

# Changes in Soil Microbial Activity and Community Composition as a Result of Selected Agricultural Practices

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## Abstract

For a constantly growing human population, healthy and productive soil is critical for sustainable delivery of agricultural products. The soil microorganisms play a crucial role in soil structure and functioning. They are responsible for soil formation, ecosystem biogeochemistry, cycling of nutrients and degradation of plant residues and xenobiotics. Certain agricultural treatments, such as fertilizers and pesticides applications, crop rotation, or soil amendment addition, influence the composition, abundance and function of bacteria and fungi in the soil ecosystems. Some of these practices have rather negative effects; others can help soil microorganisms by creating a friendlier habitat or providing nutrients. The changes in microbial community structure cannot be fully captured with traditional methods that are limited only to culturable organisms, which represent less than 1% of the whole population. The use of new molecular techniques such as metagenomics offers the possibility to better understand how agriculture affects soil microbiota. Therefore, the main goal of this review is to discuss how common farming practices influence microbial activity in the soil, with a special focus on pesticides, fertilizers, heavy metals and crop rotation. Furthermore, potential practices to mitigate the negative effects of some treatments are suggested and treatments that can beneficially influence soil microbiota are pointed out. Finally, application of metagenomics technique in agriculture and perspectives of developing efficient molecular tools in order to assess soil condition in the context of microbial activities are underlined.

## Keywords

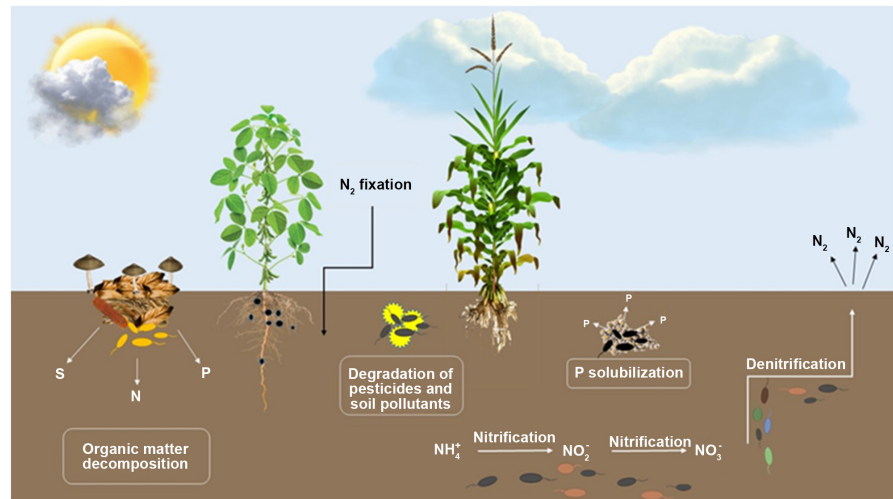
Agricultural Practices, Microbial Activity, Soil Microorganisms

## 1. Introduction

Soil is considered a non-renewable natural resource and it is of great environmental concern to keep it in a healthy and productive state. Sustainable use of agricultural lands has become one of the main interests among researchers, policy makers and farmers [1]. However, in the concept of sustainable farming is rarely the importance of soil microbiota mentioned. Microbiological aspects of soil fertility are extremely important features; however, they are often underestimated or completely neglected. Bacteria and fungi are the organisms that play a key role in the soil formation, decomposition of organic matter, ecosystem biogeochemistry and cycle of nutrients [2] [3] [4]. The nutrient cycle is considered to be a critical ecosystem function essential to life. Some symbiotic microorganisms have a direct positive impact on crop productivity by increasing bioavailability of nutrients such as phosphorus (P) by arbuscular mycorrhizal fungi (AMF) [5] or P-solubilizing bacteria [6]. Also, nitrogen (N) can be provided to the plant through biological nitrogen fixation (BNF) by soil bacteria such as rhizobium. The decomposition of organic matter into simpler molecules is another important service provided by soil microorganisms. It is estimated that up to 90% of degradation processes are carried out by bacteria and fungi [7]. Once the organic matter breaks down, excess nutrients such as N, P and S (Sulfur) are released into the soil and become available to the plants (Figure 1).

