



# Impact of Herbicide Use on Soil Microorganisms

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## Abstract

Globally, weeds are a major threat for agriculture production from time immemorial. Discovery of 2,4-D herbicide in the 1940s revolutionized the modern-day agriculture. Since then, nearly 2000 herbicide molecules have been discovered and are used worldwide for the management of weeds in different arable crops. Economic viability coupled with easy application makes it one of the most widely preferred tools for weed management in modern-day agriculture. Herbicide contributes 16% of the global pesticide industry, and in recent years, consumption of herbicide increased many folds due to increased cost of agricultural labour. Researches showed that a number of herbicides have an impact on soil microorganisms. In this chapter, an attempt has been made to document the effect of widely used herbicides on soil microorganisms, especially on mycorrhiza, bacteria and actinomycetes. A number of herbicides showed reduced population of these soil microorganisms with transient inhibition up to 7–10 days. Contrary to that, some herbicides have no effect on microbial population and even increase their population. To overcome the limitation of studies and to generalize the effect of herbicides on soil microorganisms, these studies preferably involves long-term impact assessment having a number of herbicides and variable soil environment.

## Keywords

Pesticide status · Mycorrhiza · Bacteria · Actinomycetes · Limitations

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## 11.1 Introduction

Weeds are one of the major threats to the agriculture for ages. Globally, a number of chemicals are tested and used for weed management from time immemorial. However, the major shift in the use of agricultural chemicals was observed after World War II. Now the chemists and agronomists are overly optimistic about the use of agricultural chemicals in solving the pest problems (Trappe et al. 1984). The introduction of 2,4-Dichlorophenoxyacetic acid (2,4-D) in the 1940s totally revolutionized the agriculture and started the era of chemical weed control (Choudhury et al. 2016). Up till 2016, more than 2000 herbicides belonging to 15 different modes of action were introduced in the global market (Choudhury et al. 2016). Economic viability and easy application make it one of the most common tools for the weed management in modern-day agriculture. Intensity of utilization was further increased with adoption of conservation agriculture practices and herbicide-resistant genetically modified crops.

In fact, use of herbicide increases the profitability of the farm but at the expense of ecosystem functions. It is now apparent that use of these herbicides not only has unforeseen impact on the environment but also severely impacts our soil microflora (Trappe et al. 1984). Of late, Pimentel (1995) raises the concern that only small fractions of pesticide reach to the target organisms, leading to potential impact in soil and water and ultimately affecting the human, crop and animal health. Although it is true that use of pesticide increases the crop production but at the same time it acts as a double-edged weapon, because since the onset of Green Revolution, nearly 800,000 people in the developing countries have died due to pesticides (Devi et al. 2017). Furthermore, nearly, 20,000 people in the developing countries die every year due to pesticide consumption through food (Bhardwaj and Sharma 2013).

Nowadays, the impacts of herbicide use on soil microorganisms are being questioned and at the same time the comprehensive review on this topic is lacking. Keeping these facts, in the current chapter, we review the effect of commonly used herbicides on the soil microorganism.

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## 11.2 Pesticide National and International Status

The use of chemical pesticides is an integral component of crop production in many regions of the world. It is important to note that herbicide constitutes nearly 60% of the total pesticide consumed worldwide (Sondhia et al. 2019). However, in India, the pesticide industry is dominated by insecticide (nearly 65%) followed by herbicide (16%), fungicide (15%) and other (4%) (Subash et al. 2017). In the last 10 years, herbicide consumption increased up to 25% of the total pesticide consumption (Sondhia 2019).

