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STUDY ON THE EFFECTS OF FERTILIZATION ON THE ABUNDANCE OF SOIL MICROBIAL COMMUNITY, ITS COMPOSITION AND ANTIFUNGAL EFFICACY

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Abstract

Soil microbial community is of high importance in preserving soil functions and its ecosystem goods and services. Within field experiences, regarding agricultural sustainability and the resilience of agro-ecosystems, a study was conducted to evaluate the evolution and abundance of the soil microbial community under the influence of environmental and agro-technical factors. For this purpose, periodic determinations of the microbial community on the depth of 0-20 cm were made in the plots cultivated with wheat, maize, soybean and a mixture of perennial grasses and legumes. In the experimental field, organic fertilizer materials (manure compost in doses of 15, 30 and 60 t/ha) and synthetic fertilizers (complex fertilizer in formula 20.20.0, in doses which varied with the specific consumption of crops and the amount of manure compost) were applied. At the same time, in the laboratory, biometric determinations were made regarding the antifungal efficacy of soil microorganisms after 5 days from incubation. The results of microbiological analyses showed that the microbial population from soil inhibits the in vitro development of the pathogen tested.

Key words*: soil microbial community, antifungal efficacy, sustainability, agroecosystem resilience*.

INTRODUCTION

Soils represents a complex system "being essential for food production and a key component to sustainability, through its support of important societal and ecosystem services" (Lehmann et al., 2020). Soil microorganisms play diverse and important roles in the ecosystem services (Aislabie et al., 2013) even if only recently it has been understood that their biological and functional diversity is a crucial factor for maintaining all ecosystems (Pylro et al., 2020). The most populated area is considered the soil rhizosphere, where the microbial activity is intense (Borozan et al., 2021).

Soil microbial population contribute to nutrient cycling and soil structure maintenance (Helgason et al., 2010; Tian et al., 2015; FAO et al., 2020) and are "the agents of transformation of soil organic matter, nutrients and of most key soil processes" (Powlson et al., 2001). Various studies reported that microbial biomass content, microbial diversity or soil enzymatic activity can be valuable soil quality indicators (Tian et al., 2015). Also, microbial diversity of soil is an important soil health indicator (Houskova et al., 2021). It is important to mention that the activities of soil microorganisms are influenced by the physico-chemical and ecological interactions of soil (Powlson et al., 2001).

Soil microbial composition can change depending on the soil type, vegetation, management strategy, and fertilization regime (Guan et al., 2022).

Fertilization is an indispensable agricultural practice for increasing soil organic matter, improving plant nutrition (Tian et al., 2015) but can produce changes in soil microbial activity, abundance, and community (Chu et al., 2007; Hartmann et al., 2015; Tian et al., 2015; Zhang et al., 2019).

A particular attention has been given to the effects of chemical fertilizers and organic amendments on soil microorganisms (Li et al., 2018). A meta-analysis made by Geisseler et al. (2015) through 100 datasets from long-term trials in the world showed that nitrogen fertilisation, compared with an unfertilized

control, significantly increased soil microbial biomass with an average of 15.1% and that the addition of chemical N fertilizer generally promoted the growth of fungi (Esperschütz et al., 2007; Li et al., 2018; Zhang et al., 2019). Some studies showed that "low rates of mineral fertilizers can stimulate the growth of ammonifying and nitrifying microorganisms, as well as the proliferation of spore-forming bacteria" (Bučiene, 2012; Grzyb et al., 2020; Sivojiene et al., 2021). By comparison, other studies found that the application of nitrogen fertilizer reduced the abundance or biodiversity of soil microorganisms because of soil acidification (He et al., 2007; Wang et al., 2018). Also, Ling et al. (2017) found that both N and P additions altered the bacterial community structure in a semi-arid steppe. Another research showed that long-term P input affected soil fungal and bacterial diversity but not the fungal community of arbuscular mycorrhiza in alfalfa (Beauregard et al., 2009).

In contrast with mineral fertilization, "higher soil microbial biomass and different community structures have been observed in agricultural soil with regular organic manure application" (Franciolli et al., 2016).

The type of the organic substrate influence significantly the abundance of microbial community and its functional diversity. For example, the application of organic wastes such as animal waste or poultry litter for a long period led to soil properties improvement and increase of the bacterial community diversity, especially bacteria (Gupta et al., 2022).

Ye et al. (2020) suggested that pig and chicken manure significantly increased the abundance of bacteria in soil in correlation with an increase in total soil nitrogen, but composted pig manure reduced the amount of some organotrophic bacteria. Also, the type of crop and crop rotation systems with different crop species can influence the soil microbial abundance biomass and community structure mainly through the root exudates and crop residues (Zhang et al., 2019). Plant root exudates can lead to the establishment of a different rhizosphere microbiota (Philippot et al., 2013). Regarding crop species influence on soil microbiota, previous reports showed that legume and grass have specific soil microbial community (Zhou et al., 2017). For example, the soil microbial community for *Lotus corniculatus* differed from that of *Holcus lanatus* grass (Ladygina et al., 2010). Also, "*Trifolium repens* species enriched alpha-proteobacteria abundance, but reduced beta-proteobacteria abundance, indicating the improved soil fertility" (Chen et al., 2014). Turner et al. (2013) showed that the bacterial and fungal community structure in rhizosphere were different between pea and cereal crops which was also demonstrated by Benitez et al. (2016) who reported that *Trifolium incarnatum* and *Vicia villosa* cover crops enriched arbuscular mycorrhizal fungi in soil.

