

**PUBLIC TESTIMONY  
SUBMITTED FOR  
JULY 29, 2024  
SPPDAC MEETING**

**From:** nancy webster <[REDACTED]>

**Sent:** Monday, July 29, 2024 6:45 AM

**To:** ron clemen <[REDACTED]>; Alesia Franken <[REDACTED]>; Jay Udelhoven <[REDACTED]>; lydia hess <[REDACTED]>; Jason Maxfield <[REDACTED]>; [s.l.johnson](mailto:s.l.johnson) <[REDACTED]>; [charlesmcneilly](mailto:charlesmcneilly) <[REDACTED]> City Hall <[cityhall@corb.us](mailto:cityhall@corb.us)>

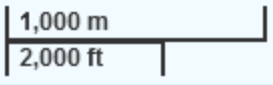
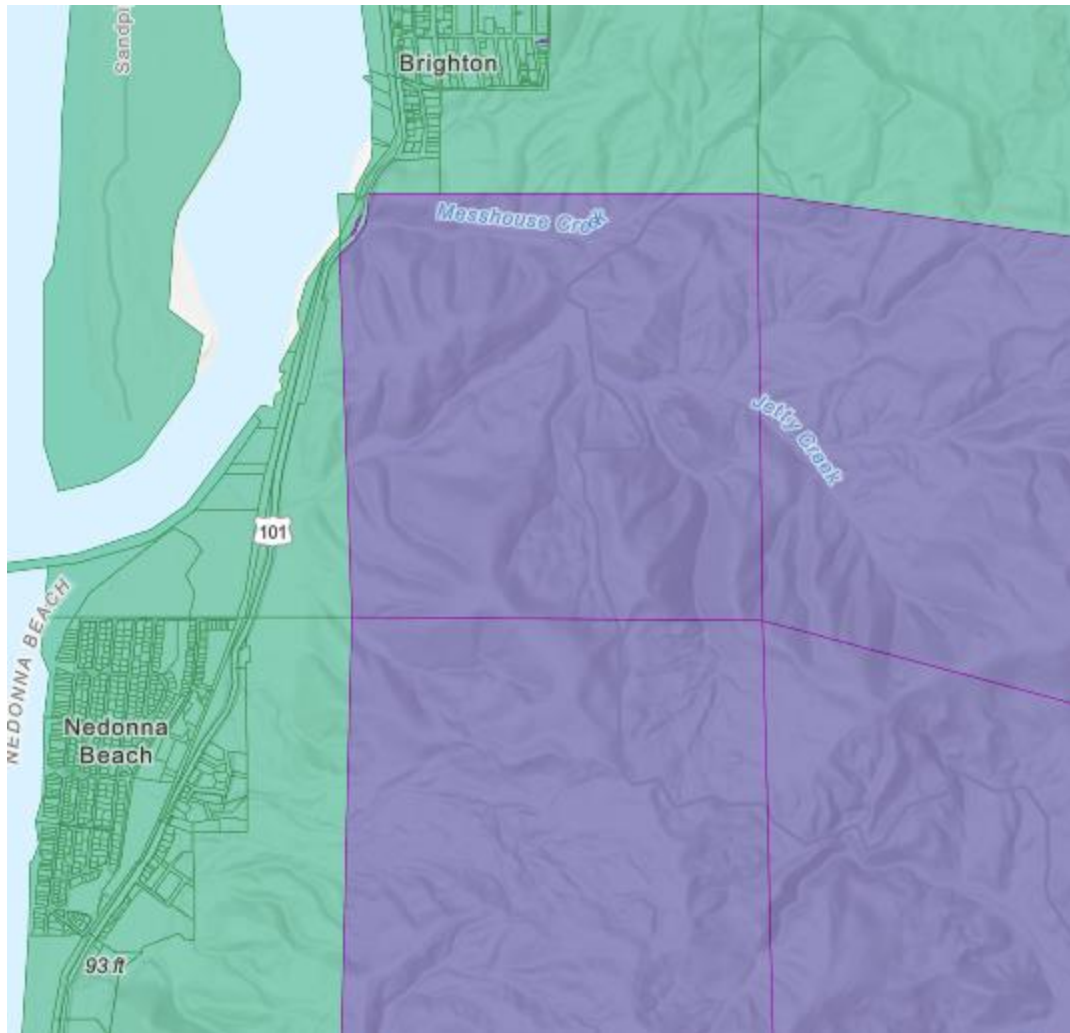
**Cc:** nancy webster <[REDACTED]>

**Subject:** Wildfire Risk Levels: Submitted to SPPDAC July 29th 2024

Rockaway Beach Source Water Protection Plan Risk Prioritization Table (GSI.)




**I question the accuracy of the stated wildfire risk for the Jetty Creek watershed. The stated risk is "2 or low." See below for my justifications for questioning this low risk for wildfires.**

