microbialinsights

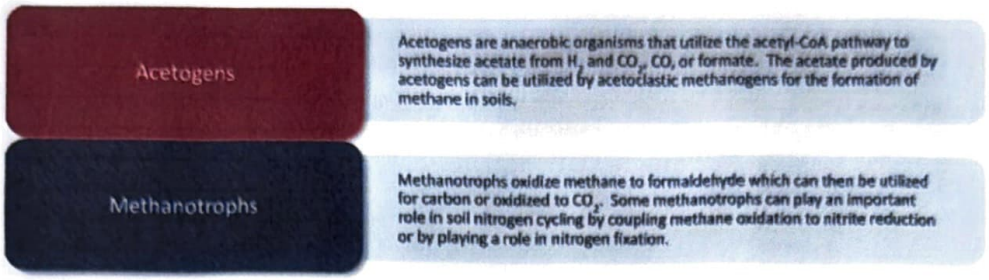
The QuantArray®-Ag Approach

Culture dependent methods like plate counts, MPNs, or Biological Activity Response Tests for heterotrophic bacteria and sulfate reducing bacteria are commonly performed to evaluate the potential for biogeochemical processes in soils. However, the overwhelming majority of microorganisms (>99%) cannot be grown in the laboratory. The soil biogeochemistry consists of diverse microbial communities which carry out processes such as sulfate reduction, sulfur oxidation, iron reduction, metal oxidation, nitrification, denitrification, nitrogen fixation, fermentation, acetogenesis, methanogenesis, and various other processes. Thus, conventional techniques and practices may vastly underestimate and oversimplify the soil biogeochemistry. The QuantArray®-Ag addresses both of these issues: (1) The QuantArray® is a molecular biological tool (MBT) based on analysis of DNA or RNA extracted directly from a field sample eliminating the biases of traditional approaches and (2) The QuantArray® platform provides simultaneous quantification of a broad spectrum of key microorganisms and functional genes for a much more accurate and comprehensive assessment of agriculture or soil related biogeochemical processes.

The QuantArray®-Ag is used to quantify specific microorganisms and functional genes to evaluate the following:

Sulfate Reduction	Sulfate reducing bacteria are capable of reducing sulfate that has been released from decaying plant material or added through fertilizers to hydrogen sulfide.
Iron Reducing Bacteria	Iron-reducing bacteria (IRB) are capable of reducing insoluble iron and manganese oxides to soluble ferrous iron and manganese byproducts which can be readily used by plants. IRB can have a strong influence on carbon, nitrogen, and sulfur cycling in soils.
Metal Oxidizing Bacteria	Iron and manganese oxidizers oxidize soluble iron and manganese to form insoluble iron and manganese oxides. They can also play a role in nitrogen cycling in soils by coupling nitrate reduction to iron or manganese oxidation.
Sulfur Oxidation	Sulfur oxidizing bacteria oxidize sulfide and sulfur that have been added to soils through fertilizers to produce sulfate which can be utilized by plants.
Nitrogen Cycle	In soils, nitrogen fixers convert N_2 from the atmosphere into a usable form for plants, ammonia. Nitrogen loss in soil begins with ammonia and nitrite oxidizers carrying out nitrification, the conversion of ammonia to nitrate. Denitrifiers then convert the nitrate back into N_2 which is released. Anammox bacteria can also anaerobically convert nitrite and ammonia into N_2 leading to nitrogen loss.
Methanogens	Methanogens utilize fermentation products formed by other anaerobes as electron donors (H_2, formate, and alcohols) and acceptors (CO_2, methanol, methylamines, and methylsulfides) to produce methane. Some methanogens can also play an important role in nitrogen fixation in soils.
Fermenters	Designed to quantify a broad spectrum of fermenting bacteria, most notably of the class Clostridia. Fermenters produce H_2 during fermentation which can be utilized by other soil organisms such as acetogens, methanogens, sulfate reducers, iron and manganese reducers, and nitrate reducers.

