# SOIL HEALTH TECH BULLETIN III

FACT SHEET

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## VitTellus Bio (Tool for Soil functional group Microbial profiling)

## Technical notes

### What is VitTellus Bio?

VitTellus Bio is an analytical package that quantifies the levels of specific functional groups of microbes in the soil that are associated with improved soil health and crop yield.

### How does it work?

VitTellus Bio measures the utilization of selective carbon sources to calorimetrically quantify the levels of specific functional microbial groups within a soil sample. This test uses customized Biolog Eco-plates that optically measure the color development of redox dye for each carbon source that are presented as numerical values as functional index.

### Introduction:

Soil is a heterogeneous matrix with a vast range of physical, chemical and biological characteristics, all of which create a variety of niches that can sustain large amounts of microbial diversity. Microbial communities in the soil play a significant role in soil health by regulating major biogeochemical cycles, thereby controlling major ecosystem functions. Agriculture is one of the most impactful anthropogenic activities that affect nearly all of the soil properties and their functions. The VitTellus Bio analytical package is designed to quantify the levels of 17 different microbial functional groups including 1. *Pseudomonas* spp., 2. Nitrogen Fixers, 3. *Rhizobium* and Relatives, 4. Total Gram-Positives, 5. Actinomycetes, 6. General Bacteria, 7. General Fungi, 8. *Trichoderma* spp., 9. Anaerobes, 10. Total major Gram Negatives, 11. Total Bacteria, 12. Total Microbial Activity, 13. Ratio of Gram-Positive to Gram-Negative Bacteria, 14. Ratio of Gram-Positive to Actinomycetes, 15. Ratio of Fungi to Bacteria, 16. Ratio of Fungi to *Trichoderma* and 17. Ratio of Aerobes to Anaerobes. All of these individual components are carefully chosen to measure the various gradations of soil health.

## Test background:

Using data collected through our Agricultural Innovation Program (AIP) on the interaction of soil & plant physical, chemical and microbial parameters on soil & plant health, we found some key interactions between microbes and soil health, overall fertility and yield. The multi-year, government funded, AIP program was carried out by A & L for five years between 2014 to 2018. The aim of the program was to understand what factors drive plant productivity and soil sustainability. We have sampled 60+ corn farms across Ontario and Quebec with multiple sample sites in each farm, measured 487 different variables that directly or indirectly influence soil health and plant productivity. By analyzing and summarizing all the data, we found the top 20 influential factors that span physical, chemical and microbial spheres. These findings have been verified with wheat and soybean crops as well. Based on this work, we developed a simple, fast, affordable test to identify the indicators of soil health by measuring 17 key microbial components described below. The VitTellus Bio test is an informative tool that complements traditionally available soil and plant tests in order to produce a complete soil health profile for effective soil management and sustainability.

Below is some brief information about each individual assessment:

### 1. *Pseudomonas* Population

Pseudomonads are one of the major groups of Gram-negative bacteria present in the soil. The majority of the *Pseudomonas* species present in soil are beneficial to plants; they have the ability to quickly colonize roots of many agricultural crops, help with growth stimulation, enhance plant immunity, supress plant diseases through antibiotics secretion and, most importantly, provide vital services to the function of the agroecosystem by improving nutrient cycling and its availability in soil. From a nutritional perspective, the presence of *Pseudomonas* species strongly indicate the optimal phosphorous saturation levels that, in turn, support high crop productivity, despite soil type. These bacteria are also linked to the balance of soil B and Fe, and the disease suppression of major soil borne fungal pathogens such as *Fusarium* Head Blight, Take-all disease, etc. Functional index values >3500 indicate stable levels of *Pseudomonas* in the system and is considered strongly optimal.

