Can Biologicals Substitute for Fertilizer Nutrients?

Kurt Steinke, Ph.D.
Associate Professor
Soil Fertility & Nutrient Mgmt.
Michigan State University

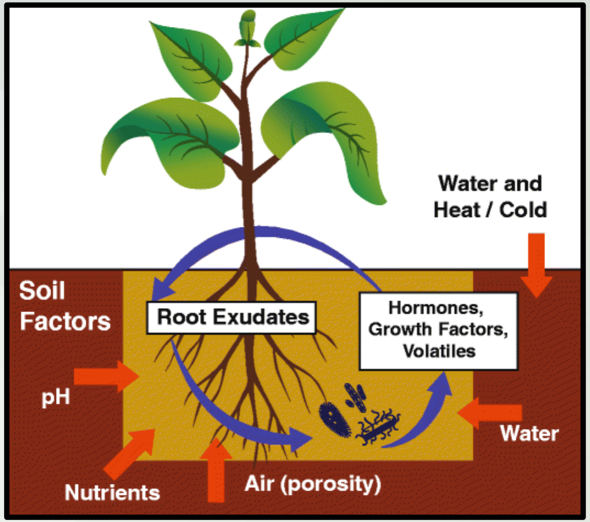
N 7 P 15 K 19
Greater yield in the field
MSU SOIL FERTILITY RESEARCH

MICHIGAN STATE UNIVERSITY EXTENSION

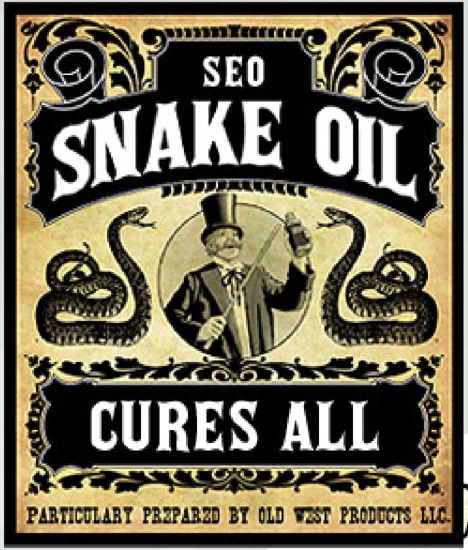
AgBioResearch
MICHIGAN STATE UNIVERSITY

Can Biologicals Substitute for Fertilizer Nutrients?

- Microbes and microbial processes are essential for productive, sustainable ag. ecosystems
  - Process waste
  - Recycle nutrients
  - Control/cause disease
  - Affect GH gas emissions
    - Impact climate
- BUT……



Vs.



Not true, why?

Agricultural Biologicals: Boost crop productivity and manage vegetation

Biofungicide

Biologicals

Microbials

Biofertilizers

Bioherbicide

Biostimulant

Bioinoculant

Bioinsecticide

Bioactivators

Bio-Control Agents

Biorational

Inoculants

Biopesticide

K. Steinke, MSU

Biologicals in Agriculture

- What are biological products for crop production
  - Derived from natural materials, contain natural materials, or use naturally-occurring processes
  - Naturally occuring compounds or synthetic (but similar structure and function as natural counterparts) (Biological Products Industry Alliance [BPIA])

- Types of biologicals
  - Microbial products (bacteria, fungi, nematodes, protozoa, and viruses)
  - Biochemical products (plant growth regulators, insect growth regulators, organic acids, plant extracts, minerals, pheromones)
  - May also consider beneficial insects

