Pesticide Effects on Soil Biology: Part I

by Jill Clapperton

SCIENCE

Jill Clapperton is a soil ecologist and land resource consultant, based in Montana.

Editors' Note: Jill Clapperton, PhD (Plant Ecophysiology), is one of only a handful of soil ecology scientists in the world. Formerly with Agri-Food Canada at Lethbridge, AB, she is now a freelance consultant in her "new life" in Montana. Her business is named Earthspirit Land Resource Consulting, earthspiritconsulting@gmail.com.

One of the biggest criticisms against no-till farming is the use of herbicides to control weeds. How many times have we all heard: "I just don't like all those chemicals that farmers use, and don't no-till farmers use far more chemicals anyways? And doesn't that sterilise the soil?" So let's look at how herbicides, fungicides, and insecticides affect the soil biology. This is the first in a series of articles addressing the question of how agricultural practices affect soil biological properties and soil ecology functions.

In this first article, I will discuss the effects of pesticides on soil micro-flora, and on the rhizosphere (the microbiologically active portion of the soil near plant roots), and how these effects can be managed. This article looks especially at the primary producers and the early-stage decomposers in a soil food web: bacteria and fungi. In future articles, I will address interactions between pesticides and the soil fauna ('animals,' such as predatory or scavenging protozoa, nematodes, mites, collembola, enchytraeids, earthworms, spiders, and beetles), and the influence of transgenic (GMO) crops on the soil biota (all organisms that live in the soil) and ecosystem processes.[1]

Photo by Kris Nichols, USDA-ARS.

Cyanobacteria from grassland soil in central North Dakota.

Before we begin, all of us should be clear on some key background information: First, what happens in the rhizosphere drives most of what happens biologically in the soil. Secondly, it is the organic material (in both quality and quantity) that feeds the soil biota, and the term 'soil organic material' includes the plant roots and root exudates (carbon-containing compounds that leak from roots). Lastly, undisturbed soil allows the biota to build a stable and continuous soil pore network, establish an interactive community, and provide key functions, such as C, N, P, and S mineralisation and nitrogen fixing that we rely on to grow nutritious foods.

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The disclaimer for these articles is that much is yet to be discovered. Science has a limited understanding of the abundance and diversity of organisms in the soil, let alone trying to figure out all the biological interactions that unite the soil's chemical and physical properties for 'soil health.' We know a lot about how pesticides influence the target, and even some of the effects on plants and other aboveground organisms that are not the targets of pesticides. But we don't know much about how pesticides interact with soils and soil organisms, and there's far greater species diversity belowground than aboveground. The following article is a summary of my understanding on how pesticides affect the soil biota, and how that could affect soil ecosystem function specifically in a no-tillage system.

[1] *Editors: The grouping of organisms into fauna and flora is a bit arbitrary at times, e.g., protozoa somewhat blur the distinction between animal and plant, while fungi are actually more closely related to animals than to green plants, and the greatest single distinction of all these life forms is prokaryote (bacterial & archaean) versus eukaryote (cells with mitochondria and a true nucleus). Protozoa are single-celled eukaryotes; all multicellular species are comprised of eukaryotic cells.*

Knowing the Rhizosphere

In undisturbed soil, most of the nutrient cycling, roots, and biological activity are found in the top 20 to 30 cm (8 to 12 inches), known as the rhizosphere. More specifically, the rhizosphere is the root and the immediately adjacent soil, which is strongly influenced by the root. It is a zone of intense microbial activity. (*Editors: As used by scientists, 'microbe' and 'microbial' encompass bacteria and fungi, and oftentimes protozoa as well. Mites and nematodes aren't included, although they often are microscopic.*)

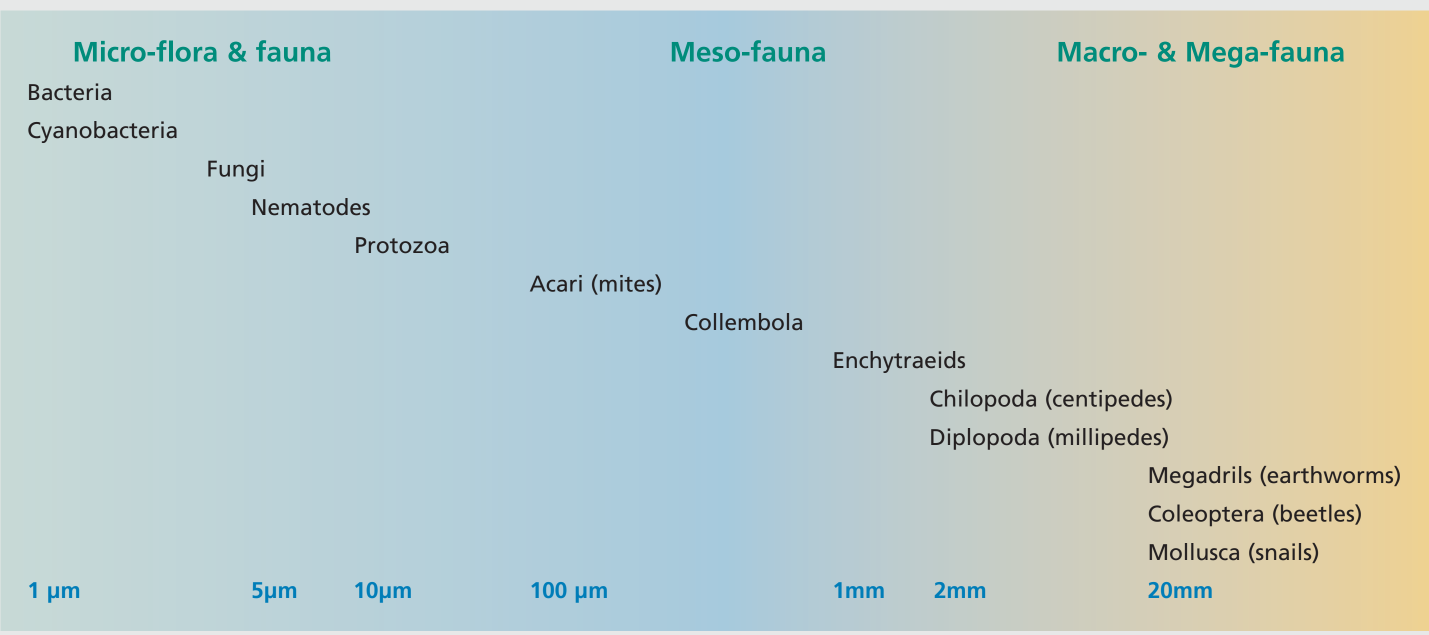
The rhizosphere is a close relationship between the plant, soil matrix, and soil organisms where any outside factor affecting one member of the triad will have consequences for the other two members. The rhizosphere is bathed in energy-rich carbon compounds, such as sugars, amino acids, and organic acids (all are products of photosynthesis) that leak from the roots, called root exudates. An example of a rhizosphere effect that many of you will know is the effect that peas, and to a lesser extent, beans, have on soil tilth. Both of these crops make the soil very soft and mellow (easy to dig), and impart a slightly sweet smell to the soil from the microbial community associated with these plants.

