Pesticide Action Network Europe

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Beneath the orange fields:
Impact of Glyphosate on soil organisms

Summary

Glyphosate is the active substance of the most used pesticide (herbicide) products globally: glyphosate-based herbicides (GBHs). They are used widely to kill plants, leading to their widespread presence in our ecosystems, surroundings and bodies. **Soils** are extremely biologically diverse and complex ecosystems, providing a wide series of essential functions, and directly interacting with groundwater, surface water and air. There is a misbelief that glyphosate-based herbicides are beneficial for agricultural production without having any negative consequences to beneficial species and soil health. This is far from the truth. Apart from killing beneficial plants and endangering important pollinators like bees, glyphosate can seriously disrupt soil health by harming the soil microbiome and earthworms.

- Soils are estimated to harbour about 59% of Earth's species, or even more, as soils are understudied. For example, **90% of fungi, 85% of plants and 50% of bacteria are living in soils.**

- Healthy soils provide a **wide variety of ecosystem services** such as biodiversity, nutrient cycling, sustainable plant production, natural pest control, good water quality, water and carbon storage and erosion management. **Soil micro- and macrofauna are essential contributors to these functions**, and harm to these organisms can impact soil functioning.

- **Glyphosate and its metabolite AMPA** are **widely present** in our environment, and the most frequently found pesticide residues in **soils** across Europe.

- The **persistence** of glyphosate in soils can vary from low to very high, depending on environmental conditions and properties.

- Glyphosate inhibits the **shikimate pathway.** This pathway is responsible for essential aromatic amino acid biosynthesis, and is **present in plants and algae, but also in fungi and bacteria in soils and in the gut microbiome of animals and humans**.

- Inhibition of this pathway leads to the **death of plants**. Given the same pathway is present in fungi and bacteria, researchers have looked at the impacts of glyphosate and GBHs on microorganisms.

- GBHs can harm the **soil microbiome:**
    - GBHs can **alter the composition and abundance of soil microorganisms** (bacteria and fungi), and for example **increase pathogenic and decrease beneficial organisms**.
    - GBHs can **reduce** the forming of mutually beneficial relationships between fungi and plant roots, called **root mycorrhization**, which can impact plant health/growth
    - GBHs can also lead to changes in **nutrient composition** in the roots, leaves, grape juice and xylem sap.
    - Scientists warn that microbiomes play an essential role in maintaining ecosystems health, and that **microbiome alterations can have unforeseen impacts on functioning of organisms and ecosystems**.

➢ Research also points at the importance of the possible links between the impact exposure to GBHs and other pesticides on the gut **microbiome of animals and humans, and impacts on animal and human health, including cancer and neurological disorders**.

- GBSs can harm **earthworms**:

➢ Research shows **glyphosate contamination is common in earthworms**.
➢ GBHs can severely **negatively impact survival, body mass, microbiome and behaviour of earthworms**
➢ Negative impacts on earthworms reach far beyond soils, for example, use of pesticides and fertilisers have been found as one of the main drivers for drastic declines in **farmland birds**, especially for invertebrate feeders.

- While there is a need for more long-term, detailed studies to further untangle possible impacts on soil life and highly complex processes, GBHs **can clearly harm organisms** and **disrupt** ecosystem functions, the **very foundation on which safe and sustainable agricultural production depends**. Furthermore, the use of GBHs poses a threat to the health of farmers and the general public.

- **Fortunately, there are viable alternatives to GBHs, that are aligned with climate and pest resilient and nature-inclusive cropping systems, offering extensive benefits for ecosystems and citizens' health, farmers wellbeing and food security.**

- The EU assessment has major shortcomings and data gaps, particularly in assessing the impact of glyphosate and glyphosate products on biodiversity and microbiome

The **soil microbiome**, encompassing bacteria, archaea, viruses and fungi, comprises the highest biodiversity within soils, and performs multiple vital functions. Many soil microbiota form symbiotic relationships with plants, supporting plant growth, regulating nutrient cycling and biogeochemical cycles, decomposing organic matter, defining soil structure and suppressing pathogens. GBHs have been shown to impact the composition and abundance of soil microbial communities, potentially increasing pathogenic fungi or decreasing beneficial soil microorganisms. These disturbances within microbial communities can lead to long-term effects on the nutrient status of the plant-root interface and impact plant health and growth. Moreover, a growing body of literature points at the possible impacts of pesticides, including glyphosate, on **the microbiome of animals and humans**. Due to the important role of the microbiome for many functions, researchers highlight the possible impacts on animal and human health, and the possible links with illnesses. **Earthworms**, rightfully called 'ecosystem engineers', are responsible for breaking down and redistributing organic material in soil, increasing soil penetrability for roots, aeration and consequently, improving overall soil fertility. Earthworms can also significantly increase water retention capacity of soils. Nevertheless, these critical roles played by earthworms are adversely impacted by GBHs, which have been shown to affect their reproduction, behaviour, growth and survival of earthworms.

The impact on soil organisms goes far beyond soils, and extends also to animals dependent on soil organisms. For example, research has shown that the drastic declines in (farmland) **birds** in Europe are linked to pesticide and fertilizer use. The effects are very outspoken for birds that are invertebrate feeders.

Although more long-term studies are needed to further untangle the highly complex soil processes, and fully understand all possible impacts of GBHs on these processes, the evidence currently available clearly indicates that **glyphosate and GBHs can negatively impact soil organisms and soil health. This, in turn, poses broader risks and implications for ecosystem health and human well-being**. **While only a minority of plant species is damaging to crop yields and considered pests, GBHs indiscriminately kill**

all plants and harm various organisms, including bees and soil organisms. Overall, GBHs disrupt ecosystem functions, the very foundation on which safe and sustainable agricultural production depends. Furthermore, the use of GBHs poses a threat to the health of farmers and the general public. Fortunately, there are viable alternatives to GBHs, that are aligned with climate and pest resilient cropping systems, offering extensive benefits for ecosystems and citizens' health, farmers wellbeing and food security.

