NARDI FUNDULEA, ROMANIA ROMANIAN AGRICULTURAL RESEARCH, NO. 38, 2021
www.incda-fundulea.ro Print ISSN 1222–4227; Online ISSN 2067–5720

THE INFLUENCE OF MICROBIAL INOCULANTS ON MICRO- AND MACRONUTRIENTS ON CALCARIC ALLUVIAL SOIL, BUZĂU, ROMANIA

Aurora Dobrin[1], Maria Mihaela Zugravu[1], Andrei Moţ[1], Marian Mușat[2], Floarea Burnichi[3], Roxana Ciceoi[1*]

[1]Research Centre for Study of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

[2]Faculty of Agriculture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

[3]Vegetable Research and Development Station Buzău, 23 Mesteacănului str., Buzău, Buzău County, Romania

[*]Corresponding author. E-mail: roxana.ciceoi@qlab.usamv.ro

ABSTRACT

The EU, having the overarching goal to become the first climate-neutral continent by 2050, plans to consolidate food chains that works for all: consumers, producers, climate, and the environment. For this, we need to reduce dependency on pesticides, reduce the excess fertilization, promote the biodiversity in agroecosystems, including on soils. Therefore, practical solutions using microbial inoculants to protect crops, restore soil health and functions, and improve plants health are enforced. Some fungal inoculants function both as entomopathogens and endophytes; colonizing a wide variety of plants, favoring plant growth, and protecting crops against pests. In this context, the present study aimed to evaluate the variation of total content of macro and micronutrients from soil in the context of application of microbial inoculants based on diverse benefic species, in an experimental field, under conversion to organic farming. The soil samples were taken from 0-20 cm depth, dried and analyzed by CHNS elemental analyzer and ICP-MS. The results shown some correlations between microbial inoculants and macro and micronutrients content, which can be further exploited inside the organic or sustainable production systems by farmers.

Keywords: micronutrients, macronutrients, microbial inoculants, calcaric alluvial soil, tomato crop.

INTRODUCTION

The 'Farm to Fork' strategy, inside the EU Green Deal, pursues the issue of food sustainability in the context of some ambitious targets by 2030, as reducing the use of pesticides by 50%, reducing the use of fertilizers by 20% and reducing nutrient loss by at least 50% (European Union, 2020).

Microbial inoculants, by their ability to act both as entomopathogens and endophytes (Canassa et al., 2019), can be successfully used to fulfill some assumed targets. Plant-microbiomes interactions manifest both at rhizosphere and phyllosphere level, being involved in plant nutrient provision and enhancement of plant defense. The microbial inoculants can enhance plant health and protect crops by reducing pest population from below and above soil even when applied as priming on seeds. Recent studies showed that two fungal isolates used as seed inoculants had the potential to suppress spider mites in bean and the strategy appears to have no conflict with use of predatory mites. Regarding the nutrients, the soil microorganisms, especially those inhabiting the rhizosphere have direct role in the supply of nitrogen, phosphorus, potassium, and micronutrients (Giagnoni et al., 2020; Qiu et al., 2021), increasing the availability of these nutrients but they can be used also for biofortification, that has been used to improve micronutrient contents in crops for human consumption (Ku et al., 2019). Microbes have direct contribution in carbon storage, nitrogen fixation, phosphorus solubilization and phytohormone production, or indirectly in antimicrobial compounds biosynthesis (Abass et al., 2020; Brucker et

Received 11 February 2021; accepted 12 February 2021. First Online: March, 2021. DII 2067-5720 RAR 2021-88

al., 2020; De Mastro et al., 2020; Yan et al., 2020; Eltlbany et al., 2019; Rashid et al., 2016; Xue et al., 2018; Singh and Arora, 2016; Zhang et al., 2016). Liu et al. (2018) found that there is a strong correlation between macronutrients, as P, K, Mg, Fe and Ca with prokaryotes and fungal community compositions, and micronutrients, as Al, Ba, Co, Hg, Mn, Cd, Sr with the diversity of prokaryotes and fungi. Otherwise said, they concluded that the microbial diversity and community composition were driven by different macro and microelements content. Microbial inoculation enhanced soil nutrients and increased their bioavailability for the plants as mentioned by Senicovscaia (2012), Abbott et al. (2018), Eke et al. (2019) and Singh et al. (2020). Analyzing the mutual influences between iron and microbial communities, Jin et al. (2014), found that the structure of microbial communities in the rhizospheres of Fe-deficient plants can be quite different from that of Fe-sufficient plants, but the exact mechanism underlying this difference remains an open question.

