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## REVIEW ARTICLE

# Elucidation Of Herbicides Induced Impacts on Soil Microbiome: A Meta-Analysis Using Soil Quality Biomarkers at Agro-Ecological Interface for Sustainable Development

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**ABSTRACT**
*Herbicides have been extensively exploited in the field of agriculture for overall agro-economic developments. Rapid globalization has necessitated the sharp rise in application of herbicides to meet the food demands of the escalating population. Extensive utilization has led to weed resistance, variation in plant tolerance and soil fertility, production of toxic metabolites, altered microbial community structure and biogeochemical processes. The review dealt with the influence of different herbicide treatment such as glyphosate, butachlor and 2, 4 D on microbial community dynamics reflecting their mode of action highlighting the impacts of different herbicides on plants, microbial population and its influence on overall soil quality. Different soil quality biomarkers have been employed to investigate the microbial community structure, offering valuable knowledge regarding ecological effects of herbicide application on soil health. The microbiological and biochemical indicators are recognized as sensitive biomarkers, thar play crucial role in soil quality assessment and elucidate functional status of herbicide treated agricultural soil. The shift in microbial community structure has been analyzed applying high-throughput sequencing and metagenomic techniques. The study emphasizes herbicide driven shift in microbial community structure, highlighting the relative distribution of herbicide metabolizing microbes that are involved in diverse degradation pathways. Overall, the review sheds light on the ecological implications of extensive application of herbicides on microbial community dynamics, which provide deeper understanding for enhancing soil quality for attaining sustainable development.*
*Keywords: Agriculture, herbicides, glyphosate, butachlor, 2,4 D, soil quality*

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## INTRODUCTION

Soil is a non-renewable resource, structured, heterogeneous and discontinuous system where perpetual interactions among available nutrients, microbes in discrete microhabitats and vegetation patterns govern the biogeochemical cycles and plant productivity in terrestrial ecosystems. Soil represents the complex microhabitat with diverse microbial populations that differ with time and space catalyzing biochemical reactions, which prevail under different environmental conditions. Soil quality is correlated with organic matter decomposition, water retention and release, nutrient mineralization and immobilization, regulation of microbial mediated soil processes. Soil variables influence the microbial mediated processes that are intimately related with microbial colonization, which substantiate the concept of soil quality involving their contributions towards crop productivity. Besides, the soil quality biomarkers are closely related with soil physico-chemical, biochemical and microbiological indices and their evaluation is associated with microbial diversity. Hence, the soil quality assessment involves the selection of sensitive indicators followed by linking these soil variables in time scale, which provides meaningful insights into vital soil processes and ecosystem functioning. Expanding global population has necessitated the agricultural intensification resulting sharp rise in usage of herbicides to fulfill food security and demands. Intention of herbicides treatment is to control weeds and unwanted vegetation

below acceptable threshold limit based on minimal negative environmental impacts without causing any significant damage to crops. However, the indiscriminate application of herbicides has negative effects on microbial communities depending on dosage, frequency of treatment and soil properties, which influence microbial community structure. Some herbicides are found to be vulnerable to volatilization, leaching and runoff, which accumulate in soil and water bodies inducing adverse impacts on unintended microbe. Herbicides applied onto foliage indirectly enter surface water through runoff or leachate leading to biological impairments. Extensive usage of herbicides promotes development of herbicides resistant plants either by natural selection or intentional resistance developed in GMO crops. Herbicides applied with ultimate objective to maximize yield and economy that act potentially at the expense of ecosystem functions. Moreover, the overutilization of herbicides leads to the gradual deposition in soil, promote biomagnification in plants and subsequently enter food chain leading to health issues. However, the effect of chemically unique and structurally distinct herbicide on the microbial community structure is different due to their differential degree of resilience to adapt the altered environmental conditions along with their ability for herbicide degradation without losing the ability to support agronomic goals. Further, two primary processes that govern the fate of herbicide application to the soil: (i) the transfer process including runoff, percolation, sorption and desorption, flora and fauna uptake; (ii) the degradation process including photodecomposition, chemical breakdown, microbial decomposition and plant-mediated detoxification. Keeping in view, the ecotoxicological studies based on the impacts of different herbicides and its persistence in soil influencing microbial community dynamics is pivotal for the implementation of effective management strategies. The study represents a holistic approach using combination of quantitative biomarkers that established connecting links between fluxes driving nutrient pool, which can be used for periodic assessment soil quality. Besides, the assessment of microbial community dynamics holds potential as complementary criteria for ecological assessment. The assessment of microbial mediated processes is prerequisite for the implementation of effective management strategies. Assuming microbial community dynamics and ecological restoration are linked, the soil quality and functional status can be evaluated through periodic assessment of microbial community dynamics and functional biomarkers. Considering the outcome based conceptual frameworks of microbial community dynamics-ecosystem functioning using the complex statistical and mathematical predictive models, there is strong recognition of metabolic capabilities exhibited by microbial community structure, their utilization regardless of the magnitude on soil quality assessment as well as these differential patterns of responses reflected by soil variables. Soil seems to be characterized by redundancy of functions and therefore there is need for selection of suitable biomarkers with relatively higher discriminating potentials.

**Herbicides induced impacts on microbial community dynamics**

Exposure of microbial community to herbicides induces microbial community dynamics with the mechanisms for tolerance. The adverse effects of herbicides vary depending on dosage, frequency and soil properties that in turn influence microbial community structure and enzyme mediated activities. Herbicides have been applied in different growth stages for effective weed control such as glyphosate (pre-planting phase) that inhibits amino acid biosynthesis; butachlor (pre-emergence phase): selective systemic herbicide imposing neurotoxic effect through cholinergic impairment; 2, 4-D (post-emergence phase) is involved in growth regulation. Indiscriminate use of herbicides leads to gradual soil accumulation that causes biomagnification in plants and subsequently enters the food chain leading to different health implications. Despite of recognition of microbial community structure in ecosystem sustainability, there is little understanding of the fundamental processes that drive, maintain and alter microbial community structure influencing soil processes. Moreover, the soil variables show differential pattern of responses to same impact reflecting multidimensional soil quality, which makes it difficult to infer functional status of soil sub-system influencing ecosystem functioning. Though various soil quality indicators are selected based on their potency and sensitivity to interpret the differential patterns of responses with efficiency and reliability are rooted to have complex relationships between different soil variables. Besides, the emerging paradigm 'microbial community dynamics-ecosystem function' supplements the soil quality assessment primarily as the functional diversity underlining active influence of microbial communities. Although several soil quality indices are applied to determine the variation in microbial community structure but the ecological significance of microbial community dynamics has not yet been investigated. Keeping in view, the ecotoxicological studies based on the impacts of herbicides and its persistence in agro-ecological interface influencing the microbial community dynamics is pre-requisite to elucidate the soil quality and functionality in terrestrial ecosystems useful for the implementation of effective soil management strategies.

**Classification of herbicides**

Herbicide classification is crucial for comprehending the mechanism of herbicide resistance along with implementation of sustainable agriculture strategies that work [1,2] This is due to the fact that certain herbicide-resistant weeds can still be susceptible to few particular herbicides if the dosage and frequency of application are suitable. Conversely, the overuse of herbicides can either damage the crop or confer resistance that is intended for control, which substantiate the optimal use of herbicides for maximal effect. Herbicides have been classified based on (a) mode of action, (b) selectivity, (c) time of application, (d) chemical structure, (e) site of action, (f) residual action, (g) spectrum of control and (h) formulations (Figure 1).

