The impact of glyphosate on soil health

The evidence to date

Glyphosate and Soil

Introduction

Over the last decade, about 6.1 billion kilograms of the herbicide glyphosate have been applied worldwide.[1] Glyphosate [N-(phosphonomethyl) glycine] is an active ingredient in a range of weed killer products, created for use in agriculture, horticulture and at amenity sites. Its use globally has risen almost 15-fold since 1996, when genetically engineered glyphosate-tolerant "Roundup Ready" crops were introduced.[2] In Great Britain in 2014, 1.9 million kilograms of glyphosate were used on agricultural and horticultural crops, on 2.2 million hectares.[3]

Despite being the most heavily applied herbicide in the world,[4] in 2015 glyphosate was classified as 'probably carcinogenic to humans' by the International Agency for Research on Cancer, the specialized cancer agency of the World Health Organization, following a review of evidence from human exposure studies and in research on laboratory animals.[5] Furthermore, in 2016, scientists raised their concerns about the safety of glyphosate based herbicides and call for new investments in epidemiological studies, biomonitoring, and toxicology studies.[6]

Whilst the effects of glyphosate on human health are coming under scrutiny, scientists are now concerned about our insufficient knowledge of the *ecological* safety of glyphosate, the way it behaves in the natural environment, how it interacts with living organisms, and the pathways through which it is degraded.[7]

Glyphosate has been considered an environmentally safe herbicide because it is assumed to be inactivated quickly after spraying due to rapid sorption onto particles in the soil, and its fast degradation by microbes.[8] In addition, the mechanism by which it kills plants (inhibiting the shikimic acid metabolic pathway)[9] is thought to be unique to plants and some micro-organisms, including bacteria, algae and fungi, and thus theoretically not a threat to mammals.[10] However, evidence from several studies now shows that glyphosate-based herbicides, via multiple mechanisms, can adversely affect the biology of mammals. Furthermore, the half-life of glyphosate, which gives an indication of its persistence in the soil and water, is believed to be longer than previously thought.[11] Recent research suggests that the herbicide persists longer with the return of crop residues containing glyphosate to the soil.[12] There is evidence to suggest that glyphosate-based herbicides can adversely affect aquatic invertebrate ecology[13] and research has also shown a negative impact on amphibian larvae (tadpoles)[14] and earthworms.[15]

As well as the active ingredient of glyphosate, there is also concern about impacts of the adjuvants (other chemical substances that are added such as solvents and surfactants) in commercial glyphosate products, where different formulations have been found to have different levels of toxicity compared to pure glyphosate.[16]

The Soil Association has reviewed the science on the impact of glyphosate on soils and soil life. For the world's most widely sold weed-killer, we found surprisingly little research has been done. What research there is shows contrasting results, significant uncertainty and some evidence that glyphosate causes harm. More research is urgently needed.

What about the impact of glyphosate on soil and soil life?

Soils are the foundation of our food security and yet a recent global scientific assessment found that 33 per cent of land is degraded due to the erosion, salinization, compaction and acidification and chemical pollution of our soils.[17] This report reviews the published and peer-reviewed scientific evidence about the impact of glyphosate-based herbicides on soils, soil micro-organisms and soil fauna.

Sorption of glyphosate onto soil and the risk of leaching

The risk of environmental pollution through the leaching of pesticides out of soils into water bodies is affected by how strongly the compound is sorbed to soil. (Sorption is the process by which one substance becomes attached to another and includes both adsorption and absorption). Compared to other pesticides, glyphosate is recorded to have strong sorption characteristics, reducing the risk of leaching. However, several studies have indicated that in some circumstances there is a risk of the leaching of glyphosate into deeper soil layers, where it could end up in ground and surface waters.[18] The level of sorption depends on several soil characteristics including mineral content and type, pH, soil redox conditions, phosphate content (that can compete with glyphosate for sorption sites) and possibly soil organic matter.[19] Rainfall and poor state of soils can increase the risk of glyphosate loss out of soils through erosion.[20]