Presently, in India, 68 herbicides are registered for broad-spectrum weed management in various arable crops (Sondhia 2019). In India, the more common herbicide application is in wheat crop (44%), followed by rice (31%), plantation crops (10%), soybean (4%) and other crops (11%) (Sondhia 2019). Trends showed that from 2009 onwards there was a significant increase in the pesticide

consumption, on both total and per hectare consumption. It is important to note the increase in pesticide consumption in recent years has been attributed to the increased consumption of herbicides because of labour scarcity in most regions and higher cost of crop production. In fact, amongst the pesticide production, the share of insecticide declines from 70% in 2003–2004 to 39% in 2016–2017 (Subash et al. 2017). Furthermore, the major herbicide products imported to India are glyphosate and atrazine, which was mainly imported from China (Subash et al. 2017). The top three importers of herbicides in India are China, Israel and Japan. Further, in the recent year, India exports the largest quantity of herbicides to Brazil (20,457.02 tonnes) and USA (6095.06 tonnes). It is noteworthy that India stands at the fourth position in the global supplier of agrochemicals, next only to the USA, Japan and China. India produces 68,490 tonnes of pesticide in 2011–2012 with the total value of Rs 8000 crore, of which worth Rs 6000 crore of pesticide consumed in domestic use. The pesticide market in India is expected to grow at the rate of 12–13% per annum (domestic growth 8–9% per annum and export 15–16% per annum) (Devi et al. 2017). Furthermore, it is important to note that in India although there was severe crop loss due to pest infestation, the intensity of pesticide consumption is lowest in the world (291.2 g/ha). Among the leading consumer of the pesticide in the world are China (14 kg/ha), Japan (11 kg/ha) and the USA (4.5 kg/ha), whereas the world average is 3.0 kg/ha.

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### 11.3 Effect of Herbicides on Soil Microorganisms

In today's world, microbial population in soil is the index of agricultural prosperity. Soil microorganisms are an important link between the soil–plant–herbicide–fauna–man relationships as they play a very vital role in the degradation of herbicides (Raj and Syriac 2017). In both the quantitative and qualitative terms, application of herbicides leads to significant change in soil microbial population (Saeki and Toyota 2004; Raj and Syriac 2017) (Table 11.1).

An ideal pesticide, including herbicide, must possess the ability to act on the target pest as well as detoxify into non-toxic substances as quickly as possible (Stanley et al. 2013). During the initial stage of herbicide application can lead to quantitative and qualitative changes in the soil microbial growth (either stimulating or depressive) and their enzymatic activities, depending on the phytotoxic nature of the herbicide (type and concentration), microbial species and environmental conditions (Latha and Gopal 2010; Zain et al. 2013a; Maheswari and Ramesh 2019). Further, these non-target effects on soil microorganisms may reduce the performance of critical soil functions include organic matter (OM) degradation, the nitrogen cycle and methane oxidation (Sebiomo et al. 2011). It is very important to note that many a time, application of herbicides, in general, reduces the microbial population, including bacteria, fungi, actinomycetes and protozoa, thereby upsetting the soil ecological balance between the plant pathogenic and beneficial organisms,

**Table 11.1** Effect of commonly used herbicides on the soil microorganisms

Herbicide	Concentration	Effect on soil microbes	References
Atrazine	3.4 kg/ha	Transient inhibition of bacterial growth during first WAA, repeated applications have no effect on viable bacteria or fungi population	Cole (1976)
Acetochlor	1.25, 1.50, 2.50, 3.125 and 5.0 L/ha	Bacterial population adversely affected followed by (fb) fungi fb actinomycetes. At crop harvest, microbial population almost equal in all treatment or even more than original level in few treatments	Tyagi et al. (2018)
Oxyfluorfen	850 mL/ha	Soil application cause transient reduction in microbial population	Adhikary et al. (2014)
Propaquizafop	750 mL/ha		
Pendimethalin	3300 mL/ha		
Atrazine	100 µg/L	Application caused complete disappearance of cyanobacteria	Herman et al. (1986)
Paraquat	4 µM	Inhibited the growth of <i>Nitrobacter agilis</i> (nitrite oxidizer), did not affect growth of <i>Nitrosomonas europaea</i> (ammonium oxidizer)	Tateo (1983)
Alachlor	2.5 L/ha, kg/ha	Stimulated the fungal and azotobacter population	Bopaiah and Rai (1979)
Simazine	4.0 L/ha	Stimulated the fungal and azotobacter population	
Propinol	3.5 L/ha	Reduced bacterial population	
Nitrofen	4.0 L/ha	Reduced bacterial population but stimulation in the azotobacter population	
2,4-D-ethylester	0.75 kg/ha	Reduced population of total heterotrophic bacteria	
Butachlor	1.0 kg/ha	Reduced population of total heterotrophic bacteria	Latha and Gopal (2010)
Pretilachlor	0.30 kg/ha	No differences in fungal population at different intervals	
Pyrazosulfuron ethyl	25 g/ha	Reduced population of total heterotrophic bacteria but no differences in fungal population at different interval	
Imazethapyr	90 g/ha	Stimulated growth of bacteria and actinomycetes; inhibited growth of fungi	
Linuron	850 g/ha	Stimulated growth of bacteria and actinomycetes; inhibited growth of fungi	Sawicka et al. (1996)
Glufosinate-ammonium	1, 10 and 100 ppm	Both stimulating and inhibitory effects on microbial populations depending on concentration of the herbicide and the period of incubation	Pampulha et al. (2007)