The EU is currently in the process of renewing the approval of glyphosate, with its current licence set to expire at the end of 2023. Unfortunately the EU assessment has major shortcomings and data gaps, particularly in assessing the impact of glyphosate and glyphosate products on biodiversity and microbiome. It incorrectly concludes that glyphosate is safe. This contradicts the provisions of the pesticide EU law and the implementation of the precautionary principle, which both prioritise a high level of human and the environment, including biodiversity and ecosystems. At the same time, the Green Deal and Farm to Fork strategy envision a 50% reduction in the use and risk of chemical pesticides in the EU by 2030, and the promotion of safer, nature-based alternatives. Given the important identified risks of the use of GBHs for human and environmental health, glyphosate use should be completely banned for use in agriculture as well as for non-agricultural uses (gardens, urban areas, railway tracks, …). To safeguard all soils, this ban should also encompass the export of glyphosate and glyphosate-based herbicides to third countries.

Introduction

Glyphosate is the active ingredient of the most used pesticide (herbicide) products globally: glyphosate-based herbicides (GBHs). It is non-selective and broad spectrum, meaning it kills all plants and trees. GBHs are used widely to eliminate plants in agriculture, towns and cities, peoples' homes and gardens and even in nature-protected areas. Consequently, glyphosate and its metabolites are widespread in our environment, including in soils and have been detected to be systematically present in animals and humans. Glyphosate also exhibits anti-parasitic properties with antibiotic effects. This briefing looks below the surface and provides an overview of important findings from scientific literature on **the impact of glyphosate and GBHs on soil health**.

Soils are estimated to harbour about 59% of Earth's species, or even more, as soils are understudied. For example, 90% of fungi, 85% of plants and 50% of bacteria are living in soils (Anthony et al., 2023). Healthy soil ecosystems provide a wide variety of ecosystem services, which are central to human health, such as biodiversity, nutrient cycling, sustainable plant production, natural pest control, good water quality, water and carbon storage and erosion management. Soils are characterised by highly complex processes and interactions, of which many still need to be further explored. Soils are composed of minerals (clay, silt and sand), soil organic matter, gas and water, and are home to plants, mammals, birds, macrobiota (worms, insects, …) and microbiota (bacteria, archaea, viruses, and eukaryotes like fungi, …).

Microbiota represent the greatest biodiversity in soils, and play a crucial role in supporting plant growth, regulating nutrient cycling, decomposing organic matter, defining soil structure and suppressing diseases. Microbiota are key to biogeochemical cycles. Many of these processes take place at the rhizosphere, the root-plant interface: the area around a plant root which is characterised by a specific population of microbiota. Although external to the plant, it is vital to the survival and health of the plant. It can be seen as a plant's external gut (Shamayim et al., 2012). There are many similarities between the human gut and the rhizosphere (Mendes and Raaijmakers, 2015). For example, like the bacteria in our guts, microbiota around the root help digest food and absorb nutrients for the plant, and protect the root against pathogenic microbes (Berg and Koskella, 2018). Soil macrobiota, such as earthworms, known as 'ecosystem engineers', perform essential functions such as shredding and redistributing organic material, nutrient cycling, stimulating microbial activity, improving soil structure, root penetrability, aeration and water retention capacity (Blouin et al. 2013). Depending on the location, earthworms can bring 40 tonnes/ha to 1000 tonnes/ha of fertile and nutrient rich soils to the surface every year (Feller et al., 2003; Coleman et al. 2018).

Glyphosate in soils

Glyphosate and its metabolite, AMPA, are the most frequently found pesticide residues in soils across Europe (Silva et al., 2018; Silva et al., 2019). The persistence of glyphosate and its most frequent metabolite AMPA in soils is greatly influenced by environmental conditions. The half-life of glyphosate and AMPA (the time required to reduce to half of its initial concentration) can vary significantly, ranging from just a few days up to one or two years, depending on factors such as temperature and soil moisture.

According to EFSA (EFSA, 2015), the persistence of glyphosate varies from low to very high (DT50 2.8 to 500.3 days). For AMPA, laboratory studies found moderate to high persistence (DT50 38.98 to 300.71 days), with field studies showing a high to very high persistence (DT50 288.4 - 374.9 days).

Per the findings of Bento et al. (2016), glyphosate is 30 times more persistent in soil under cold and dry, than under warm and moist conditions. Meaning that its persistence is influenced by climate conditions and environmental properties. In this study, the half-life (*DT50 (Disappearance Time 50)*) of glyphosate was found to be between 1.5 and 53.5 days (DT90 8.0-280 days). AMPA was shown to be more persistent than glyphosate, with a half life in soil at 30 °C between 26.4 and 44.5 days (DT90 87.8-148 days).

Silva et al. (2018) found that glyphosate and/or its metabolite AMPA were present in 45% of 317 topsoil samples from eleven EU countries, with the highest observed concentration being 2 mg/kg. In general higher levels were detected under permanent crops and lower concentrations under dry pulses and fodder crops. Silva et al. reported half-life times of 143.3 days for glyphosate and 514.9 days for AMPA.

Impact of glyphosate on microbiomes

Impacts on soil microbiota

Several studies investigated the effects of glyphosate and GBHs on the microbiomes of a variety of organisms, as well as on the soil microbiome. A variety of studies show that soil microbial communities are impacted by GBHs, while others found no significant impacts. More (long-term) studies are needed to adequately assess all potential effects of the herbicide. GBHs inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) of the shikimate pathway, which is responsible for essential aromatic amino acid biosynthesis, which are key plant nutrients. The inhibition of this pathway leads to the death of plants. This pathway is not only available in plants, but also in certain fungi and bacteria, which makes it likely that glyphosate and GBHs can impact microbiomes through the same mechanism (Klátyik et al., 2023).

Part of the studies have shown no difference in relative number of microorganisms and overall microbial community composition between glyphosate-treated plots and plots without glyphosate under field condition in studies well replicated in time and space (Kepler et al., 2020). A variety of studies of short and long-term experiments under field conditions, greenhouses and laboratory conditions, show that the use of GBHs can impact the microbial communities (Klátyik et al., 2023). Glyphosate can act in soils as an additional nutrient source, leading to stimulation of soil biochemical parameters, such as for example dehydrogenase and β-glucosidase activity, as well as carbon and nitrogen content of microbial biomass. Different studies have shown that after biodegradation, glyphosate is used as a source of available carbon (Brühl and Zaller, 2021; Zaller and Brühl, 2021; Panettieri et al., 2013; Singh et al., 2020).