Microorganisms also play a very important role in the soil structure and aggregates formation [8], as well as functions related to plant health and pathogens suppression. In the soil system soil-born pathogenic bacteria and fungi are kept under control through competition, predation and parasitism by healthy soil microbial communities [9]. Some bacteria belonging to *Pseudomonas* spp. are known to be able to control plant diseases such as root-rot fungi by rapid root colonization, production of antifungal antibiotics and induction of systemic resistance in the plant [10]. Soil microorganisms are capable of degrading some pollutants and pesticides [11] which are vital for the functioning of agricultural systems. Therefore, it is essential to consider microbiological aspects in the concepts of sustainable land use and responsible agriculture. There is an urgent need for better understanding of the distribution and behavior of microbial communities and their functions in the soil as well as their response to agricultural treatments. Arable lands are highly disturbed through numerous treatments that affect soil physicochemical properties, soil structure and biological activity. Use of mineral fertilizers and pesticides in the intensive crop production causes many environment and health concerns. There are also reasons to believe that agricultural chemicals and intensive farming can negatively affect soil microbiota and reduce soil biodiversity.

On the other hand, there is some evidence suggesting that soil amendments such as green and animal manure, biochar as well as crop rotation (plant diversity) may improve soil microbial activity and diversity [12] [13] [14]. As it is already known, only a small portion of microorganisms are cultivable in *in-vitro*



**Figure 1.** The role of microorganisms in the nutrient cycling in agricultural soils.

conditions. The development of metagenomics techniques opens all range of possibilities to identify and quantify soil microbiota directly from an environmental sample. We can better identify soil and rhizospheral microbial communities and understand how agricultural practices affect specific groups of microorganisms. Therefore, the main objective of this review is to discuss the response of soil microbiota to different agricultural treatments such as pesticides, fertilizers and soil amendment applications as well as plant rotation and GMO (genetically modified organisms) crop cultivation. The special focus is on the soil microbial activity which is an important indicator of soil health and fertility. Furthermore, the importance of metagenomic techniques in agricultural microbiology as a tool to better understand an impact of agriculture on soil microbial communities has been underlined.

## 2. Effect of Selected Farming Practices on Soil Microorganisms

A gram of soil contains billions of microorganisms and an undetermined diversity of them. It is estimated that a microbial diversity of soil might be composed of several hundreds or thousands different taxa [15]. Some estimations of the number of species of bacteria per gram of soil go as high as 8.3 million [16]. Although plant-microbe interactions and ecosystem functions have received much attention in ecology and agricultural sciences [17], the relationship between an abundance of microorganisms and their function become focus of scientists only recently. Less than 1% of microorganisms can be cultured in the classical plate count method [11]. Although culture-based techniques on single organisms may not give field relevant information about the soil functions, some of that research did provide valuable insights on some microorganisms e.g. rhizobia N fixation. Nevertheless, the pure-culture approach, although commonly used in environmental biology, seriously restricts the holistic image of the microbial communities colonizing the soil ecosystem. The soil ecosystem is believed to be

the least understood among all ecosystems. Soil microbiology is still a challenge to scientists trying to understand the way microorganisms metabolize substrates, participate in the nutrient cycle and decompose organic matter.

It was shown that DNA concentration can vary from 0.1 to 7  $\mu\text{g}\cdot\text{g}^{-1}$  in the agricultural soils using different extraction methods, which is lower compared to forest soils where DNA concentration can range from 0.1 to 35  $\mu\text{g}\cdot\text{g}^{-1}$  [18]. Agricultural practices affect microbial activity in the soils, particularly in the soils with long agricultural history. Soil disturbance as a result of different agrotechnical treatments might be the reason why agricultural soils are microbiologically less rich compared to the natural undisturbed soils. Anthropogenic activities can directly or indirectly influence the function and diversity of microorganisms in the soil system [19]. Already decades ago, it was shown that different management systems affect soil microbial composition and abundance. Martyniuk and Wagner [20] showed using standard plate counts methods that microbial populations differ between growing systems. Microbial abundance was significantly lower for untreated soils, intermediate for the soils where chemical fertilizers were applied and higher on the plots treated with manure. In another study it was demonstrated that the biomass of bacteria in two agricultural soils were higher than in the natural shrubs formation while the biomass of fungi was higher in undisturbed natural soils compared to cultivated soils [21]. Tillage destroys the fungal hyphae network and incorporates organic waste into deeper layers of the soil; therefore, it affects negatively the fungi but increase the abundance of bacteria.