The interest in using microbial-based methods for improving crop yield and soil health was constantly growing in the last decade. The present work analyzed the content variation of the most important macro and microelements in soil, in the context of fertilization using microbial inoculants, in one organic tomato crop, with the aim of establishing good practices for tomato organic technology.

MATERIAL AND METHODS

Crop conditions

The experiment was conducted in the organic research plot from Vegetable Research and Development Station Buzău, România (VRDSB), in 2019. The crop, represented by Florina 44, an open field tomato variety, was cultivated using the standard crop technology developed by the VRDSB. In addition, the plot was treated with a mix of commercial products including microbial inoculants and fertilizers produced by Microspore (Microspore, 2021). The fertilization scheme included 16 applications, four before planting the seedlings, at 36, 39, 47, and 56 days after sowing and 12 after seedlings planting, at 1, 3, 17, 26, 33, 40, 54, 61, 63, 70, 84, and 104 days after planting. The products used before planting included inoculants from *Beauveria bassiana, Metarhizium anisopliae, Glomus* spp., *Bacillus* spp., *Trichoderma* sp., *Poconia chlamidosporia* and *Arthrobotrys* sp. while those after planting included *Poconia chlamidosporia, Arthrobotrys*, *Streptomyces*, *Trichoderma* sp. The control samples were taken from two plots, one that was in the same field, but not cultivated with tomatoes and one from the tomato crop that was not fertilized following the above-described scheme.

Soil preparation

The soil samples were taken from topsoil (0-20 cm) in July and in November 2019, from the Florina 44 tomato crop. Samples preparation and physico-chemical analyses have been performed in the Research Centre for Study of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest. Soil samples were dried at room temperature; soil subsamples were homogenized, milled, and sieved through a 250 µm sieve.

Analysis of soil micro- and macroelements

Microwave-assisted digestion procedure was used for soil samples preparation using 0.1 g sample and Suprapur solvents: 65% HNO_3 and 37% HCl. Elemental analysis of micro- and macroelements was performed by inductively coupled plasma mass spectrometry (ICP-MS), according to Dobrin et al. (2020), using an Agilent 7700x ICP-MS instrument with Mass Hunter 4.3 Workstation software for ICP-MS, version C.01.03 (Agilent Technologies, Tokyo, Japan). A multi-element ICP-MS calibration standard was used for the calibration curve.

The total soil nitrogen and carbon content was determined using CHNS elemental analyzer. An amount of 5-10 mg of sample was used to determine the nitrogen and carbon content. The analysis was performed using the CHNS elemental analyzer (EA3100

Elemental Analyzer). Cystine was used as standard reference material. All determinations were performed in three repetitions.

Statistical analysis

The chemical analysis (micro- and macroelements) was performed in three repetitions. All the results were expressed as the mean values. The obtained data were subjected to ANOVA using the statistical package IBM SPSS Statistics for Windows (Version 27.0, SPSS, Ireland), and the effects of treatments on the soil elements were evaluated. The calculated mean values were compared using Duncan's multiple range test with significance defined at $P \leq 0.05$. Pearson correlation analysis was used to estimate correlations between K, Mg and Na.

RESULTS AND DISCUSSION

Macroelements analysis

Microbial inoculants (MI) have different and specific action on each of the macroelements, time dependent. Their content variation is illustrated in the Figure 1.

