Bio-Prime®

We sell “Do It Yourself” Seed Treatment material to farmers.

What is the purpose of Bio-Priming Seed?

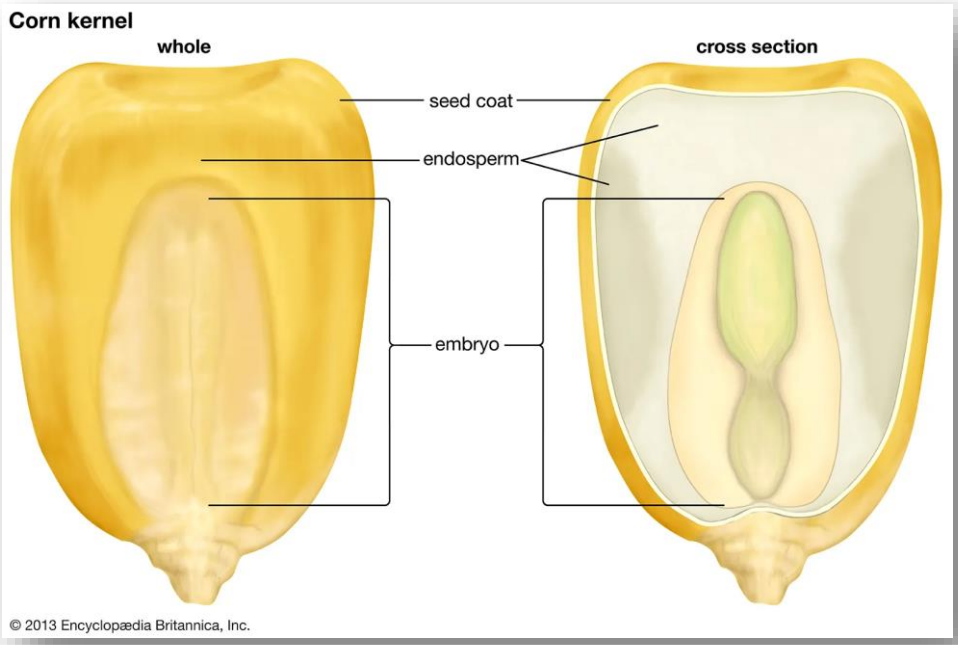
I. 20-40% higher yields.

II. 20-40% increased plant hardiness and stress handling ability in drought/cold/heat.

III. 20-40% improved seed/fruit quality.

The first stage of Biological Priming of seed is discussed on this www.bioprime.co.za webpage in detail, with its logic chain, value chain and background. There is a Second stage Seed Coating Phase, which is continued on www.vermicoat.com for second stage coating.

Introduction to Biological Priming of Seed:



Bio-Priming is when a farmer adds living Microbes into water, and have dry seed suck up the moisture and biology into the endosperm, so that the Microbes live inside the seed. The seed is only partially germinated during this process in preparation for planting. The seed has enough moisture for the microbes to live, but it is dry enough for commercial planting. Upon germination, these microbes interact with soil microbes, greatly improving nutrient uptake efficiency. Bio-Priming improves plant growth due to better microbial nutrient acquisition and more efficient plant nutrient uptake.

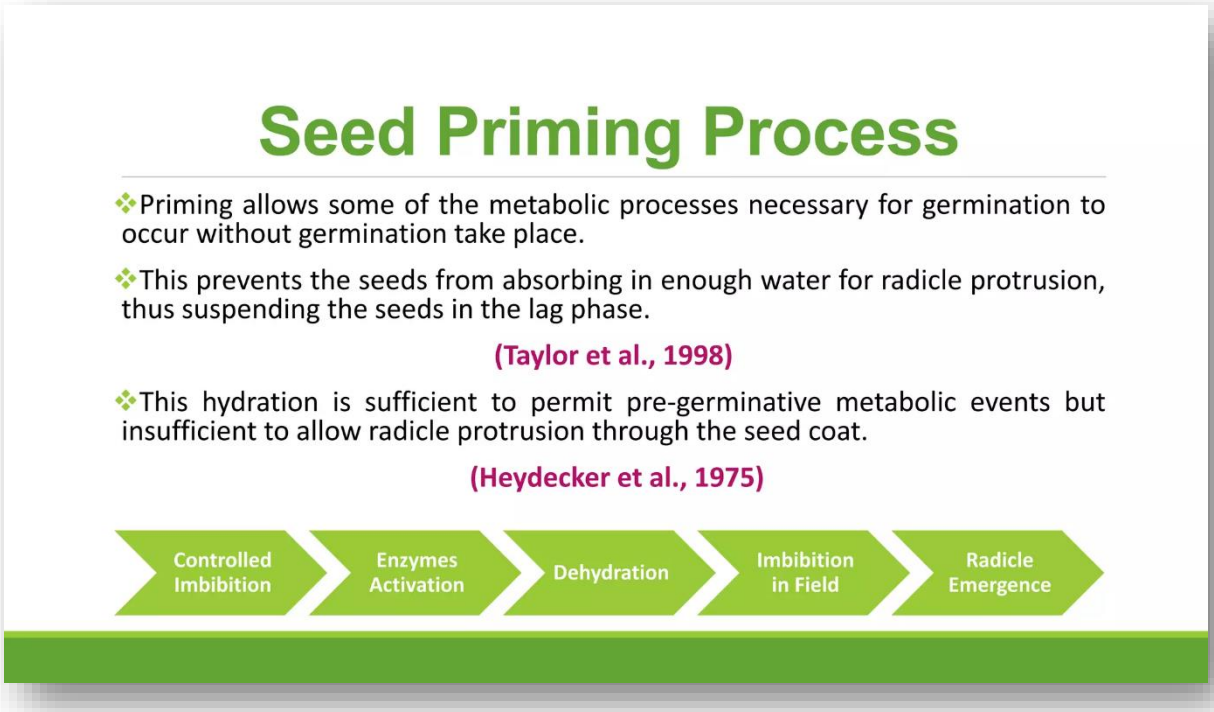
Seed Germination water requirement:

Mature seeds are often extremely dry and need to absorb, through a process of imbibition, a significant quantity of water, relative to the dry weight of the seed. Generally, the minimum water content required in the grain for germination is 35% to 45% by weight.

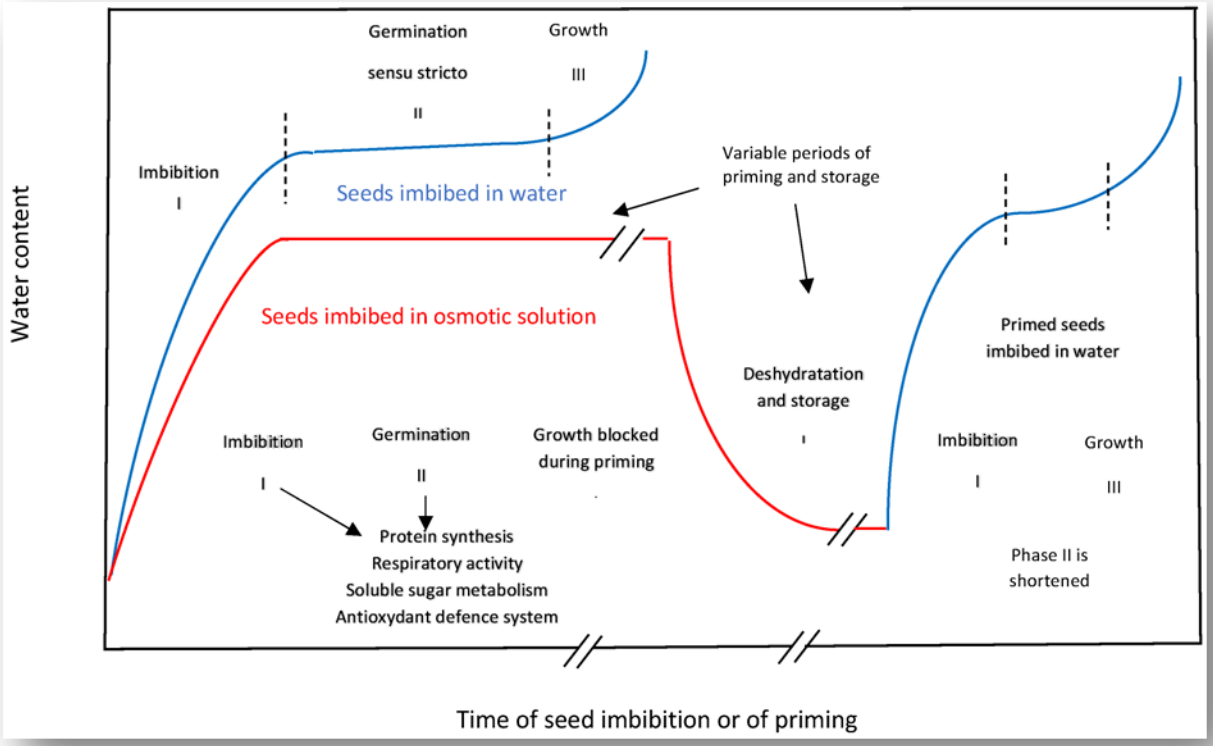
During Bio-Priming we typically add 10-20% water (roughly 50% of what is needed for full germination). This means that after planting a seed only required the other 50% water for the germination process to complete. As such, germination happens quicker.

Seed Priming:

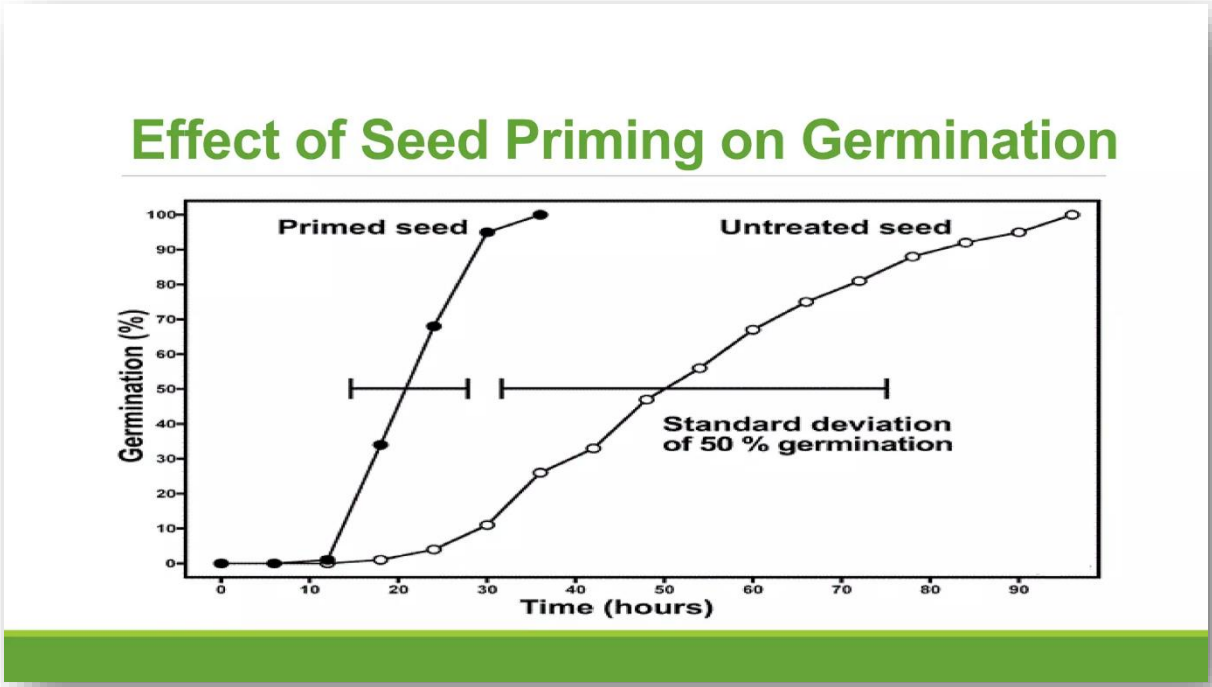
“Seed priming is a controlled hydration technique in which seeds are soaked in a water solution to a point where germination related metabolic activities begin in the seeds, but radical emergence does not occur.”



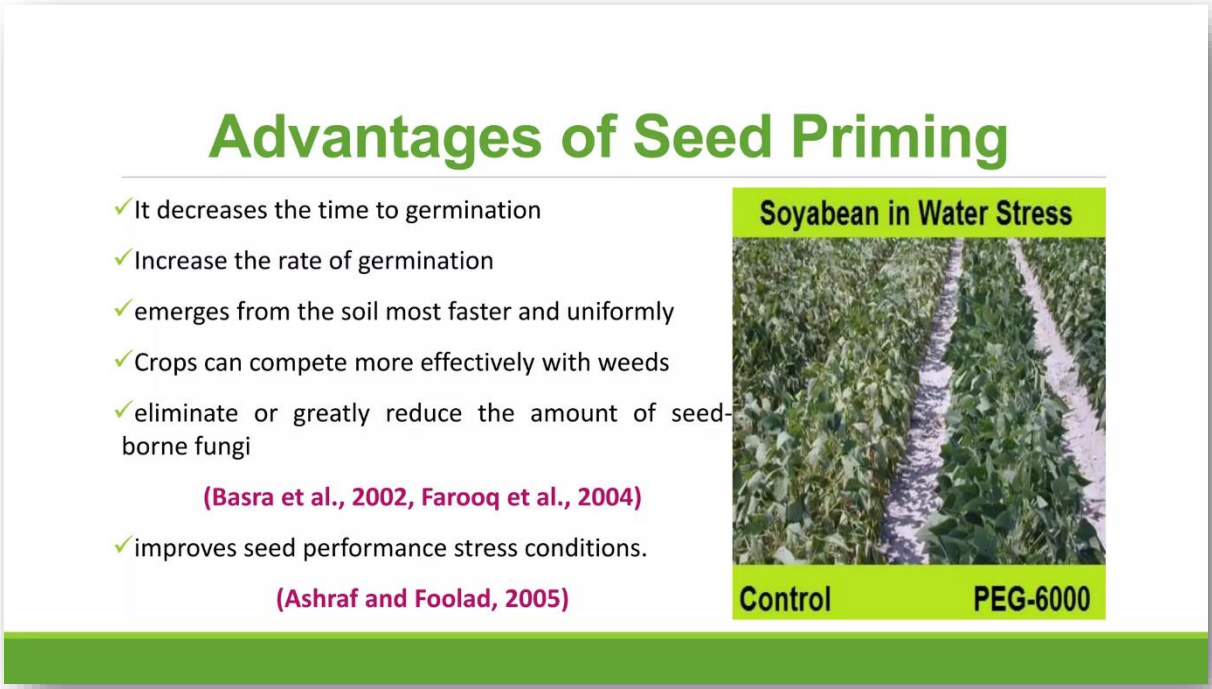
Definition of Seed Priming.