### 3. Effect of Pesticides on Soil Microorganisms

To sustain plant growth and protect the crops from diseases and pest infestations, use of pesticides is currently a very common practice. Pesticides are foreign substances in the soil environment, they might disturb microorganisms, affect their behavior and in consequence influence the cycle of nutrients and lead to serious ecological consequences [22]. Degradation pathways and mobility of pesticides depend on biotic and abiotic factors [23] and their lifetime in the soil is strongly related to their physical and chemical properties (Table 1). Many of commonly use pesticides have the capacity to accumulate and stay in the soil ecosystem or leach to the ground water [24]. Contamination with the pesticidal chemicals due to their non-target specification and mobility became one of the main environmental issues in agriculture. Pesticides can get to the soil systems directly from the plant treatment or with the plant organic matter which is later decomposed by the microorganisms. Many of the soil microorganisms have bioremediation qualities and are capable of degrading most pesticides in the soil [23]. A number of studies conducted on agricultural and forest soils demonstrated a positive correlation between the soil microbial biomass and the degradation rate of pesticides such as metribuzin, linuron, glyphosate, alachlor, 2,4-dinitroaniline and dicamba [25] [26] [27] [28]. It was suggested that microbial

**Table 1.** Effect of selected pesticides on microbial activity.

Pesticide	Type	Half-life in the soil <sup>a</sup>	Effect on soil microorganisms	Source
Glyphosate	Herbicide	>35 days	Stimulate soil microbial activity measured by C and N mineralization. Do not affect soil microbial biomass	[42]
			Reduce microbial biomass at higher concentration (3.84 L·ha <sup>-1</sup> ) Temporary inhibitory effect recommended field doses or higher	[43]
Atrazine	Herbicide	60 to 100 days	Decrease dehydrogenase activity	[44]
Paraquat	Herbicide	16 months to 13 years	Decrease dehydrogenase activity	[44]
Captan	Fungicide	10 days	Inhibits denitrifying bacterial activity	[45]
Cypermethrin	Insecticide	2 to 8 weeks	At the rates 10 - 80 µg·g <sup>-1</sup> soil no significant effect on soil enzyme activities ( $\beta$ -glucosidase, urease, acid-phosphatase, and dehydrogenase)	[46]
			Slightly toxic short-term effect on soil biomass, respiration and FDHA activity at the recommended dose	[47]
Acetamiprid	Insecticide	16 days	Strong negative effect on soil respiration and phosphatase activity	[39]

biomass could be a suitable indicator of the soil pesticide degradation capacity [27]. Many researches have been conducted in order to assess the impact of pesticide on microbial communities, soil biomass, respiration, enzymatic activities and other microbial parameters which were reviewed by Chowdhury and colleagues [24]. Generally, most of the pesticides have rather negative impact on microorganisms and they decrease microbial biomass in the soils [29]-[35]. However, the effect of pesticides on the microbial structure and function depends on many factors. The most important factor among them, which can be controlled by the farmer, is the type of pesticide, dosage and time of application. The fluorescein diacetate hydrolyzing activity (FDHA) is a method which is widely used to assess the primary decomposers, bacteria and fungi activity [36]. Zelles *et al.* [37] using FDHA method, studied the effects of some herbicides (4-chloroaniline, atrazine, pentachlorophenol and chloroacetamide), fungicides (zineb and captan) and insecticides (4-nitrophenol and lindane) on the soil microbiota over 48 days. Atrazine, captan and lindane showed insignificant effect. The remaining pesticides induced changes in the activity of microorganisms. In most cases, higher concentrations of pesticides caused reversible or irreversible reductions of FDHA, while the low concentrations sometimes produced a stimulating effect.

Soil respiration, which is the metabolic activity of soil microorganisms quantified by CO<sub>2</sub> evolution, is also used to assess pesticides effects on the microorganisms [24]. Soil respiration is considered to be an efficient bioindicator of microbial condition and of general soil health and quality. The degree of inhibition effect caused by the pesticides depends mostly on the intensity of the stress but also on the time of exposure to the pesticides [38]. Several studies have been conducted to investigate the impact of various pesticides on the soil respiration. The results showed a strong variation mostly between different application doses of a

pesticide. Yao *et al.* [39] tested field concentration (0.5 mg·kg<sup>-1</sup> dried soil) and increased concentration (5 and 50 mg·kg<sup>-1</sup> dried soil) of acetamiprid on the soil enzymes activities and soil respiration. The results demonstrated that the pesticide had a strong inhibiting effect on the soil respiration and enzymes activities at the high application rate; however, no negative effect was recorded at the normal field concentration. Studies conducted by Bartha and colleagues [40] showed that initially, pesticides can increase CO<sub>2</sub> production and subsequently, after a longer time period, decrease the CO<sub>2</sub> evolution in the soil. On the other hand, certain investigations clearly demonstrated the pattern of increased CO<sub>2</sub> evolution after treatment with different types of pesticides [35] [37] [41].