Every plant species leaks a unique signature of compounds from its roots. The quantity and qualities of these compounds depend to a certain extent on the soil chemical and physical properties; these exudates largely determine the microbial community of the rhizosphere. Symbionts (such as mycorrhizas, and the nitrogen-fixing *Rhizobia* in legume root nodules) as well as disease-causing pathogens may be particularly attuned to the composition and quantity of root exudates attracting, and/or activating, them to a particular plant species.

Many types of bacteria produce plant-growth-promoting substances.

More generally, bacteria and fungi use root exudates (and the dead sloughed cells from the root) as a food source to grow and reproduce. Many types of bacteria that live in the rhizosphere will produce plant-growth-promoting substances that increase root growth, thereby providing themselves with increased root area to colonise, and more exudates for food. (Self-serving manipulation is ancient indeed!)

Rhizosphere interactions often produce changes in soil structure. Sticky secretions from bacteria, the glomalin from mycorrhizas, and hyphae from these and other fungi, along with exudates and dead root cells, will bind soil particles to create aggregates and a unique habitat for other soil organisms. As scavengers and/or predators, various species of protozoa, nematodes, and mites feed on the large numbers of bacteria and fungi near the root, as well as the organic substances secreted. In turn, the faecal pellets from these microscopic animals add to the



Typical sizes of various groups of soil biota, some categories of which span a considerable range of sizes among their members (e.g., from miniscule fungal hyphae of 3 microns in diameter, up to 20+ mm for some species such as mushrooms). Note that the scale isn't linear (it's roughly logarithmic). In the next article (Part II), we will be focusing on fauna, and splitting them into micro-, meso- and macro-groups for the discussion. You can see where we have started and where we will end. 1 µm is a micrometer (a.k.a. micron), or one-millionth of a meter (a meter is ~ 39 inches). A human hair is 50 – 80 microns in diameter. Adapted from M.J. Swift, O.W. Heal & J.M. Anderson, 1979, *Decomposition in Terrestrial Ecosystems*, Univ. Calif. Press Berkeley.

structure of the soil and are a rich source of nutrients for bacteria, fungi, and plants.

To summarize, the rhizosphere is a close relationship between the plant, soil, and soil organisms. Plants produce photosynthate which is the food source for organisms that build soil aggregates and recycle nutrients, and the soil provides habitat, water, and mineral nutrients for both soil organisms and plants. Any factor that changes the amount and quality of carbon- and nitrogen-based compounds going into the soil as either residue or root exudates will alter the soil biological community.

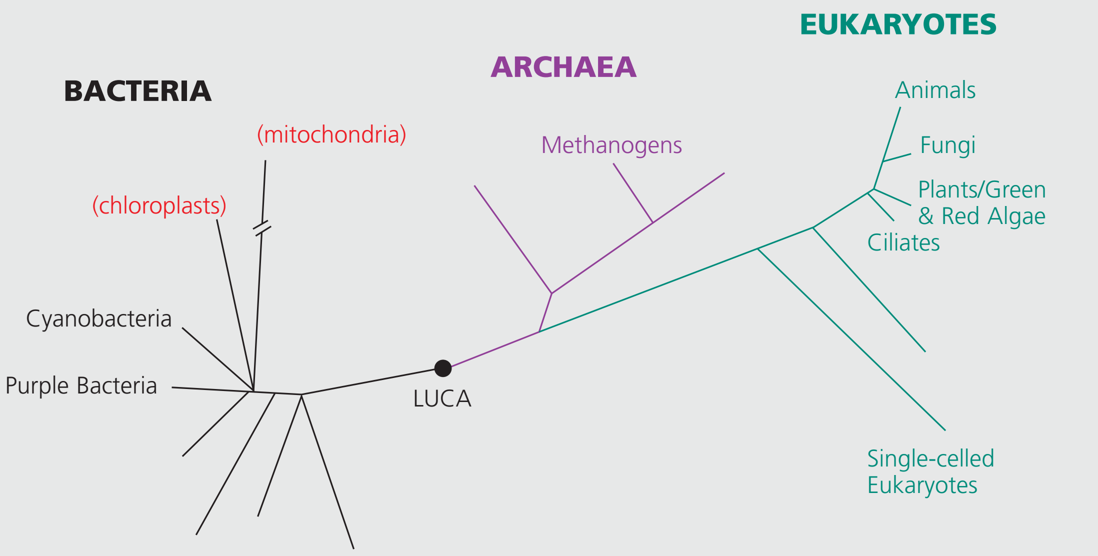
Building the Soil Habitat

Tillage directly affects soil porosity. Porosity determines the amount of air and water the soil can hold, as well as providing passage for roots and other organisms. (*Editors: See Schumacher & Riedell, 'Soil Structure,' Jan. '08.*) Tillage collapses the naturally occurring pores and tunnels, and alters the water-holding capacity as well as the gas and nutrient exchange capacities of the soil. It then becomes somewhat necessary to continue tillage, as there is a net loss of soil organisms that can perform the nutrient-cycling functions and maintain natural porosity.