10515 Research Drive
Knoxville, TN 37932
Phone: 865.573.8188
Fax: 865.573.8133



10515 Research Drive
Knoxville, TN 37932
Phone: 865.573.8188
Fax: 865.573.8133

microbialinsights

Results

Table 1: Summary of the QuantArray®-Ag results obtained for sample Fish Fin Trout Boost

Sample Name Sample Date	Fish Fin Trout Boost 02/02/2026
Soil Microbiology	cells/mL
Total Bacteria (EBAC)	**5.61E+07**
Total Archaea (ARC)	**3.10E+04**
Sulfate Reducing Bacteria (APS)	**8.80E+04**
Sulfate Reducing Archaea (SRA)	<2.80E+00
Iron Reducing Archaea (IRA)	<2.80E+00
Iron Reducing Bacteria - Other (IRB)	**4.70E+04**
Iron Reducing *Geobacter* (IRG)	<2.80E+00
Iron Reducing *Shewanella* (IRS)	**7.20E+03**
Iron Oxidizing Bacteria (FEOB)	**8.20E+04**
Manganese Oxidizing Bacteria (MnOB)	**1.80E+02**
Sulfur Oxidizing Bacteria (SOB)	**1.80E+05**
Ammonia Oxidizing Bacteria (AMO)	**1.30E+03**
Ammonia Oxidizing Archaea (AOA)	<2.80E+00
Nitrite Oxidizing Bacteria (NOR)	<2.80E+00
Anaerobic Ammonia Oxidizers (AMXNIRK)	<2.80E+00
Anaerobic Ammonia Oxidizers (AMXNIRS)	<2.80E+00
Nitrogen Fixing Bacteria (NIF)	**5.90E+05**
Denitrifying Bacteria (nirK)	**6.30E+04**
Denitrifying Bacteria (nirS)	**6.20E+02**
Denitrifying Archaea (ANIRK)	<2.80E+00
Denitrifying Archaea (ANIRS)	<2.80E+00
Methanogens (MGN)	<2.80E+00
Fermenters (FER)	**2.10E+06**
Acetogens (AGN)	**3.60E+02**
Burkholderia cepacian exopolysaccharide (BCE)	<2.80E+00
Deinococcus spp. (DCS)	<2.80E+00
Meiothermus spp. (MTS)	**6.30E+01**

Legend:

NA = Not Analyzed NS = Not Sampled J = Estimated Gene Copies Below PQL but Above LQL

I = Inhibited < = Result Not Detected

10515 Research Drive
Knoxville, TN 37932
Phone: 865.573.8188
Fax: 865.573.8133

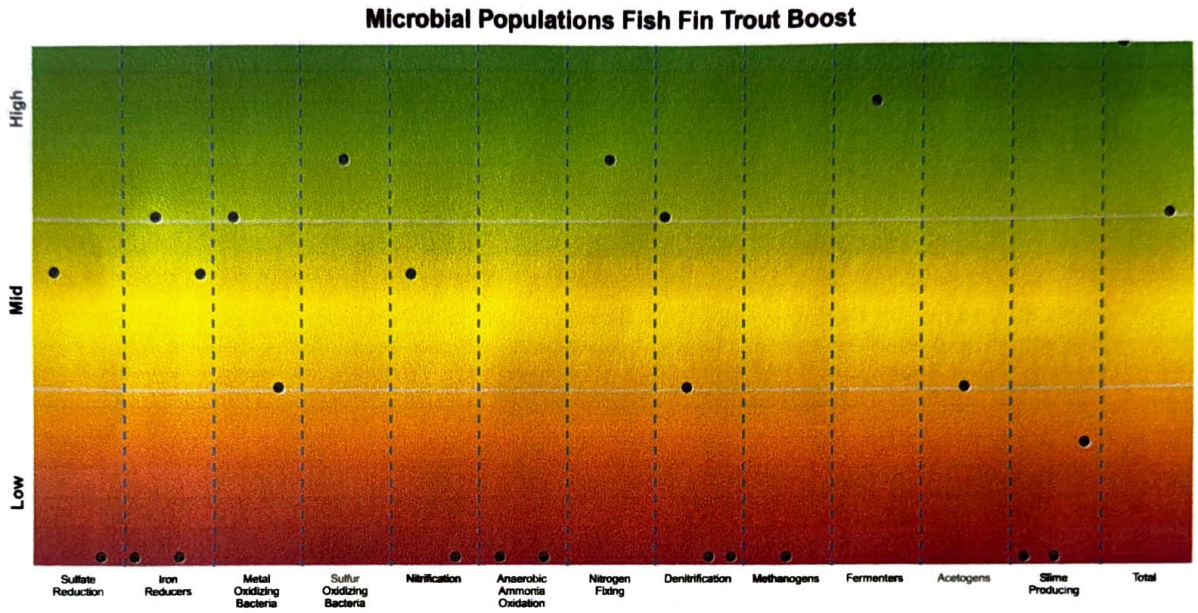


Figure 1: Microbial population summary to aid in understanding soil health conditions. See below to understand the correlation between contaminants and their associated qPCR assays.