It was also shown that a much higher dose of pesticide compared to the normal field application rate would cause a temporary decrease of respiration rates in the agricultural soil; however the effect was of little ecological importance [31].

More accurate parameter to assess the degree of the disturbances in soil is the microbial metabolic quotient (qCO<sub>2</sub>), which is the respiration rate per unit of biomass. This index is considered to be a more sensitive indicator of the toxicity than soil respiration or microbial biomass alone [48]. Anderson and Domsch [49] suggested that microbial qCO<sub>2</sub> rises due to pesticide application which might force microorganisms to use more of their energy for the cell maintenance. Furthermore, Jones *et al.* [50] showed that the fungicide metalaxyl and the herbicide propachlor application on the soil from the arable and pasture ecosystem caused the disturbance of microbial metabolic quotient for 21 and 15 days respectively. Moreno *et al.* [51] conducted the research to investigate the effect of atrazine on microbial activity. He found that qCO<sub>2</sub> was significantly higher in the soil treated with a higher dose of herbicide than those of untreated soil. Zhang *et al.* [52] studied phospholipid fatty acids (PLFAs) after foliar application of cypermethrin on the pepper phyllosphere microbial community. The results demonstrated that the treatment significantly increased the total and bacterial biomass compared with the control; however, the fungal fatty acids importantly decreased after the pesticide application. DNA and RNA quantification method were used by Bælum, *et al.* [53] in an experiment where the herbicide was applied repetitively to the soil. The results showed that the population of microorganisms that could degrade the pesticide was increased. It could suggest that overall changes caused by the pesticides application are of minor importance perhaps due to fast adaptation of microbial communities to the changing conditions.

The influence of the pesticides on soil microbiota is a very complex process and it depends on multiple factors such as the pesticide structure and concentration, but also environmental factors such as temperature, moisture, soil pH, type, salinity as well as the content of the organic matter [24]. Although most of studies show rather negative effect of pesticides on the soil microbiota, a better comprehension of the soil ecosystem and soil disturbance caused by the soil management is needed. To reduce the negative influence of pesticides on the soil

microorganisms it is necessary to know the mechanisms of action and understand how it affects the microbial function and activities. Furthermore, most of the studies were performed in laboratory setups which poorly reflect real field conditions. To avoid the negative effect of pesticides on the soil microorganisms recommended application rates should not be exceeded.

#### **4. Effect of Fertilizers and Soil Amendments on Microorganisms**

In present days' crop production is almost impossible without the application of mineral fertilizers. Fertilizers can increase productivity and, in a result, increased quality of products in agricultural systems [54]. However, the overuse of fertilizers, which is often observed in modern intensive agriculture, is often the source of many undesirable effects on the environment as well as on human health. Moreover, organic as well as inorganic fertilizers can significantly affect structure and function of soil microbiota which is directly linked to soil fertility and health.

Numerous studies have been conducted to evaluate the effect of different types of fertilizers on soil microbiota. Since a long time, it is known that soil microorganisms respond to the organic matter introduced to the soil system [55]. Therefore, it was not unexpected that bacterial density, CO<sub>2</sub> evolution and the enzymatic activities were significantly increased in poultry dung amended soils compared to untreated soil [56]. Likewise, Bol *et al.* [57] showed the positive effect of poultry slurry and farmyard manure on the enzyme activities in the temperate climate grassland. Zhong *et al.* [14] using PLAF soil profile demonstrated that long-term application of organic manure increases soil microbial biomass, activity and diversity. Lazcano and coworkers [13] compared the effect of mineral and organic (rabbit manure and vermicompost) fertilizers on microbial communities' structure and function. Results showed that manure led to a fast increase in the abundance of PLAF biomarkers for gram-negative bacteria compared to inorganic fertilizer. Also, microbial biomass was higher when the organic fertilizers were applied and generally manure-amended soils showed higher microbial activity than the inorganic fertilizer treatment at harvest time. Moreover, it was demonstrated that both manure and vermicompost increased activity of the soil enzymes that are responsible for degrading organic C, N and P compounds between 12% and 22% compared to soils where, inorganic fertilizers were applied.