(continued)

**Table 11.1** (continued)

Herbicide	Concentration	Effect on soil microbes	References
Nicosulfuron	0.3, 1.5, 3.0 and 15.0 mg/kg	Reduced population of actinomycetes in soil and in vitro at highest concentrations of herbicides (10× and 50×)	Šantrić et al. (2016)
Metribuzin	12.0, 60.0, 120.0 and 600 mg/kg		
Atrazine	750 kg/ha	Long-term application altered soil community structure, particularly methanotrophic bacteria	Seghers et al. (2003)
Metolachlor	2000 kg/ha		

thus the disease causing organisms to become a problem (Kalia and Gupta 2004). In fact, change in the soil microflora has been listed as one of the possible causes of the decline in productivity in rice cropping systems (Reichardt et al. 1998).

### 11.3.1 Effect on Mycorrhiza

Mycorrhiza is a mutualistic symbiotic relationship between plants and fungi, located in roots and root-like organs, which act as a bridge for the flow of energy between plant and soils (Traquair 2002; Naher et al. 2013). Of the seven types of mycorrhizae described (arbuscular, ecto, ectendo-, arbutoid, monotropoid, ericoid and orchidaceous mycorrhizae), arbuscular mycorrhizae and ectomycorrhizae are the most abundant and widespread (Siddiqui and Pichtel 2008). Globally, arbuscular mycorrhizal fungi (AMF) formed mutualistic association with more than 80% vascular plant species in the ecosystem (Huang et al. 2009). AMF form mutualistic relationship, where the plant supplying sugar to obligate biotrophic fungus and fungus supplies organic mineral nutrition to plants, particularly immobile nutrients such as phosphorus and zinc (Singh and Singh 2019). In general, forest species are completely dependent on the symbiotic association with ectomycorrhizae for the mobilization of minerals to the plant. These ectomycorrhizae have limited capability to degrade and utilize the complex carbohydrate from the organic detritus. Thus, they rely on the tree for the supply of nutrients (Siddiqui and Pichtel 2008).

To study the effect of herbicides on ectomycorrhizal formation in three conifer species (*Pinus ponderosa*, *Pseudotsuga menziesii* and *Abies concolor*), three herbicides applied at recommended rates and double the recommended rates, i.e. sulfometuron and triclopyr at 4.5 and 9.0 kg a.i./ha and imazapyr 1.1 and 2.1 kg a.i./ha. Irrespective of all the herbicide treatments, ectomycorrhizae were observed on 91% of the root tips and hardly, 7 out of 69 treatment combination showed significant reduction in ectomycorrhizae. One of the reasons for the less effect of these herbicides on mycorrhizal growth might be due to acidic nature of experimental soil and weak acidic nature of herbicides; herbicide molecules are weakly adsorbed on clay micelle and remain active in the soil solution, until degraded or leached (Busse et al. 2004).

