/k) for organic matter from beech and Douglas fir (ANOVA, n=5; ***p<0.001, *p<0.05) (Kostić et al., 2016).

A prognosis of the decomposition intensity of the organic matter from the studied species based on Olson's decomposition model suggested that 95% of organic matter from beech will decompose after 7.6 years and from Douglas fir after 10.8 years, while for 99% decomposition, 12.7 years will be necessary for beech organic matter and almost 18 years, i.e. an additional 5 years, for Douglas fir organic matter (Figure 4).

CONCLUSION

On the basis of the results of this study, it can be concluded that there is a difference in the amount and composition of humus in the soil of the examined ecosystems, as well as in the decomposition intensity of the organic matter of the edificators. The results of the spatial and temporal analysis of soil humus levels lead us to the conclusion that the clearcutting of beech and the organic matter from Douglas fir have contributed to a reduction in the amount of humus in beech forests on Mt. Maljen. A qualitative analysis of humus indicates that the chemical composition of the humus substances that form in the soil under Douglas fir is unfavourable compared to that under beech, which may in the future lead to the destruction of acid brown soil through further acidification and a reduction in its productivity. Specifically, the slower decomposition of Douglas fir organic matter and its more modest requirements for nutrients compared to beech will mean a significant amount of nutrients will be excluded from the cycling process for a lengthy period of time. In this way, the processes of beech habitat degradation, which began with the clearcutting of beech trees and the removal of large amounts of beech organic matter, will continue and will probably culminate after the cutting of the Douglas fir.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia, Grants 451-03-68/2020-14/200007

REFERENCES

Albers D, Migge S, Schaefer M, Scheu S (2004): Decomposition of beech leaves (*Fagus sylvatica*) and spruce needles (*Picea abies*) in pure and mixed stands of beech and spruce. *Soil Biol. Biochem*. 36: 155–164.

Augusto L, Ranger J, Binkley D, Rothe A (2002): Impact of several common tree species of European temperate forests on soil fertility. *Ann. For. Sci.* 59: 233–253.

Avdalović V (1971): *Geneza i osobine kiselih smeđih zemljišta SR Srbije* (The genesis and properties of acid brown soils in Serbia). Doctoral dissertation, Faculty of Forestry, Belgrade University, Belgrade.

Beljčikova NP (1975): Opredelinie gumusa počvi po metodu I.V. Tjurina (Определение гумуса почвы по методу И.В. Тюрина). In: *Agrohimičeski metodi issledovania počv*. (Агрохимические методы исследования почв). Moscow: Nauka, 56–62.

Binkley D (1995): The influence of tree species on forest soils processes and patterns. In: Mead DJ, Conforth IS (eds.), *Proceedings of the Tree and soil Workshop*, Agronomy Society of New Zealand, Canterbury, 1–33.

Bonifacio E, Santoni S, Cudlin P, Zanini E (2008): Effect of dominant ground vegetation on soil organic matter quality in a declining mountain spruce forest of central Europe. *Bor. Env. Res.* 13: 113–120.

Bot A, Benites J (2005): *The importance of soil organic matter – Key to drought-resistant soil and sustained food production*. Food and agriculture organisation of the United Nations (FAO), Rome.

Chen Y, Clapp CE, Magen H (2004): Mechanisms of Plant Growth Stimulation by Humic Substances: The Role of Organo-Iron Complexes. *Soil Sci. Plant Nutr.* 50: 1089–1095.

Cornelissen JHC (1996): An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. *J. Ecol.* 84: 573–582.

Ćirić M (1984): *Pedologija*. Sarajevo: Svijetlost.

Flanagan PW, VanCleve K (1983): Nutrient cycling in relation to decomposition and organic matter quality in taiga ecosystems. *Can. J. Botany* 55: 1632–1640.

Guggenberger G (2005): Humification and Mineralization in Soils. In: Buscot F, Varma A (eds.), *Soil Biology, Microorganisms in Soils: Roles in Genesis and Functions*, Volume 3, Berlin–Heidelberg: Springer-Verlag.

Kawahigashi M, Sumida H (2006): Humus composition and physico-chemical properties of humic acids in tropical peat soils under sago palm plantation. *Soil Sci. Plant Nutr.* 52: 153–161.

Kostić O (2007): *Uticaj monokultura smrče i duglazije na pedogenezu i svojstva zemljišta na Maljenu* (The influence of spruce and Douglas fir monocultures on pedogenesis and soil properties on Mt. Maljen). Master's thesis, Faculty of Forestry, Belgrade University, Belgrade.

Kostić O, Jarić S, Gajić G, Pavlović D, Marković M, Mitrović M, Pavlović P (2016): The effects of Douglas fir monoculture on stand characteristics in a zone of montane beech forest. *Arch. Biol. Sci.* Belgrade 68: 753–766.

Kostić O, Mitrović M, Jarić S, Djurdjević L, Gajić M, Pavlović M, Pavlović P (2012): The effects of forty years of spruce cultivation in a zone of beech forest on Mt. Maljen (Serbia). *Arch. Biol. Sci. Belgrade* 64: 1181–1195.

Kostić O, Pavlović P, Knežević M, Mitrović M, Djurdjević L, Dinić A (2003): Decomposition of beech and spruce leaf litter in natural conditions. *Third International Balkan Botanical Congress*, 18–24 May 2003, Sarajevo, Abstract 397.

Marques R, Ranger J, Villette S, Granier A (1997): Nutrient dynamics in a chronosequence of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) stand on the Beaujolais Mounts (France). 2. Quantitative approach. *For. Ecol. Man.* 92: 167–197.

Martinik A (2003): Possibilities of growing Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) in the conception of sustainable forest management. *Ekologija* (Bratislava) 22: 136–146.

McKeague JA, Cheshire MV, Andreux F, Berthelin J (1986): Organo-mineral complexes in relation to pedogenesis. In: Huang PM, Schnitzer M (eds.). *Interactions of soil minerals with natural organics and microbes*, Soil Sci. Soc. Amer. Spec. Publ. (Madison), 549–592.

Millar SC (1974): Decomposition of Coniferous Leaf Litter. In: Dickinson CH, Pugh GJF (eds.), *Biology of Plant Litter Decomposition*, London: Academic Press, I: 105–128.

Moukoumi J, Munier-Lamy C, Berthelin J, Ranger J (2006): Effect of tree species substitution on organic matter biodegradability and mineral nutrient availability in temperate topsoil. *Ann. For. Sci.* 63: 763–771.

Mudrick AD, Hoosein M, Hicks RR, Townsend CE (1994): Decomposition of leaf litter in an Appalachian forest: effects of leaf species, aspect, slope position and time. *Forest Ecol. Manag.* 68: 231–250.

Nardi S, Pizzeghello D, Muscolo A, Vianello A (2002): Physiological effects of humic substances on higher plants. *Soil Biol. Biochem.* 34: 1527–1536.

Nielsen KE, Ladekarl UL, Nørnberg P (1999): Dynamic soil processes on heathland due to changes in vegetation to oak and Sitka spruce. *For. Ecol. Manage.* 114: 107–116.

Olson JS (1963): Energy storage and the balance of producers and decomposers in ecological systems. *Ecology* 44: 322–331.

Pavlović P (1992): *Analiza stanja sastava stelje i humusa u šumskim zajednicama na planini Cer* (An analysis of the state of litter and humus composition in forest communities on Mt. Cer). Master's thesis, Faculty of Biology, Belgrade University, Belgrade.

Pavlović P (1998): *Pedološke komponente metabolizma nekih šumskih zajednica na planini Maljen* (Pedological components of the metabolism of various forest communities on Mt. Maljen). Doctoral dissertation, Faculty of Biology, Belgrade University, Belgrade.

Pavlović P, Mitrović M, Djurdjević L, Kostić O, Gajić G (2003): Leaf litter decomposition in different forest ecosystems at Maljen mountain. *Third International Balkan Botanical Congress*, 18–24 May 2003, Sarajevo, Abstract 407.

Pavlović P, Mitrović M, Popović R (1998a): Prognosis of litter decomposition rate in different forest ecosystems at Cer mountain. *Arch. Biol. Sci.* 50: 109–118.

Pavlović P, Mitrović M, Popović R (1998b): Decomposition of *Quercus frainetto* Tenn. and *Quercus cerris* L. leaf litter under natural forest conditions. In: J. Tsekos, M. Moustakas M (eds.), *Progress in Botanical Research*, Kluwer Academic Publishers, 361–365.

Pavlović P, Mitrović M, Popović R, Kostić O (1997): Prostorna i vremenska distribucija količine humusa distričnog kambisola u različitim šumskim zajednicama na planini Cer (Spatial and temporal distribution of the amount of humus in dystric cambisol in different forest communities on Mt. Cer). *Ekologija* 32: 63–72.

Pavlović P, Kostić N, Karadžić B, Mitrović M (2017): *The Soils of Serbia*. World Soils Book Series (Series editor Hartemink, A.E.). Springer Science and Business Media Dordrecht, The Netherlands, p. 225.

Pena-Mendez EM, Havel J, Patočka J (2005): Humic substances–compounds of still unknown structure: applications in agriculture, industry, environment and biomedicine. *J. Appl. Biomed.* 3: 13–24.

