Bioscience

Analysis, Research and Consulting

SOIL BIOLOGY HANDBOOK

Bioscience ARISA

Using the power of genetics
for land management

bioprime

Managing Soil Biology

Bioscience

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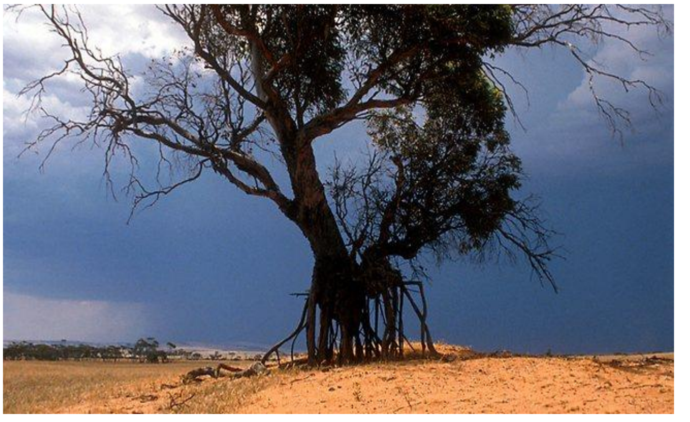
Ecology is Important for Understanding Your Soil



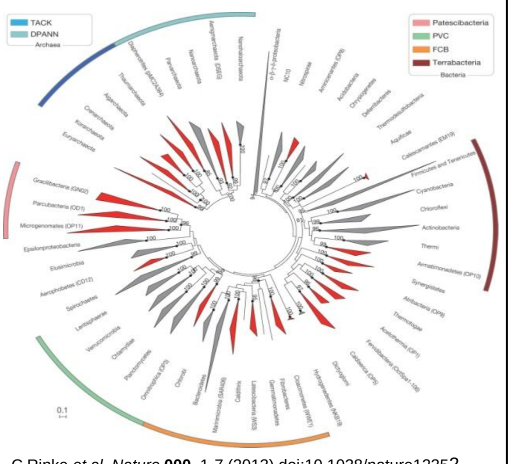
- Ecology aims to understand the distribution and abundance of organisms, and their function in a community
- An organism’s ecology is moderated by interactions with both the non-living environment and with other organisms
- An ecosystem, for example a soil ecosystem, is a community of interacting organisms
- Species-rich ecosystems with many interactions are more stable

Healthy Soils are Diverse Ecosystems

- Healthy soils are complex ecosystems containing many species
- Specific soil fungi and bacteria are associated with plant roots
- Rhizosphere fungi and bacteria are functionally important to their plants

Healthy soils have a highly diverse microbiota. Recent research estimates that a typical 1 gram soil sample contains 10,000 species of bacteria or 10^{10} bacterial cells/cm^3 (Fierer et al 2011). The latest genetics techniques are allowing us to discover soil microbiota that could not previously be studied.

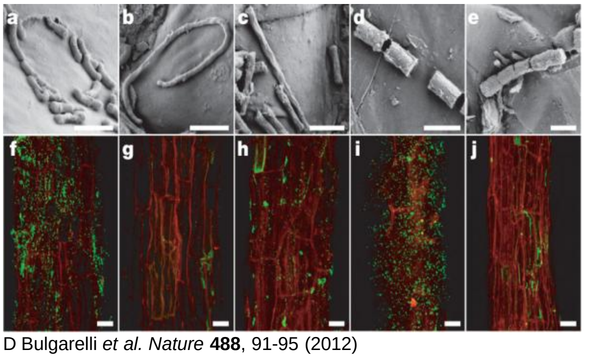
Bacterial Tree of Life



C Rinke *et al. Nature* **000**, 1-7 (2013) doi:10.1038/nature12352

A unique microbiota are specifically associated with plant roots. Plant rhizosphere soil contains a very different community of bacteria and fungi compared to the soil matrix. Many rhizosphere bacteria require metabolically active plant root cells to persist, and many fungi live symbiotically within plant roots.

Root-inhabiting bacteria (top, SEM) and visualised on the root surface (bottom, green)



D Bulgarelli *et al. Nature* **488**, 91-95 (2012)

Root-associated microbiota have functional significance for plants:

- Mycorrhizal fungi increase plant mobilisation and uptake of nutrients, especially phosphate

- Mycorrhizal fungi have a large surface area and can uptake water efficiently, this is transferred to plants

- Bacteria release many soil nutrients in a bioavailable form for plant uptake

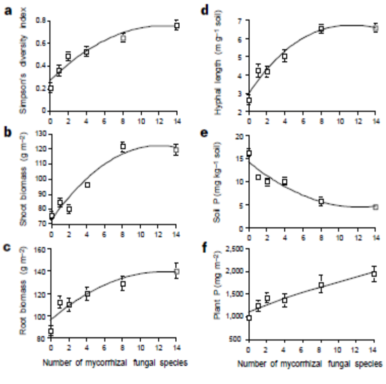
- Specialized bacteria fix atmospheric nitrogen for plants use