The study of microbial communities and their relationship with the environment is essential for understanding ecosystem dynamics. So, it is necessary to investigate the modification of soil microbial activity, abundance, and community under different agricultural practices to maintain and enhance the fertility and productivity of soils and protecting soil ecosystems against disturbances (Gupta et al., 2022).

The main objectives of this study were to evaluate the evolution and abundance of soil microbial community under the influence of environmental and agro-technical factors as well as the antifungal efficacy of soil microorganisms.

MATERIALS AND METHODS

Study Site and Experimental Design

The experiment was carried out in Moara Domneasca Teaching and Research Station of the USAMV Bucharest, situated in Sylvo-steppe ecological area, on a red preluvosoil which characteristics are presented in Table 1. The preceding crop was alfalfa. Because of the drought installed in the fall, the soil was scarified 2 times to a depth of 40 cm, being very dry. The seed bed was prepared by two passes with the cultivator, the average soil moisture being of 19.5%.

The experiment was organized into 4 blocks, each of them being organized into 32 plots. The area of one plot was of 15 m^2. A plant species was cultivated in each block, and 8 fertilization treatments were carried out in 4 replicates.

Table 1. The physico-chemical characteristics of the red preluvosoil from Moara Domneasca Experimental Field

Soil parameters	Mean values
Ph	5.89
C_org. (%)	1.94
N_{total} (%)	0.26
$N\text{-}NO_3$ (mg/kg d.m.)[1]	11.84
$N\text{-}NH_4$ (mg/kg d.m.)	12.08
P_{AL} (mg/kg d.m.)	65.46
K_{AL} (mg/kg d.m.)	231.65

[1]mg/kg dry matter

The crops included in the experiment were as follows: winter wheat - *Triticum aestivum* L., mixture of grasses (rye grass - *Lolium perenne* L., blue grass - *Poa pratensis* L. and meadow fescue - *Festuca pratensis* L.) and perennial legumes (white clover - *Trifolium repens* L., bird's foot trefoil - *Lotus corniculatus* L.), soybean (*Glycine max* L.) and maize (*Zea mays* L.).

There were organized 8 experimental variants (V1.....V8) in 4 replicates and the treatments were the following:

V1 - control (soil);
V2 - complex chemical fertilizers (NPK);
V3 - 15 t/ha compost;
V4 - 15 t/ha compost + complex chemical fertilizers (NPK);
V5 - 30 t/ha compost;
V6 - 30 t/ha compost + complex chemical fertilizers (NPK);
V7 - 60 t/ha compost;
V8 - 60 t/ha compost + complex chemical fertilizers (NPK) (Photo 1).

Photo 1. Different fertilization regime of manure compost in Moara Domneasca Experimental Field plots

The complex fertilizers 20:20:0 were applied fractionally (Table 2*)*. In September 2021 – July 2022 period, the climatic conditions of Moara Domneasca Experimental Field fluctuated as can be observed in Figure 1.

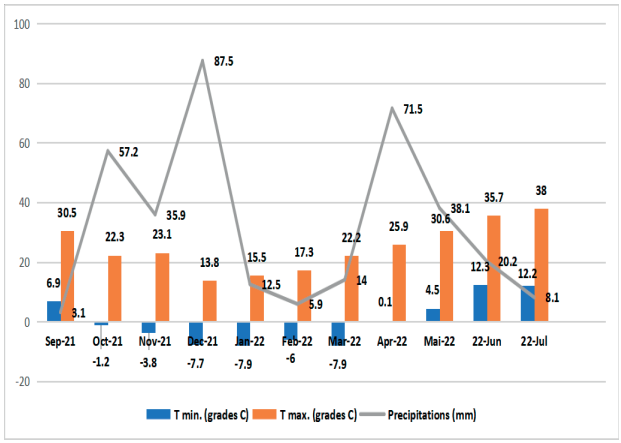


Figure 1. Climatic conditions in Moara Domneasca Experimental Field, September 2021 - July 2022 period

Soil microbiological analysis

From each experimental block, soil samples were taken from 0-20 cm depth both before the application of chemical fertilizers, compost and crop cultivation as well as in crops vegetation period. In laboratory, serial decimal dilutions were prepared to quantify the microbial load and perform quantitative and qualitative microbiological analysis.

In case of 10^{-1} dilutions, 10 g of soil were processed, which were put to infuse for 1 hour, at room temperature, under stirring at 120 rpm, then statically overnight.

Depending on the type of analysis, $10^{-4} \div 10^{-6}$ dilutions were subsequently used.

Different agar substrates were used for quantitative and qualitative microbiological tests such as:

i) *Plate Count Agar (PCA)* medium for determining the cultivable bacterial load;
ii) *Pink-Bengal Chloramphenicol Agar (RBC)* medium for isolation and enumeration of fungal load;
iii) *Eosine Methylene Blue (EMB) Agar* medium for the isolation, enumeration, and differentiation of *Enterobacteriaceae*.