Piene H, Van Cleve K (1978): Weight loss of litter and cellulose bags in a thinned white spruce forest in interior Alaska. *Can. J. Forest Res.* 8: 42–46.

Podrazsky V, Remeš J, Hart V, Moser WK (2009): Production and humus form development in forest stands established on agricultural lands – Kostelec nad Černymi lesy region. *J. Forest Sci.* 55: 299–305.

Ponomareva VV, Plotnikova TA (1975a): Opredelenie sostava organičeskogo veščestva torfjano-bolotnih počv po sheme i metodu Ponomarevoj i Nikolaevoj. In: *Metodičeskie ukazanija po opredeleniju sodržaja i sostava gumusa v počvah (mineralnih i torfjanih)*, Vsejuznaja Akademija Selskohozjajstvenih nauk V. I. Lenina i Centralnij Muzej Počvovedenija V. V. Dokučaeva, Leningrad, 92–101.

Ponomareva VV, Plotnikova TA (1975b): Opredelenie sostava gumusa po sheme Tjurina v modifikacii Ponomarevoj i Plotnikovoj. V: Metodičeskie ukazanija po opredeleniju sodržaja i sostava gumusa v počvah (mineralnih i torfjanih). Vsejuzna Akademija Selskohozjajstvenih nauk V. I. Lenina i Centralnij Muzej Počvovedenija. V. V. Dokučaeva, Leningrad, 48–66.

Prescott CE, Blevins LL, Staley C (2004): Litter decomposition in British Columbia forests: Controlling factors and influences of forestry activities. BC *J. Ecosyst. Manag.* 5: 44–57.

Prescott CE, Zabek LM, Staley CL, Kabzems R (2000): Decomposition of broadleaf and needle litter in forests of British Columbia: Influences of litter type, forest type and litter mixtures. *Can. J. Forest Res.* 30: 1742–1750.

Ranger J, Allie S, Gelhaye D, Pollier B, Turpault MP, Granier A (2002): Nutrient budgets for a rotation of a Douglas fir plantation in Beaujolais (France) based on a chronosequence study. *Forest Ecol. Manag.* 171: 3–16.

Ranger J, Bonnaud P, Bouriaud O, Gelhaye D, Picard JF (2008): Effects of the clear-cutting of a Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) plantation on chemical soil fertility. *Ann. For. Sci.* 65: 303.

Ranger J, Marques R, Colin-Belgrand M (1997): Nutrient dynamics during the development of Douglas fir (*Pseudotsuga menziesii* Mirb.) stand. *Acta Oecol.* 18: 73–90.

Raulund-Rasmussen K, Vejre H (1995): Effect of tree species and soil properties on nutrient immobilization in the forest floor. *Plant Soil* 179: 1–7.

Riise G, Van Hees P, Lundström U, Tau Strand L (2000): Mobility of different size fractions of organic carbon, Al, Fe, Mn and Si in podzols. *Geoderma* 94: 327–247.

Rovira P, Vallejo VR (2002): Labile and recalcitrant pools of carbon and nitrogen in organic matter decomposing at different depths in soil. An acid hydrolysis approach. *Geoderma* 107: 109–141.

Stefanović K, Pavlović P (1991): Uporedna proučavanja zemljišta u brdskoj bukovoj šumi (*Fagetum montanum serbicum* Rud.) i kulturi borovca (*Pinus strobus* L.) podignutoj na staništu bukve (Comparative research of soil in a hilly beech forest (*Fagetum montanum serbicum* Rud.) and a culture of Weymouth pine (*Pinus strobus* L.) planted in a beech habitat). *Zemljište kao prirodni resurs i faktor razvoja*: u spomen na akademika Milivoja Ćirića, XCVIII, Sarajevo. ANUBiH, 155–161.

Swift MJ, Heal OW, Anderson JM (1979): *Decomposition in terrestrial ecosystems*. Oxford: Blackwell; 372 p.

Škorić A, Sertić V (1963): Prilog poznavanju i primeni kompleksometrijskih analiza u pedokemiji (Understanding and applying complexometric analysis in pedochemistry). *2nd Congress of the Yugoslav Society of Soil Science*, Ohrid. 175–181.
Ussiri DA, Johnson CE (2001): Effects of clear-cutting on structure and chemistry of soil humic substances of the Hubbard Brook experimental forest, New Hampshire, USA. In: EA Ghabbour, G. Davies (eds.), *Humic Substances, Structures, Models and Functions*. Royal Society of Chemistry (Great Britain), Royal Society of Chemistry, 387 p.
Velasco MI, Campitelli PA, Ceppi SB, Havel J (2004): Analysis of humic acid from compost of urban wastes and soil by fluorescence spectroscopy. *Agriscientia* 21: 31–38.
Vesterdal L, Dalsgaard M, Felby C, Raulund-Rasmussen K, Jørgensen B (1995): Effects of thinning and soil properties on accumulation of carbon, nitrogen and phosphorus in forest floor of *Norway spruce* stands. *Forest Ecol. Manag.* 77: 1–10.
Vogt KA, Grier CC,Vogt DJ (1986): Production, turnover and nutrient dynamics of above and below ground detritus of world forests. *Adv. Ecol. Res.* 15: 303–378.
Wollum AG, Schubert GH (1975): Effect of thinning on the foliage and forest floor properties of ponderosa pine stands. *Soil Sci. Soc. Am. Proc.* 39: 968–972.
World Reference Base for Soil Resources (2006): FAO, ISRIC and ISSS, Rome.

УТИЦАЈ ДУГЛАЗИЈЕ НА ДИНАМИКУ И САСТАВ ХУМУСА У ЗЕМЉИШТУ АУТОХТОНЕ ШУМЕ БУКВЕ У ЗАПАДНОЈ СРБИЈИ

Олга А. КОСТИЋ, Мирослава М. МИТРОВИЋ, Павле Ж. ПАВЛОВИЋ

Универзитет у Београду,
Институт од значаја за Републику Србију
Институт за биолошка истраживања „Синиша Станковић”,
Одељење екологије
Булевар деспота Стефана 142, Београд 11060, Србија

РЕЗИМЕ: У раду је истраживан утицај органске материје дуглазије (*Pseudotsuga menziesii* [Mirb.] Franco) на количину и састав хумуса киселог смеђег земљишта климарегионалне букове шуме (*Fagetum moesiacae montanum* B. Jov. 1967 s.l.) на Маљену. За постизање наведеног циља обављен је једногодишњи *in situ* експеримент, коришћењем “litter bag” методе/технике са стељом букве (*Fagus moesiaca*) и дуглазије (*Pseudotsuga menziesii*). Анализиран је квантитативан и квалитативан садржај хумуса и интезитет процеса разлагања органске материје букве и дуглазије. Утврђена је нижа количина хумуса, уочен је тренд смањивања количине хумуса током истраживања и опадање квалитета хумуса (неповољнији хемијски састав) у култури дуглазије у односу на контролну површину под аутохтоном буквом. Закључено је да ће ове промене, изазване ефектима чисте сече букве и у будућности дуглазије, и успорено разлагање органске материје дуглазије, допринети даљој деградацији буковог станишта у погледу продуктивности на планини Маљен.

КЉУЧНЕ РЕЧИ: нарушени екосистеми, *Fagus moesiaca*, *Pseudotsuga menziesii* силвикултура, хумус, хуминске и фулво киселине, разлагање стеље

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. Novi Sad, № 138, 97—108, 2020

UDC 631.42:552.527(497.113 Veliko Gradište
https://doi.org/10.2298/ZMSPN2038097P

Radmila N. PIVIĆ[1*], *Zoran S. DINIĆ*[1], *Jelena S. MAKSIMOVIĆ*[1], *Dobrivoj Ž. POŠTIĆ*[2], *Ratibor T. ŠTRBANOVIĆ*[2], *Aleksandra B. STANOJKOVIĆ-SEBIĆ*[1]

[1]Institute of Soil Science,
Teodora Drajzera 7, Belgrade 11000, Serbia
[2]Institute for Plant Protection and Environment,
Teodora Drajzera 9, Belgrade 11000, Serbia

EVALUATION OF TRACE ELEMENTS MPC IN AGRICULTURAL SOIL USING ORGANIC MATTER AND CLAY CONTENT

ABSTRACT: The aim of this paper is to investigate the contribution of the influence of organic matter and clay content on the value of maximum permissible concentrations (MPC) of trace elements Pb, Ni, Cr, and Cd. The investigation was conducted on agricultural soil in the territory of Veliko Gradište Municipality. There were analyzed 82 samples of eutric cambisol type soil, 17 samples of chernozem soil, and 32 samples of sandy soil. In the composite soil samples, taken from a depth of 0–30 cm, main parameters of soil fertility (pH, P_2O_5, K_2O, $CaCO_3$, soil organic matter – SOM), the content of the clay fraction, and total forms of Pb, Ni, Cr, and Cd were determined. Interpretation of the obtained results was carried out in relation to the MPC of trace elements defined in the Regulations (*Official Gazette*, 88/2018). Based on the ratio of the defined MPC and corrected values whose calculation includes the values of the organic matter and clay content, there was determined the correlation concerning the content of organic matter and the content of clay fractions, respectively, in the tested samples. In addition, the content of Cr and Pb in tested types of soil still did not exceed the adjusted MPC value. As for Cd and Ni, there was no deviation from the established and modified values of MPC. Concludingly, the research should be continued and supplemented by data for other types of soils, which would represent a base for a further assessment of the applicability of the existing regulations taken from Dutch sources and incorporated into Serbian Regulations (*Official Gazette*, 88/2018).

