

# Roots to Shoots Trial

#### Background

RhizeBio uses shotgun metagenomics, a method for studying microbial communities in soil by sequencing all DNA present, and providing a comprehensive view of soil biodiversity. The testing program in the Roots to Shoots trial included Haney, M-III, and TND soil testing, Rhizosphere metagenomics, and plant tissue and sap analysis.

RhizeBio combines shotgun metagenomic sequencing with patented bioinformatics to assess the structure, health, and functional capacity of the root-associated microbial communities (the rhizosphere) which play a central and important role in crop growth, nutrient availability and uptake, resistance to diseases and pests, and ultimately crop yield.

The purpose of this study was to use soil chemistry data, rhizosphere metagenomics data, and plant tissue nutrient data together to evaluate the effect of treatment with EnSoil Algae<sup>™</sup>, a live cell biostimulant (Chlorella vulgaris). This analysis was conducted to assess row-cropping systems across different crop growth stages and field conditions, and to evaluate nutrient uptake efficiency, soil carbon, plant stress, and overall crop health.

#### **Results Summary**

- Nitrogen Cycling Improvement
  - Nitrogen cycling metrics (nodulating bacteria, denitrification, and organic nitrogen breakdown,) potassium solubilization, plant stress adaptation, and soil oxygenation improved in corn, soybean, and milo crops with EnSoil Algae<sup>™</sup> application.

#### Increase in Nutrient Movement

- Data combined from the Haney test and plant tissue analyses showed an increase in nutrient movement into the treated crops.
- Increase in Carbon Cycling Potential
  - Carbon cycling potential increased mid-season in treated corn, suggesting a more active microbial community response to crop demands during active growth.

#### Increase in Total Organic Carbon

• Total organic carbon increased in corn at silking, revealing improved carbon sequestration and overall soil health.



## Corn V4 - V6: <u>JRH Grain Farms</u> | <u>Janski Farms</u> | Purdue University

- Improved microbial respiration, oxygen availability, carbon fixation (12%), and nitrogen cycling (18% increase in nitrogen fixation genes,) and iron acquisition (6%)
- Significant increases in available soil N (17.6%), P (8%), and K (35%), particularly nitrate (32% increase) and inorganic P (13% increase). As peak N and P demand will not occur until later, these increases represent "storage" of inorganic nutrients in soil for later consumption by plants.
- High levels of Cu, Fe, and Al in the untreated plant were decreased by 34%, 73%, and 25%, respectively, reducing the potential for toxicity.

#### Corn V7 - V9: Purdue

## Corn at Silking: Purdue | <u>7 Gen Ag</u>

- Improved soil organic matter, microbial respiration (39%), oxygen availability, and plant stress adaptation (microbial production of phytohormones)
- Higher levels of P (13%), K (25%), and S (29%) cycling in the microbiome
- Higher levels of N, P, and K in the tissue than untreated plants, and sufficient or high levels of all other nutrients tested

## Soybeans: <u>Schwindt</u> | <u>Byrdland</u>

- Significantly improved microbial respiration, oxygen availability, carbon fixation, and nitrogen cycling
- Slightly decreased Organic Carbon (13%), but significantly increased proportion of Microbially Available Carbon (90%)
- Higher levels of N, S, Ca, Zn, Mn, and B in the plant tissue
- High levels of Fe and Al in the untreated plants were decreased by 55% and 65% respectively, reducing the potential for toxicity

## Milo: <u>Stegman</u>

## Key Takeaways: Nutrient Uptake, Uptake Efficiency, and Reduced Toxicity

- Increased microbiological activity in soils treated with EnSoil Algae™ is leading to improved nutrient uptake and improved nutrients in plant tissue.
- The table below shows P and K cycling in corn at silking. Reductions in soil nutrients correspond to increased nutrient levels in plant tissue, and to increased levels of microbial cycling.
- Increased microbial cycling of P and K in the root rhizosphere (13% and 7% respectively) corroborate this trend, indicating improved microbial nutrient acquisition in response to treatment with EnSoil Algae™ is leading to the increase in plant tissue nutrients