On the other hand, numerous studies report the negative effect of mineral fertilizers on soil microorganisms. In a literature review, Allison and Martiny [58] found that from 38 articles as much as 84% reported that microbial communities are sensitive to nitrogen (N), phosphorus (P) and potassium (K) fertilizers. Some meta-analysis [59] based on unmanaged ecosystems found that N application can suppress soil microbiota. However, the responsiveness of the microorganisms in the agricultural land is expected to differ from natural ecosystems. In an agricultural land, the N concentration surpasses the amount of N in a natural

ecosystem due to large application of the fertilizer during the year. High application rates of N fertilizers can temporarily increase levels of osmotic potential, furthermore, some concentrations of N forms can be potentially toxic [60]. Additionally, increased application of N fertilizers is known to cause pH changes in the agricultural soils which strongly influence microbial composition [61]. Fierrer and Jackson [62] found (using ribosomal DNA-fingerprinting) that in 98 samples of soil collected from North and South America, microbial diversity was not related to temperature, latitude and other factors that usually affect plant and animal diversity. The diversity and richness of soil microbial communities depend mostly on the ecosystem type, and the differences between ecosystems are greatly related to soil pH. Generally, it was observed that bacterial diversity was higher in neutral soils and lower in acidic soils. The nitrogen fertilizer application was reported in many long-term field experiments to significantly acidify and change the soil chemical properties [63] [64] [65]. For example, Juo and coworkers [66] demonstrated that ammonium sulfate decreased soil pH from 5.8 to 4.5 during five years of continuous maize cropping. To prevent acidification of agricultural soil and thereby protect soil microorganisms from the stress conditions, common agricultural treatment such as liming is recommended. In tempered climate it can raise the soil pH into the range of 5.7 to 6.5; additionally lime application is considered to have beneficial effects on the soil physical conditions [67].

Geisseler and Scow [61] in their meta-analysis review reported that mineral fertilization significantly increased soil organic carbon ( $C_{org}$ ) content compared to untreated control, by an average of 12.8%. It is explained by the increased plant productivity caused by the use of fertilizers in agricultural systems which increases organic material such as aboveground residues, decomposing roots and exudates which constitute a great source of C available for soil microorganisms. In the same study it was found that mineral fertilizers tend to decrease  $qCO_2$  level and significantly increase enzymatic activity of  $\beta$ -glucosidase and acid phosphatase. Also, microbial carbon biomass ( $C_{mic}$ ) was significantly higher (15.1%) compared to unfertilized plots. It is believed that the major factor contributing to the overall increase in  $C_{mic}$  and enzyme activity is the result of higher content of  $C_{org}$ . Nonetheless, when the fertilizer decreases soil pH below certain level  $C_{mic}$  does not respond to the fertilizer or may even be reduced.

In recent years, biochar has gained importance as a way to deal with global climate changes, by sequestering C into soils, but also as a soil amendment. Biochar and its highly porous structure can provide a suitable habitat for many microorganisms by protecting them from predation and desiccation, providing carbon (C) as a source of energy and mineral nutrients [68]. Głodowska *et al.* [69] showed that some biochars can sustain viability of plant growth promoting bacteria for more than 6 months and that biochar can be a suitable carrier for bacterial inoculation. Anderson *et al.* [70] using TRFLP and new generation sequencing (NGS) techniques, investigated the effect of biochar on the bacterial communities in the pot experiment. The study revealed that biochar amended



soil positively affected the abundance of *Bradyrhizobiaceae* (~8%), *Hyphomicrobiaceae* (~14%), *Streptosporangineae* (~6%) and *Thermomonosporaceae* (~8%) compared to the control. It is hypothesized that biochar application as a soil conditioner alter microbial communities and function mainly through changes in physicochemical properties of soil and introduction of metabolically available C-compounds [71].

Microbial inoculants and so-called bio-fertilizers are promising tools to mitigate the negative effect of mineral fertilizers on the environment due to its plant growth promoting properties and capacity to enhance nutrient availability and uptake. Although there is a lack of consistent evidence that inoculants could replace mineral fertilizers, many studies showed that they are a good supplement to mineral fertilizers [72]. Vázquez [73] found that application of microbial inoculation caused important changes in the microbial community structure in the rhizosphere as well as in the bulk soil of corn. It was shown that esterase activity was increased by *G. mosseae* (256%), chitinase by *G. mosseae* (197%), *G. deserticola* (152%) and trehalase by *G. deserticola* (444%) inoculation. Microorganisms from inoculants are not always able to compete with the native strains in the natural environment; hence, it is believed that bacteria isolated from the soil will perform better in a habitat similar to the one they were isolated from, since they are better adapted to ecological stresses typical for this environment. Introducing foreign microorganisms in the soil environment might bring undesirable ecological effects; therefore, it is important that the environmental fate of inoculant organisms, as well as any effects of their release, is previously assessed [74].