The nitrogen (N) is one the most important and most debated macroelement of soils, especially in a cropping system frame. Due to the environmental (water pollution, human safety etc.) and economic (production boost by fertilizers) issues, the need to better understand the role and fate of nitrogen in crop production increased significantly. Most often, N is considered a deficient nutrient and the farmers use it extensively in the hope of substantial economic returns. Still, a balance between profit and environmentally tolerable levels of NO_3 should be achieved, and deeper knowledge about the role of microbial organisms in nitrogen cycle is required. Plants require more N than any other nutrient but 98% of the nitrogen in soil is in organic forms, which cannot be used by plants, with few exceptions. Soil microorganisms are the one to convert the organic forms of nitrogen to mineral forms when they decompose organic matter and fresh plant residues.

In our tested soils, the total nitrogen content varied between 0.145-0.169% for the summer period and 1.78-1.97% in the autumn, indicating a soil with medium N content (Madjar, 2008). The values were significantly higher in November compared with July in all three variants. The highest value of total nitrogen content during summer was found in the soil cultivated with untreated tomatoes.

The Carbon to Nitrogen Ratio (C:N) is a soil indicator providing important information on crop residue decomposition and soil and crop nutrient cycling, being one decisive factor in designing crop rotations and the use of cover crops in agricultural systems (USDA, 2011). The C:N values of our soils varied from 13.32:1 to 16.13:1, for the different treatments and months, indicating an overall very fast relative decomposition rate, quite close to the Ideal Microbial Diet rate, which is 24:1. Research showed that soil microorganisms have a C:N ratio near 8:1, but they actually require a diet with a C:N ratio near 24:1, as 16 parts of carbon is used as energy supplier and eight parts for body integrity maintenance. Since our soil contains a lesser proportion of carbon to nitrogen than the 24:1, we have an indication that the microbial communities within our soils will be able to release the excess nitrogen in the soil, making it more fertile. This additional nitrogen in the soil will be available for the next crops and for other soil microorganisms to use it to decompose other organic residues with a C:N ratio greater than 24:1 (USDA, 2011). We can conclude that the studied soils are providing a high-quality habitat for soil microorganisms. Regarding the characterization of soil fertility according to the C:N ratio, according to Madjar and Davidescu (2015), values of 12-14:1 indicates a soil with medium fertility.

The potassium and phosphorus had the significantly highest content in the test treated with microbial inoculants. Potassium content varied between 6170 mg/kg and 7244 mg/kg, still illustrating a low soil content (Madjar, 2008). Phosphorus content varied between 976 and 1141 mg/kg, indicating a good soil content.

Calcium content varied between 2.13-2.33%, indicating a high content, above the usual limit, despite the sandy texture (Madjar, 2008). A light decrease of this element was observed in November compared to July, for the uncultivated test. This change of calcium content evolution may be due to microbial treatment.