Biologicals in Agriculture

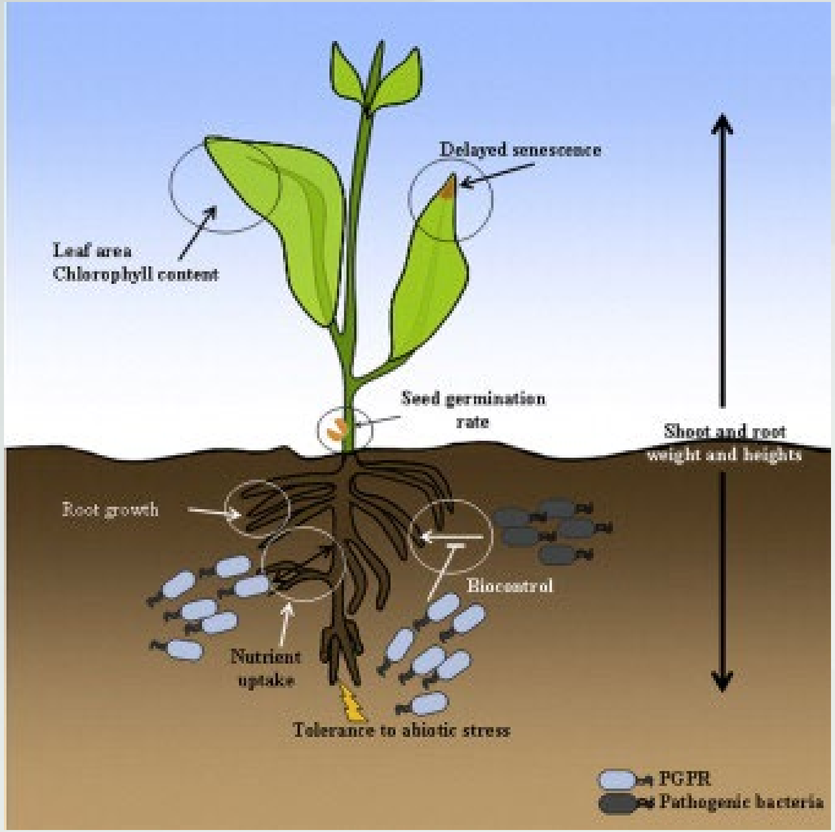
- Two categories
  - Biopesticides (plant or crop protection)
    - Protect against or directly control fungal and bacterial pathogens, insects, weeds (direct pathogen efficacy, plant incorporated protectants (Bt-corn)
    - REGULATED BY THE EPA
  - Biostimulants (plant growth promotion and plant nutrition)
    - Biofertilizers/Bioinoculants regarded as subcategory of Biostimulants
    - NOT REGULATED BY EPA
    - Products stimulating plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more characteristics of the plant 1) nutrient use efficiency, 2) tolerance to abiotic stress, 3) crop quality traits

How do biostimulants help plant fitness?

- Crop enhancement
  - Plant growth promotion
    - Biomass, root structure and growth, germination
  - Abiotic stress tolerance
    - Water use efficiency, reduce stress tolerance (drought, temp., salinity, pH)
  - Plant nutrient availability
    - Better synchronize availability with plant needs
  - Plant nutrient uptake
    - Better synchronize availability with plant needs
  - Pathogen suppression (antibiotics)
- **BUILD RESILIENCY!**

< 10% of products we know how function

PGPR Plant Fitness Enhancement



Plant benefits:

- Germination rate
- Root growth
- Yield
- Leaf area
- Chlorophyll
- Nutrient uptake
- Hydraulic activity
- Abiotic stress tolerance
- Bio-control
- Delayed senescence

Figure adapted from: Pérez-Montaño et al., 2014. Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. Micro. Res. 169:325-336.

Where are bacteria found in soil?