In forest nursery of *Pinus sylvestris* and *P. nigra*, application of simazine at recommended rates did not inhibit the growth of ectomycorrhizae and even under some conditions it will enhance the growth of the mycorrhizae (Smith and Ferry

1979). Similarly, application of simazine at the rate of 500–1250 mg/m<sup>2</sup> applied annually did not negatively impact mycorrhizal growth on coniferous seedlings (Uhlir 1966). Later on, it was hypothesized that simazine-induced release of sugar and amino acids from roots would lead to an increase in mycorrhizal growth (Schwab et al. 1982). Furthermore, application of bifenox (3.4 and 6.7 kg a.i./ha), DCPA (11.80 and 23.50 kg a.i./ha) and napropamide (3.4 and 6.7 kg a.i./ha) also showed no significant reduction in ectomycorrhizal growth (Harvey et al. 1985).

A study was conducted to evaluate the effect of differential concentration of prometryn and acetochlor, i.e. 0.1, 1.0 and 10 mg/L, on dual monoxenic culture of *Glomus etunicatum* with Ri T-DNA carrot hairy roots. Both the herbicides negatively affect the AM fungi as well as symbiosis at the higher concentration; in fact, prometryn was apparently more toxic as compared to acetochlor. Furthermore, the spore formation was not affected with the application of irrespective concentrations of acetochlor; however, a significant decrease was noted with higher concentration of prometryn (Li et al. 2013). In pot culture experiment, atrazine added in soil at a concentration of 0.0, 0.5, 2.0 and 5.0 mg/kg soil; interestingly mycorrhizal root colonization decreased with a concentration from 0.0 to 2.0 mg/kg but was increased at a concentration of 5.0 mg/kg. It was hypothesized that enhanced mycorrhizal colonization at higher concentration might be due to the development of tolerance to the pollutant by the fungus (Huang et al. 2007). Moreover, mycorrhizal growth played a significant role in degradation of atrazine applied in maize (Huang et al. 2009).

Later on, application of fluazifop-*p*-butyl [187.5 g a.i./ha] and fomesafen [250 g a.i./ha] in *Phaseolus vulgaris* affected the mycorrhizal colonization under conventional-till system at 12 days after application (DAA), whereas no such effects observed in no-till system (Santos et al. 2006). Trappe et al. (1984) and Paula Jr and Zambolim (1994) were also in opinion that application of herbicide affects the mycorrhizal growth.

In pot culture experiment, repeated extreme exposure of nicosulfuron ( $\times 0$ ,  $\times 10$ ,  $\times 100$ ,  $\times 1000$  the recommended dose) significantly reduced the mycorrhizal colonization and AMF richness. It was hypothesized that limiting establishment of AMF could be the result of either direct toxicity of herbicide on the AMF growth and colonization or indirect effect of maize plant to detoxify the herbicide (Karpouzias et al. 2014). However, Trappe et al. (1984) were in opinion that herbicide possibly alters the metabolism of plants, reduced the photosynthate production, thereby limiting the establishment of AM symbiosis. However, under the field condition, application of nicosulfuron even at the  $\times 5$  level did not significantly change the colonization ability or community structure of AMF (Karpouzias et al. 2014); contrary to this, application of paraquat at the recommended rates (Ramos-Zappata et al. 2012) or chlorsulfuron and glyphosate at higher than recommended rates (Mujica et al. 1999) significantly inhibited the mycorrhizal colonization. Sheng et al. (2012) observed almost similar AMF richness under glyphosate-treated and glyphosate-free plot. Furthermore, there was no significant effect on the rate of colonization in pea roots or wheat roots under both glyphosate-treated and glyphosate-free plots.