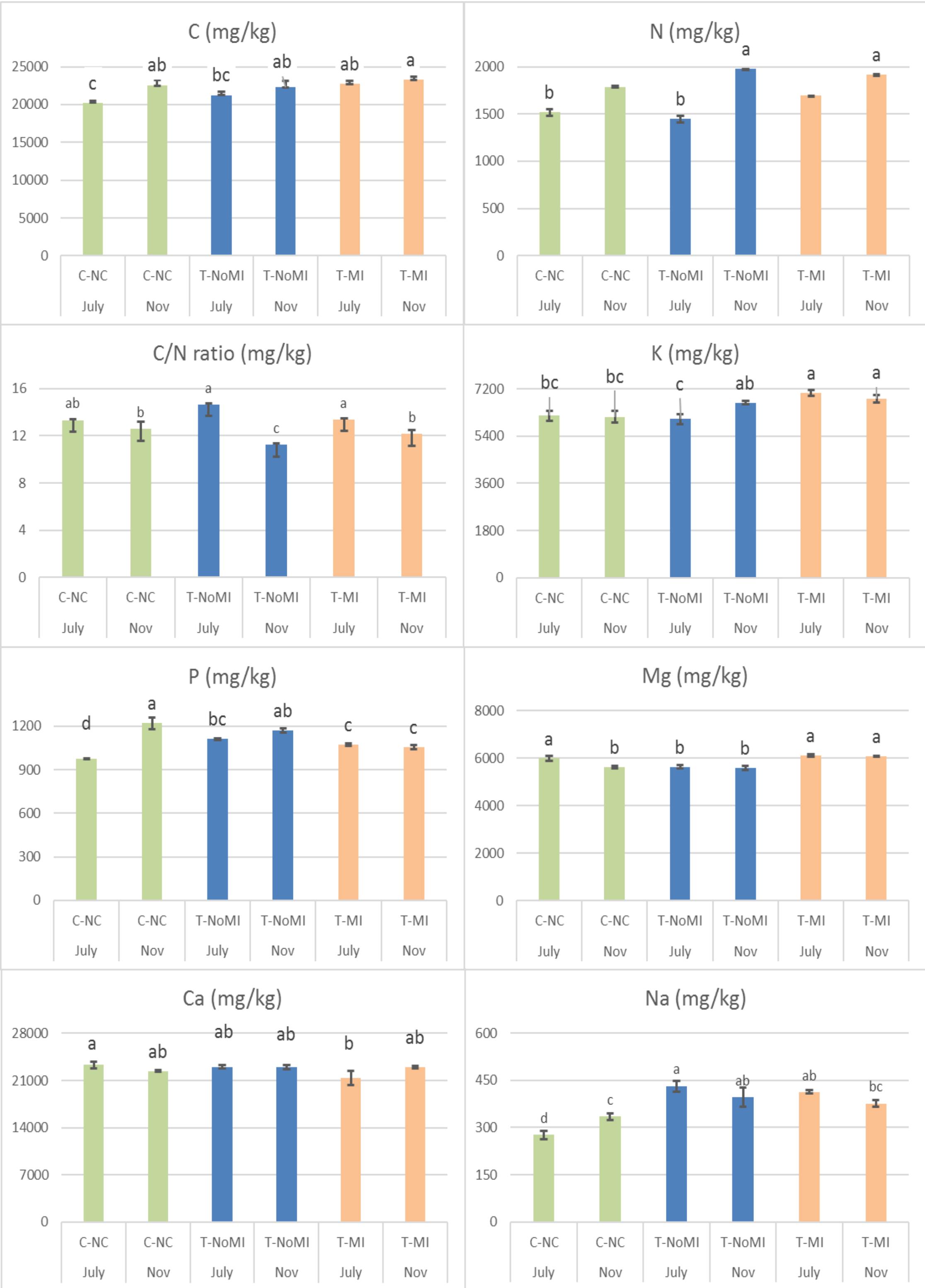

Figure 1. Macronutrients content variation in soil fertilized with microbial inoculants.
C-NC - control, no crop on the land (previous crop was peas); T-NoMI - tomato crop, no microbial inoculants used; T-MI - tomato crop with presented scheme of fertilization using microbial inoculants.
Error bars represents standard error of the mean. Bars with the same letter are not significantly different at $P<0.05$, according to Duncan multiple range test.

The sodium content was lower on the treated variant compared to the control and it was higher in both tomato fields, regardless of the microbial treatment, while in not-cultivated plot the values were significantly lower.

Magnesium content was significantly higher in the inoculated variant in both sampling periods, if compared with the untreated tomatoes field.

As correlation between macronutrients, a very good correlation was found between K and Mg (r=0.988) (Figure 2a), and a very good correlation also for K and Na (r=0.960) (Figure 2b).