No-till farming has generally been shown to build populations of soil animals such as protozoa, mites, and earthworms because this system retains and builds the integrity of the soil pore network.[2] It also builds the surface residues (mulch cover), creating a litter layer that provides a habitat for all the organisms as well as a continuous food supply. No-tillage systems function better as they age, and when the diversity of crops is increased. Together these practices improve the soil habitat and build the quantity and quality of the organic matter, thereby allowing increased abundance and diversity of soil organisms.

Pesticides in the Soil Ecosystem

There is no doubt that in the last couple of decades production agriculture has relied more heavily on applying pesticides. In many cases, weeds, insects, and diseases have developed resistance to chemistries that were once effective at controlling or suppressing these organisms. Broad-spectrum pesticides (in particular) kill the target pest or disease but likely also affect similar natural enemies and beneficial non-target organisms despite the best efforts of chemists to be very specific. Thus, the use of pesticides, even the more specific ones, can lead to decreased biodiversity, which often causes the 'flare up' of other weeds, damaging insects, and pathogens. Further, there is legitimate concern that pesticide use may inadvertently be damaging to various soil organisms, which may compromise soil aggregation or porosity (by suppressing earth-



The tree of life showing the three domains, based on comparisons of ribosomal RNA, with the length and branching of the lines proportional to genetic similarities (except the line length for mitochondria, which is shortened here). (Labels omitted for some lines.) LUCA is the Last Universal Common Ancestor. Note that the entire animal kingdom—from sponges and jellyfish to nematodes and humans—represents rather little diversity of genetics and metabolism. Chloroplasts are organelles within the cells of algae and green plants; chloroplasts perform photosynthesis and are most closely related to free-living cyanobacteria. Mitochondria are organelles within the cells of all eukaryotes, and carry out respiration (oxidizing of sugars), and again are most closely related to bacteria. The consensus emerging among biologists is that extended symbiosis eventually resulted in a (unicellular) archaean methanogen acquiring/internalizing a bacterium which became mitochondria and chloroplasts (these organelles contain their own genetic material, and divide independently of the cell itself). Diagram derived in part from N. Lane, 2002, *Oxygen: The Molecule that made the World*, Oxford Univ. Press; A.H. Knoll, 2003, *Life on a Young Planet*, Princeton Univ. Press; W.F. Doolittle, Feb. 2000, Uprooting the Tree of Life, *Scientific American* 90-95 (the original ribosomal RNA analysis was by Carl Woese at Univ. of Illinois).

[2] See, e.g., D.C. Coleman, D.A. Crossley Jr. & P.F. Hendrix, 2004, *Fundamentals of Soil Ecology*, 2d ed., Elsevier.

worms, fungi, or other organisms), N-fixation (by suppressing rhizobial symbionts and/or free-living N-fixing organisms), or nutrient cycling or plant uptake (by suppressing mycorrhizas).

Degradation by Bacteria

Bacteria strains isolated from soils that have been contaminated with various biochemicals, including pesticides, are increasingly being used for bioremediation (reclaiming the soil by inoculating it with organisms able to degrade certain compounds which are detrimental to other life forms).[3] In other words, the bacteria are using specific pesticides to meet their energy needs, i.e., using them as food. For example, bacteria were isolated from soil contaminated with triazines, then inoculated onto charcoal (to bind the chemical and deliver the bacteria), and reintroduced into contaminated soil. The results indicated that these bacteria degraded the triazines in 4 – 9 days. Similarly, it has been shown that four weeks after simazine and atrazine were applied, there was an increase in the population of organisms known to degrade those chemicals.[5] Generally speaking, some bacteria types appear to adapt to degrade regularly used herbicides such as atrazine, 2,4-D, sulfentrazone (Authority, Spartan), and glyphosate,[6] as well as insecticides such as chlorpyrifos (Lorsban), hexachlorocyclohexane (HCH, a.k.a. lindane), imidacloprid (Gaucho), and carbofuran (Furadan),[7] and fungicides including chlorothalonil (Bravo) and metalaxyl / mefenoxam (Apron, Ridomil).[8] (*Editors: Examples of trade names registered in the USA are provided if the compound is commonly used in agriculture. A few others are included for historical purposes, or because they are used in forestry or turf care*.) Here is the caveat to all that: Just because the bacteria break it down doesn't mean there are no effects on other soil biota.

Photo by Jill Clapperton.

Bacillus thuringiensis ('*Bt*') bacteria, which occur naturally in many soils.

No-till farming has generally been shown to build populations of soil animals such as protozoa, mites, and earthworms.

Herbicides

Glyphosate is one of the most successful and acceptable herbicides used today, for reasons including: broad-spectrum weed control, benign characteristics for handling and application, environmental safety, and relatively good crop safety when used on transgenic glyphosate-resistant crops.[9] While glyphosate poses no direct threat to crops after coming in contact with the soil (due to being strongly bound to soil particles), the compound itself persists in the soil for some time. Glyphosate contains a carbon-phosphorus bond (C-P bond) resistant to chemical breakdown. However, the C-P bond is susceptible to biodegradation by any bacterium with the

3 M. Hernández, P. Villalobos, V. Morgante, M. González, C. Reiff, E. Moore & M. Seeger, 2008, Isolation and characterization of a novel simazine-degrading bacterium from agricultural soil of central Chile, *Pseudomonas* sp. MHP41, *FEMS Microbiol. Letters* 286: 184-190.