### 11.3.2 Effect on Bacteria

Bacteria are minute size (0.5–1.0  $\mu\text{m}$  in diameter and 1.0–10.0  $\mu\text{m}$  in length); unicellular organisms are the most abundant among the soil microflora. Most of the bacteria are adsorbed on the clay particles and humus component present in soil and their number varies with the type of soil, climatic condition and other environmental factors (Biswas and Mukherjee 1987). Reviews written in the early 1960s by Audus (1964), Bollen (1961), Fletcher (1960, 1961), and Smith and Fletcher (1964) were in opinion that most of the herbicides applied at recommended field rates did not bring significant change in soil microbial populations. However, repeated application of 2,4-D (4.48 kg/ha for five times/annum) and trifluralin (1.12 kg/ha, once in a year) over a 5-year period resulted in significant reduction in bacterial population in the soil (Breazeale and Camper 1970). Similarly, in aqueous culture of nitrifying bacteria, low rate of application of paraquat (1  $\mu\text{g}/\text{mL}$ ) leads to complete inhibition of ammonium and nitrite oxidation up to 40 days. However, atrazine (1 and 2  $\mu\text{g}/\text{mL}$ ) leads to transient inhibition of ammonium oxidizing activity for short period, which can be resumed after 16–18 days, whereas the rate of nitrite oxidation was increased at 1  $\mu\text{g}/\text{mL}$  (Gadkari 1988). However, in soil culture even at higher concentration, paraquat (100  $\mu\text{g}/\text{mL}$ ) showed no influence of nitrification; this might be due to the fact that paraquat is strongly adsorbed on the negatively charged clay particles; thus, it may not be accessible to micro-organisms for interaction (Mathur et al. 1976). However, later studies showed that application of paraquat and atrazine at recommended and half-the-recommended rate significantly reduced the bacterial population, diversity and distribution (Stanley et al. 2013). Sebiomo et al. (2011) also noticed that up to 20th day of soil application of atrazine, paraquat, glyphosate and ready-mix atrazine + metolachlor reduced the bacterial population. Even soil application of 2,4-D Ethyl Ester (EE), butachlor, pretilachlor and pyrazosulfuron ethyl at differential rates showed reduction in bacterial population and the highest reduction was observed with butachlor. Further, the decline in bacterial population was enhanced with an increased concentration of herbicide (Latha and Gopal 2010).

Contrary to these experiments, long-term application (9 years) of atrazine (3.4 kg/ha) in maize crop showed transient inhibition during the first week of application and thereafter showed no effect on viable bacteria, as well as relative abundance of bacteria producing hydrolytic enzyme and soil enzyme level (Cole 1976). Later on, Seghers et al. (2003) observed that long-term application of atrazine and metolachlor brought changes in soil community structure; however, these changes did not decrease community function; this might be due to total abundance of methanotrophs in soil was preserved. Similarly, application of pendimethalin, oxyfluorfen and propaquizafop at recommended rates in chilli crop inhibited the soil microbial populations up to 15 DAA; thereafter, treated plots exhibited a significant increase as compared to control. Maximum inhibition was noticed in oxyfluorfen followed by pendimethalin and propaquizafop (Adhikary et al. 2014).

Furthermore, interestingly, it was observed that application of ioxynil, dalapon, mecoprop, MCPA + dichlorprop and amitrole at normal and tenfold rates increased

the population of bacteria after the 2 and 4 weeks after application (WAA) as compared to the control; however, these stimulatory effects are not observed after 20 WAA. However, these herbicides at recommended rates specifically reduced the population of *Azotobacter chroococcum* at 2 and 4 WAA (van Schreven et al. 1970). A similar stimulatory effect was noted with repeated application of dalapon at much higher rates than recommended (Magee 1958). Imazethapyr and linuron applied in soil under legume crops increased the bacteria count by utilizing these herbicides as an additional source of food.

Glyphosate is one of the widely used non-selective herbicides. Earlier studies showed that its application would lead to a temporary increase in bacterial population and overall microbial activity (Wardle and Parkinson 1990a, b). However, later studies confirmed slight reduction in bacterial population (Araújo et al. 2003; Sebiomo et al. 2011).

