Scientific Papers. Series B, Horticulture. Vol. LXVIII, No. 1, 2024
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

COMPARISON OF THE MICROBIAL COMMUNITY OF ORCHARD SOILS IN THE NORTH-EAST PART OF ROMANIA

Andrei-Mihai GAFENCU, Andreea-Mihaela FLOREA, Florin-Daniel LIPȘA, Eugen ULEA

"Ion Ionescu de la Brad" Iași University of Life Sciences,
3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: andrei.gafencu@iuls.ro

Abstract

Orchard crop species can influence the soil microbial population by releasing root exudates, which are compounds released by plant roots into the soil. Some crop species exude compounds that are more beneficial for certain groups of microbes, while others exude compounds that inhibit the growth of certain groups. Additionally, different crop species have different root systems, which can affect the physical structure of the soil and the availability of water and nutrients, which in turn can influence the soil microbial population. This study aimed to determine the soil microbial diversity of some orchards during different seasons to advance knowledge about the role of microbes in the balance of the orchard ecosystem. The research was carried out in a mixed orchard in Iasi county. Effects of season (i.e. autumn, spring and summer) and land use (apple orchard, plum orchard and cherry orchard) on soil microbial populations and microbial community structures were determined using the Petri dish culture method on different culture media.

Key words*: soil microbiome, soil microbial community, orchard soil.*

INTRODUCTION

Fruits are among the most attractive and delicious horticultural species, and together with vegetables, they represent important components for a healthy and balanced diet (Bvenura & Sivakumar, 2017; Mandal et al., 2021). The fruit tree species, such as apple, plum, or cherry trees, can exert various influences on the soil in which they are cultivated and on the physical, chemical, and biological properties of the soil (Montanaro et al., 2017). Among the physical properties of the soil, those strongly influenced by fruit trees are represented by soil structure, soil temperature, soil aeration, as well as soil erosion (Letey, 1985; Van Quang et al., 2012). The roots of fruit trees directly influence the soil structure, thereby affecting soil porosity and facilitating the circulation of air and water around the roots. Additionally, the well-developed root system of fruit trees reduces the risk of soil erosion. Fruit trees also influence soil temperature due to the shading provided by their canopy, which helps maintain soil temperature at a lower value during hot periods, thus avoiding soil overheating in the summer (Pregitzer et al., 2000). Besides the physical and chemical properties, soil undergoes a series of biological changes when fruit tree species are cultivated. Fruit trees consume significant amounts of essential nutrients from the soil (such as nitrogen, phosphorus, potassium), leading to changes in nutrient concentrations in the soil and potentially influencing their availability to soil microorganisms (Qian et al., 2014). Another important aspect is the chemicals secreted by the roots of fruit trees into the soil, leading to changes in the soil environment near the roots through modifications in pH, the presence of acids, or other substances that can influence soil microorganisms (Si et al., 2018). Additionally, fruit trees also influence the biological properties of the soil through interactions established between soil microorganisms and the roots of fruit trees or as a result of the indirect influence exerted by the roots of trees or the modification of the physical and chemical properties of the soil.

The substances secreted by plant roots into the soil can serve as food for various groups of soil microorganisms, stimulating the growth and activity of soil microbiota (Hayat et al., 2010). Certain species of fruit trees are capable of forming symbiotic relationships with nitrogen-fixing bacteria (e.g. actinorhizal plants with *Frankia* spp.). Fruit trees can contribute to increasing the biodiversity of soil microorganisms through various ecological interactions established between fruit trees and soil microbiota (Schwintzer, 2012; Van Nguyen & Pawlowski, 2017).

Understanding the soil microbiota of fruit trees is important due to the multiple roles microorganisms play, such as decomposing organic matter, protecting plants against diseases and pests, and enhancing soil fertility through nutrient cycling (Mercado-Blanco et al., 2018; Castellano-Hinojosa & Strauss, 2020).

Given the aforementioned factors, soil samples were collected from a mixed orchard of apple, plum, and cherry trees between April and September 2023 to assess the influence these three fruit tree species have on the soil microflora.

MATERIALS AND METHODS

The research sites were established in an orchard (apple, plum and cherry) at S.C. Agralmixt S.A. farm (Figure 1), located in the North-Eastern Romania, Iasi county, Andrieseni village ($47^0$34'9" N, $27^0$20'38" E, 60 m above sea level).