GBHs also affect the composition of soil microorganisms and fungi (Mandl et al., 2018; Zaller et al., 2018). For example, after treatment with GBH Roundup Powerflex (application rate of 4.0 L ha^{-1} corresponding to 0.8 kg GLY AI ha^{-1}), a significantly lower number of viable microorganisms or 'colony-forming units' (CFUs) were observed. A colony-forming unit is a unit which estimates the number of microbial cells in a sample which are viable and able to multiply under the controlled condition (Zaller et al., 2018). The effect of Roundup PowerFlex, applied at recommended rate (3.75 L ha–^{1} or 0.75 kg GLY AI ha^{-1}), on individual fungal species groups was shown to increase growth of some species groups (e.g. *Colletotrichum sp., Cunninghamella sp.,* and *Mortierella sp.*), while other species, e.g. *Mucor*, were found to be absent (Mandl et al., 2018). Compared to mechanical weeding, GBHs have been shown to reduce root mycorrhization, the forming of symbiotic relationships between plant roots and fungi, in grapevines. GBHs can also change nutrient composition in the roots, leaves, grape juice and xylem sap (Zaller et al., 2018). Also, the rhizosphere-associated bacterial communities of soybean and corn were shown to be altered by GBHs, with relative abundance of some groups increasing, and other decreasing, suggesting that the nutrient status of the rhizosphere may be impacted (Newman et al. 2016).

An increase in the root colonisation of certain *Fusarium* species (fungi) has been found after glyphosate application (Zobiole et al., 2011), which would increase the production of *Fusarium* mycotoxins (Ekwomandu et al., 2021), which can significantly influence crop productivity and quality. *Fusarium spp*. can produce mycotoxins which are harmful to animal and human health (Ferrigo et al., 2016).

The metabolome, the complete set of small-molecule chemicals, of beneficial bacteria such as *Pseudomonas* and *Bacillus* species have been shown to be disturbed by glyphosate (Aristilde et al, 2017; Yu et al., 2015). Mendonca et al. (2019) also found that the addition of the co-formulant POEA led to a reduction of biomass growth of beneficial *Pseudomonas* species by up to 60%, while a mixture of POEA and glyphosate also resulted in growth inhibition. GBH proved also to be toxic to the fungus *Aspergillus nidulans* at doses 100 times lower than the recommended application rate with glyphosate alone (Nicolas et al., 2016). Negative effects on soil fungal biomass were observed following the application of glyphosate formulations. Following two doses of GBH or after long-term GBH application, there were also impacts on the species diversity and molecular profiles of soil fungal communities (Vázquez et al., 2021).

Given the central role that microbiomes play in eco evolutionary adaptations and in maintaining the health of ecosystems today, scientists warn that certain alterations in microbiomes can have unforeseen impacts on organismal and ecosystem functioning as well as evolutionary consequences. The health of microbial communities is a prerequisite for the health of ecosystems. Therefore, microbiome-mediated herbicide effects must be addressed and considered in pesticide assessments (Ruuskanen et al., 2023).

To further assess all potential effects of glyphosate and GBHs on soil microbial communities in detail, further research is needed. Multiple studies provide conflicting results, probably linked to varied study designs, experimental conditions, testing methods and different GBHs with unknown co-formulants. Van Bruggen et al. (2021) also notice that the observation of glyphosate effects on microbial communities in soil, rhizosphere and animal guts is dependent on the level of taxonomy studied. While at levels of Phyla, Order or Classes often no effects are found, negative effects on the composition of microorganisms have been shown at genus and species levels, as well as on biological processes. Notably, beneficial rhizobacteria in the soil and beneficial intestinal bacteria in animals are often adversely affected, while pathogenic bacteria and fungi are enhanced (van Bruggen et al., 2021). Furthermore, there is an important lack of studies on the long-term effects of GBHs on soil parameters. It is evident that environmental parameters, weather conditions and soil characteristics influence the impact of glyphosate and GBHs on soil microbiota

Impacts on microbiomes of mammals

As mentioned above, GBHs inhibit the shikimate pathway, which is present in plants and algae, but also in fungi and bacteria. Several studies have already investigated if glyphosate and GBHs can affect microbiomes in the gut of mammals, including humans. During the last years, the relationships between human or animal health and the microbiomes have been more intensively studied. The microbiome is central to the health of the host, due to its important functions such as protection against pathogenic microorganisms, conversion of nutrients and detoxification and interaction with the nervous and endocrine systems. At the same time, the indirect effects of pesticides, including glyphosate, on animal and human health has received more attention.

Van Bruggen et al. (2021) point in their review at the increased susceptibility of birds and mammals to toxic *Clostridium* and *Salmonella* species. For example, lactic acid producing bacteria, which can produce antibiotics and suppress pathogenic bacteria, were mostly negatively affected by Roundup® (Krüger et al. (2013); Rodloff and Krüger (2012)). The incidence of botulism was also increased in cows with high concentrations of glyphosate in their feed and urine (Gerlach et al. (2014); Krüger et al. (2013); Krüger et al. (2014)). Mesnage and Antoniou (2020) have found in their analysis of DNA sequences available from the Human Microbiome Project that gut microbiome EPSPS enzymes are predicted to be sensitive to glyphosate, although the degree to which glyphosates really perturb the human gut microbiota is debated. They concluded that further research with more advanced molecular profiling techniques is needed to assess whether glyphosate and GBHs can alter gut microbiome functioning leading to health impacts. Also Puigbò et al. (2022) concluded that more than half of the human microbiome is sensitive to glyphosate. They find that, while further experimental and epidemiological studies are needed, their research as well as an increasing number of other studies point to the herbicide's potential to disrupt healthy microbiomes, including the human microbiome. Van Bruggen et al. (2021) recommend further interdisciplinary research on low level chronic glyphosate exposure, changes in microbial communities at species level, and the emergence of diseases, including intestinal cancer (Davoren and Schiestle (2018)). A recent paper by Matsuzaki et al. (2023) highlights the potential links between pesticide exposure and the microbiota-gut-brain axis. On the one hand, exposure studies show that pesticides can negatively impact microbiota, physiology and health of the host. At the same time, an increasing amount of studies show that pesticide exposure can lead to behavioural impairments in the host. They stress the need for further exploration of the mechanistic connection between gut microbiota and behavioural changes observed after exposure to pesticides.

Impact of Glyphosate on Earthworms

A 2022 study by Pelosi at al. showed that glyphosate contamination is common in both soil and earthworms on French arable land. Glyphosate and AMPA were detected in 88% and 85% of soils samples, and in 74% and 38% of the earthworm samples respectively. In this study, the highest glyphosate concentration measured in soil was 0.598 mg/kg. In earthworms, 2 to 3 times higher concentrations were measured. The authors acknowledged that "*bioaccumulation of glyphosate and AMPA in earthworms was higher than expected according to the properties of the molecules*".