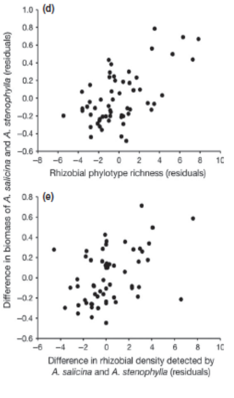
- Bacteria and fungi can suppress competing pathogens that cause plant diseases

A Diverse Soil Microbiota Results in Improved Soil Ecosystem Services

- Soils with high microbial diversity suppress plant diseases spread in soil
- Higher soil bacterial and fungal diversity increases soil functioning:
  - Higher soil nutrient cycling
  - Increased soil decomposition rates
  - Higher plant productivity

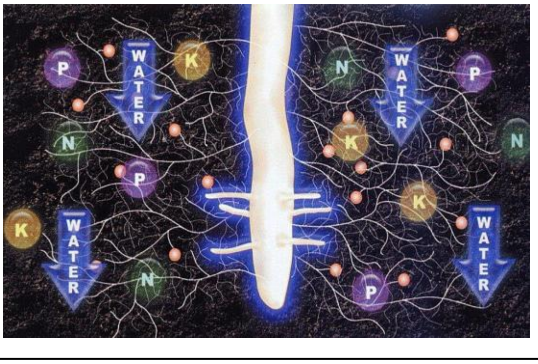
Increased mycorrhizal fungal species diversity caused higher plant shoot biomass, root biomass, increased overall plant phosphate uptake, and plant productivity.



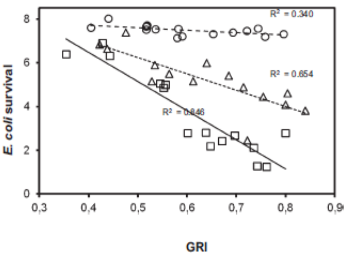


Van der Heijden et al (1998), Nature 396, 69-72.

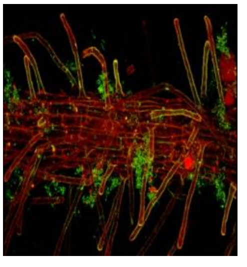
Bevan et al (2013), Ecology Letters 16, 167-74.

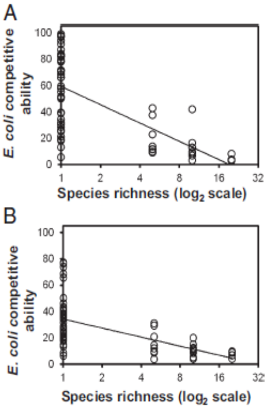


Diverse soils can be disease-suppressive. Increased soil microbial diversity reduced both the size of a soil bacterial plant pathogen population, and its competitive ability (so also its likelihood of survival).

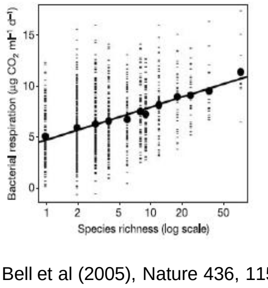


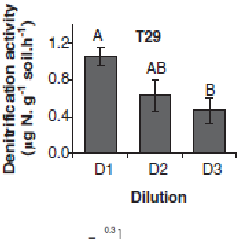
Van Elsas (2012)
PNAS 109, 1159-64.

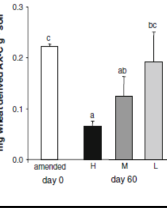




Increased microbial diversity increases bacterial nutrient (nitrogen) cycling rates and wheat straw decomposition rates.







Bell et al (2005), Nature 436, 1157-60.
Philippt et al (2013), ISME Journal 1-11.
Baumann et al (2013), Biogeochemistry 114, 21-21.

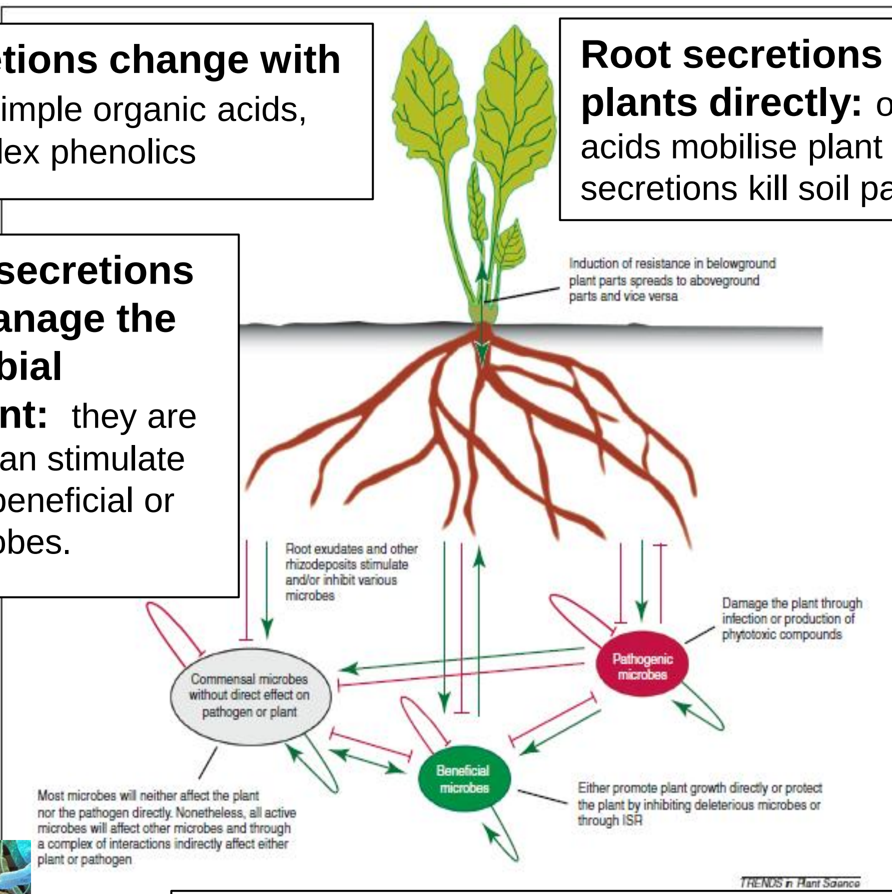
The Rhizosphere – A Complex and Diverse Interacting Ecosystem