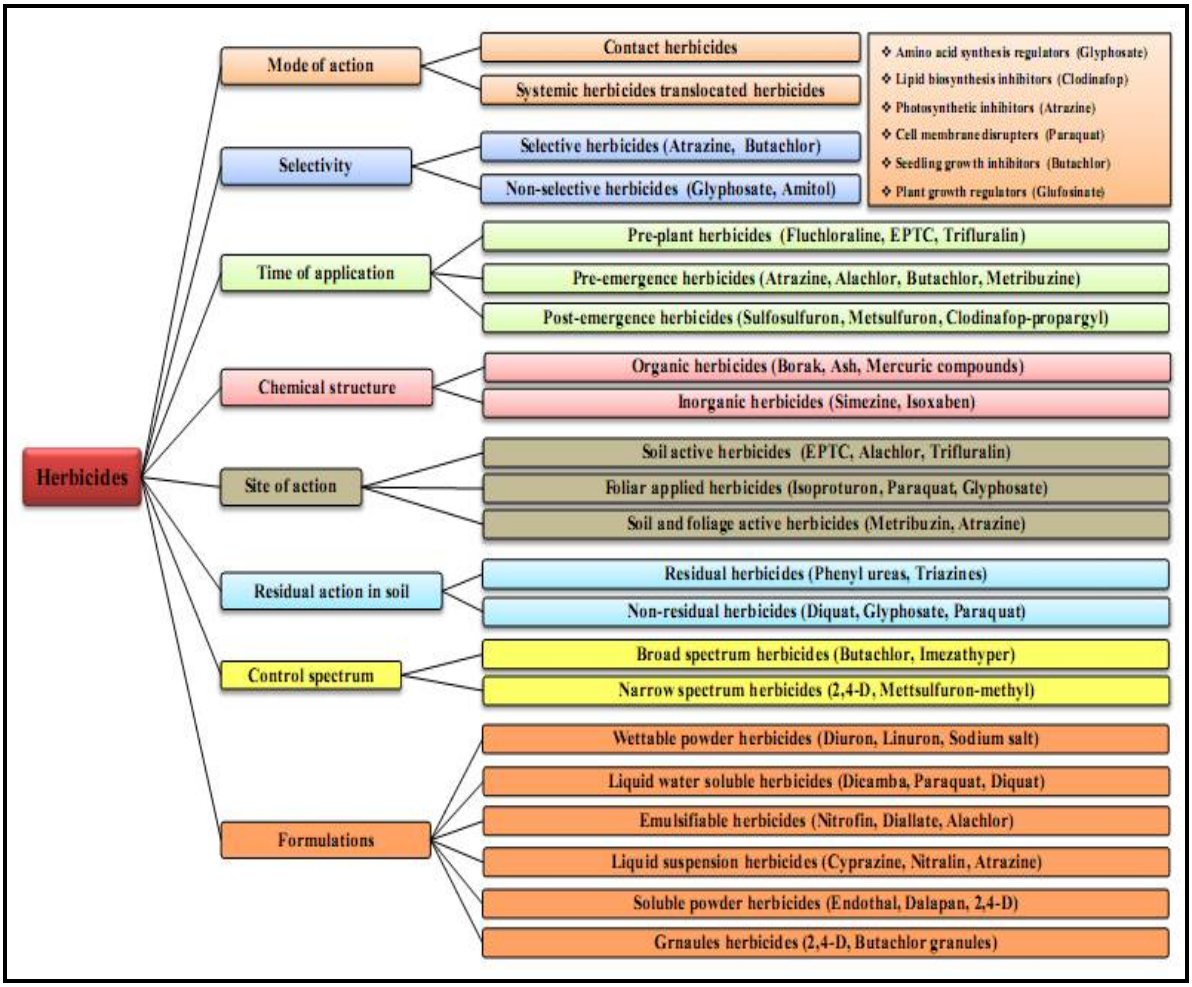



**Figure 1. Schematic presentation of herbicides classified on the basis of their (a) mode of action, (b) selectivity, (c) time of application, (d) chemical structure, (e) site of action, (f) residual action, (g) spectrum of control and (h) formulations.**

Herbicides are classified into contact herbicides and systemic/translocated herbicides based on their mechanism of action. Contact herbicides (paraquat, sulphuric acid, sodium arsenite, ammate) are mostly involved in killing or inhibiting plant growth through direct contact, whereas the systemic herbicides (dalapon, acid arsenical, sodium chloride, glyphosate) are absorbed by plant parts and exert adverse effects on plant growth and metabolism by moving to untreated areas through xylem and phloem of weed depending on the nature of herbicides. Besides, several studies have substantiated different mechamism of action of herbicides through (i) amino acid synthesis inhibitors (glyphosate), (ii) lipid biosynthesis inhibitors (clodinafop), (iii) photosynthetic inhibitors (atrazine, sencor, hyvar, karmex, buctril), (iv) cell membrane disrupters (paraquat), (v) seedling growth inhibitors (butachlor), (vi) plant growth regulators (glufosinate, 2,4-D) and (vii) pigment inhibitors (balance, callisto). Besides, selectivity is the capacity of herbicide to kill target plant without damaging non-target plant species. Few selective herbicides (atrazine, butachlor, 2,4-D, pendimethalin) are highly specific that disrupt the metabolic machinery or biosynthetic pathways of few specific weeds while mostly sparing intended crop. The non-selective

herbicides (paraquat, glyphosate) are formulated to control weeds and toxic to all vegetation. However, the selectivity of herbicide depends on the soil topography, soil fertility, environmental conditions, timing and rate of application [2,3,4,5]. Herbicides are classified into three major categories based upon the time of application: pre-plant, pre-emergence and post-emergence (Figure 1). The pre-planting herbicides (paraquat, basalin, trifluralin, fluchloralin, EPTC) are applied before cropping, which are incorporated into soil to reduce volatility and photo decomposition. The pre-emergence herbicides (simazine, atrazine, alachlor, butachlor, metribuzine) are most effective, which are applied to soil before emergence of crop and weeds. Post-emergence herbicides (paraquat, glyphosate, sulfosulfuron, 2,4-D) are sprayed following the emergence of crop and weeds or herbicides applied after crop emergence but before weeds grow. Moreover, herbicides are classified by their chemical composition such as inorganic and organic herbicides. Inorganic herbicides (simezine, isoxaben, arsenic acid, sulphuric acid, borax, copper sulfate, copper nitrate) contain no carbon actions in their molecules and used initially for weed control before the introduction of organic herbicides. Organic herbicides include polycyclic oils, acrolein, butachlor, alachlor, chloramben, paraquat, carabamates, triazines, urea, uracils, 2,4-D, fluchlorine, bromoxylin *etc*. The plants exhibit differential patterns of interactions with herbicides, which solely based on their absorption, translocation, metabolism and mechanism of action (Table 1). Accordingly, herbicides are either applied to soil during plowing (soil active herbicides) or directly to foliage (foliar applied herbicides). Soil active herbicides (alachlor, butachlor, trifluralin, ETPC) applied to soil are sufficiently active to kill weeds through vertical or horizontal translocation movement, which mostly include pre-emergence herbicides. On the other hand, foliar applied herbicides (paraquat, glyphosate, isoproturon, 2,4-D, sulfosulfuron, metribuzin) are applied to plant foliage that kill upon contact and does not require translocation, which mostly includes post-emergence herbicides. Some herbicides exhibit both function (soil active and foliar applied) such as atrazine and metribuzin. Additionally, the herbicides are classified on the basis of their residual action in soil either residual herbicides or non-residual herbicides. Residual herbicides (triazines, phenyl ureas) are resistant to degradation and remain in soil in active form for longer period allowing longer lasting influence on weed control. In contrast, the non-residual or zero persistence herbicides (diquat, glyphosate, paraquat) are easily degraded or subjected to microbial mediated metabolism after application and therefore do not have long lasting effects on weed control. Herbicides that control particular group of weeds rather than all the weed of particular group are referred to as narrow spectrum herbicides (2,4-D, mettsulfuron-methyl). The 2,4-D specifically kills broad leaf weeds and sedges rather than grasses. In contrast, the broad-spectrum herbicides (alschlor, butachor, imezathyper, atrazine, pendimethalin) control weeds including grasses, broad leaf weeds and sedges. Herbicides are applied with different formulations that influence their efficacy towards weeds control. The wettable powder herbicides (2,4-D, diuron, linuron) exhibit low water solubility and hence grounded into fine powder is used as water suspension. Liquid water-soluble herbicide formulations (2,4-D, dicamba, diquat, paraquat) are used in the form of soluble liquids. Emulsifiable herbicides (nitrofin, diallate, alachlor) are active ingredients dissolved in solvent followed by emulsification, which helps in uniform distribution of toxic chemicals without stirring mostly useful for spraying. Liquid suspension herbicides (atrazine, cyprazine, nitralin) involve the solubilization of active ingredients in organic solvents that are not soluble in water for spraying in form of liquid suspension. Soluble powder herbicides (endothal, dalapan, 2,4-D) include water soluble powders that form homogenous solution in water for spraying. Granules herbicides (butachlor, 2,4-D) are granules with inert clays, the solution of toxicant is sprayed on these granules in desired quantity and dried.