Seed priming process as related to seed water content. Priming consists of partial seed imbibition to a point where germination (phase II, germination sensu stricto) occurs but is not completed by radicle growth. The moisture content of the seeds is maintained during priming at 10–20% fresh weight basis, corresponding to about 50% of the moisture content that allow radicle growth. During phase I (imbibition), controlled water uptake allows protein synthesis and induces respiratory activity. Phase II is associated to various physiological, biochemical, and molecular activities such as protein synthesis, respiratory activity, metabolism of soluble sugars and repair processes, but the radicle emergence is prevented.



The benefit of general Seed Priming is that Germination is much quicker as only moderate moisture is required to fully complete the germination process. This means that the treated crop germinates before weeds and competition plants enabling a head start early season.



Advantage of Seed Priming -the ability to emerge first in the race for sunlight and nutrients.

Germination of Primed Seeds

IRREGULARITIES IN SEED GERMINATION
POOR QUALITY SEEDS

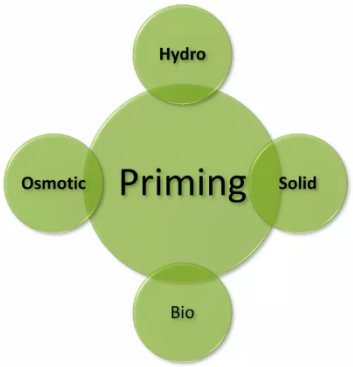
UNIFORMITY IN THE GERMINATION OF
PRIMED SEEDS

The seeds on the left did not all get enough moisture for full germination, unlike those on the right.

Seed Priming Methods

There are four common methods utilized for priming seeds:

1) hydro-priming
2) osmotic priming
3) solid matrix priming
4) Bio Priming



1) Hydro-priming

Hydro- priming also involves soaking in water and drying back to storage moisture prior to sowing of the seeds.

(Harris et al., 1991)

This decreases the time that the seed spends in the seedbed simply imbibing water.

(Halmer, 2006)

Seed can be primed to 95% of its germination requirement.

Effect of Hydro-priming on Wheat Seed

❖Hydro-priming of Wheat seed improves:

1) Vigor
2) Germination percentage
3) Seedlings Establishments
4) Uniform Growth
5) Water use efficiency
6) Grain yield

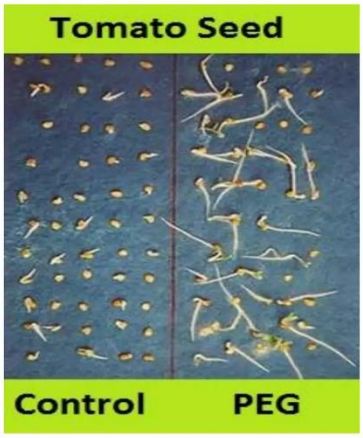
(Raj Pal Meena, Sendhil R, S. C. Tripathi, 2013)

2) Osmotic priming

Osmotic priming is the soaking of seeds in solutions containing chemicals such as :

1) mannitol
2) potassium nitrate (KNO_3)
3) potassium chloride (KCl)
4) polyethylene glycol (PEG)
5) sodium chloride (NaCl)

(Halmer, 2006)



Osmotic Priming typically speed up germination using chemical stimulation.

3) Solid matrix priming

Solid matrix priming involves the incubation of seeds in a solid, insoluble matrix, such as vermiculite, diatomaceous earth, or another highly water absorbent polymer, with a limited amount of water, allowing for slow imbibition.

(McDonald, 2000)

Solid Matrix Priming is typically to slow down germination so that only very large rainfall events trigger germination when there is a lot of soil moisture.

4) Bio-Priming

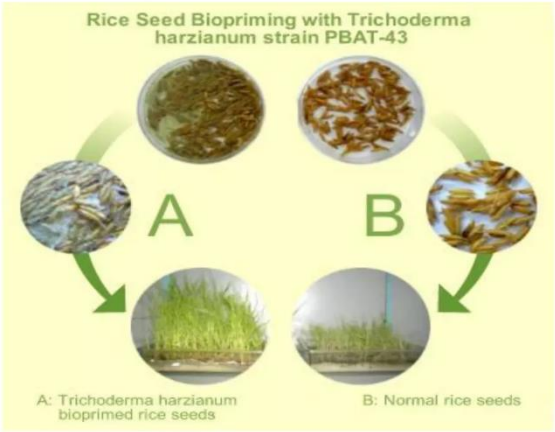
Bio-priming is a process of biological seed treatment that refers combination of seed hydration (physiological aspect of disease control) and inoculation (biological aspect of disease control) of seed with beneficial organism to protect seed

We use Bio-Priming to increase yield, quality, and stress handling capability by locating Microbes inside and on top of the seed.

Effect of Bio-priming on Rice Seed

It is an ecological approach using selected fungal antagonists against the soil and seed-borne pathogens.

(Halmer, 2006)



Bio-Priming is mostly used to prevent disease. It is an approach to positively dominate the seed with good microbes so that pathogens do not get any foothold, and to have the microbes acquire nutrients and feed and protect the plant.

What do we use Bio-priming for in South African Commercial Agriculture?

I. Chemical/Mineral Fertilizer and pesticide use is bad for soil microbial life.
II. We use Bio-Prime® to restore Soil Microbial Diversity and Soil Function -it is a form of regenerative agriculture restoring biodiversity unto every single seed in order that the plant is able to take up the maximum amount of nutrients available in the full soil profile.
III. It means that just as crop-rotation is good for soil microbes, as just as minimum-till and no-till is good for soil structure, so Bio-Priming is another tool to improve soil health and soil function thanks to a complete diverse microbial population of soil bacteria and fungi.

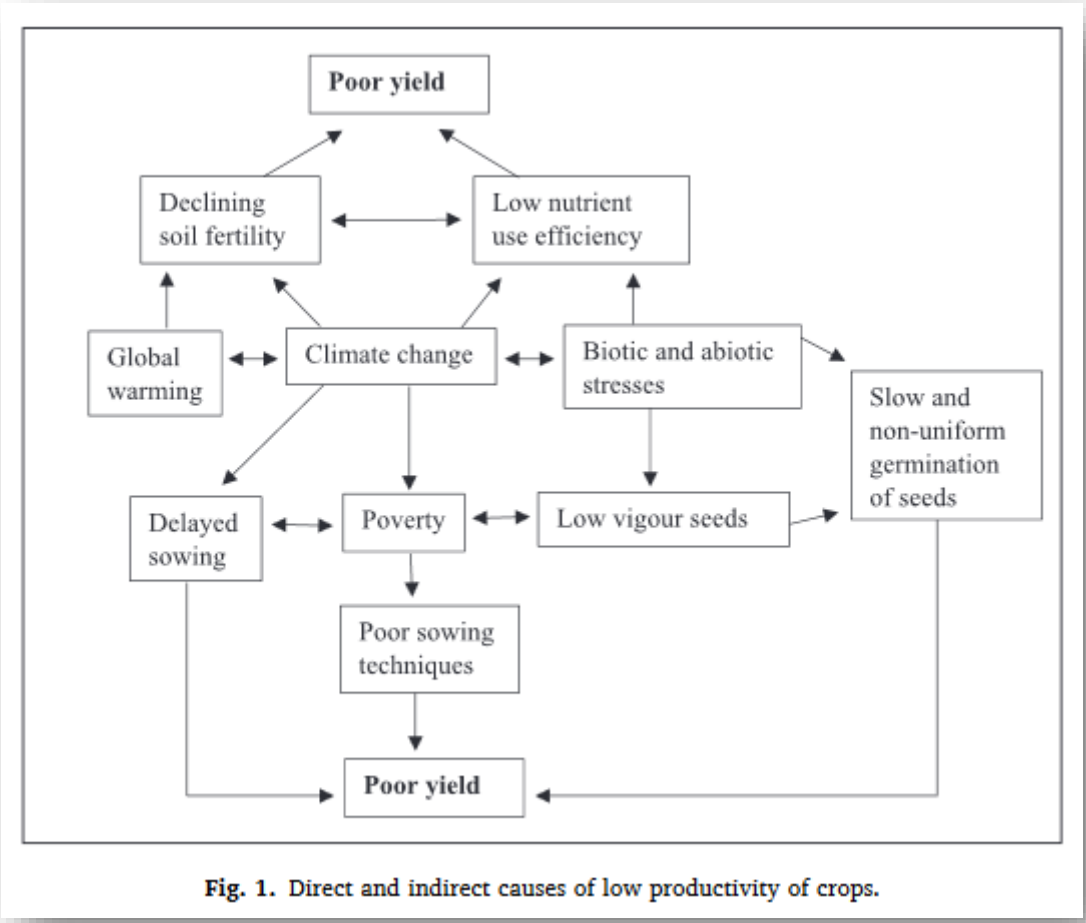


Fig. 1. Direct and indirect causes of low productivity of crops.

The greatest cause of low soil fertility in agricultural soils are because of long-term herbicide and chemical fertilizer use lead to a loss of soil microbial diversity and thus less soil function. This leads to low nutrient use efficiency -with plants unable to fully make use of all the mineral fertilizer given because of a lack of soil microbes able to make nutrients plant available.

Bio-Priming is a form of microbial inoculation of seed which restores soil microbial diversity, re-enables full soil function by putting the life back into the soil. If you have dead soil, you need to Bio-Prime® the life back into it.

What Happens when you Bio-Prime® any type of Seeds?

I. You increase the Microbial Biodiversity; restoring back the specific type of soil bacteria and fungi which pesticide and mineral fertilizer kills specifically. This enables biological trading and acquisition of plant available nutrients from all the other soil microbes, especially those microbes in the deeper layers where there is more leached moisture and nutrients.

II. You thus increase the nutrient use efficiency, because putting back the missing microbes required for trade re-enables the plant to acquire and fully take up more of the nutrients in the soil.

III. The added microbes restore soil function, typically by making existing nutrients available in a plant available form.

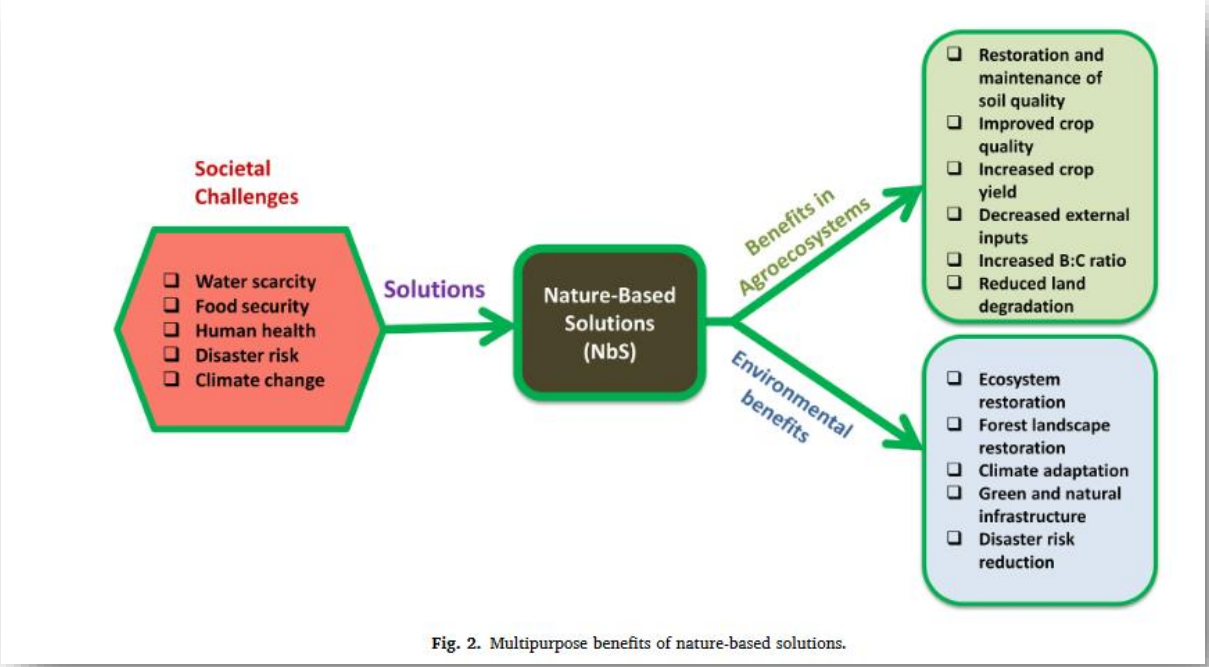


Fig. 2. Multipurpose benefits of nature-based solutions.

Bio-Priming is a Nature Based Solution Whereby In-soil earthworm microbes (bacteria and fungi) are used to inoculate seed. It is a form of "consortium priming" whereby hundreds of Billions of Microbes are used from all different earthworm classes in the full soil profile, to restore Soil Microbial Biodiversity for full on biological trade to a deep soil depth.