Sulfate Reduction	APS, SRA	Denitrification	nirK, nirS, ANIRK, ANIRS
Iron Reducers	IRA, IRB, IRG, IRS	Methanogens	**MGN**
Metal Oxidizing Bacteria	FEOB, MnOB	**Fermenters**	**FER**
Sulfur Oxidizing Bacteria	**SOB**	**Acetogens**	**AGN**
Nitrification	**AMO**, NOR	Slime Producing	BCE, DCS, MTS
Anaerobic Ammonia Oxidation	AMXNIRK, **AMXNIRS**	**Total**	EBAC, ARC
Nitrogen Fixing	NIF		

10515 Research Drive
Knoxville, TN 37932
Phone: 865.573.8188
Fax: 865.573.8133

Interpretation

Total Bacteria: Soil biogeochemical processes are carried out by a wide array of bacteria. Monitoring total bacteria provides a general measure for evaluating the overall growth of bacteria at the site.

Total Archaea: Archaea are another domain of single-celled microorganisms which, like bacteria, can play important roles in the biogeochemical processes. Depending upon types and environmental conditions, total archaea can outnumber total bacteria and be a more important factor in soil biogeochemistry.

Sulfate Reduction: Sulfate reducers can utilize sulfate as a terminal electron acceptor and reduce it to hydrogen sulfide utilizing hydrogen produced by other organisms as an electron donor.

Sulfate Reducing Bacteria (APS): In anoxic zones in agricultural soils SRB utilize the APS gene to reduce sulfate that has been released from decaying plant material or added through fertilizers to hydrogen sulfide. Some SRB also possess genes for the reduction of thiosulfate to hydrogen sulfide. The hydrogen sulfide produced by SRB is then volatized or re-oxidized to elemental sulfur and sulfate in oxic zones [1-3].

Sulfate-Reducing Archaea (SRA): Some genera of Archaea including *Archaeoglobus*, *Caldivirga*, and *Vulcanisaeta* spp. are capable of sulfate reduction. In addition, sulfate-reducing archaea will often reduce elemental sulfur and thiosulfate to hydrogen sulfide [4-6].

Sulfur Oxidizing Bacteria (SOB): Sulfur and sulfide are often introduced to agricultural soils through fertilizers to replace sulfur stores that have been depleted due to continuous land tillage and repeated extractions of crops. Elemental sulfur and sulfide must first be converted to sulfate before they can be utilized by plants. Sulfur oxidizing bacteria oxidize sulfide from organic fertilizers and elemental sulfur to sulfate which can be taken up by plants and incorporated into essential biological molecules. Some sulfur oxidizers can also oxidize thiosulfate through the SOX gene pathway [3, 7-10].

Iron-Reducing Bacteria (IRB): Iron-reducing bacteria (IRB) can reduce insoluble iron oxides, the major form of iron in soils, to soluble ferrous iron byproducts which allows the iron to be taken up and utilized by plants. Some IRB can also reduce insoluble manganese oxides to soluble manganese by products to allow uptake of manganese by plants. Many IRB can also reduce elemental sulfur to sulfide. Iron reduction can have a strong influence on carbon, nitrogen, and sulfur cycling, and IRB can also compete with methanogens for available electron donors. The IRB assay targets IRB including *Defferibacter*, *Geopsychrobacter*, *Geothrix*, and *Rhodoferax* [11-16].

IRB *Geobacter* spp. (IRG): Common genus of iron-reducing bacteria. In addition to utilizing hydrogen, some species are capable of utilizing acetate as an energy source and elemental sulfur as an electron acceptor, producing sulfide. Some *Geobacter* spp. can also reduce insoluble manganese oxides. *Geobacter* have been identified as one of the top iron reducing genera in rice paddy soils [17-18].

IRB *Shewanella* spp. (IRS): Another genus of common and metabolically versatile iron-reducing bacteria. *Shewanella* spp. can utilize hydrogen as an energy source reducing ferric iron to ferrous iron and elemental sulfur, sulfite, and thiosulfate to sulfide. Some *Shewanella* spp. can also reduce insoluble manganese oxides [19].