For detecting the specific microorganisms load, six nutrient substrates were used as follows:

i) *King B medium* for detection of fluorescent siderophore producing *Pseudomonas* load under UV light;

Table 2. Fraction of mineral fertilizers applied to crops during the study

Treatment	Wheat		Mixture of grasses		Maize			Soybean		
	Fraction 1 (24-03-2022)	Fraction 2 (15-04-2022)	Fraction 1 (24-03-2022)	Fraction 2 (15-04-2022)	Fraction 1 (15-04-2022)	Fraction 2 (13-05-2022)	Fraction 3 (30-05-2022)	Fraction 1 (15-04-2022)	Fraction 2 (13-05-2022)	Fraction 3 (30-05-2022)
V1 - control (soil)	-	-	-	-	-	-	-	-	-	-
V2 – NPK	57 kg/ha N + 57 kg/ha P_2O_5 + 0 K_2O	28 kg/ha N + 28 kg/ha P_2O_5 + 0 K_2O	40 kg/ha N + 40 kg/ha P_2O_5 + 0 K_2O	40 kg/ha N + 40 kg/ha P_2O_5 + 0 K_2O	40 kg/ha N + 40 kg/ha P_2O_5 + 0 K_2O	46 kg/ha N + 46 kg/ha P_2O_5 + 0 K_2O	29 kg/ha N + 29 kg/ha P_2O_5 + 0 K_2O	18 kg/ha N + 18 kg/ha P_2O_5 + 0 K_2O	20 kg/ha N + 20 kg/ha P_2O_5 + 0 K_2O	13 kg/ha N + 13 kg/ha P_2O_5 + 0 K_2O
V3 - 15 t/ha compost	-	-	-	-	-	-	-	-	-	-
V4 - 15 t/ha compost + NPK	42 kg/ha N + 42 kg/ha P_2O_5 + 0 K_2O	21 kg/ha N + 21 kg/ha P_2O_5 + 0 K_2O	29 kg/ha N + 29 kg/ha P_2O_5 + 0 K_2O	29 kg/ha N + 29 kg/ha P_2O_5 + 0 K_2O	29 kg/ha N + 29 kg/ha P_2O_5 + 0 K_2O	34 kg/ha N + 34 kg/ha P_2O_5 + 0 K_2O	21 kg/ha N + 21 kg/ha P_2O_5 + 0 K_2O	16 kg/ha N + 16 kg/ha P_2O_5 + 0 K_2O	18 kg/ha N + 18 kg/ha P_2O_5 + 0 K_2O	11 kg/ha N + 11 kg/ha P_2O_5 + 0 K_2O
V5 - 30 t/ha compost	-	-	-	-	-	-	-	-	-	-
V6 - 30 t/ha compost + NPK	27 kg/ha N + 27 kg/ha P_2O_5 + 0 K_2O	13 kg/ha N + 13 kg/ha P_2O_5 + 0 K_2O	18 kg/ha N + 18 kg/ha P_2O_5 + 0 K_2O	18 kg/ha N + 18 kg/ha P_2O_5 + 0 K_2O	19 kg/ha N + 19 kg/ha P_2O_5 + 0 K_2O	22 kg/ha N + 22 kg/ha P_2O_5 + 0 K_2O	14 kg/ha N + 14 kg/ha P_2O_5 + 0 K_2O	13 kg/ha N + 13 kg/ha P_2O_5 + 0 K_2O	15 kg/ha N + 15 kg/ha P_2O_5 + 0 K_2O	9 kg/ha N + 9 kg/ha P_2O_5 + 0 K_2O
V7 - 30 t/ha compost	-	-	-	-	-	-	-	-	-	-
V8 - 30 t/ha compost + NPK	In V_8, for winter wheat, mixture of perennial grasses and maize, according to the calculation, the amount of manure compost should have ensured the nutrient requirements (NPK) and it was decided not to supplement it with chemical fertilizers.							8 kg/ha N + 8 kg/ha P_2O_5 + 0 K_2O	10 kg/ha N + 10 kg/ha P_2O_5 + 0 K_2O	6 kg/ha N + 6 kg/ha P_2O_5 + 0 K_2O

ii) *NBRIP medium (National Botanical Research Institute's phosphate growth medium*) based on tricalcium phosphate for the differentiation of microorganisms capable of solubilizing inorganic phosphate from poorly soluble compounds;
iii) *Chitin-Agar medium* for the isolation and enumeration of chitinolytic microorganisms;
iv) *Carboxymethylcellulose-Agar (CMC-Agar) medium* for the isolation and enumeration of cellulolytic microorganisms;
v) *Skim Milk Agar (SMA) medium* for the isolation and enumeration of caseinase-producing proteolytic microorganisms;
vi) *Yeast Mannitol Agar medium (YMA)* supplemented with Congo red for the differentiation of *Rhizobium*-like bacteria.
For testing, the seeding method in the lawn was used on the surface of the agar using 100µl suspension from the $10^{-3} \div 10^{-6}$ dilutions.
The antifungal activity of soil microbial communities against different phytopathogenic fungus was also analysed. For this, the double culture method was used against *Fusarium graminearum*, *Fusarium culmorum* (soil key pathogens for cereal crops), *Sclerotium bataticola* (soil pathogen for grain legumes) and *Sclerotinia sclerotiorum* (soil pathogen with varied host spectrum).
The tests were carried out *in vitro*, on *PDA culture medium (Potato-Dextrose-Agar)* which is specific for the development of fungi, and in comparison with some control cultures. Antimicrobial activity was assessed biometrically by measuring the growth rays of the tested phytopathogenic fungus and by determining the effectiveness of inhibiting phytopathogenic mycelial growth.