Degradation of glyphosate

Micro-organisms in soil (bacteria and fungi)[21] are responsible for the degradation of glyphosate through two chemical pathways. One pathway produces a compound known as AMPA (aminomethylphosphonic acid) which is found in soils treated with glyphosate. This is thought to be mildly toxic to plant growth. The second pathway produces the compound sarcosine. The micro-organisms responsible for the degradation use enyzmes to break down glyphosate, to obtain a source of phosphorus, nitrogen and carbon for themselves.[22] Studies examining the rate of glyphosate degradation showed some variability in results, and the process can depend on a range of factors. There is some evidence for the rate of degradation being correlated with the population size of bacteria in the soils.[23] Overall, sorption of glyphosate onto soil particles is thought to decrease degradation, but glyphosate that has been sorbed can still be degraded by micro-organisms. Rates will vary with topographical features that effect water availability,[24] soil type, and increase with temperature.[25]

Effect of glyphosate on soil micro-organisms

Micro-organisms are a major portion of the biodiversity and biomass of soils and play a key role in maintaining soil processes, and thus the functioning of ecosystems. They are considered 'crucial to life', and are present in very large numbers.[26] 'Ecosystem services' is a term to measure the (monetary) 'benefits provided by ecosystems that contribute to making human life both possible and worth living'.[27] Microbial communities in the soil form the basis of ecosystem services such as the transformation of pollutants and the nutrient cycling[28] and underpin all provisioning and regulating services.[29] Several groups of scientists working in the field are calling for further research on the impact of glyphosate on microbial soil communities given the critical role that these organisms play in ecosystem services, including in maintaining plant health in agricultural systems.[30]

To date, scientific studies about the impact of glyphosate on soil micro-organisms have provided contrasting results. Some soil-based studies have not found any threat to soil micro-organisms from glyphosate.[31] Indeed, it is understood that glyphosate increases soil microbial activity when the herbicide is added because microbes break it down and use it as a source of carbon, nitrogen or phosphorus (as discussed above).[32] However, this is thought to be a short-term effect only.[33] One study found no effect on bacteria numbers from the use of glyphosate, but fungi and Actinomycetes (bacterium) numbers increased.[34]

In the forestry context, it has been found that in ponderosa pine plantations glyphosate has no consequential effects on soil communities in soil based tests.[35] Another study found a benign effect on microbial community structure when the commercial formulation of glyphosate was applied to soil samples at the recommended field rate, and produces a non-specific, short-term stimulation of bacteria at a high concentration.[3]6 A further study found that glyphosate has only small and transient effects on soil microbial community structure, function and activity on field scale experiments in agricultural soils.[37]

However, the potential non-target effects of glyphosate on soil micro-organisms are still of much concern amongst scientists.[38] There is concern about the diverse effects now reported about the impact of glyphosate on the biology and ecology of rhizosphere micro-organisms, and on their interactions with plant roots when released into the rhizosphere, the region of soil that surrounds, and is influenced by, plant roots.[39]

Effects on soil microbial community population, function and structure

A recent study suggested that glyphosate may have an indirect effect on the soil microbial community function and structure in arable ecosystems which should be further evaluated. This research, that looked at the impact of glyphosate (as RoundUp) on the soil bacterial communities in the rhizosphere of glyphosate-treated barley, found that the abundance of the culturable bacterial community, and the total bacterial composition were affected, and there was a proliferation of protists (a varied group of single celled organisms). This is likely due, at least in part, to an increased availability of easily degradable carbon compounds from the roots killed by the glyphosate.[40]

Another study examining the effects of glyphosate (as RoundUp) on soil bacteria, found that proteobacteria increased in relative abundance for corn and soya crops, following glyphosate exposure, whilst the relative abundance of Acidobacteria decreased in response to glyphosate exposure. This is a significant observation because Acidobacteria are believed to be highly involved in biogeochemical processes, such as for cellulose degradation. Decreases in the abundance of these bacteria over the long-term could impair the ability of soil to perform certain biogeochemical reactions performed by these organisms.[41]

Recent research demonstrated that a Roundup formulation (R450) was toxic to the soil fungus *Aspergillus nidulans*, at doses far below the recommended agricultural application rate, and concluded that the herbicide might potentially impair agricultural soil ecosystems.[42]