## 5. Effect of Heavy Metals on Soil Microorganisms

The various physiological groups of microorganisms showed a remarkable correlation with relevant soil chemical elements. Because of the agricultural treatments such as the application of sewage sludge, livestock manures, inorganic fertilizers and other agrochemicals, soils are constantly exposed to elevated concentrations of heavy metals [75]. Therefore, it is not a surprise that agricultural soils often show a higher concentration of heavy metal compared to natural, untreated lands. Soil chemical and physical properties such as pH, clay and organic matter content can alter the effect of certain metals on soil microbiota [76]. Increased concentration of some metals has been shown to have unfavorable effects on the diversity, size, as well as the activity of microbial populations in the soil [77]. The danger comes from the fact that these elements accumulate in the soil perpetually often in toxic concentrations and cannot be degraded. Cd, Pb, As, Cu, Ni has the most deleterious effect on living organisms, including microorganisms. Donkova and Dinev [78] conducted the study on a heavily polluted area of non-ferrous metals factory where concentration of heavy metals such as Cd, Pb, Cu and Zn overpassed the Maximum Permitted Concentrations. The results showed that the abundance of bacteria and of the cellulose degrading mi-

croorganisms decreased to 80%, that of the actinomycetes to 50%; on the other hand, a stimulating effect was observed in the case of fungi. The most common case in the arable lands is a combination of several metals together with other pollutants. Therefore, some scientists attempted to combine different factors and tried to understand the pollution complex and its influence on the soil microflora. Wang and Zhou [79] studied the combined effects of cadmium (Cd, 10 mg/kg of soil) and herbicide of the acetanilide class, butachlor (5, 10 and 50 mg/kg of soil) on soil enzymatic activities (urease and phosphatase) and the microbial community structure. It was demonstrated that the effect was strongly related to the ratio of Cd and butachlor added to the soil. When Cd was applied alone, phosphatase activities in soils were decreased, whereas urease activities were insensitive to the Cd addition. On the other hand, phosphatase and urease activities were significantly reduced when a high butachlor concentration (50 mg/kg of soil) was added. Maliszewska-Kordybach [80] proved that the combined effect of some polycyclic aromatic hydrocarbons (PAH) such as anthracene, flourene, chrysene and pyrene with Zn, Pb and Cd can have a stronger effect on the activity of soil microorganisms than the application of these pollutants separately. It was also observed that the reaction of the tested organisms was related to the soil properties as well as the PAHs concentration.

Numerous studies showed that soil microorganisms have different sensitivity to heavy metals. The experiment where the contaminated sewage sludge was applied to the soil and then inoculated with rhizobium demonstrated that after a few weeks of inoculation, the number of *R. leguminosarum* bv. *trifolii* significantly decreased in the contaminated soils [81]. The same was observed with *R. loti* but a *Sinorhizobium meliloti* strain appeared to be considerably less sensitive to heavy metals in the soil. Although some of bacteria show resistance to the higher concentration, generally heavy metals pollution tends to reduce microbial diversity by the extinction of a particular group and enrichment of another which survives in the metal stress conditions [82]. This is considered to be true for other factors that can create stressful conditions for microorganisms, such as mineral fertilizers or different types of pesticides.

## 6. Effect of Crop Rotation on Microbial Communities

The plant and its surrounding soil create a very complex system. There is a close relationship between plants and microorganisms that allows them to coexist [83]. A lot of attention was focused on the endophytes, symbionts, plant growth promoting rhizobacteria and pathogens. However, these microorganisms are just a small portion of the whole microbiome. The remaining, unknown part of the soil microbiome is the majority of the organisms that exist in the soil system. Clearly plant diversity affects soil microbial communities by providing suitable habitat and a source of nutrients. Some microorganisms increase the availability of nutrients in the soils. The most studied example is the legumes which enhance soil quality through their symbiotic relation with nitrogen-fixing bacteria [84]. A

number of studies suggest that the effect of aboveground vegetation is significant to soil microorganisms. Zak *et al.* [85] conducted a long-term field experiment focusing on plant diversity, microbial communities and their functions. It was shown that microbial community biomass, respiration, fungal abundance and N-mineralization rate, significantly increased with greater plant diversity. Similarly, Carney and Matson [86] with the use of the PLFA method showed that plant diversity had a significant effect on the communities' composition. It was found that microbial communities' composition varied with the plant diversity gradient and this was a factor strongly related to the soil catabolic potential. Spehn *et al.* [87] also demonstrated that plant biodiversity affected the microbial communities. When the plant species' richness was reduced, a decrease of microbial biomass was observed. It was also noticed that the presence of specific plants communities such as legumes stimulated microbial population size and function [87] [88].