KEYWORDS: soil, trace elements, maximum permissible concentration (MPC), soil organic matter (SOM), clay

[*] Corresponding author. E-mail: drradmila@pivic.com

INTRODUCTION

It is generally recognized that the climate, especially air temperature and precipitation, presents the most important factor which regulates the level of organic matter in the soil (Alvarez and Lavado, 1998). The study of Lemenih and Itanna (2004) indicates that the content of soil organic matter increases with the amount of precipitation and decreases with the increases in temperature.

In similar climatic conditions, the organic matter content in the soils of fine texture (clayey soils) is two to four times higher than in the rough texture (sandy) soils (Prasad and Power, 1997). Clayey soils accumulate organic carbon rather quickly, while the sandy soils cannot accumulate it even after 100 years of a high input of organic matter (Freibauer et al., 2004).

Soil organic matter (SOM) is a complex matter system and is of great importance for all processes that occur in the soil, as the nutrients are accumulated within it. It is a source of fertility, contributing to soil aeration in a way that it reduces the soil density, improves the infiltration of the soil, and increases its water and air capacity (Vidojević, 2016). SOM content is of vital importance for the functioning of the ecosystem and has an important effect on the soil structure, the capacity for water retention in the soil, the capacity for the exchange of the cation, and the ability of the soil to form complexes with metal ions, as well as to keep the nutrients (Van Keulen, 2001).

The organic matter in soil affects not only the color of soil, its physical properties, and adsorption capacity of the mineral soils cations, but also the supply of plant accessible nutrients through the expressed exchange of nutrients and release of N, P, and S from organic forms, the extraction of elements from soil minerals, etc. (Miljković, 1996).

Soil can be structural and non-structural. The structural aggregates are formed by joint action of a number of factors which may be classified into five groups: soil, air, biological factors, anthropogenic factors, and time (Dugalić and Gajić, 2012).

In sandy soils or sands, the mechanical elements are generally not interconnected. Loam and clay soils can be structural, non-structural, or slightly structural. In general, at elevated content of the clay particles, the content of organic carbon in the soil tends to be higher. The reason for this is the connection between the surface of the clay particles and organic matter which inhibits the degradation process. Soils with higher clay content have a greater potential for the formation of aggregates.

Sandy soils typically contain less organic matter than the soils with a more delicate texture, loam or clay (Van-Camp et al., 2004). This is because, generally, low moisture content and high aeration in sandy soils result in faster oxidation of the organic matter in comparison with heavier soils.

The level of organic carbon in the soil is closely related to the soil structure and is one of the major factors of aggregation (Bronick and Lal, 2005). Likewise, the content of organic matter plays an important role in soil aggregation. This primarily refers to freshly formed and multivalent cation-saturated humic and ulmic acids that, after the coagulation, bind particles of the mechanical fractions of clay, silt, and sand in stable structural units. Aggregate stability and preservation of the organic carbon levels in the soil depend greatly on the soil texture. The effect of soil organic carbon on the soil structural stability is enhanced in soils containing a low percentage of the clay fraction (Wuddivira and Camps-Roach, 2007).

The content of organic matter and clay fractions, as well as soil pH, affects the mobility of trace elements. In soils of pH range from 5.50 to 8.00 chromium (Cr) is nearly insoluble, and the solubility of cadmium (Cd) decreases with the increased pH values so that pH values above 7.50 lead to its immobilization (Kabata Pendias, 2011). In soils with pH values from 3.80 to 7.1 Cd is less mobile than nickel (Ni) (Adriano, 2001).

High concentrations of trace elements in the soil can affect the soil's fertility and may represent an ecological and human health risk if they enter the food chain or leach into receiving waters (Daskalopoulou et al., 2014).

Contamination by trace elements is a potential risk to the crops, animals, and humans because of their toxicity, persistence, bioaccumulation and biomagnification in the food chain (Rao et al., 2011). By incorporating the influence of organic matter content and clay fraction into the MPC (maximum permissible concentrations) calculation, the potential danger of determined trace elements content for the environment is more realistically considered. This concept was taken from the Netherlands (MvV, 2000).

The main difference between the two existing regulations of the Republic of Serbia (23/1994 and 88/2018) classification 23/94 and 88/2018 is in the reported values of certain trace elements. Also, for the interpretation of whether a trace element is below or above the MPC at a given location, the modified limit values are used according to Regulation 88/2018, which presents the result of the content of clay fraction and organic matter influence.

Based on the data obtained in a previous study (Institute of Soil Science, 2014), this study aimed to determine the influence of the organic matter and clay content in the soil and evaluate the established maximum permissible concentrations of trace elements (*Official Gazette RS*, 23/1994) following the Regulation (*Official Gazette RS*, 88/2018).

MATERIALS AND METHODS

The Municipality of Veliko Gradište is located in the northeastern part of Serbia, in the foothills of the Carpathians and Homolje mountains, at the entrance to the Đerdap Gorge (Iron Gate). In the west, it borders with the Municipality of Malo Crniće, in the southeast with the Municipality of Kučevo, and in the east with the Municipality of Golubac (X: 534771, Y: 4948075). In the north, the municipality is bordered by the Danube that separates it from neighboring Romania in a length of 20 km. The municipality belongs to Braničevo district and covers an area of 344 km^2.

The study territory belongs to the area of moderate continental climate with clearly derived seasons and almost no difference in the characteristics between the lower and higher terrains (Stanojković-Sebić et al., 2015).

In the area of study and according to the sampled soil distribution (Figure 1), the most represented soil type was eutric cambisol – brown forest soil, which is primarily the result of the favorable climate, the substrate on which the soil is formed, and the influence of the relief and vegetation present at its creation. This type is mainly formed on Miocene sediments of the lighter mechanical composition, or loess and old alluvial deposits. After the composition, it is slightly heavier loam, plastic and sticky.

The production value of eutric cambisol is high because this soil is among the very deep soil types of neutral and weakly acidic reaction. It has good physical properties when it was formed on the loess and has medium content of nutrient elements, except the available phosphorus.

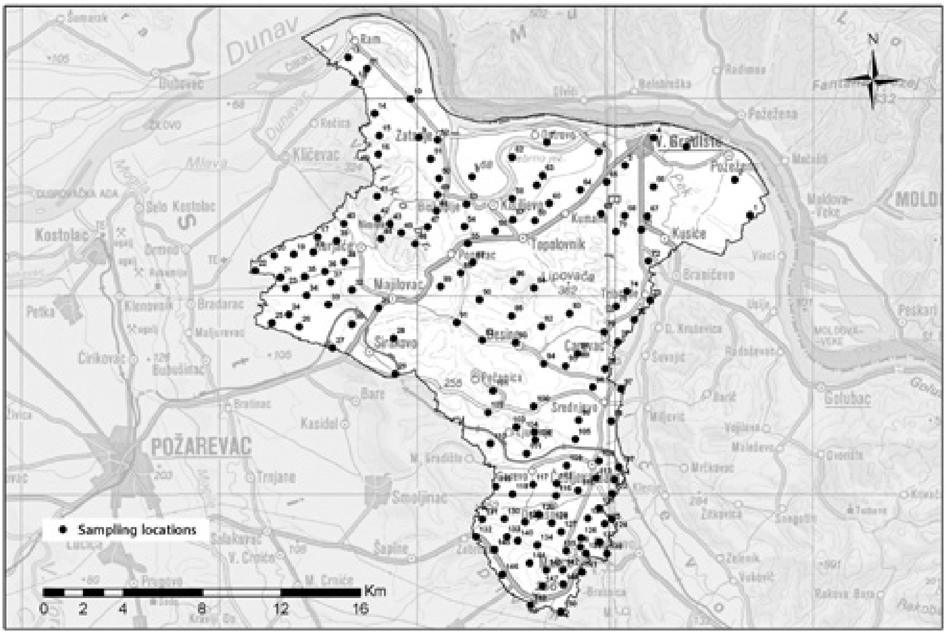


Figure 1. The position of soil samples

In the study area, chernozem soil occurs on smaller areas, on the loess deposits, and the contact zone of loess and sand. Particle size distribution varies from loam to sandy clay loam. The usual chemical analyses show that the chernozem is calcareous throughout the whole depth and that the share of carbonates increases with the depth, although an acid reaction can occur in leached chernozem. The soil reaction is usually slightly alkaline with adsorptive complex highly saturated with cations, mainly calcium. The share of humus is over 3%, while the share of available phosphorus and potassium varies from plot to plot.