Impact on mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) improve water access and soil minerals for plants, improve drought tolerance and help with resistance against pathogens. Recent research has found that glyphosate (and/or its metabolite AMPA) reduces the spore viability and root colonisation of AMF, and could reduce plant diversity.[43] Another study found a 40% reduction of mycorrhization after the application of Roundup in soils that had been amended with the mycorrhizal fungi, *Glomus mosseae*.[44]

Impact of repeated glyphosate applications

There is evidence now to suggest that repeated glyphosate applications can impact on soil microbial communities as they adapt to repeated glyphosate applications.[45] One study found that a single exposure to soils of glyphosate (technical grade) caused only minor changes to microbial community function or structure. However, in soils where there had been no previous application of glyphosate, microbial respiration increased in response to glyphosate exposure. This potentially reflects a stress response of species sensitive to glyphosate. In contrast, in soils that have been chronically exposed to glyphosate, the microbes did not have this response. This is most likely due to the gradual elimination of glyphosate sensitive species.[46]

Another study, found a negative impact of glyphosate (RoundUp) on non-pathogenic soil borne microfungi species in boreal forest soil, and suggested that repeated herbicide applications caused a shift in the fungal species towards those more resistant to exposure.[47] This effect was not found in a study looking at the impact of long-term glyphosate use on soils in Argentina on soil microbial communities. One possible reason for this lack of difference could be explained by the adsorption of glyphosate to soil which would make it unavailable for microbial communities.[48]

Increase in micro-organisms causing disease in crops

It has been reported that using glyphosate as a weed control in agricultural systems has led to the increased severity or re-emergence of crop diseases.[49] There is concern over how the use of glyphosate increases the potential for the development of pathogen levels that affect crop health, altering the communities of rhizosphere microbes involved in nutrient transformation, and shifting the balance between micro-organisms that are beneficial and detrimental to plant health.[50] For example, one study found that the disease severity and frequency of the soil borne fungus *Fusarium solani* f. sp. *Glycines*, the cause of Sudden Death Syndrome, in glyphosate-tolerant soya beans was higher after application of glyphosate compared to no herbicide application.[51]

There is now evidence to suggest that it is not just the direct disruption of the shikimic acid metabolic pathway which is responsible for the herbicidal properties of glyphosate. It is now believed by some scientists that the herbicidal efficacy of glyphosate is largely due to colonization of roots of affected plants by soil-borne pathogens and that glyphosate somehow compromises the ability of plants to defend against pathogens that inhabit the rhizosphere. Many of plants defences are reliant on the shikimic acid pathway, and as glyphosate blocks this pathway, it is conceivable that glyphosate would render plants more susceptible to pathogens.[52]

Research on glyphosate-resistant soybeans found that glyphosate altered particular rhizosphere micro-organisms.[53] In one study, the colonisation of roots by Fusarium fungi increased steadily as soybean growth progressed and as the rate of glyphosate increased. This suggests that glyphosate affects the ability of plants to suppress potential pathogen colonisation and root infection. Further, by suppressing Fluorescent pseudomonads bacteria and Mn-reducing rhizobacteria, glyphosate lowers two plant defence mechanisms for warding off pathogens.[54] A different laboratory study, found no effect of glyphosate (Roundup) on Trichoderma or Gliocladium genera of fungi. However, both Fusarium and Pythium fungi genera populations increased proportionally to the increase in glyphosate concentrations, a concern given that both genera contain plant pathogens.[55]

Conversely, one study has looked at the impact of glyphosate, active ingredient and glyphosate commercial formulations, in laboratory tests, at field concentrations, on four types of entomopathogenic fungi – fungi that are understood to play a *positive role in controlling* pest insects in agricultural systems. They found that glyphosate active ingredient had no impact on the fungi, but that the glyphosate formulations (different brands of Roundup) did have a negative impact. The authors say that it is important that the impact of the supposedly inert ingredients in these formulations is further studied.[56]

Impact on soil fauna: Earthworms

Earthworms act as 'ecosystem engineers' by shredding plant litter, mineralising it and soil organic matter in their guts, and producing casts that enhance soil nutrient availability and promote plant productivity. Their burrowing enhances soil root penetration and water infiltration.[57]

