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Fall 2005

ES/RP 531
Fundamentals of Environmental Toxicology

Lecture 17

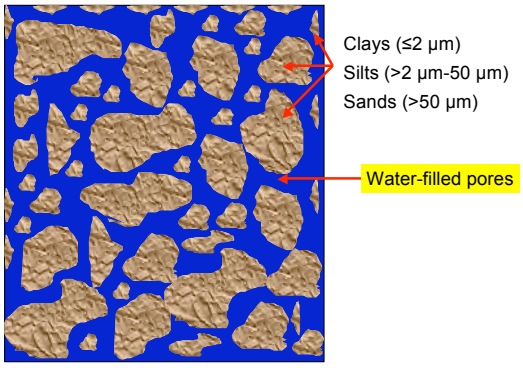
Microbial and Plant Toxicity

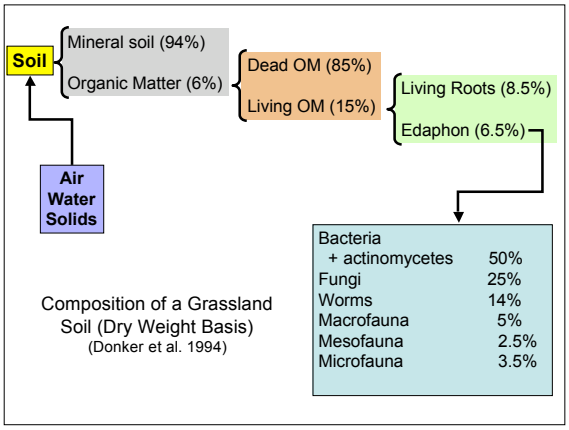
Concerns Over Soil Microbial Toxicity

- 'Soil quality' (or health) has become an issue as population has increased but arable soils are limited in area
  - Furthermore erosion, lack of water, and accumulation of salts limits use of soils
- Soil quality defined as:
  - "Capacity of soil to function as vital living system within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance quality of air and water environments, and support human health & habitation" (Winding et al. 2005, Ecotox. Environ. Safety 62:230)
- Microbial communities and their biochemical ecology is crucial to the optimal ecological functions of soil

Soil Composition

- Solid, water, air phases
- Solids
  - Inorganic minerals and "polymers" (clays)
  - Organic polymers (organic matter)
  - Characterized by particle sizes (texture)
  - Organized into a network that creates pores between solid materials
  - Determines how much water the soil can hold



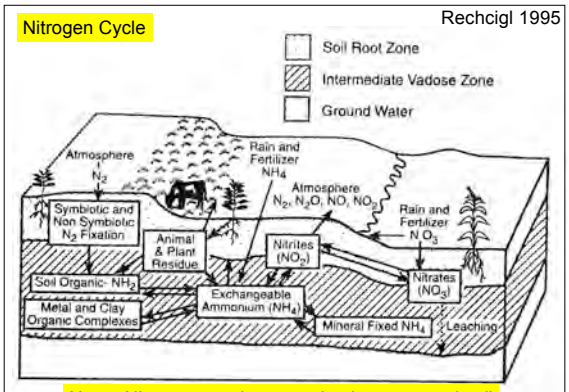


Composition of a Grassland Soil (Dry Weight Basis)
(Donker et al. 1994)

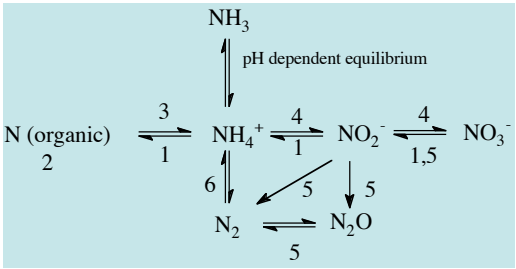
Important Soil Microbial Functions

- Nutrient Cycling
  - Carbon, nitrogen, phosphorus, sulfur
- Contribute to structure of soil through organic matter decomposition and synthesis
- Degrade contaminants