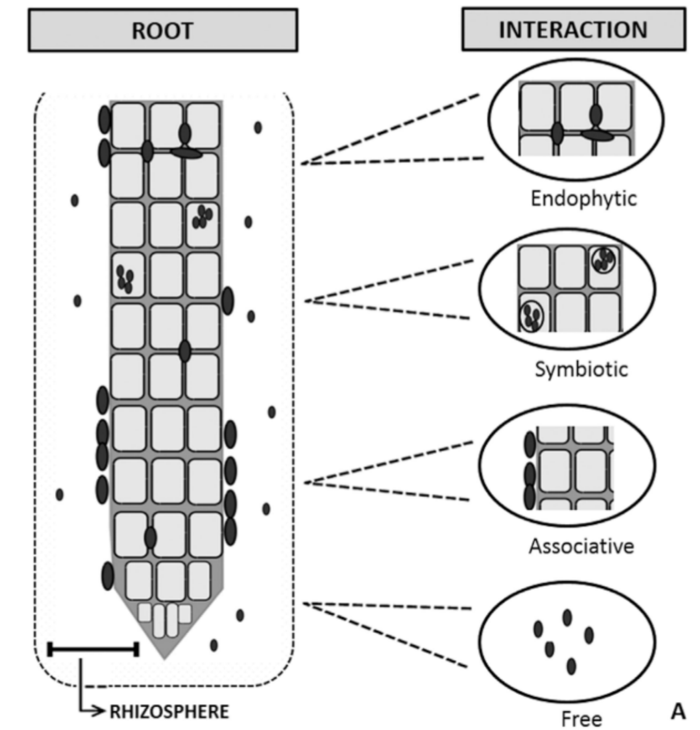


Figure adapted from Souza et al., 2015. Plant growth-promoting bacteria as inoculants in agricultural soil. Genetics and Molecular Biology 38:401-419.

Bulk soil

Rhizosphere

- soil influenced by, or in association with plant roots and plant-produced material
- 1-2mm from root
- Bacterial hotspot
- Rhizodeposits by plant roots attract bacteria
    - Corn ~1,000 lbs exudates/A/yr

How many are there?

- 1×10^{30} bacteria on planet (nonillion)
  - 1,000,000,000,000,000,000,000,000,000,000
  - Number above is 1,000,000,000 times greater than stars in universe
- <1% bacteria classified
- "The soil is an ocean of well-established bacteria" (C. Rice, KSU)

Why the interest?

- Consumer interest in qualitative food
  - Demand for organic food
  - Growing health conscience individuals
  - European tendency to narrow or eliminate pesticide use
- Environmental concerns
  - Agriculture ‘sustainability’
  - N_2O emissions
- USDA support to help shift growers from ‘traditional’ to organic farming

- Demand for new innovation

Why The Interest?

- $$$$$ may be the key driving factor
- Cost of Development, Regulatory, and Labeling of "New" Products
  - Pesticide
    - ~$255 million
  - Seed Traits
    - ~$135 million
  - Biologicals
    - ~$5 - $15 million
- Similar trends with fertilizers and amendments
- Biostimulant industry value (2019) – $2.1 billion (factory value)
- Biofertilizer industry value (2025) - $3.9 billion (factory value)
- Retail values >2x numbers above

Two Schools of Thought on How to Manipulate Soil Bacteria Populations

1) Inoculants
   - Seed or seedling coatings, soil-applied
   - Bacteria
   - Bacteria + fungi
   - Bacteria + sucrose (bacteria stimulant) + crop protectant (i.e., fungicide)
   - Bacteria + nutrients
2) Soil carbon (C) additions
   - Manures, compost
   - Cover crops
   - Crop rotation

K. Steinke, MSU

What are Biostimulants?

- Substances
  - Humic and fulvic acids, humins (not soil extractable) (LARGEST Market Share)
    - Extracted from humified organic matter, composts, or mineral deposits (e.g., leonardite).
    - Root growth, nutrient uptake (increase CEC), P_2O_5 availability, phenylpropanoid metabolism for stress response (drought, salinity)
  - Protein hydrolysates, N-containing compounds
    - Amino-acids and peptides
    - Regulates TCA cycle enzymes used to modulate N uptake and assimilation
    - Micronutrient chelation
    - Impact to soil microbial biomass, activity, and respiration
    - Induce plant defense response and increase tolerance to salinity, drought, temperature, and oxidative stress

What are Biostimulants (cont.)

- Seaweed extracts and botanicals (Second largest market share)
  - Unique to algal source (brown algae or red seaweeds)
  - Mixture of polysaccharides, micro's, growth hormones
  - Soil or foliar applied
  - Enhance root growth
  - Supply macro- and micro- nutrients
  - Chelators
  - Stimulate plant growth via hormones (cytokinins, auxins, abscisic acid, gibberellins
  - Stimulatory to soil microflora
  - Improve soil structure and aeration

What are Biofertilizers or Bioinoculants?