A significant area of study is occupied by sandy soils, as follows: Aeolian quicksand, loess sand, and brown sand. Production value of sandy soils is generally low, but it can vary depending on the involvement of pedogenetic processes that go towards the creation of brown steppe soils and shallow chernozem on the sand. Soil profiles on sands are distinguished by the surface parts color and the share of humus in them. Substantially, their soil reaction in surface layers is neutral or slightly alkaline.

Composite random sampling (15–20 single samples) of an agricultural soil was carried out at pre-defined locations (Figure 1), from the depth of 0–30 cm. There were analyzed 82 samples of eutric cambisol soil, 17 samples of chernozem soil, and 32 samples of sandy soil. The soil typology was taken from the pedological map published in a previous study (Institute of Soil Science, 1975; WRB, 2015).

A total number of 131 composite soil samples were prepared for analyses in accordance with SRPS ISO 11464: 2004 – Pretreatment of samples for physical-chemical analyses, and sieved through a sieve of 2 mm in diameter. Soil acidity (pH in 1M KCl, v/v-soil: 1M KCl = 1:5) was analyzed potentiometrically, using glass electrode (SRPS ISO 10390, 2007); available phosphorus (P_2O_5) was analyzed spectrophotometrically, and available potassium (K_2O) by flame emission photometry, using AL-method according to Egner-Riehm (Riehm, 1958); calcium carbonate ($CaCO_3$) was determined using the volumetric method (SRPS ISO 10693: 2005); SOM (soil organic matter) was calculated using the following formula: SOM content (%) = total organic C (%) x factor 1.724 (Džamić et al., 1996), using the total C content obtained on elemental CNS analyzer Vario EL III (Nelson and Sommers, 1996) and carbonate content.

The granulometric composition was analyzed by determination of particle size distribution in mineral soil material, using the standardized method by sieving and sedimentation (ISO 11277: 2009(E), 2009).

Determination of the total trace elements forms (Pb, Ni, Cr, and Cd) was done by inductively coupled plasma-atomic emission spectrometry – THERMO iCAP 6300 Duo (radial/axial view versions) ICP-OES, after the digestion of the samples with aqua regia (ISO 11466:1995, 1995; ISO 22036:2008, 2008).

Reference soil NCS ZC 73005, Soil Certificate of Certified Reference Materials approved by China National Analysis Center Beijing China, and reagent blanks were used as the quality assurance and quality control (QA/QC) samples during the analysis.

The results of the conducted soil analysis represent the arithmetic means of three replicates of each sampling. Statistical methods that were applied in the data processing were descriptive statistics and correlation, using the statistical program SPSS 18.0.

The interpretation of the content of trace elements in the soil samples was done using the Rule book of permissible concentrations of dangerous and hazardous materials in soil and water for irrigation and methods for analysis, in which MPC for the analyzed trace elements are as follows: Cd = 3 mg kg^{-1}, Cr = 100 mg kg^{-1}, Ni = 50 mg kg^{-1}, Pb = 100 mg kg^{-1} (*Official Gazette of RS*, 23/1994).

These values were used in the formula for calculating the limit and remediation values defined in the Regulation on the program of systematic monitoring of soil quality via indicators for the assessment of soil degradation risk and methodology for the creation of remediation programs (*Official Gazette of RS*, 88/2018).

RESULTS AND DISCUSSION

The results of the present study showed that the soil reaction of chernozem ranged from highly acidic (in one sample of leached chernozem) to alkaline (pH in 1 M KCl was 4.00–7.35), of sands ranged from acidic to alkaline (pH in 1 M KCl was 5.10–7.65), and of eutric cambisol from highly acidic to neutral (pH in 1 M KCl was 3.60–7.05). According to the carbonate content, the chernozem soil samples were in the range of non-calcareous to slightly calcareous (below detection limit of the method, BDLM–2.94%); sands soil samples were in the range of non-calcareous to medium carbonate (BDLM–9.33%), and eutric cambisol soil samples ranged from non-calcareous to slightly calcareous (BDLM–1.91%). The content of organic matter in the chernozem (2.69–4.75%), sands (1.38–4.33%), and eutric cambisol (2.09–5.06%) soil samples were in the range of medium to high.

Table 1. Main chemical parameters of the tested soils and the clay fraction content

Soil type	Statistical parameter	pH 1M KCl	$CaCO_3$	SOM	P_2O_5	K_2O	Clay fraction (<0.002 mm)
			%	%	mg 100 g^{-1}	mg 100 g^{-1}	%
Chernozem	Min	4.00	BDLM	2.69	2.14	15.98	23.60
	Max	7.35	2.94	4.75	37.05	38.65	33.20
	Average	5.92	0.60	3.68	8.18	20.81	29.84
	STDEV	1.11	1.05	0.62	7.95	5.45	2.41
	CV	0.64	0.61	1.08	4.59	3.15	4.17
Sand	Min	5.10	BDLM	1.38	2.32	8.94	6.20
	Max	7.65	9.33	4.33	85.89	38.65	46.10
	Average	6.63	1.27	2.75	17.49	17.94	20.12
	STDEV	0.77	2.05	0.65	17.41	7.78	10.13
	CV	0.44	1.18	1.13	10.05	4.49	17.55
Eutric cambisol	Min	3.60	BDLM	2.09	0.55	9.33	14.20
	Max	7.05	1.91	5.06	78.48	38.65	47.60
	Average	4.67	0.03	2.79	7.52	18.86	29.92
	STDEV	0.67	0.22	0.50	12.78	6.16	4.37
	CV	0.39	0.13	0.87	7.38	3.56	29.92

SOM – soil organic matter; BDLM – below the detection limit of the method (0.04%)

The determined content of available phosphorus in all tested soils was in the range from very low to very high, as follows: 2.14–37.05 mg 100 g^{-1} in chernozem, 2.32–85.89 mg 100 g^{-1} in sands, and 0.55–78.48 mg 100 g^{-1} in eutric cambisol. The content of available potassium was in the range of medium to high in chernozem (15.98–38.65 mg 100g^{-1}) and sands (8.94–38.65 mg 100 g^{-1}), and within the levels of a very low to a high value in eutric cambisol (9.33–38.65 mg 100 g^{-1}) (Table 1).

All these obtained data on the main parameters of soil fertility are in accordance with the ones obtained in a previous study (Institute of Soil Science, 1975).

By analyzing the content of total trace elements in soil samples (Figure 2), the content of Pb, Cr, and Cd in all samples of all three types of soil was below the maximum permissible concentrations (*Official Gazette of RS*, 23/1994). Ni content in chernozem and eutric cambisol was also below the MPC, while in the sandy soils the content of this element exceeded the MPC at four localities.

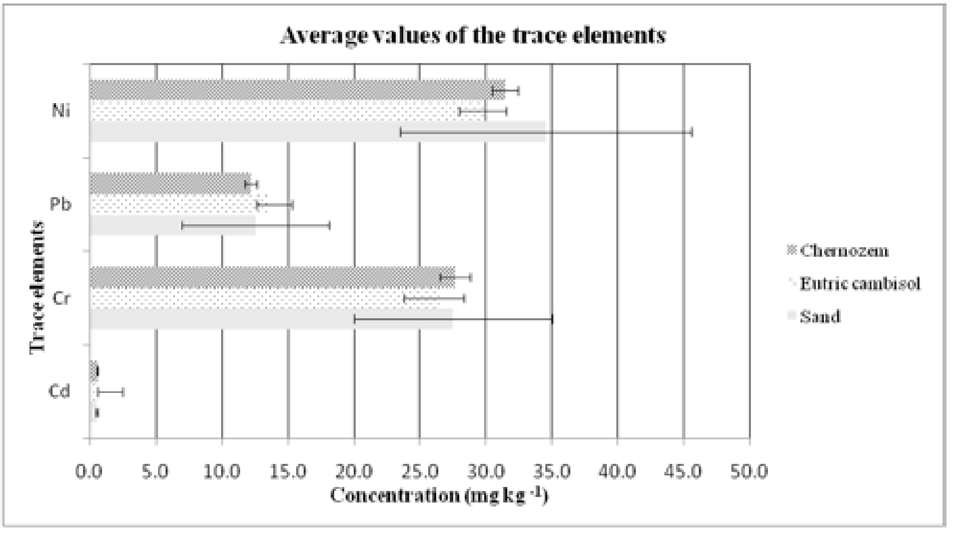


Figure 2. The levels of analyzed total forms of trace elements

For adjusted established MPC (*Official Gazette RS* 23, 1994) it was applied the equation defined by *Official Gazette RS* 88 (2018):

$$(SW, IW)_b = (SW, IW)_{sb} \times \frac{A + (B \times \%\ clay) + (C \times \%\ SOM)}{A + (B \times 25) + (C \times 10)}$$

By applying the defined equation below, where $(SW, IW)_b$ is corrected threshold or remediation value for a particular soil, and $(SW, IW)_{sb}$ is threshold or remediation value from the table of *Official Gazette of RS* (88/2018), which determines the corrected MPC value, it was found that in all soil samples of chernozem the Cd content was below the defined limit value (0.8 mg/kg of dry matter); the Cd content in sandy soils was above the limit values in two samples, while in eutric cambisol the content of this element was above the limit values in even 25 samples.