RESULTS AND DISCUSSIONS

To understand the microorganism's response to chemical and organic fertilization, it is important to determine the initial microbial load and the properties of the cultivable microorganisms in the soil before fertilizer application and before the cultivation of winter wheat, maize, soybean, and the mixture of perennial herbs and legumes. Through the microbiological analysis there have been quantified the communities of microorganisms (bacteria and fungi) which can have beneficial properties for agricultural crops (Table 3).

The microbiological tests showed a bacterial load of 10^7 CFU/g soil, a fungal load of 10^5 CFU/g soil (Photo 2) and a high load of microorganisms that can have a beneficial impact on cultivated crops growth. For example, the number of CFU/g soil of *Pseudomonas fluorescent* varied from 2.9 x 10^5 to 7 x 10^6.

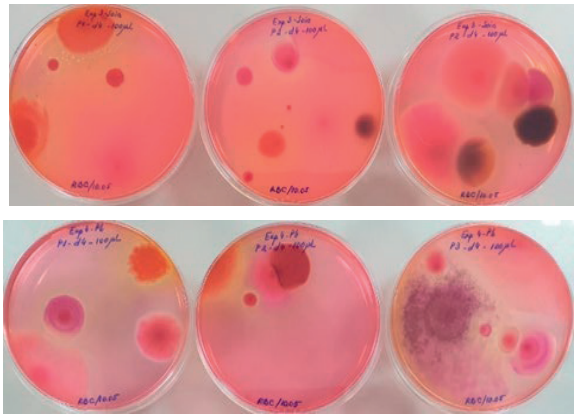
Table 3. The initial microbial composition and abundance in the soil before winter wheat, mixture of perennial grasses and legumes, soybean and maize cultivation

Microbial composition (mean values)	Soil - winter wheat	Soil - mixture of perennial grasses and legumes	Soil - soybean	Soil - maize
Cultivable bacteria (CFU/g)	3.3x 10^7	4.1x 10^7	5.7 x 10^7	3.6x 10^7
Fungus (CFU/g)	2 x 10^5	2.1 x 10^5	3 x 10^5	2.8x 10^5
Pseudomonas fluorescent (CFU/g)	6 x 10^6	7 x 10^6	2.2 x 10^6	2.9x 10^5
Microorganisms solubilizing inorganic phosphorus (CFU/ml)	8 x 10^6	1.1 x 10^7	7 x 10^6	5.3x 10^6
Chitinolytic microorganisms (CFU/ml)	8 x 10^6	7 x 10^6	1.2 x 10^7	1.3x 10^7
Cellulosic microorganisms (CFU/ml)	5 x 10^6	4 x 10^6	1.1 x 10^7	6.5x 10^6
Proteolytic microorganisms (CFU/ml)	7 x 10^6	9 x 10^6	1 x 10^7	2.5x 10^6
Rhizobium like bacteria (CFU/ml)	nd*	nd	1.5 x 10^6	1.3x 10^6

*nd-indeterminate

Many strains of *Pseudomonas fluoresecent* are known to enhance plant growth and some strains have been shown to be potential agents for the biocontrol, suppressing plant diseases by protecting the seeds and roots from the fungal infection (Ganeshan et al., 2007), aspect proved by the antagonistic tests realized *in vitro* in our experiments.
The number of microorganisms that solubilize the phosphorus from the mineral compounds was between 5.3 x 10^6 in the soil that was to be cultivated with maize and 1.1 x 10^7 in the soil

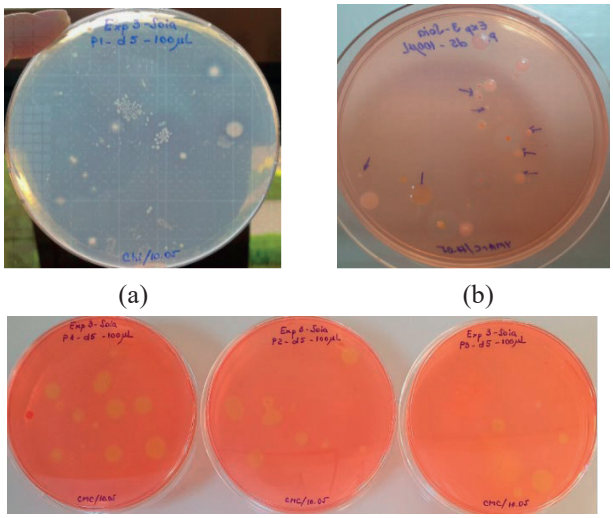
to be cultivated with the mixture of perennial grasses and legumes.



(a)

(b)

Photo 2. Aspects on the cultivation of fungi on *Pink-Bengal Cloramfenicol Agar* medium from the soil samples - before cultivation of soybean (a) and maize (b) crops



(a) (b)

(c)

Photo 3. Enumeration of (a) chitinolytic, (b) Rhizobium bacteria and (c) cellulosic microorganisms on different substrates

Also, the microbiological analysis showed a high number of chitinolytic, proteolytic and cellulosic microorganisms (10^6-10^7) that are active in the rhizosphere zone and that are producing mycolytic enzymes, especially chitinases, proteases and cellulase, which are known to hydrolyze chitin (a major component of fungal cell walls) (Brzezinska et al., 2014), protein and cellulose (one of the most widespread biomolecules of the biomass) (Nivesse et al., 2021).

In samples taken from the soil where maize and soybean were to be cultivated, there was found a high load of *Rhizobium* bacteria (10^6) which have a great importance for the biological nitrogen fixation in grain legume crops (Photo 3).