- Plant roots and soil microbiota interact via chemicals secreted by plant roots
- Plant root secretions provide rhizosphere fungi and bacteria with abundant carbon-based food
- Plant root secretions can stimulate or suppress soil fungi and bacteria

Root secretions change with root age: Simple organic acids, then to complex phenolics

Root secretions benefit plants directly: organic acids mobilise plant nutrients, secretions kill soil pathogens.

Plant root secretions actively manage the root microbial environment: they are signals that can stimulate or suppress beneficial or harmful microbes.



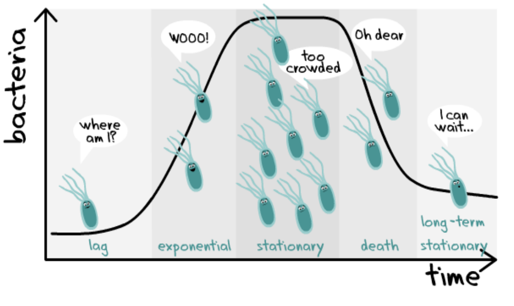
Berendsen et al (2012), Trends in Plant Science 17, 478-86.

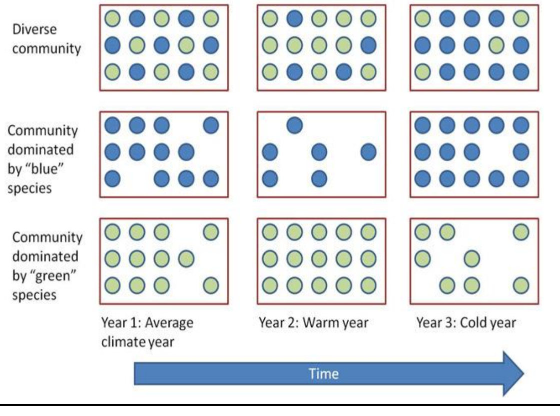
Rhizosphere soil compared to bulk soil: up to 40% of plant carbon photosynthates are secreted into soil via roots, so there can be abundant food for microbes and fungi here compared to bulk soil.

Diversity and Stability in Microbial Populations

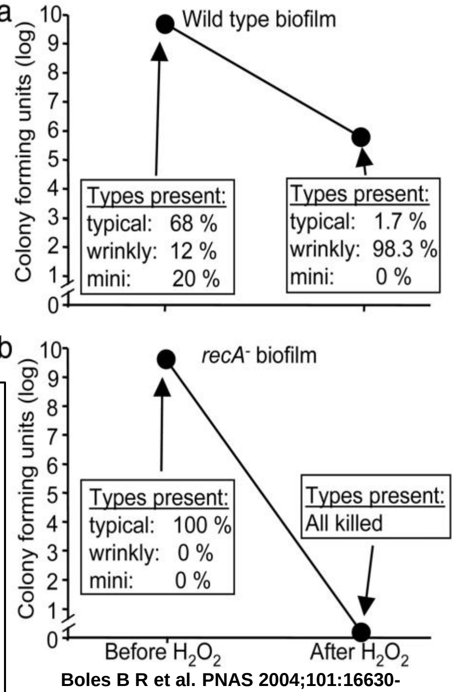
- Microbes usually occupy subtly different microhabitats (niches) in soil
- Some bacteria have resistant spores that can remain in soil to wait for a burst of food availability
- If conditions becomes suitable, the population size of microbe(s) that can consume available food will grow rapidly and exponentially
- Soils with high microbial species diversity are more resilient to environmental change because they are more likely to contain species able to grow in any novel conditions

The lifecycle of a bacterial species: adding food results in very rapid growth until food is exhausted, at which point some "resting" forms survive and remain dormant to wait for another food burst. In soil, roots can provide food bursts.