Whilst at least two studies have not indicated any negative effects of glyphosate on earthworms,[58] at least six other studies found damaging effects of the herbicide. One study found that the earthworm *Eisenia fetida* avoids soil contaminated by the glyphosate based herbicide Groundclear, and this impact on locomotor activity could compromise the survival of the worms.[59] In another study, the number of hatched *Eisenia fetida* Andrei cocoons was significantly reduced in earthworms exposed to Roundup treated soils, and the number of juveniles was also significantly lower, indicating that glyphosate has a deleterious effect on the viability of cocoons. This study too found that the earthworms also avoided the soil treated with the herbicide. Earthworms have chemoreceptors and sensory turbercles and present a high sensitivity to chemicals in the soil.[60]

Another laboratory study on *Eisenia foetida* earthworms demonstrated severe effects on the development and reproduction caused by glyphosate (active ingredient only) at a range of concentrations, indicating that it may have significant toxic effects on soil biota. There was a decrease in the mean weight of the earthworms, and no cocoons or juveniles were found in the soil containing the herbicide.[61]

In a different earthworm greenhouse experiment, Roundup application initially stimulated surface casting activity of *Lumbricus terrestris* L. However the number of produced casts ceased dramatically about one week after herbicide application; cumulative cast mass produced by *L. terrestris* four weeks after herbicide application was reduced by 46% compared to the area not treated by herbicides. The activity of soil dwelling earthworms, *Aporrectodea caliginosa*, was not affected. The reproduction success of both earthworm species substantially decreased after herbicide application. The hatching rate of cocoons decreased from 43% to 17% for *L. terrestris* and from 71% to 32% for *A. caliginosa* when cocoons were collected from soil without herbicide or with herbicide treatment, respectively.[62]

Another study looked at the impact of Roundup on ecological interactions between the earthworm species, *Lumbricus terrestris*, and symbiotic arbuscular mycorrhizal fungi (AMF). The applications of Roundup reduced earthworm activity (as measured by the disturbance of toothpicks) in areas which contained AMF only. Earthworm activity (as measured by surface cast production) was not influenced by the application of Roundup or the presence of AMF. The application of Roundup led to earthworms that were heavier and that were less active at the surface. This is probably because there was abundant food in form of dead roots, or AMF in the soil that prohibited the earthworms from foraging from the surface.[63]

A recent 2016 study found that in the long term (132 days), the continuous consumption of leaf litter contaminated with glyphosate (Cheminova) decreased the earthworms *(Pontoscolex corethrurus)* growth rate, with a clear decline in their total biomass. However, no consistent effect was seen on cocoon production.[64]

Research into the glyphosate on other soil fauna is extremely limited. A meta-analysis of the impact of a range of herbicides on soil nematodes concluded that herbicides do affect soil webs with a variety of impacts on different nematode assemblages.[65] The only study looking specifically at glyphosate and nematodes made a comparison with 'conventional herbicide' and not with soil that did not receive any herbicide treatment.[66]

Further research required

The scientific evidence on the impact of glyphosate on the soil and soil life is far from conclusive. Research indicates potential impacts in increasing crop diseases, changing the composition and functioning of soil micro-organism species and ecosystems, and recently published studies are showing a negative impact on earthworms. Scientists working in this field are calling for future research to be carried out. This is urgent given the widespread and heavy use of glyphosate worldwide.

Recommendations for future research

- Research should examine the impact of glyphosate on other soil fauna in addition to earthworms; nematodes, ants, beetles, termites, spiders, anthropods, molluscs and protozoa.
- Research should look at both the impact on specific species of soil fauna and microflora but also the 'knock-on' succession effects of changes in the soil ecosystem.
- Research should consider the differences between the impact of glyphosate on soils as an active ingredient only, and when it is combined with other ingredients in a range of commercial products.
- Many of the studies described in this report have looked at glyphosate in the context of its use in relation to genetically-modified glyphosate resistant crops. In the UK context, where GM crops are not commercially grown, it is just as important to consider the use of glyphosate in relation to non-GM crops, and in amenity situations.
- Research should consider whether there is a significant build-up of AMPA in soils, which is produced when glyphosate is broken down, and whether this is problematic or not, as it is considered mildly toxic to plants.