In the equation, A, B, and C represent the constants dependent on the type of trace element.

The Ni content in chernozem and eutric cambisol was below the limit values (35 mg/kg of dry matter), while in sandy soils from five sites the content of this element exceeded the limit value.

By applying the regulative 88/2018 (*Official Gazette of RS*, 88/2018) and the related correction formula, and by replacing the limit values from that regulative with the limit values from regulative 23/1994 (*Official Gazette of RS*, 23/1994) new modified limit values were obtained. By analyzing the content of trace elements from given locations with newly modified values, no values were found that were above the modified limit values.

The number of samples in which the corrected MPC value for Cd was found increased by one in eutric cambisol, and in sandy soils by two. The Ni content above the corrected MPC was higher in sandy soils only at three localities.

The value of the content of organic matter and clay fractions in a ratio of 1:3 affects the mobility of Cd to a negligible extent. The mobility of Cr and Ni was not affected by the content of organic matter in the soil, while the mobility of Pb was equally affected by the content of clay and organic matter (MvV, 2000).

It was also determined the determination coefficient of the relationship between the adjusted MPC value for each element and each soil type and the MPC value defined in Official Gazette of Republic of Serbia (*Official Gazette of RS*, 23/1994) and the content of organic matter and clay fractions, respectively (Tables 2 and 3). The results show a better relationship for clay than SOM. This should be first noted and then discussed.

Table 2. Relationship between the adjusted and unadjusted value of trace elements and organic matter content

Soil type	Average content of trace elements ± standard deviation (mg kg^{-1}) – R^2 (the correlation coefficient)			
	Ni	Pb	Cr	Cd
Chernozem	31.45±1.72 $y = 0.0139x + 3.4848$ $R^2 = 0.0239$	12.18±0.80 $y = 0.0788x + 3.815$ $R^2 = 0.1483$	27.63±1.98 $y = 0.0199x + 3.4848$ $R^2 = 0.0239$	0.54±0.03 $y = 0.146x + 5.517$ $R^2 = 0.4687$
Eutric cambisol	29.74±3.10 $y = 0.0182x + 2.5393$ $R^2 = 0.2046$	13.52±1.51 $y = 0.0497x + 2.9287$ $R^2 = 0.2881$	27.72±4.77 $y = 0.026x + 2.5393$ $R^2 = 0.2046$	0.67±0.17 $y = 0.0719x + 3.8651$ $R^2 = 0.4443$
Sand	34.55±19.13 $y = 0.0169x + 2.9889$ $R^2 = 0.5644$	12.53±9.65 $y = 0.0404x + 3.33$ $R^2 = 0.6041$	27.52±12.99 $y = 0.0241x + 2.9889$ $R^2 = 0.5644$	0.48±0.16 $y = 0.0512x + 3.9686$ $R^2 = 0.6715$

Table 3. Relationship between the adjusted and unadjusted value of trace elements and the clay fraction content

Soil type	Average content of trace elements ± standard deviation (mg kg^{-1}) – R^2 (the correlation coefficient)			
	Ni	Pb	Cr	Cd
Chernozem	31.45±1.72 y = 0.35x + 25 R^2 = 1	12.18±0.80 y = 0.7712x + 31.185 R^2 = 0.9434	27.63±1.98 y = 0.5x + 25 R^2 = 1	0.54±0.03 y = 0.6834x + 38.449 R^2 = 0.6822
Eutric cambisol	29.74±3.10 y = 0.35x + 25 R^2 = 1	13.52±1.51 y = 0.8003x + 32.071 R^2 = 0.9906	27.72±4.77 y = 0.5x + 25 R^2 = 1	0.67±0.17 y = 0.9058x + 43.405 R^2 = 0.9338
Sand	34.55±19.13 y = 0.35x + 25 R^2 = 1	12.53±9.65 y = 0.8096x + 31.67 R^2 = 0.9984	27.52±12.99 y = 0.5x + 25 R^2 = 1	0.48±0.16 y = 0.9679x + 43.094 R^2 = 0.9878

The obtained results indicate that the C constant, which refers to the organic matter content and is associated with the product used in the calculation of the corresponding element (C = 0 for Ni; C = 1 for Pb; C = 0 for Cr; C = 0.021 for Cd), has a higher dependency in sandy soils, as expected, considering the source of formula which is primarily intended for similar soil types. For the content of Cd in the chernozem soil, it was registered the lowest coefficient of determination for the clay fraction content, which can be explained by the lowest coefficient of variation for the tested parameter which was determined by processing the observed number of samples.

The correlative dependence which refers to the clay fraction content showed a high correlation, which is in correspondence with the assigned B constants for the tested elements (B = 1 for Ni; B = 1 for Pb; B = 2 for Cr; B = 0.007 for Cd).

CONCLUSION

Using the equation for determination of the adjusted MPC value defined in *Official Gazette of RS* (2018), which in the calculation includes the coefficients of the content of organic matter and clay fractions individually associated with the tested trace element and the limit MPC value, for a determined number of samples with trace elements value above the MPC, defined in Official Gazette of Republic of Serbia (*Official Gazette of RS*, 1994), it was found that the content of Cr and Pb in tested types of soil still does not exceed the adjusted MPC value. As for Cd and Ni, there was no deviation from the established and modified values of MPC.

Research should be continued and supplemented by data for other types of soils, based on which there would be done a further assessment of the applicability of the existing regulations taken from Dutch sources (MvV, 2000) and incorporated into the Regulative *Official Gazette* (88/2018).

ACKNOWLEDGMENT

This research was carried out with the support of the Ministry of Education, Science and Technological Development of the Republic of Serbia and financed from the Project TR-37006. The study was partially financed by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia (Study "Amelioration of soils in the municipality of Veliko Gradište with an aim of high-value food production", realized by the Institute of Soil Science during 2014).

REFERENCES

Adams RS (1975): Variability in mineral and trace element content of dairy cattle feeds. J. *Dairy Sci*. 58: 1538–1548.

Adriano DC (2001): *Trace Elements in Terrestrial Environments. Biogeochemistry, Bioavailability, and Risks of Metals*. 2nd edition, New York – Berlin – Heidelberg: Springer.

Alvarez R, Lavado RS (1998): Climate, organic matter and clay content relationships in the Pampa and Chaco soils, Argentina. *Geoderma* 83: 127–141.

Bronick CJ, Lal R (2005): Soil structure and management: a review. *Geoderma* 124: 3–22.

Daskalopoulou K, Calabrese S, Milazzo S, Brusca L, Bellomo S, D'Alessandro W, Kyriakopoulos K, Tassi F, Parello F (2014): Trace elements mobility in soils from the hydrothermal area of Nisyros (Greece). *Ann. Geophys*., 57: 1–6. (DOI: https://doi.org/10.4401/ag-6623).

Dugalić G, Gajić B (2012): *Pedologija*. Univerzitet u Kragujevcu, Agronomski fakultet u Čačku, Srbija.

Džamić R, Stevanović D, Jakovljević M (1996): *Praktikum iz agrohemije*. Poljoprivredni fakultet Univerziteta u Beogradu.

Freibauer A, Rounsevell MDA, Smith P, Verhagen J (2004): Carbon sequestration in European agricultural soils. *Geoderma* 122: 1–23.

Institute of Soil Science (1975): *Zemljišta Braničevsko-zviške oblasti Homolja*. Beograd: Institut za proučavanje zemljišta.

Institute of Soil Science (2014): Melioracije zemljišta na području opštine Veliko Gradište u cilju proizvodnje visokovredne hrane (Studija istraživanja). Beograd: Institut za zemljište. Available to: http://velikogradiste.rs/wp-content/uploads/2015/07/Projekat-V-G.pdf.

ISO 11466:1995 (1995): Soil quality – Extraction of trace elements soluble in aqua regia. International Organization for Standardization, Geneva, Switzerland.

ISO 22036:2008 (2008): Soil quality – Determination of trace elements in extracts of soil by inductively coupled plasma-atomic emission spectrometry (ICP-AES). International Organization for Standardization, Geneva, Switzerland.

ISO 11277:2009 (2009): Soil quality – Determination of particle size distribution in mineral soil material – Method by sieving and sedimentation. International Organization for Standardization, Geneva, Switzerland.

Kabata-Pendias A (2011): *Trace Elements in Soils and Plants*. 4th edition. Boca Raton, Fl, USA: CRC Press, Taylor & Francis Group.

Lemenih M, Itanna F (2004): Soil carbon stocks and turnovers in various vegetation type and arable lands along an elevation gradient in southern Ethiopia. *Geoderma* 123: 177–188.

McKone TE, Maddalena RL (2007): Plant uptake of organic pollutants from soil: a critical review of bioconcentration estimates based on models and experiments. *Environ. Toxicol. Chem*. 26: 2494–2504.

Miljković N (1996): *Osnovi pedologije*. Novi Sad: Prirodno-matematički fakultet, Institut za geografiju.

MvV (2000): Circular on Target Values and Intervention Values for Soil Remediation. Dutch Target and Intervention Values (the New Dutch List). Annexes. Ministerie van Volkshulsvesting, Ruimtelijke

Ordeningen Milieubeheer; Ministry of Housing, Spatial Planning and the Environment: Barendrecht, The Netherlands; Available to: http://esdat.net/Environmental%20Standards/Dutch/annexS_I2000Dutch%20Environmental%20Standards.pdf (accessed on 30. 1. 2020).