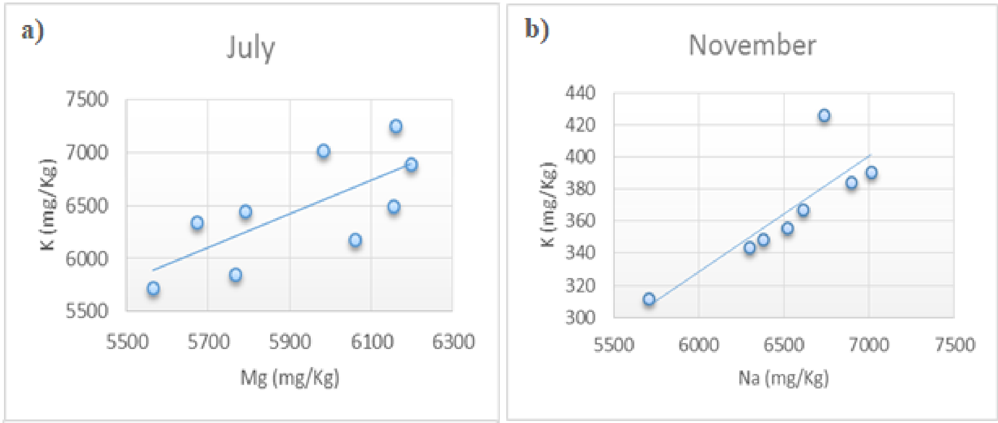

Figure 2. Correlations between potassium and magnesium (a) and sodium (b)

Microelements analysis

The microelements taken into consideration for the present study were represented by iron, selenium, and silver (Figure 3). Other microelements having some roles in plant growing and development, as Zn, Ni, Mo, Cr, were assessed in a study of heavy metals (Pb, Cd, Ni, Co, Zn, Cu, Mo, As, and Cr) content in the same soil from Buzău County, that aimed to establish the compliance of that organic soil with the Romanian and European legislation (Moţ et al., 2020).

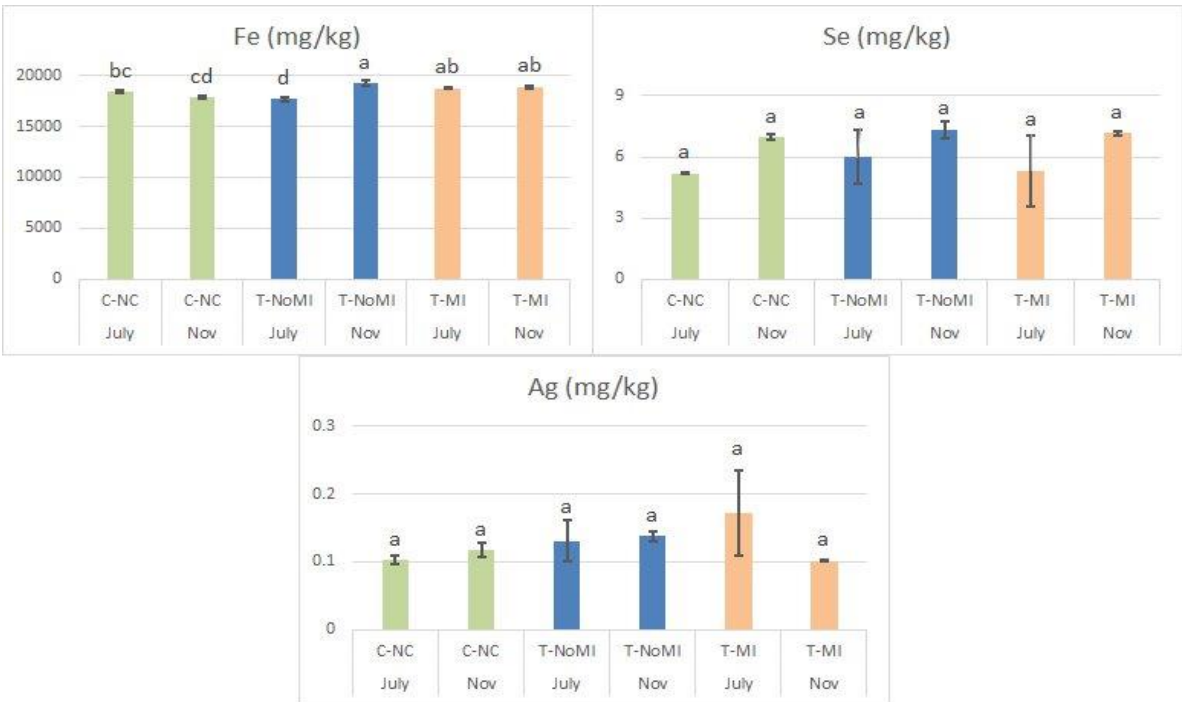

Figure 3. Micronutrients content variation in soil fertilized with microbial inoculants.
Error bars represents standard error of the mean. Bars with the same letter are not significantly different at P<0.05, according to Duncan multiple range test.