4 K. Yamazaki, K. Takagi, K. Fuji, A. Iwasaki, N. Harada & T. Uchimura, 2008, Simultaneous biodegradation of chloro- and methylthio-s-triazines using charcoal enriched with a newly developed bacterial consortium, *J. Pesticide Sci.* 33: 266-270.

5 M.A. Dinamarca, F. Cereceda-Balic, X. Fadic & M. Seeger, 2007, Analysis of s-triazine-degrading microbial communities in soil using more probable number enumeration and tetrazolium-salt detection, *Int. Microbiol.* 10: 209-215.

6 E. Sandmann & M.A. Loos, 1988, Aromatic metabolism by a 2,4-D degrading *Arthrobacter* sp., *Can. J. Microbiol.* 34: 125-130; A.E. Smith & A.J. Aubin, 1991, Transformation of 14C-2,4-dichlorophenol in Saskatchewan soils, *J. Agricult. Food Chem*. 39: 801-804; C.O. Martinez, C.M.M. de Souza Silva, E. Francisconi Fay, R.B. Abakerli, A. de H.N. Maia & L.R. Durrant, 2008, The effects of moisture and temperature on the degradation of sulfentrazone, *Geoderma* 147: 56-62; A.L. Gimsing, O.K. Borggaard, O.S. Jacobsen, J. Aamand & J. Sorensen, 2004, Chemical and microbial characteristics controlling glyphosate mineralization in Danish surface soils, *Appl. Soil Ecol.* 27: 233-242; M.A. Weaver, L.J. Krutz, R.M. Zablotowicz & K.N. Reddy, 2007, Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil, *Pest Manag. Sci.* 63: 388-393.

7 C. Vischetti, E. Monaci, A. Candinalie & P. Perucci, 2008, The effect of initial concentration, co-application and repeated applications on pesticide degradation in a biobed mixture, *Chemosphere* 72: 1739-1743 (chlorpyrifos degradation); L. Xiao Hui, J. Jian Dong, S.W. Ali, H. Jian & L. Shun Peng, 2008, Diversity of chlorpyrifos-degrading bacteria isolated from chlorpyrifos-contaminated samples, *Int. Biodeterioration & Biodegrad*. 62: 331-335; M.J. Sainz, B. González-Penalta & A. Vilariño, 2006, Effects of hexachlorocyclohexane on rhizosphere fungal propagules and root colonization by arbuscular mycorrhizal fungi in *Plantago lanceolata*, *Eur. J. Soil Sci.* 57: 83-90; P.S. Kidd, A. Prieto-Fernández, C. Monterroso & M.J. Acea, 2008, Rhizosphere microbial community and hexachlorocyclohexane degradative potential in contrasting plant species, *Plant & Soil* 302: 233-247; M. Soudamini, P. Meera, A.K. Ahuja, S.S. Venna & R. Sandhya, 2008, Degradation of lindane and imidacloprid in soil by *Calocybe indica*, *Pesticide Res. J.* 20: 143-145; S.L. Trabue, A.V. Ogram & L.T. Ou, 2001, Dynamics of carbofuran-degrading microbial communities in soil during three successive annual applications of carbofuran, *Soil Biol. & Biochem.* 33: 75-81.

8 W.V. Sigler & R.F. Turco, 2002, The impact of chlorothalonil application on soil bacterial and fungal populations as assessed by denaturing gradient gel electrophoresis, *Appl. Soil Ecol.* 21: 107-118; S.G. Pai, M.B. Riley & N.D. Camper, 2001, Microbial degradation of mefenoxam in rhizosphere of *Zinnia angustifolia*, *Chemosphere* 44: 577-582; W.J. Jones & N.D. Ananyeva, 2001, Correlations between pesticide transformation rate and microbial respiration activity in soil of different ecosystems, *Biol. & Fertility Soils* 33: 477-483; Vischetti et al., 2008 (metalaxyl degradation).

9 J.P. Quinn, J.M.M. Peden & R.E. Dick, 1988, Glyphosate tolerance and utilization by the microflora of soils treated with the herbicide, *Appl. Microbiol. Biotech*. 29: 511-516. K.N. Reddy, 2001, Glyphosate resistant soybean as a weed management tool: opportunities and challenges, *Weed Biol. Manag*. 1: 193-202.

enzyme C-P lyase, such as *Pseudomonas* species.[10] Other organisms capable of breaking the C-P bond and using the phosphonate as an energy source include some of the cyanobacteria[11] (depending on your age, you might know these organisms as "blue-green algae") which are microscopic filament-forming, free-living (non-symbiotic), nitrogen-fixing, photosynthesizing bacteria. It appears that soil bacteria are the principal degraders of glyphosate in the environment.

The effects of glyphosate on the soil biological community generally are benign, but with some mixed results. A number of studies have shown that glyphosate, when used at recommended rates, has insignificant effects on the microbial community,[12] although there can be a short-term stimulation of bacterial populations at higher concentrations.[13] One group of researchers concluded that glyphosate likely results in minor effects on soil biological and chemical properties.[14] They further suggested that the effect of greater amounts of soil carbon and plant residues retained on the soil surface with no-till and conservation farming practices likely mitigated any negative effects of glyphosate usage.

Just because the bacteria break down a pesticide doesn't mean there are no effects on other soil biota.

Still, there have been negative indirect effects of glyphosate on soil microbiology. Glyphosate-tolerant soybean (Roundup Ready) seedlings that have been treated with glyphosate are more susceptible to *Fusarium solani* infections which cause Sudden Death Syndrome.[15] This is likely related to the finding that glyphosate is released into the rhizosphere in root exudates, and that the *Fusarium* fungus is actually attracted to the increase in glyphosate concentration in the rhizosphere.[16] This is consistent with the finding that glyphosate tends to stimulate fungal growth in the short term.[17] Now if we think about our rhizosphere model, let's speculate as to what goes on underground. The balance between beneficial bacteria and fungi has changed, damage to the root by the pathogen further unbalances populations and diversity in the rhizosphere community, and pathogenic nematodes are attracted to the root damage. The plant reduces photosynthesis, and puts more of its energy into blocking the root damage. The root exudates change dramatically, affecting populations, diversity, and function of the rhizosphere community of microorganisms. Eventually, the population of fungal-feeding nematodes (non-pathogenic to plants) increases opportunistically in response to the flourishing fungi (non-mycorrhizal), and the entire ecosystem finds a new balance. However, some plants may succumb to the combination of *Fusarium*, pathogenic nematodes, and other diseases. Obviously, there are many other scenarios given all the interactions in the rhizosphere.