Iron-Reducing Archaea (IRA): Iron reducing archaea are capable of reducing insoluble ferric iron to soluble ferrous iron which is more readily absorbed by plants. The IRA assays target two genera of thermophilic iron-reducing archaea, *Ferroglobus* and *Geoglobus*. *Ferroglobus placidus* has been shown to couple anaerobic oxidation of aromatic compounds to iron reduction. *Geoglobus* can utilize hydrogen as an electron donor or couple iron reduction to growth on long-chain fatty acids and acetate [20-21].

Metal-Oxidizing Bacteria: As the name suggests, metal-oxidizing bacteria oxidize reduced metal ions (FE^{2+} and Mn^{2+}) and form insoluble metal oxides. To utilize insoluble forms of iron and manganese, plants can either release protons to make the soil more acidic and the metals more soluble or release chelating agents which bind the metals and make them soluble [22].

Iron-Oxidizing Bacteria (FeOB): Microaerophilic iron-oxidizing bacteria gain energy from the oxidation of ferrous iron to ferric iron often resulting in the formation of dense tubercles or filamentous rusticles of iron oxides. The

10515 Research Drive
Knoxville, TN 37932
Phone: 865.573.8188
Fax: 865.573.8133

Interpretation

Total Bacteria: Soil biogeochemical processes are carried out by a wide array of bacteria. Monitoring total bacteria provides a general measure for evaluating the overall growth of bacteria at the site.

Total Archaea: Archaea are another domain of single-celled microorganisms which, like bacteria, can play important roles in the biogeochemical processes. Depending upon types and environmental conditions, total archaea can outnumber total bacteria and be a more important factor in soil biogeochemistry.

Sulfate Reduction: Sulfate reducers can utilize sulfate as a terminal electron acceptor and reduce it to hydrogen sulfide utilizing hydrogen produced by other organisms as an electron donor.

Sulfate Reducing Bacteria (APS): In anoxic zones in agricultural soils SRB utilize the APS gene to reduce sulfate that has been released from decaying plant material or added through fertilizers to hydrogen sulfide. Some SRB also possess genes for the reduction of thiosulfate to hydrogen sulfide. The hydrogen sulfide produced by SRB is then volatized or re-oxidized to elemental sulfur and sulfate in oxic zones [1-3].

Sulfate-Reducing Archaea (SRA): Some genera of Archaea including *Archaeoglobus*, *Caldivirga*, and *Vulcanisaeta* spp. are capable of sulfate reduction. In addition, sulfate-reducing archaea will often reduce elemental sulfur and thiosulfate to hydrogen sulfide [4-6].

Sulfur Oxidizing Bacteria (SOB): Sulfur and sulfide are often introduced to agricultural soils through fertilizers to replace sulfur stores that have been depleted due to continuous land tillage and repeated extractions of crops. Elemental sulfur and sulfide must first be converted to sulfate before they can be utilized by plants. Sulfur oxidizing bacteria oxidize sulfide from organic fertilizers and elemental sulfur to sulfate which can be taken up by plants and incorporated into essential biological molecules. Some sulfur oxidizers can also oxidize thiosulfate through the SOX gene pathway [3, 7-10].

Iron-Reducing Bacteria (IRB): Iron-reducing bacteria (IRB) can reduce insoluble iron oxides, the major form of iron in soils, to soluble ferrous iron byproducts which allows the iron to be taken up and utilized by plants. Some IRB can also reduce insoluble manganese oxides to soluble manganese by products to allow uptake of manganese by plants. Many IRB can also reduce elemental sulfur to sulfide. Iron reduction can have a strong influence on carbon, nitrogen, and sulfur cycling, and IRB can also compete with methanogens for available electron donors. The IRB assay targets IRB including *Defferibacter*, *Geopsychrobacter*, *Geothrix*, and *Rhodoferax* [11-16].

IRB *Geobacter* spp. (IRG): Common genus of iron-reducing bacteria. In addition to utilizing hydrogen, some species are capable of utilizing acetate as an energy source and elemental sulfur as an electron acceptor, producing sulfide. Some *Geobacter* spp. can also reduce insoluble manganese oxides. *Geobacter* have been identified as one of the top iron reducing genera in rice paddy soils [17-18].

IRB *Shewanella* spp. (IRS): Another genus of common and metabolically versatile iron-reducing bacteria. *Shewanella* spp. can utilize hydrogen as an energy source reducing ferric iron to ferrous iron and elemental sulfur, sulfite, and thiosulfate to sulfide. Some *Shewanella* spp. can also reduce insoluble manganese oxides [19].