**Table 1. Mode of action of herbicides along with representatives under different categories [6].**

| Mode of action | Target site and effects | Herbicides |
|---|---|---|
| ACCase inhibitors | Few herbicide classes (phenylpyrazolin aryloxyfenoxypropionates and cyclohexanediones) that inhibit the acetyl coenzyme A carboxylase (ACCase), which cause disruption of lipid synthesis. | Clethodim, cyhalofop, diclofop, fluazifop, pinoxaden, sethoxydim |
| ALS inhibitors | Herbicide classes (imidazolinones and sulfonylureas) inhibit acetolactate synthase (ALS) enzyme that disturb biosynthesis of branched-chain amino acids. | Bensulfuron, halosulfuron, imazamox, imazethapyr, metsulfuron |
| Amino acid synthesis inhibitors | Herbicides classes such as imidazolinone, sulfonylurea, sulfonylamino, carbonyltrozolinone that inhibit the biosynthesis of amino acids. | Glufosinate, glyphosate, chlorosulfuron, imazethpyr, flumetsulam |

| | | |
|---|---|---|
| Carotenoid synthesis inhibitors | Herbicides that block enzymes involved in synthesis of carotenoids and chlorophyll. Carotenoids shield plants from oxidative energy, whereas lack of carotenoids cause disruption in protein and membrane. | Amitrole, clomazone, fluridone, mesotrione, norflurazon, topramezone |
| Cell membrane disrupters | Herbicides classes including diphenylether, imine, N-phenylphthalimide, pyrimidinedione, triazolinone and bipyridylium that not only disrupt cell membrane, but also inhibit the synthesis of cell membrane to certain extent. | Bifenox, lactofen, chlornitrofen, fomesafen, flumioxazin, butafenacil, thidiazimin, azafenidin, sulfentrazone |
| Cellulose inhibitors | Herbicides represent heterogeneous groups affecting the assembly and deposition of cellulose, as well as inhibit the cell wall (cellulose) synthesis. | Dichlobenil, indaziflam, isoxaben, quinclorac, flupoxam |
| Fatty acid and lipid synthesis inhibitors | Few chemical classes such as the thiocarbamates, inhibit reactions vital in fatty acid/lipid biosynthesis disturbing the synthesis of biomembrane, proteins, hormones and other cellular components. | Bensulide, butylate, EPTC, molinate, triallate, vernolate |
| Glutamine synthetase inhibitors | Phosphonic acid herbicides inhibit enzyme glutamine synthetase, which lead to increase of ammonia in plants and inhibits PSI and PSII. | Glufosinate |
| Mitosis inhibitors | Chemical families affect various processes involved in cell division, which mostly include chloroacetemides, dinitroaniline and pyridine herbicides. | Alachlor, dimethenamid, metolachlor, oryzalin, pendimethalin, pronamide |
| Photosystem I (PSI) inhibitors | PSI inhibitors disturb electrons during photosynthesis, generate free radicals that disrupt cell membranes and lead to cell and tissue desiccation. | Paraquat, diquat |
| Photosystem II (PSII) inhibitors | Herbicide classes such as triazines, uracils, amides that disturb photosynthesis by blocking the electron transport in PSII. Protein and lipid oxidation generates free radicals that causes plant death. | Atrazine, bromacil, diuron, hexazinone, linuron, propanil, simazine, tebuthiuron |
| Plant growth regulators | Herbicides classes phenoxyacetic acid, benzoic acid and pyridines mostly disrupt the hormone balance and protein synthesis thereby inhibit plant growth, but also induce various plant growth abnormalities | 2,4-D, dicamba, clopiralid, picloram, triclopyr, |
| Seedling growth inhibitors | Herbicides classes dinitroaniline, chloroacetamide and isoxazoline, thiocarbamate, benzofuran inhibit growth of seedlings of annual and perennial weeds. | Butachlor, trifluralin, alachlor, EPTC, benefin |
| Synthetic auxins | Phenoxycarboxylic acids, benzoic acids, pyrachlor and pyridine carboxylic acids mimic endogenous auxins. At higher concentration, the growth regulator herbicides lead to the uncontrolled cell division stimulate ethylene production and growth. | 2,4-D, aminocyclopyrachlor, aminopyralid, clopyralid, dicamba, MCPA, quinclorac, triclopyr, |

The strategic usage of herbicides targeting specific weed need to be controlled, which solely depend on the time and frequency of application, dosage regimes, mode of action, route of application, rate of absorption and retention of herbicides based on the formulations [7,8,9]. Herbicides having higher absorption and retention capacity need less volume and potency in comparison to other herbicides. Efficacy of herbicides depends upon efficient contact (soil or foliage), degree of absorption, translocation and degree of toxicity [6,10,11] keeping pace with time of application and mode of action.

**Advantages of herbicides treatment**

- Application of herbicides as pre-plant and pre-emergence treatment prevents extensive weeds before they emerge from soil thereby minimizing competition during seedling stage.
- Herbicides usage is effective for crop improvement compared to mechanical methods.
- Selective herbicides not only target specific weed control without damaging crops, but also withhold weeds for longer period after application.
- Unlike mechanical methods, the translocated herbicides are used to control deep rooted or vegetatively propagated weeds. However, the combinational approach (chemical and mechanical) is more efficient in controlling weeds.

**Limitations of herbicides treatment**

- Excessive use of herbicides makes differentiation between success or failure of weed control substantiating environmental pollution.
- Herbicide family that belongs to specific group have similar mechanism of action even though

belonging to different chemical families.

- Long residual effects mediated by certain herbicides limit the choice of crop selectivity.
- Acquisition of herbicides resistance by weeds through natural selection or gene transfer leads to the long-term devastating economic consequences.
- Genetic drifts mediated by herbicides influence the productivity of neighboring crops
- Cross-resistance is another alarming issue in agriculture as few weeds may acquire resistance to different herbicides with similar mode of action.
- Rationale of using the herbicide-resistance management strategy compared to passive approach should be quantified based on cost-effectiveness and crop productivity.

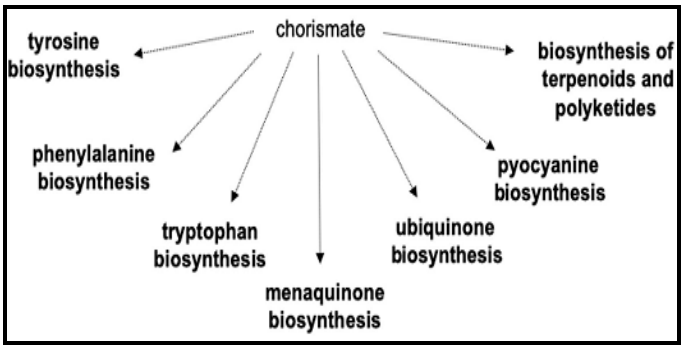
**Glyphosate ($C_3H_8NO_5P$)**

Glyphosate [N-(phosphonomethyl)glycine) is broad-spectrum, non-selective systemic herbicide, which is effective in killing wide varieties of plants, annual broadleaf weeds, grasses and woody plants that compete with existing crops [12]. Glyphosate is aminophosphonic glycine analogue exists in different ionic states based on medium pH. Glyphosate is nonvolatile and does not undergo photochemical degradation. Glyphosate application is relatively safe due to rapid inactivation through adsorption and microbial mediated degradation [13,14,15]. The phosphonic acid and carboxylic acid moieties undergo ionization whereas the amine group is protonated therefore exists as chain of zwitterions. Being acid molecule, glyphosate are developed with isopropylamine, diammonium, monoammonium or potassium as counterion. Glyphosate is formulated using various surfactants/adjuvants such as polyoxyethylene amine to improve the uptake followed by translocation of active ingredient by plants [16,17]. Glyphosate is primarily used before planting of agricultural crops applicable in horticulture, viticulture, silviculture and garden maintenance specifically used for the eradication of invasive species, habitat restoration and enhancement of native plant establishment [18,19].

**Mechanism of action of glyphosate**

Glyphosate is absorbed primarily by foliage of plants whereas minimal through roots, which suggested that glyphosate is effective on actively growing plants and unable to prevent seeds from germination.