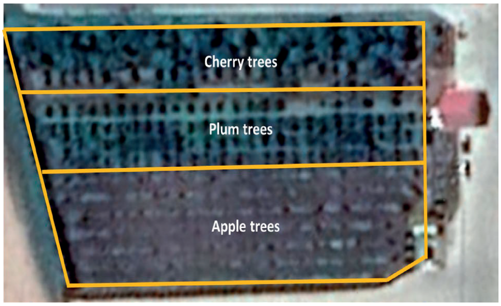


Figure 1. Orchard within the farm S.C. Agralmixt S.A.

The climate of this region is a temperate continental with an average annual air temperature and precipitation of 9.5°C and 520 mm, respectively (Gafencu et al., 2023; Nazare et al., 2023).

The average air temperature and precipitation during the sampling months were 8.7°C and 99.8 mm in April, 20.6°C and 36.4 mm in June, and 19.5°C and 23.2 mm in September (Figure 2).

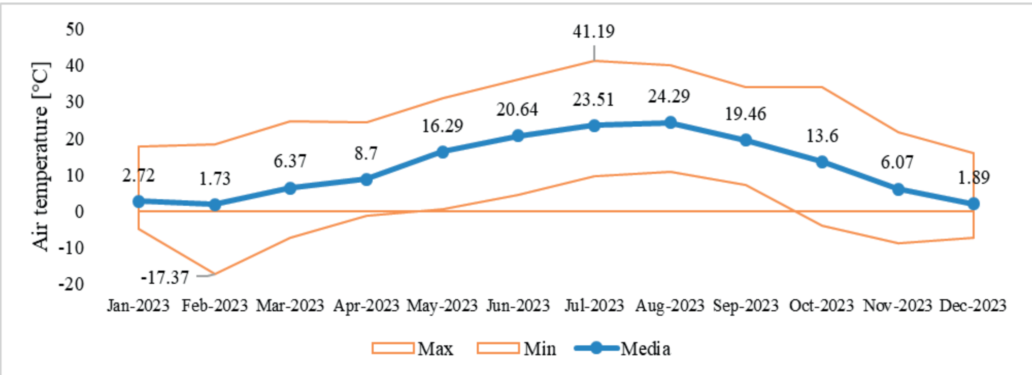


Figure 2. The air temperature (average, minimum and maximum monthly) recorded in the year 2023 within the farm S.C. Agralmixt S.A. at weather station 00001CED

The average monthly air temperature, in the studied area, did not register negative values during the analyzed period, but during the winter months the daily air temperature drops below the 0°C threshold. The lowest value of the air temperature was -17.37°C, recorded on February 10, 2023. In the summer months, the average monthly air temperature exceeds 20°C, in the July-August period there were days when air temperature values over 40°C were recorded, for example July 2 and 4, 2023 (Figure 3).

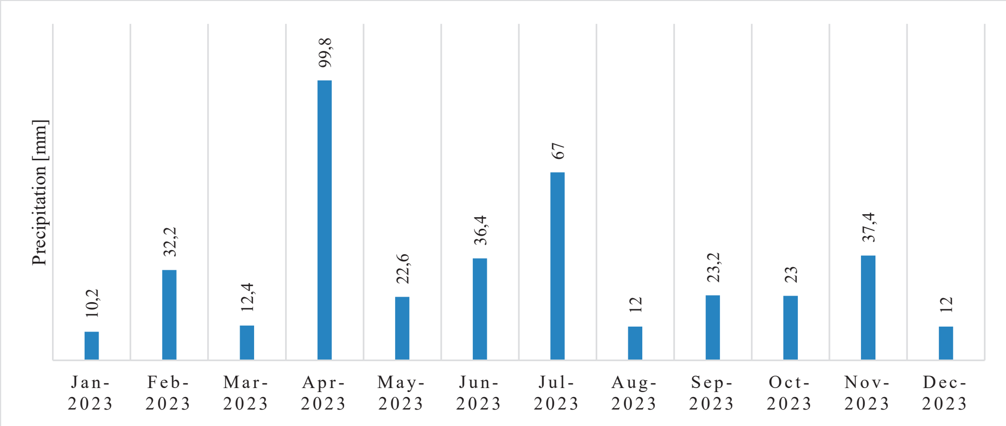


Figure 3. The monthly amount of atmospheric precipitation recorded in 2023 within the S.C. Agralmixt S.A. farm at weather station 00001CED

At the Agralmixt farm, in the orchard, within the framework of applied technology, large amounts of chemical fertilizers and pesticides are not used. Treatments are carried out only in case of strong attacks by pathogens or pests.