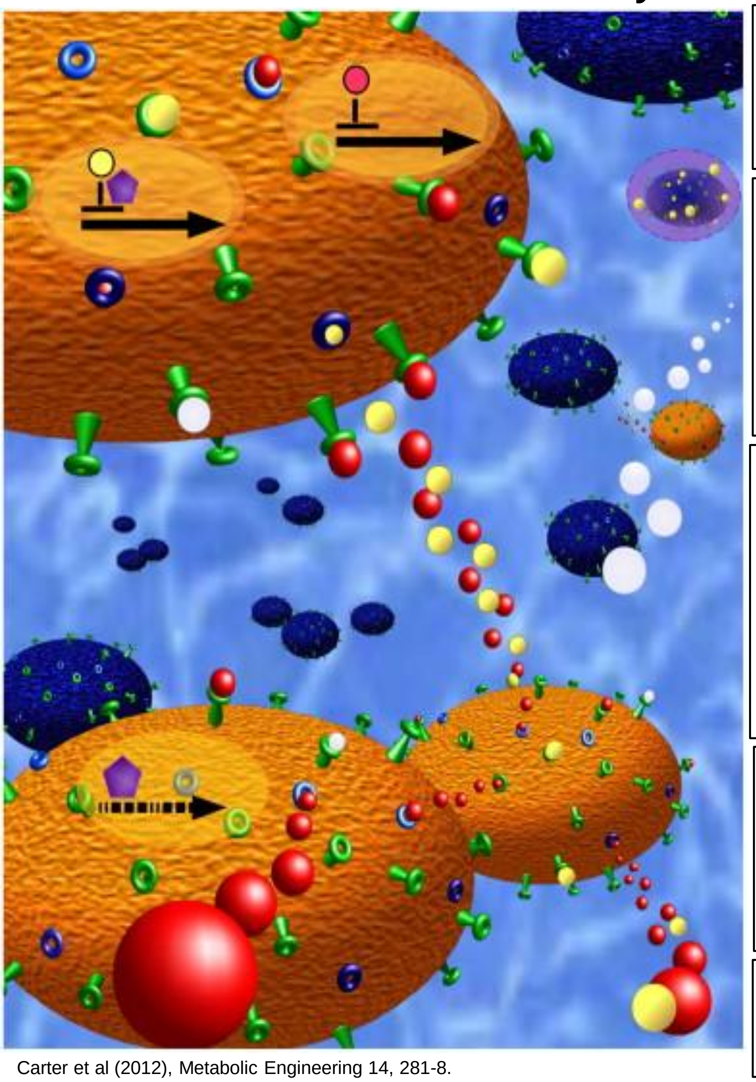
Diversity and stability: a healthy soil microbial community has many species including many rare ones; a diverse community continues to function well in changed environmental conditions because it contains many species that might grow well in the new environment.



Boles B R et al. PNAS 2004;101:16630-16635

A Diversity of Bacterial Chemical Signalling

- Microbes secrete small molecules into their environment, and respond to molecules secreted by others
- Some responses to signalling molecules (“quorum sensing”) result in adaptation to new conditions, which benefit select groups over others
- Signalling molecules from other organisms can block the signal cascade (called “quorum quenching”)
- Plant roots encourage beneficial associations with certain bacteria and fungi by secreting “elicitor” molecules, these lead to symbiotic associations



Carter et al (2012), Metabolic Engineering 14, 281-8.

Green and Blue cell surface receptors: Specific signal receptors.

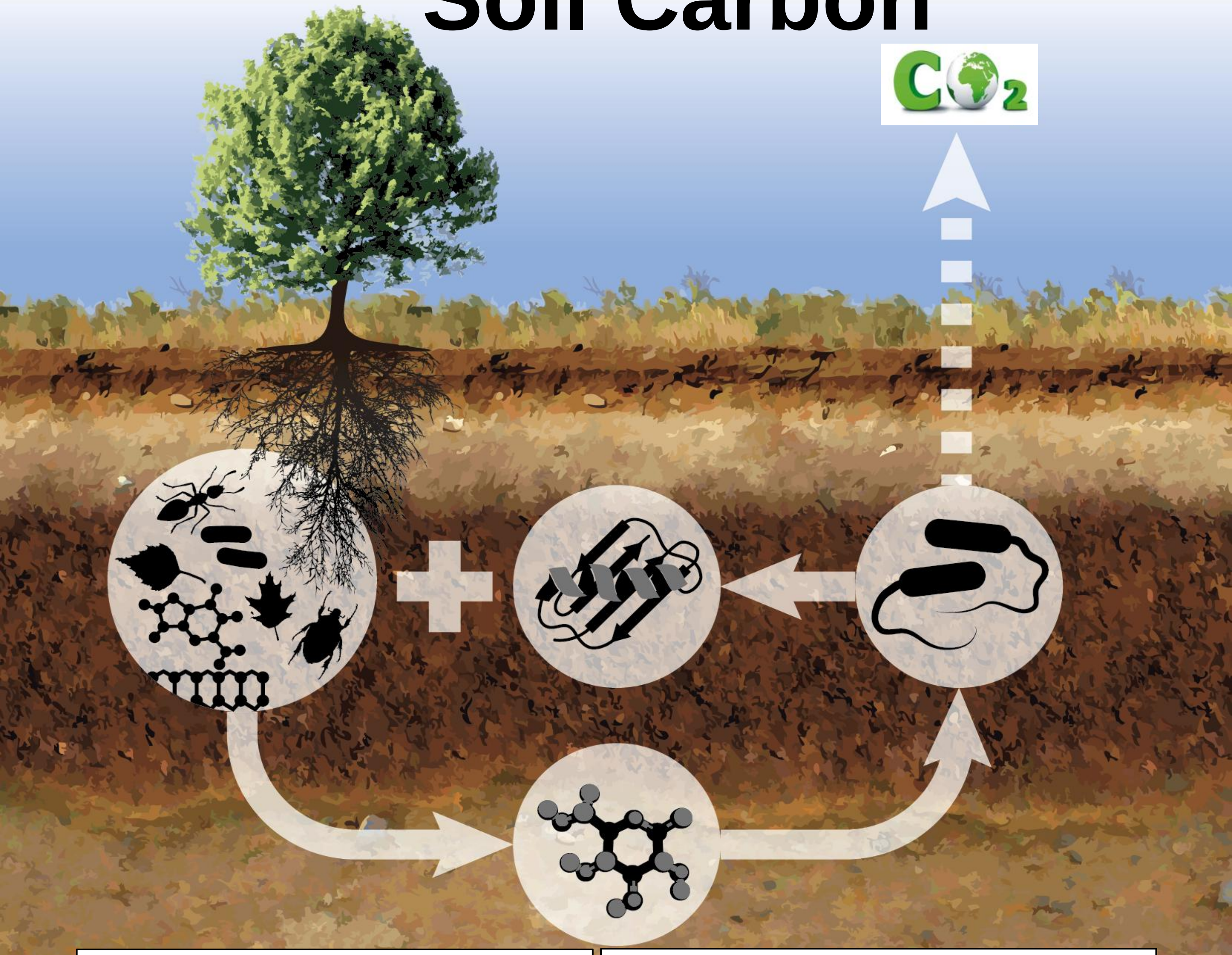
Red: signalling molecules from the same bacterial species (accelerating signal cascade, “quorum sensing”).