Unfortunately, sometimes the byproducts of bacterial degradation are more toxic than the original chemical.[18] Researchers studied the breakdown of two selective triketonic herbicides, sulcotrione (in Europe: Mikado) and mesotrione (Callisto), and compared them with the known toxicity of the commercial products.[19] They concluded that it was necessary to assess the potential toxicity of the intermediate byproducts of biodegradation as well as the active ingredients and additives in commercial herbicide formulations.

The general consensus among soil ecologists is that the commonly used herbicides do not greatly affect the diversity and general function of the soil microbial community.[20] However, applied pesticides can favour the

[10] Gimsing et al., 2004.

[11] G. Forlani, M. Pavan, M. Gramek, P. Kafarski & J. Lipok, 2008, Biochemical bases for a widespread tolerance of cyanobacteria to the phosphonate herbicide glyphosate, *Plant Cell Physiol.* 49: 443-456.

[12] D.A. Wardle & D. Parkinson, 1990, Influence of the herbicide glyphosate on soil microbial community structure, *Plant & Soil* 122: 29-37; M.D. Busse, A.W. Ratcliff, C.J. Shestak & R.F. Powers, 2001, Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities, *Soil Biol. Biochem*. 33: 1777-2789; R.L. Haney, S.A. Senseman, L.J. Krutz & F.M. Hons, 2002, Soil carbon and nitrogen mineralization as affected by atrazine and glyphosate, *Biol. Fert. Soils* 35: 35-40; A.W. Ratcliff, M.D. Busse & C.J. Shestak, 2006, Changes in microbial community structure following herbicide (glyphosate) additions to forest soils, *Appl. Soil Ecol*. 34: 114-124; Weaver et al., 2007; M.A. Locke, R.M. Zablotowicz & K.N. Reddy, 2008, Integrating soil conservation practices and glyphosate-resistant crops: impacts on soil, *Pest Manag. Sci.* 64: 457-469.

[13] Ratcliff et al., 2006.

[14] Locke et al., 2008.

[15] S. Sanogo, X.B. Yang & P. Lundeen, 2001, Field response of glyphosate tolerant soybean to herbicides and sudden death syndrome, *Plant Disease* 85: 773-779.

[16] R.J. Kremer, N.E. Means & S. Kim, 2005, Glyphosate affects soybean root exudates and rhizosphere microorganisms, *J. Environ. Analyt. Chem.* 15: 1165-1174.

[17] A.S.F. Araújo, R.T.R. Monteiro & R.B. Abarkeli, 2003, Effect of glyphosate on the microbial activity of two Brazilian soils, *Chemosphere* 52: 799-804.

[18] C. Tkaczuk & R. Mietkiewski, 2005, Effects of selected pesticides on the growth of fungi from *Hirsutella* genus isolated from phytophagous mites, *J. Plant Protect. Res.* 45: 171-179.

[19] J.L. Bonnet, F. Bonnemoy, M. Dusser & J. Bohatier, 2008, Toxicity assessment of the herbicides sulcotrione and mesotrione toward two reference environmental organisms: *Tetrahymena pyriformis* and *Vibrio fischeri, Arch. Environ. Contam. Toxicol.* 55: 576-583.

[20] N.Z. Lupwayi, K.N. Harker, G.W. Clayton, T.K. Turkington, W.A. Rice & J.T. O'Donovan, 2004, Soil microbial biomass and diversity after herbicide application, *Can. J. Plant Sci.* 84: 677-685.

growth of specific bacterial degraders that are able to use the various molecular components of the chemical. This modifies the overall function and population of the community in favour of degrading the chemical, but generally leaves the diversity of the community intact.[21] In freshwater microbial communities exposed to herbicide-contaminated runoff, researchers found that primary production had increased twofold, while cyanobacteria populations increased 4.5-fold, and picocyanobacteria increased 40-fold, although populations of plankton *decreased*.[22]

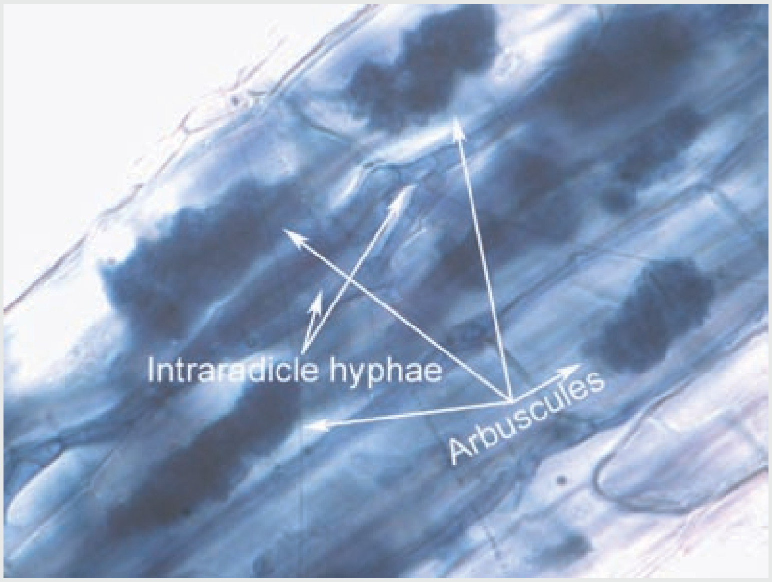


Photo by Kris Nichols, USDA-ARS.