The activity of the microbial communities in the analyzed soil samples was biometrically determined to quantify the effectiveness of inhibiting phytopathogenic mycelial growth. Thus, the studies carried out in the laboratory have shown that the microbial load in the soil before cultivation of winter wheat and the mixture of perennial grasses and legumes inhibits the *in vitro* development of the tested pathogens (Figure 2).

A strong antifungal efficacy against *Fusarium culmorum*, *Fusarium graminearum* and *Sclerotium bataticola* (87.16%, 86.23% and 84.83%) and a moderate efficacy against *Sclerotinia sclerotiorum* (74.36%) was observed at isolates from soil before cultivation of winter wheat.

Isolates from soil before cultivation of mixture of perennial grasses and legumes showed lower antifungal activities against the tested fungi (78.2% against *Fusarium graminearum* and 73.06% against *Fusarium culmorum*).

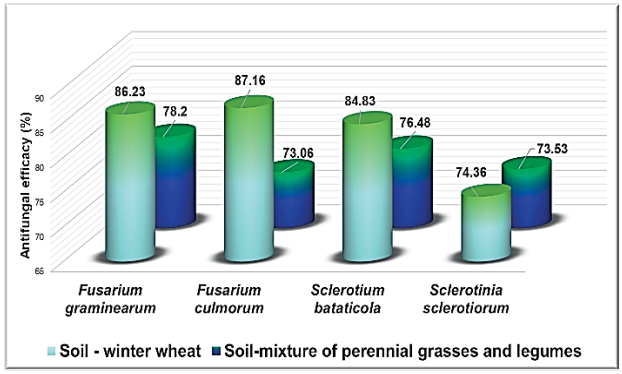


Figure 2. The efficacy of microbial communities to inhibit the phytopathogenic mycelial growths in soil before cultivation of winter wheat and the mixture of perennial grasses and legumes

The microbial load from the soil where soybean and maize where to be sown didn't influenced considerably the *in vitro* development of pathogens tested, the percentages of inhibition of phytopathogenic fungal growth being reduced (Figure 3).

However, in case of pathogens that are specific to cereal crops, i.e *Fusarium culmorum* and *Fusarium graminearum*, a decrease in the growth potential of the fungi in the presence of the microbial community from the soil sample that was to be sown with soybean was

observed. This aspect is less relevant since *Fusarium culmorum* and *Fusarium graminearum* would not have caused economic damage to soybean.

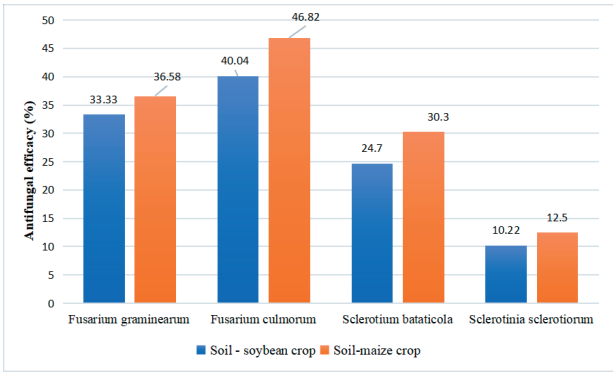


Figure 3. The efficacy of microbial communities to inhibit the phytopathogenic mycelial growths in soil before cultivation of soybean and maize

It is well documented that a good soil quality, which can be characterized by a diverse and abundant microbial community and activity, is a pre-requisite for plant growth and for crop production (Paz-Ferreiro et al., 2016). Both chemical and organic fertilizers can stimulate the growth of specific microorganisms by supplying nutrients and consequently can lead to an increase of microbial abundance and diversity (Dincă et al., 2022).

Cultivable bacteria (10^7 CFU/g soil), chitinolytic, proteolytic, cellulosic microorganisms (10^6-10^7 CFU/ml) and *Rhizobium* bacteria (10^6 CFU/ml) were the most abundant groups in soil samples (Table 4).

The fungi load was lower (10^5) in all treatments and experiments, those results being in line with those obtained by Murphy et al. (1998) who reported a higher bacteria population than fungal one in the soil. The highest number of fungus cells was registered in soil from the mixture of perennial grasses and legumes, in variants V6 (11.5 x 10^5 CFU/g soil), V7 (6.7 x 10^5 CFU/g soil) and V5 (6.6 x 10^5) respectively. The lowest number of fungi was observed in V3 from winter wheat experiment and V4 from maize experiment (1 x 10^5). According to the results obtained, fertilization with 30 t/ha compost and NPK in mixture of perennial herbs can be an option for increasing the fungal population in soil.

Pseudomonas fluorescent bacteria load in the soil from all treatments was lower (10^3-10^4 CFU/g soil), the number of cells varying from 6.8 x 10^4 in V3 (winter wheat experiment) to less than 10^3, in all variants of mixture of perennial grasses and legumes and maize experiments, as well as in V1, V3, V5, V7 of soybean experiment.

The highest cultivable bacteria number (8.9 x 10^7 CFU/g soil) was determined in variant V5 of winter wheat experiment (30 t compost/ha), and the lowest number, in V2 of soybean experiment (1 x 10^7 CFU/g soil). In the mixture of perennial grasses and legumes and maize experiments, there was observed a raised number of cultivable bacteria in variants with chemical fertilizers. This increase may be caused by the direct effect of chemical fertilizer on microbial population (Kanazawa et al., 1988) or because the soil microorganisms may be carbon or nitrogen limited. However, the increase of microbial biomass is significant if soil pH is > 5 (Dincă et al., 2022).