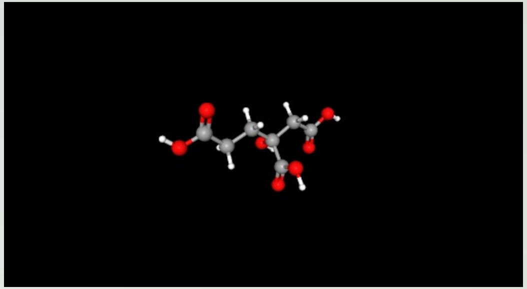
- Regarded as subcategory of biostimulants
- Contain living microorganisms
  - Isolated from soil, plant residues, water, composted manures
  - Single strains (e.g., *Bacillus subtilis*) or mixtures for additive or synergism
  - Genetically engineered (many seed inoculants)
  - Free-living bacteria, fungi, arbuscular mycorrhiza
- Used as compliment to mineral fertilizer
  - Increase nutrient supply (e.g., N_2 fixation)
  - Increase root biomass (volume of soil accessed)
  - Increased nutrient uptake capacity
  - Increased nutrient solubilization

When are bacterial inoculants applied

- At planting
  - Seed or seedling coatings, soil-applied
  - Bacteria
  - Bacteria + fungi
  - Bacteria + sucrose (bacteria stimulant) + crop protectant (i.e., fungicide)
  - In-furrow w/wo starter fertilizer
  - Fertigation, drip tape
- Mid-season
  - Foliar applications (product specific)

Conditions for N Fixation to Occur (Symbiotic, Non-Symbiotic, or Industrial)

1) Source of energy for reducing power
   - Ex. H gas to combine with N2 to produce a fixed form of N
2) Source of energy to keep N fixation process moving
   - In plant – CHOs
   - In soil – root exudates, OM, decay compounds
   - Industrial – natural gas
3) A catalyst or stimulus to start process and maintain
   - Heat, pressure, and metal catalyst
   - Nitrogenase enzyme and metalloproteins (fixation facilitators)
4) An O2-free environment for fixation to take place
5) System to remove fixed N as accumulation will stop process



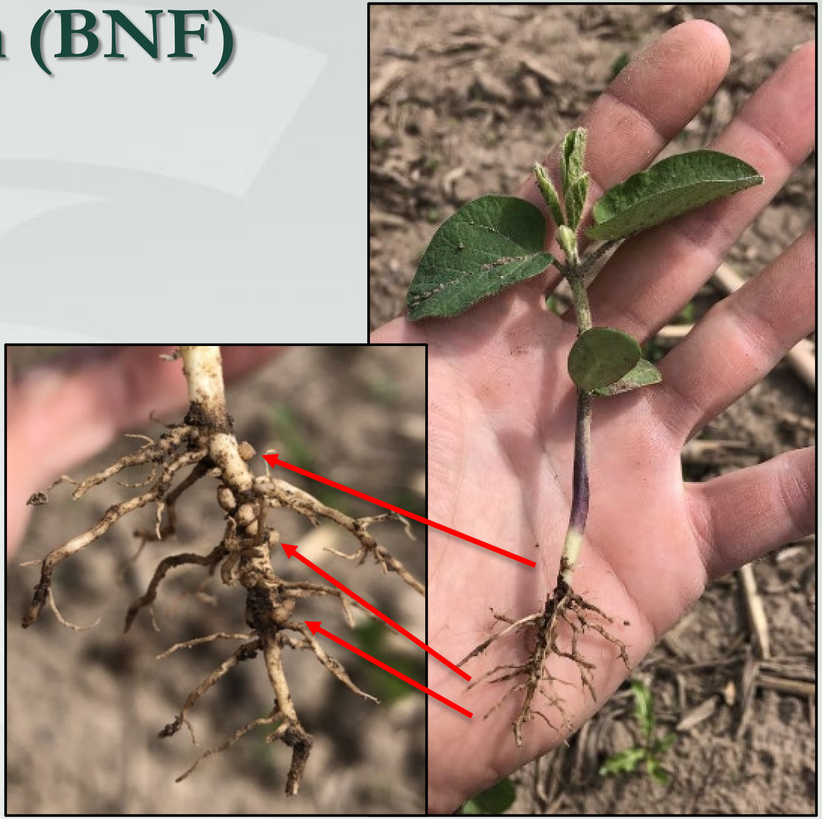
$C_7H_{19}Fe_7MoNO_7S_{9-12}$ (Nitrogenase enzyme)

Symbiotic Biological N Fixation (BNF)

- Nodulation results from a symbiosis between roots and soil-borne rhizobium (*Bradyrhizobium japonicum* L.)