3.3. *Consortium priming*

Increasing reports regarding the use of multiple microorganisms in the inoculation process for harnessing the maximum benefits in plant productivity as compared to the single inoculation (Meena et al., 2010; Badawi et al., 2011; Mäder et al., 2011; Jain et al., 2012; Yadav et al., 2013; Masciarelli et al., 2014; Kumar et al., 2016; Sarkar and Rakshit, 2021; Sarkar et al., 2021b), depicts the need to readdress the issue. Inconsistencies of single inoculum might be due to higher resource competition with native microbes or survival problems in diverse ecological conditions (Singh, 2015; Rashid et al., 2016). Compatible microbes may produce a more synergistic effect on plant growth and development. Meena et al. (2010) documented that single inoculation of *Pseudomonas striata* and *Piriformospora indica* had a negative influence on the growth and yield of chickpea, while synergistic results were obtained with the combined use of two bio-agents. Seed bio-priming with PGPR consortium (*Azotobacter chroococcum* + *Azospirillum lipoferum*) significantly augmented several yield attributes of barley including dry matter accumulation, thousand-grain weight (test weight), harvest index, biological yield, and grain yield (Mirshekari et al., 2012). In a recent review, Rashid et al. (2016) concluded that the contribution of both bacteria and fungi (co-inoculation) with or without organics in restoring the soil fertility and/or organic matter status is greater than the application of single inoculum. There is a paradigm shift in food production systems using single microbe to microbial consortia because of a better exploration of the diverse PGP traits consolidated in consortium package.

Benefits of Consortium Bio-Priming.

https://www.researchgate.net/publication/355104447_Seed_bio-priming_with_microbial_inoculants_A_tailored_approach_towards_improved_crop_performance_nutritional_security_and_agricultural_sustainability_for_smallholder_farmers/link/616ff451750da711ac5a4518/download

Table 1
Role of seed priming in sustainable food production.

Microbe or microbes (concentration)	Treatment rate	Host crop	Mechanisms	Beneficial impacts	References
Single species priming					
Trichoderma harzianum (1×10^8 CFU g^{-1})	10 g kg^{-1} seeds	Maize	Rapid multiplication of antagonists (*T. harzianum*) in soil, root colonization, production of growth regulators	Enhanced seed germination, vigour index, field emergence, 1000 seed weight, and yield; reduced *Fusarium verticillioides* and fumonisin infection	Chandra Nayaka et al. (2010)
Trichoderma harzianum (2.1×10^7 spores g^{-1})	8 and 10 g kg^{-1} seeds	Sunflower	Nutrient mobilization and thereby increase the nutrient uptake of plants, root colonization, activation of induced systemic resistance	Growth promotion, reduction of disease incidence (downy mildew caused by *Plasmopara halstedii*) under greenhouse and field conditions	Nagaraju et al. (2012)
Azosprilium sp. (1×10^7 CFU mL^{-1})	7 g kg^{-1} seeds	Barley	Production of plant growth regulators, root elongation	Increased plant height, spike length, number of spikes per unit area, number of grains per spike, 1000-grain weight, and grain yield	Shirinzadeh et al. (2013)
Trichoderma harzianum (1×10^8 spores mL^{-1})		Wheat		Increased leaf area, ear length, ear weight, test weight, and grain yield; reduced chemical fertilization	Meena et al. (2017)
Pseudomonas fluorescence (1×10^8 CFU mL^{-1}), *Trichoderma harzianum* (1×10^7 CFU mL^{-1})		Cumin	Increase in antioxidant enzyme activities (APX, CAT) in seeds, soluble protein in leaf, root length	Improved seed germination, seedling establishment, shoot length under drought stress	Piri et al. (2019)
Bacillus amyloliquefaciens (1×10^7 CFU mL^{-1})		Rice	Induction of systemic resistance, increase in antioxidant enzyme activities (PO, PPO)	Reduced disease severity of blast caused by *Magnaporthe oryzae*, increased grain yield under field conditions	Amruta et al. (2019)
Trichoderma asperellum (1×10^7 CFU mL^{-1}), *Ochrobactrum* sp. (1×10^8 CFU mL^{-1})		Tomato	Increased accumulation of total phenol and antioxidant enzyme activities such as PAL, PO, PPO, and Chi in plants; induction of defense genes such as *PAL*, *Chi*, *LOX*, and *PR*	Improved seed germination, reduction of disease incidence (Fusarium Wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*)	Singh et al. (2020)
Enterobacter spp. (1×10^8 CFU mL^{-1})		Okra	Root colonization, P and K solubilization, secretion of organic acids such as gluconic acid, malic acid, and citric acid	Increased leaf surface area, SPAD chlorophyll index, plant P and K uptakes, soil fertility	Roslan et al. (2020)
Trichoderma viride (2×10^5 CFU mL^{-1})	10 g kg^{-1} seeds	Soybean	Root elongation, soil P solubilization by acid phosphatase activity	Increased P acquisition in plants, reduced application of P-fertilizer dose	Paul and Rakshit (2021)
Pseudomonas fluorescens, *Pseudomonas gessardii*, *Bacillus subtilis*, *Bacillus mojavensis* (1×10^8 CFU mL^{-1})	5 mL g^{-1} seeds	Pepper	Synthesis of phytohormones	Increased seed germination and seedling emergence	Yildirim et al. (2021)
Bacillus cereus, *B. amyloliquefaciens*, *Bacillus megaterium*		Spinach	Reduction in metal uptake, biosorption, biotransformation, complexation, increased SOD activity in plants	Growth promotion (root and shoot length, fresh and dry root and shoot weight) under the stress of heavy metals like As, Cr, Ni, and Pb	Renu et al. (2021)
Consortium priming					
Staphylococcus epidermidis +		Rice and	Induction of amylase activity in plants,	Increased seed germination, plant	Duarah et al.

Commercial benefits of Bio-priming with different Plant Growth Promoting Rhizobacteria over different crops. You can use Bio-Prime® with any crop and any type of seed.

Negative Impact of active herbicide ingredients on Soil Microbial Diversity and Function:

Glyphosate:

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7602795/
- https://academic.oup.com/jambio/article/134/2/lxad006/6987274

Glufosinate:

- https://pubmed.ncbi.nlm.nih.gov/17647211/
- https://swr.agriculturejournals.cz/pdfs/swr/2015/04/10.pdf

Sulfonylurea:

- https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2017.00378/full
- https://www.sciencedirect.com/science/article/pii/S2667064X21000518

Research Background and further downloads:

Table 1. Different herbicides with their reported effects on soil microorganisms and biochemical reactions.

Herbicides	Effects on Microorganism and Associated Process	References
2,4-D	Adversely affects the activities of *Rhizobium* sp.	[122]
2,4-D	Reduces nitrogenase, phosphatase, and hydrogen photoproduction activities of purple non-sulfur bacteria.	[123]
2,4-D and 2,4,5-T	Adversely affects node-expression disrupting plant *Rhizobium* signaling. 2,4-D also reduces fixation by blue-green algae and nitrifying process impacting *Nitrosomonas* and *Nitrobacter* sp.	[124]
2,4-D, Agroxone, and Atranex	Inhibits activities of *Rhizobium phaseoli* and *Azotobacter vinelandii* (most sensitive).	[122]
2,4-D, Bromoxynil, and Methomyl	Reduces CH_4 oxidation to CO_2.	[125]
Bensulfuron methyl and Metsulfuron-methyl	Decreases N-mineralization.	[126]
Bentazone, Prometryn, Simazine, and Terbutryn	Inhibits N-fixation and decreases the number of nodules and N content overall.	[127]
Isoproturon, Triclopyr	Adversely impacts *Nitrosomonas*, *Nitrobacter*, urea hydrolyzing bacteria, nitrate reductase activity, and growth of actinomycetes and fungi.	[128]
Glyphosate	Glyphosate produces a non-specific, short-term stimulation of bacteria at a high concentration.	[137]
Isoproturon	Affects the proliferation of *Sphingomonas* spp.	[138]
Butachlor	Negatively affects the general bacterial communities; the diversities ranged from 28% to 52%.	[139]
Diuron or Linuron	Removal of dominant acidobacterium.	[135]
Glyphosate	Increased relative abundance of β-Proteobacteria (*Burkholderia*).	[140]
Napropramide	Initial decrease in bacterial and fungal abundance followed by an increase in abundance of Gram-negative bacteria and fungi.	[141]
Pretilachlor	Decreased activity of phosphatase, urease, and dehydrogenase	[111]
Mesotrione	No response of the soil microbial communities in soil spread with field rate applications. Soil microbial activity stimulated by 100× FRA of pure Mesotrione.	[142]
Isoproturon	Treatment-induced changes in community composition	[109]
Imazetapir	Decreases nitrogenase activity in *Rhizobium leguminosarum*. *R. trifolii, Bradyrhizobium* sp., and *Sinorhizobium meliloti*.	[143]

Table 2. Different fungicides with their reported effects on soil microorganisms and biochemical reactions.

Fungicides	Effects on Microorganism and Associated Process	References
Fenpropimorph	Fenpropimorph inhabited the growth of active fungi and calculable bacteria.	[144]
Iprodione	Affects the soil bacterial communities.	[145]
Apron, Arrest, and Captan	Reduces viable counts of *Rhizobium cicero*.	[146]
Benomyl	Impacts mycorrhizal associations and nitrifying bacteria.	[147]
Benomyl, Mancozeb	Arrests activity of dehydrogenase, urease, and phosphatase.	[148]
Captan	Inhibits aerobic N-fixing, nitrifying, denitrifying bacteria, nitrogenase activity, phosphate solubilization, and other fungi.	[149]
Captan and Thiram	Decreases cell growth and nitrogenase activity in *Azospirillum brasilense*.	[150]
Captan and Carbendazim	Decreases the activity of nitrogenase enz	123]
Captan, Carboxin, Thiram	Inhibits the activity of bacteria responsible	151]
Carbendazin and Thiram	Inhibits nodulation in legumes and thus N-	143]
Chlorothalonil	Affects bacteria associated with nitrogen cycling.	[147]
Chlorothalonil, Azoxystrobin	Affects biocontrol agent(s) used against Fusarium wilt.	[152]
Copper fungicides	Decreases population of bacteria, cellulolytic fungi, and *Streptomycetes*.	[153]
Dimethomorph	Inhibits nitrification and ammonification process.	[154]
Dinocap	Inhibits the activity of ammonifying bacteria.	[155]
Dithianon	Destroys bacterial diversity.	[156]
Fenpropimorph	Slows down bacterial activity.	[151]
Fludioxonil	Toxic to algal activities.	[157]
Funaben, Baytan, Oxafun	Inhibits nitrogenase activity of methylotrophic bacteria.	[158]
Hexaconazole	Impacts bacteria involved in N cycling.	[159]
Mancozeb	Impacts on bacteria involved in the N & C cycle.	[155]
Mancozeb, Chlorothalonil, Metal dithiocarbamates	Reduces nitrification process.	[160]
Metalaxyl	Reduces urease activity continuously while phosphatase activity seems stimulated but then reduces.	[161]
Metalaxyl	Disturbs activity of ammonifying and nitrifying bacteria.	[162]
Oxytetracycline	Acts as bactericide.	[163]

Chen, S.K.; Edwards, C.A.; Subler, S. Effects of the fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations. *Soil Biol. Biochem.* **2001**, *33*, 1971–1980. [**Google Scholar**] [**CrossRef**]

Mancozeb	Impacts on bacteria involved in the N & C cycle.	[155]
Mancozeb, Chlorothalonil, Metal dithiocarbamates	Reduces nitrification process.	[160]
Metalaxyl	Reduces urease activity continuously while phosphatase activity seems stimulated but then reduces.	[161]
Metalaxyl	Disturbs activity of ammonifying and nitrifying bacteria.	[162]
Oxytetracycline	Acts as bactericide.	[163]
Pencycuron	Short-term impact on metabolically active soil bacteria.	[164]
Propiconazole	Retards PGP effects of *Azospirillum brasilense* on its host plant.	[165]
Triadimefon	Deleterious to long-term soil bacterial community.	[166]
Triarimol and Captan	Reduces frequency of *Aspergillus* sp.	[167]
Azoxystrobin, Chlorothalonil, Tebuconazole	None of the fungicides affected bacterial community structure. Chlorothalonil negatively affect the ciliate protozoan *Arcuospathidium* sp., or *Bresslaua vorax*. Azoxystrobin affect the Flagellate protozoan *Paraflabellula hoguae*, while ascomycete fungus *Cladosporium tenuissimum* was affected by tebuconazole.	[162]
Cobber	Bioavailable Cu positively correlated with relative abundances of phylums *Acidobacteria* and negatively correlated with the phylums Proteobacteria and Bacteroidetes.	[168]
Cobber	Decrease in abundance of acidobacteria and increase of Firmicutes. *Bacillus* community highly resistant to high cobber concentrations.	[169]
Mancozeb	Enhanced activity of alkaline phosphatase, protease, amidase. Decreased activity of urease and asparaginase	[170]
Propiconazole	Decreased activity of phosphatase, urease, and dehydrogenase.	[111]
Chlorothalonil	More transient and weaker negative effects on soil micro-organisms.	[171]
Thiram	Diversity decrease at 200 mg kg^{-1}.	[172]
Tebuconazole, Metalaxyl	Perturbation of bacterial community structure compared to control.	[173]
Carbendazim, Thiram	Decreases nitrogenase activity in *Rhizobium leguminosarum. R. trifolii, Bradyrhizobium* sp., and *Sinorhizobium meliloti*.	[143]
Metalaxyl and Mefenoxam	Decreases nitrogen-fixing bacteria and microbial biomass.	[174]

Table 3. Different insecticides with their reported effects on soil microorganisms and biochemical reactions.