Regarding microelements, the iron content varied 1.78-1.92%, a low content, specific for sandy soils (Madjar, 2008). The iron content was almost constant in the three variants, both in July and November, with no significant difference between variants.

Selenium (Se) content was significantly higher in November then in July in all three experimental variants, with no significant differences between treated or untreated variants. Matos et al. (2016) found that Se concentration in soil correlated significantly and positively with Fe and that Se adsorption it is influence by Fe, Al, and Mn. Kita et al. (2008) observed that selenium affects the uptake, distribution, and accumulation of nutrient elements in the plant tissues. Microorganisms play an important role in transformation of Se in soils, especially in soils that have a high content in carbon (Manojlović, 2003). The plants have an iron content decrease in the roots in the presence of selenium in medium. Zhou et al. (2020) revealed that N-fertilizer applications can lead to decreases in Se concentration due to soil acidification, having as results less plant-available ferric-iron-selenite complexes.

The silver content, at levels of 0.1-0.17 mg/kg soil, was higher in July in the treatment variant then November in opposite untreated variants had higher values in November. According to Yang et al. (2014), there is a recent concern regarding the activity of some metals used as nanoparticles in different inputs, as high concentration of Ag proved to have a negative impact on microbial communities. Hänsch and Emmerling, 2010, showed that silver nanoparticles at concentrations of 32-320 µg/kg significantly decreased microbial biomass in soils.

CONCLUSIONS

The use of microbial inoculants becomes more and more a common practice, with new species or lines selected, patented, and marketed every year. According to EU Missions in Horizon Europe, the soils are essential for all life-sustaining processes on Earth and they provide a high range of ecosystem services. As it can take up to 1,000 years to produce 1 cm of soil, and approximately 33% of our soils are degraded globally, new methods of increasing soil health are increasingly sought after. For most nutrients, our preliminary study showed that the microbial inoculants increase the total content, when compared with untreated variant and the control plot, and between July and November sampling periods, for N, P, K, Se. The main macronutrients (N, P, K) had the highest values in the inoculated variants, suggesting that microbial inoculants can be used successfully also for the reduction of the use of fertilizers, besides the use in plant health.

Beneficial effects of microbial inoculation of soil on plant nutrition may depend on the soil properties and growth environment, as well as plant and mycorrhiza species. It is important to determine what soil proprieties, growth environments, plant species and microorganism can have an influence to improve plant nutrition, information that may help to improve crop production in alluvial soils.

Still, the influence of any management practice on soil quality will be first reflected in the microbial related indicators and soil enzyme content, long before measurable changes in soil chemical properties can be measured (Samuel and Ciobanu, 2018), therefore our study represent only a first step into quantification of microbial inoculants effect on soil physico-chemical properties.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI-UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0301/ 28PCCDI, within PNCDI III.

REFERENCES

Abbas, F., Hammad, H.M., Ishaq, W., Farooque, A.A., Bakhat, H.F., Zia, Z., Fahad, S., Farhad, W., Cerdà, A., 2020. *A review of soil carbon dynamics resulting from agricultural practices*. Journal of Environmental Management, 268. doi:10.1016/j.jenvman.2020.110319

Abbott, L.K., Macdonald, L.M., Wong, M.T.F., Webb, M.J., Jenkins, S.N., Farrell, M., 2018. *Potential roles of biological amendments for profitable grain production - A review.* Agriculture, Ecosystems and Environment, 256: 34-50. doi:10.1016/j.agee.2017.12.021

Brucker, E., Kernchen, S., Spohn, M., 2020. *Release of phosphorus and silicon from minerals by soil microorganisms depends on the availability of organic carbon*. Soil Biology and Biochemistry, 143. doi:10.1016/j.soilbio.2020.107737