There were no significant differences between treatments in soil cultivated with winter wheat, mixture of perennial grasses and soybean regarding the proteolytic microorganism's presence. In the soil cultivated with maize, the proteolytic microorganisms number varied from 1 x 10^6 CFU (V6 and V8) to 8 x 10^6 CFU (V2).

The highest load (1 x 10^7) for cellulosic microorganisms was observed in variant V5 (winter wheat blocks), followed by V8 (9.3 x 10^6) from soybean blocks and variants V1, V2, V4, V5, V8 (9 x 10^6) in mixture of perennial grasses and legumes and the lowest load (3.5 x 10^6), in V6 (mixture of perennial grasses and legumes experiment).

Rhizobium bacteria were highlighted in the soil from all four experiments.

Thereby, in soybean experiment, V6 registered the highest number of *Rhizobium* colonies (4.3 x 10^6), followed by V3 and V1 (3.8 x 10^6) and the lowest number (1 x 10^6) was observed in the V7, where 60 t compost/ha was applied. In maize experiment, the number of *Rhizobium* bacteria was higher in V3, V6 and V8.

In the mixture of perennial grasses and legumes experiment, a high number of *Rhizobium* bacteria colonies was found in variants V3 and V5 (3 x 10^6 CFU/g soil), followed by V7 (2.5 x 10^6 CFU/g soil) and in the other variants, there were registered 2 x 10^6 CFU/g soil.

Table 4. The microbial composition and abundance in soil cultivated with winter wheat, mixture of perennial grasses and legumes, soybean, and maize under various fertilization treatments

Microbial composition	V1	V2	V3	V4	V5	V6	V7	V8
Winter wheat experiment - mean values								
Fungi (CFU/g)	2×10^5	4.5×10^5	1×10^5	3×10^5	$2.8x\ 10^5$	2.3×10^5	3.5×10^5	3.7×10^5
Cultivable bacteria (CFU/g)	2.2×10^7	2.5×10^7	5.7×10^7	3.4×10^7	8.9×10^7	2.3×10^7	3.8×10^7	2.1×10^7
Fluorescent Pseudomonas (CFU/g)	2.5×10^4	5.7×10^4	6.8×10^4	3×10^4	6.4×10^4	3×10^4	3.5×10^4	4×10^4
Microorganisms that solubilize inorganic phosphorus (CFU/ml)	1×10^6	9×10^5	9.5×10^5	$9.6x\ 10^5$	1.5×10^6	1×10^6	1×10^6	3.5×10^6
Chitinolytic microorganisms (CFU/ml)	5×10^6	8×10^6	2×10^6	1×10^6	3×10^6	4×10^6	1×10^6	8×10^6
Cellulosic microorganisms (CFU/ml)	8.5×10^6	1.5×10^7	4×10^6	7×10^6	1×10^7	6×10^6	4×10^6	8×10^6
Proteolytic microorganisms (CFU/ml)	6×10^6	9.8×10^6	6×10^6	9×10^6	6×10^6	5×10^6	4×10^6	6.5×10^6
Rhizobium like (CFU/g soil)	2×10^6	2×10^6	2.5×10^6	3×10^6	3×10^6	2×10^6	2×10^6	2.5×10^6
Soybean experiment - Mean values								
Fungi (CFU/g)	3.0×10^5	3.8×10^5	3.4×10^5	3.8×10^5	2.7×10^5	3×10^5	4.4×10^5	2×10^5
Cultivable bacteria (CFU/g)	5.5×10^7	1×10^7	3×10^7	4×10^7	2.5×10^7	3×10^7	3×10^7	2×10^7
Fluorescent Pseudomonas (CFU/g)	1×10^4	$\leq 10^3$	1×10^4	$\leq 10^3$	1×10^4	$\leq 10^3$	1×10^4	$\leq 10^3$
Microorganisms that solubilize inorganic phosphorus (CFU/ml)	1.5×10^6	$\leq 10^5$	2.5×10^6	$\leq 10^5$	1×10^6	$\leq 10^5$	1×10^6	$\leq 10^5$
Chitinolytic microorganisms (CFU/ml)	6.5×10^6	2.5×10^6	7.5×10^6	5×10^6	3.5×10^6	3.5×10^6	3.5×10^6	5×10^6
Cellulosic microorganisms (CFU/ml)	4.5×10^6	9.1×10^6	9.2×10^6	9.2×10^6	9.2×10^6	8.5×10^6	9.2×10^6	9.3×10^6
Proteolytic microorganisms (CFU/ml)	7×10^6	7×10^6	5×10^6	6×10^6	6×10^6	6×10^6	6×10^6	5×10^6
Rhizobium like (CFU/g soil)	3.8×10^6	2×10^6	3.8×10^6	3.1×10^6	3.1×10^6	4.3×10^6	1.0×10^6	1.7×10^6
Mixture of perennial grasses and legumes experiment - Mean values								
Fungi (CFU/g)	3×10^5	5.2×10^5	5.9×10^5	$4.4x\ 10^5$	6.6×10^5	11.5×10^5	6.7×10^5	4.3×10^5
Cultivable bacteria (CFU/g)	2.5×10^7	5.6×10^7	3×10^7	5×10^7	4×10^7	4×10^7	5×10^7	5×10^7
Fluorescent Pseudomonas (CFU/g)	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$
Microorganisms that solubilize inorganic phosphorus (CFU/ml)	$\leq 10^5$	$\leq 10^5$	$\leq 10^5$	$\leq 10^5$	$\leq 10^5$	$\leq 10^5$	$\leq 10^5$	$\leq 10^5$