Iron-Reducing Archaea (IRA): Iron reducing archaea are capable of reducing insoluble ferric iron to soluble ferrous iron which is more readily absorbed by plants. The IRA assays target two genera of thermophilic iron-reducing archaea, *Ferroglobus* and *Geoglobus*. *Ferroglobus placidus* has been shown to couple anaerobic oxidation of aromatic compounds to iron reduction. *Geoglobus* can utilize hydrogen as an electron donor or couple iron reduction to growth on long-chain fatty acids and acetate [20-21].

Metal-Oxidizing Bacteria: As the name suggests, metal-oxidizing bacteria oxidize reduced metal ions (FE^{2+} and Mn^{2+}) and form insoluble metal oxides. To utilize insoluble forms of iron and manganese, plants can either release protons to make the soil more acidic and the metals more soluble or release chelating agents which bind the metals and make them soluble [22].

Iron-Oxidizing Bacteria (FeOB): Microaerophilic iron-oxidizing bacteria gain energy from the oxidation of ferrous iron to ferric iron often resulting in the formation of dense tubercles or filamentous rusticles of iron oxides. The

10515 Research Drive
Knoxville, TN 37932
Phone: 865.573.8188
Fax: 865.573.8133

QuantArray®-Ag targets a variety of iron oxidizers including *Gallionella*, *Leptothrix*, *Sphaerotilus*, and *Mariprofundus* spp [23-24].

Manganese-Oxidizing Bacteria (MnOB): Although the physiological function of manganese oxidation remains unclear, functional genes encoding proteins related to multicopper oxidases have been linked to manganese oxidation. As with iron oxidation, manganese oxidation leads to the formation of insoluble manganese oxides [24-25].

Ammonia Oxidizing Bacteria (AMO): Ammonia oxidizing bacteria catalyze the conversion of ammonia to nitrite, the first step in the nitrification process. In AOB, ammonia is first converted to hydroxylamine by the ammonia monooxygenase enzyme, and then it is oxidized by hydroxylamine oxidoreductase to nitrite. Some ammonia oxidizers also possess genes for denitrification and can convert the nitrite to nitrogen gas. AOB have been identified as the predominant ammonia oxidizers in most fertilized agricultural soils where they compete with plants for bioavailable nitrogen and are primarily responsible for nitrogen loss and N_2O emissions. AOB also have the ability to fix CO_2 through the use of the Calvin cycle. The AMO assay targets the ammonia monooxygenase gene that encodes the enzyme responsible for the initial oxidation of ammonia in the nitrification process [26-28, 30].

Ammonia Oxidizing Archaea (AOA): Ammonia oxidizing archaea catalyze the conversion of ammonia to nitrite, the first step in the nitrification process. In AOA the first step of this process is carried out by the ammonia monooxygenase enzyme, but the enzyme responsible for the second step has yet to be elucidated. AOA do not possess genes for hydroxylamine oxidoreductase which suggests that they do not utilize the same pathway as AOB to complete the conversion of ammonia to nitrite. AOA are typically the dominant ammonia oxidizers in neutral and acidic soils where they compete with plants for bioavailable nitrogen. Some AOA are also capable of autotrophically fixing CO_2 through the hydroxypropionate-hydroxybutyrate cycle [26, 28-30].

Nitrite Oxidizing Bacteria (NOR): Nitrite oxidizing bacteria (NOB) catalyze the conversion of nitrite to nitrate, the last step in the nitrification process, utilizing the nitrite oxidoreductase enzyme. Soil NOB play a major role in nitrogen cycling and the removal of bioavailable nitrogen [26, 31].

Anaerobic Ammonia Oxidizing Bacteria (ANAMMOX): Anammox bacteria are responsible for anaerobically converting nitrite and ammonia directly into nitrogen gas. In this process nitrite and ammonia are transported into the anammoxosome where the anammox nitrite reductase genes nirS and nirK reduce the nitrite to nitric oxide. The nitric oxide is then condensed with ammonia to form hydrazine by the enzyme hydrazine synthase (hzsA). The hydrazine is then oxidized to molecular nitrogen by the enzyme hydrazine dehydrogenase/hydrazine oxidoreductase (hdh/hzo). The assays target the genes encoding two types of nitrite reductase enzymes (nirS and nirK) for quantification of anammox bacteria [32-34].