- Fixes triple bonded N_2 from the atmosphere into plant-available NH_4
  - BNF provides 44-72% of soybean "N requirements"
  - **Estimated that BNF requires 5-7% more ATP than the assimilation of NH_4 or NO_3- from the soil solution**

- Can begin anytime between VE to V6 depending on the environment, high soil N content, cool soil temps, soil pH, moisture, etc.



K. Steinke, MSU

Symbiotic Biological N Fixation (BNF)

- Rhizobia
  - One example of microbe involved in N fixation, others exist
  - Invade root hairs, form tumor-like structures (nodules) on the root surface
  - Use the nitrogenase enzyme & energy to break the strong triple bond ($N \equiv N$) and produce NH_4^+ for plant growth
  - Require C from roots for survival
    - To fix N, nitrogenase requires anaerobic conditions which occur within the root nodule
    - Leg-hemoglobin (vitamin B12) creates the low O_2 envt and give nodules pink color

Non-Symbiotic N-Fixing Organisms

- Fix atmospheric N_2, transform to NH3, and attach to C to mobilize
- N-fixation
  - Still requires LOTS of energy
  - To convert 1 N_2 to **1 NH_3** requires **16 ATP** molecules (produced during photosynthesis) and 8 electrons
  - 1 peptide bond in protein synthesis requires only 5 ATP
- Generally limited by shortage of energy-yielding materials in soil

Organisms or system	N_2 fixed lb. per A/yr.
Legumes	
Soybean	50-85
Cowpea	75
Clover	95-145
Alfalfa	115-535
Lupin	135-150
Nodulated non-legumes	
Alnus	35-265
Hippophae	2-160
Ceanothus	55
Coriaria	135
Plant-algal associations	
Gunnera	10-20
Azollas	280
Lichens	35-75
Free-living microorganisms	
Blue-green algae	20-80
Azotobacter	0.3
Clostridium pasteurianum	0.1-0.5

Non-Symbiotic N-Fixing Organisms

- Energy sources for asymbiotic N-fixing organisms
  - In soil: exudates near roots including organic matter and residue decay intermediary compounds in the bulk soil
  - In plant: organisms receive energy from within the plant in which reside
- Generally limited by shortage of energy-yielding materials in soil
- Asymbiotic N-fixing bacteria already in most soils
  - Activity increases: tillage decreases, moist, warm conditions
  - **Activity decreases: dry soil, wet soil, cold soil**
  - Access to inorganic N reduces N fixation
- New strains being developed and discovered
  - Growth stimulating hormones, root systems, etc.

Regional studies on commercial asymbiotic N-fixation products

- Marketed products tested
  - Envita®, Azotic North America
    - *Gluconacetobacter diazotrophicus*
  - Utrisha™, Corteva Agrisciences
    - *Methylobacterium symbioticum*
  - ProveN™, ProveN40™, PivotBio
    - *Klebsiella variicola, Kosakonia sacchari* & *Klebsiella variicola*
- Compared N rates with and without biological

K. Steinke, MSU

NDSU EXTENSION — EXTENDING KNOWLEDGE » CHANGING LIVES

SF2080 (April 2023)

Performance of Selected Commercially Available Asymbiotic N-fixing Products in the North Central Region

Asymbiotic or non-symbiotic N-fixing organisms, usually species of bacteria, have the ability to 'fix' nitrogen (N) from the atmosphere where it is abundant as N_2 gas, and produce NH_3 without being associated with plants or fungi. Fossil evidence indicates that asymbiotic N-fixers were present and active 1.5 billion years ago (Boyd and Peters, 2013). This compares to the appearance only about 59 million years ago of the more commonly known symbiotic N-fixing bacteria associated with legumes (Sprent and James, 2007).

Dave Franzen, North Dakota State University
and the following members of the NCERA-103 committee on non-conventional additives and amendments, and additional contributors:

Overall Findings

- **2 of 61 site-years** observed a yield increase from inclusion of N-fixing bioinoculant beyond N rate increases
  - 10 states, 4 crops
- Further on-farm testing required

WHY?