Mycorrhizal colonization inside a switchgrass root. The fungal hyphae run between the plant cells as well as intruding into the cells (arbuscules).

Fungicides and Insecticides

As compared to herbicides, there are still fewer research papers studying the effects of fungicides and insecticides on the soil biota. As we will see in the next article, fungicides and insecticides tend to have a greater effect on the soil fauna ('animals'), often negatively. But, generally speaking, we are encouraged by the fact that it appears most of these chemicals can be degraded by soil bacteria, making bioremediation a reality. (Many pesticides are also degraded by sunlight and by non-biological chemical reactions in the soil.) Given enough time, the soil ecology appears capable of recovering from applied fungicides and insecticides, although the recovery may take months or years and the economics of crop production may be negatively impacted in the meantime.

The general consensus among soil ecologists is that the commonly used herbicides do not greatly affect the diversity and general function of the soil microbial community.

Fungicides are used to prevent fungal disease as seed treatments, or to actually treat (or prevent) a particular disease when foliarly or soil applied. Potentially the worst side effect of using a fungicide is that it kills most of the fungi in the soil or around the seed, many of which could actually protect the seedling from pathogens, and/or confer other benefits. In the worst case, a fungicide would prevent beneficial mycorrhizal fungi from colonising the plant. However, it appears that mycorrhizas are only temporarily inhibited from colonising the new root until the seed treatment is diluted or broken down sufficiently (3 – 4 weeks, usually), with remarkably only a small effect on the overall amount of mycorrhizal fungi that eventually establish on a more mature plant.

Mycorrhizal fungi (also referred to as 'vesicular-arbuscular mycorrhizas,' or simply 'arbuscular mycorrhizas') form symbiotic relationships with their host plants, increasing plant establishment and growth, so you want to encourage these fungi. Mycorrhizas cannot grow in the absence of a host plant, and are known to colonise more than 85 percent of all vascular land plants. Mycorrhizal fungi increase plant uptake of mineral nutrients that are less mobile in the soil such as phosphorus (P), zinc (Zn), and copper (Cu), as well as more mobile ones such as calcium (Ca). In exchange, the plant supplies the mycorrhizas with photosynthates such as amino acids, organic acids, and sugars. Once a plant is colonised by mycorrhizas, the rhizosphere microbial community changes in favour of plant-growth-promoting rhizobacteria ('PGPR'), and the plant increases photosynthesis. The ability of a mycorrhizal fungus to colonise a host plant is affected by the phosphorus status of the plant and soil. It is thought that the extra P acts to tighten the plant cellular membranes, thereby decreasing the amount of photosynthate leaked from the root, which means less of a signal is received by the mycorrhizas and making the host less attractive. So using too much P fertiliser can have the undesired consequence of reducing mycorrhizal colonisation, thus also reducing the population and species diversity of

[21] L.V. Gonod, F. Martin-Laurent & C. Chenu, 2006, 2,4-D impact on bacterial communities, and the activity of 2,4-D degrading communities in soil, *FEMS Microbiol. Ecol.* 58: 529-537.

[22] G.L. Pérez, A. Torremorell, H. Mugni, P. Rodríguez, M. Solange Vera, M. do Nascimento, L. Allende, J. Bustingorry, R. Escaray, M. Ferraro, I. Izaguirre, H. Pizarro, C. Bonetto, D.P. Morris & H. Zagarese, 2007, Effects of the herbicide Roundup on freshwater microbial communities: a mesocosm study, *Ecol. Appl.* 17: 2310-2322.

A fungal hyphae has grown across this earthworm channel (note the size difference). Mycorrhizal hyphae can extract water and nutrients from a soil volume far surpassing the plant roots by themselves.

mycorrhizas. (Mycorrhizal fungi are relatively complex organisms, with reproductive life cycles spanning weeks or months, in sharp contrast to bacteria and protozoa which double or quadruple their population in a matter of hours with favourable conditions.) Most of the original wild types ("land races") of the major cereal grains were dependent on mycorrhizas. However, many of our modern cereal grain varieties are much less dependent on mycorrhizas, likely as an inadvertent consequence of plant breeding on well-fertilised soils. The lowest level of P availability at which plants can grow without mycorrhizas indicates the dependency of that plant species (or varietal) on mycorrhizas. Thus, crops that can grow at low P levels and without mycorrhizas have low dependency. Plants that do not form mycorrhizal associations are non-hosts.

The good news is that many fungicides that have been studied are not a serious problem for directly reducing colonisation by mycorrhizas,[23] except for benomyl and particularly the new benomyl, Topsin-M,[24] and the highest rate of carbendazim (an old chemistry, no longer labeled in the USA).[25] However, many widely used foliar fungicides haven't been studied at all for their effect on mycorrhizas, including propiconazole (Tilt), azoxystrobin (Quadris), and pyraclostrobin (Headline).

Fungicides in general may also affect mycorrhizas indirectly, via a slightly negative but transient influence on rhizobacteria.[26] Any fungicide-induced increase in bacterial biomass is likely a result of surges in chemical-degrading bacteria,[27] while tending to decrease the beneficial bacterial populations such as PGPR as well as free-living, N-fixing bacteria.[28] Indeed, in a number of cases it appears that adding PGPR or root-disease-antagonistic bacteria as a seed treatment can be as effective as a fungicide seed treatment, or overcome any adverse affects on the microbial community by the fungicide. By far the best ways to build populations of bacteria, including PGPR, is to use diverse crop rotations and cover crops. To partly overcome poor crop rotations (e.g., corn – soybean) or long fallow periods, food sources such as molasses have a small but arguably worthwhile effect, by supplying a mixture of amino acids, organic acids, and complex sugars that approximates root exudates.[29] And you certainly won't hurt anything with molasses, which may not be true of some of the soil bio-stimulants being sold. But generally, if you have good crop rotations that provide lots of high-quality mulch on the surface, then the soil organisms will flourish in that habitat, as opposed to adding living organisms

The persistence of insecticides and their effect on the rhizosphere microbial community are more negative as compared with both herbicides and fungicides.