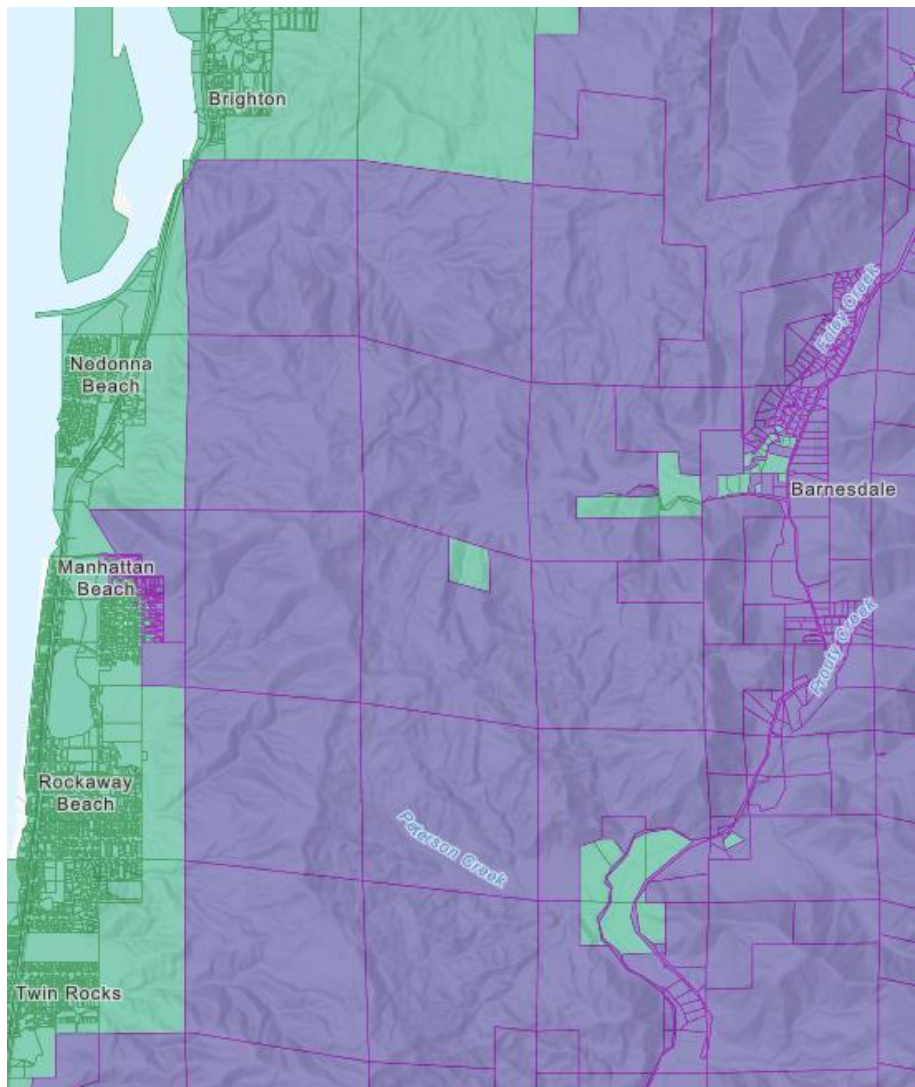
1. Overall, the current State fire maps show the Jetty Creek watershed as a "moderate fire risk." Please see tables below.
2. Conifer tree plantations burn hotter than natural forests that are of a mixed age and type of trees, having both deciduous trees (such as alders) and conifers. (Professor Beverly Law from Oregon State University) Alder trees can become fire breaks. (Research from Canada and U.S.)
3. In the last twelve years, North Coast Communities for Watershed Protection members have observed a number of wildfires resulting from slash pile burning after logging on tree plantations in Tillamook and Clatsop Counties.
4. NCCWP has observed wildfires above Short Sand Beach resulting from aerial spraying of "Easy Light" (Napalm) after logging.
5. Those of us living in the Nedonna Beach neighborhood have concerns that fires starting in the private forestland east of us could easily jump Highway 101. Our sem-forested neighborhood could easily go up in flames, and there is only one exit to the highway for all 370 houses. But still more housing is proposed to be built in our neighborhood. Conversely, a major fire in this neighborhood could easily cross Highway 101 to the privately-owned forestlands. The Nedonna Beach neighborhood is defined by DEQ as a source drinking water protection area for the groundwater backup wells for Rockaway Beach.



ESRI, NACA, NCA, USGS, FEMA, LG

### Statewide Wildfire Hazard Map

-  Low Hazard
-  Moderate Hazard
-  High Hazard



Thank you.  
Nancy Webster  
Nedonna Beach Neighborhood  
P.O. Box [REDACTED]  
Rockaway Beach, Oregon  
Phone: [REDACTED]

**Sent:** Monday, July 29, 2024 7:02 AM

**Subject:** New message from SPDAC Comment Form

**Attachments:** 66a7a0c9a01c4.pdf

Nancy Webster  
 POB [REDACTED] Rockaway Beach Oregon 97136  
 [REDACTED]

More Information on Pesticides Submitted to SPPAC 7-29-24

Rockaway Beach Source Water Protection Plan Risk Prioritization Table.

Let's take a closer look at the pesticides being sprayed in both our surface and groundwater DEQ protection areas. Pesticides have been used regularly in the "tree plantation" that the Jetty Creek watershed (primary source for RB drinking water) has become. Pesticides are also being used by homeowners and others in Nedonna Beach, which is within the DEQ groundwater protection area (location of City backup wells). Potentially, we are exposed to harmful pollutants through the air we breathe and the water we drink according to DEQ, OHA, and EPA.

Here is more information from toxicologist, Dr. Deke Gundersen, Professor Emeritus of Environmental Science at Pacific University. Since 2015, North Coast Communities for Watershed Protection has been consulting with Dr. Gundersen about public and habitat health concerns regarding the spraying of pesticides. NCCWP recently asked Dr. Gundersen for an update about research on the pesticides being sprayed on industrial forest land. On June 22, 2024, Dr. Gundersen spoke at an NCCWP forum.