Nitrogen Cycle

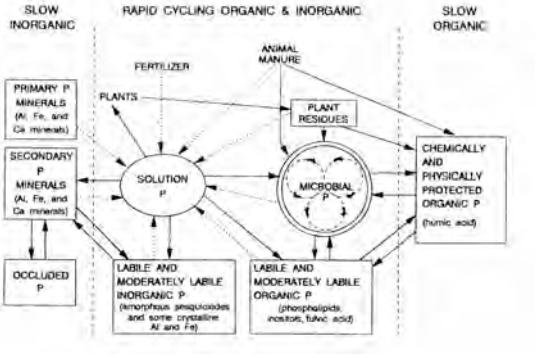


Note: Nitrogen species occur in air, water, and soil



1. Assimilation of inorganic N
2. Heterotrophic conversions
3. Mineralization
4. Nitrification
5. Dentrification
6. Biological Nitrogen Fixation

Reactivity of Nitrogen



Note: Phosphorus cycle does not include an air phase

Microbial Activity Largely Restricted to the Soil Surface

Numbers of Organisms (Cells) x 1000 per gram soil

Depth (cm)	Aerobic Bacteria	Anaerobic Bacteria	Actinomycetes	Fungi	Algae
3-8	7800	1950	2080	119	25
20-25	1800	379	245	50	5
35-40	472	98	49	14	0.5
65-75	10	1	5	6	0.1
135-145	1	0.4	--	3	--

Eijsackers 1994

Concerns Over Microbial Toxicity

- Waste situations (including spills)
- Routine application of pesticides
  - Fumigants
  - Fungicides
  - Herbicides

Measuring Microbial Toxicity

- "In Vitro" Tests
  - Culture microbes in liquid or agar culture
    - In liquid culture, can use turbidity to get relative measure of population size
    - Can count colony forming units (CFUs) on agar plate
    - Add toxin to culture and look at effect on turbidity or CFUs

Measuring Microbial Toxicity

- "In Vivo" Tests
  - Whole soils tested for microbial activity and numbers
    - Respiration (dehydrogenase enzyme activity); indicative of general oxidative metabolic activity
    - Carbon mineralization (CO2 evolution; indicative of biomass)
    - Nitrification
    - Specific enzyme tests: nitrogenase; urease; phosphatase
  - Most probable number methods

Measuring Microbial Toxicity

- "Ecological" Tests
  - Decomposition of litter
    - Use of leaf material in buried litter bags
      - Weigh biomass after period of time
      - Examine appearance of material

Routine Applications of Pesticides

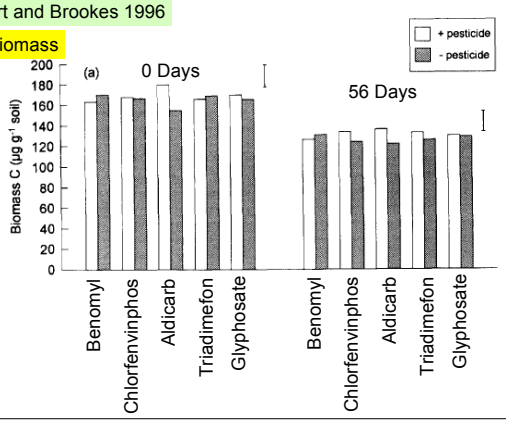
- Work of C.M. Tu circa ~25 years ago
  - Measured dehydrogenase activity (general respiratory enzyme for overall bioactivity)
  - Nitrogen fixation (nitrogenase)
  - Denitrification
  - Carbon Mineralization (CO_2 evolution)
  - Most Probable Number method
- No effect beyond a few days