Arrows: Genetic response cascade to signalling molecules, resulting in specific bacterial products.
eg. molecules that increase the availability of micronutrients (-> enhance bacterial and plant growth), that produce biofilms (aggregate bacterial networks), or that attack a plant host (-> plant disease)

Yellow: signalling molecules from different bacteriaL species (“quorum quenchers”)

White: Signalling molecules from plants.

Soil Carbon



Forms of carbon in soil:

- Largest part: Short lived – root exudates, dead root hairs, microbial biomass.
- Big part: Longer life – litter, dead roots and stems, spores, sporangia etc.
- Smaller part: Very long life – refractory carbon, humic substances
- Charcoal – essentially inert
- All are food for specific groups of microbes, and have different impacts on soil physical and chemical properties.
- Soil biology is the main driver of carbon dynamics.

Measuring soil carbon:

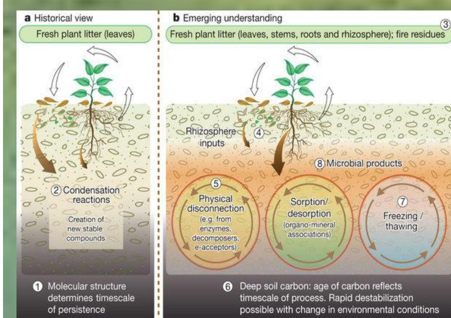
- Carbon occurs in different forms at different soil depths.
- Some particles are microscopic, others are larger
- Sampling collection, preparation, fractionation and analysis methods are critically important for meaningful results which are compliant with carbon trading protocols
- Analysis methods: direct (most accurate) and indirect
- Bioscience measures soil carbon to international protocols using LECO induction furnace (direct) methods

A Primer on Soil Priming

- Priming occurs when the addition of modest amounts of soil organic materials have a strong short-term effect on soil microbial activity and on organic carbon breakdown
- Priming occurs naturally when organic matter derived from photosynthesis is pumped into the rhizosphere soil via plant root secretions
- Some compounds have significant soil priming effects, even at low concentrations

Priming with organic matter: addition of organic matter to the soil can cause an exponential increase in specific groups of soil microbes. These can rapidly consume both the organic matter added to the soil plus existing soil organic matter when added material runs out.

Most natural organic matter additions into soil: via plant secretions into the soil rhizosphere, rather than plant litter



Simple organic matter addition: sugars, organic acids = priming via many microbes