Glyphosate is rapidly translocated to the regions of active growth within plant (systemic activity), which is necessary for its effectiveness [20,21]. Glyphosate inhibits 5-enol-pyruvyl-shikimate-3-phosphate synthase [22,23] that catalyzes conversion of shikimate- 3-phosphate and phosphoenolpyruvate to synthesize 5-enolpyruvyl-shikimate-3-phosphate (Figure 1.2). Hence, the biosynthesis of aromatic amino acids (such as tyrosine, phenylalanine, tryptophan) synthesized through shikimate pathway is inhibited [24, 25, 26] that leads to accumulation of shikimate under plant tissues diverting energy and other resources from metabolic processes lead to plant death. The plants treated with glyphosate and its derivatives usually die within (1-3) weeks period because of its even distribution in plant.



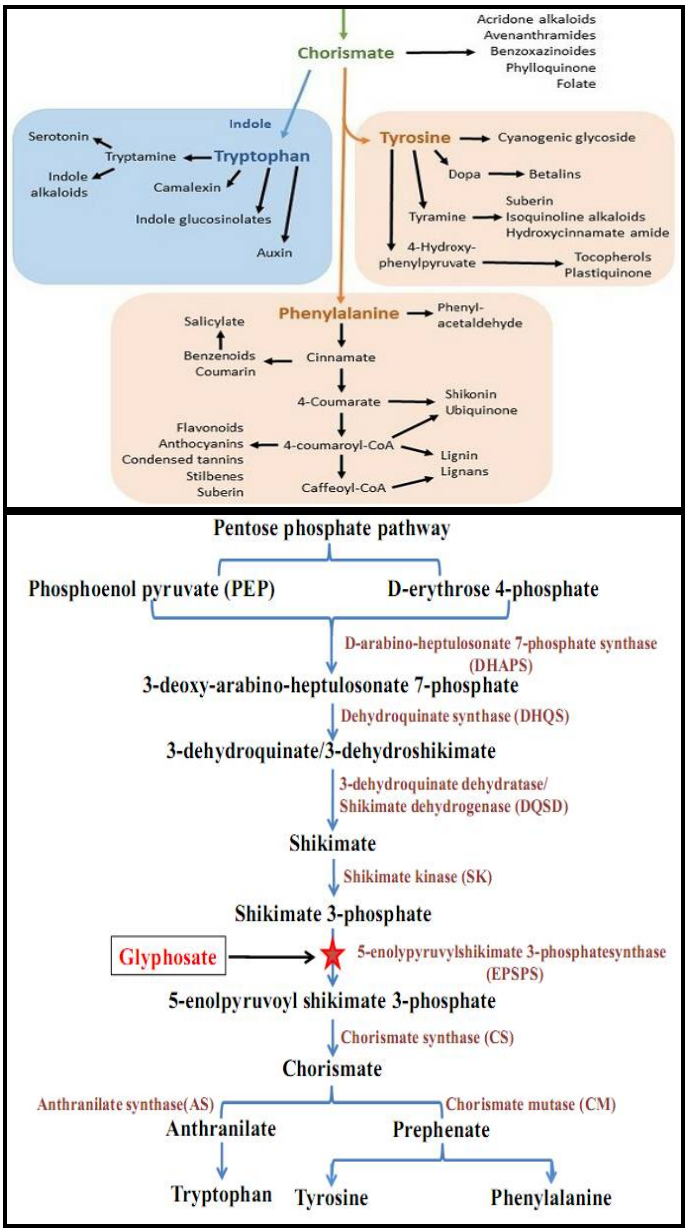



**Figure 2. Glyphosate inhibits 5-endopyruvylshikimate 3-phosphate synthase (EPSPS) in shikimic acid pathway inhibiting the biosynthesis of aromatic amino acids (tyrosine, tryptophan, phenylalanine) [27, 28].**

**Effects of glyphosate on plants and microorganisms**

The sublethal dose of glyphosate soil residues induce underappreciated consequences, which show direct impacts on target species and also cascading effects on non-target organisms influencing different physiological processes including plant defense mechanism with their potential indirect effects on biotic interactions. Glyphosate and its byproduct aminomethyl phosphonic acid inhibit antioxidant enzyme activities that promote the deposition of ROS influencing physiological dysfunctions and cellular damage [29]. Glyphosate is reported to increase chlorophyll degradation whereas AMPA alters chlorophyll biosynthesis resulting in necrosis of plant foliage and plant death [17, 29]. Sub-lethal dose of glyphosate modulates plant defense-related metabolic pathways including (i) the role of phytohormone concentration in regulating biosynthetic pathways; (ii) regulating synthesis of different compounds; (iii) alternations in species-specific plant defense mechanisms; (iv) influencing the plant-associated microbes involved in defense mechanism; (v) plant-microbial community dynamics; and (vi) emission of volatile compounds mediating impacts on plant defense responses. Synthesis of phenylpropanoids involved in plant defense responses, its interactions with abiotic and biotic factors is influenced by chorismate, which is inhibited by glyphosate [30, 31]. Majority of the plant derived secondary metabolites synthesized

through phenylpropanoid pathway reveal decline production following exposure with glyphosate doses [32]. Synthesis of salicylic acid that mediates plant defense responses to pathogens, insects is influenced by chorismate synthesized through shikimate pathway [33, 34]. Besides, glyphosate represses $FAD_2$ mediated enzymatic conversion of oleic acid to linolenic acid through lipoxygenase pathway, which decline the linolenic acid responsible for enzyme metabolization of polyunsaturated fatty acids (Jasmonic acid, oxylipins) essential for defense mechanism [35]. Glyphosate decline the synthesis of phenolic-based volatile compounds (indole, phenethyl acetate) that are essential signalling components involved in indirect plant defense responses [36]. Indole derived from tryptophan plays significant role in volatile emission and indirect plant defense responses decline with sublethal exposure of glyphosate [31]. Sublethal glyphosate dose decline plant growth is correlated with lower level of IAA leading to their faster breakdown [37, 38]. Sublethal glyphosate dose induces higher synthesis of phytohormones (ethylene, abscisic acid) that are directly linked to plant induced defense mechanism and correlated well with increased susceptibility to plant attackers [39]. Adverse impacts of glyphosate-based herbicide led to alternations in soil and aquatic microbial communities influencing their activities through biogeochemical cycling and nutrient turnover [40, 41, 42]. Glyphosate is considered as an antimicrobial agent [43] that targets EPSPS enzyme inhibiting shikimate pathway [44, 45]. Some microbes use glyphosate as the major carbon, nitrogen and phosphorous source for their development [46, 47]. Besides, glyphosate undergoes degradation through two metabolic processes mediated by the glyphosate degrading bacteria such as (a) glyphosate oxidoreductase degrades carboxymethylene N-bond of glyphosate to form AMPA and glyoxylate; (b) the C-P lyases including phosphoenolpyruvate hydrolase, phosphonoacetate hydrolase, phosphonoacetaldehyde hydrolase cleave C-P bond of AMPA leads to the synthesis of inorganic phosphate and sarcosine [47, 48, 49].

**Butachlor ($C_{17}H_{26}ClNO_2$)**

Butachlor [N-(butoxymethyl)-2-chloro-N-(2,6-diethylphenyl) acetamide] is chloroacetanilide class of herbicide, which is used as pre-emergence, selective and systemic herbicide [50, 51]. Besides, it is a non-ionic herbicide mainly used for the pre-emergent control of annual grasses and broadleaf weeds particularly in paddy fields both seeded and transplanted [52]. Butachlor is absorbed majorly by germinating shoot and by root, translocated throughout the plant with relatively greater accumulation in vegetative parts with half-life period (1.65- 2.48) days in field water and about (2.67 – 5.33) days in soil [53]. Because of short half-life period and biodegradability of butachlor substantiates its usage in rice cultivation [54, 55]. Butachlor is degraded by photochemically formed hydroxyl radicals and removed at particulate-phase by wet deposition. Butachlor has no mobility with minimal volatilization in moist soils and hence it is absorbed to soil sediment [56, 57].

**Mechanism of action of butachlor**

Butachlor has gained worldwide recognitions as highly selective and effective acetamide herbicide with efficient weed management strategies. Mode of action mediated by butachlor includes its influence on lipid metabolism, seed germination, gibberelic acid biosynthesis, cell division, cell permeability, amino acids incorporation during protein synthesis, mineral uptake due to alternation in selective absorption [58, 59, 60]. Butachlor alters cell division and cellular metabolism in due to the inhibition in elongase enzyme responsible for long chain fatty acids biosynthesis [61, 62, 63]. Chloroacetanilide herbicides including butachlor also inhibit the gibberellins biosynthesis [64], secondary metabolites [63] as well as enzymes involved in geranylgeranyl pyrophosphate cyclization [50, 65]. The negative impact of butachlor including, growth, chlorophyll fluorescence and photosynthesis of aquatic plants with toxic impacts increasing with concentration have been reported by several workers [63, 66, 67].