Several independent studies demonstrate that glyphosate and GBH products in applied concentrations pose a risk to earthworms. The wellbeing of earthworms is a key factor to soil health, as they are the major decomposers of organic matter, while also having an important role in soil aeration, infiltration, structure, nutrient cycling and water movement.

Toxicity, Mortality and Body mass loss

Already in 2010, Correia & Moreira exposed that in glyphosate-treated soil, the weight of earthworms *Eisenia foetida* reduced by 50%. The toxicity of GBHs highly depends on the specific formulated product and the co-formulants. Research by Piola et. al. (2013) underlined the tangible difference in the toxicity of various formulated glyphosate products. Under laboratory conditions, the LC50 (Lethal Concentration 50: the concentration at which 50% of organisms die) to *Eisenia andrei* was 4.5-fold higher for Roundup FG than the LC50 of the product Mon 8750. Roundup FG caused DNA and lysosomal damage already at 14.4 µg ae cm^{-2}, what the authors consider as close to the applied environmental concentrations. Furthermore "*sublethal concentrations caused a concentration-dependent weight loss, consistent with the reported effect of glyphosate as an uncoupler of oxidative phosphorylation*" (Piola et. al., 2013).

Decreasing survival rate and drastic decline in the number of cocoons was also observed for several earthworm species by Stellin et al. in 2018, after exposure to Roundup 360® (0.59, 2.9, 5.79 g/m2 of glyphosate) in comparable concentrations as applied in vineyards in the North-East of Italy. For example, severe effects were shown on the deep-burrowing earthworm species *Octodrilus complanatus*, with the lowest levels of survival rate 33% and 7% after respectively 21 and 42 days of exposure. *Lumbricus terrestris* had lowest survival rates of 36% and 12% after 21 and 41 days, while for *Aporrectodea caliginosa* lowes survival rates of 32% and 12% were observed. Also significant reductions in cocoons numbers were found, for example, a 70% reduction in cocoons number for *L. terrestris* and *A. caliginosa*.

A 2020 study from Pochron et.al. found that exposure of earthworms (*Eisenia fetida*) in soil compost to 26.3 mg/kg glyphosate (in the form of isopropylamine salt per kg compost) caused 14.8 - 25.9% loss in body mass. Furthermore, the exposure caused worms to die 22.2–33.3% faster in a stress test. However, exposure to Roundup Ready-to-Use III® and Roundup Super Concentrate® did not show loss of body mass nor increased mortality in the stress test. These results reflect that differences in the exact composition and circumstances can result in different effects. The authors suggest it is possible that the nitrates and phosphates in the formulations contribute to the worm growth and increase glyphosate degradation.

A 2019 study by Pochron *et al.* found that earthworm (*Eisenia fetida*) sensitivity to Roundup-Ready-To-Use III® (yielding 26.3 mg glyphosate per kg dry soil, 29 days) depends on soil temperature and worm characteristics (e.g. initial body mass). Earthworms in unheated soil survived significantly fewer minutes during the stress test, with herbicide-exposed worms in unheated soil surviving the shortest. Pochron *et al.* (2021) also found in a later study that, after one-week exposure to Roundup (60.7 mg glyphosate per kg of soil), earthworms *Eisenia fetida* demonstrated the strongest decline. Two-weeks post exposure, soil microbes demonstrated the strongest decline. Both worms and soil microbes recovered by the third week.

The impact on soil organisms such as earthworms goes far beyond soils, and extends also to animals dependent on soil organisms. For example, Rigal et al. (2023) have shown that the drastic declines in (farmland) **birds** in Europe are linked to pesticide and fertilizer use. The effects are very outspoken for birds that are invertebrate feeders.

Toxicity, Behaviour

Nuutinen et al. (2020) treated *Eisenia fetida* with Rodeo® XL (1080 g active ingredients/ha) and found that "*the straw incorporation was slightly but not significantly lower in glyphosate-treated soil*".

Oxidative stress was observed by Hackenberger *et al.* (2018) in the short term after exposure of GBH products (glyphosate; 0.3; 3 and 30 µg kg dw/soil), but earthworm *Dendrobaena veneta* species recovered after a 28 days period.

Research from Brazil by Niemeyer *et al.* (2018), with different GBH products at recommended-use dose (Roundup Original®, Trop®, Zapp®, Crucial®), investigated the effect on the behaviour of different soil invertebrates: earthworms (*Eisenia andrei*), collembolans (*Folsomia candida*) and isopods (*Porcellio dilatatus*). The authors found that the different formulations had distinct effects: "*Non-avoidance behaviour was observed in standard tests (earthworms) in soil, neither in multispecies tests (earthworm + isopods) using oat straw, while for collembolans it occurred for the product Zapp® Qi 620 even at the recommended dose.*" On a bait lamina test, Crucial® treatment showed impaired feeding activity.

Glyphosate and its formulation (ROUNDUP) caused increased seizure-like behaviour in *C. elegans*. The convulsions were not recovered in the ROUNDUP exposed nematodes at 300-fold less concentration than the recommended dosage. The observed physiological changes show that glyphosate targets GABA-A receptors. The dysregulatory effect of glyphosate on inhibitory neurological circuits was highlighted as well. The study from Naraine et al. (2022) states, that: "*Our findings characterize glyphosate's exacerbation of convulsions and propose the GABA-A receptor as a neurological target for the observed physiological changes. It also highlights glyphosate's potential to dysregulate inhibitory neurological circuits.*"

Microbiome of earthworms

Glyphosate can disrupt earthworms' health through impacts on their gut microbiome. Owagboriaye et al. (2021) found that, when soils were sprayed with 115.49 mL/m² of Roundup® Alphée, this altered the bacterial population in all the three studied earthworm species significantly (*Alma millsoni*, *Eudrilus eugeniae* and *Libyodrilus violaceus*). Proteobacteria became the dominant phylum, as their populations were significantly enhanced by the GBH. As stated by the authors "*Affected bacteria were mostly from the genus Enterobacter, Pantoea and Pseudomonas, which together represented approximately 80 % of the total abundance assigned at the genus level in exposed earthworms, while they were present at a minor abundance (~1%) in unexposed earthworms.*"

Earthworm activity and soil health

A 2020 study by Owagboriaye et al. found that when earthworms species (*Alma millsoni*, *Eudrilus eugeniae* and *Libyodrilus violaceus*) were exposed to Roundup® Alphée (115.49 ml/m2), tomatoes planted with the casts of the exposed earthworms were unable to set fruit. Earthworms that remained unexposed improved the performance of the tomato plants, resulting in higher Vitamin C and β-carotene contents in those fruits .