Studies conducted by Ahmad and Malloch (1995) noticed that the application of phosphinotricin, an active ingredient of glufosinate-ammonium considered as a microbial toxin, significantly decreases the bacterial population. However, later studies conducted for three consecutive years found only 5% of the 300 species of bacteria are sensitive to this herbicide (Bartsch and Tebbe 1989). Moreover, up to 40 days, transient enhancement in bacterial population was noticed at different concentrations (1, 10 and 100 ppm), where the maximum increment noticed with an increase in concentration (Pampulha et al. 2007).

### 11.3.3 Effect on Actinomycetes

Actinomycetes were having the characteristics transitional between bacteria and fungi and are often referred to as fungi-like bacteria that constitute a major group of soil microorganism (Biswas and Mukherjee 1987). Repeated soil application of 2,4-D and trifluralin over the year showed that application of trifluralin increased the actinomycetes population by 89% over the control; however, no significant difference observed in actinomycetes population with the application of 2,4-D (Breazeale and Camper 1970), propinol, alachlor and simazine (Bopaiah and Rai 1979) as compared to the control plots.

Application of the most prominent herbicides in paddy, such as 2,4-D (EE), butachlor, pretilachlor and pyrazosulfuron ethyl revealed that butachlor recorded significantly lower population of actinomycetes as compared to pyrazosulfuron ethyl, pretilachlor and 2,4-D (EE). In fact, the transient inhibition was noticed for 7 days, whereas maximum population was observed at 30 days (Latha and Gopal 2010). Previous experiments also showed a significant reduction in actinomycetes population at variable concentrations (5.5–22.0 µg/g dried soil) of butachlor (Min et al. 2001) and no effect of 2,4-D(EE) on actinomycetes after 40 days (Deshmukh and Srikhande 1974).

Earlier studies revealed that repeated application of glyphosate over the year resulted in reduced population of actinomycetes (Araújo et al. 2003). However, later laboratory study on impact assessment of nicosulfuron, metribuzin and glyphosate applied at four rates, i.e. 1× (recommended), 5×, 10× and 50× revealed that



application of herbicide caused transient inhibitory effect on actinomycetes in soil. Furthermore, the 10× and 50× of herbicides caused a significant inhibition of the number of actinomycetes in soil and growth of the isolates *in vitro*. Glyphosate caused highest inhibitory effect and the results were more pronounced at higher concentration (Šantrić et al. 2016). Similar transient inhibition of actinomycetes population was noticed for 7-DAA of metribuzin (Mohiuddin and Mohammed 2013; Lone et al. 2014) and 15-DAA of pendimethalin, oxyfluorfen and propaquizafop (Adhikary et al. 2014). Application of glyphosate also reduced the actinomycetes population on seventh day, whereas the highest population was noticed on 28th day of treatment (Baboo et al. 2013). Similar reduction in actinomycetes population in soil was recorded with the application of atrazine, atrazine + metolachlor, paraquat, glyphosate (Sebiomo et al. 2011), glufosinate-ammonium (Pampulha et al. 2007) and nitrofen (Bopaiah and Rai 1979).

It is important to note that the inhibitory effect of herbicides on actinomycetes was more under direct exposure (*in vitro*) than their growth in soil treatment (Zain et al. 2013b). Furthermore, the microbes using the herbicide as a source of carbon might be the reason for increased population after second to sixth WAA (Sebiomo et al. 2011).

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## 11.4 Effect of Herbicide on Soil Functions

Application of herbicides affects not only the target organisms but also the soil microorganism. These non-target impacts on soil microorganisms many a time adversely affect the performance of important soil functions. One of the probable side effects of herbicides usage involves disturbance in soil biochemical process occurring in the soil. In fact, many a time, these herbicides hamper the rate of biochemical processes, interfering with the soil enzymatic activity and microbial growth (Maheswari and Ramesh 2019). Soil microbial population plays a significant role in the cycling of nitrogen, sulphur, and phosphorus, and the decomposition of organic residues (Nielsen and Winding 2002). Any alterations in the soil microorganism population or its activity disturb the biological equilibrium in the soil, which may adversely affect the soil fertility.