**Effects of butachlor on plants and microorganisms**

Butachlor has gained recognition as selective acetamide herbicide specifically used for rice cultivation targeting weeds with minimal adverse effects [63]. Efficacy of butachlor in controlling weeds varies with plant species, exposure concentration, phonological period, frequency and duration of treatment [50, 52]. Exposure of butachlor not only targets weeds but also plants upon exposure with significant phytotoxicity causing death of non-target plants. Plant species exhibited differential patterns of responses to butachlor *i.e.* some species are susceptible inhibiting the plant growth whereas the detrimental impacts on some species even at lower concentration. The dose dependent response of butachlor exhibits concomitant link with inhibition of somatic cell division in plant [52, 68]. Butachlor absorb by plants exert herbicidal effects belowground and induce toxicity upon root tip spindle formation [69] and reduction in shoot length [70] led to its pervasive effect on different plant species. Besides, the variations in total chlorophyll under varying butachlor concentration indicated the dose dependent decline in photosynthetic efficiency of aquatic plant species [63]. Butachlor enhance antioxidant effect by enabling them to prevent oxidative stress within the plant cells [71]. The morbidity caused by butachlor is mutagenic and genotoxic, their

mode of action is unspecific to non-target microbes influencing microbial community dynamics [72]. Being the persistent pollutant in agricultural soil, butachlor influence microbial community structure and enzyme activities [73]. Butachlor negatively impacts the growth and metabolism of beneficial microbes in agricultural soils [74]. Microbial mediated transformation is considered as the efficient route for butachlor degradation in soil [75, 76, 77]. *Bacillus altitudinis* use butachlor as the sole carbon source and involved in butachlor degradation [78]. Besides, the *Sphingobium sp.* and *Mycobacterium sp.* isolated from paddy soil were reported to be involved in butachlor degradation [79]. Several microorganisms have the ability to degrade butachlor [80] including different microorganisms such as *Paracoccus* sp. [81], *Catellibacterium caeni* sp. [82], *Rhodococcus* sp. [83], *Sphingomonas chloroacetimidivorans* sp. [84]. The process of microbial mediated butachlor degradation mostly occurs by hydrolysis via two pathways (a) deacylation and (b) dealkylation (Figure 3). Butachlor was initially deacylated leading to the loss of acetyl group to form N-(butoxymethyl)-N-(2-chloroethyl)-2,6-diethylaniline. Enzymatic formation of N-(butoxymethyl)-2,6-diethyl-N-propylaniline and N-(butoxymethyl)-2-ethylaniline due to hydrolysis of chlorine atoms and dealkylation of nitrogenous alkyl chains. Dechlorination form (2,6-diethylphenyl) (ethoxymethyl) carbamic acid before diacylation [80]. Secondly, the dealkylation leads to the formation of most common degradation products such as 2-chloro-N-(2,6-diethylphenyl) acetamide and butoxymethanol, then subsequently hydrolyzed followed by dealkylation of butachlor catalyzed by different enzymes so as to form catechol and muconic acid, which were eventually converted into $H_2O$ and $CO_2$ [80].

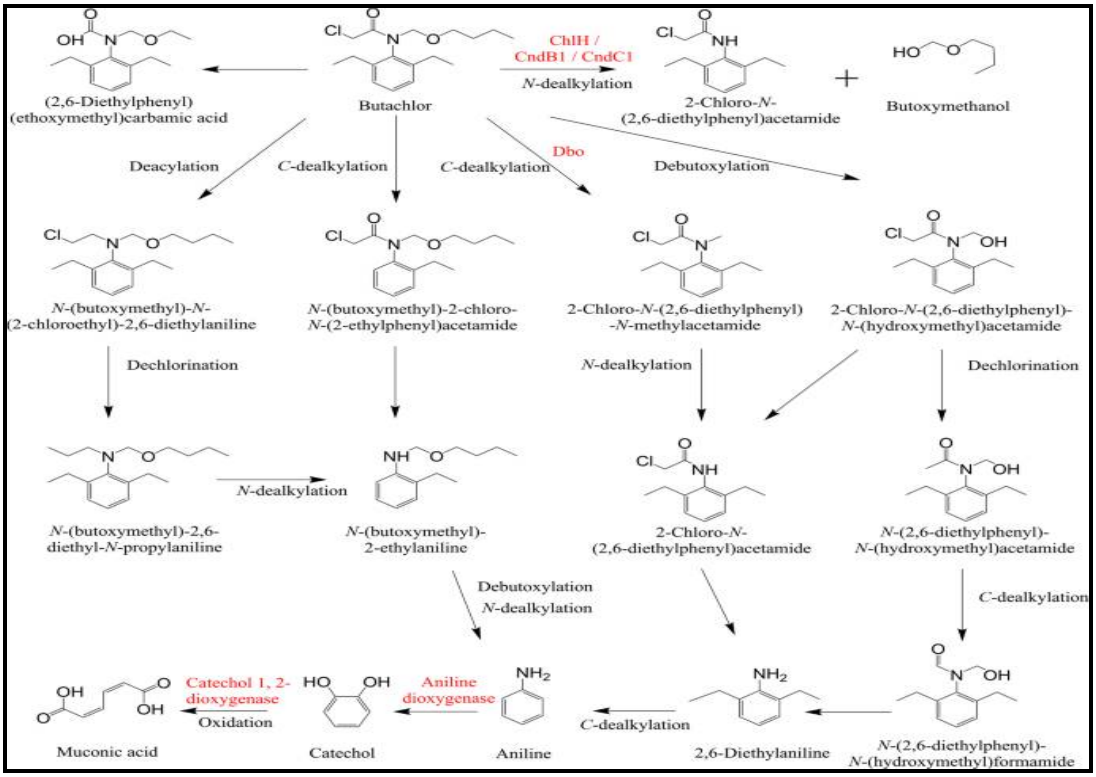



**Figure 3. Microbial mediated pathway involved in butachlor degradation through (a) deacylation and (b) dealkylation [80].**

### 2,4-Dichlorophenoxyacetic acid ($C_8H_6Cl_2O_3$)

The (2,4-dichlorophenoxy) acetic acid (2,4-D) is a post-emergence systemic widely applied herbicide due to selectivity, broad spectrum efficacy in controlling the broadleaf weeds and unwanted vegetation by causing the uncontrolled growth but not applicable for grasses [85, 86, 87, 88]. Basically, 2,4-D is used to inhibit the spread of invasive and non-native annual or perennial weeds thereby minimizing the crowding of native species. Widespread occurrence of the esters and amines of 2,4-D in aqueous ecosystem is due to the presence carboxyl group in soil subsystem [89].

Besides, the commercial formulations of 2,4-D are water soluble that makes herbicide more effective due to its rapid penetration through leaves and roots of plant species [90, 91]. However, efficacy of phenoxy herbicide (2,4-D) is influenced not only by the dynamic interplay between physical, chemical and

biological processes, but also based on their chemical formulations, soil properties, microbial community structure, soil dissipation, bioleaching and its availability to plant species [92, 93, 94].

**Mechanism of action of 2,4-D**

The 2,4-D is an auxin analogue that suppress growth of weeds based on uncontrolled and lethal growth of target plants [95, 96]. Mode of action of 2,4-D is mediated through activation of auxin receptor system inducing variation in actin cytoskeleton and by up-regulation of auxin responses [ 97] and produce higher concentration of reactive oxygen species, which mediates cell wall dismantling and leaky membrane leading to cell necrosis [98, 99]. The 2,4-D induce expression of auxin genes leading to abscisic acid and ethylene biosynthesis and (Figure 4), which inhibit cell division and plant growth, stimulate the leaf senescence leading to plant death [100, 101, 102]. At low concentration, the 2,4-D mimics auxin to promote cell division and elongation whereas controls the broadleaf growth at relatively higher concentration [95]. Different formulations of 2,4-D show diverse effects such as the dimethylamine salts (2,4-D DMA) are polar non-volatile compounds that are absorbed through roots whereas the butylester salts (2,4-D BE) being volatile are absorbed by leaves [96, 102].