### 2. Nitrogen Fixers

This assay quantifies the functional levels of heterotrophic, free living nitrogen fixers in the soil, including *Azotobacter*, *Pseudomonas*, *Azospirillum*, *Aquaspirillum*, *Klebsiella*, *Burkholderia*, Cyanobacteria (*Nostoc*, *Anabaena*), *Clostridium* and some *Bacillus* spp. The most optimal functional index is >3000. This value also indicates how future nitrogen fertilizer applications should be handled: lower values in this index implies an excess in nitrogen amendments and cautions for an immediate change in fertilization practices.

### *3. Rhizobium* and Relatives

This index quantifies the functional levels of symbiotic (both nodule & non-nodule forming) diazotrophs. *Rhizobium* spp. and their relatives have symbiotic relationships with over 120 legume plants and several other non-legume plants, such as corn, wheat, etc. Rhizobia can fix nitrogen after becoming established inside the plant host since they cannot fix nitrogen independently. The minimum required functional index level is ~1500. These functional levels indicate optimal soil B levels, which must be at least 2 ppm to get this microbial population in the optimal range.

### 4. Gram-positives

Gram-positive *Bacilli* are spore-forming rods that can survive in environments for many years; they adapt to extreme soil and environmental conditions unlike Gram-negatives. These bacteria are generalists and can survive in wider soil types and pH ranges. Excessive values of this index (>4000) with very low levels of *Pseudomonas*, Nitrogen fixers and *Rhizobium* and Relatives (indices 1–3) indicates a problem with microbial imbalances in the soil. This reverse relationship has been observed in many low productive sites that have a physical and/or chemical imbalance. It is still unknown if this is the indication of poor soil conditions or one of the causes involved in depletion of other important microbial functional groups by taking advantage of the situation with its resilient & recalcitrant nature and the antimicrobial proteins it produces that directly inhibit the proliferation of Gram-negative bacteria by increasing the permeability of the microbial cell membrane. Levels between 2000–4000 are beneficial.

### 5. Actinomycetes

Actinomycetes are a group of Gram-positive bacteria that form branched filamentous hyphae, similar to fungi but smaller in diameter. They are the hardiest in changing soil conditions, next to saprophytic soil fungi. The functional index values should be below 2000–2500 in highly productive soil with a good physical and chemical balance. These values should be approximately 1/3 to 2/3 of the Gram-positive index, approximately 1/4 of Total Gram-negative index, and can be equivalent or lower than the fungal index in productive soils.

### 6. General Bacteria

This index is used for the quantification of all bacteria that can metabolize standard carbon sources. The general bacterial load in an agriculture ecosystem will be at least 2–4 log units higher than the fungal load. In our indexing system, you will see a range of at least 2500–5000 in soils with decent physical and chemical properties, with proper agronomic practices. Lower index values (1200–2500) are possible during the fall and early spring snow melt, where the fungal activity would be slightly higher during the major residual decomposition occurs.

### 7. General Fungi

Fungi are an integral part of ecosystem processes. They are filamentous, multicellular, eukaryotic organisms that have important roles in nutrient cycling and decomposition of crop residues in soil. The fungal load index should be >2500–4000 during the growing season for an agricultural ecosystem to perform at optimal levels, with activity peaking (4000–6000) during spring and fall.

### *8. Trichoderma*

*Trichoderma* are opportunistic soil fungi that provide an array of beneficial functions to a healthy agricultural ecosystem, including the biological control of other pathogenic fungi, plant growth promotion, and beneficial nutrient (micro & macro) solubilization. Their stable presence (a functional index >500) indicate a strong contribution to soil function.

### 9. Anaerobes

Anaerobic organisms are present in oxygen-limiting conditions and are often pathogenic organisms, mainly bacteria. Higher the levels of anaerobes, higher the problem that the soil indicates. When soil conditions support anaerobic growth, the aerobic population will quickly diminish, so a lower anaerobic functional index value (<2000) is best for soil health.