[23] P.F. Schweiger, N.H. Spliid & I. Jakobsen, 2001, Fungicide application and phosphorus uptake by hyphae of arbuscular mycorrhizal fungi into field-grown peas, *Soil Biol. Biochem*. 33: 1231-1237; V.J. Allison, T.K. Rajaniemi, D.E. Goldberg & D.R. Zak, 2007, Qualifying direct and indirect effects of fungicide on an old-field plant community: experimental null community approach, *Plant Ecol.* 190: 53-69.

[24] G.W.T. Wilson & M.M. Williamson, 2008, Topsin-M: the new benomyl for mycorrhizal-suppression experiments, *Mycologia* 100: 548-554.

[25] Schweiger et al., 2001.

[26] L. Thirup, A. Johansen & A. Winding, 2003, Microbial succession in the rhizosphere of live and decomposing barley roots as affected by the antagonistic strain *Pseudomonas fluorescens* DR54- BN14 or the fungicide imazalil, *FEMS Microbiol. Ecol*. 43: 383-392.

[27] J. Demanou, S. Sharma, U. Dörfler, R. Schroll, K. Pritch, T. Njine, U. Bausenwein, A. Monkiedje, J.C. Munch & M. Schloter, 2006, Structural and functional diversity of soil microbial communities as a result of combined applications of copper and mefenoxam, *Soil Biol. Biochem*. 38: 2381-2389.

[28] F.I. Ekundayo & M.K. Oladunmoye, 2007, Influence of benomyl on ability of *Fusarium oxysporum* and *Fusarium solani* to produce beauvericin and rhizosphere organisms of cow pea, *Int. J. Soil Sci.* 2: 135-141 (decreases in N-fixing bacteria); M. Attia, N.M. Awad & A.S. Turky, 2002, Associative action of growth promoting rhizobacteria and phytoremediation on the biodegradation of certain pesticides in soil, *Bulletin – National Research Centre (Cairo)* 27: 469-480 (decreases in PGPR bacteria).

[29] Root exudates include complex sugars, but not simple sugars. C4 plants leak far more sugars from their roots than cool-season C3 plants, which may be why C4 plants tend to be more mycorrhizal.

that may not have previously existed in your soil. As for the existing organisms in your soil (which are already adapted to it), their populations usually will expand much more rapidly under favourable conditions than what you could ever hope to accomplish by applying them to the soil.

Introducing legume inoculants can increase the populations of *Rhizobium* or *Bradyrhizobium* regardless of whether these are applied on a legume crop or not: These bacteria won't colonise non-legumes, but they are free-living in the soil and act as PGPR (they don't fix N until they infect a legume, however). *Rhizobium* and *Bradyrhizobium* will both increase their populations completely independent of legume roots, so long as other conditions in the soil are conducive.

***Rhizobium* and *Bradyrhizobium* will both increase their populations completely independent of legume roots, so long as other conditions in the soil are conducive (they don't fix N until they infect a legume, however).**

The persistence of insecticides and their effect on the rhizosphere microbial community are more negative as compared with both herbicides and fungicides. Again, most insecticides are rapidly degraded by soil bacteria. Chlorpyrifos (Lorsban), a widely used neurotoxin insecticide, can degrade in as few as 20 days,[30] and although there can be effects on soil bacteria and fungi during that time, these organisms recover in a few weeks (however, effects on soil fauna are more persistent).

Generally, the strongest negative effect of the more commonly used insecticides is on the nitrogen cycle, which seems especially true in tropical soils. For instance, imidacloprid (Gaucho, Senator) directly inhibited N-fixation in mung beans.[31] Chlorpyrifos along with quinalphos and a pyrethroid were all shown to have negative effects on the N-fixing ability of the free-living bacteria *Azospirillum* spp.[32] However, many of the other side effects of insecticide treatments of seed or soil are indirect. For example: Seed treatment with diazinon, imidacloprid, and lindane increased the plant uptake of P.[33] Another study showed that mycorrhizal fungi and low P soil concentrations interact to enhance plant growth that increased the rate of microbial degradation of lindane in the soil.[34] However, some of the most problematic effects of insecticides, especially when broadcast-applied, are on soil fauna, which will be discussed in the next article.

Managing the Rhizosphere & Pesticide Use

Studying mycorrhizas is tricky work in the laboratory because of the difficulty of culturing them (mycorrhizas do not grow well without a host plant), so scientists con-

Crop Dependency on Mycorrhizal Colonisation

Dependent		Intermediate	Non-host
alfalfa	medic	oats	canola
alsike clover	millet, foxtail	barley	lupin
chickling vetch (*Lathyrus* spp.)	millet, proso	annual ryegrass	mustard, oriental or brown
chickpea, desi	millet, pearl	*Crotalaria* spp.	mustard, tame yellow
chickpea, kabuli	onions	safflower	radish
corn	potatoes	wheatgrass	sugarbeet
cotton	red clover	rye	turnip
cowpea (*Vigna unguiculata*)	sorghums & sudan	triticale	
field pea	soybean	wheat	
flax	sunflower		
hairy vetch	sweetclover		
lentil	white clover		

Plant species termed 'obligate mycotrophs' are dependent on mycorrhizas for various aspects of growth (e.g., nutrient uptake, drought resistance). For instance, tropical trees, warm-season rangeland grasses (bluestems, switchgrass, etc.), and most legumes require mycorrhizal colonisation for normal growth. Plants that are 'facultative mycotrophs,' such as cool-season grasses (fescue, timothy, wheat, and barley) do benefit from colonisation but will also establish and grow reasonably well without it, although this somewhat depends on the conditions. Some plant species fend off the mycorrhizas almost entirely, such as lupin and all members of the brassica family. The disclaimer for the categories is that many factors affect the degree of mycorrhizal association, including plant genetics (varietal), number of appropriate mycorrhizal spores in the soil (different species of mycorrhizal fungi have different capabilities or 'preferences' for host plants), nutrient status, chemical residuals, etc.