Canassa, F., Tall, S., Moral, R.A., de Lara, I.A.R., Delalibera, I., Meyling, N.V., 2019. *Effects of bean seed treatment by the entomopathogenic fungi Metarhizium robertsii and Beauveria bassiana on plant growth, spider mite populations and behavior of predatory mites*. Biological Control, 132: 199-208. doi:10.1016/j.biocontrol.2019.02.003

De Mastro, F., Traversa, A., Brunetti, G., Debiase, G., Cocozza, C., Nigro, F., 2020. *Soil culturable microorganisms as affected by different soil managements in a two year wheat-faba bean rotation*. Applied Soil Ecology, 149. doi:10.1016/j.apsoil.2020.103533

Dobrin, A., Zugravu, M., Moţ, A., Muşat, I.B., Ciceoi, R., 2020. *Macro and micronutrients distribution in calcaric aluviosoil.* Scientific Papers, Series A, Agronomy, LXIII(1): 68-73.

Eke, P., Wakam, L.N., Fokou, P.V.T., Ekounda, T.V., Sahu, K.P., Kamdem Wankeu, T.H., Boyom, F.F., 2019. *Improved nutrient status and Fusarium root rot mitigation with an inoculant of two biocontrol fungi in the common bean (Phaseolus vulgaris L.).* Rhizosphere, 12, 100172. doi:10.1016/j.rhisph.2019.100172

Eltlbany, N., Baklawa, M., Ding, G.C., Nassal, D., Weber, N., Kandeler, E., Neumann, G., Ludewig, U., van Overbeek, L., Smalla, K., 2019. *Enhanced tomato plant growth in soil under reduced P supply through microbial inoculants and microbiome shifts*. FEMS Microbiology Ecology, 95(9), fiz124. doi:10.1093/femsec/fiz124

European Union, 2020. *Farm to fork strategy. For a fair, healthy and environmentally-friendly food*. system.https://ec.europa.eu/food/sites/food/files/safety/docs/f2f_action-plan_2020_strategy-info_en.pdf

Giagnoni, L., Taiti, C., León, P., Costa, C., Menesatti, P., Espejo, R., Gómez-Paccard, C., Hontoria, C., Vázquez, E., Benito, M., Mancuso, S., Renella, G., 2020. *Volatile organic compound emission and biochemical properties of degraded Ultisols ameliorated by no tillage and liming*. Pedosphere, 30, Issue 5. doi:10.1016/S1002-0160(20)60024-8

Hänsch, M., and Emmerling, C., 2010. *Effects of silver nanoparticles on the microbiota and enzyme activity in soil*. Journal of Plant Nutrition and Soil Science, 173(4): 554-558. doi:10.1002/jpln.200900358

Kita, A., and Pietruszka, M., 2008. *Effect of Selenium on Magnesium, Iron, Manganese, Copper, and Zinc Accumulation in Corn Treated by Indole-3-acetic Acid*. Communications in Soil Science and Plant Analysis, 39(15-16): 2303-2318. doi: 10.1080/00103620802292343

Ku, Y.S., Rehman, H.M., Lam, H.M., 2019. *Possible roles of rhizospheric and endophytic microbes to provide a safe and affordable means of crop biofortification*. Agronomy, 9, 764: 1-26. doi:10.3390/agronomy9110764

Liu, K., Ding, X., Tang, X., Wang, J., Li, W., Yan, Q., Liu, Z., 2018. *Macro and microelements drive diversity and composition of prokaryotic and fungal communities in hypersaline sediments and saline-alkaline soils*. Frontiers in Microbiology, 9.

Madjar, R., 2008. *Agrochemistry*. Plant and soil, Ed. INVEL Multimedia. (In Romanian)

Madjar, R., and Davidescu, V., 2015. *Agrochemistry*. Horticulture faculty, Ed. ExTerra Aurum, USAMV Bucharest. (In Romanian)

Manojlović, M., 2003. *Selenium in soil*. Proceedings for Natural Sciences, Matica Srpska Novi Sad, 104: 23-37.