### 10. Total Gram-negatives

Gram-negatives often use plant derived carbon sources and are therefore more labile to population fluctuations if agronomic practices are not properly followed. They are very hard to repopulate in soils after depletion, which encourages the maintenance of a minimal standard population. Based on our research, we recommend that the Total Gram-negatives index should be >7000, where the higher the index, the better the health of the soil.

### 11. Total Bacteria

The Total Bacterial index refers to the measured total bacterial functional activity. To have adequate biological activity and soil functioning is to have a good balance between total fungal and bacterial activity. The functional index should be >7000.

### 12. Total microbial activity

The soil microbiome is an essential part of nutrient cycling. It plays several vital ecological and physiological functions: crop residue and soil organic matter decomposition, soil structure enhancement, ameliorating soil physical and chemical conditions, regulation of mineral nutrient availability, atmospheric nitrogen fixation, partial involvement in carbon sequestration as soil organic matter and the reduction of $CO_2$, production of biologically active substances that stimulate plant growth and health, disease suppression, etc. The total microbial functional activity index should be at least 10000 to keep the soil healthy.

### 13. Ratio of Gram-positive to Gram-negative Bacteria (Gram+:Gram-)

Soil pH, aerobic or anaerobic soil conditions, CEC and the nutrient balance affect the selective colonization of different microbial communities in soil. Gram-positives are hardier microbes that include Actinobacteria (*Actinomyces*, *Bacillus*, *Enterococcus*, *Streptomyces*). These have larger cells with a thicker cell wall, the capacity to produce endospores and survive under wider pH ranges, drought, water stress and metal toxicity conditions. However, Gram-negatives, including Proteobacteria (*Acetobacter*, *Azospirillum*, *Burkholderia*, *Enterobacter*, *Flavobacterium*, *Klebsiella*, *Nitrobacter*, *Pseudomonas*, *Pantoea*, *Rhizobium*, *Rahnella*, *Serratia*, *Thiobacter*) are smaller and sensitive to extreme conditions, and can only survive at near neutral pH. The Gram+:Gram- should be <1.0 to maintain the diversified functional activity in the soil.

### 14. Ratio of Gram-positive to Actinomycetes (Gram+:Actinomycetes)

As mentioned earlier, the Actinobacteria are a sub-group of Gram-positive bacteria that produce fungal-like mycelial appendages. A Gram+:Actinomycetes ratio >3.0 is optimal. When the ratio drops, depending on the agronomic practices and crop type, one must pay particular attention to these populations since a few species of *Streptomyces* (Actinomycetes) are plant pathogenic.

### 15. Ratio of Fungi to Bacteria (Fungi:Bacteria)

Depending on the agronomic practices, some soils are fungal-dominated and others are bacterial-dominated. In general, fungal populations are slightly higher during spring, and significantly higher during fall crop residue degradation. We suggest the healthy Fungi:Bacteria ratio should be 0.3–0.6. We caution that a ratio outside this range may indicate problems in soil health, depending on the management practices.

### 16. Ratio of Fungi to *Trichoderma* (Fungi:*Trichoderma*)

The presence of *Trichoderma* contributes greatly to soil health and disease suppression. Therefore, having any minimal stable activity is good for healthy, active soil. This means the ratio anywhere below 50 indicates the presence of a stable *Trichoderma* population.

### 17. Ratio of Aerobic to Anaerobic Bacteria (Aerobe: Anaerobe)

This is a quick indicator of a soil's physical condition. An aerobe: anaerobe ratio >5.0, indicates average–good quality soil, where <5.0 may indicate problems with soil physical conditions. If this ratio drops below 4.0, attention should be paid to improve soil physical conditions (aeration, tiling, etc.).