Cover image: Wheat being sprayed with glyphosate immediately before harvest.

References

[1] Benbrook, C.M. (2016) Trends in glyphosate herbicide use in the United States and globally, *Environmental Sciences Europe*, 28:3, DOI 10.1186/s12302-016-0070-0.

[2] Ibid.

[3] FERA (2016) *Pesticide Usage Statistics* available at *https://secure.fera.defra.gov.uk/pusstats/myindex.cfm* accessed 4/3/16.

[4] Myers, J.P., Antoniou, M.N., Blumberg, B., Carroll, L., Colborn, T., Everett, L.G.,
Hansen, M.,, Landrigan, P.J., Lanphear, B.P., Mesnage, R., Vandenberg, L.N., vom Saal, F.S., Welshons, W.V., Benbrook, M.C.(2016) Concerns over use of glyphosate-based
herbicides and risks associated with exposures: a consensus statement, *Environmental Health*, 15:19 DOI 10.1186/s12940-016-0117-0.

[5] World Health Organisation (WHO) (2015) *International Agency for Research on Cancer (IARC) Monographs, Volume 112: Evaluation of five organophosphate insecticides and herbicides*, 20th March 2015 available at *https://www.iarc.fr/en/media-centre/iarcnews/pdf/MonographVolume112.pdf* accessed 4/3/16.

[6] Myers et al (2016).

[7] Sviridov, A.V., Shushkova, T.V., Ermakova, E.V., Ivanova, D.O., Epiktetov, D.O., and Leontievsky, A.A. (2015) Microbial Degradation of Glyphosate Herbicides (Review), *Applied Biochemistry and Microbiology*, Vol. 51, No. 2, pp. 188–195.

[8] Hagner, M., Hallman, S., Jauhiainen, L., Kemppainen, R., Ram, S., Tiikkala, K., and Setal, H. (2015) Birch (Betula spp.) wood biochar is a potential soil amendment to reduce glyphosate leaching in agricultural soils, *Journal of Environmental Management*, 164, pp. 46 – 52.

[9] 'Glyphosate is a unique inhibitor of 5enolpyruvylshikimate3phosphate synthase (EPPS), the key enzyme of the shikimate pathway of aromatic compound biosynthesis in plants and some microorganisms. EPPS inhibition suppresses the synthesis of proteins and secondary metabolites, e.g., flavonoids, lignin, or coumarins, and deregulates energy metabolism' (Sviridov et al 2015, pp.188).

[10] Gaupp-Berghausen, M., Hofer, M., Rewald, B., and Zaller, J.G. (2015) Glyphosate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations, *Nature: Scientific Reports*, 5:12886, DOI: 10.1038/srep12886.
Tanney, J.B., and Hutchison, L.J. (2010) The effects of glyphosate on the in vitro linear growth of selected microfungi from aboreal forest soil, *Canadian Journal of Microbiology*, 56, pp. 138–144, DOI:10.1139/W09-122.

[11] Myers et al (2016).

[12] Mamy, L., Barriuso, E., Gabrielle, B., (2016) Glyphosate fate in soils when arriving in plant residues, *Chemosphere* 154, pp.425-433

[13] Cuhra, M., Traavik, T., and Bohn, T. (2013) Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in Daphnia magna, *Ecotoxicology*, 22, pp. 251–262, DOI 10.1007/s10646-012-1021-1.

[14] Relyea, R.A. (2005) The Lethal Impacts of Roundup and Predatory Stress on Six Species of North American Tadpoles, *Archives of Environmental Contamination and Toxicology*, 48, pp351-357), DOI: 10.1007/s00244-004-0086-0.
For a discussion and rebuttal of these results see Thompson, D.G., Solomon, K.R., Wojtaszek, B.F., Edington, A.N., Stephenson, G.R. and Relyea, R.A. (2006), The Impact of Insecticides and Herbicides on the Biodiversity and Productivity of Aquatic Communities: [with Response], *Ecological Applications*, Vol. 16, No. 5 (Oct., 2006), pp. 2022-2034.

[15] Gaupp-Berghausen et al 2015.