Shown in italics below is an email from Dr. Gundersen:


Basically, the safety of Roundup and other pesticides cannot be confirmed, because very little testing has been done on the FORMULATIONS. However, recent studies on HUMAN CELLS are showing that the toxicity of the formulation (e.g. Roundup) is much more toxic (in some cases 1000 times more toxic) than the pure compound (e.g. glyphosate) on human cells. We know nothing about the effects of pesticide mixtures (e.g. Roundup + 2,4-D, + triclopyr) on humans. Here are links to the articles I talked about in my presentation on June 22. Just focus on the information in the abstract (a good summary of the paper). <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9699558/> <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3955666/pdf/BMRI2014-179691.pdf> <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4808927/> <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5786549/> This is a great article on the toxicity of formulations: <https://www.theguardian.com/business/2020/jan/23/formulations-glyphosate-based-weedkillers-toxic-tests#:~:text=Anotherinternalemailwrittenin,doesthedamage.> Monsanto emails showing they have not tested the formulation: <https://www.wisnerbaum.com/documents/pdf/monsanto-documents/27-internal-monsanto-email-you-cannot-say-that-roundup-is-not-a-carcinogen.pdf> <https://corporateeurope.org/sites/default/files/attachments/37-monsanto-executive-admits-studies-demonstrate-formulated-roundup-does-the-damage.pdf> I have also attached a review

article discussing the impacts of pesticides on the forest microbiome. I hope all of this is useful to you. Thanks for all you do. Deke  
Submitted by Nancy Webster

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Opinion

# Ecosystem consequences of herbicides: the role of microbiome

Suvi Ruuskanen <sup>1,2,\*</sup> Benjamin Fuchs,<sup>3</sup> Riitta Nissinen,<sup>1</sup> Pere Puigbò,<sup>2,4,5</sup> Miia Rainio,<sup>2</sup> Kari Saikkonen,<sup>3</sup> and Marjo Helander<sup>2</sup>

**Non-target organisms are globally exposed to herbicides. While many herbicides – for example, glyphosate – were initially considered safe, increasing evidence demonstrates that they have profound effects on ecosystem functions via altered microbial communities. We provide a comprehensive framework on how herbicide residues may modulate ecosystem-level outcomes via alteration of microbiomes. The changes in soil microbiome are likely to influence key nutrient cycling and plant–soil processes. Herbicide-altered microbiome affects plant and animal performance and can influence trophic interactions such as herbivory and pollination. These changes are expected to lead to ecosystem and even evolutionary consequences for both microbes and hosts. Tackling the threats caused by agrochemicals to ecosystem functions and services requires tools and solutions based on a comprehensive understanding of microbe-mediated risks.**

## Microbes in the Anthropocene

Overexploitation and chemicalization are major drivers of accelerating biodiversity loss – one of the greatest global threats to functions and services in natural and agricultural ecosystems [1]. The heavy use of agrochemicals, such as **herbicides** (see [Glossary](#)), plays a critical role in the contamination, exposing non-target plants, animals, and humans [2]. While many herbicides were initially considered safe for **non-target taxa** as their mechanism of action was thought to be absent in these organisms, it has been understood only recently that herbicides may have profound effects on non-target taxa via alterations of microbial communities and microbial function in soil, plants, and animals [3,4] ([Table 1](#)). Given the imperative role of microbes in driving ecoevolutionary adaptations since the origin of life, and that microbes and their hosts comprise coevolving, multipartite entities, holobionts [5], a comprehensive understanding of the risks associated with altered **microbiomes** is needed [6]. Here, we propose that herbicides can influence natural and agricultural ecosystem functioning due to soil- and host-associated microbiome alteration ([Figure 1](#)) and may have evolutionary consequences. Further, we discuss the limitations in the current literature to address these questions. We focus mainly on terrestrial ecosystems where herbicides are intended to be used, although agrochemicals are known to escape to aquatic environments as well [7]. We address ecosystem processes from molecular biology through physiology to ecological and evolutionary ecosystem processes. Thus, our Opinion, providing a conceptual framework on how sublethal herbicide residues modulate ecological and evolutionary changes in ecosystems, is important to fundamental ecological understanding as well as to applications in agroecological and environmental management.

## Risks of herbicides: global habitat contamination

Global herbicide usage has increased drastically over the past decades, with 1 Mt used every year [8]. Glyphosate is globally the most-used herbicide [9]. Glyphosate is used in agriculture, but, importantly, also in horticulture, silviculture, and urban environments [9], leading to global

## Highlights

Microbes have driven ecoevolutionary adaptations since the origin of life and maintain the welfare of ecosystems today.

Global contamination with herbicides, initially considered safe for non-target taxa, is shown to influence soil, plant, and animal microbiomes.

Changes in microbiomes can have unforeseen effects on organismal and ecosystem functioning and have evolutionary consequences.

A comprehensive understanding of the risks associated with agrochemical-altered microbiomes is needed.

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contamination of manmade and natural ecosystems. Other widely used herbicides are, for example, triazines (e.g., atrazine), acetochlor and metolachlor, paraquat, and dicamba [8,10] (Table 1). Residues of herbicides are found in soil, water, non-target plants, animals, and humans [3]. In addition to **active ingredients**, commercial herbicides include **co-formulants**, which can be even more toxic to non-target organisms (Box 1). Herbicides can have non-microbiome-mediated effects on non-target plants and animals, but the effects via altered soil and host-associated microbes are less understood and therefore are our focus.