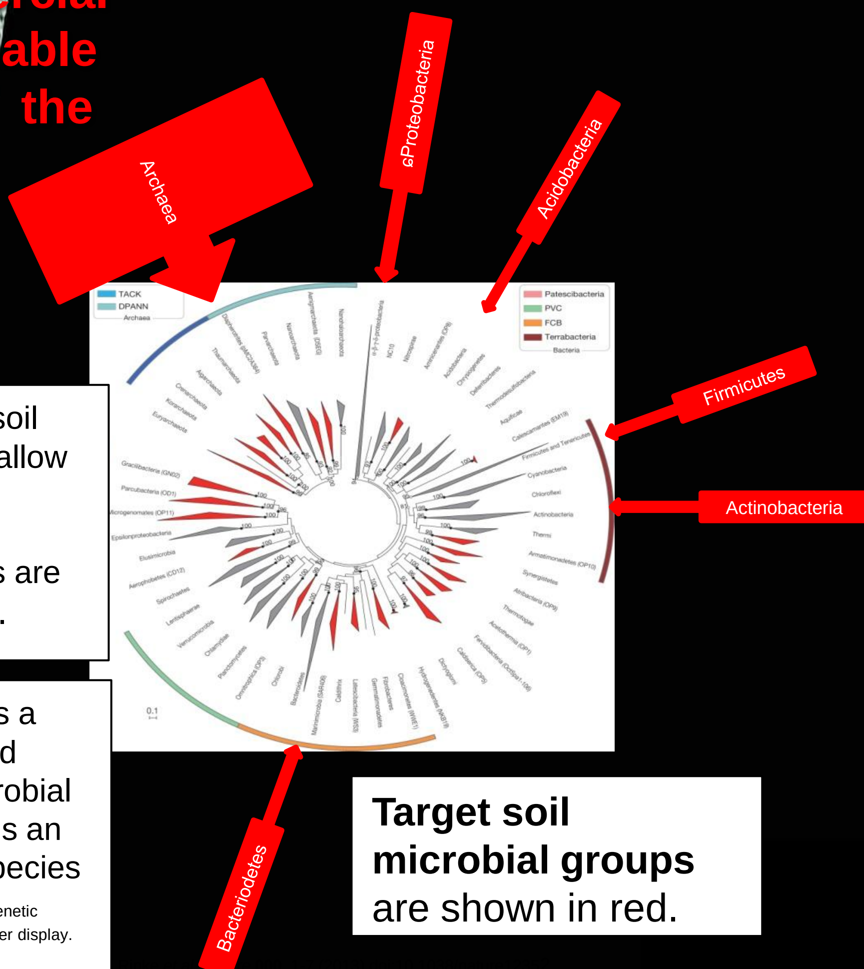
Complex organic matter addition: straw, wood cellulose, lignin = priming via specialist microbes

Different organic materials: different soil priming effect, because different groups of microbes are stimulated.

Is My Soil Healthy?
Bioscience Can Measure the Microbial Diversity of Your Soil

- **An estimate of microbial species richness can assist in land management.**
- **Bioscience measures microbial diversity by estimating species richness for up to 10 groups of important soil organisms: fungi, archea, all bacteria, groups of important soil bacteria.**
- *Bioscience ARISA* **assay can compare microbial diversity across fields, plots, treatments, management approaches and amendment types.**
- **This is the first such commercial service available anywhere in the world.**

Bacterial Diversity Analysed by Bioscience Plotted on the Bacterial Tree of Life



Why? An estimate of soil microbial diversity can allow land managers to test objectively whether soil management strategies are effective and beneficial.

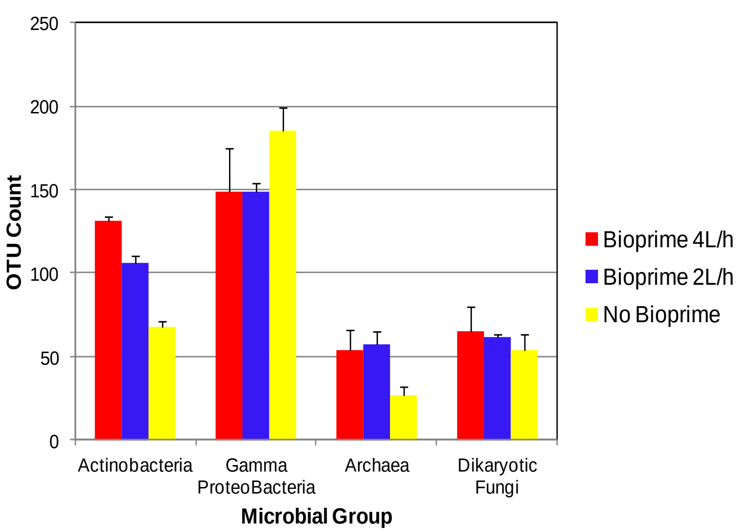
How? Bioscience uses a genetic technique called ARISA to estimate microbial diversity. ARISA returns an index proportional to species richness. For further details of the genetic technologies involved, please see the computer display.

Target soil microbial groups are shown in red.

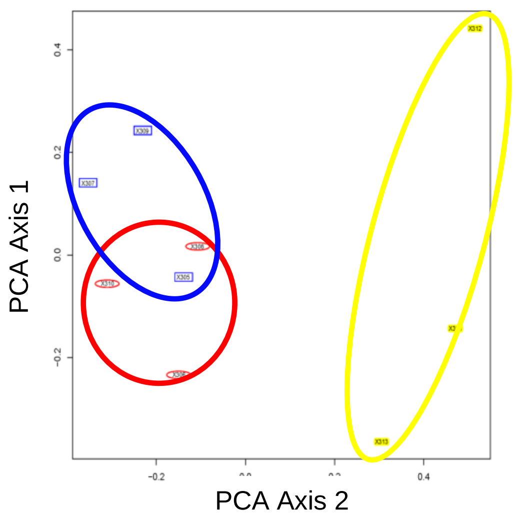
Bioscience ARISA

Using the power of genetics for land management

- A Bioscience ARISA assay requires as little as 2g of soil
- The assay returns data for up to 10 microbial groups
- OTU ("operational taxonomic unit") counts are an index of the number of species of that group in the soil sample
- PCA ("principle components analysis") reveals how the composition of OTUs (equivalent to species) in the microbial communities have shifted in response to a treatment



Typical OTU count results returned to a client. OTU counts (index of species diversity) are shown for four selected microbial groups in a factorial field trial with three levels of Bioprime. Both stimulatory (eg. Actinobacteria, Archea) and suppressive (eg. Gamma Proteobacteria) effects were observed.