[16] Newman, M.N., Hoilett, N., Lorenz, N., Dick, R.P., Liles, M.R., Ramsier, C., and Kloepper, J.W. (2016) Glyphosate effects on soil rhizosphere-associated bacterial communities, *Science of the Total Environment*, 543, pp. 155 –160;
Seralini, G.E. (2015) Why glyphosate is not the issue with Roundup: A short overview of 30 years of our research, *Journal of Biological Physics and Chemistry*, Vol. 15, Issue 3, pp.111-119;
Tsui, M.T.K., and Chu, L.M. (2003) Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors,
Chemosphere 52, pp. 1189 –1197;
Sihtmäe, M., Blinova, I., Künnis-Beres, K., Kanarbik, L., Heinlaan, M., Kahru, A., (2013) Ecotoxicological effects of different glyphosate formulations, *Applied Soil Ecology*, 72, pp.215 –224.

[17] Food and Agriculture Organisation (FAO) (2015) Status of the World's Soil Resources available at *http://www.fao.org/documents/card/en/c/c6814873-efc3-41db-b7d3-2081a10ede50/* accessed on 4/3/16.

[18] Hagner et al (2015).

[19] See for a review Borggaard, O.K., and A.L. Gimsing. 2008. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: A review, *Pest Management Science*, 64, p441–456. DOI:10.1002/ps.1512;
Kanissery, R.G., Welsh, A., and Sims, G.K. (2015) Effect of Soil Aeration and Phosphate Addition on the Microbial Bioavailability of Carbon-14-Glyphosate, *Journal of Environmental Quality*, January 8, 2015, Technical Reports, Biomediation and Biodegradation;
Okada, E., Costa, J.L., Bedmar, F., (2016) Adsorption and mobility of glyphosate in different soils under no-till and conventional tillage, *Geoderma*, 263, pp. 78 –85;
Aparicio, V.C., De Gerónimo, E., Marino, D., Primost, J., Carriquiriborde, P., Costa, J.L., (2013)

Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins, *Chemosphere* 93, pp.1866 –1873;
Rampazzo, N., Rampazzo Todorovic, G., Mentler, A., and Blum W.E.H. (2013)
Adsorption of glyphosate and aminomethylphosphonic acid in soils, *International Agrophysics*, 27, pp. 203-209 DOI: : 10.2478/v10247-012-0086-7.
[20] Todorovic, G.R., Rampazzo, N., Mentler, A., Blum, W.E.H., Eder, A., Strauss, P. (2014)
Influence of soil tillage and erosion on the dispersion of glyphosate and aminomethylphosphonic acid in agricultural soils, *International Agrophysics*, 28, pp.93 –100.
Yang, X., Wang, F., Bento, C.P.M., Meng, L., van Dam, R., Mol, H., Liu, G., Ritsema, C.J., and Geissen, V. (2015) Decay characteristics and erosion-related transport of glyphosate in Chinese loess soil under field conditions, Science of the Total Environment, 530 –531, pp.87 –95.
[21] See Sviridov et al (2015) for a full list of glyphosate degrading micro-organisms and their sources (pp.191).
[22] Ibid.
[23] Gimsing, A.L., Borggaard, O.K., Jacobsen, O.S., Aamand, J and Sorensen, J. (2004)
Chemical and microbiological soil characteristics controlling glyphosate mineralization in Danish surface soils. *Applied Soil Ecology*, 2, pp. 233–242. DOI:10.1016/j.apsoil.2004.05.007.
[24] Stenrod, M., Charnay M.P., Benoit, P. and Eklo, O.M., (2006) Spatial variability of glyphosate mineralization and soil microbial characteristics in two Norwegian sandy loam soils as affected by surface topographical features, *Soil Biology and Biochemistry*, 38, pp.962–971.
[25] Borggaard and Gimsing (2008).
[26] UK National Ecosystem Assessment (2011) *The UK National Ecosystem Assessment: Technical Report*. UNEP-WCMC, Cambridge. Chapter 4: Biodiversity in the Context of Ecosystem Services. pp. 80. Available at accessed 4/3/16.
[27] Ibid.
[28] Zabaloy, M.C., Gomez, E., Garland, J.L., Gomez, M.A. (2012) Assessment of microbial community function and structure in soil microcosms exposed to glyphosate, *Applied Soil Ecology*, 61, 333–339.
[29] UK National Ecosystem Assessment (2011).
[30] Zabaloy et al (2012); Newman et al (2016); Sviridov, 2015;
Allegrini, M., Zabaloy, M.C., Gomez, del V. (2015) Ecotoxicological assessment of soil microbial community tolerance to glyphosate, *Science of the Total Environment*, 533, pp.60 –68.
Meriles, J. M., Vargas Gil, S., Haro, R.J., March, G. J., and Guzman, C. A. (2006)
Glyphosate and Previous Crop Residue Effect on Deleterious and Beneficial
Soil-borne Fungi from a Peanut–Corn–Soybean Rotations, *Journal of Phytopathology*, 154, 309–316.
[31] Araujo, A.S.F, Monteiro, R.T.R., Abarkeli, R.B., (2003) Effect of glyphosate
on the microbial activity of two Brazilian soils, *Chemosphere* 52, pp.799–804.
Haney, R.L., Senseman, S.A., Hons, F.M., (2002) Effect of roundup ultra
on microbial activity and biomass from selected soils, *Journal of Environmental Quality* 31, pp. 730–735.
Hart, M.M., Powell, J. R., Gulden, R.H., Dunfield, K. E., Pauls, K, P,, Swanton,
C.J., Kliroromos, J. N., Antunes, P. M. , Koch, A. M. , Trevors, J.T. (2009)
Separating the effect of crop from herbicide on soil microbial
communities in glyphosate-resistant corn, *Pedobiologia*, 52, pp.253–262.
Lane, M., Lorenz, N., Saxena, J., Ramsier, C., Dick, R. P. (2012) The effect of glyphosate on soil microbial activity, microbial community structure, and soil potassium. *Pedobiologia*, 55, pp. 335–342.
[32] Haney and Hons (2002); Araujo et al (2003); Mijangos, I., Becerril, J.M., Albizu, I., Epelde, L., Garbisu, C. (2009) Effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions by cultivation-dependent and independent methodologies, *Soil Biology & Biochemistry*, 41 , pp505–513.
[33] Mijangos et al (2009).
[34] Araujo et al (2003).
[35] Busse, M.D., Ratcliff, A. W. , Shestak, C. J., Powers, R. F. (2001) Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities, *Soil Biology and Biochemistry* 33, pp.1777–1789.
[36] Ratcliff, A.W., Busse, M.D., Shestak, C.J. (2006) Changes in microbial community structure following herbicide (glyphosate) additions to forest soils, *Applied Soil Ecology*, 34, pp.114–124.
[37] Weaver, M.A, Krutz, L.J., Zablotowicz, R.M., and Reddy, K.N. (2007) Effects of glyphosate on soil microbial communities and its mineralisation in a Mississippi soil, *Pest Management Science*, 63, pp.388-393.
[38] Mijangos et al (2009).
[39] Kremer, R.J., Means, N.E. (2009) Glyphosate and glyphosate resistant crop interactions with rhizosphere micro-organisms, *European Journal of Agronomy* 31, pp. 153–161.