Microbial physiological groups that use specific nutrient resources are associated with specific plant species, and the rhizosphere is the most colonized region of the plant [89]. Although plants are known to have specific mycorrhizal and nodulation associations developed with the microorganisms at the level of rhizosphere, there are also other relationships at the level of the phyllosphere and endosphere. Plant rhizosphere colonization by the genera such as *Rhizobium* sp., *Azospirillum* sp., *Bacillus subtilis* sp., and *Pseudomonas* sp. has been investigated extensively and the communication patterns are already well understood [84] [90]. From an economical point of view, some of the best know and important nitrogen-fixing symbiosis occurs in the Fabaceae family, for example between soybean and *Bradyrhizobium japonicum* [91], common bean and *Rhizobium etli* [92], lentils and *Rhizobium leguminosarum* bv. *viciae* [91]. The surface properties of the root tissue, nutrient and water availability in the root exudes, metabolites and signaling compounds released by plant roots are factors that attract specific groups of soil microorganisms to the rhizosphere [93]. The range of carbon compounds, such as amino acids and sugars, released by the plant makes the rhizosphere much more abundant in the microorganisms, enhance microbial biomass and activity compared to the bulk soil [94]. For comparison rhizosphere/bulk soil ratio for bacteria, actinomycetes and fungi are commonly in the ranges 2 - 10, 5 - 10 and 10 - 20, respectively [95].

Plant growth promoting rhizobacteria (PGPR) have the potential to increase plant growth by a variety of mechanisms. The most important of them are nitrogen fixation, siderophore production, phosphate solubilization as well as phytohormones production. Because of the environmental concerns the use of PGPR in agriculture is growing regularly offering some alternatives to mineral fertilizers, pesticides and other supplements [96]. Interestingly, phylogenetically distinct microbial profiles in different rhizosphere zones of the same plant are not unusual. For example, it was shown using 16S rDNA profiles generated by PCR-DGGE, different root zones of the same plant can create the habitat for dis-

tinct bacterial communities which is most probably caused by differences in quality and quantity of root exudes [97].

Like all living organisms, microorganisms need energy to conduct their basic metabolic activities. Plant exudes are usually a great source of carbon used by soil microbiota in their metabolic processes. Some of these compounds attract specific group of bacteria. Therefore, there is no surprise that crop rotation significantly influences the soil microorganisms. Bernard *et al.* [12] found that rapeseed green manure rotation had a great impact on soil microbial communities. It caused an increase in the total population of culturable bacteria. Also, observable shifts in microbial communities were determined by sole carbon-source substrate utilization and fatty acid methyl ester (FAME) profiles. Similarly, Hilton *et al.* [98] found that rapeseed rotation had a significant effect on the fungal community. Using TRFLP technique and sequencing, it was shown that continuously grown oilseed rape increased the abundance of pathogenic fungi, compared to the oilseed rape and wheat rotation. Jiang and coworkers [99] studied how crop rotation altered bacterial and fungal diversity in rice paddies. Their survey demonstrated remarkable differences in the diversity and composition of microbial operational taxonomic units (OTUs) among four different crop rotations. They also revealed that Rhizobiales bacteria (genus *Bradyrhizobium*) and Hypocreales fungi are the most cosmopolitan groups, found among four crop systems.

Nowadays, the transgenic plants are gaining a lot of attention; therefore, study has been conducted to evaluate the effects of the GMO and non-GMO crops on soil and rhizospheric bacteria and fungi. Milling *et al.* [100] recorded some differences in the microbial communities of the rhizosphere between transgenic potatoes and its parental cultivar. Similarly, Sessitsch and coworkers [101] found that rape rhizosphere bacteria were affected by genetic modification and herbicide application. However, the effect depended on the plant development stage. Vilvert *et al.* [102] showed significant increase in the microbial biomass carbon and the total microbial biomass in the soil where transgenic soybean was cultivated, in contrast to non-transgenic crops. Reduction in the microbial metabolic quotient ( $qCO_2$ ) in the soil under transgenic crops was also observed. This evidence suggests that GMOs crops are other important aspects to consider in agricultural systems that can affect microbial communities and activities.