Typical ARISA PCA result returned to a client: In this example of the Dikaryotic fungi, clusters of soil samples corresponding to different treatments are plotted. Bioprime shifted the composition of the species in the community.

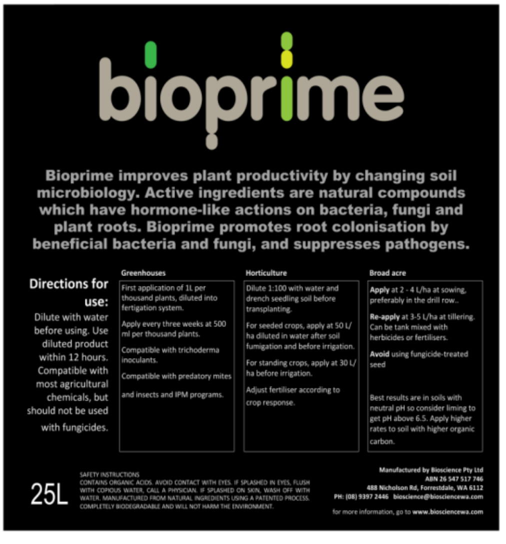
No Bioprime

Bioprime 2L/h

Bioprime 4 L/h

bioprime

Managing Soil Biology

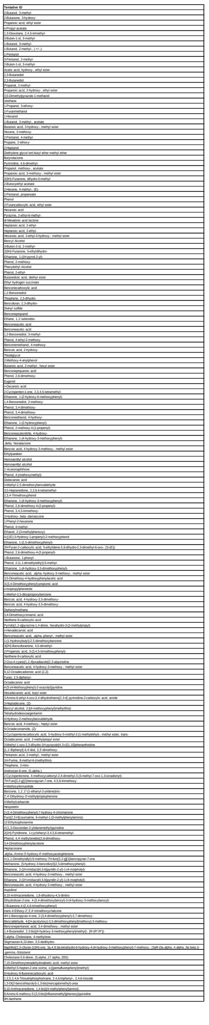


- Bioprime: a soil priming agent
- Bioprime: effective through changing soil microbiology
- Unique form of soil priming
- Composed of a huge range of signalling molecules
- When applied to soil, even in very small amounts, it changes diversity within a wide range of important soil microbial groups
- Bioprime promotes the development and diversity of beneficial microbes
- The outcome is a stronger plant, better root growth and ultimately, a more efficient system
- Greater efficiency equals better profits
- Bioprime: raw materials are natural products, manufactured using a patented process
- Bioprime: non-toxic and completely biodegradable

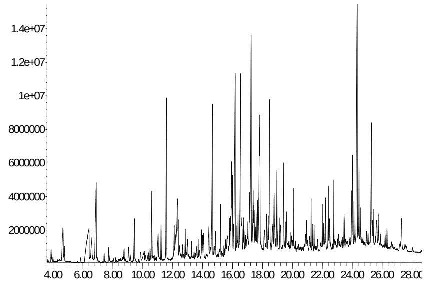
How Does It Work?

What is in Bioprime?:

- Typical list of components returned from an analysis (top)
- Typical analytical plot showing component peaks by size (bottom)



- Bioprime contains a wide range of components, including diverse soil signalling molecules
- Some components (“quorum quenchers”) act to suppress harmful bacteria consortia
- Some components act as soil priming agents and “quorum sensors”, stimulating microbial groups known to be beneficial to plant roots
- Others components are identical to the organic acids and phenolics that plant roots secrete to attract beneficial microbes
- Yet other components are precursors to plant steroids
- When applied to soil, Bioprime changes microbial communities
- In trials, Bioprime:
  - Increased species diversity within Actinobacteria (known disease-suppressors) and Archea (nitrogen metabolisers)
  - Changed species diversity within Gamma Proteobacteria
  - Shifted the composition of the Dikaryotic fungal community
- Bioprime produces a healthier soil with greater diversity of beneficial microbes and a reduction in pathogens
- Most importantly for grain growers, only small volumes are required (2 – 4 L per hectare) to produce changes, and it is inexpensive



bioprime

How Do We Know It Works?