Matos, R., Lima, V., Windmöller, C., Nascentes, N., 2016. *Correlation between the natural levels of selenium and soil physicochemical characteristics from the Jequitinhonha Valley (MG), Brazil*. Journal of Geochemical Exploration, 172. doi:10.1016/j.gexplo.2016.11.001

Microspore, 2021. *GREENSPORE product line*. Last accessed 02.08.2021. http://www.microspore.com/wp-content/uploads/2015/02/greenspore.pdf

Moţ, A., Dobrin, A., Zugravu, M., Muşat, I.B., Ciceoi, R., Madjar, R., 2020. *Assessment of heavy metals content in calcaric alluvial soil from Buzău County under restriction of organic farming*. Scientific Papers, Series A, Agronomy, LXIII(1): 98-103.

Rashid, M.I., Mujawar, L.H., Shahzad, T., Almeelbi, T., Ismail, I.M.I., Oves, M., 2016. *Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils*. Microbiological Research, 183: 26-41. doi: 10.1016/j.micres.2015.11.007

Samuel, A.D., and Ciobanu, C., 2018. *Enzyme activities in a preluvosoil as affected by crop rotation and fertilization systems*. Romanian Agricultural Research, 35: 183-191.

Senicovscaia, I., 2012. *Biota of degraded soils and methods for its restoration*. AgroLife Scientific Journal, 1(1): 78-83.

Singh, D.P., Singh, V., Shukla, R., Sahu, P., Prabha, R., Gupta, A., Gupta, V.K., 2020. *Stage-dependent concomitant microbial fortification improves soil nutrient status, plant growth, antioxidative defense system and gene expression in rice*. Microbiological Research, 126538.

Singh, R., and Arora, N.K., 2016. *Bacterial formulations and delivery systems against pests in sustainable agro-food production*. Reference Module in Food Science, First Edition, 1-11. doi:10.1016/B978-0-08-100596-5.03068-7

Qiu, X., Peng, D., Tian, H., Wang, H., Liu, X., Cao, L., Li, Z., Cheng, S., 2021. *Soil ecoenzymatic stoichiometry and microbial resource limitation driven by thinning practices and season types in Larix principis-rupprechtii plantations in North China*. Forest Ecology and Management, 482. doi:10.1016/j.foreco.2020.118880

USDA, 2011. *Carbon to nitrogen ratios in cropping systems*. USDA NRCS East National Technology Support Center, Greensboro, NC. www.nrcs.usda.gov› PA_NRCSConsumption

Xue, P., Carrillo, Y., Pino, V., Minasny, B., McBratney, A.B., 2018. *Soil properties drive microbial community structure in a large scale transect in South Eastern Australia.* Sci. Rep., 8, 11725.

Yan, G., Xing, Y., Han, S., Zhang, J., Wang, Q., Mu, C., 2020. *Long-time precipitation reduction and nitrogen deposition increase alter soil nitrogen dynamic by influencing soil bacterial communities and functional groups*. Pedosphere, 30, Issue 3. doi:10.1016/S1002-0160(19)60834-9

Yang, Y., Bi, X., Westerhoff, P., Hristovski, K., McLain, J.E., 2014. *Engineered nanomaterials impact biological carbon conversion in soils*. Environmental Engineering Science, 31(7): 381-392. doi:10.1089/ees.2013.0421

Zhang, H., Ding, W., Luo, J., Bolan, N., Yu, J., Zhu, J., 2016. *Temporal responses of microorganisms and native organic carbon mineralization to 13C-glucose addition in a sandy loam soil with long-term fertilization*. European Journal of Soil Biology, 74: 16-22. doi:10.1016/j.ejsobi.2016.02.007

Zhou, X., Yang, J., Kronzucker, H.J., Shi, W., 2020. *Selenium biofortification and interaction with other elements in plants: A review*. Frontiers in Plant Science, 11. doi:10.3389/fpls.2020.586421