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#### Corn V7 - V9: Purdue

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- Increases in genes involved in cycling of N, P, K, S, and Ca
- Increases in N, P, S, Zn, Mb, Ca, Mo, and B in plant tissue, including significant increases in N (13.7%) and P (10%)

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Sample Type	Untreated			Treated			% Change		
	Haney	Rhize	Tissue	Haney	Rhize	Tissue	Haney	Rhize	Tissue
Phosphorus	23.9	42%	0.18	16.7	56%	0.21	-30.2%	13.2%	17.1%
Phosphorus Solubilization		42%			56%			31.1%	
H3A Total Phosphorus	10.4			7.3			-30.2%		
H3A Inorganic Phosphorus	7.5			5.2			-31.7%		
H3A Organic Phosphorus	2.9			2.1			-26.3%		
Organic P Release	2.9			2.1			-26.3%		
Organic P Reserve	0.0			0.0					
Available P	23.9			16.7			-30.2%		
Phosphorus, % P			0.18			0.21			17.1%
Potassium	47.6	25%	1.31	47.4	32%	1.52	-0.6%	6.5%	16.3%
Potassium Solubilization		25%			32%			25.7%	
H3A ICAP Potassium	39.7			39.5			-0.6%		
Available K	47.6			47.4			-0.6%		
Potassium, % K			1.31			1.52			16.3%

- In some cases, a key benefit of EnSoil Algae™ application is improved nutrient uptake efficiency, rather than high overall nutrient levels in plant tissue
- In Corn (V4 V6), plant tissue nutrients in the untreated samples were already close to desired levels. However, pH levels in these soils were low (5.6, 5.7) leading to increased chemical mobility of Fe, Cu, and Al, and decreased solubility of N, P, K, S, Ca, and Mg. This likely led to elevated Fe, Cu, and Al levels in untreated plant tissue, above desired level and possibility leading to toxicity.
- The treated samples showed significant decreases in Fe, Cu, and Al below potential toxicity. Meanwhile N, P, K, Na, S, Ca, Nn, B, and Mg saw only minor changes, and all remained in sufficiency range.

Sample Type	Untreated			Treated			% Change		
	Haney	Rhize	Tissue	Haney	Rhize	Tissue	Haney	Rhize	Tissue
Health Overview	12.2	58%	27	10.6	60%	61	-13%	3%	34.51
Community Structure	12.2	64%		10.6	65%		-12.7%	2.2%	
Respiration	93.2	76%		78.2	79%		-16.1%	4.4%	
Environmental Stressors		38%			37%			-2.8%	
Carbon	122.0	66%		119.7	71%		-1.8%	7.7%	
Nitrogen	66.8	65%	4.50	78.6	67%	4.48	17.6%	1.8%	0.4%
Phosphorus	32.2	59%	0.44	35.0	58%	0.41	8.6%	-0.9%	8.0%
Potassium	40.5	47%	3.55	54.7	46%	3.00	35.0%	-1.6%	15.6%
Sulfur	5.0	45%	0.27	4.9	44%	0.22	-1.6%	-1.4%	20.3%
Calcium	319.7	32%	0.45	333.9	35%	0.42	4.4%	9.8%	6.8%
Iron	67.3	66%	428.30	68.4	72%	279.94	1.6%	9.4%	34.6%
Zinc	0.9		36.59	0.6		36.81	-36.7%		-0.6%
Manganese	7.3		70.17	6.7		67.05	-7.5%		4.4%
Magnesium	102.3		0.28	111.3		0.31	8.9%		-8.7%
Sodium	7.6		0.020	7.2		0.016	-5.7%		-19.7%
Copper	0.2		45.64	0.2		12.14	-4.2%		73.4%
Aluminum	155.0		221.53	149.7		166.21	-3.4%		-25.0%
Molybdenum			0.24			0.24			2.7%
Boron			11.09			10.17			8.3%

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