Soil samples were collected separately for each species (apple, plum, and cherry), representing the analyzed plots (Figure 3).

The soil samples were collected at three different times (April, June, and September 2023). Within each plot, samples were taken from 10 randomly selected points. The soil was collected from near the roots of the fruit trees, at a depth of about 10 cm.

The soil samples were transported to the Microbiology Laboratory of the Iasi University of Life Sciences for preparation, where they were stored overnight at 4°C, dried at room temperature, and sieved before undergoing microbiological analysis.

To determine the total number of bacteria in the soil, expressed as CFUs (colony-forming units) per gram of dry soil, the culture method in Petri plates was employed, involving a series of serial dilutions prior to plating (Gafencu & Ulea, 2023). To determine the total number of bacteria in the soil, the Potato Dextrose Agar (PDA) culture medium (Scharlau, Spain, 39 g l^{-1}) was used (Ulea et al., 2017). Streptomycin (35 mg l^{-1}) was incorporated into the media to inhibit the growth of Gram-negative (G^-) bacteria, thereby enabling the enumeration of Gram-positive bacteria (G^+). Streptomycin was thoroughly mixed into the PDA media post-autoclaving (15 minutes at 121°C), at a temperature of approximately 48°C. For the assessment of filamentous fungi, the PDA medium was also employed, with the addition of Bengal Rose at a concentration of 35 mg l^{-1}. The Rose Bengal was used to limit the size of faster-growing molds (Smith & Dawson, 1944).

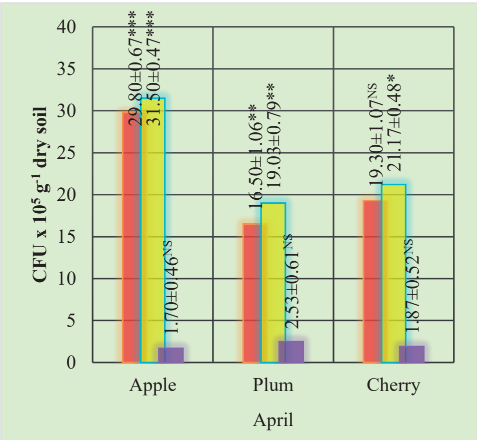
For each sample, the method of successive dilutions was employed. To determine bacteria and fungi in the soil, one ml of the suspension from the 10^{-3} and 10^{-4} dilutions, respectively, was transferred to a Petri dish. Subsequently, 17 ml of media were added at a temperature of 48°C and homogenized by orbital movements. After solidification of the culture media, the Petri dishes were incubated at a temperature of 28°C. After 24 hours, the number of bacterial colonies that developed was determined for both the PDA media and the PDA media containing Streptomycin. The Scan® 1200 automatic colony counter (Interscience, France) was used to determine the number of colonies that developed.

To determine the total number of bacterial colonies per gram of soil, the values obtained from each analyzed Petri dish were multiplied by the inverse value of the dilution factor. The results were expressed in colony forming units per gram of dry soil (CFU x 10^5 g^{-1} dry soil). Filamentous fungi were assessed after 5 days, and identification was based on morphological characteristics (Gilman, 1957; Barnett, 1960; Ellis & Ellis, 1985; Seifert & Gams, 2011; Guarro et al., 2012). Fungi that did not form spores during the 5-day period and could not be identified were grouped together (Other species). Statistical analysis of the data obtained in the experiments was performed using the SPSS program (IBM SPSS Statistics 26). For the statistical analysis of the data, a benchmark represented by the average value was utilized for comparison with the obtained values. Additionally, mean values were determined for each sampling moment and for each parameter, including the total number of bacteria, Gram-positive bacteria, and Gram-negative bacteria.

RESULTS

Some recent studies show that fruit species such as apple, plum or cherry can influence the soil microbiota (Franke-Whittle et al., 2015; Si et al., 2018).

The data obtained from the conducted research indicate that in April, the total number of bacteria in soil sampled from the apple orchard was significantly higher compared to soil sampled from plum and cherry orchards (Figure 4).