Zaller et al. found in 2014 that GBH Roundup Speed in a mesocosm greenhouse experiment disrupted belowground interactions between earthworms and symbiotic arbuscular mycorrhizal fungi. The GBH application substantially decreased root mycorrhization, soil AMF (Arbuscular Mycorrhizal Fungi) spore biomass, vesicles and propagules. GBH application in interaction with AMF in the mesocosm experiment led to slightly heavier but less active earthworms. The negative effects on AMF can have wide consequences for crop cultivation, given the important role of earthworms and AMFs regarding plant nutrition. For example, conclude the authors, declines in AMF could require more fertilization, with economical and ecological impacts for farm management.

Zaller et al. (2021) concluded that GBH products (Roundup LB Plus, Roundup PowerFlex, Touchdown Quattro) and their corresponding active ingredients (salts of glyphosate isopropylammonium, potassium, diammonium), in their recommended dosages, decreased earthworm (*Lumbricus terrestris*) casting and movement activity. The authors found "*no consistent pattern that formulations had either higher or lower effects on earthworm activity than their active ingredients; rather, differences were substance-specific.*". According to the research, water infiltration was affected by both weed control types as soil organic matter (SOM). For example, the amount of leachate was higher when formulations were applied, rather than active ingredients in itself, and was also higher when SOM was low.

Conclusion and recommendations

Pollution of glyphosate, GBHs and their metabolites in soils is widespread. While the damage that glyphosate causes to plants is often highly visible aboveground, the negative impacts below ground remain mostly hidden for the eye. Soils are characterised by a very high biological diversity and complex processes, with soil organisms such as soil microbiota and earthworms performing vital functions for soil health and plant growth. While among the available body of research, contradictory results are found and further, long-term, detailed studies are needed, a variety of studies show negative impacts of GBHs on soil organisms and soil health. Glyphosate and GBH can impact the abundance and composition of certain **microbial communities**, such as rhizosphere-associated bacterial communities and arbuscular mycorrhizal fungi. GBHs can also cause increased colonisation of specific plant pathogens on plant roots, which can lead to increased release of mycotoxins, with possible impacts for crop productivity and food safety. Glyphosate negatively affects (e.g. growth inhibition) specific soil bacteria (e.g. *Bacillus* and *Pseudomonas* families), which perform vital functions such as increasing nutrient availability and suppressing pathogenic fungi. Glyphosate negatively impacts **earthworms** in multiple ways -including their survival, growth and behaviour-, which can in turn detrimentally impact **soil health and fertility**. GBHs can also affect the **microbiome of animals and humans**, with a growing amount of research pointing at the potential links between impacts on the microbiome and a variety of health impacts and illnesses.

In view of the potential extension of the authorisation of glyphosate at the end of 2023, it is concerning to note that the EU risk assessment does not adequately assess the impact of glyphosate on earthworms. In a recent article, Céline Pelosi of the French National Research Institute for Agriculture, Food and the Environment (Inrae), rightfully denounces that the effects of glyphosate on earthworms are underestimated because not adequately taken into account in the EU assessment. As Pelosi argues, over 60 studies have been published in the public scientific literature on the effects of glyphosate on earthworms, and virtually none were included in EFSA's peer review. The ones that have been retained concern compost worms, which are not found in natural soils where glyphosate is used. This bias is not insignificant as a study from Pelosi & al. (2013) showed that these compost worms were up to four times less sensitive to pesticides than the earthworms actually present in agricultural soils.

The risk assessment of EFSA is characterised by significant shortcomings and data gaps, which also relate to the assessment of the impact of glyphosate, GBH and their metabolites, on biodiversity and microbiome health. This approach contradicts the objective of the EU Pesticide Law (1107/2009 EC) that the protection of human and animal health and the environment should always take priority over the objective of improving yield production. In case of uncertainty about an identified health or environmental harm the EU Regulators should evoke the precautionary principle to ensure this high level of protection. At the same time, the Green Deal and Farm to Fork strategy envision a 50% reduction in the use and risk of chemical pesticides in the EU in 2030, through the proposal for the Sustainable use of Pesticides Regulation (SUR), and the promotion of safer, nature-based alternatives.

Scientific briefing

Alternatives to glyphosate use are available, as demonstrated in PAN Europe's report on alternatives to the use of glyphosate early this year. These alternatives enable weed management practices that retain the beneficial 'Aliae Plantae', enhancing the resilience and ecosystem functioning of agricultural areas. This includes promoting healthy soil functioning, increasing climate resilience and natural pest control. **These alternatives can and should be widely used to protect the health of farmers, citizens, soils and the wider environment, and to safeguard long-term sustainable food production. A complete ban on glyphosate** use on agricultural land and a general ban on glyphosate for non-agricultural uses (rails tracks, invasive species, urban areas, water banks etc) **is urgently needed**. To safeguard all soils, it is imperative that this ban also extends to the export of glyphosate and glyphosate-based herbicides to third countries.

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About PAN Europe:

Pesticide Action Network (PANEurope) is a network of NGOs working to reduce the use of hazardous pesticides and have them replaced with ecologically sound alternatives. We work to eliminate dependency on chemical pesticides and to support safe sustainable pest control methods. Our network brings together over 45 consumer, public health and environmental organisations and women's groups from across Europe.