Chitinolytic microorganisms (CFU/ml)	2 x 10^6	1 x 10^6	4 x 10^6	3.5 x 10^6	5 x 10^6	3 x 10^6	3 x 10^6	2 x 10^6
Cellulosic microorganisms (CFU/ml)	7 x 10^6	6 x 10^6	8.5 x 10^6	6 x 10^6	6.5 x 10^6	3.5 x 10^6	4.5 x 10^6	9.5 x 10^6
Proteolytic microorganisms (CFU/ml)	9 x 10^6	9 x 10^6	8 x 10^6	9 x 10^6	9 x 10^6	8 x 10^6	7 x 10^6	9 x 10^6
Rhizobium like (CFU/g soil)	2 x 10^6	2 x 10^6	3 x 10^6	2 x 10^6	3 x 10^6	2 x 10^6	2.5 x 10^6	2 x 10^6
Maize experiment – mean values								
Fungi (CFU/g)	2.3 x 10^5	4.8 x 10^5	4.1 x 10^5	1 x 10^5	2.6 x 10^5	1.3 x 10^5	2.8 x 10^5	1.5 x 10^5
Cultivable bacteria (CFU/g)	2 x 10^7	6 x 10^7	5 x 10^7	5 x 10^7	3 x 10^7	5 x 10^7	2.5 x 10^7	2.3 x 10^7
Fluorescent Pseudomonas (CFU/g)	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$
Microorganisms that solubilize inorganic phosphorus (CFU/ml)	6 x 10^5	7 x 10^5	5 x 10^5	3 x 10^5	9 x 10^5	8 x 10^5	1 x 10^5	1 x 10^5
Chitinolytic microorganisms (CFU/ml)	7 x 10^6	5 x 10^6	4 x 10^6	4 x 10^6	2 x 10^6	1.8 x 10^6	3 x 10^6	2.5 x 10^6
Cellulosic microorganisms (CFU/ml)	6 x 10^6	6 x 10^6	4 x 10^6	5 x 10^6	7 x 10^6	6 x 10^6	4 x 10^6	7 x 10^6
Proteolytic microorganisms (CFU/ml)	6 x 10^6	8 x 10^6	5 x 10^6	3 x 10^6	4 x 10^6	1 x 10^6	3 x 10^6	1 x 10^6
Rhizobium-like (CFU/g soil)	1 x 10^6	2.5 x 10^6	3.6 x 10^6	1.4 x 10^6	2.7 x 10^6	3.1 x 10^6	2.6 x 10^6	3.1 x 10^6

In all cultivated blocks and in all variants, the antifungal activity of soil microbial communities against *Fusarium culmorum, Fusarium graminearum, Sclerotinia sclerotiorum and Sclerotium bataticola* was determined and expressed as a percentage of the effectiveness of inhibiting pathogenic growth (Figures 4 A., B., C., D.).

So, the microorganisms isolated from the soil samples taken from winter wheat blocks showed a medium to high antagonistic efficacy on *Fusarium graminearum* strains, the percentages varying from 52.3 % in V7 (60 t/ha compost) to 79.3 % in variants V2 (NPK) and V8 (Compost 60 t/ha + NPK) (Figure 4 A).

The inhibitory effects against *Sclerotium bataticola* were week in all variants (15.2% in V6 to 48.5% in V4) and against *Fusarium culmorum* and *Sclerotinia sclerotiorum,* week to medium in all variants.

The isolates from the soil within soybean experiment showed week to medium antagonistic activity on all fungi that were tested. The highest percentage for antifungal efficacy was determined against *Fusarium graminearum* (64.5%) in V7 and the lowest, against *Sclerotinia bataticola,* in control variant (V1). The antifungal effect of soil microbes against *Sclerotium bataticola* (which cause infection especially in grain legumes) was very low and varied from 41.6% in V5 (30 t/ha compost) to 11.3% in V1 (control) (Figure 4 B).

In case of microorganisms isolated from soil samples taken from the mixture of perennial grasses and legumes, the antifungal activity against *Sclerotinia sclerotiorum* and *Sclerotium bataticola* was low and against *Fusarium graminearum* was medium, the values varying from 66.7 % in V1 and V3 and 38.9% in V7. For *Fusarium culmorum*, the antagonistic activity was medium in V5 (68.8%) and low in V1 (21.6%) (Figure 4 C).