### How herbicides affect microbes and microbial communities

The effects of herbicides on soil- and host-associated microbes can be either (i) **direct**, influencing microbe **function and survival** or (ii) indirect, via the environment or host, depending on the mode of action of the herbicide [4] (Table 1). Glyphosate can influence microbial survival directly as it inhibits the enzyme **5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)** of the **shikimate pathway**, which produces essential amino acids in both plants and the majority of microbes [15]. Other herbicides directly inhibiting microbial metabolic processes include **acetolactate synthase (ALS)** inhibitor herbicides altering the biosynthesis to branch-chained amino acids, **acetyl-CoA carboxylase (ACC)** inhibitors interfering with fatty acid synthesis, and glutamine inhibitors interfering with nitrogen metabolism [4]. In another group of herbicides, the mode of action does not directly target microbes but targets plant cellular metabolism, such as photosynthesis and plant hormone biosynthesis. Any changes in plant traits, however, can alter the microbiota interacting with plants [16]. Herbicide exposure can ultimately change microbial communities via multiple processes [17] (Figure 1). (i) Microbes differ in their intrinsic susceptibility to herbicides. For example, certain amino acid markers determine the affinity of glyphosate for the target enzymes and thereafter the microbial sensitivity [18,19]. Differences in microbe sensitivity can lead to changes in their abundance under herbicide exposure. (ii) Many microbes can metabolize herbicides and use them as sources of nutrients. Examples include atrazine and glyphosate, which are metabolized by *Pseudomonas* and *Arthrobacter*, acetochlor and dicamba metabolized by *Sphingomonas*, and paraquat metabolized by *Lipomyces* yeast [20]. Therefore, herbicide residues can increase the abundance of herbicide-metabolizing microbes in the community. (iii) Herbicides can cause functional changes in microbes that could cascade to community effects. (iv) All of the abovementioned alterations can further alter microbe–microbe interactions. Healthy microbial communities are able to maintain potential for self-regulation in the long term [21]. Thus, changes due to herbicide exposures can negatively impact community functionality.

### Consequences of herbicide residues for soil processes

Soil- and **rhizosphere**-associated microbes are essential for the capacity of soil to support vital ecosystems. The consequences of herbicide use for soil processes depend on the herbicides' chemical composition, their mode of action, and the microbes' susceptibility to them, as well as edaphic factors and climate. The fate of herbicide and its legacy in soil are difficult to predict because they depend on multiple and/or partly counteracting forces; herbicides affect microbial communities and microbes degrade herbicides, and two processes can be interactively determined by the physical and chemical characteristics of the soils, agricultural management practices, and climatic conditions. Accordingly, the findings on herbicide effects on soil microbiomes have been variable [7,44]. For example, glyphosate (active ingredient) negatively affects shikimate pathways present in the majority of microbes, but their genetic resistance to glyphosate varies [18]. Therefore, some of the resistant and glyphosate-degrading microbes that can use glyphosate as a nutrient source may become prevalent in the microbial community [26]. Similarly, in some environments atrazine may not affect the overall microbial community ([45]; active ingredient), while in other environments it can decrease soil microbial biomass or increase atrazine-degrading bacteria ([46,47]; active ingredient) due to strong selection favoring them, thus leading to atrazine degradation.

### Glossary

#### 5-Enolpyruvylshikimate-3-phosphate synthase (EPSPS):

enzyme that is inhibited by glyphosate in many microbes.

**Acetolactate synthase (ALS):** some herbicides inhibit this enzyme pathway also in microbes.

#### Acetyl-CoA carboxylase (ACC):

some herbicides inhibit this enzyme pathway also in microbes.

**Active ingredient:** chemical element or compound in a herbicide having specific effects on plant metabolism resulting in plant death.

**Co-formulants:** non-active ingredients in herbicides to increase the efficiency of the product. For example, surfactants are added to reduce surface tension, increasing the emulsifying, spreading, dispersibility, and wetting properties of the liquid.

**Herbicide:** plant protection product that is used to eradicate undesired plants.

**Microbiome:** microorganisms (bacteria, archaea, fungi) and their genes in a particular habitat.

**Mycorrhiza:** symbiotic association between a plant root and a fungus.

**Non-target taxa:** microbe, plant, or animal species that are not targeted with a herbicide.

**Phyllosphere:** aboveground plant surfaces; the largest biologically active surface on Earth, plant–atmosphere interface, and habitat for diverse microbes.

**Rhizosphere:** soil surrounding plant root impacted by plant root exudates and associated microbiota.



Table 1. Examples of studies on the effects of herbicides with different modes of action on soil, plant, and animal microbiomes<sup>a</sup>