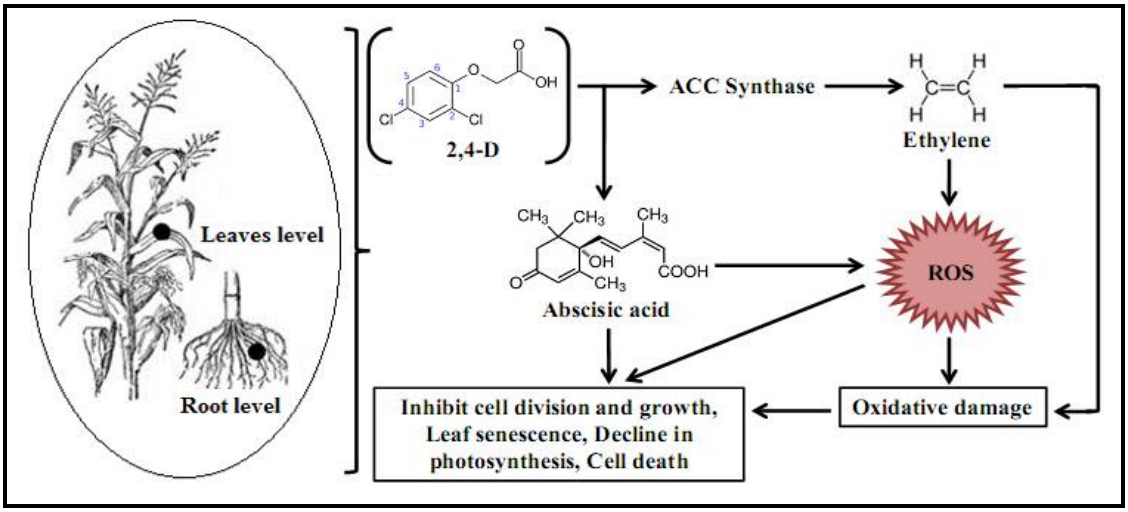


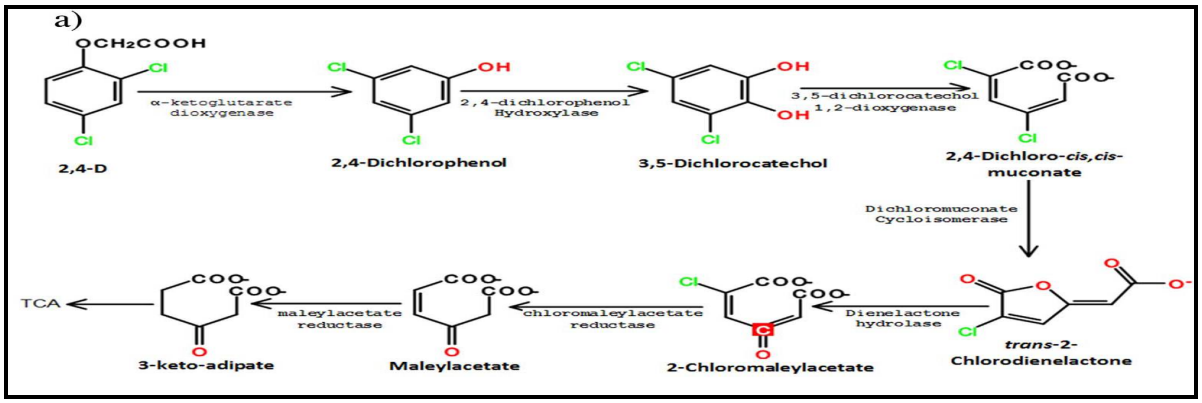

**Figure 4. Mode of action of 2,4-dichlorophenoxyacetic acid mediated by up-regulation of ACC synthase, biosynthesis of abscisic acid and ethylene production, which prevent cell division, promote leaf senescence and oxidative damage due to higher level of ROS leading to cell death [96].**

**Effects of 2,4-D on plants and microorganisms**

The 2,4-D is a moderate persistent herbicide having half-life (20 - 30) days with low adsorption coefficients and high-water solubility [103, 104]. Several factors including plant age, species and soil quality influence the development and maturity of cuticle influencing the herbicide uptake and translocation. The differential pattern of responses induced by 2,4-D showed significant decline in photosynthetic rate, stomatal conductance and transpiration in plants compared to plants without the herbicide exposure [105]. Besides, the reduction in stomatal conductance stimulates ethylene production and abscisic synthesis, which accumulates in leaves and subsequently translocated in plants leading to the stomatal closure thereby limiting the $CO_2$ assimilation and reducing plant yield [106]. Besides, 2,4-D imposes higher risk of drift and causes higher phytotoxicity even with lower dose in non-target crops affecting photosynthesis and plant growth [107]. Plant mediated degradation of 2,4-D herbicide takes place majorly by the direct conjugation, ring hydroxylation followed by side-chain cleavage to smaller extent [108]. The degradation of 2,4-D in an attenuation process influenced by closer interactions of microbial community structure with abiotic and biological processes [109, 110]. Efficacy of 2,4-D biodegradation varies with the relative distribution of microbial community structure, their nature of interactions and catabolic activities (Table 2).

**Table 2. Microbial degradation of 2,4-dichloropehnoxyacetic acid with their degradative potentials [87, 96].**

| Bacterial strains | Isolation habitat | Degradative potential | References |
|---|---|---|---|
| *Delftia acidovorans* | Polluted river in Buenos Aires, Argentina | 200 g $L^{-1}$ of 2,4-D within 24 hrs | 111 |
| *Burkholderia, Dyella, Mycobacterium,* | Agricultural lands in Guanajuato, México | 27.7 mg $L^{-1}$ in 150 days | 112 |
| *Achromobacter sp.* QXH | Mixture of soil and activated sludge | 144 mg $L^{-1}$ in 18 days | 113 |
| *Burkholderia cepacia, Pseudomonas sp.* DS-2, *S. paucimobilis* DS-3 | 2,4-D contaminated soil, Poland | 34.72 mg $L^{-1}$, 36.76 mg $L^{-1}$, 27 mg $L^{-1}$ in 10 days respectively | 114 |
| *Cupriavidus campinensis* BJ71 | Wheat fields, Beijing, China | 200 mg $L^{-1}$ 2,4-D in 3 days | 115 |
| *Cupriavidus necator* N-1 | Agricultural soil, Buenos Aires, Argentina | 250 mg $L^{-1}$ in 3 days | 116 |
| *Pseudomonas sp., Stenotrophomonas sp.* | Wheat fields in Beijing, China | 185 mg $L^{-1}$ of 2,4-D during 7 days | 115 |
| *Achromobacter anxifer* LZ35 | 2,4-D-contaminated soil in Suzhou, China | 200 mg $L^{-1}$ 2,4-D within 2 days | 117 |
| *Corynebacterium humireducens* MFC-5 | Wastewater contaminated with 2,4-D | 9 µmol $L^{-1}$ of 2,4-D in 5 days | 118 |
| *Novosphingobium sp.* DY4 | Paddy field site in Jiangsu province, China | 200 mg $L^{-1}$ 2,4-D within 5 days | 119, 120 |
| *Rhodococcus ruber* | Wastewater treatment plant, Ciudad Real, Spain | 0.071 mg $L^{-1}$ 2,4-D per day | 121 |
| *Bradyrhizobium elkanii* USDA94 | Soybean root-nodulating | 20 mg $L^{-1}$ 2,4-D within 10 day | 122 |
| *Burkholderia cepacia* | 2, 4-D-contaminated soil, Suzhou, China | 60 mg $L^{-1}$ 2,4-D within 3 days | 123 |
| *Cupriavidus gilardii* | 2, 4-D-contaminated soil, Shandong, China | 10 mg $L^{-1}$ 2,4-D per day | 124 |
| *Achromobacter anxifer* LZ35 | 2, 4-D-contaminated soil, Suzhou, China | 50 mg $L^{-1}$ 2,4-D within 12 day | 117 |
| *Cupriavidus sp.* CY1 | Pristine forest soil, Muroran, Japan | 500 mg $L^{-1}$ 2,4-D within 3 day | 119 |