Insecticides	Effects on Microorganism and Associated Process	References
Cypermethrin	Increase in Gram-negative bacteria and decrease in firmicutes.	[175]
Amitraz, Aztec, Cyfluthrin, Imidachlorpid, and Tebupirimphos	Reduces activities of urease and phosphatase enzymes.	[176]
Arsenic, DDT, and Lindane	Decreases microbial biomass and microbial and enzymatic activities.	[177]
Bensulfuron methyl and Metsulfuron-methyl	Reduces soil microbial biomass.	[178]

Insecticides	Effects on Microorganism and Associated Process	References
Carbamate	Inhibits several soil microorganisms, enzymes, and nitrogenase activity of *Azospirillum*.	[130,179]
Carbofuran, Ethion	Inhibits nitrogenase activity of *Anabaena doliolum*.	[180]
Chlorinated hydrocarbons	Inhibits methanogenesis.	[181]
Chlorpyrifos, Dichlorvos, Phorate, Monocrotophos, Methyl parathion, Cypermethrin, Fenvalerate, Methomyl and Quinalphos	Increases phosphatase activity initially and later reduces gradually. Phorate reduces the total bacterial population and N-fixing bacteria.	[182]
Chlorpyrifos, Profenofos, Pyrethrins, and Methylpyrimifos	Reduces the population of aerobic N-fixing, nitrifying and denitrifying bacteria, and several fungi. Profenofos and Pyrethrins decrease the activity of urease enzyme and nitrate reductase.	[183]
Chlorpyrifos, Quinalphos	Reduces the ammonification process.	[182]
Cyfluthrin, Fenpropimorph, and Imidacloprid	Decreases the nitrification and denitrification process. Stimulates sulfur oxidation.	[176]
Diazinon and Imidacloprid	Inhibits a urease-producing bacterium (*Proteus vulgaris*).	[184]
Lindane, Malathion, Diazinon, and Imidacloprid	Lindane inhibit state of nitrification, N-availability, P-solubilization, and activity of phosphomonoesterase enzyme, while the opposite effect is observed in the case of Diazinon and Imidacloprid.	[177]
Methamidophos	Reduces microbial biomass by 41–83%.	[185]
Neemix-4E	Reduces urease enzyme activity.	[186]
Organophosphate insecticide	Impacts the activity of soil enzymes, several beneficial soil bacteria, and fungal population and reduces N-mineralization rate.	[179]
Pentachlorophenol	Reduces nitrification.	[187]
Quinalphos	Reduces activity of phosphomonoesterase.	[188]
Diflubenzuron	Diflubenzuron (100–500 µg/g) stimulates dinitrogen-fixing bacteria (*Azotobacter vinelandii*).	[189]
Methylpyrimifos, Chlorpyrifos	Methylpyrimifos (100–300 µg/g) or chlorpyrifos (10–300 µg/g) significantly decreased aerobic dinitrogen-fixing bacteria. Fungal populations and denitrifying bacteria were not affected.	[190]

Methamidophos	High concentrations of methamidophos (250 mg/kg) stimulate fungal populations. DGGE fingerprinting patterns showed a significant difference between the responses of culturable and total fungi communities under the stress of methamidophos.	[192]
Methamidophos	Methamidophos at 0.031 g/pot/week and 0.31 g/pot/week significantly decreases microbial biomass by 41–83% compared with the control.	[185]
Methylparathion	Induced the community of γ-porteobacteria (*Pseudomonas stutzeri* and *Pseudomonas putida*).	[193]
Carbaryl, Carbofuran	Carbary (10 µg/g) had almost no effect on nitrogenise; however, carbofuran (2 µg/g) reduced the population of *Azospirillum* and anerobic nitrogen fixers. Carbofuran (4 µg/g) stimulated the population of *Azospirillum* and other anaerobic nitrogen fixers.	[133]
Profenofos	Decreased activity of phosphatase, urease, and dehydrogenase	[189]
	Higher activities at lower dosage, greater toxic effects at higher dosage.	[194]

Table 4. Different soil fumigants with their reported effects on soil microorganisms and biochemical reactions.

Soil Fumigants	Effects on Microorganism and Associated Process	References
Metam sodium	Dose-dependent shift in community structure (after 5 weeks).	[195]
Methyl Bromide	Increased abundance of Gram-positive bacteria.	[196]
Methyl isothiocyanate	Increased abundance of Gram-positive bacteria.	[196]
Metam sodium	Inhibitory effect on Gram-negative bacteria and fungi in both field and laboratory studies.	[197]
1,3-dichloropropene	Initial inhibition of dehydrogenase activity (at 500 mg kg^{-1}). Bacterial community diversity decreased with higher concentration.	[126]

https://www.mdpi.com/1660-4601/19/5/3141

Long term use of Pesticides, Herbicides, Fungicides, and mineral fertilizers thus leads to a loss of soil microbial diversity without the farmer even being aware. Bio-Prime® restores the missing biology back into the soil and plant Rhizosphere.

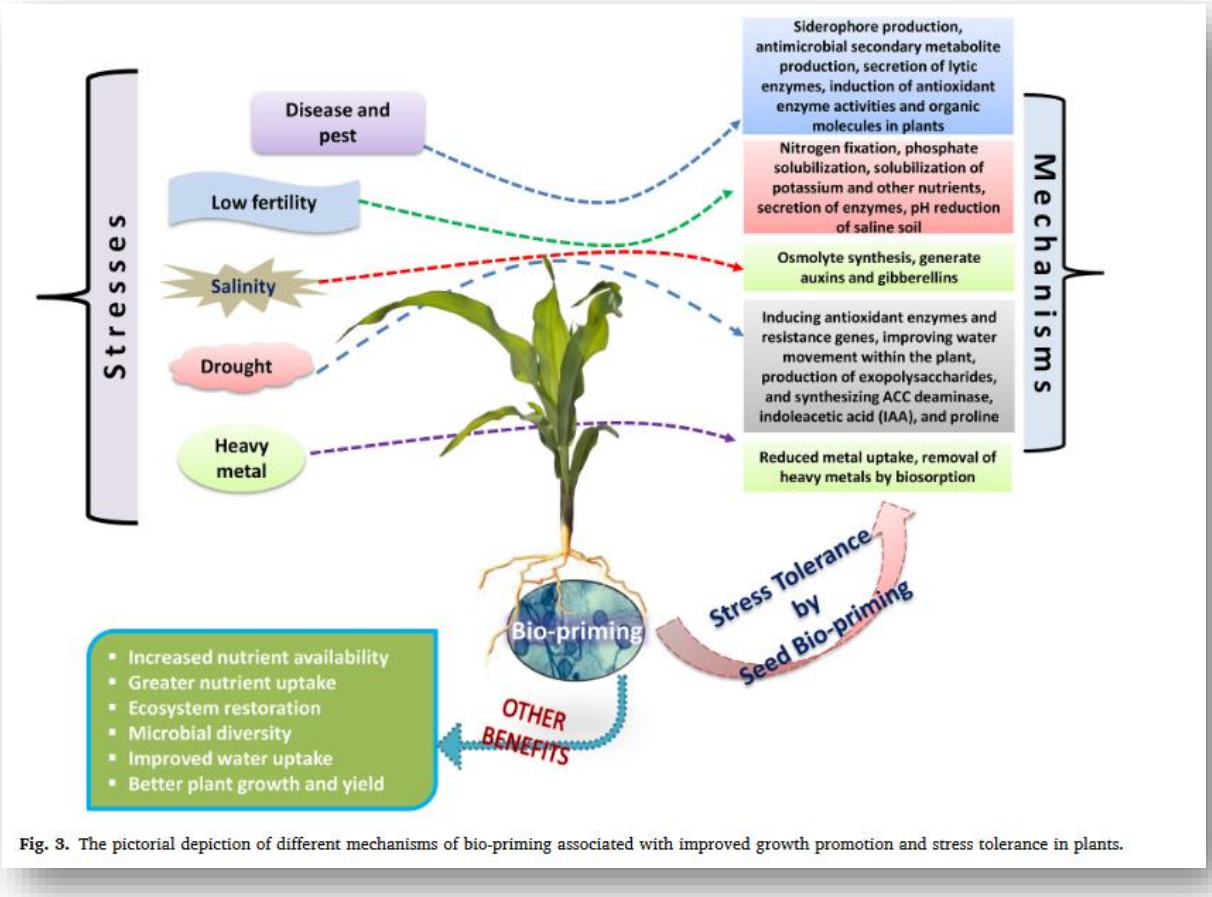
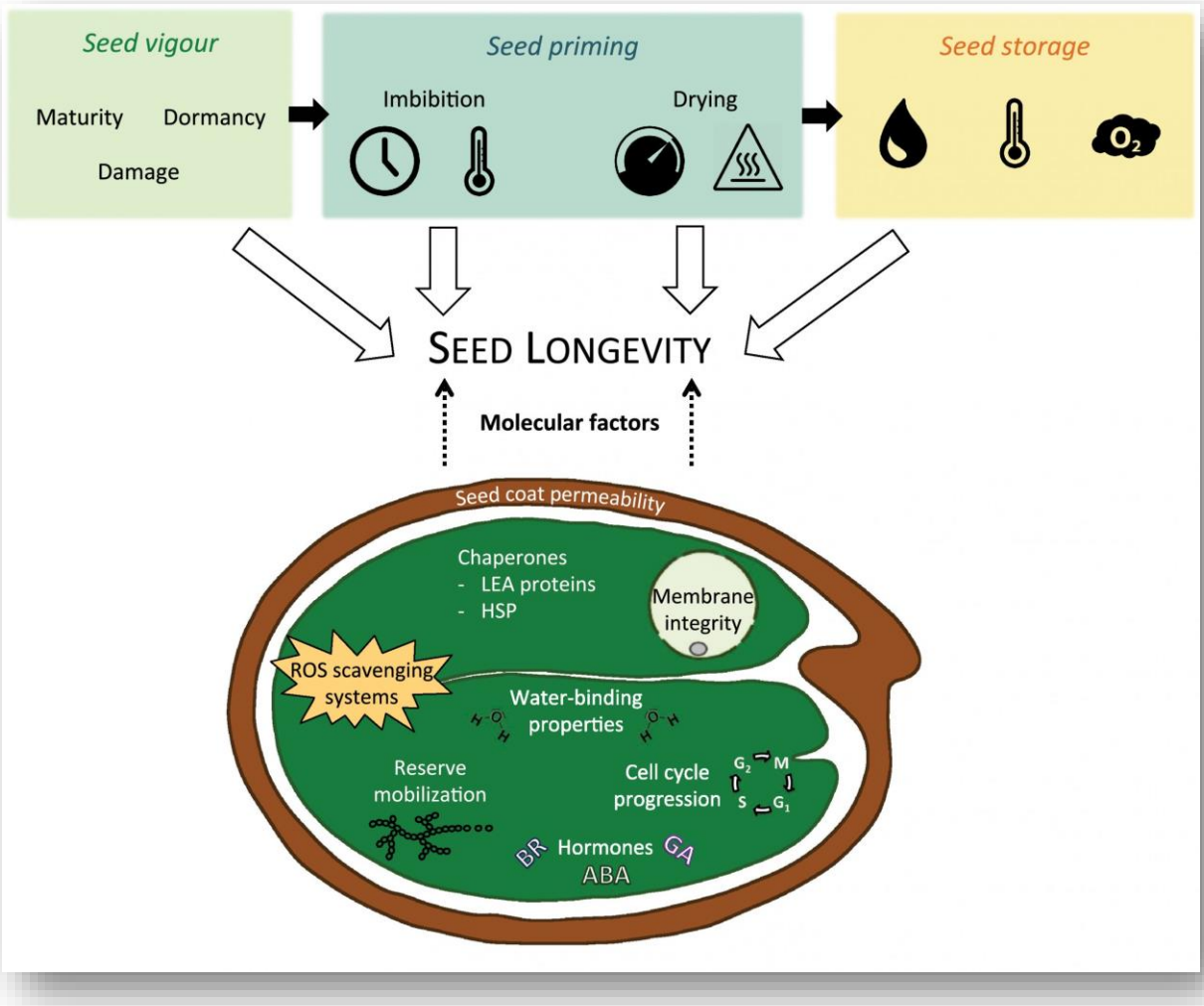


Fig. 3. The pictorial depiction of different mechanisms of bio-priming associated with improved growth promotion and stress tolerance in plants.

With restored soil microbial diversity, the plant can handle stress of all types again. This relates to 20-40% increased plant hardiness.



The reason existing seed companies do not do Seed priming is because it shortens the seed longevity and amount of time which seeds can be kept in storage, from years to months or weeks. With our Do-it-Yourself Bio-Prime® Vermicoat® process this is not an issue because you Prime, Coat and Plant within days if not weeks. The shorter the period between priming and planting the higher the microbial population and the bigger the beneficial effect.

What we do is to place the exact right Seed Priming and Coating material into the farmers hands for on farm priming, just prior to planting. This restores soil biology, and triggers yield gains.