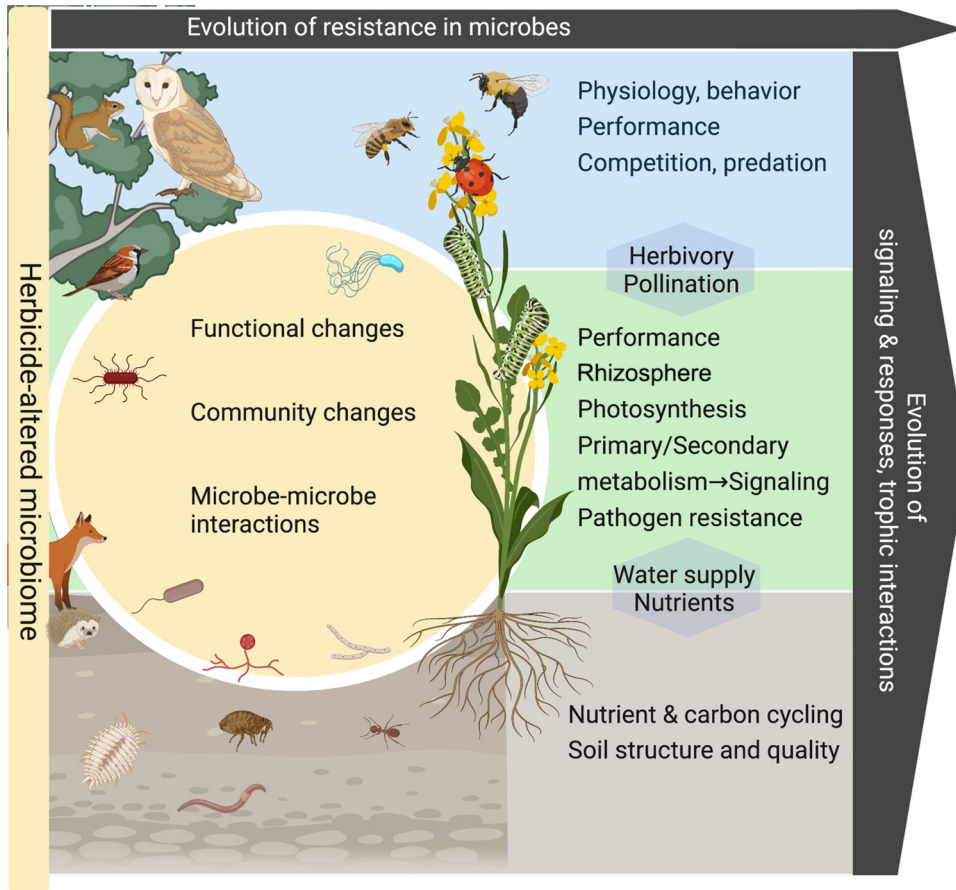
	Herbicide class/model of action	Example of chemical	Effect on microbiome		
			Soil	Plant	Animal
Direct effect on microbes	ACC inhibitors	Diclofop, haloxyfop	Composition, including Sulfur-cycling-associated bacteria [22] <b>F</b>	Rice, <i>Oryza sativa</i> [23] <b>A</b>	–
	ALS inhibitors	Sulfonylureas, imidazolinones, triazolopyrimidines	Community composition [24] <b>A</b>	<i>Arabidopsis</i> [25] <b>A</b>	–
	EPSPS inhibitors	Glyphosate	Microbial functions [26] <b>AF</b>	<i>Arabidopsis</i> [27] <b>A</b>	Japanese quail <i>Coturnix japonica</i> [28] <b>F</b>
	Glutamine synthetase inhibitors	Glufosinate	Community functional response [29] <b>A</b>	Oilseed rape <i>Brassica napus</i> [30] <b>F</b>	Mouse <i>Mus musculus</i> [31] <b>A</b>
Indirect effect on microbes	Auxin-like herbicides	2,4-D, aminopyralid, dicamba	Bacterial diversity [32] <b>F</b>	Rice <i>Oryza sativa</i> <i>L. japonica</i> [33] <b>A</b>	Mouse <i>M. musculus</i> [34] <b>A</b>
	Photosystem II inhibitors	Triazine herbicides	Community structure [35] <b>A</b>	Millet <i>Pennisetum americanum</i> [36] <b>A</b>	Black spotted frog <i>Pelophylax nigromaculatus</i> [37] <b>A</b>
	Photosystem I inhibitors	Bipyridinium herbicides (e.g., diquat, paraquat), diphenyl ether	Nitrogen-fixing bacteria [38] <b>F</b>	Rice <i>O. sativa</i> [39] <b>F</b>	Mouse <i>M. musculus</i> [40] <b>A</b>
	Gibberellin inhibitors	Acetochlor, metolachlor, pendimethalin	Composition, nitrogen-fixing bacteria [41] <b>A</b>	Wheat <i>Triticum aestivum</i> [42] <b>A</b>	Ground beetle <i>Pterostichus melas italicus</i> [43] <b>F</b>

<sup>a</sup>The first four have a mode of action with direct antimicrobial effects, while the latter four affect plant traits, and therefore microbes only indirectly. Our aim is to demonstrate the breadth of microbial processes affected and provide examples across taxa when available, but also to point to missing information in the literature, to our knowledge (marked –). We have further provided information on whether the study used an active ingredient (**A**) of the herbicide or a commercial formulation (**F**).

The potential ecological and evolutionary consequences of glyphosate and other herbicides for microbial soil communities are insufficiently understood [19,48]. Nevertheless, recent studies indicate a negative correlation between pesticide use and beneficial soil- and root-associated microbes ([12,49,50]; active ingredient and commercial formulation) and herbicide-modulated nutrient cycling in soils [13]. For example, the glyphosate molecule contains phosphorus (P) that adds an extra P load to the ecosystem [13]. In addition, herbicides may further affect soil P cycling by competing with phosphate ions for the same binding sites. However, the outcome of this glyphosate–phosphorus interaction in the soil again depends greatly on the soil properties and biotic factors (vegetation type and soil microbial communities), as well as climate and weather conditions.