Long-Term Research at Rothamsted Experimental Research Stn., UK

- Plot delineated and treated with single or multiple combinations of herbicides and insecticides
- Crop cultivated for 20 years
- After the last application of herbicides, monitored microbial activity and function right after application and in the fall
- Found no differences among plots due to pesticide use
  - One exception: microbial biomass was augmented in association with use of the pesticide aldicarb

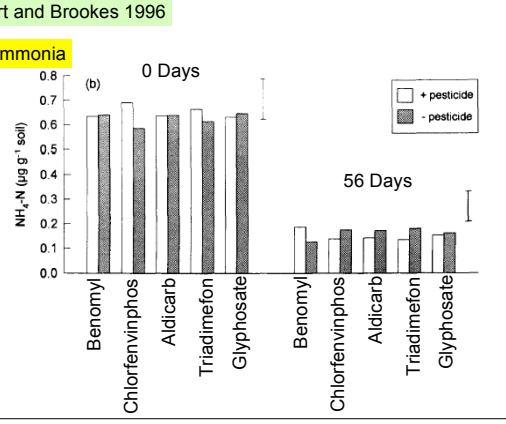
Hart and Brookes 1996

Biomass

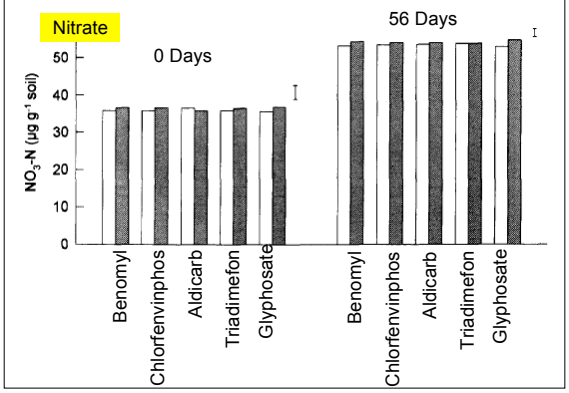


Hart and Brookes 1996

Ammonia



Hart and Brookes 1996



Waste & Microbial Toxicity

- Chemical spills and waste produce extremely high concentrations that are toxic
- At lower concentrations the same chemicals are not toxic
  - Petroleum hydrocarbons
  - Pesticides

Plant Toxicity

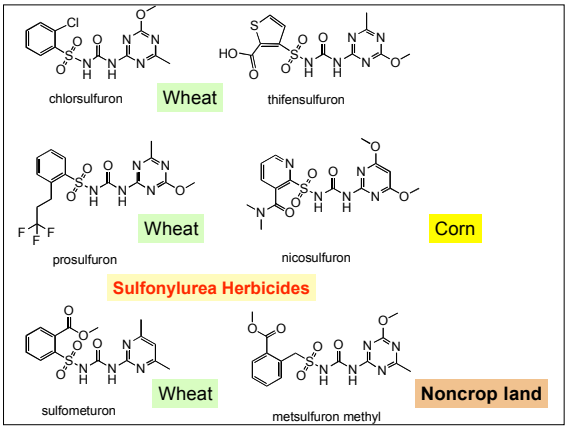
- Concerns
  - Agricultural use of pesticides, especially herbicides
  - Restoration of waste sites
  - Air pollution (acid rain in the U.S. is a more minor concern now than 15 years ago)

Plant Toxicity

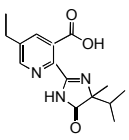
- Herbicides not likely to be selective for native species
- Problems with drift of sprays onto native species (natural lands) as well as drift into nontarget crops
  - Historical problems of 2,4-D use in cereals and consequent effects on grapes
- Although herbicides tend to be biodegradable in soil, low levels of residues could affect subsequent susceptible crops grown in rotation with tolerant crop ("carry-over")

Selected Herbicide Examples (Specific Modes of Action)

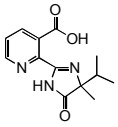
- Sulfonylurea herbicides & imidazolinone herbicides
  - Inhibit acetolactate synthase in pathway for branched chain amino acid synthesis
    - Valine, leucine, isoleucine
  - Application rates @ g/ha (ounces/acre)