- 20 year history of use in intensive horticulture for disease suppression
- Despite lack of knowledge of how it worked, leading growers kept purchasing the product and stayed disease free
- Four years of intensive DNA-based investigations on broad acre soils
- Molecular biology showed us how microbial group diversity shifted after application
- R&D focus on fermentation technology increased levels of active ingredients for lower production costs
- Pot trials lead to small field trials, larger field trials, then on-farm trials
- Work is ongoing: especially finding optimal timing and application rates to give the best returns to our growers

bioprime Field Trials 2013

Bioscience ARISA

Bioscience Trials:

Liebe Group

Bioscience living farm

Wheat, longterm trial, factorial design:
Bioprime application rate
Bioprime application time
Nitrogen fertiliser

Forrestdale

Bioscience

Wheat, sandy soil
Factorial design:
Bioprime application rate
Bioprime application time

Horticulture Grower Trials:

Berrysweet

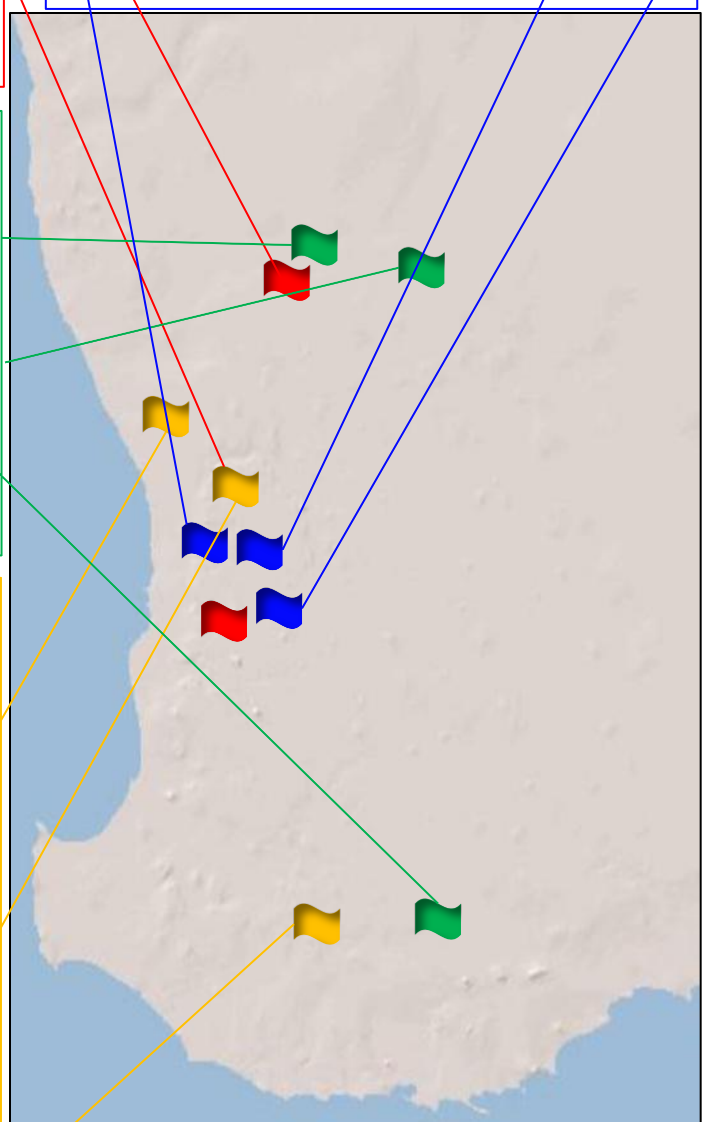
Bioprime for strawberry *Fusarium* control

Pan Pty Ltd

Bioprime for *Pythium* and *Sclerotina* control in leafy vegetables

Zaldeesh Pty Ltd

Bioprime for Greenhouse Capsicums



Broad Acre Grower Trials:

McAlpine Research

Biological farming, product interactions
Bioprime factorial trial

CailBros

Bioprime on wheat

Glenvar

Bioprime on wheat and oats

Independent Trials:

Evergreen

Perennial pastures
Alternative biological
ARISA monitoring

Evergreen FARMING

The University of Western Australia

UWA/DAF

Department of Agriculture and Food

Bioprime and ARISA
Control and monitoring of stableflies
Vegetable crop residue breakdown

Blackwood Basin Group

Blackwood Basin Group

Stubble breakdown trials
ARISA monitoring

Bioscience

Analysis, Research and Consulting

Analysis

- Soil
- Water
- Leaf Tissue
- Plant pathology
- Microbial
- Genetic
- Geotechnical
- Hydrology

Enabling Technologies

- Hydroponic consumables
- Liquid fertilisers
- Calibration standards
- Bioprime

An Australian Government Initiative

AusIndustry

Certificate of Registration

R&D Tax Incentive Program

Research Service Provider

Bioscience Pty Ltd

RSP No. 89461

Registration valid from 1/07/2013 to 30/06/2014

1 May 2013

Date

Consultancy

- Horticulture
- Arboriculture
- Agriculture
- Rehabilitation
- Groundwater licensing
- Stormwater and groundwater management
- Contaminated sites
- Urban development

Research & Development

- Extensive grant-funded R&D history
- Fully equipped laboratories
- Contract R&D service provider to many leading growers.
- **AusIndustry Registered Research Service Provider**: clients can qualify for tax concessions to offset costs. This can substantially change the risk profile of undertaking farm trials