References

Anthony, M. A., Bender, S. F., & van der Heijden, M. G. (2023). Enumerating soil biodiversity. *Proceedings of the National Academy of Sciences*, *120*(33). https://doi.org/10.1073/pnas.2304663120

Aristilde, L., Reed, M. L., Wilkes, R. A., Youngster, T., Kukurugya, M. A., Katz, V., & Sasaki, C. R. (2017). Glyphosate-induced specific and widespread perturbations in the metabolome of Soil pseudomonas species. *Frontiers in Environmental Science*, *5*. https://doi.org/10.3389/fenvs.2017.00034

Bento, C. P. M., Yang, X., Gort, G., Xue, S., van Dam, R., Zomer, P., Mol, H. G. J., Ritsema, C. J., & Geissen, V. (2016). Persistence of glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness. *Science of The Total Environment*, *572*, 301–311. https://doi.org/10.1016/j.scitotenv.2016.07.215

Berg, M., & Koskella, B. (2018). Nutrient- and dose-dependent microbiome-mediated protection against a plant pathogen. *Current Biology*, *28*(15). https://doi.org/10.1016/j.cub.2018.05.085

Blouin, M., Hodson, M. E., Delgado, E. A., Baker, G., Brussaard, L., Butt, K. R., Dai, J., Dendooven, L., Peres, G., Tondoh, J. E., Cluzeau, D., & Brun, J. -J. (2013). A review of earthworm impact on soil function and ecosystem services. *European Journal of Soil Science*, *64*(2), 161–182. https://doi.org/10.1111/ejss.12025

Brühl, C. A., & Zaller, J. G. (2021). Indirect herbicide effects on biodiversity, ecosystem functions, and interactions with global changes. *Herbicides*, 231–272. https://doi.org/10.1016/b978-0-12-823674-1.00005-5

Coleman, D. C., Callaham, M. A., & Crossley, D. A. (2018). *Fundamentals of Soil Ecology*. Elsevier/Academic Press.

Correia, F. V., & Moreira, J. C. (2010). Effects of glyphosate and 2,4-D on earthworms (Eisenia foetida) in laboratory tests. *Bulletin of Environmental Contamination and Toxicology*, *85*(3), 264–268. https://doi.org/10.1007/s00128-010-0089-7

Davoren, M. J., and Schiestl, R. H. (2018). Glyphosate-based herbicides and cancer risk: a post-IARC decision review of potential mechanisms, policy and avenues of research. *Carcinogenesis* 39 (10), 1207–1215. https://doi.org/10.1093/carcin/bgy105

Ekwomadu, T. I., Akinola, S. A., & Mwanza, M. (2021). Fusarium mycotoxins, their metabolites (free, emerging, and masked), food safety concerns, and health impacts. *International Journal of Environmental Research and Public Health*, *18*(22), 11741. https://doi.org/10.3390/ijerph182211741

European Food Safety Authority. (2015). Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. *EFSA Journal 2015;13(11):4302.* https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2015.4302

European Food Safety Authority. (2023, July 6). Glyphosate: No critical areas of concern; data gaps identified. https://www.efsa.europa.eu/en/news/glyphosate-no-critical-areas-concern-data-gaps-identified

Feller, C., Brown, G. G., Blanchart, E., Deleporte, P., & Chernyanskii, S. S. (2003). Charles Darwin, earthworms and the natural sciences: Various lessons from past to future. *Agriculture, Ecosystems & Environment*, *99*(1–3), 29–49. https://doi.org/10.1016/s0167-8809(03)00143-9

Ferrigo, D., Raiola, A., & Causin, R. (2016). Fusarium toxins in cereals: Occurrence, legislation, factors promoting the appearance and their management. *Molecules*, *21*(5), 627. https://doi.org/10.3390/molecules21050627

Gerlach, H., Gerlach, A., Schrödl, W., Schottdorf, B., Haufe, S., Helm, H., et al. (2014). Oral application of charcoal and humic acids to dairy cows influences Clostridium botulinum blood serum antibody level and glyphosate excretion in urine. *J. Clin. Toxicol. 4*, 186. https://doi.org/10.4172/2161-0495.186

Hackenberger, D. K., Stjepanović, N., Lončarić, Ž., & Hackenberger, B. K. (2018). Acute and subchronic effects of three herbicides on biomarkers and reproduction in earthworm Dendrobaena Veneta. *Chemosphere*, *208*, 722–730. https://doi.org/10.1016/j.chemosphere.2018.06.047

Kepler, R. M., Epp Schmidt, D. J., Yarwood, S. A., Cavigelli, M. A., Reddy, K. N., Duke, S. O., Bradley, C. A., Williams, M. M., Buyer, J. S., & Maul, J. E. (2020). Soil microbial communities in diverse agroecosystems exposed to the herbicide glyphosate. *Applied and Environmental Microbiology*, *86*(5). https://doi.org/10.1128/aem.01744-19

Klátyik, S., Simon, G., Oláh, M., Mesnage, R., Antoniou, M. N., Zaller, J. G., & Székács, A. (2023). Terrestrial ecotoxicity of glyphosate, its formulations, and co-formulants: Evidence from 2010–2023. *Environmental Sciences Europe*, *35*(1). https://doi.org/10.1186/s12302-023-00758-9

Krüger, M., Shehata, A. A., Schrödl, W., and Rodloff, A. (2013). Glyphosate suppresses the antagonistic effect of *Enterococcus* spp. on *Clostridium botulinum*. *Anaerobe* 20, 74–78. https://doi.org/10.1016/j.anaerobe.2013.01.005

Krüger, M., Schledorn, P., Schrödl, W., Hoppe, H.-W., Lutz, W., and Shehata, A. A. (2014a). Detection of glyphosate residues in animals and humans. *J. Environ. Anal. Toxicol. 4*, 2. https://doi.org/10.4172/2161-0525.1000210

Lescano, M. R., Masin, C. E., Rodríguez, A. R., Godoy, J. L., & Zalazar, C. S. (2020). Earthworms to improve glyphosate degradation in biobeds. *Environmental Science and Pollution Research*, *27*(21), 27023–27031. https://doi.org/10.1007/s11356-020-09002-w

Mandl, K., Cantelmo, C., Gruber, E., Faber, F., Friedrich, B., & Zaller, J. G. (2018). Effects of glyphosate-, glufosinate- and flazasulfuron-based herbicides on soil microorganisms in a vineyard. *Bulletin of Environmental Contamination and Toxicology*, *101*(5), 562–569. https://doi.org/10.1007/s00128-018-2438-x

Matsuzaki, R., Gunnigle, E., Geissen, V., Clarke, G., Nagpal, J., Cryan, J.F. (2023). Pesticide exposure and the microbiota-gut-brain axis. *The ISME Journal - Multidisciplinary Journal of Microbial Ecology - Nature, 17,* 1153-1166*.* https://www.nature.com/articles/s41396-023-01450-9