[40] Imparato, V., Santos, S.S., Johansen, A., Geisen, S., and Winding, A. (2016) Stimulation of bacteria and protists in rhizosphere of glyphosate-treated barley, *Applied Soil Ecology*, 98, pp.47–55.
[41] Newman et al (2016).
[42] Nicolas, V., Oestreicher, N., and 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, pp 1-12 DOI 10.1007/s11356-016-6596-2.
[43] Druille, M., Cabello, M.N., Omacini, M., and Golluscio R.A (2013) Glyphosate reduces spore viability and root colonization of arbuscular mycorrhizal fungi, *Applied Soil Ecology*, 64, pp.99–103.
[44] 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 Reprorts*, 4, 5634. DOI: 5610.1038/srep05634.
[45] Lane (2012); Cherni, A.E., Trabelsi, D, Chebil, S., barhoumi, F., Rodriguez-Llorente, I.D., Zribi, K. (2015) Effect of Glyphosate on Enzymatic Activities, Rhizobiaceae and Total Bacterial Communities in an Agricultural Tunisian Soil, *Water Air Soil Pollution*, pp. 226:145 DOI 10.1007/s11270-014-2263-8.
[46] Zabaloy et al (2012).
[47] Tanney and Hutchison (2010).
[48] Allegrini et al (2015).
[49] Johal, G.S., and Huber, D.M. (2009) Glyphosate effects on diseases of plants, *European Journal of Agronomy*, 31, pp. 144–152.
[50] Kremer and Means (2009).
[51] Sanogo, S., Yang, X. B. and Scherm, H. (2000) Effects of Herbicides on *Fusarium solani* f. sp. Glycines and Development of Sudden Death Syndrome in Glyphosate-Tolerant Soybean, *Disease Control and Pest Management*, Vol. 90, No. 1.
[52] Johal and Huber (2009).
[53] Fusurim spp. have been used in several studies, because they are a good indicator of the microbial ecology of the soybean rhizosphere because they can dominate microbial communities and become pathogenic, often in response to root exudation.
[54] 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, pp. 118–127.
[55] Meriles, J. M., Vargas Gil S., Haro R. J., March G. J. and Guzman C. A. (2006) Glyphosate and Previous Crop Residue Effect on Deleterious and Beneficial Soil-borne Fungi from a Peanut–Corn–Soybean Rotations, *Journal of Phytopathology*, 154, pp.309–316.
[56] Morjan, W.E., Pedigo, L.P. and Lewis L.C. (2002) Fungicidal Effects of Glyphosate and Glyphosate Formulations on Four Species of Entomopathogenic Fungi, *Environmental Entomology*, 31(6): pp. 1206-1212.
[57] Gaupp-Berghausen, M., Hofer, M., Rewald, B., and Zaller, J.G. (2015) Glyphosate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations. *Scientific Reports*, 5, 12886, DOI: 10.1038/srep12886.
[58] Bon, D., Gilard, V., Massou, S., Peres, G., Maler-Martino, M., Martino, R., and Desmoulin, F. (2006) In vivo 31P and 1H HR-MAS NMR spectroscopy analysis of the unstarved Aporrectodea caliginosa (Lumbricidae), *Biology and Fertility of Soils*, 43, pp191–198; Pereira, J.L., Antunes, S.C., Castro, B.B., Catarina, R.M., Goncalves, A.M.M. Goncalves, F., and Pereira, R (2009) Toxicity evaluation of three pesticides on non-target aquatic and soil organisms: commercial formulation, *Ecotoxicology*, May 2009, Vol. 18, 4, pp 455-463.
[59] Verrell, P. and Van Buskirk, E. (2004) As the worm turns: Eisenia fetida avoids soil contaminated by a glyphosate-based herbicide, *Bulletin of Environmental Contamination and Toxicology*, 72, pp. 219–224.
[60] Casabe´, N Piola, L., Fuchs, J., Oneto, M.L., Pamparato, L., Basack, S., Gimenez, R., Massaro, R., Papa, J.C., and Kesten, E. (2007) Ecotoxicological assessment of the effects of glyphosate and chlorpyrifos in an Argentine soya field, *Journal of Soils and Sediment* 8, pp.1–8.
[61] 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, pp.264–268.
[62] Gaupp-Berghausen et al (2015).
[63] Zaller et al (2014).
[64] García-Pérez, J.A., Alarcón, E., Hernández, Y., Christian Hernández, C. (2016) Impact of litter contaminated with glyphosate-based herbicide on the performance of Pontoscolex corethrurus, soil phosphatase activities and soil pH, *Applied Soil Ecology*, available online 15 March 2016.
[65] Zhao, J., Neher, D.A., Shenglei, F., Li, Z., and Wang, K. (2013) Non-target effects of herbicides on soil nematode assemblages, *Pest Management Science*, 69: pp. 679–684.
[66] Liphadzi, K.B., Al-Khatib, K., Bensch, C.N., Stahlman, P.W., Dille, J.A., Todd, T., Rice, C.W., Horak, M.J., and Head, G. (2005) Soil Microbial and Nematode Communities as Affected by Glyphosate and Tillage Practices in a Glyphosate-Resistant Cropping System, *Weed Science*, Vol. 53, No. 4 (Jul. - Aug., 2005), pp. 536-545.