### Consequences of herbicides on plant–microbe and plant–animal interactions

Herbicide residues may affect a plant and its associated microbes, either individually or in concert as a metagenomic unit. The consequences of sublethal herbicide doses, especially glyphosate, for soil processes are now increasingly recognized, but the effects on plant associated microbiota, plant physiology and subsequent consequences for species interactions remain poorly understood (Figure 1). The negative impact of glyphosate (active ingredient and formulations) on root infectivity, colonization, and the arbuscule density of arbuscular mycorrhizal fungi (AMFs) has been repeatedly observed [13,14,49,51]. This has consequences for the water and nutrient economy of plants and may also impact AMF diversity and ecosystem functioning [14]. Far less is known about the impacts of herbicides on ectomycorrhizal fungi, although the majority are potentially sensitive to glyphosate [18] and are known to play a key role in boreal and taiga forest ecosystems. As root-associated



#### Trends in Ecology & Evolution

**Figure 1. Consequences of herbicides for plant-microbe and plant-animal interactions.** Herbicides can directly affect non-target organisms (grey, green, blue), especially microbes (yellow), and cause ecosystem-level changes. Microbes are a significant component of all healthy living organisms. Thus, herbicides can indirectly affect the behavior and performance of organisms. These changes can further shape species interactions and ecosystems (ecological processes: blue arrows) as well as evolutionary processes (black arrows). This figure was created using BioRender (<https://biorender.com/>).

fungi impact their hosts' fitness [52], the selective suppression of fungal associates has the potential to shape plant communities and ecosystems based on them.

Likewise, plant rhizosphere bacterial communities are shaped by herbicide exposure ([50,53]; active ingredient and formulations). While the findings on gross community structures are variable and dependent on the plant host, the experimental system, and exposure levels, studies show a consistent reduction in the relative abundance of nitrogen-fixing bacteria ([54,55]; commercial formulations) and repression in plant-beneficial microbial functions (nitrogen fixation, 1-aminocyclopropane-1-carboxylic acid deaminase, and antifungal enzymes [53]; commercial formulations). As many plant traits, including growth, phenology, and resistance to abiotic stressors and pathogens, are modulated by rhizosphere microbiome, changes in rhizosphere composition and functioning are likely to be reflected in host fitness and growth [50].

In plants, the compounds derived from physiological pathways interfered with by herbicides, such as the shikimate pathway, are essential precursors for many plant defense and signaling metabolites. Therefore, sublethal doses of glyphosate (active ingredient and formulations) can potentially disrupt

### Box 1. Active ingredients and co-formulants

Herbicides used in the field are a combination of the active ingredient and a complex mixture of co-formulants. A compound is classified as active when it is intentionally added for toxicity to target species. However, the active ingredient (e.g., glyphosate) of a herbicide is effective only if it can persist on the leaf surface long enough to penetrate the plant epidermis in variable weather conditions; thus, co-formulants are added to the commercial product to increase the efficiency of the active ingredient. Under the current regulations and laws, usually only the active ingredients are required to be tested for their toxicity to non-target organisms [11]. Furthermore, the co-formulants in a commercial product can vary geographically and over time and are regarded as confidential information.

An increasing number of studies is showing that the effects of commercial herbicide formulations on microbes and other non-target organisms are stronger than the effects of the active ingredient alone [8,12–14]. However, often it is not possible to differentiate whether the results are the outcome of the effect of the active ingredient, the co-formulants, or their combination, because many studies do not adequately explain what commercial formulations they have used. To better understand the ecological and evolutionary consequences of herbicides in natural ecosystems and agroecosystems, we need more well-replicated, field-realistic, and long-term experiments using active ingredients and various commercial formulants.

virtually all plant aboveground (**phyllosphere**) interactions with other coevolving organisms such as pathogens, plant-mutualistic microbes, herbivores, and pollinators [16,50,56,57] (Figure 1). For example, in *Arabidopsis thaliana* glyphosate altered the plant metabolome, causing a change in the core phyllosphere microbiome ([27]; active ingredient). Thus, microbial changes in the phyllosphere may be caused indirectly by changes in the plant metabolome, which may have a substantial impact on plant resilience and is likely to affect ecoevolutionary processes at the field scale [52]. Phytohormones are key regulators of plant metabolite biosynthesis in response to herbivory or microbial infections. Several plant-associated bacteria modulate plant phenotype by biosynthesis and the regulation of phytohormones such as auxins and ethylene [58]. Thus, residues of glyphosate-based herbicides in soil may disrupt the phytohormone homeostasis of plants directly or indirectly via altered microbiome [59]. On a field scale, the persistent effects of pesticides on the plant metabolome may have cascading effects in multitrophic and multispecies networks, with unknown consequences for entire ecosystems and the coevolution of plant–microbe and plant–insect dynamics [16].

Besides altering herbivory, herbicides can reduce pollinator visitation; both may be explained by changes in the volatile organic compounds (VOCs) released by plants [16,59] attracting pollinators and predatory insects. Some VOCs derived, for example, from the shikimate pathway are affected by low glyphosate doses ([60]; active ingredient). It remains to be elucidated to what extent glyphosate-mediated changes in the plant volatilome impact pollination or pest control, but global studies indicate general trends towards decreased ecosystem multifunctionality on agricultural fields managed using pesticides compared with organic farming [61].

### Consequences of herbicides for animal hosts and species interactions

Both gut and skin microbiomes are known to influence animal health, playing key roles in digestion, pathogen resistance, and even neurobehavioral coordination in both invertebrates and vertebrates [5]. Herbicides with direct antimicrobial effects have been shown to influence microbiome composition in invertebrate and vertebrate hosts [3] (Table 1). So far, the studies in terrestrial invertebrates have mainly concentrated on bees (but see [62,63] for effects on beetles and mosquitoes using both active ingredients and formulations). For example, glyphosate (active ingredient) has been shown to increase pathogenic and decrease symbiotic bacteria [64,65], which may affect the susceptibility of bees to viral and fungal pathogens [56,66] with survival effects cascading to the ecosystem level. In addition to direct herbicide exposure, herbicide-altered plant microbiomes and/or metabolomes in plant leaves, pollen, and nectar may alter the exposure and consumption of pollinators and herbivores, which can have cascading effects on their gut microbiomes and, therefore, the health of the pollinators and herbivores [86]. Herbicides also lead to consistent compositional and functional changes in vertebrate models (mice and poultry [3,28,31,56,67]; both active ingredients

and formulations) with associated effects on, for example, endocrine and immune function [3,25]. Furthermore, herbicides proclaimed to lack antimicrobial function have been revealed to affect animal host gut microbiomes (e.g., in beetles, *Drosophila*, frogs, and mice [68–70]) (Table 1; both active ingredients and formulations). For example, low-dose paraquat (active ingredient) exposure remodeled the microbiome of *Drosophila Melanogaster*, simultaneously influencing the adult lifespan [70]. We propose that such effects of non-antimicrobial herbicides could be mediated via alterations of the host physiology, which then drives the changes in the microbiome.