## 7. Metagenomics—To Better Understand Soil Microbial Communities

Metagenomics is a new field that combines elements of molecular biology and genetics which allows identifying and characterizing soil microorganisms in the soils through analyses of genetic materials isolated directly from this soil sample. Furthermore, *in situ* analysis of rRNA permits to identify the active taxa and mRNA allow detecting an expression of functional genes in soil which gives a complete image of the structure and function of the soil microbial communities

[11]. Metagenomics studies are considered the most efficient way to see the complete microbiological profile of the bulk soil as well as rhizosphere [103] and the plant. Metagenomic approach allows to look at the diversity of microorganisms present in a sample and to characterize their taxonomic structure and richness. There is an increasing amount of data obtained by these techniques which give much more complete image of the soil diversity and abundance.

In a 13-year field trial in southern Brazil, Souza *et al.* [104] used the shotgun sequencing approach to study the effect of different soils and crop management practices on soil biodiversity. Approximately 1 million reads per treatment were obtained which revealed a very high level of diversity. This could never be achieved using classical methods.

Fierer *et al.* [105], using pyrosequencing-based analyses of 16S rRNA genes, revealed no significant effects of N fertilization on the bacterial diversity, however significant changes were observed in the bacterial community composition. For example, it was observed that the number of copiotrophic taxa (*Proteobacteria* and *Bacteroidetes* phyla) usually increased in the high N plots, with oligotrophic taxa (*Acidobacteria*) demonstrating the opposite pattern. Similarly, Carbonetto and coworkers [106], using the metagenomic approach, observed that microbiomes of cultivated soils of Argentinian Pampas presented tendencies to copiotrophy while non-cultivated soils more oligotrophic lifestyle. Furthermore, the study confirmed the hypothesis that agricultural practices such as tillage, rotation as well as P and N fertilization affect the microbial structure and composition. The phyla such as *Gemmatimonadetes*, *Nitrospirae* were more abundant in cultivated soils while *Verrucomicrobia*, *Plactomycetes*, *Actinobacteria*, and *Chloroflexi* were more abundant in non-cultivated soils. The results suggest that agronomical land use and the tillage induced the changes in the life strategies of soil microorganisms. Metagenomic data provides a great amount of information. However, the interpretation and application of this information in the agriculture are still challenging. There is no doubt that the new cutting-edge molecular techniques can help us to understand the role played by different microbial groups within various ecosystems. Knowledge of the taxonomic groups that dominate in certain types of soil or agricultural systems can be precious information and might help to follow the changes caused by the agricultural practices.

## 8. Conclusions and Perspectives

The evidence reviewed above suggests that soil microbiota is strongly affected by agricultural treatments. To maintain the fertility and productivity of the soil it is extremely important to protect not only the aboveground plant communities but also the underground microbial soil ecosystem. It is very difficult to investigate the behavior and composition of soil microorganisms in agricultural soils which are constantly disturbed and affected by different treatments. Some of them such as the application of organic amendments are known to have a positive influence

on soil microflora. Others, when used inappropriately, cause changes in the microbial profile which can lead to serious ecological consequences. The soil system is extremely complex; therefore, it is continuous work for microbiologists to understand the function and structure of the soil microbiota and how the anthropogenic and natural occurring activities can change it. These shifts in the microbial community structure cannot be fully captured with traditional methods; however, metagenomics approaches can differentiate changes within specific groups and can improve our understanding of phylogenetic and functional changes in the soil. Molecular methods can be used in order to establish key species crucial for maintaining ecosystem services and to determine effects of agricultural treatments on the soil bacterial diversity and abundance. Metagenomic analysis of soil microbiota might be an easy tool to determine soil condition by assessment of microbial relative abundance, biodiversity and phylogenetic structure. Data generated by molecular study can provide very useful and complete information of soil microbiology that can be easily correlated to agricultural treatments as well as soil chemistry. As a result, the understanding of how agriculture affects soil microorganisms should be much easier and assessment of soil health and fertility in the context of microbial activity should be facilitated.

Although many agricultural activities cause environmental problems and are known to have negative effects on soil microorganisms, there are some practices that can help soil microbiota by creating suitable conditions. Maintaining or increasing the soil organic matter by frequent applications of organic fertilizers such as compost, farm yard manure or green manure, is an example of such practices. To reduce the negative effect of mineral fertilizer and pesticides on soil microbiota the integrated farming system is recommended, as well as alternating with microbial inoculants and bio-fertilizers. To overcome side effects of mineral fertilizers such as soil acidification, simple treatments such as lime application is recommended. Additionally, use of soil amendments such as biochar can help to maintain the bacterial abundance and activities by providing an appropriate habitat for soil microorganisms. Finally, crop rotation should be applied in all agricultural systems. It can help to maintain a high abundance as well as a diversity of microbial communities, and by growing legumes it can increase the amount of plant growth promoting rhizobacteria such as N-fixing bacteria.

### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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