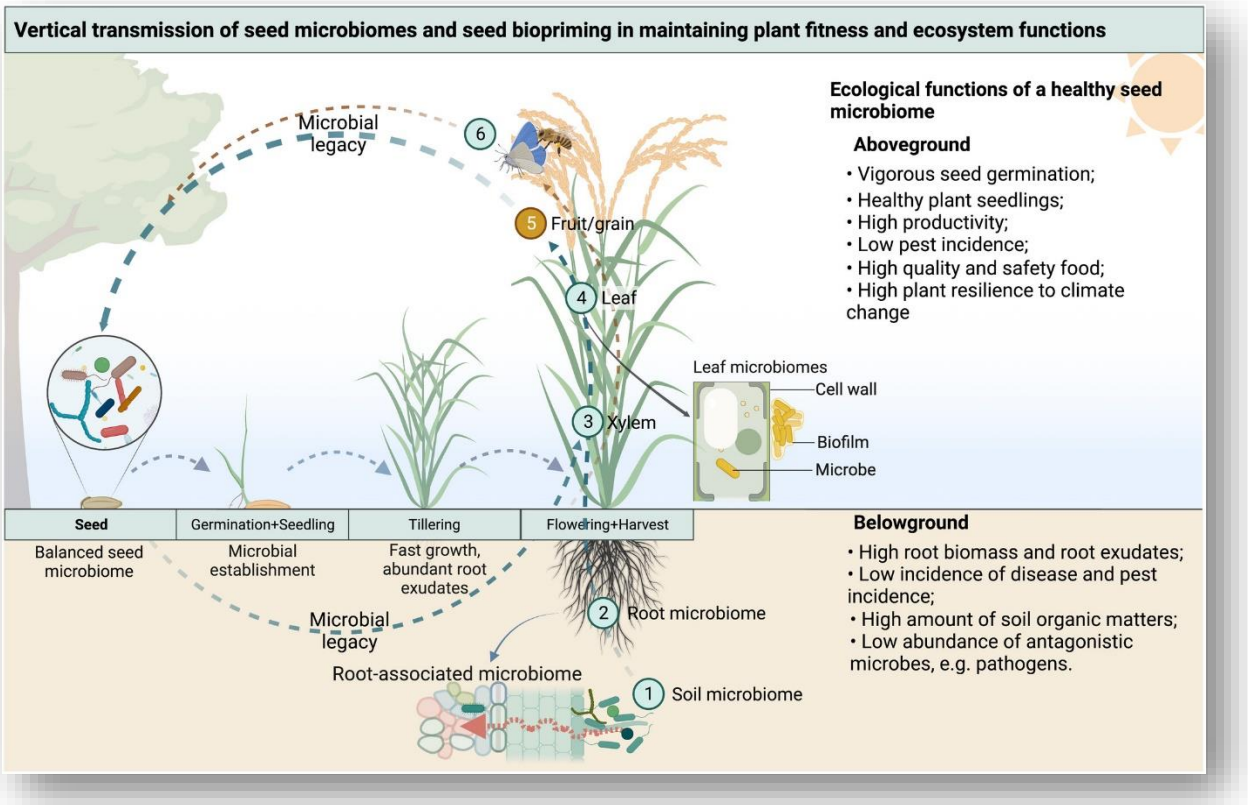
Section 2:

Vertical transmission of Seed Microbes

Did you know that in nature, a mother plant places 2 billion microbes of its root microbiome in the seed for the future generation- so that upon germination the new plant can enjoy the full microbial benefit that the mother plant built up in terms of soil microbial diversity and soil function. Kindly see https://enviromicro-journals.onlinelibrary.wiley.com/doi/full/10.1111/1751-7915.14322

What happens in nature in terms of vertical seed microbiome transmission:

- *Earthworms visit the roots of plants to feast on healthy soil microbes.*
- *Soil Microbial diversity increases with each visit.*
- *The mother plants take up the Soil Microbes with its root system and deposit them in a newly formed seed endosperm -with about 2 billion microbes per seed.*
- *The seed drops and resides in the soil seed bank and germinates much later, with a complete consortia of diverse soil microbes.*
- *Upon germination the number of soil microbes in the seed increase by 1000% immediately.*

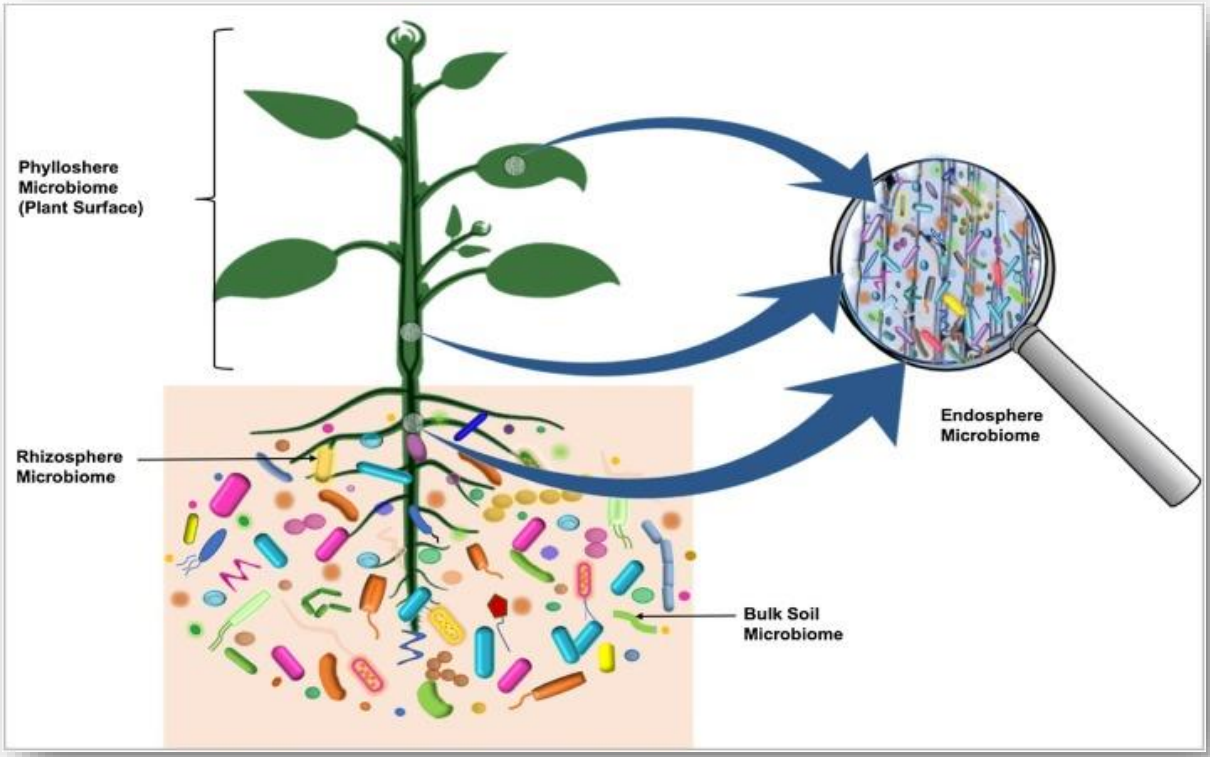


Transmission of seed microbiomes across generations: implications for ecosystem function and legacy effects. Seed microbiomes serve as the origin of plant microbiomes, encompassing both endophytic and surface-attached microorganisms. These microbes maintain intimate associations with plants and their ecological functions, which are becoming increasingly

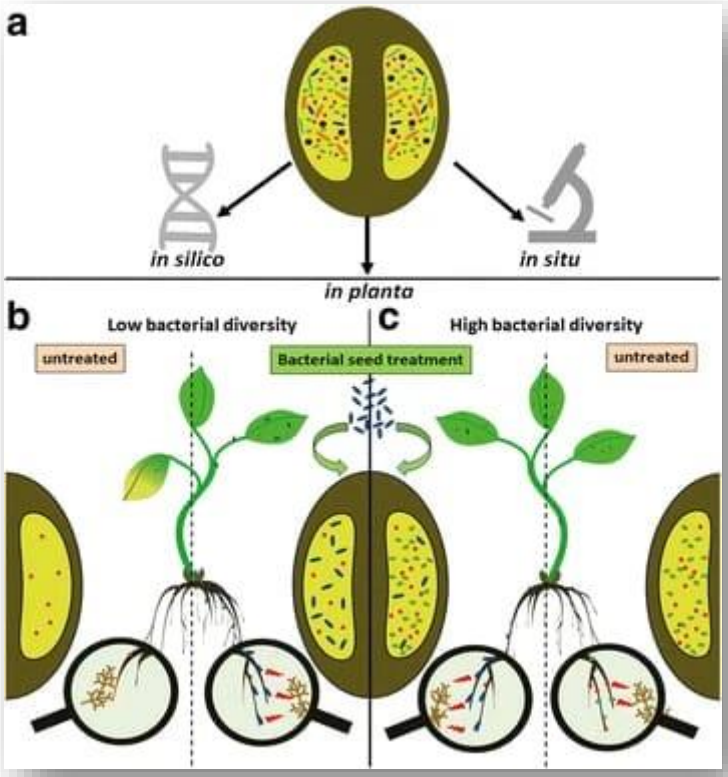
evident in terms of plant and ecosystem health. They contribute to seed health, seed germination and overall plant vitality. For instance, seeds contaminated by fungal infections due to fungal spores and high humidity of the environment can result in infection-induced reduction in germination rates. Seed microbiomes not only contribute to the plant microbiome in the roots and phyllosphere but also that in the flowers and fruits, along with significant contribution from soil microbiomes, as demonstrated in processes (1) through (5) in the figure. Crucially, certain plant and soil microbial components are retained and transmitted to seeds, which are then carried over to subsequent plant life cycles. A healthy seed microbiome is also vital for maintaining the functionality of both above- and below-ground ecosystems. This includes ***promoting plant biomass production, improving food quality*** *and safety,* ***preserving high soil organic matter content****, and* ***regulating the abundance of soil pathogens****.*

Plant Microbiome:

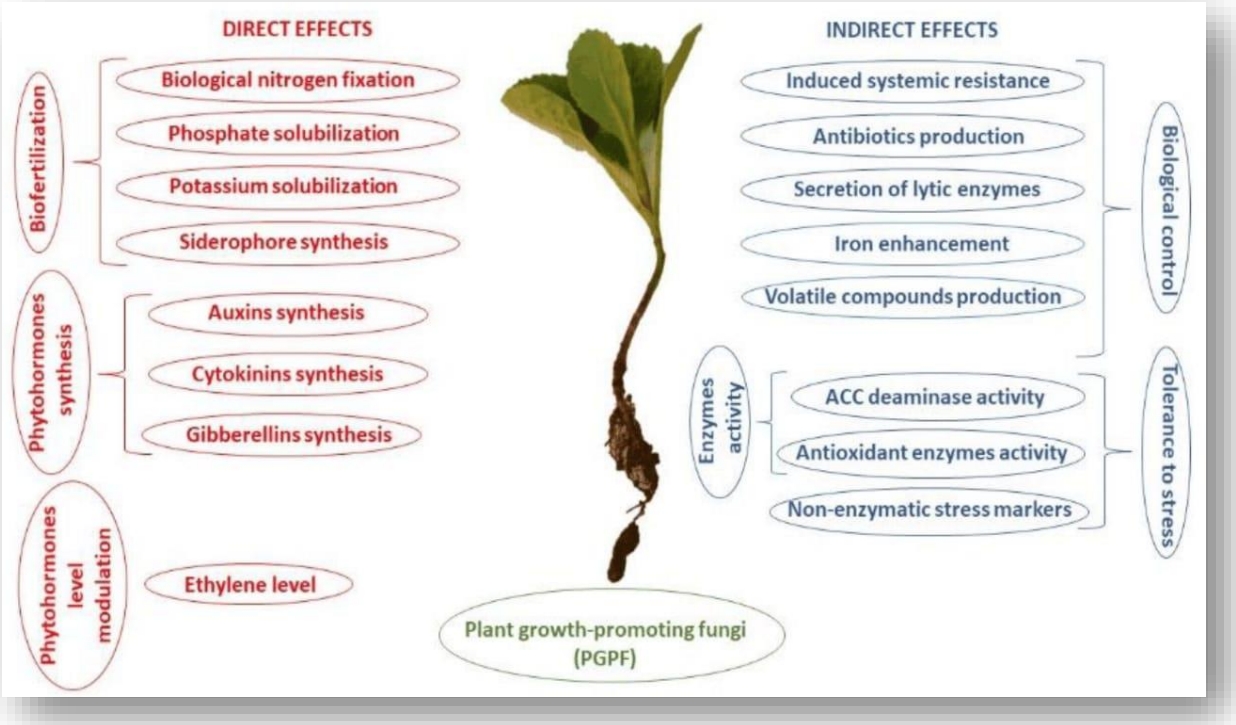
The plant microbiome is ***an assembly of microorganisms that live together in and near a plant and interact to form a microbial ecosystem****. Microbiomes of all sorts interact closely with the living and non-living parts of their environment.*



Diverse microbial communities of characteristic microbiota are part of plant microbiomes and are found on the outside surfaces and in the internal tissues of the host plant, as well as in the surrounding soil.



After Biologically Priming a Seed, the number of beneficial and Plant Growth Promoting Microbes (both Bacterial and Fungal) increases, assisting the plant in various functions.

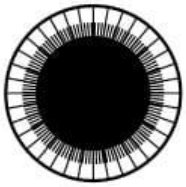


Plant Growth Promoting Fungi and Plant Growth Promoting Rhizobacteria in the Microbiome aid plant growth and soil function. Biopriming is the process of enhancing the seed microbiome with a wider diversity of earthworm derived plant beneficial plant growth promoting microbes to increase plant performance.

Bio-Priming:

Bio-Priming is a new technique of seed treatment that integrates biological (inoculation of seed with beneficial organism to protect seed) and physiological aspects for disease control and yield increase. https://enviromicro-journals.onlinelibrary.wiley.com/doi/full/10.1111/1751-7915.14322 . It simulates the visit of earthworms to the mother plant and vertical transmission of beneficial soil microbiome organisms to the new generation.

Different types of Seed Coating:

	Bioprimed	*Film coated*	*Slurry coated*	*Pelleted*
	 *Inoculant within seed*	 *Inoculant in thin layer on seed surface*	 *Inoculant in (peat) carrier stuck to outside of seed*	 *Inoculant applied to seed along with conventional seed additives*
Method	Seed soaked in saline / inoculant suspension	Inoculant suspended (e.g. sugar, methyl cellulose) and dried	Inoculant grown in solid carrier medium applied to seed using sticker Often dusted with lime to ensure flowability	Typical commercial process
Utility	Experimental, limited commercial use	Mainly for experimental use only	Widely used for rhizobial inoculants prior to sowing	Not yet but desired by seed companies and growers
Inoculant survival	Good long term survival	Short term survival	Variable	Poor survival unless resistant (spore-former) inoculants used

What we do is the first two, with Bio-Prime® and Vermicoat®.