A

B

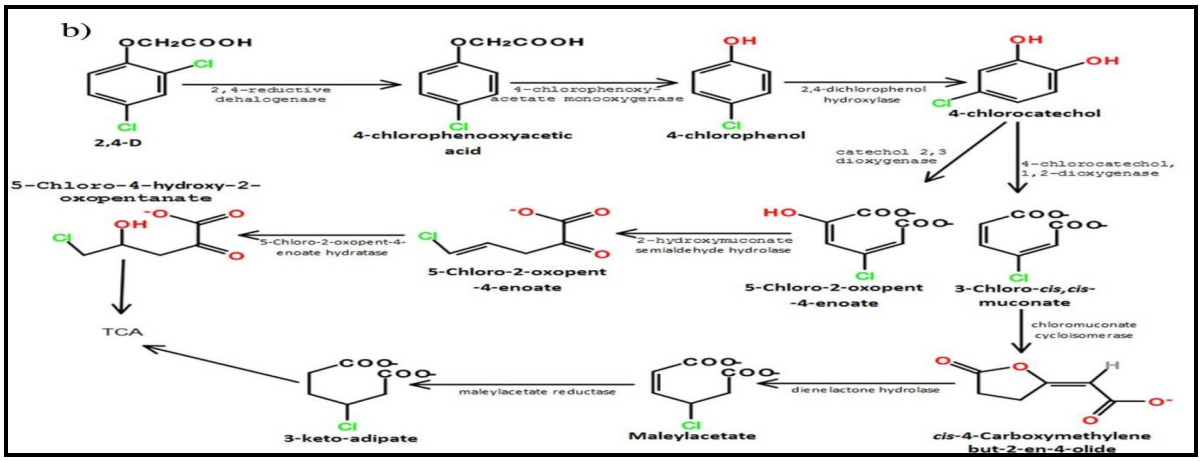



**Figure 5. Biochemical pathways involved in degradation of 2,4-D herbicide mediated by (a) *Cupriavidus necator* JMP 134 (b) *Azotobacter chroococcum* [87].**

The 2,4-D degrading bacteria are categorized into: (a) Group-I includes γ- and β-proteobacteria for predominance of α-ketoglutarate (α-KG) dioxygenase (TfdA) gene catalyzing the initial process in 2,4-D degradation [125, 126, 127], which is sub-divided into three sub-groups (class I–III) based on the tfdA sequences similarities; (b) Group II includes α-proteobacteria have tfdAα gene; (c) Group III includes microbes that are phylogenetically similar to the genus *Sphingomonas* harbouring the tfdAα/cadAB gene [115, 128]. Microbial degradation involves the degradation of 2,4-D by breakdown of ether bond to synthesize 2,4-dichlorophenol or 4-chloro-2-methylphenol by α-ketoglutarate dependent 2,4-D dioxygenase encoded by tfdA gene (Figure 5). Next step includes hydroxylation of phenol compounds to form catechol catalyzed by 2,4-DCP hydroxylase encoded by tfdB gene. Catechol is catalyzed by 3,5-dichloro–catechol dioxygenase encoded by tfdC to form 2,4-dichloro-cis, cis-muconate, which is catalyzed by dichloromuconate cycloisomerase encoded by tfdD into cis 2-dichlorodienelactone. Dienelactone hydrolase encoded by tfdE gene catalyzes conversion of chlorodienelactone to chloromaleylacetate involved in 2,4-D degradation by *Cupriavidus necator* JMP134 [129]. The maleylacetate reductase encoded by tfdF yields 3-oxoadipate that enters intermediary metabolism (Figure 5a). Alternative pathways of 2,4-D degradation (Figure 5b) involving *Azotobacter chroococcum* leading to dehalogenation and formation of 4-chlorophenol instead of 3,5-chlorocatechol through the pJP4 pathway [130].

**Soil quality biomarkers**

Soil represents the structured, heterogeneous and discontinuous complex microhabitat that governs ecosystem functioning based on (a) the shift in physico-chemical, microbiological properties, (b) differential patterns of metabolism and herbicides biodegradation, (c) efficiency of biotransformation, and (iv) microbial community structure [131, 132, 133, 134]. Being difficulties in inferring ecosystem functioning directly due to differential pattern of responses revealed by soil variables to same impact, sensitive biomarkers should be selected to determine the variation in herbicides treated soil over time with equal efficiency and reliability. The concept of pedodiversity has emerged as the functional determinants of ecosystem functioning. The criteria for quality assessment of herbicides treated soil focuses on microbial community dynamics including their interactions with plant-soil system [135, 136, 137] as the patterns observed aboveground is regulated by microbial community structure associated soil processes. Ecological assessment of herbicides treated soil reflect holistic approach involving periodic assessment using reliable soil quality indicators [138, 139]. Such recognition emphasizes the need of sensitive soil quality biomarkers elucidating their intimate relationships with soil physico-chemical and microbiological indices and hence the periodic assessment is necessary to explore their contribution in time scale influencing the dynamic changes in microbial community structure [140]. Besides, the quantitative assessment involves the selection sensitive soil quality biomarkers reflecting the sign of improvement in soil genesis, microbial mediated herbicide degradation through periodic monitoring of the changes in soil nutrients and microbial activities [141, 142]. Effective monitoring requires the sensitive biomarkers sufficiently simple, robust for periodic assessment that provide information about the state of soil [143]. Further, the use of sensitive ecological biomarkers is pre-requisite to explore the site-specific ecology mediated by the adverse impacts of herbicides using sensitive ecological biomarkers at ecological interface for agro-ecosystem sustainability.

**Soil organic matter**

The soil quality assessment of herbicide treated soil from the ecological standpoints encompass physico-chemical characterization including organic C, total N and extractable P as the sensitive indicators reflecting dynamic changes in microbial community structure [144, 145, 146]. SOM promotes aggregate stability and increase hydrological regimes, which acts as the source of nutrients and energy to support vegetation. Soil represents largest organic carbon sink influencing carbon mobilization on the basis of size and breakdown of soil organic matter that promotes nutrient flow through mineralization [147, 148]. Besides, nitrogen is an important soil nutrient mostly derived from organic matter, which prevents growth of vegetation and shift in microbial community structure [149, 150]. Implications towards improving soil quality, the extractable phosphorous is essential to plants as macronutrient, is extracted in organic and inorganic forms through mineralization [150, 151]. Soil carbon C, total N and extractable P are major soil quality indicators that limit soil functioning [152, 153] and shift in microbial community structure [154, 155].

**Microbial biomass pool**

Microbial biomass is defined as the viable component that comprises of living organisms, small standing stock of nutrients reflecting quantity and quality of SOM [145]. Microbial biomass pool is involved in biotransformation, nutrient turnover and structural stability in herbicides treated soil, which led microbial ecologists to use it as sensitive indicators for soil quality assessment and keystone biological driver for ecosystem functions [156, 157]. Pool size of microbial biomass in herbicides treated soil is the functional index of accessing the soil quality [158, 159], biological marker of soil health [160] and labile pool of the plant available nutrients, which determines microbial community structure involved in nutrient conserving mechanisms through immobilization and mineralization [160, 161]. Higher microbial biomass pool size supplements the greater functional diversity among microbial communities in herbicides treated soil [145, 162] and hence considered as the valuable designator useful for soil quality assessment in terrestrial ecosystems. Microbial biomass carbon is referred as viable components of organic carbon through decomposition of organic matter and control nutrient dynamics influencing primary productivity in soil [145, 163, 164]. MB-C is considered as the sensitive index of changes in organic C and hence responds quickly to environmental changes compared to soil organic matter because of its high turnover rate [165, 166, 167]. Microbial biomass nitrogen acts as reservoir of significant part of the potentially mineralizable nitrogen and serves both as transformation matrix and source-sink of nitrogen in herbicides treated soil [168,169]. Hence, the importance of quantifying nitrogen dynamics based on the MB-N assessment reflects nitrogen availability and overall nitrogen cycling in herbicides treated soil [170, 171]. Rapid turnover of microbial biomass P accounts for 2-10% of total phosphorous, which represents the structural stability influenced by microbial mediated phosphorus cycling and availability for plant uptake in herbicides treated soil [168; 169, 172). Immobilization of inorganic P mediated by soil microbes prevent the available P from physico-chemical fixation [173, 174].

**Microbial basal soil respiration**

Basal soil respiration is defined as consistent rate of soil microbial respiration originate through organic matter mineralization and considered as sensitive indicator of biological activity [165, 175], which is estimated based on (a) autotrophic respiration involving $CO_2$ release from plants, and (b) heterotrophic respiration mediated $CO_2$ release due to microbial mediated organic matter decomposition [176, 177]. BSR is influenced by the dynamic interplay between plant root-microbe-soil interactions, available soil nutrients, microbial community structure, microbial biomass and activity [178, 179, 180]. Moreover, the microbial respiration per unit of microbial biomass carbon is defined as 'microbial metabolic quotient' ($qCO_2$), which reflects the eco-physiological status of soil quality [181], as well as microbial activity in herbicides treated soil [157, 182, 183, 184, 185].