Total number of bacteria – Yellow bars
Gram positive bacteria (G^+) – Purple bars
Gram negative bacteria (G^-) – Red bars

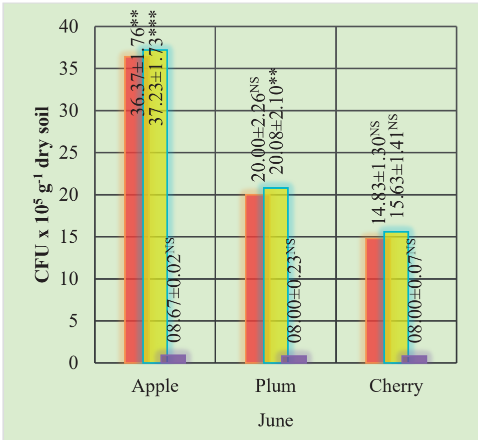
NS – $P > 0.05$
* – $P \leq 0.05$
** – $P \leq 0.01$
*** – $P \leq 0.001$

Figure 4. Soil bacterial communities under the influence of tree species - in April 2023

According to the results, in the apple orchard, the total number of bacteria was 31.50±0.47 CFUx$10^5$$g^{-1}$dry soil, whereas in soil sampled from the plum and cherry orchards, the total number of bacteria was 19.03±0.79 CFUx10^5 g^{-1} dry soil and 21.17±0.48 CFUx$10^5$$g^{-1}$ dry soil, respectively.

In the case of Gram-negative bacteria, the same trend was observed regarding the number of bacteria in the soil, with significant differences noted between the values obtained from soil sampled from the apple orchard compared to soil sampled from the cherry and plum orchards. However, concerning Gram-positive bacteria, the data obtained indicate that in April, the number of Gram-positive bacteria in soil sampled from the apple orchard was lower compared to the number of Gram-positive bacteria identified in soil sampled from the cherry and plum orchards.

Following the second sampling in June, it was observed that the number of bacteria in the soil increased in the apple and plum orchards, while in the cherry orchard, the number of bacteria decreased (Figure 5).



Total number of bacteria – Yellow bars
Gram positive bacteria (G^+) – Purple bars
Gram negative bacteria (G^-) – Red bars

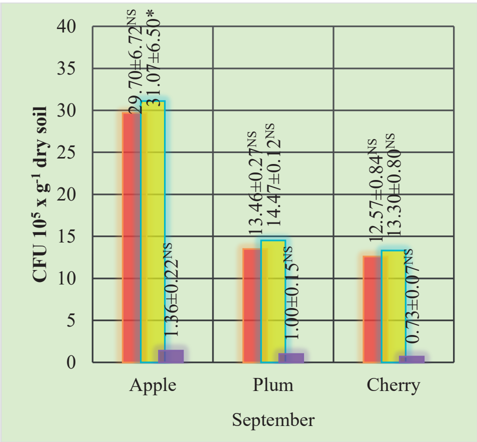
NS – $P > 0.05$
* – $P \leq 0.05$
** – $P \leq 0.01$
*** – $P \leq 0.001$

Figure 5. Soil bacterial communities under the influence of tree species - in June 2023

Following this sampling, it was observed that the apple orchard recorded the highest values of the total number of bacteria in the soil, specifically measuring 37.23±1.73 CFUx$10^5$$g^{-1}$ dry soil. In the case of the cherry orchard, a total bacterial count of 15.63±1.41 CFUx$10^5$$g^{-1}$ dry soil was determined.

Towards the end of the vegetative period of the fruit tree species, the number of soil bacteria slightly decreased in all three orchards. At this sampling moment, the highest bacterial count was still observed in the soil sampled from the apple orchard, with a count of 31.07±6.50

CFUx10^5g^{-1} dry soil (Figure 6). At every sampling moment and across all three species, it was observed that the majority of soil bacteria are Gram-negative, constituting approximately 95% of the total bacterial population in the soil.



Total number of bacteria – Yellow bars
Gram positive bacteria (G$^+$) – Purple bars
Gram negative bacteria (G$^-$) – Red bars

NS – P > 0.05
* – P ≤ 0.05
** – P ≤ 0.01
*** – P ≤ 0.001

Figure 6. Soil bacterial communities under the influence of tree species - in September 2023

The ratio between Gram-positive and Gram-negative bacteria in soil is an important aspect of microbial diversity in soil. Generally, the majority of bacteria in soil are Gram-negative, with their proportion often exceeding 90% of the total bacterial population.