Section 3:

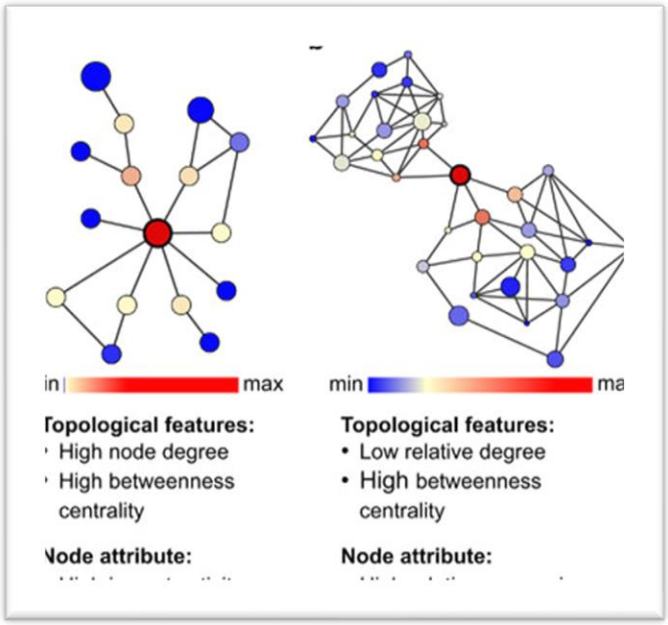
Specific Case Studies:

Canola Microbiome Perspective:

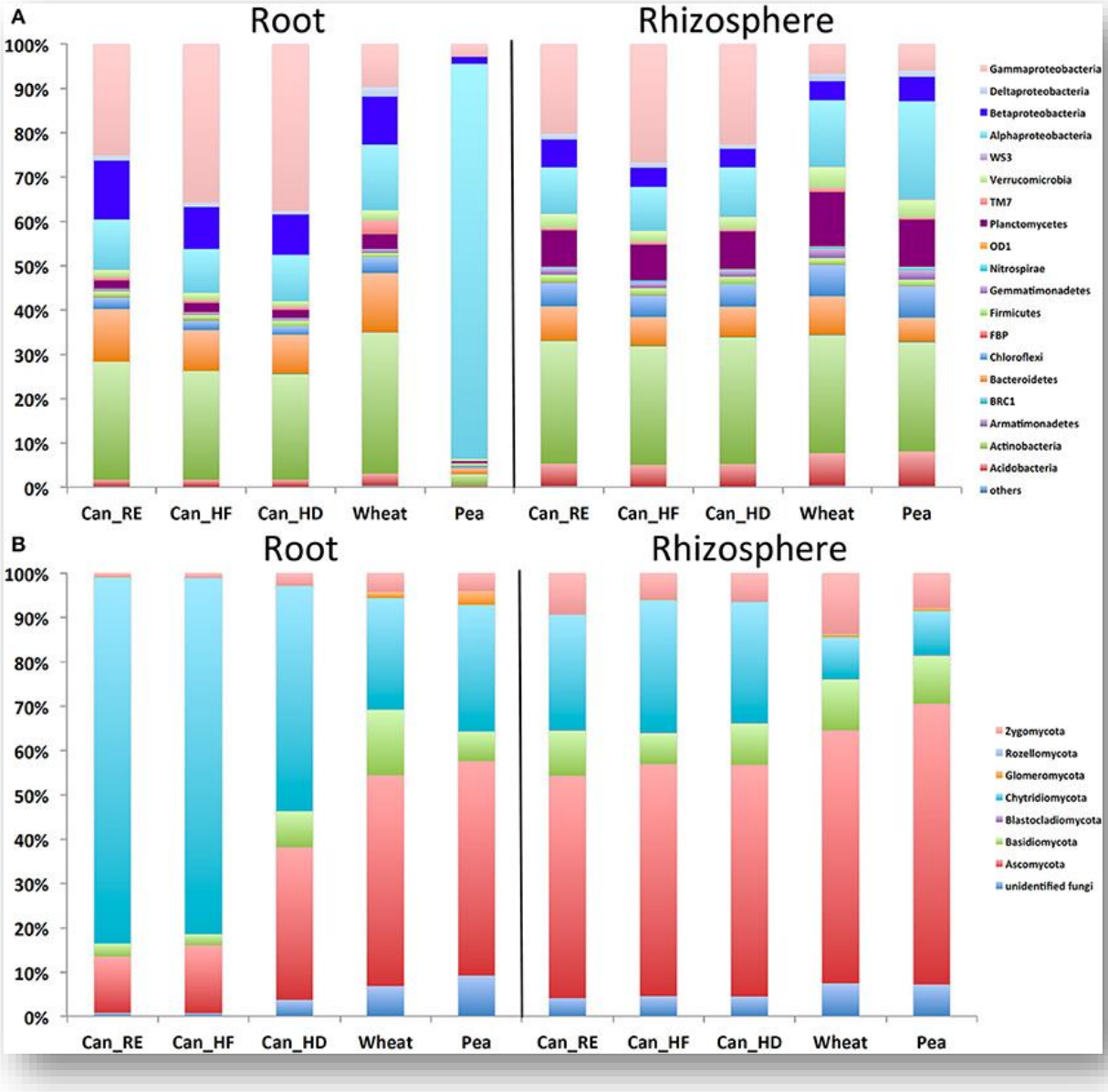
1. Canola plants have a very specific microbiome and we Bio-Prime® these specific microbes into and unto the seed.
2. The Canola plant discriminates between different soil microbes and select specific fungi and microbes as Keystone species which act as gateways during plant-soil microbe interactions.

What are the keystone species?

A keystone species is an organism that helps define an entire ecosystem. Without its keystone species, the ecosystem would be dramatically different or cease to exist altogether. Keystone species have low functional redundancy-if they are gone everything collapses.

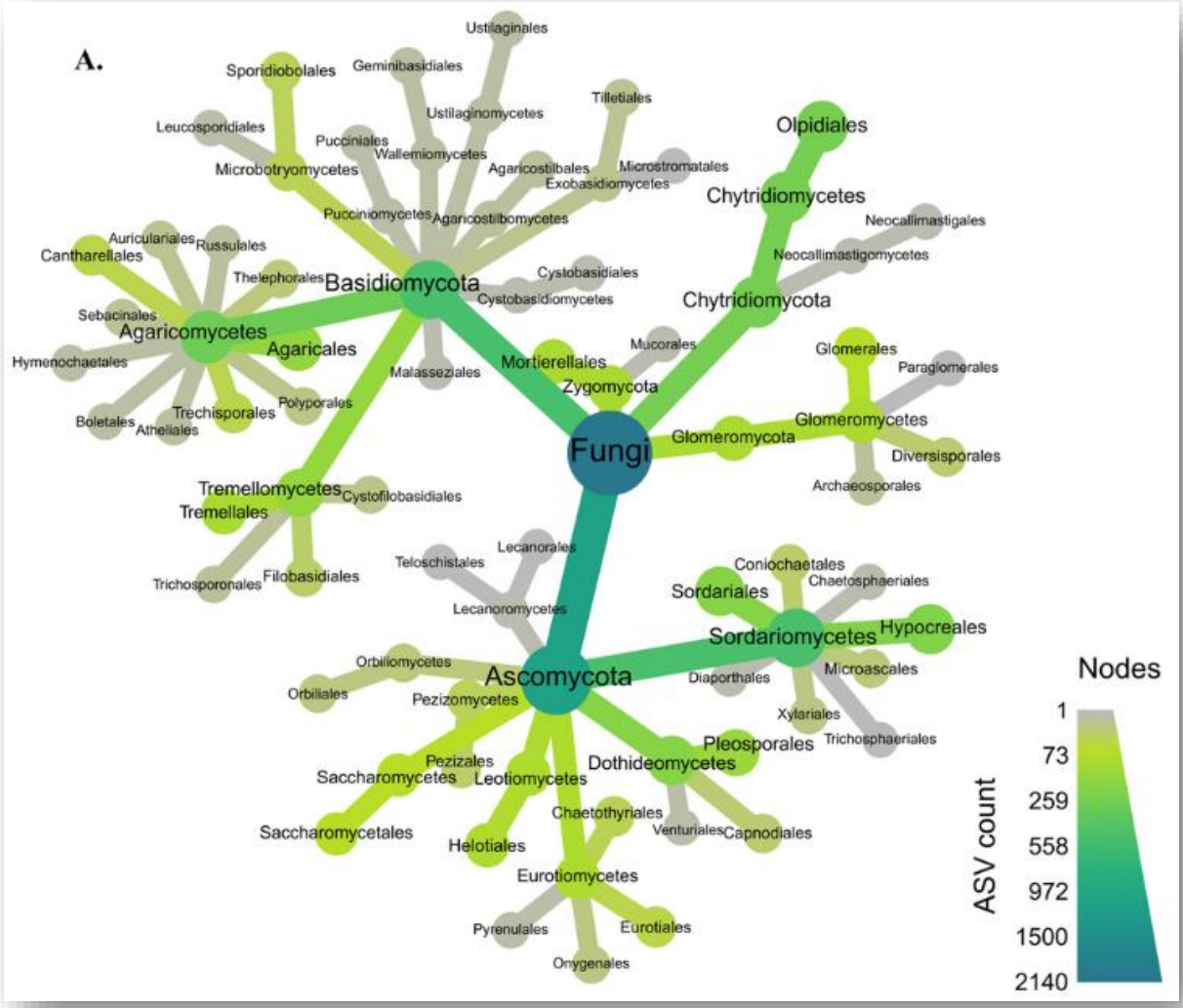


The two microbes in red would typically qualify as Keystone species as they act as functional gateways linking clusters of microbial bacteria and fungae with each other, enhancing soil function and nutrient uptake. Without they key gateway species the nutritional growth benefits of entire microbial cluster activity would not be plant available. In Canola the plant has selected certain keystone species as the gateway through which it takes up nutrients. If you do not have these vital keystone species in abundance around a seed, it retards nutrient uptake.



Mean (n = 12) relative abundances of (A) bacterial taxa based on the 16S ribosomal RNA (rRNA) gene fragments, and (B) fungal taxa based on the internal transcribed spacer (ITS) sequences in the roots and rhizospheres of canola, wheat, and pea. For canola, three different treatments were applied: canola grown as recommended (Can_RE), canola fertilized at 150% of the recommended rate (Can_HF), and canola seeded at 150% of the recommended rate (Can_HD).

https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2018.01188/full



This is what the Canola fungal Microbiome looks like.

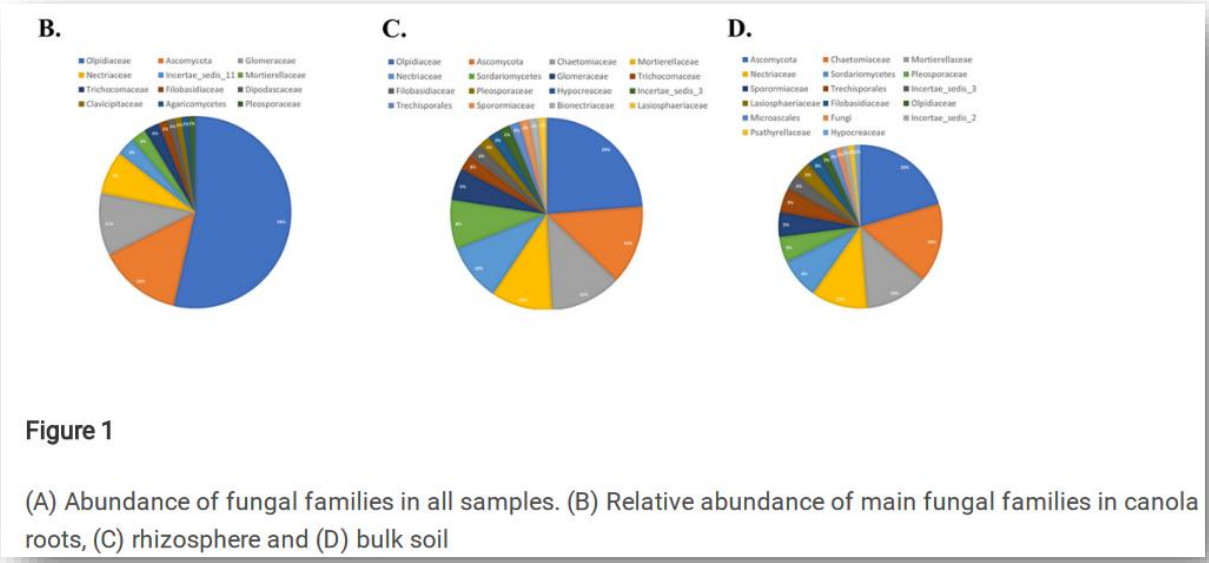
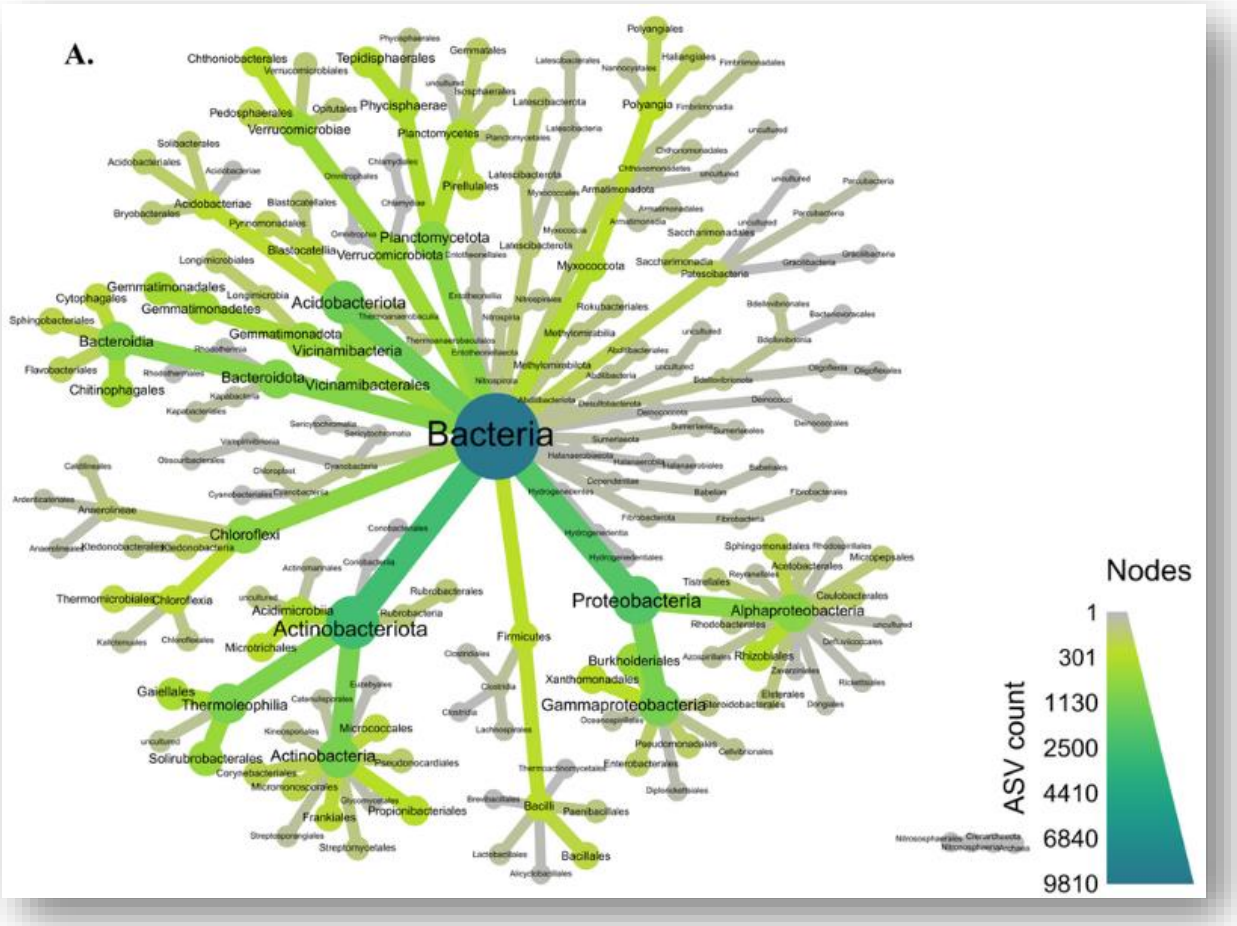