Mendes, R., & Raaijmakers, J. M. (2015). Cross-kingdom similarities in microbiome functions. *The ISME Journal*, *9*(9), 1905–1907. https://doi.org/10.1038/ismej.2015.7

Mendonca, C. M., Reed, M. L., Kukurugya, M. A., & Aristilde, L. (2019). Adverse metabolic outcomes in soil *pseudomonas* species exposed to polyethoxylated tallow amine and glyphosate. *Environmental Science & Technology Letters*, *6*(8), 448–455. https://doi.org/10.1021/acs.estlett.9b00363

Mesnage, R., & Antoniou, M. N. (2020). Computational modelling provides insight into the effects of glyphosate on the shikimate pathway in the human gut microbiome. *Current Research in Toxicology*, *1*, 25–33. https://doi.org/10.1016/j.crtox.2020.04.001

Naraine, A. S., Aker, R., Sweeney, I., Kalvey, M., Surtel, A., Shanbhag, V., & Dawson-Scully, K. (2022). Roundup and glyphosate's impact on GABA to elicit extended proconvulsant behavior in Caenorhabditis elegans. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-17537-w

Newman, M. M., Hoilett, N., Lorenz, N., Dick, R. P., Liles, M. R., Ramsier, C., & Kloepper, J. W. (2016). Glyphosate effects on soil rhizosphere-associated bacterial communities. *Science of The Total Environment*, *543*, 155–160. https://doi.org/10.1016/j.scitotenv.2015.11.008

Nicolas, V., Oestreicher, N., & Vélot, C. (2016). Multiple effects of a commercial Roundup® formulation on the soil filamentous fungus aspergillus nidulans at low doses: Evidence of an unexpected impact on energetic metabolism. *Environmental Science and Pollution Research*, *23*(14), 14393–14404. https://doi.org/10.1007/s11356-016-6596-2

Niemeyer, J. C., de Santo, F. B., Guerra, N., Ricardo Filho, A. M., & Pech, T. M. (2018). Do recommended doses of glyphosate-based herbicides affect soil invertebrates? field and laboratory screening tests to risk assessment. *Chemosphere*, *198*, 154–160. https://doi.org/10.1016/j.chemosphere.2018.01.127

Nuutinen, V., Hagner, M., Jalli, H., Jauhiainen, L., Rämö, S., Sarikka, I., & Uusi-Kämppä, J. (2020). Glyphosate spraying and Earthworm Lumbricus terrestris L. Activity: Evaluating short-term impact in a glasshouse experiment simulating cereal post-harvest. *European Journal of Soil Biology*, *96*, 103148. https://doi.org/10.1016/j.ejsobi.2019.103148

Owagboriaye, F., Dedeke, G., Bamidele, J., Bankole, A., Aladesida, A., Feyisola, R., Adeleke, M., & Adekunle, O. (2020). Wormcasts produced by three earthworm species (alma millsoni, Eudrilus eugeniae and Libyodrilus violaceus) exposed to a glyphosate-based herbicide reduce growth, fruit yield and quality of tomato (lycopersicon esculentum). *Chemosphere*, *250*, 126270. https://doi.org/10.1016/j.chemosphere.2020.126270

Owagboriaye, F., Mesnage, R., Dedeke, G., Adegboyega, T., Aladesida, A., Adeleke, M., Owa, S., & Antoniou, M. N. (2021). Impacts of a glyphosate-based herbicide on the gut microbiome of three earthworm species (alma millsoni, Eudrilus eugeniae and Libyodrilus violaceus): A pilot study. *Toxicology Reports*, *8*, 753–758. https://doi.org/10.1016/j.toxrep.2021.03.021

Panettieri, M., Lazaro, L., López-Garrido, R., Murillo, J. M., & Madejón, E. (2013). Glyphosate effect on soil biochemical properties under conservation tillage. *Soil and Tillage Research*, *133*, 16–24. https://doi.org/10.1016/j.still.2013.05.007

PAN Europe. (2023a). GLYPHOSATE IS POLLUTING OUR WATERS - ALL ACROSS EUROPE. PAN EUROPE'S WATER REPORT, SEPTEMBER 2023. https://www.pan-europe.info/sites/pan-europe.info/files/public/resources/reports/Glyphosate%20is%20polluting%20our%20waters%20all%20across%20Europe.pdf.

PAN Europe. (2023b).GLYPHOSATE BASED HERBICIDES & THEIR IMPACT ON BEES' HEALTH. https://www.pan-europe.info/sites/pan-europe.info/files/public/resources/briefings/Glyphosate%20based%20herbicides%20and%20their%20impact%20on%20bees%27%20health.pdf.

PAN Europe. (2023c). WEED MANAGEMENT: ALTERNATIVES TO THE USE OF GLYPHOSATE. https://www.pan-europe.info/sites/pan-europe.info/files/public/resources/reports/Weed%20management%20Alternatives%20to%20the%20use%20of%20glyphosate%20Report_09032023.pdf.

PAN Europe. (2023d). Expert meeting shows that glyphosate is not safe for health and environment. https://www.pan-europe.info/expert-meeting-shows-glyphosate-not-safe-health-and-environment#.

Pelosi, C., Bertrand, C., Bretagnolle, V., Coeurdassier, M., Delhomme, O., Deschamps, M., Gaba, S., Millet, M., Nélieu, S., & Fritsch, C. (2022). Glyphosate, ampa and glufosinate in soils and earthworms in a French arable landscape. *Chemosphere*, *301*, 134672. https://doi.org/10.1016/j.chemosphere.2022.134672

Pelosi, C., Joimel, S., & Makowski, D. (2013). Searching for a more sensitive earthworm species to be used in pesticide homologation tests - a meta-analysis. Chemosphere, 90(3), 895–900. https://doi.org/10.1016/j.chemosphere.2012.09.034

Piola, L., Fuchs, J., Oneto, M. L., Basack, S., Kesten, E., & Casabé, N. (2013). Comparative toxicity of two glyphosate-based formulations to Eisenia Andrei under laboratory conditions. *Chemosphere*, *91*(4), 545–551. https://doi.org/10.1016/j.chemosphere.2012.12.036

Pochron, S. T., Mirza, A., Mezic, M., Chung, E., Ezedum, Z., Geraci, G., Mari, J., Meiselbach, C., Shamberger, O., Smith, R., Tucker, W. J., & Zafar, S. (2021). Earthworms Eisenia fetida recover from Roundup Exposure. *Applied Soil Ecology*, *158*, 103793. https://doi.org/10.1016/j.apsoil.2020.103793