**Enzyme activities**

The catalytic efficacy of microbial enzymes involved not only in decomposition of SOM, nutrient cycling and nutrient turnover but also degradation of xenobiotic compounds [186], which quickly respond to anthropogenic activities and hence used as sensitive biomarkers for soil quality assessment [187, 188]. Biochemical reactions are responsive to changes in microbial mediated soil processes influenced by the microbial community structure in herbicides treated soil [189, 190, 191] and hence the microbial community dynamics is likely to be reflected based on the functional integrity of soil [192, 193]. Enzyme catalyzed reaction either intracellular or extracellular reflect nutrient availability in herbicides treated soil due to their rapid response based on soil quality [153, 194, 195]. Nevertheless, the co-existence of differential vegetation patterns with the complexity of microbial community dynamics lead to the increasing heterogeneity in enzyme mediated catalysis in herbicides treated soil [196, 197]. Amylases are involved by hydrolyzing glycogen, starch and polysaccharides to produce oligosaccharides that are

involved in microbial mediated cellular metabolism [198, 199]. Invertase enzyme hydrolyzes sucrose into glucose and fructose useful for soil quality assessment [145, 200]. Proteases degrade proteins and release $NH_4$-N vital for maintenance of N-mediated soil processes including mineralization that regulates plant available N promoting plant growth [196, 201, 202]. Ureases are extracellular enzymes involved in hydrolysis urea to form $NH_3$ regulating nitrogen cycle [155, 194, 203]. Dehydrogenases is used as sensitive biomarker reflecting overall estimate of microbial activity and oxidative index in soil [204, 205]. Being intracellular, dehydrogenase activity provides potential indications governing cellular and biochemical processes essential not only for the assessment of soil quality, but also exhibits closer relationships with microbial community dynamics in different herbicides treated soil profiles [195, 205, 206].

**Microbial community structure**

Being sensitive to environmental and human activities, the contribution microbial community structure in metabolic processes including SOM decomposition, nitrogen fixation, nutrient turnover, biogeochemical cycling and plant growth gain considerable recognition as the sensitive biomarker for soil quality assessment. Soil microbiome varies according to the physiological and nutritional status of herbicides treated soil that directly or indirectly respond to changes in soil system determining functional integrity through changes in taxonomic richness and microbial community dynamics prerequisite in governing ecosystem functioning. Besides, the chronosequence assessment of herbicides treated soil over multiple time scale provides an opportunity to explore microbial community dynamics useful for monitoring soil quality.

**Strategies used to explore microbial community structure**

Due to difficulties in microbial culturing, the culture-independent technique is applied to determine microbial community dynamics using phospholipid fatty acid profiling that reveal not only viable microbial biomass but also physiological soil status [207, 208, 209]. PLFAs confined to microbial membrane are used to unravel microbial community composition reflecting as 'microbial community fingerprint' [210, 211, 212]. PLFA profiling discriminate microbial communities by providing set of molecular markers specific for different microbial taxa, which is used as biomarkers to elucidate microbial community dynamics in soil profiles from diverse origin and land management practices [213, 214]. Besides, the adaptive variations in microbial community structure in various soil profiles were analyzed by community level physiological profiling (CLPP) applying 'BIOLOG system' [214, 215, 216]. Being metabolically heterogeneous with varying metabolic pathways and microbial soil processes, it is imperative to elucidate metabolic profiling of different herbicides treated soil on different days after treatment to analyze functional diversity among microbial communities through community level physiological profiling [217, 218, 219]. Moreover, the shift in average well colour development based on metabolic catabolism of substrate utilization reflects overall changes in microbial community structure that can be correlated with soil quality assessment [214, 220].

**High throughput screening of microbial community structure**

The high throughput sequencing provides platform to elucidate microbial community composition and diversity owing to multiplexing and the meta-data analysis at higher scale [221, 222, 223, 224]. High-throughput sequencing has revolutionized microbial ecology studies due to accurate identification of taxa without culture-dependent approaches [225]. Metagenomic approach provides complete picture of microbial diversity, taxonomic structure and interactions between microbes to reveal their relationships with ecological factors and functional diversity among microbes [226, 227, 228, 229]. High-throughput sequencing is potent technique used to explore the taxonomic profiling, which provides unbiased insights and facilitates the comprehensive metagenomic studies on microbial community dynamics [230, 231, 232, 233]. High-throughput sequencing based impact assessment of glyphosate treated soil was explored by 16 rRNA gene profiles of soil microbiomes [234, 235, 236, 237]. Impacts of butachlor induced shift in microbial communities were analyzed using 16S rDNA sequencing [238]. Microbial community dynamics associated with 2,4-D treated soil using the high throughput 16S rRNA amplicon sequencing was substantiated by several workers [239, 240, 241].

**Microbial community dynamics in herbicides treated soil**

Microbial community dynamics reflects the ecologically significant endpoint used to evaluate the soil quality in relation to the herbicide treatment, which is reflected through the microbial communities at multiple spatial scales influenced by environmental heterogeneity. The shift in microbial community composition occurs even when the size of the microbial community as a whole remains constant that indicates their role as sensitive biomarker of soil quality compared to either their overall microbial community size or metabolic processes. Difference in microbial community structure is correlated with soil organic matter and long-lasting land use practices that influence in shaping microbial community

structure. The functional outcomes of microbial mediated interactions, triggered by the short-term or long-term herbicides applications through competitive and cooperative mechanisms, play significant role in supporting the ecosystem functioning [241, 242]. The shift in microbial community structure upon the long-term glyphosate exposure followed by stimulation of microbial respiration was suggested by several workers [163, 237, 243, 244]. Glyphosate mediated stimulation in microbial activity and functional diversity of cultivable heterotrophs involved in glyphosate biodegradation was reported [245, 246, 247]. The distribution of glyphosate and its byproduct aminomethyl phosphonic acid not only induce oxidative stress influencing microbial community dynamics, but also alter nutrient cycling [248, 249, 250, 251]. The sorption and desorption dynamics of glyphosate with soil matrix and physico-chemical properties determine its bioavailability thereby influencing microbial community structure [252, 253]. Repeated use of butachlor followed by its soil persistence inducing functional diversity, microbial metabolic activities, enzyme activities and microbial respiration because of the shift in microbial community structure [254, 255, 256], which poses potential impacts to agro-ecology and health issues through food chain [257, 258]. Being pre-emergence herbicide, butachlor influences nitrogen fixing abilities of soil microbes altering microbial community structure [259]. Higher exposure of butachlor alters soil enzyme activities, microbial respiration [260, 261, 262], which were proved to be powerful ecotoxicological tool for soil quality assessment. The dose dependent response with elevated level of butachlor towards the shift in microbial metabolic quotients and microbial community structure has been suggested [262]. The dose-dependent response of 2,4-D influencing microbial community dynamics was substantiated by several workers [96, 263, 264, 265]. The frequent use of 2,4-D triggers the degradation pathways with existing microbes leading to structural and functional shifts in overall microbial community structure in soil [266, 267, 268]. Exploration of the multidimensional applications of 2,4-D as prominent herbicide, it is crucial to explore microbial mediated degradation and subsequent elimination of 2,4-D through complex processes involving metabolism, conjugation and efflux pump transport mechanism lead to variation in microbial community structure [269, 270, 271].

## CONCLUSION

The heterogeneity in soil variables within landscape in different days after herbicide application influence microbial community dynamics across the sites. Such variations may be attributed by gradual shift in microbial community composition, which highlights the approach prerequisite for investigating soil quality status at agroecological interface over time. Microbial community structure responds to herbicide persistence associated with toxicity, which induced decline in available soil nutrients and soil functional changes through the alternations in microbial biomass pool, basal soil respiration and enzyme activities. Due to inherent difficulties in soil quality assessment, the real-time ecological assessment necessitated the selection of the potential soil quality biomarkers to determine adverse impacts of different herbicides influencing soil functional status over time. The review emphasized the integrative measure of microbial community dynamics influenced by indiscriminate use of herbicides. Being sensitive to the persistence of herbicides mediated toxicity, the exploration of microbial community dynamics through catabolic profiling on the basis of available nutrients and enzyme activities is essential, which is considered as effective approach to elucidate functional diversity influencing soil quality.

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