Nelson DW, Sommers LE (1996): Total carbon, organic carbon, and organic matter. In: DL Sparks (ed.), *Methods of Soil Analysis*, Part 3, Madison, Wisconsin: SSSA, 961–1010.

Official Gazzete of RS / Službeni glasnik Republike Srbije (1994): Rulebook on permitted quantities of dangerous and harmful substances in soil and water for irrigation and methods of their testing, 23/94.

Official Gazzete of RS / Službeni glasnik Republike Srbije (2018): Regulation on the program of systematic monitoring of soil quality, indicators for assessing the risk of land degradation and methodology for developing remediation programs, 88/2018.

Prasad R, Power JF (1997): *Soil Fertility Management for Sustainable Agriculture*. New York, USA: Lewis Publishers.

Puget P, Chenu C, Balesdent J (2000): Dynamics of soil organic matter associated with particle-size fractions of water-stable aggregates. *Eur. J. Soil Sci.* 51: 595–605.

Rao MM, Kumarmeena A, Galib M (2011): Detection of toxic heavy metals and pesticide residue in herbal plants which are commonly used in the herbal formulations. *Environ. Monit. Assess* 181: 267–271.

Riehm H (1958): Die Ammoniumlaktatessigsaure-Methode zur Bestimmung der leichtloslichen Phosphorsure in Karbonathaltigen Boden. *Agrochimica*, 3: 49–65.

Soltanpour PN, Johnson GW, Workman SM, Bentonjones JJ, Miller RO (1996): Inductively coupled plasma emission spectrometry and inductively coupled plasma mass spectrometry. In: DL Sparks (ed.), *Methods of Soil Analysis*, Part 3, Madison, Wisconsin SSSA, 91–139.

Stanojković-Sebić A, Dinić Z, Maksimović J, Ferdinando M, Jaramaz D, Pivić R (2015): Stanje i predlog mera popravke zemljišta opštine Veliko Gradište, Naučno-stručni skup „Održivo korišćenje zemljišta", Institut za ratarstvo i povrtarstvo u Novom Sadu, Rimski Šančevi (zbornik radova), 123–129.

SRPS ISO 11464:2004 (2004): Soil quality – Pretreatment of samples for physico-chemical analyses. Institute for Standardization of Serbia, Belgrade.

SRPS ISO 10693:2005 (2005): Soil quality – Determination of carbonate content. Institute for Standardization of Serbia, Belgrade.

SRPS ISO 10390:2007 (2007): Soil quality – Determination of pH. Institute for Standardization of Serbia, Belgrade.

Van-Camp L, Bujarrabal B, Gentile A-R., Jones RJA, Montanarella L, Olazabal C, Selvaradjou S-K (2004): Reports of the technical working groups established under the thematic strategy for soil protection. Office for Official Publications of the European Communities, Luxembourg, EUR 21319 EN/3: 872.

Van Keulen H (2001): (Tropical) soil organic matter modelling: problems and prospects. *Nutr. Cycl. Agroecosys*. 61: 33–39.

Vidojević D (2016): *Procena rezervi organske materije u zemljištima Srbije*. Poljoprivredni fakultet Univerziteta u Novom Sadu, Srbija (Doctoral dissertation).

Wuddivira MN, Camps-Roach G (2007): Effects of organic matter and calcium on soil structural stability. *Eur. J. Soil Sci*. 58: 722–727.

WRB (2015): World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps. Update 2015. Food and Agriculture Organization of the United Nations, Rome. Available to: http://www.fao.org/3/i3794en/I3794en.pdf.

ПРОЦЕНА MDK ЕЛЕМЕНАТА У ТРАГОВИМА У ПОЉОПРИВРЕДНОМ ЗЕМЉИШТУ ПОМОЋУ САДРЖАЈА ОРГАНСКЕ МАТЕРИЈЕ И ГЛИНЕ

Радмила Н. ПИВИЋ[1], Зоран С. ДИНИЋ[1], Јелена С. МАКСИМОВИЋ[1], Добривој Ж. ПОШТИЋ[2], Ратибор Т. ШТРБАНОВИЋ[2], Александра Б. СТАНОЈКОВИЋ-СЕБИЋ[1]

[1] Институт за земљиште,
Теодора Драјзера 7, Београд 11000, Србија
[2] Институт за заштиту биља и животну средину,
Теодора Драјзера 9, Београд 11000, Србија

РЕЗИМЕ: Циљ овог рада је истраживање доприноса утицаја садржаја органске материје и глине на вредност максимално дозвољених концентрација (МДК) елемената у траговима Pb, Ni, Cr и Cd, које је спроведено на пољопривредном земљишту територије Општине Велико Градиште. Анализирано је 82 узорка на земљишту типа еутрични камбисол, 17 узорака на земљишту типа чернозем и 32 узорка на песковитим земљиштима. У композитним узорцима земљишта, узетих са дубине 0–30 cm, одређени су основни параметри плодности (pH, P_2O_5, K_2O, $CaCO_3$, органска материја), садржај фракције глине и садржај укупних форми Pb, Ni, Cr и Cd. Тумачење добијених резултата испитивања спроведено је у односу на МДК испитиваних микроелемената дефинисаних Правилником (*Службени гласник РС*, 88/2018). На основу односа дефинисаних МДК и коригованих вредности које у обрачун узимају и вредности садржаја органске материје и глине, утврђена је корелациона зависност у односу на садржај органске материје, односно садржај фракције глине у испитиваним узорцима. Поред тога, садржај Cr и Pb у испитаним типовима земљишта и даље не прелази прилагођену вредност МДК. За вредности Cd и Ni, није било одступања од утврђених и модификованих вредности МДК**.** Закључак је да истраживања треба наставити и допунити подацима и за остале типове земљишта на основу чега би се дала коначна оцена примењивости постојеће регулативе која је преузета из холандских извора а у 2018. години су унета у Правилник (*Службени гласник РС*, 88/2018) .

КЉУЧНЕ РЕЧИ: земљиште, елементи у траговима, максимално дозвољене концентрације (МДК), органска материја, глина

EDITORIAL POLICY

The journal MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES is dedicated to publishing original scientific papers presenting new results of fundamental and applied research, review articles as well as brief communications from all scientific fields as referred to in the title of the journal. Review articles are published only when solicited by the editorial board of the journal. Manuscripts that have already been published in extensor or in parts or have been submitted for publication to other journal will not be accepted. The journal is issued twice a year.

The manuscripts should be written in correct English language regarding the grammar and style. The manuscripts should be submitted electronically as a separate file to vnikolic@maticasrpska.org.rs and enclosed with the author's written consent for the publishing of the manuscript.

Upon the reception of the manuscript, the author shall be assigned with a manuscript code, which has to be referred to in any further correspondence. The authors will be notified about the manuscript reception within seven days and about the reviewers' opinion within two months from submission. All submitted manuscripts are reviewed and proofread.

MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES is an Open Access journal.

Contributions to the Journal should be submitted in the English language, with an abstract also in Serbian.

MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES is available in full-text at the web site of Matica Srpska and in the following data bases: Serbian Citation Index, EBSCO Academic Search Complet, abstract level at Agris (FAO), CAB Abstracts, CABI Full-Text, Thomson Reuters Master Journal List and DOAJ (Directory of Open Access Journals).

Editorial responsibilities

The Editor-in-Chief and the Editorial Board are responsible for deciding which articles submitted to MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES will be published. The Editor-in-Chief and the Editorial Board are guided by the Editorial Policy and constrained by legal requirements in force regarding libel, copyright infringement and plagiarism.

The Editor-in-Chief reserves the right to decide not to publish submitted manuscripts in case it is found that they do not meet relevant standards concerning the content and formal aspects. The Editorial Staff will inform the authors whether the manuscript is accepted for publication within two weeks after receiving from authors manuscript revised following reviewers comments.

The Editor-in-Chief and members of Editorial Board must hold no conflict of interest with regard to the articles they consider for publication. If an Editor feels that there is likely to be a perception of a conflict of interest in relation to their handling of a submission, the selection of reviewers and all decisions on the paper shall be made by the Edittor-in-Chief.

The Editor-in-Chief and members of Editorial Board shall evaluate manuscripts for their intellectual content free from any racial, gender, sexual, religious, ethnic, or political bias.

The Editor-in-Chief and the Editorial Staff must not use unpublished materials disclosed in submitted manuscripts without the express written consent of the authors. The information and ideas presented in submitted manuscripts shall be kept confidential and must not be used for personal gain.

The Editor-in-Chief and members of Editorial Board shall take all reasonable measures to ensure that the reviewers remain anonymous to the authors before, during and after the evaluation process.

Author's responsibilities

Authors warrant that their manuscript is their original work, that it has not been published before, and is not under consideration for publication elsewhere. Parallel submission of the same paper to another journal constitutes a misconduct, and eliminates the manuscript from consideration by MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES.