However, Gram-positive bacteria also play a crucial role in the soil ecosystem, contributing to nutrient cycles and organic matter decomposition processes. The balance and diversity between these two types of bacteria are crucial for soil health and its proper functioning.

The findings concerning filamentous fungi capable of cultivation on synthetic culture media reveal that morphological assessments identified a total of 10 genera of micromycetes in the soil samples. However, certain fungi remained unidentified due to their lack of spore production during the 5-day observation period, thus being classified under the group labelled as "Other species" (Figure 7).

Predominantly, the most abundant colonies of fungi developed on the PDA with Rose Bengal culture media belonged to the *Penicillium* genus. Over half of the fungi colonies that developed on the culture media belonged to this genus. This trend was consistent across all three species and in all three sampling moments, with one exception noted in the plum orchard, where *Penicillium* represented 35.90% of the total identified micromycete genera.

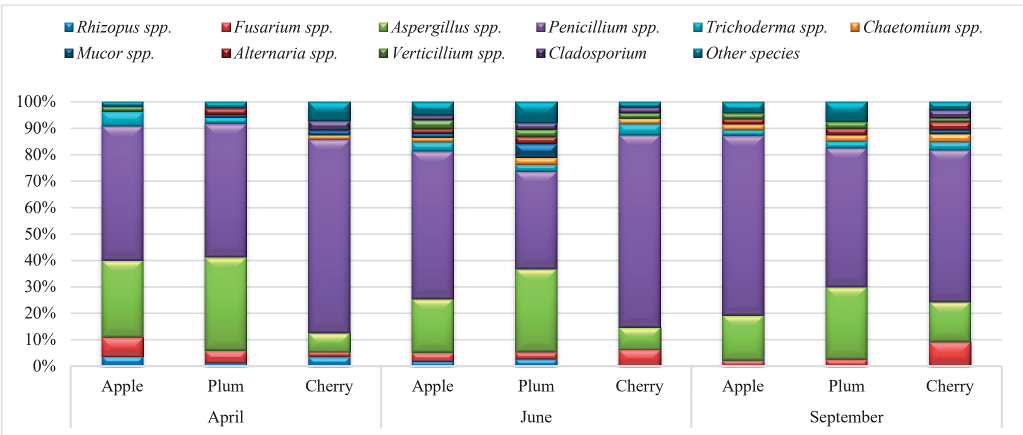


Figure 7. Changes of soil fungi structure during the vegetation period of fruit trees

The second most prevalent genus of micromycetes was *Aspergillus*. This genus represented between 7% and 35% of the fungal colonies that developed on the culture media. Analyzing the number of colonies developed in Petri dishes revealed that approximately 75-80% of the developed colonies belonged to these two genera. In addition to *Penicillium* and *Aspergillus*, other fungi belonging to the genera *Rhizopus*, *Fusarium*, *Trichoderma*, *Chaetomium*, *Mucor*, *Alternaria*, *Verticillium*, and *Cladosporium* were identified on the culture media. Additionally, there were fungi that could not be identified based on morphological characteristics (either due to sterile mycelium or lack of spore formation within the 5-day observation period). All other identified genera represented between 1% and 9% of the total micromycetes identified.

CONCLUSIONS

The results obtained from the research conducted throughout the year 2023 on the soil microbiota of the three pomological species (apple, plum, and cherry) indicate a higher number of bacteria in soil sampled from the apple orchard compared to the other two species. This suggests a significant difference in soil microbiota depending on the fruit tree species. It is possible that this observation is linked to specific soil conditions and requires further investigation to understand the underlying reasons for this discrepancy.

Regarding the micromycetes in the soil, it was observed that the majority of micromycetes belong to the genera *Penicillium* and *Aspergillus*. However, alongside these, other genera of fungi with high importance for the biological processes in soil are also present.

REFERENCES

Barnett, H. L. (1960). *Illustrated genera of imperfect fungi*. Illustrated genera of imperfect fungi.

Bvenura, C., & Sivakumar, D. (2017). The role of wild fruits and vegetables in delivering a balanced and healthy diet. *Food Research International*, 99, 15-30.