Ultimately, herbicide-driven changes in animal-host gut microbiomes may lead to ecosystem-level changes. For example, altered gut microbiomes may directly affect pathogen resistance, endocrine disruption, and, therefore, the survival/reproduction of animals or cause changes indirectly by altering species–species interactions including pollination/herbivory, competition, or predation. These could result from altered behavior driven by modifications of gut microbiomes [71]. For example, altered gut microbiome and impaired locomotor activity and memory formation were reported in rats exposed prenatally to glufosinate ([31]; active ingredient). Understanding how these physiological and behavioral changes might contribute to organism performance is therefore a key future research challenge. To summarize, any herbicide-mediated change in host microbiome can have complex and unforeseen effects on species associations.

### Evolutionary consequences for microbiomes and how they feed back to ecosystem level

In addition to the resistance of plants to herbicides, which can have cascading effects on ecoevolutionary dynamics [17], a widely known evolutionary consequence of repeated herbicide exposure is selection for increased herbicide resistance in free-living soil bacteria [19] (Box 2; see Figure 1 in Box 2). This can further feed back to the ecosystem level, as the resulting changes in the community composition may influence soil processes; for example, nitrogen and carbon flows [72]. Long-term exposure to herbicides may influence not only microbial evolution but also the evolution of the animal hosts driven via microbes. For example, atrazine (active ingredient) exposure for 85 generations in the wasp *Nasonia vitripennis* led to adaptive changes in the gut and exerted selective pressure on the host genome [68]. These results indicate that herbicide-mediated host–microbiome coadaptation is leading to a new host–genome–microbiome equilibrium. The effects of herbicides on animal-host gut microbiomes can also feed back into soil processes when they influence soil fauna, such as earthworms, contributing to detoxification [73], decomposition, and nutrient cycling [74]. Several herbicides have been found to decrease earthworm microbiome gut diversity ([75,76]; active ingredient and formulations), which may lead to both impaired soil processes and selection on hosts.

### What limits current understanding of ecosystem-level effects?

The lack of published studies limits our understanding of the extent and complexity of the ecosystem and the evolutionary effects of herbicides. First, the co-formulants in herbicides can have additive or synergistic effects that complicate the predicted effects of active ingredients (Box 1) but have not been thoroughly quantified. Second, most studies are conducted in the laboratory or on agriculturally important/model species, which poses several constraints. Model systems in the laboratory fail to capture the breadth of variability inherent in wild coevolving microbes, plants, and animals, their interactions, and their responses to variable environments. Thus, the ecoevolutionary consequences on ecosystems are impossible to quantify in the laboratory. Although mechanisms can be examined in laboratory studies, the dosages and durations of herbicide exposure are challenging to adjust equivalent to the exposure in the natural environment. This holds true, especially, in long-term studies with low chronic exposure levels. Although knowledge on the complex and indirect effects of

### Box 2. Evolution of resistance to glyphosate

Some species have evolved a variety of mechanisms resistant to glyphosate, including target-site and non-target-site mechanisms [77] (Figure I). Target site susceptibility can be determined based on bioinformatic analyses of amino acid markers in the target protein sequence. The evolution of target site sensitivity to glyphosate has been thoroughly studied through the identification of amino acid markers in the EPSPS active site [18]. Although the phylogenetics and lifestyles of bacteria may determine the potential sensitivity to the glyphosate, the status may easily change by single mutations in the EPSPS active site or via horizontal gene transfer [19]. Moreover, glyphosate may affect additional metabolic pathways, such as the mitochondrial electron transport chain [78–80]; thus, some species may be sensitive to the herbicide even in the presence of the EPSPS-resistant copy of the protein. The exposure hypothesis (i.e., free-living bacteria that are more exposed tend to be more resistant to the herbicides than host-associated and parasitic bacteria) has been suggested for glyphosate based on the analysis of target site mechanisms [19]. However, literature-mining studies suggest that pathogenic bacteria are likely to be more resistant to the herbicide than free-living and host-associated bacteria [3,81], which may be explained by their greater genomic plasticity [82]. Moreover, there are differences in target site sensitivity to glyphosate among animal organs and plant tissues [81]. Further empirical studies are needed to disentangle the association between target and non-target site mechanisms as well as the role of glyphosate and other herbicides in the selection for antimicrobial-resistant bacteria [83–85].