N is Unpredictable

Factors that affect soil bacterial survival

- Biotic
  - Predation (protozoa)
  - Competition (starvation, pathogen antibodies)
  - Root growth (exudates enhance survival)
- Abiotic
  - Clay minerals (microhabitats, moisture)
  - Water tension
  - Organic carbon (copiotrophic vs. oligotrophic)
  - Inorganic nutrients (N, P)
  - pH (nutrient release [e.g., P], or Al^{3+} toxicity)
  - Temperature chemicals

Why do inoculants that show positive results in laboratory conditions sometimes fail under field conditions?

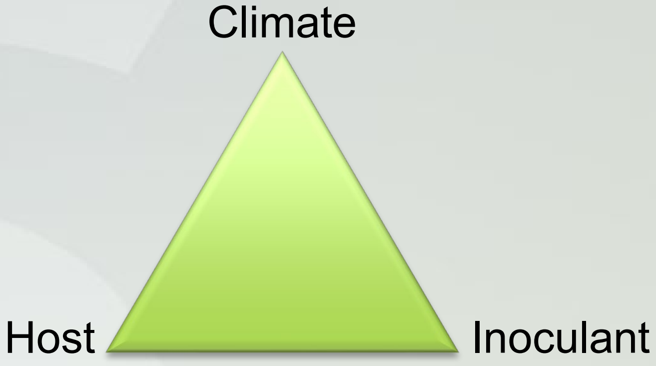
- Microbiostasis: the survival and growth inhibitory effect of the soil on inoculum
- Reduced biological diversity, favorable climate range, exclusion of natural enemies, substrate food sources, functional redundancy (i.e., multiple bacteria performing the same function)
- **The soil environment is hostile!**
  - Decline in inoculant active cell population
  - Lack of root surface colonization
  - "One size fits all" approach – same strain across geographies and soil types
  - **Current microbiome results from past and present environment**
    - **Organisms adapt and compete to survive**
    - **Natural selection**

Laboratory vs. Field Results

- Much inconsistency in field results
- Why? Unpredictable soil environment
  - Soils are heterogeneous
  - Texture, temperature, salinity, pH, moisture, heavy metals
- **Functional redundancy of bacteria**
  - High diversity: 1g soil = 10,000 to 50,000 species
  - Multiple species carry our similar function
  - Other organisms (e.g., nitrification by archaea)
- Isolation from rhizosphere vs. bulk soil
  - Bacteria must be compatible with host
- Fungal propagation difficult – biotrophic (needs host)
- Most crop systems already contain organisms

What influences inoculation success?

- Host (growth stage, health)
- Environment
  - Soil type and structure (housing)
  - Geography
  - Climate
- Rhizocompetence
  - Biofilm formation (survival)
  - Siderophore production (Fe^{2+} for microbes)
  - Antagonism (synthesis of antibiotics and enzymes)
  - Food sources (low vs. high molecular weight compounds)
  - Competition with bulk soil populations
  - Motility (bacteria can only move in water films)
  - Protease activity (ability to hydrolyze N compounds)



Focus on 2 items:

1) Better protect the microbe
2) Better dispersion of the microbe

A product sounds too good to be true?
It probably is

Growers must ask themselves if the product:

1. Claims to be a ‘fix-all’ solution, work in all soils and all situations
2. Contains scientific evidence vs. grower testimonials
3. Has proven mechanism by which it performs

General Comments

- Marketing is well ahead of the science!
  - Burden of research falls upon the user
- Companies are good at 'development', meaning marketing
  - BUT research is sparse and results from University researchers may be controlled by the restrictions of signed confidentiality agreements
- Try them on **replicated strips and multiple N rates** on the farm
  - N rates must not all be > recommended N rates
- Refer to L. Thompson, 2022 from proceedings of the North Central Extension-Industry Soil Fertility Conf. for ideas regarding on-farm testing and data analysis

MICHIGAN STATE UNIVERSITY EXTENSION

Thank you!

Michigan State University

Soil Fertility and Nutrient Management

For more information

ksteinke@msu.edu

soil.msu.edu

AgBioResearch
MICHIGAN STATE UNIVERSITY

N 7 P 15 K 19
Greater yield in the field
MSU SOIL FERTILITY RESEARCH