Figure 1

(A) Abundance of fungal families in all samples. (B) Relative abundance of main fungal families in canola roots, (C) rhizosphere and (D) bulk soil

Fungal diversity in Canola Microbiome. These are the Fungae you must have for optimum nutrient uptake. It is these same fungae which herbicide, pesticide and fungicide destroy.

https://www.researchgate.net/publication/353303077_Inter-Kingdom_Networks_of_Canola_Microbiome_Reveal_Bradyrhizobium_as_Keystone_Species_and_Underline_the_Importance_of_Bulk_Soil_in_Microbial_Studies_to_Enhance_Canola_Production/link/60f22e7516f9f313008bcf26/download



Canola Bacterial Microbiome required for optimum nutrient uptake. If you do not have the entire keystone species cluster with the sub-clusters then Canola yield will suffer,

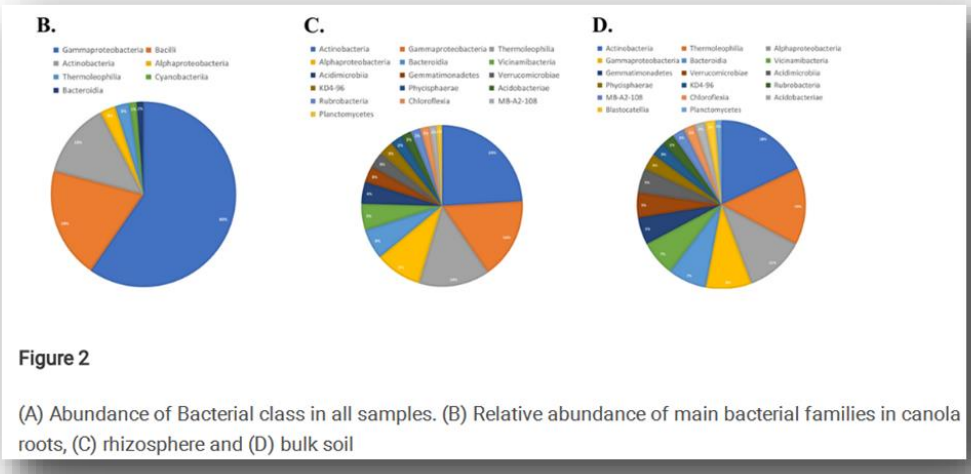


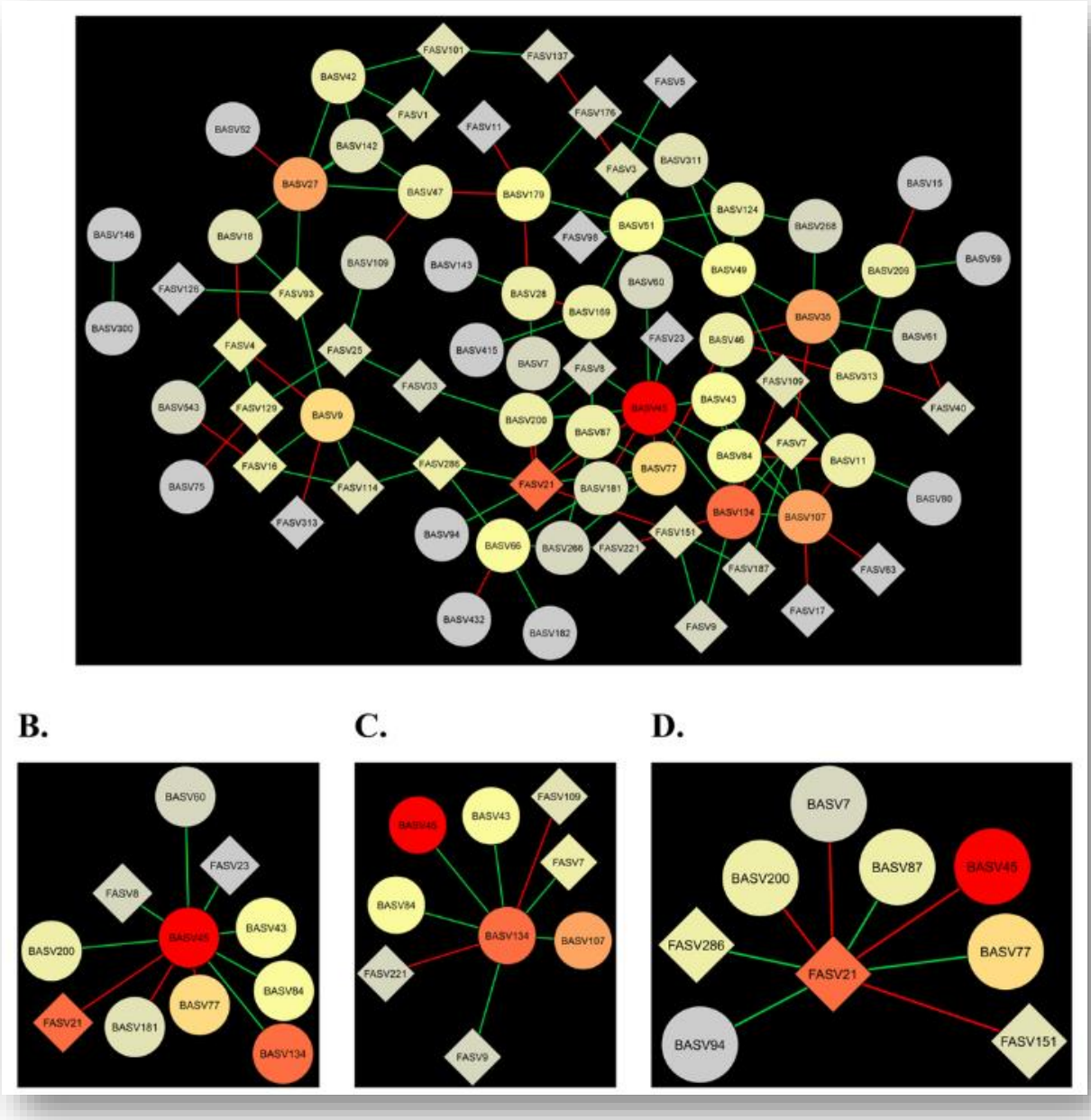
Figure 2

(A) Abundance of Bacterial class in all samples. (B) Relative abundance of main bacterial families in canola roots, (C) rhizosphere and (D) bulk soil

Canola Microbiome bacteria at different depths. You need them all at the correct depth.

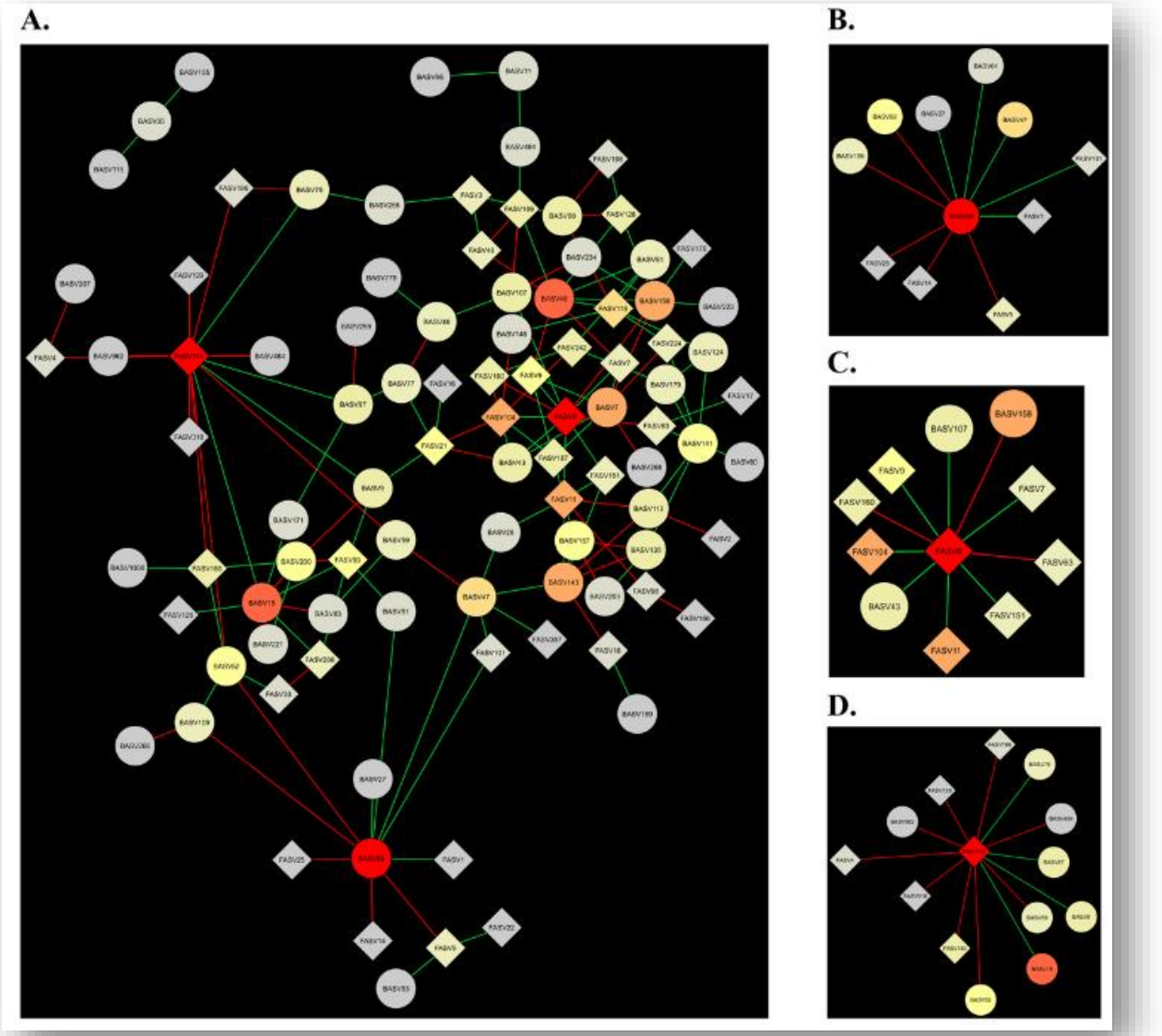
What does Bio-Prime® and Vermicoat® do to these Canola Fungal and Microbial Clusters?

1. It ensures you have the keystone species (diversity) on all seeds in enough quantity.
2. The keystone species act as your nutrient gateways.
3. It builds out these clusters around the nutrient gateway keystone species so that more microbes feed more plant available nutrients to your plants root system.

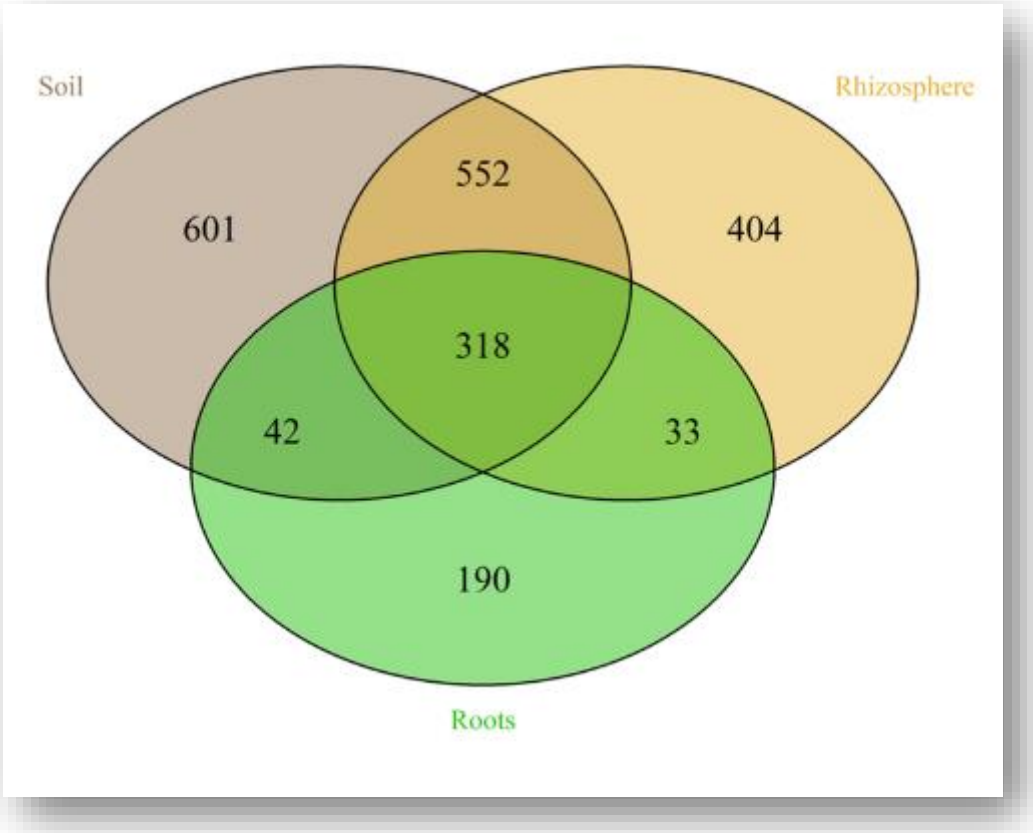


(A) Network of inter-kingdom interactions between the bacteria and fungi forming the microbiome of canola rhizosphere. Node shades indicate the degree of connectivity: ASVs with warm colours are more connected with the other members of the network than the cold colored ones. Green edges indicate positive relationships and red edges, negative relationships. (B)