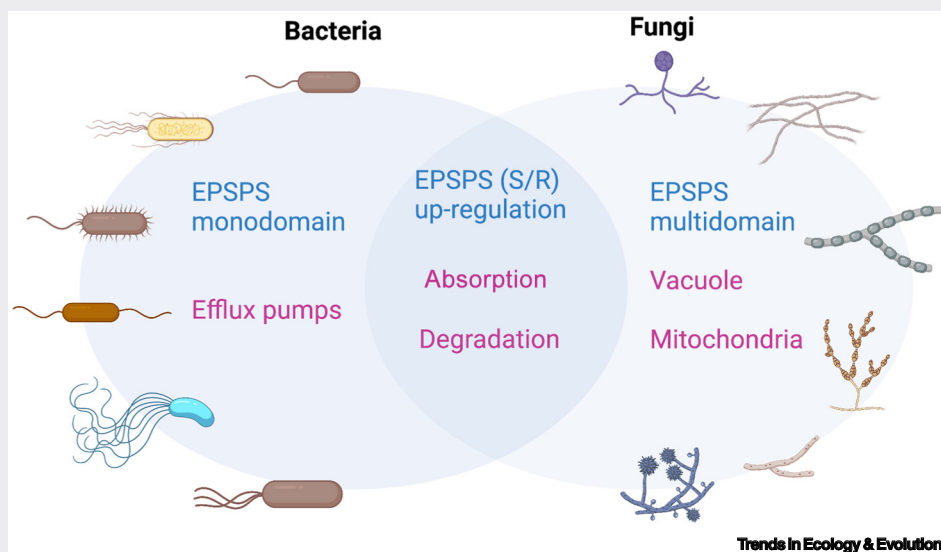


Figure I. Target-site (blue) and non-target-site (purple) mechanisms of sensitivity/resistance (S/R) to glyphosate. This figure was created using BioRender (<https://biorender.com/>). Abbreviation: EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase.

herbicides on microbes is emerging, the empirical studies on the wider ecoevolutionary consequences of the long-term use of herbicides remain lacking.

### Concluding remarks

The health of microbial communities is extremely important, since they maintain the well-being of ecosystems. By altering microbial communities, herbicides can have far-reaching, long-term, and unforeseen impacts on ecosystems. Therefore, tackling the threats caused by agrochemicals requires tools and solutions based on a comprehensive understanding of microbe-mediated risks (see [Outstanding questions](#)). To truly address and control microbiome-mediated herbicide effects, these must be considered in future assessments of the registration of pesticides that currently do not involve microorganisms.

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### Outstanding questions

What are the effects of co-formulants in commercial products on microbes and microbial communities?

How do herbicide residues affect soil ecosystem functions, such as nutrient cycling, via changes in microbiomes?

How do herbicide effects on direct and indirect (microbiome mediated) pathways influence species interactions in wild and agricultural ecosystems?

What are the effects of herbicide-modulated microbiomes on ecosystem functions and services?

What are the evolutionary consequences of herbicide-altered changes in ecosystem functions?

## Declaration of interests

The authors declare no interests.

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Jetty Creek July 2024 Measurement under .7

Rockaway Beach Source Water Protection Plan Risk Prioritization Table.

To make good decisions regarding protecting *all* the drinking water used in Rockaway Beach (both surface water from Jetty Creek *and* groundwater from the Nedonna wells), we need to know what is the monthly streamflow in Jetty Creek. Broken down by month, how much of the water used comes from Jetty Creek and how much comes from the wells? How have these numbers changed in the



last twenty years? My understanding is that the wells are used when Jetty Creek is experiencing heavy turbidity and in the summer months when the creek water flow is low. Before the water is treated, what are the measured levels of turbidity? How much chlorine is being used to treat the raw water?

On Saturday, July 27, I took a look at the Jetty Creek stream monitor that's on the southwest side of the treatment plant, and it measured slightly below 0.70. How is the monitor calibrated? How much water flow does 0.70 represent? The water level looked very low. Is there enough water there for the coho salmon which are "listed?" In Aug. 2023 the same monitor read .6 ( please see attachment "Coastal Water Crises.") Presently, at the end of July, are we using the wells and, if so, how much? Please see the attachment that shows incomplete RB water usage tables.

The entire Nedonna Beach neighborhood is in the DEQ-defined source water protection area for Rockaway Beach's groundwater (the Nedonna wells). Nedonna Beach already has about 370 houses in this area at high risk from tsunamis, earthquakes, floods, king tides, rising sea levels, extreme weather, and wildfires. Seventy-five percent of these homes use septic systems and the remainder are on the RB sewer system. These septic systems, many of them aging, are poorly regulated and inspected. The Nedonna wells are shallow (only about 50 feet below the surface) and, thus, are not far below the septic systems. More stress may be put on these wells by additional home construction and fluids from vehicles using the roads; pesticides, fertilizers, and chemicals from personal care products; and storm water overflow. These wells are in close proximity to the ocean; so, they are naturally subject to salt water infiltration.

If more houses are built in a high-density PUD (planned unit development) proposed to be built in close proximity to McMillan Creek, more stress will be placed on these wells, further degrading them. McMillan Creek, and the surrounding area, is home to beaver, fish, and other wildlife. It flows into the Nedonna Marsh estuary, a saltwater and tidal water area that flows into Nehalem Bay and the ocean. Jetty Creek also flows into this estuary which is designated as salmon migration and nursery waters.

In 2008, approximately twenty percent of the Jetty Creek watershed had been clearcut and sprayed with pesticides. Sixteen years later, approximately ninety percent of that watershed has been clearcut and sprayed numerous times with pesticides. This has seriously compromised drinking water quantity and quality. Now because of these industrial forestry practices, we have lower stream flows in the summer, resulting in less drinking water available. It should be noted that Rockaway Beach does have water rights to McMillan Creek; therefore, everything should be done to protect it.

In summary, we do need to protect the Jetty Creek watershed, but we also need to protect our groundwater source area (the Nedonna wells) that face many stressors. These stressors seriously threaten our water quality and quantity.

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# A Coastal Water Crisis in Jetty Creek and Beyond