[30] C.V. Lakshmi, M. Kumar & S. Khanna, 2008, Biotransformation of chlorpyrifos and bioremediation of contaminated soil, *Int. Biodeterioration & Biodegrad.* 62: 204-209.

[31] A. Kaur & A. Kaur, 2005, Impact of imidacloprid on soil fertility and nodulation in mung bean (*Vigna radiata*), *Asian J. Water & Environ. Pollution* 2: 63-67.

[32] R.S. Gadagi, Tongmin Sa & J.B. Chung, 2004, Chemical insecticide effects on growth and nitrogenase activity of *Azospirillum* sp OAD-2, *Comm. Soil Sci. & Plant Analysis* 35: 495-503.

[33] J. Singh, N. Sabir, D.K. Singh & M. Singh, 2008, Plant available phosphorus and total phosphorus as affected by diazinon, imidacloprid and lindane treatments in a ground nut field, *Pesticide Res. J.* 20: 146-150.

[34] Sainz et al., 2006.

tinually look for indicator species that respond similarly to mycorrhizal fungi. Indicator species are used to gauge not just mycorrhizal well-being, but also the entire spectrum of soil biota. For example, one group of organisms that are reasonably good indicators for both fungi and bacteria are the micro-algae (photosynthesizing, soil-dwelling, unicellular or colony-forming eukaryotes).

When several commonly used herbicides were ranked separately for their effects on micro-algae, the most toxic were diuron (Karmex), propanil, and atrazine, while chlorpropham was intermediate, and MCPA and glyphosate were the least toxic.[35] The key finding is that it appears that most microorganisms are capable of tolerance to glyphosate and, to a lesser extent, 2,4-D or atrazine. Even in soils that had no previous history of glyphosate or 2,4-D use, many glyphosate- and 2,4-D-tolerant microorganisms were isolated.[36] This is the good news, because once again it means that if herbicides are used judiciously and at the appropriate time, they are likely to be broken down relatively quickly, limiting the potential for negative effects in the field as well as in runoff. Pesticides and other chemicals break down much faster when the soil conditions favour high biological activity, such as in the spring when soils are moist.

Given enough time, the soil ecology appears capable of recovering from applied fungicides and insecticides, although the recovery may take months or years and the economics of crop production may be negatively impacted in the meantime.

Let's think about the rhizosphere model again. We can isolate the effects of glyphosate on plants by using glyphosate-resistant soybeans. When sprayed with glyphosate, glyphosate-resistant soybeans had higher protein, greater N assimilation, less oil content (more oleic and less linoleic), and changes in C and N metabolism compared with glyphosate-resistant soybeans that were not sprayed.[37] These metabolic influences will also be manifest in roots, and glyphosate itself is exuded from the roots. These changes would no doubt affect the community composition of the rhizosphere, having direct and indirect effects on soil biota and plant growth. Once again, use the lower rates if at all possible to minimize the effects on soil ecosystem diversity and function.

Fungicides and insecticides used as seed treatments are generally safer for soil ecosystems as compared with soil applications (banded or broadcast) of the same chemistries, due to the much smaller volume of soil affected. The problem is that if you kill soil fungal pathogens with fungicides, you also kill most other soil fungi. Fungi are an important food source for many soil animals, and often contribute directly to plant vigour as well as soil aggregation.

So, although fungicides and insecticides are eventually broken down by soil microorganisms, still they are definitely more toxic to other organisms in the soil food web compared with herbicides. I recommend avoiding prophylactic use of fungicides and insecticides, and instead nurturing the build-up of a biologically diverse rhizosphere to compete with pathogens and damaging insects. In my experience, most healthy plant rhizospheres have an adequate population of *Bacillus* and other bacterial species that provide some protection from insect larval grazing. However, when an insect population is out of balance and threatening the crop, then using an insecticide may become necessary. Whenever feasible, use the somewhat more targeted insecticides (e.g., synthetic pyrethroids) instead of broad-spectrum chemistries (e.g., carbofuran) that tend to be more disruptive. Following a crop that has had significant insecticide use with a cover crop, or any kind of green cover, will speed the degradation of the chemical and allow some recovery of the damage to the soil ecology. Having substantial diversity of plants (including cover crops), growing them well, providing adequate nutrients (but not surplus P), and retaining very high levels of mulch cover will allow your soil ecology to flourish, which in turn minimizes many problems with pathogens and damaging insects.

Thus far, I have confined the discussion to bacteria and fungi. Now just imagine the direct and indirect effects that pesticides have at the next level, when we start talking about soil animals such as protozoa, mites, collembola, earthworms, and carabid beetles. To be continued . . . .

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[35] A. Maule, 1984, Interactions of micro-algae with soil herbicides, with particular reference to chlorpropham, Dissertation Abstracts International, C 9 European Abstracts 45: 84.

[36] V. López-Rodas, A. Flores-Moya, E. Maneiro, N. Perigones, F. Marva, M.E. García & E. Costas, 2007, Resistance to glyphosate in the cyanobacterium *Microcystis aeroginosa* as a result of pre-selective mutations, *Evolutionary Ecol.* 21: 535-547; L.J. Merini, V. Cuadrado, C.G. Flocco & A.M. Giulietti, 2007, Dissipation of 2,4-D in soils of the Humid Pampa region, Argentina: A microcosm study, *Chemosphere* 68: 259-265.

[37] N. Belloui, R.M. Zablotowicz, K.N. Reddy & C.A. Abel, 2008, Nitrogen metabolism and seed composition as influenced by glyphosate application in glyphosate-resistant soybean, *J. Agricult. Food Chem.* 56: 2765-2772.