## Soil physical and chemical factor relations with biological activity:

### Soil Health Index and soil biological activity

*(Total Gram-negatives, Pseudomonas Population, Gram-positives, Gram+:Gram- ratio and Fungi:Bacteria ratio)*

Based on years of research into soil fertility and crop productivity, A&L has developed a Soil Health Index (SHI) numbering system to categorize overall fertility and nutritional balance of the soil. Then, The Agricultural Innovation Program has assessed the relationship of the SHI with soil biological activity. Soil with fertility index values over 40 have a >70% positive correlation with Total Gram-negatives and *Pseudomonas* population, >70% negative correlation with Gram-positives if the functional activity of Gram-positive population is >5000. If the functional index drops down to 2500–4000, that correlation becomes positive, and there is no correlation if the functional index activity falls <2500. There is a strong positive relationship between SHI and Gram+:Gram- ratio when the ratio is <1.0, and there is a negative correlation if index values are >1.0. Optimal SHI (>40) indicates the Fungi:Bacterial ratio falls between 0.3–0.6. The relationship will be stronger when both numbers are within this range. Otherwise, a negative or no relationship will be seen. If the soil chemistry is optimal and the microbiome is not in the optimal range, this could be easily corrected or enriched. If there is issue with microbial imbalance, the chemistry should be corrected and balanced before balancing and enriching the microbiome and its functional activity.

### pH and soil biological activity *(Fungi:Bacteria ratio, Total Gram-negatives)*

Soil pH is the one of the main factors of soil health, largely affected by chemical crop inputs that in turn significantly influence the nutritional availability, crop growth, and microbial diversity. There is an optimal pH range where different microbes selectively colonize and multiply in soil. Bacteria prefers pH 5–9, Actinomycetes pH 6.5–9.5, Fungi pH 2–7, and blue-green bacteria pH 6–9. In the same way, pathogenic microbes are dominant/recessive in certain pH ranges. In general, lower pH favours fungi and higher pH favours most bacteria, and the increase/decrease of the fungal or bacterial density five-fold in the lower or higher pH, respectively. Among the bacteria, Gram-positives, the Actinobacteria (*Actinomyces*, *Bacillus*, *Enterococcus*, *Streptomyces*), are hardier, with larger cells, thicker cell walls, can produce endospores to survive in wider pH ranges, drought, water stress, and high metal toxicity conditions. However, Gram-negatives, the Proteobacteria (*Acetobacter*, *Azospirillum*, *Burkholderia*, *Enterobacter*, *Flavobacterium*, *Klebsiella*, *Nitrobacter*, *Pseudomonas*, *Pantoea*, *Rhizobium*, *Rahnella*, *Serratia*, *Thiobacter*), comprise the majority of beneficial soil bacteria. They are smaller-celled and sensitive to extreme conditions; only surviving near neutral pH and can be easily out-competed by more resilient Gram-positive microbes. Any changes in pH will significantly affect most beneficial microbes. By providing a conducive environment for these beneficial microbes to increase their abundance and diversity, one can keep pathogenic organisms suppressed, conserve nutrients from wastage and enhance soil health & sustainable productivity. If the pH is between 6–7, the fungi:bacteria ratio will be between 0.3–0.6 and the total Gram-negative functional index will be >6000. With all these ranges the relationship would be positive and >70%.

### Organic Matter vs Fungi:Bacteria ratio

Organic matter (OM) plays a strong role in keeping microbial populations healthy and alive, especially when the soil is extremely light or heavy. Values of at least 3% OM is very important in any soil to feed the microbiome and maintain functional activity. Data collected from years of study indicated that OM values above 3% in mainly loamy soils did not seem influence any major shifts in microbial population. OM up to 6% is more influential in heavy and light soils to support active microbial levels. Stronger relationships with the ideal Fungi to bacterial ratios will be observed at these levels of OM in specific soil types.

### Percent P vs *Pseudomonas* population

The functional activity of *Pseudomonas* populations shows a very strong relationship with phosphorus (P) saturation and Potassium (K) levels. The survival and the sustainability of Pseudomonads are dependent on the level of carbon resources available in heavy or light soils. The proliferation of Pseudomonads helps to optimize initial P and the K levels and this shapes their population through a feedback mechanism to be resilient enough to survive in soil extremes. The stronger the positive relationship between Percent P & *Pseudomonas* population, the better the soil fertility balance will be.