Pochron, S., Choudhury, M., Gomez, R., Hussaini, S., Illuzzi, K., Mann, M., Mezic, M., Nikakis, J., & Tucker, C. (2019). Temperature and body mass drive earthworm (Eisenia fetida) sensitivity to a popular glyphosate-based herbicide. *Applied Soil Ecology*, *139*, 32–39. https://doi.org/10.1016/j.apsoil.2019.03.015

Pochron, S., Simon, L., Mirza, A., Littleton, A., Sahebzada, F., & Yudell, M. (2020). Glyphosate but not roundup® harms earthworms (Eisenia fetida). *Chemosphere*, *241*, 125017. https://doi.org/10.1016/j.chemosphere.2019.125017

Puigbò, P., Leino, L. I., Rainio, M. J., Saikkonen, K., Saloniemi, I., & Helander, M. (2022). Does glyphosate affect the human microbiota? *Life*, *12*(5), 707. https://doi.org/10.3390/life12050707

Ramírez-Puebla, S. T., Servín-Garcidueñas, L. E., Jiménez-Marín, B., Bolaños, L. M., Rosenblueth, M., Martínez, J., Rogel, M. A., Ormeño-Orrillo, E., & Martínez-Romero, E. (2013). Gut and root microbiota commonalities. *Applied and Environmental Microbiology*, *79*(1), 2–9. https://doi.org/10.1128/aem.02553-12

Rodloff, A. C., and Krüger, M. (2012). Chronic Clostridium botulinum infections in farmers. Anaerobe 18, 226–228. https://doi.org/10.1016/j.anaerobe.2011.12.011

Ruuskanen, S., Fuchs, B., Nissinen, R., Puigbò, P., Rainio, M., Saikkonen, K., & Helander, M. (2023). Ecosystem consequences of herbicides: The role of microbiome. *Trends in Ecology & Evolution*, *38*(1), 35–43. https://doi.org/10.1016/j.tree.2022.09.009

Silva, V., Mol, H. G. J., Zomer, P., Tienstra, M., Ritsema, C. J., & Geissen, V. (2019). Pesticide residues in European agricultural soils – a hidden reality unfolded. *Science of The Total Environment*, *653*, 1532–1545. https://doi.org/10.1016/j.scitotenv.2018.10.441

Silva, V., Montanarella, L., Jones, A., Fernández-Ugalde, O., Mol, H. G. J., Ritsema, C. J., & Geissen, V. (2018). Distribution of glyphosate and aminomethylphosphonic acid (AMPA) in Agricultural Topsoils of the European Union. *Science of The Total Environment*, *621*, 1352–1359. https://doi.org/10.1016/j.scitotenv.2017.10.093

Singh, S., Kumar, V., Gill, J. P., Datta, S., Singh, S., Dhaka, V., Kapoor, D., Wani, A. B., Dhanjal, D. S., Kumar, M., Harikumar, S. L., & Singh, J. (2020). Herbicide glyphosate: Toxicity and microbial degradation. *International Journal of Environmental Research and Public Health*, *17*(20), 7519. https://doi.org/10.3390/ijerph17207519

Stellin, F., Gavinelli, F., Stevanato, P., Concheri, G., Squartini, A., & Paoletti, M. G. (2018). Effects of different concentrations of glyphosate (roundup 360®) on earthworms (octodrilus complanatus, Lumbricus Terrestris and aporrectodea caliginosa) in vineyards in the north-east of Italy. *Applied Soil Ecology*, *123*, 802–808. https://doi.org/10.1016/j.apsoil.2017.07.028

Van Bruggen, A. H., Finckh, M. R., He, M., Ritsema, C. J., Harkes, P., Knuth, D., & Geissen, V. (2021). Indirect effects of the herbicide glyphosate on plant, animal and human health through its effects on microbial communities. *Frontiers in Environmental Science*, *9*. https://doi.org/10.3389/fenvs.2021.763917

Vázquez, M. B., Moreno, M. V., Amodeo, M. R., & Bianchinotti, M. V. (2021). Effects of glyphosate on soil fungal communities: A field study. *Revista Argentina de Microbiología*, *53*(4), 349–358. https://doi.org/10.1016/j.ram.2020.10.005

Yu, X. M., Yu, T., Yin, G. H., Dong, Q. L., An, M., Wang, H. R., & Ai, C. X. (2015). Glyphosate biodegradation and potential soil bioremediation by bacillus subtilis strain BS-15. *Genetics and Molecular Research*, *14*(4), 14717–14730. https://doi.org/10.4238/2015.november.18.37

Zaller, J. G., & Brühl, C. A. (2021). Direct herbicide effects on terrestrial nontarget organisms belowground and aboveground. *Herbicides*, 181–229. https://doi.org/10.1016/b978-0-12-823674-1.00004-3

Zaller, J. G., Cantelmo, C., Santos, G. D., Muther, S., Gruber, E., Pallua, P., Mandl, K., Friedrich, B., Hofstetter, I., Schmuckenschlager, B., & Faber, F. (2018). Herbicides in vineyards reduce grapevine root mycorrhization and alter soil microorganisms and the nutrient composition in grapevine roots, leaves, xylem sap and grape juice. *Environmental Science and Pollution Research*, *25*(23), 23215–23226. https://doi.org/10.1007/s11356-018-2422-3

Zaller, J. G., Heigl, F., Ruess, L., & Grabmaier, A. (2014). Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem. *Scientific Reports*, *4*(1). https://doi.org/10.1038/srep05634

Zaller, J. G., Weber, M., Maderthaner, M., Gruber, E., Takács, E., Mörtl, M., Klátyik, S., Győri, J., Römbke, J., Leisch, F., Spangl, B., & Székács, A. (2021). Effects of glyphosate-based herbicides and their active ingredients on earthworms, water infiltration and glyphosate leaching are influenced by soil properties. *Environmental Sciences Europe*, *33*(1). https://doi.org/10.1186/s12302-021-00492-0

Zobiole, L. H. S., Kremer, R. J., Oliveira, R. S., & Constantin, J. (2010). Glyphosate affects micro-organisms in rhizospheres of glyphosate-resistant soybeans. *Journal of Applied Microbiology*, *110*(1), 118–127. https://doi.org/10.1111/j.1365-2672.2010.04864.

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