The soil application of herbicides is toxic to the microbial population, which in turn resulted in reduced microbial biomass, soil heterotrophic respiration and activity of OM decomposing and nutrient-cycling microbes (Rose et al. 2016). In contrast, many a time, it was noticed that the microbial populations and enzyme activities are recovered after initial transient inhibition; this might be due to the fact that the microbe gets adapted to these herbicides or due to their degradation. Simultaneously, where the plants die following herbicide application, the plant debris provides an increased supply of nutrients resource to support microbial growth and activity (Latha and Gopal 2010; Vandana et al. 2012; Sondhia et al. 2013; Maheswari and Ramesh 2019). The increment in soil dehydrogenase (DH) activity in herbicide applied soil after 7th day to 28th day could be attributed to an increase in microbial community having capabilities of utilizing the herbicides as carbon (C) source

(Vandana et al. 2012), whereas the activity of protease depends on the distribution of proteolytic bacteria (Subrahmanyam et al. 2011).

Application of glyphosate at recommended rate did not significantly affect the respiration (Rose et al. 2016), contrary to that; application of pretilachlor enhances the respiration activity as well as biomass (Kumar et al. 2012). The conventional rates of application of alachlor, metolachlor, and butachlor did not affect soil dehydrogenase activity (Dzantor and Felsot 1991; Subhani et al. 2002). Even the application of butachlor (from 5 to 100 mg/kg) significantly reduced the methane production in alluvial rich soil (Mohanty et al. 2004).

Furthermore, application of sulfonylurea herbicides at the recommended rates did not have a significant effect on respiration (Rose et al. 2016). Application of herbicidal mixture of nicosulfuron, atrazine, and dimethenamide did not significantly change the soil methane oxidation rate or the abundance of methane oxidizers (Seghers et al. 2005). Application of imazaquin (0.14 kg/ha) in field-grown soybean had no effect on soil microbial biomass, soil DH, or hydrolase activity (Seifert et al. 2001). However, application of imazethapyr (0.05 kg/ha) decreased the DH activity and increased hydrolyase, protease and catalase activity (Perucci and Scarponi 1994).

Application of atrazine at the rate greater than 100 mg/kg would lead to an increase in soil microbial activity such as respiration and dehydrogenase activity (Moreno et al. 2007). However, application of atrazine to five different soils at recommended rates (5 mg/kg) showed no significant effect on  $\beta$ -glucosidase activity (Mahía et al. 2011). Application of related herbicide terbuthylazine (4 kg/ha) to two different apple orchard soils showed no effect on soil respiration (Hartley et al. 1996); even higher rates of application (10 kg/ha) did not influence the soil respiration or straw decomposition (Hantschel et al. 1994).

Application of 2,4-D at low rates (0.5 mg/kg) produced minor effects on microbial respiration; however, application at higher rates (5 mg/kg) showed transient effects on inhibiting hydrolase activity and stimulating DH activity in the short term, i.e. <24 days (Rose et al. 2016). In another study, Niemi et al. (2009) applied linuron at the recommended rate (0.7 kg/ha) and also at 7 kg/ha, results showed negligible effect on the variety of soil enzyme activity. However, application of linuron and metoxuron at variable rates (5, 50, and 500 mg/kg) produced inhibitory effects on CO<sub>2</sub> evolution at 500 mg/kg, with some minor reduction also observed for linuron at 50 mg/kg. Furthermore, metoxuron 500 mg/kg greatly reduced nitrification, whereas linuron 500 mg/kg showed no effect on mineralization of nitrogen (N) (Grossbard and Marsh 1974).

Several studies revealed that application of prominent herbicides at the recommended rates, such as pendimethalin and difenzoquat (both 0.5–5 mg/kg), or thiobencarb (2.5–25 mg/kg) (Atlas et al. 1978); mesotrione (0.45 mg/kg) (Crouzet et al. 2010); propanil (5 mg/kg) (Kyaw and Toyota 2007); and dalapon at 2.6 or 26 mg/kg (Greaves et al. 1981) has a limited effect on the microbial activity. Moreover, Lewis et al. (1978) surveyed the impact of 25 herbicides, applied at recommended rates and observed no effect of these herbicides on soil microbial respiration and DH activity. In general, the herbicides at the recommended rate of application have non-inhibitory effects on the DH activity (Rao and Raman 1998).