### CEC vs soil biological activity *(Total microbial activity, Total Gram-negatives, Total Fungi)*

The soil's Cation Exchange Capacity (CEC) is a main factor affecting biological community shifts. Average CEC levels of 10–20 with a minimal level of OM (2–3%) is enough to support the microbial diversity, if very drastic agronomic practices have not been followed. If soils have average CEC levels but don't have a good balance of microbial diversity and functions, there are ways to modify a negative trajectory before it becomes worse. In heavier and lighter soils, building and maintaining the microbial balance will require additional inputs, but it is possible. The biological community is constantly going through the impact of weather extremes (water (logged) stress, drought stress, heat), soil clumping, slower/faster OM decomposition, chemical fertilizer burn, over/under use of N fertilizer, etc. Based on the relationship between CEC to one or all of these factors (Total microbial activity, Total Gram-negatives & Total fungi), and the measured functional activity, we can tweak agronomic practices to get them at optimal levels.

### Boron vs Rhizobium and Relatives

We know that Boron (B) plays a key role in the nutritional interactions between soils and plants, enhancing source & sink relationships, providing forecasting signals to the plants of upcoming weather events. The implications of boron over application is often told to the growers, which resulted in growers stopping B amendments, which resulted in poor nutrient use efficiency. Analysing 1000+ soil & plant samples across Ontario, we have learned that B has role in establishing and maintaining the *Rhizobium* & *Rhizobium*-related organisms, such as nitrogen fixers, stress ameliorating and nodule & non-nodule farming bacteria. Strong positive relationship (>70%) with boron and Rhizobium communities will be seen if the soil is measured with at least 2ppm B.

### K:Mg vs Total Gram-negatives

The ratio of K to Mg at the optimal range (0.2–0.35) has strong role in supporting the beneficial Gram-negative bacterial population. The deterioration of these communities will happen on either side of this range, despite soil type. At this optimal K:Mg range, a strong positive relationship (>70%) will be seen between K:Mg and Total Gram-negatives communities.

## Advantages of our test:

√ Directly measures various functional groups of active microbial populations using different carbon sources

√ Relates the activity of various groups of microbes with soil nutritional indices

√ The functional microbial groups are selected based on years of study, and from our studies we have identified that these functional groups better represent the microbial communities of agricultural soils that show a stronger relationship to yield and soil fertility

√ Provides more usable soil biological information, compared to other biologicals tests in the market

√ Is combined with soil chemistry data to get complete soil health information

√ We can provide expert advice to create biologically inclusive soil health/ environmental farm plans and to bring resilience in soil biome by promoting beneficial soil biological activity

## Taking Action - Improving the Soil Microbial Profile:

- √ Review the soil chemistry results to identify soil parameters which are not in the optimal ranges. Making changes to fertility programs is fundamental to moving soil chemistry metrics into ideal ranges. This will ensure plants are properly nourished and providing carbon sources for the microbes in the soil.
- √ Consider management practices in your farm operation such as suitable cover crops. Cover crops offer multiple benefits such as providing an additional carbon source for soil microorganisms to ensure they are maintained throughout the year after the primary crop is removed.
- √ Continue to build soil organic matter for the purpose of moisture retention and release of mineralizable Nitrogen. In very dry or drought conditions, microbial activity can be significantly reduced and impact crop yields. Building organic matter will help mitigate the risk of lower microbial activity in less ideal situations.
- √ Ensure you are using Best Management Practices such as good crop rotation, ensuring adequate sub-surface drainage, and utilizing erosion control measures to prevent wind or water erosion.
- √ Regular soil testing will help assess the effect of management changes and improvement of soil health parameters. Analyze your soil data to help improve soil health and monitor progress on your plan.

***Note: This document will be updated as we get additional research data.***