The Authors also warrant that the manuscript is not and will not be published elsewhere (after the publication in MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES) in any language without the consent of the Editorial Board.

In case a submitted manuscript is a result of a research project, or its previous version has been presented at a conference in the form of an oral presentation (under the same or similar title), detailed information about the project, the conference, etc. shall be provided in Acknowledgements. A paper that has already been published in another journal cannot be reprinted in MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES.

It is the responsibility of each author to ensure that papers submitted to MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES are written with ethical standards in mind. Authors affirm that the article contains no unfounded or unlawful statements and does not violate the rights of third parties. The Publisher will not be held legally responsible should there be any claims for compensation.

Reporting standards

A submitted manuscript should contain sufficient detail and references to permit reviewers and, subsequently, readers to verify the claims presented in it. The deliberate presentation of false claims is a violation of ethical standards. Book reviews and technical papers should be accurate and they should present an objective perspective.

Authors are exclusively responsible for the contents of their submissions and must make sure that they have permission from all involved parties to make the data public.

Authors wishing to include figures, tables or other materials that have already been published elsewhere are required to be referred on the reference used, or to have permission from the copyright holder(s). Any material received without such evidence will be assumed to originate from the authors.

Authorship

Authors must make sure that only contributors who have significantly contributed to the submission are listed as authors and, conversely, that all contributors who have significantly contributed to the submission are listed as authors. If persons other than authors were involved in important aspects of the research project and the preparation of the manuscript, their contribution should be acknowledged in a footnote or the Acknowledgments section.

Acknowledgment of Sources

Authors are required to properly cite sources that have significantly influenced their research and their manuscript. Information received in a private conversation or correspondence with third parties, in reviewing project applications, manuscripts and similar materials must not be used without the express written consent of the information source.

Plagiarism

Plagiarism, where someone assumes another's ideas, words, or other creative expression as one's own, is a clear violation of scientific ethics. Plagiarism may also involve a violation of copyright law, punishable by legal action.

Plagiarism includes the following:

• Word for word, or almost word for word copying, or purposely paraphrasing portions of another author's work without clearly indicating the source or marking the copied fragment (for example, using quotation marks);

• Copying equations, figures or tables from someone else's paper without properly citing the source and/or without permission from the original author or the copyright holder.

Please note that all submissions are thoroughly checked for plagiarism.

Any paper which shows obvious signs of plagiarism will be automatically rejected and those authors will not be permitted to submit papers to MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES for three years.

In case plagiarism is discovered in a paper that has already been published by the journal, it will be retracted in accordance with the procedure described below under Retraction policy, and authors will not be permitted to submit papers to MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES for three years.

Conflict of interest

Authors should disclose in their manuscript any financial or other substantive conflict of interest that might have influenced the presented results or their interpretation.

Fundamental errors in published works

In the event that an author discovers a significant error or inaccuracy in his/her own published work, it is the author's obligation to promptly notify the journal Editor or publisher and cooperate with the Editor to retract or correct the paper.

By submitting a manuscript the authors agree to abide by the MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES's Editorial Policies.

Reviewer responsibilities

Reviewers are required to provide written, competent and unbiased feedback in a timely manner on the scholarly merits and the scientific value of the manuscript.

The reviewers assess manuscript for the compliance with the profile of the journal, the relevance of the investigated topic and applied methods, the originality and scientific relevance of information presented in the manuscript, the presentation style and scholarly apparatus.

Reviewers should alert the Editor to any well-founded suspicions or the knowledge of possible violations of ethical standards by the authors. Reviewers should recognize relevant published works that have not been cited by the authors and alert the Editor to substantial similarities between a reviewed manuscript and any manuscript published or under consideration for publication elsewhere, in the event they are aware of such. Reviewers should also alert the Editor to a parallel submission of the same paper to another journal, in the event they are aware of such.

Reviewers must not have conflict of interest with respect to the research, the authors and/or the funding sources for the research. If such conflicts exist, the reviewers must report them to the Editor without delay.

Any selected referee who feels unqualified to review the research reported in a manuscript or knows that its prompt review will be impossible should notify the Editor without delay.

Reviews must be conducted objectively. Personal criticism of the author is inappropriate. Reviewers should express their views clearly with supporting arguments.

Any manuscripts received for review must be treated as confidential documents. Reviewers must not use unpublished materials disclosed in submitted manuscripts without the express written consent of the authors. The information and ideas presented in submitted manuscripts shall be kept confidential and must not be used for personal gain.

Peer review

The submitted manuscripts are subject to a peer review process. The purpose of peer review is to assists the Editor-in-Chief, Editorial Board and Consulting Editors in making editorial decisions and through the editorial communications with the author it may also assist the author in improving the paper. Scientific papers, review papers, short communications are subjected to the review process.

Reviews are anonymous (single-blind procedure), and at least one positive review should be obtained for a positive decision – acceptance of the paper after minor or major revision. In case MAJOR changes are demanded by reviewer(s), the revised version of the paper will be sent again to the reviewer(s) for their FINAL OPINION. Reviewers are not paid for their reviews.

The choice of reviewer(s) is at the discretion of the Editor-in-Chief and members of Editorial Board. The reviewers must be knowledgeable about the subject area of the manuscript; they must not be from the authors' own institution and they should not have recent joint publications with any of the authors. Editors and the Editorial Staff shall take all reasonable measures to ensure that the reviewers remain anonymous to the authors before, during and after the evaluation process.

All of the reviewers of a paper act independently and they are not aware of each other's identities. If the decisions of the two reviewers are not the same (accept/reject), the Editor may assign additional reviewers.

During the review process Editors may require authors to provide additional information (including raw data) if they are necessary for the evaluation of the scholarly merit of the manuscript. These materials shall be kept confidential and must not be used for personal gain.

The Editorial team shall ensure reasonable quality control for the reviews. With respect to reviewers whose reviews are convincingly questioned by authors, special attention will be paid to ensure that the reviews are objective and high in academic standard. When there is any doubt with regard to the objectivity of the reviews or the quality of the review, additional reviewers will be assigned.

Procedures for dealing with unethical behavior

Anyone may inform the editors and/or Editorial Staff at any time of suspected unethical behavior or any type of misconduct by giving the necessary information/evidence to start an investigation.

Investigation

• Editor-in-Chief will consult with the Subject Editors on decisions regarding the initiation of an investigation.

• During an investigation, any evidence should be treated as strictly confidential and only made available to those strictly involved in investigating.

• The accused will always be given the chance to respond to any charges made against them.

• If it is judged at the end of the investigation that misconduct has occurred, then it will be classified as either minor or serious.

Minor misconduct

Minor misconduct will be dealt directly with those involved without involving any other parties, e.g.:

• Communicating to authors/reviewers whenever a minor issue involving misunderstanding or misapplication of academic standards has occurred.

• A warning letter to an author or reviewer regarding fairly minor misconduct.

Major misconduct

The Editor-in-Chief, in consultation with the Subject, and, when appropriate, further consultation with a small group of experts should make any decision regarding the course of action to be taken using the evidence available. The possible outcomes are as follows (these can be used separately or jointly):

• Publication of a formal announcement or editorial describing the misconduct.

• Informing the author's (or reviewer's) head of department or employer of any misconduct by means of a formal letter.

• The formal, announced retraction of publications from the journal in accordance with the Retraction Policy (see below).

• A ban on submissions from an individual for a defined period.

• Referring a case to a professional organization or legal authority for further investigation and action.

When dealing with unethical behavior, the Editorial Staff will rely on the guidelines and recommendations provided by the Committee on Publication Ethics (COPE).

Retraction policy

Legal limitations of the publisher, copyright holder or author(s), infringements of professional ethical codes, such as multiple submissions, bogus claims of authorship, plagiarism, fraudulent use of data or any major misconduct require retraction of an article. Occasionally a retraction can be used to correct errors in submission or publication. The main reason for withdrawal or retraction is to correct the mistake while preserving the integrity of science; it is not to punish the author.

Standards for dealing with retractions have been developed by a number of library and scholarly bodies, and this practice has been adopted for article retraction by MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES: in the electronic version of the retraction note, a link is made to the original article. In the electronic version of the original article, a link is made to the retraction note where it is clearly stated that the article has been retracted. The original article is retained unchanged; save for a watermark on the PDF indicating on each page that it is "retracted."

Open access policy

MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES is an Open Access Journal. All articles can be downloaded free of charge and used in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International (CC BY-NC-ND) licence.

The MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES journal is funded by Matica srpska and does not charge any fees to authorsis free of charge for authors.

Self-archiving Policy

The MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES allows authors to deposit Author's Post-print (accepted version) and Publisher's version/PDF in an institutional repository and non-commercial subject-based repositories, such as arXiv or similar) or to publish it on Author's personal website (including social networking sites, such as ResearchGate, Academia.edu, etc.) and/or departmental website, at any time after publication. Full bibliographic information (authors, article title, journal title, volume, issue, pages) about the original publication must be provided and a link must be made to the article's DOI.

Disclaimer

The views expressed in the published works do not express the views of the Editors and Editorial Staff. The authors take legal and moral responsibility for the ideas expressed in the articles. Publisher shall have no liability in the event of issuance of any claims for damages. The Publisher will not be held legally responsible should there be any claims for compensation.