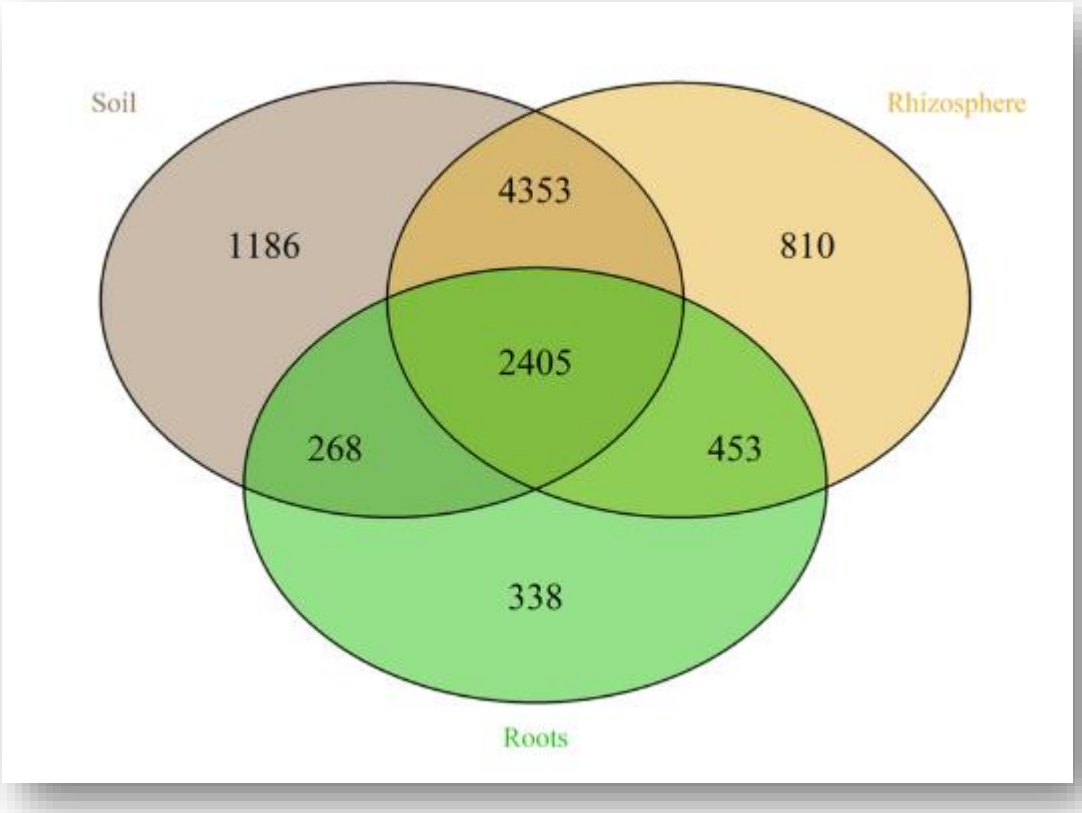
Sub-network centered around BASV45. (C) Sub-network centered around BASV134. (D) Sub-network centered around FASV21.



(A) Network of inter-kingdom interactions between the bacteria and fungi forming the microbiome of bulk soil in canola field. Node shades indicate the degree of connectivity: ASVs with warm colors are more connected with the other members of the network than the cold colored ones. Green edges indicate positive relationships and red edges, negative relationships. (B) Sub-network centered around BASV69. (C) Sub-network centered around FASV8. (D) Sub-network centered around FASV114.



Venn diagram of the ASV of the fungal community shared between root, rhizosphere, and bulk soil, taking all sites in account.



Venn diagram of the ASV of the bacterial community shared between root, rhizosphere and bulk soil, taking all sites in account.

What else does Bio-Prime® and Vermicoat® Do to the Plant Microbiome?:

I. We use Earthworm microbes from the soil surface, Rhizosphere soil; and bulk soil to build out the soil microbial diversity in each seeds microbiome.
II. A single Canola seed in nature has about 2 Billion Microbes, and after germination this increases by up to a 1000%. As the plant roots expand and grow so the microbial population expands with it.
III. We make sure that we have enough Keystone species diversity and population density to ensure that there are enough nutrient pathways to the root system of the Canola so that maximum nutrient uptake efficiency take place.

Amount of Nutrients required for Canola for 1960 kg per ha:

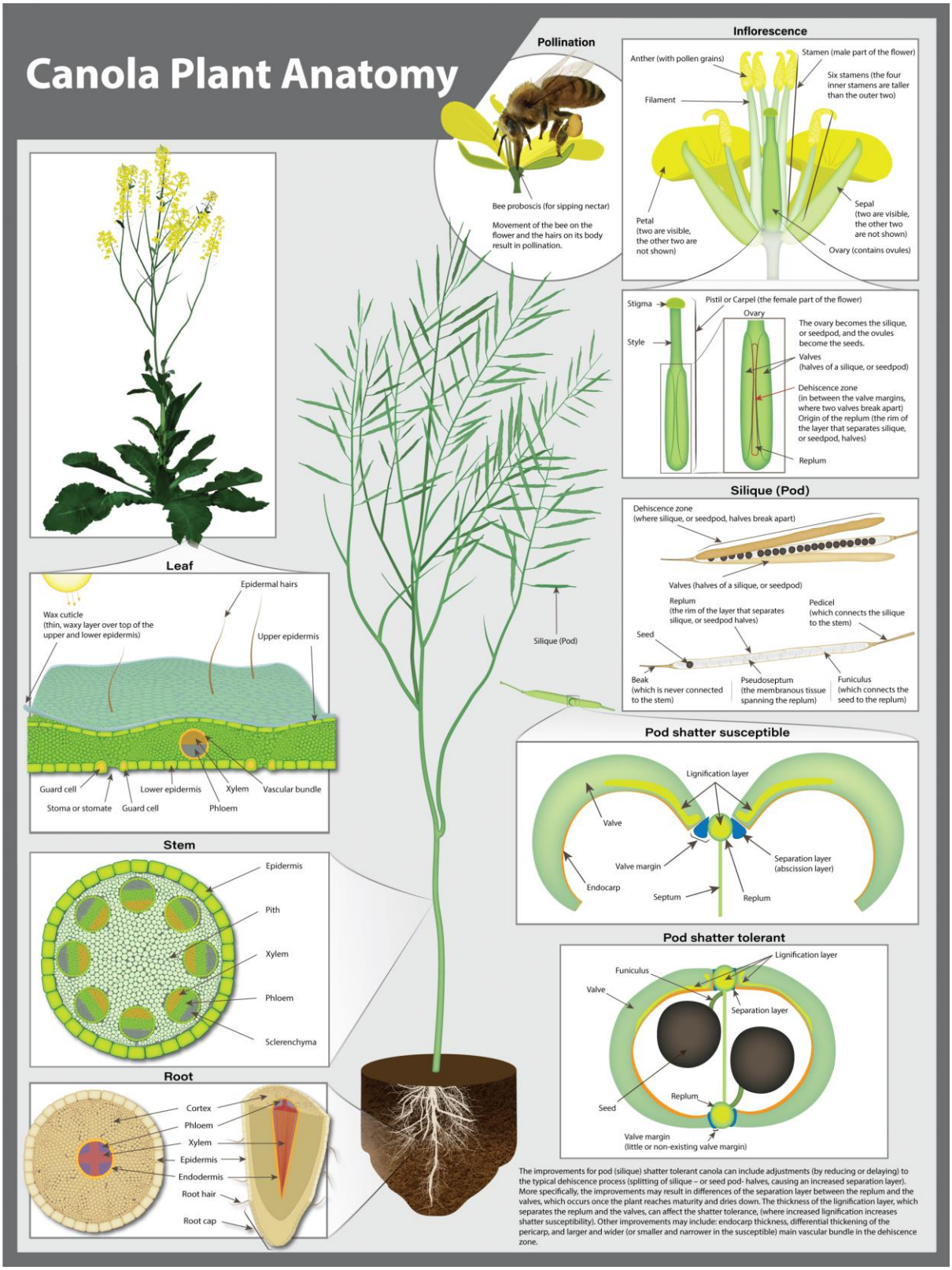
https://www.canolacouncil.org/canola-encyclopedia/fertility/basic-plant-nutrition/

Table 1. Approximate Amounts of Nutrients in the Above- Ground Portion of a 1,960 kg/ha (35 bu/ac) Canola Crop

Print Excel CSV Copy

Element	kg/ha	lb/ac
Nitrogen (N)	112-134	100-120
Phosphorus (P)	17-28	15-25*
Potassium (K)	67-134	60-120**
Sulphur (S)	22-28	20-25
Calcium (Ca)	45-67	40-60
Magnesium (Mg)	13-20	12-18
Iron (Fe)	~1	~1
Chlorine (Cl)	~0.8	~0.7
Manganese (Mn)	~0.2	~0.2
Zinc (Zn)	~0.2	~0.2
Boron (B)	~0.2	~0.2
Copper (Cu)	~0.7	~0.6
Nickel (Ni)	~0.004	~0.004
Molybdenum (Mo)	~0.004	~0.004

If you have all the keystone gateway microbes with full microbial activity it is possible to have most efficient nutrient uptake. Plants require a 2:1:1 ratio of nutrients, water and oxygen otherwise there is a rate limitation suppresing yield. Our Bio-Prime® and Vermicoa®t microbes help make these available in the correct ratio so that rate limitations do not take place. If the ratio is not correct then optimum plant growth does not take place -instead it happens at the rate of the lowest limiting factor. A small rate adjustment by a keystone cluster can make all the difference between low growth and higher growth. A key issue is that the soil microbes also make water and oxygen molecules available to the plant as and when required -neither of which are available from the mineral fertilizer industry nor from the pesticide companies.In a growth pinch, oxygen and moisture molecules will take you further than fertilizer or herbicide.



The 500kg to 1000 kg yield gains possible with Bio-Prime® and Vermicoat® flows from the following value chain enhancement:

I. *Keystone microbial species acting as gateways to root nutrient uptake with extensive cluster consortia of Microbes and Fungi.*

II. *Root system up to 700 times larger due to expanded fungal and microbial networks.*
III. *Biological Trade Network exchanging nutrients between plants.*
IV. *Biological Nitrogen fixation from Free Living Nitrogen Microbes and Fungi.*
V. *Full soil functionality down the entire soil profile.*

Why do Commercial Agriculture have such low yields?

- Very high Herbicide and Pesticide use lead to a decrease in soil microbial diversity, with the size of microbial clusters declining and delinking and the loss of entire keystone taxa.
- Very high chemical fertilizer uses also lead to declining soil microbial diversity -lowering the population density of vital microbes throttling the available nutrient gateways.

Frequently asked questions:

1. Can Bio-Prime® be used on any seed? *Yes.*
2. Can you do it yourself? *Yes.*
3. What type of water should be used? *Borehole or rainwater.*
4. What does Bio-prime® cost? *Typically, R1000 per ha for everything, Bio-Prime and Vermicoat® included. The one does the inside of the seed and the other the outside. You need get and use both.*
5. How long does it take to Bio-Prime® and Vermicoat® about 25 kg of seed? *Typically, 15 to 20 minutes the first time, and when proficient, as short as 5 minutes per ha of seed, depending on seed type. Larger seeds such as Soya are easier than fineseed such as grass seeds, while round seeds like Canola coat very easily.*
6. Can you do it outside? *Yes, in the shade and not in direct sunlight as UV light is bad for microbes.*
7. What are the most common mistakes? *Using municipal or chlorinated water which kills all microbes. Mixing seed with lime to calibrate a planter. Putting seed in direct sun. Mixing Bio-Primed seed with pesticide or herbicide.*
8. If you Bio-Prime you seed, can you still use broadleaf herbicide after emergence. *Yes, but you detract from optimal performance and typically kill 25%+ of your microbial population due to leaves taking up herbicide and leaking it via the roots where it negatively impacts the microbes inside the protective film coating covering the roots.*
9. Should you replace your mineral nitrogen fertilizer with Bio-Prime® Nitrogen microbes fixing 80 N per ha? *Yes, you can. As a rule of thumb, keep all your other phosphate potash and micronutrient fertilizer the same but swop out 100% of your nitrogen fertilizer. From year 2 onwards, depending on soil analysis, you can cut a portion of your other fertilizer as per the soil analysis. Better nutrient uptake efficiency will make this possible but eventually you need to add what you remove once alarge buffer has been mined.*
10. Should the Biological Nitrogen microbes be mixed with mineral fertilizer application? *No.*
11. Is this a scam? *No. We developed this technology for our own seed production industry and based on results expanded availability to friends and family and eventually other industries. It takes 15 years to get 15 years' worth of experience as a pioneer, so listen*

carefully and learn. We treat Millions of Rands worth of seed and know what we are doing, from doing. It is much easier to only know the right way with the right materials than to make all the mistakes that can be made mastering things.

12. Can we produce the priming and coating technology cheaper ourselves? *Yes, you can, if you only do it and nothing else, such as farm full-time.*
13. Why do results vary? *Soil varies, herbicide and fertilizer history vary, application success vary because personnel training varies. Sometimes it is the jockey and not the horse*.
14. Can we mix conventional and biological organic farming practises? Yes, you can, but results will vary. Using Bio-Prime will counter at least some negative effects of herbicide application and it is better than not doing it. Use herbicide if you must, but try to keep it to pre-emergence as far as possible in real life.
15. What risks are there? *Shelf life of treated seed is shorter so don't store primed seed for the next season but plant it as soon as possible*. 2-4 weeks storage is fine. Do trials, start small.

Contact:

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