Contrary to that, application of glufosinate-ammonium at variable rates (1, 10 or 100 mg/kg) drastically reduced the DH activity and that too it cannot be recovered even after 40 days of soil incubation (Pampulha et al. 2007).

In a nutshell, we can say that application of herbicides affects the number of soil biological functions, such as respiration, C and N mineralization, OM decomposition, enzymatic activity as well as nutrient cycling in the soil. The magnitude of impact and its direction depend on the number of factors, such as rate and time of application of herbicide and agro-climatic conditions (includes soil properties, temperature and moisture). In fact, a more number of researches on various herbicidal responses under variable conditions is required to estimate the precise impact of the herbicide on soil biological functions and underpinning its mechanisms.

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## 11.5 Limitation and Future Research Needs

It is worth mentioning that in most of the herbicidal impact studies, the experiment was either conducted as auxinic culture, also called soil microcosm, a small-scale experiment containing soil microfauna of field communities offering higher resolution of ecotoxicological effects of chemicals in soil environments (Benton et al. 2007; Adhikary et al. 2014) or the herbicides are directly applied to the soil, and then their final impact was accessed. In diversity assessment study, the primary drawback of the soil microcosm is that the results are biased towards those species which are dominant and fast growing (Hill et al. 2000). However, under the field conditions, a number of other parameters are also acting and modify the herbicide behavior and their impact on the soil microflora, which are not normally taken into account during these experimentations, like the soil physico-chemical property which plays a vital role in fixation of herbicide molecules with the clay particles, temperature and moisture which help in dissipation and alterations in the microfloral population. Pampulha et al. (2007) opine that low clay and OM lead to minimal adsorption of herbicides as well as ensure maximal bioavailability of the herbicides to microbes. Furthermore, the time of application is also not taken into account in these studies; for example, it is true that soil-applied herbicide is applied as either pre-plant incorporated (PPI) or pre emergence (PE) herbicide, where there are more chances of herbicide interaction with the soil microflora but in the foliar applied herbicides, which is generally applied as post-emergence (POE), where the large proportion of herbicides are retained and subsequently absorbed by the foliage, if admixed with surfactants and thus very less quantity of the active toxicant reaches to the ground and interact with the soil microflora. Most importantly, most of the prominent herbicides used nowadays belong to the POE group, such as sulfosulfuron, clodinafop and fenoxaprop *p*-ethyl for monocot weed management and metsulfuron and halauxifen-methyl for dicot weed management in wheat; bispyribac-Na in paddy; imazethapyr in soybean; and mesotrione in maize. Thus, it is better to conduct a comparative study to assess the impact of PE with POE herbicides for quantification of the actual amount of toxicants that reach to the ground and interact with the soil microflora.

## 11.6 Conclusion

In modern-day agriculture, herbicides are the integral component of weed management. In recent years, the consumption of herbicide increased significantly. Herbicidal impact assessment studies involve two methodologies: viz. herbicide is applied in auxinic culture or applied directly in field. Application of herbicide leads to change in soil microbial population, in both the quantitative and qualitative terms. The most widely studied microorganisms affected by the herbicide are mycorrhiza, bacteria and actinomycetes. In general, most of the herbicides at the recommended rate of application either showed no negative effect or transient inhibition for the initial period, with slight contradiction with the few herbicides. Similarly, the herbicide also showed an impact on soil biological function, such as the soil microbial respiration, various enzymatic activities and nutrient recycling. Nevertheless to mention that, in general, the effects of herbicides on biological functions are more pronounced at higher rates of application as compared to the recommended rates. To generalize the effect as well as for precise understanding of the mechanism, more number of long-term studies, involving more number of herbicides and variable environment, are required.

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