Nitrogen Fixing Bacteria (NIF): Nitrogen fixers take N_2 from the atmosphere and convert it to ammonia, a bioavailable form that can be assimilated by plants. Some nitrogen fixers are capable of forming a symbiotic nitrogen fixing relationship inside the root nodules that are produced by some plants. This relationship helps promote the efficient uptake of fixed nitrogen by the plants which improves the growth of the plant. Nitrogen fixers are also capable of producing phytohormones that are another important factor for promoting plant growth [35-37].

Denitrifying Bacteria (DNF): Denitrifying bacteria are responsible for converting nitrate from nitrification into nitrous oxide and nitrogen gas. The first step is the conversion of nitrate to nitrite utilizing the dissimiliatory nitrate reductase genes. Nitrite is then reduced to nitric oxide by the dissimilatory nitrite reductase enzymes (nirS and nirK) genes. The nitric oxide is converted to nitrous oxide by the nitric oxide reductase enzyme (norB). Finally, the nitrous oxide is converted to nitrogen gas by the nitrous oxide reductase enzyme (nosZ), and the nitrogen gas is released into the atmosphere. The assays target the genes encoding two types of nitrite reductase enzymes (nirS and nirK) for quantification of denitrifying bacteria [38-39].

Denitrifying Archaea (ANIRK and ANIRS): Targets the genes encoding two dissimilatory nitrite reductase genes (nirS and nirK) in archaea which are responsible for the conversion of nitrite to nitric oxide during denitrification [38].

Methanogens: Methanogens utilize fermentation products formed by other anaerobes as electron donors (H_2, formate, and alcohols) and acceptors (CO_2, methanol, methylamines, and methylsulfides) to produce methane. There are three

10515 Research Drive
Knoxville, TN 37932
Phone: 865.573.8188
Fax: 865.573.8133

main methanogenic pathways H_2 and CO_2 (hydrogenotrophic), acetate (acetoclastic), and methylated C_1 compounds (methylotrophic). Most of the methane produced by methanogens is through the acetoclastic pathway [40-42].

Acetogens (AGN): Acetogens are anaerobic organisms that utilize the acetyl-CoA pathway to synthesize acetate from H_2 and CO_2, CO, or formate. The acetate produced by acetogens can be utilized by acetoclastic methanogens for the formation of methane [43-44].

Fermenters (FER): Designed to quantify a broad spectrum of fermenting bacteria, most notably of the class Clostridia. Fermenters produce H_2 during fermentation which can be utilized by other organisms such as acetogens, methanogens, sulfate reducers, Iron and manganese reducers, and nitrate reducers [45].

Biofilm-Forming Bacteria: Biofilms are commonly found in soils and water, and within the rhizospheres of various plants where they can play important roles in agricultural biogeochemical cycling by aiding in protection of microorganisms, nutrient cycling, and metabolite exchange. Biofilms can also be found on various other surfaces in agricultural environments. Biofilms often contain exopolysaccharides (EPS) which bacteria can adhere to which provides a natural form of protection for bacteria especially in harsh environments. These protective biofilms can in be found within soils and associated with the roots of plants where they serve as a structured environment that supports the growth of microorganisms that are important for improving soil quality and promoting plant growth through the cycling of carbon, nitrogen, phosphorus, sulfur, and metals [46-48]. However, some biofilms can have a negative impact on agriculture by promoting the growth of potentially pathogenic microorganisms [49].

***Burkholderia cepacia* exopolysaccharide (BCE):** Targets a gene involved in exopolysaccharide (EPS) production by biofilm-forming *Burkholderia cepacia* which are commonly found in soils, water, and rhizospheres of various plants. Burkholderia biofilms can be beneficial to plants because they can harbor Burkholderia species that aid in nitrogen cycling including nitrogen fixation and denitrification and that can act as biocontrol agents against pathogenic bacteria [50-53]. However, some Burkholderia biofilms can also be detrimental to plants by harboring pathogenic microorganisms.

***Deinococcus* spp. (DCS):** *Deinococcus* spp. are capable of forming robust biofilms in environments with extreme environmental conditions such as soils in hot and arid environments [54]. Deinococcus rhizophilus is found in rhizosphere soil where it associates with the roots of plants and may play a role in plant microbe interactions and nutrient cycling [55].

***Meiothermus* spp. (MTS):** Along with Deinococcus, Meiothermus are considered primary biofilm formers functioning as an adhesion platform for secondary biofilm bacteria. Meiothermus biofilms can be found in soils and water, including agricultural runoff where they can play a role in the breakdown of some pesticides and other organic materials [56].

10515 Research Drive
Knoxville, TN 37932
Phone: 865.573.8188
Fax: 865.573.8133