Castellano-Hinojosa, A., & Strauss, S. L. (2020). Impact of cover crops on the soil microbiome of tree crops. *Microorganisms*, 8(3), 328.

Ellis, M. B., & Ellis, J. P. (1985). *Microfungi on land plants. An identification handbook*. Macmillan, New York.

Franke-Whittle, I. H., Manici, L. M., Insam, H., & Stres, B. (2015). Rhizosphere bacteria and fungi associated with plant growth in soils of three replanted apple orchards. *Plant and Soil*, 395, 317-333.

Gafencu, A. M., Florea, A. M., Lipșa, F. D., & Ulea, E. (2023). Influence of different agricultural management practices on soil microbiome. *Scientific Papers. Series A. Agronomy*, 66(2), 47-52.

Gafencu, A.M., & Ulea, E. (2023). *Îndrumător practic de microbiologie generală*. Iasi, RO: Pim.

Gilman, J. (1957). *A manual of soil fungi*. Soil Science, 84(2), 183.

Guarro, J., Gené, J., Stchigel, A. M., & Figueras, M. J. (2012). *Atlas of soil ascomycetes*. CBS Biodiversity Series 10, Utrecht.

Hayat, R., Ali, S., Amara, U., Khalid, R., & Ahmed, I. (2010). Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of microbiology*, 60, 579-598.

Letey, J. (1985). Relationship between soil physical properties and crop production. *Adv. Soil Sci*, 1, 277-294.

Mandal, D., Wermund, U., Phavaphutanon, L., & Cronje, R. (Eds.). (2021). *Temperate Fruits: Production, Processing, and Marketing*. CRC Press.

Mercado-Blanco, J., Abrantes, I., Barra Caracciolo, A., Bevivino, A., Ciancio, A., Grenni, P., Hrynkiewicz, K., Kredics, L., & Proença, D. N. (2018). Belowground microbiota and the health of tree crops. *Frontiers in Microbiology*, 9, 1006.

Montanaro, G., Xiloyannis, C., Nuzzo, V., & Dichio, B. (2017). Orchard management, soil organic carbon and ecosystem services in Mediterranean fruit tree crops. *Scientia Horticulturae*, 217, 92-101.

Nazare, A. I., Stavarache, M., Samuil, C., Sîrbu, C., & Vîntu, V. (2023). The influence of the applied management on the phytodiversity of a *Festuca valesiaca* Schleich. ex Gaudin permanent meadow. *Scientific Papers. Series A. Agronomy*, 66(1), 457-466.

Pregitzer, K. S., King, J. S., Burton, A. J., & Brown, S. E. (2000). Responses of tree fine roots to temperature. *New Phytologist*, 147(1), 105-115.

Qian, X., Gu, J., Sun, W., Li, Y. D., Fu, Q. X., Wang, X. J., & Gao, H. (2014). Changes in the soil nutrient levels, enzyme activities, microbial community function, and structure during apple orchard maturation. *Applied soil ecology*, 77, 18-25.

Seifert, K. A., & Gams, W. (2011). The genera of Hyphomycetes–2011 update. *Persoonia-Molecular Phylogeny and Evolution of Fungi*, 27(1), 119-129.

Si, P., Shao, W., Yu, H., Yang, X., Gao, D., Qiao, X., & Wu, G. (2018). Rhizosphere microenvironments of eight common deciduous fruit trees were shaped by microbes in northern China. *Frontiers in microbiology*, 9, 3147.

Schwintzer, C. R. (2012). The biology of Frankia and actinorhizal plants.

Smith N. R., & Dawson V. T. (1944). The bacteriostatic action of Rose Bengal in media used for the plate counts of soil fungi. *Soil science*, 58(6), 467-472.

Ulea, E., Lipșa, F. D., Bălău, A. M., Filipov, F., & Morari, E. C. (2017). Diversity of soil bacteria as indicator of soil pollution in Moldavia region, Romania. *Environmental Engineering & Management Journal (EEMJ)*, 16(4): 879-889.

van Nguyen, T., & Pawlowski, K. (2017). Frankia and actinorhizal plants: symbiotic nitrogen fixation. *Rhizotrophs: plant growth promotion to bioremediation*, 237-261.

van Quang, P., Jansson, P. E., & Guong, V. T. (2012). Soil physical properties during different development stage of fruit orchards. *Journal of Soil science and Environmental management*, 3(12), 308-319.