Articales are available in full-text at the web site of Matica Srpska and in the following data bases: Serbian Citation Index, EBSCO Academic Search Complet, abstract level at Agris (FAO), CAB Abstracts, CABI Full-Text and Thomson Reuters Master Journal List.

INSTRUCTION TO AUTHORS

1. General remarks

1.1. Matica Srpska Journal for Natural Sciences (short title: Matica Srpska J. Nat. Sci.) publishes manuscripts and review articles as well as brief communications from all scientific fields as referred to in the title of the journal. Review articles are published only when solicited by the editorial board of the journal. Manuscripts that have already been published in extenso or in parts or have been submitted for publication to other journal will not be accepted. The journal is issued twice a year.

1.2. The manuscripts should be written in correct English language regarding the grammar and style. The manuscripts should be submitted electronically as a separate file to vnikolic@maticasrpska.org.rs and enclosed with the author's written consent for the publishing of the manuscript.

1.3. Upon the reception of the manuscript, the author shall be assigned with a manuscript code, which has to be referred to in any further correspondence. The authors will be notified about the manuscript reception within seven days and about the reviewers' opinion within two months from submission. All submitted manuscripts are reviewed and proofread.

2. Planning and preparing of the manuscript

2.1. Type the manuscripts electronically on A4 (21 x 29.5 cm) format with 2.5 cm margins, first line indent, and 1.5 line spacing. When writing the text, the authors should use Times New Roman size 12 font and when writing the abstract, key words, summary, and footnotes use font size 10.

2.2. First name, middle initial and last name should be given for all authors of the manuscript and their institutional affiliations, institution name, and mailing address. In complex organizations, a full hierarchy should be mentioned (e.g. University of Novi Sad, Faculty of Sciences – Department of Biology and Ecology). The institution of employment of each author should be stated below the author's name. The position and academic degrees should not be cited. If there is more than one author, indicate separately institutional affiliation for each of the authors. Put the name and mailing address (postal or e-mail address) of the author responsible for correspondence at the bottom of the first page. If there is more than one author, write the address of only one author, usually the first one.

2.3. Structure the text of the original articles into Abstract, Key Words, Introduction, Material or Methods, or Material and Methods, Results or Results and Discussion, Discussion, Conclusion, References, Summary and Key Words in Serbian language, and Acknowledgement (if there is one). Original articles should not be longer than 10 pages, including the references, tables, legends, and figures.

2.4. Titles should be informative and not longer than 10 words. It is in the best interest of the authors and the journal to use words in titles suitable for indexing and electronic searching of the article.

2.5. The authors should submit the title of the article with last name and the initials of the first author.

(if the article has more than one author, et al. should be used for other authors) and running title of not more than five words.

2.6. List up to 10 key words using words and phrases that describe the content of the article in the best way and that allow indexing and electronic searching of the paper. List the key words alphabetically and divided by commas.

2.7. The Abstract in English language and Summary in Serbian language should be a short and informative presentation of the article. Depending on the length of the article, the Abstract may have from 100 to 250 words. Summary written in Serbian language can be 1/10 length of the article and should contain the title of the article, first, middle initial, and last names of the authors, authors' institutional affiliation and address, and key words.

2.8. Write the information about financial support, advices, and other forms of assistance, if necessary, at the end of the article under the Acknowledgement. Financial support acknowledgement should contain the name and the number of the project, i.e. the name of the program from which the article originated, and the name of the institution that provided the financial support. In case of other forms of assistance the author should submit the first name, middle initial, last name, institutional affiliation, and the address of the person providing the assistance or the full name and the address of the assisting institution.

3. Structure the Review articles in Abstract, Key Words, Text of the manuscript, Conclusion, and References; submit Summary and Key Words in Serbian language. Review articles should not be longer than 12 pages, including references, tables, legends, and figures.

4. Write brief communication according to the instructions for original articles but not be longer than five pages.

5. References

5.1. List the References alphabetically. Examples:

(a) Articles from journals: Last name CD, Last name CD (2009): Title of the article. Title of the journal (abbreviated form) 135: 122–129.

(b) Chapters in the book: Last name ED, Last name AS, Last name IP (2011): Title of the pertinent part from the book. In: Last name CA, last name IF (eds.), Title of the book, Vol.4, Publisher, City

(c) Books: Last name VG, Last name CS (2009): Title of the cited book. Publisher, City

(d) Dissertations: Last name VA (2009): Title of the thesis. Doctoral dissertation, University, City

(e) Unpublished articles: designation "in press" should be used only for papers accepted for publishing. Unpublished articles should be cited in the same way as published articles except that instead of journal volume and page numbers should write "in press" information.

(f) Articles reported at scientific meetings and published in extenso or in a summary form: Last name FR (2011): Proceedings, Name of the meeting, Meeting organizers, Venue, Country, 24–29.

(g) World Wide Web Sites and other electronic sources: Author's last name, Author's initial. (Date of publication or revision). Title, In: source in Italics, Date of access, Available from: <Available URL>. Use n.d. (no date) where no publication date is available. Where no author is available, transfer the organization behind the website or the title to the author space.

5.2. References in the text should include author's last name and the year of publishing. When there are two authors both should be cited, but in case of three or more authors, cite the first author only and follow with et al.

5.3. If two or more articles of the same author or authors published in the same year are cited, designate the publishing years with letters a, b, c, etc., both in text and reference list.

5.4. The names of the periodicals should be abbreviated according the instructions in the Bibliographic Guide for Authors and Editors (BIOSIS, Chemical Abstracts Service, and Engineering Index, Inc.).

5.5. Do not translate references to the language of the article. Write the names of cited national periodicals in their original, shortened form. For example, for the reference in Serbian language, put (Sr) at the end of the reference.

6. Units, names, abbreviations, and formulas

6.1. SI units of measurement (Système international d'unités) should be used but when necessary use other officially accepted units.

6.2. Write the names of living organisms using Italics font style.

6.3. Abbreviated form of a term should be put into parenthesis after the full name of the term first time it appears in the text.

6.4. Chemical formulas and complex equations should be drawn and prepared for photographic reproduction.

7. Figures

7.1. Authors may use black-and-white photographs and good quality drawings.

7.2. A caption with the explanation should be put below each figure.

8. Tables

8.1. Type tables on separate sheet of papers and enclosed them at the end of the manuscript.

8.2. Number the tables using Arabic numerals.

8.3. Above each table, write a capture with table explanation.

8.4. On the left margin, indicate the place of the tables in the text.

9. Electronic copy of the article

9.1. After the acceptance of the article, send a CD with final version of the manuscript and a printed copy to facilitate technical processing of the text. Articles should be written in Microsoft Word format and sent to the Editorial office of the Matica Srpska Journal for Natural Sciences, 1 Matica Srpska Street, 21000 Novi Sad (Uredništvo Zbornika Matice srpske za prirodne nauke, Matice srpske 1, 21000 Novi Sad).

9.2. Before printing, the manuscripts shall be sent to the authors for the approval of final version. Corrections of the text prepared for printing should be restricted to misspelling and printing errors as much as possible. For major changes of the text, a fee will be charged. Corrected manuscript should be returned to the Editorial office as soon as possible.

Зборник Матице српске за природне науке издаје Матица српска
Излази двапут годишње
Уредништво и администрација:
Нови Сад, Улица Матице српске 1
Телефон: (021) 6615798

Matica Srpska Journal for Natural Sciences
Published twice a year
Editorial and publishing office:
1 Matica Srpska Street, 21000 Novi Sad, Serbia
Phone: +381 21/6615798

E-mail: vnikolic@maticasrpska.org.rs
zmspn@maticasrpska.org.rs
www.maticasrpska.org.rs

The editors of the *Matica Srpska Journal for Natural Sciences*
completed the selection for Issue 138 (1/2020) on Mart 18, 2020

For Publishers: Prof. Dragan Stanić, PhD
Editorial Staff Secretary: Vladimir M. Nikolić
Managing editor: Prof. Slavka Gajin, PhD
Language Editor: Ljiljana Tubić and Olivera Krivošić
Proof Reader: Vladimir M. Nikolić
Technical design: Vukica Tucakov

Published in mart 2020
Computer set: Vladimir Vatić, GRAFIT, Petrovaradin
Printed by: SAJNOS, Novi Sad

Публиковање овог *Зборника* помогло је
Министарство просвете, науке и технолошког развоја Републике Србије
Publication of this volume was supported by the Ministry of Education,
Science and Technological Development of the Republic of Serbia

CIP – Каталогизација у публикацији
Библиотека Матице српске, Нови Сад

5/6(082)

ЗБОРНИК Матице српске за природне науке = Matica Srpska Journal for Natural Sciences / главни и одговорни уредник Ивана Максимовић. – 1984, св. 66– . – Нови Сад : Матица српска, Одељење за природне науке, 1984– . – 24 cm

Два пута годишње. – Наставак публикације: Зборник за природне науке. – Текст на енг. језику, резимеи на енг. и на срп. језику.

ISSN 0352-4906

COBISS.SR-ID 5845250