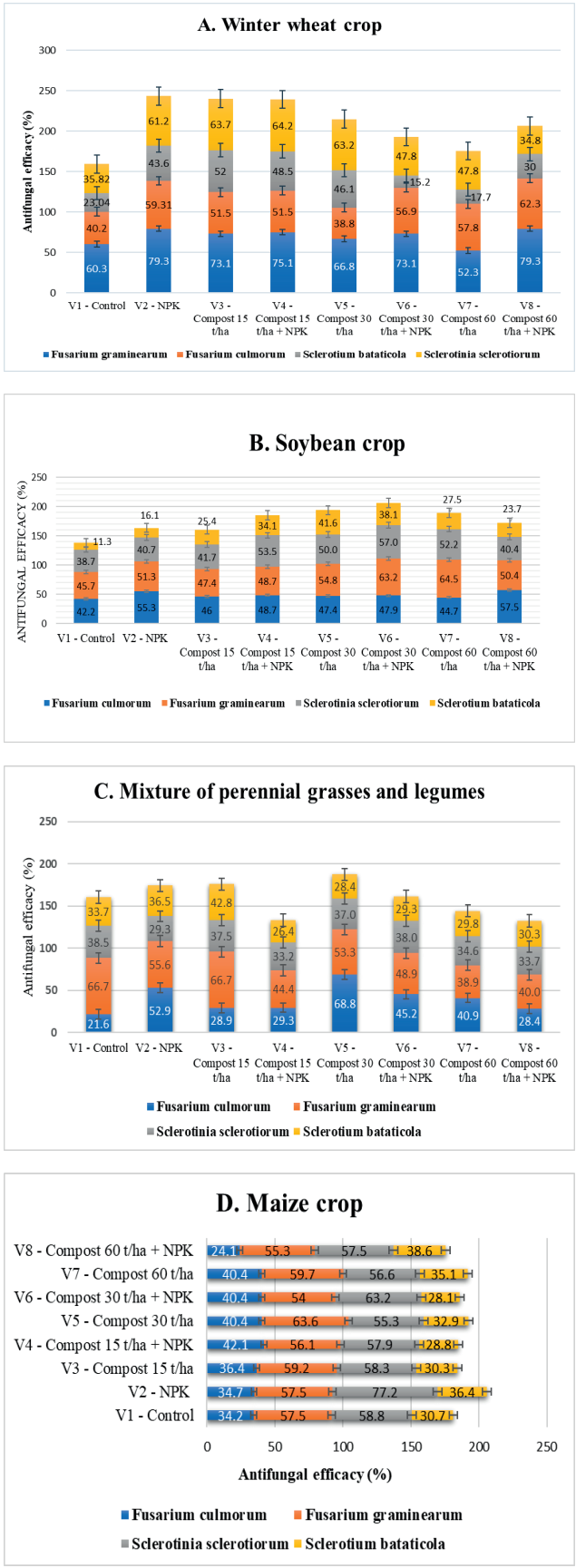


Figure 4 (A., B., C., D.). Mean antifungal efficacy of soil microorganisms from winter wheat, soybean, mixture of perennial grasses and legumes and maize experiments and under different fertilization treatments

In the soil from maize experiment, the antagonistic activity of isolated microorganisms showed a medium inhibition against *F. graminearum* and *Sclerotinia sclerotiorum* and low to medium efficacy against *Fusarium culmorum and Sclerotium bataticola* (Figure 4 D). The values do not differ significantly between the variants, exception for V2 (NPK), where the antifungal efficacy against *Sclerotinia sclerotiorum* was of 77.2%.

CONCLUSIONS

Various studies showed that fertilization affect the microbial community through the influence on soil nutrient contents. In our study, the microbial abundance was influenced both by fertilization regime and by cultivated species, but without significant differences between variants. This correspond to other previous studies in which was shown that changes in the microbial community structure are not always observed on short-term (Stark et al., 2007; Geisseler and Scow, 2014).

The number of bacteria in soil was larger compared with the fungal population. The highest bacterial load was registered in soil cultivated with winter wheat and fertilized with 30 t/ha compost whereas in the soil cultivated with mixture of perennial grasses and legumes and maize, the number of bacteria/g soil raised in variants with NPK fertilization. The increase of microbial number in chemical fertilized plots can be caused by the direct effect of chemical fertilizer.

The fungal load registered a slightly raise in soil from winter wheat experiment in variants where only NPK or 60 t/ha compost and 60 t/ha compost + NPK were applied. Other studies showed as well that organic fertilization increases the fungal abundance in soils (Xiang et al., 2020) and NPK fertilization could increase (Zhou et al., 2016) or decrease it (Xiang et al., 2020). In the soil samples from soybean blocks, fertilization treatments did not significantly change the number of fungi in soil.

Rhizobium bacteria was not significantly influenced by fertilization regimes, except for soybean experiment, where fertilization with high doses of compost determined a slightly decrease of population.

Also, application of different doses of compost and compost + NPK lead to changes on the benefic microbial population in soil cultivated with winter wheat, mixture of perennial grasses and legumes, soybean and maize. A higher number of cellulosic microorganisms were observed in soybean plots, where both organic and mineral fertilizers were used. The proteolytic microorganism's population raised in chemical fertilized variants from winter wheat and maize experiments. The number of chitinolytic microorganisms was larger in winter wheat experiment, when NPK and 60 t/ha compost + NPK were applied.

Although a rich load of microorganisms (including producers of lytic enzymes from the category of those capable of degrading the cellular constituents of phytopathogenic fungi) was found in the soil both before organic and mineral fertilization, and during plants vegetation period, the antifungal efficacy it turned out to be medium to low, exceptions being the winter wheat experiment, where the efficacy of soil microorganisms against *Fusarium graminearum* was medium to high. Agricultural practices such as fertilization can influence the microbial communities that are involved in the suppression of soil-borne plant pathogens, but further investigations are necessary to understand this process. Also, it is necessary to monitor the microbial abundance changes in response to fertilization regime on long-term to develop optimum fertilization strategies.

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