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Pesticide Effects on Soil Biology

Part 1

By Jill Clapperton, Montana Regen. No-till on the Plains, Leading Edge, Volume 8, Number 1 (www.notill.org)

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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.

Cyanobacteria from grassland soil in central North Dakota.

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]

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.

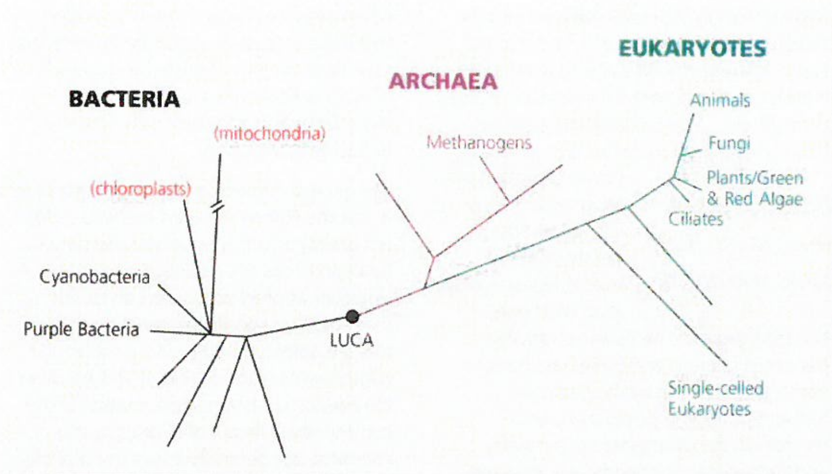
Knowing the Rhizosphere

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

[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.*



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).

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 earthstructure 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 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.

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

[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).*

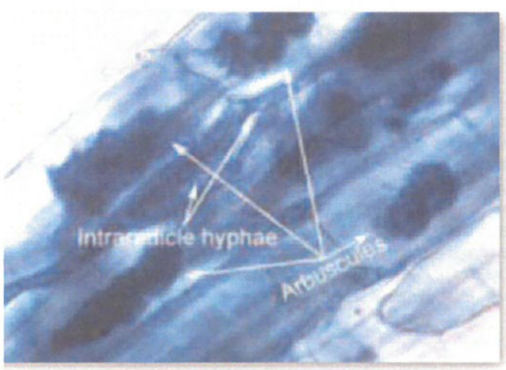
[9] *J.P. Quinn, J.M.M. Peden & R.E. Dick, 1988, Glyphosate tolerance and utilization by the microflora of soils treated with the herbicide, Appl. Microbiol. Biotech. 29: 511-516. K.N. Reddy, 2001, Glyphosate resistant soybean as a weed management tool: opportunities and challenges, Weed Biol. Manag.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

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.

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 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 nonhosts.

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.



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

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 fungicideinduced 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

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).*

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 glyphosateand 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.

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.

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.

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 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...

[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.*

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