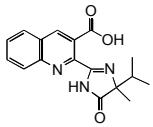
Imidazolinone Herbicides



imazethapyr



imazapyr

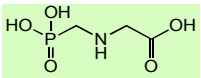


imazaquin

Use in soybeans

Selected Herbicide Examples (Specific Modes of Action)

- Glyphosate
  - Inhibits EPSPS enzyme (enolpyruvylshikimic acid phosphate synthase) in biosynthetic pathway for aromatic acids
    - tryptophan, phenylalanine, tyrosine
  - Use rates ~kg/ha (lbs/acre)
  - Used in 'Roundup Ready' Crops
    - Soybean, cotton, canola, corn

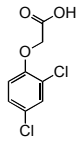


glyphosate

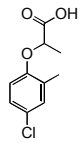
Selected Herbicide Examples (Specific Modes of Action)

- Auxin agonists
  - Mimic indole-3-acetic acid
  - Control cell elongation and growth
  - Includes phenoxyacetic acids (2,4-D) and picolinic acids (picloram, clopyralid)
  - Application rates ~kg/ha

'Phenoxys'

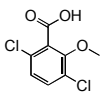


2,4-D

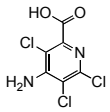


mecoprop, MCPP

Benzoic Acid

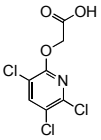


dicamba



picloram

clopyralid

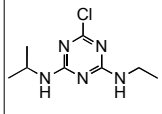


triclopyr

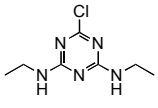
Picolinic Acids

Selected Herbicide Examples (Specific Modes of Action)

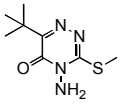
- Photosynthesis inhibitors
  - Atrazine
  - Most frequently detected pesticide residue in water



atrazine



simazine



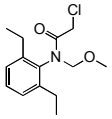
metribuzin

Symmetrical triazines

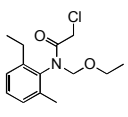
Asymmetrical triazine

Selected Herbicide Examples (Non-Specific Modes of Action)

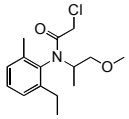
- Acylanilides (chloroacetamides)
  - Includes alachlor and metolachlor, frequently found in water
    - Acetochlor was marketed in the 90's as a substitute for alachlor that was supposed to be less likely to be found in water
  - Affect lipid metabolism
  - Rates: kg/ha



alachlor



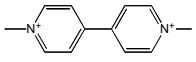
acetochlor



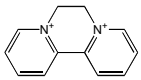
metolachlor

Selected Herbicide Examples (Non-Specific Modes of Action)

- Bipyridiniliums (paraquat & diquat)
  - Cationic
  - Generate free radicals, oxidizing the cell
  - Contact toxicant (not active from soil)
    - Used as a 'burn-down' & defoliator herbicide (potatoes)
  - Rates: kg/ha



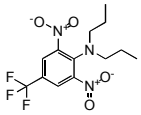
paraquat
1,1'-dimethyl-4,4'-bipyridinium



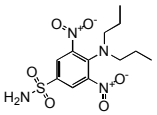
diquat
9,10-dihydro-8a,10a-diazoniaphenanthrene

Selected Herbicide Examples (Non-Specific Modes of Action)

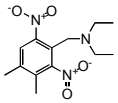
- Dinitroanilines (triluralin; oryzalin; pendimethalin)
  - Inhibit mitotic spindle formation; cells don't divide; roots don't elongate
  - Rates: sub kg/ha



trifluralin



oryzalin



pendimethalin

Assessing Plant Toxicity

- Germination tests
- Root elongation test
- Growth response tests
- Under consideration: reproductive studies

GR50: Concentration of herbicide reducing growth or biomass by 50% (compared to a control)