



Report Manual



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1. Why soil microbial performance is important?

In modern agriculture, it's easy to forget the importance of soil health and the role the microbiome plays in maintaining it. The soil microbiome is an essential component of soil health, yet it is often overlooked due to a lack of appropriate tools to measure and assess it. If farmers are to succeed in the transition to regenerative agriculture, they must find more efficient ways to manage soil health.

Regenerative farmers understand the importance of a high-quality, diverse soil microbiome. They use organic inputs and create conditions that encourage microbial communities in their soils, which in turn leads to improved fertility and nutrient cycling. Understanding the importance of the soil microbiome and its critical role in maintaining soil health is critical. Analyzing the soil microbiome can provide insight to help select the most effective practices to maintain soil fertility, increase crop yields and improve overall agricultural success. It is essential for farmers to understand how their soils interact with microorganisms to ensure that their soils are healthy and able to sustain long-term production.

A good example of a microbial process is the decomposition of plant residues, a vital process in soil ecosystems that contributes to nutrient cycling, soil fertility and carbon sequestration. This process is primarily facilitated by a wide range of soil microorganisms, including bacteria, fungi and other decomposers. They work to break down complex organic matter in plant residues into simpler forms, ultimately converting it into nutrients that plants can absorb.

That relates to many of known regenerative practices among others:

Adding Organic Amendments: Enhances microbial activity by adding decomposable organic matter, improving soil fertility and structure.

Reduced Tillage: Protects soil microbial communities by preserving soil structure, supporting nutrient cycling and organic matter breakdown.

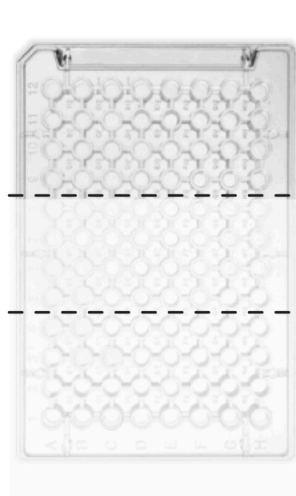
Cover Cropping: Increases soil organic matter, providing food for soil microbes which turn it into nutrients for crops.

Crop Rotation and Diversity: Helps maintain a diverse soil microbiome, preventing the buildup of plant-specific pathogens.

2. BIOTREX technology

BIOTREX technology is a novel indicator designed to assess the activity and functional diversity of soil microorganisms. It was invented in Japan by Professor Kazunari Yokoyama and is based on the well-established and scientifically recognised BIOLOG system. Microplates called EcoPlate's are used in the BIOTREX 31 test to monitor environmental changes in soil microbiomes. Microbes are often the first to respond to any changes in their environment. When the microbiome starts to behave differently, it is often an early sign that the whole environment may be at risk. A method called Community-Level Physiological Profiling (CLPP) has been proven to be useful in tracking these changes over time and across different locations. It can be used to monitor a healthy population of microbes and detect changes when something new is introduced into the environment. EcoPlate's have been documented in over 500 scientific publications using BIOLOG's technology to analyse microbial communities.

The BIOTREX method is designed to quantify two crucial microbiome parameters: activity and diversity. It offers a comprehensive view of soil microbial performance by measuring biochemical processes at 193 time points during 48 hours within 31 distinct reaction vessels called "wells". These wells each contain a different carbon source, creating an environment in which only those microorganisms capable of metabolizing a specific carbon substrate can thrive and generate violet-coloured signals visible on the microplate photos. Among the substrate groups are carbohydrates, amino acids and polymers in general resembling those found in plant residues, root exudates and soil particles.



Substrates list

Carbohydrates:

- β-Methyl-D-Glucoside
- D-Galactonic Acid γ-Lactone
- D-Xylose
- i-Erythritol
- D-Mannitol
- N-Acetyl-D-Glucosamine
- D-Cellobiose
- Glucose-1-Phosphate
- α-D-Lactose
- D,L-α-Glycerol Phosphate

Carboxylic Acids:

- Pyruvic Acid Methyl Ester
- D-Galacturonic Acid
- γ-Amino Butyric Acid
- D-Glucosaminic Acid
- Itaconic Acid
- α-Keto Butyric Acid
- D-Malic Acid

Polymers:

- Tween 40
- Tween 80
- α-Cyclodextrin
- Glycogen

Amino Acids:

- L-Arginine
- L-Asparagine
- L-Phenylalanine
- L-Serine
- L-Threonine
- Glycyl-L-Glutamic Acid

Amines and Amids:

- Phenylethylamine
- Putrescine

Phenolic compounds:

- 2-Hydroxy Benzoic Acid
- 4-Hydroxy Benzoic Acid

EcoPlate contain three identical sets of 31 substrates. In BIOTREX method one set of substrates is used to analyse one sample.

To understand how this relates to regenerative practices, we need to go back to the decomposition of plant residues. These residues are rich in complex organic polymers such as cellulose, hemicellulose and lignin. Breaking down these complex structures into simpler, more absorbable forms requires a range of different enzymes. These simpler forms then become nutrients for the plants.

The BIOTREX 31 analysis includes measurements of substrates utilisation and therefore the microbes and key enzymes involved in these biological processes. By promoting a rich diversity of soil microbes, farmers can improve the fertility and structure of their soil, enhance nutrient cycling, and promote healthier, more productive crops.

Some of the most significant soil microorganisms:

- Fungi

Trichoderma species (well-known for its use in industrial cellulase production), *Aspergillus* species, and many species of white- and brown-rot fungi.

- Bacteria

Bacillus species, *Streptomyces* species, *Cellulomonas* species, *Clostridium* species, *Thermobifida fusca* (a thermophilic actinomycete).

Examples of enzymes produced by these microbes involved in the breakdown of cellulose (the main component of plant residues):

- Cellobiohydrolases

These enzymes, which break down cellulose into cellobiose, are produced by many soil fungi and bacteria. The substrate is D-Cellobiose.

- β -Glucosidases

These enzymes, which break down cellobiose into glucose, are widespread among soil microorganisms. The substrates are β -Methyl-D-Glucoside and D-Cellobiose.

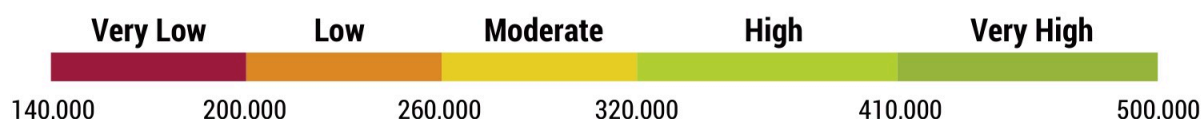
- Xylanases

These enzymes, which break down xylan (a type of hemicellulose), are produced by a range of soil microorganisms. The substrate is D-Xylose.

3. BIOTREX Score

The BIOTREX Score, a measure of soil microbial performance, is derived from our analytical results. This performance scale was established based on the analysis of over 20,000 soil samples. We've implemented a color-coded interpretation system to ensure the results are easily comprehensible.

BIOTREX Score scale



Very high BIOTREX Score (410,000 - 500,000; very high microbial performance)

The excellent condition of your soil is probably the result of careful application of regenerative farming practices. To maintain this high quality, stick to your crop rotation and cover cropping strategies while limiting soil disturbance. Maintain biodiversity by routinely incorporating organic matter, which in turn nurtures the abundant microorganisms.

High BIOTREX Score (320,000 - 410,000; high microbial performance)

Your soil has a healthy balance of microbial activity, so keep it up. To increase biodiversity, consider varying your sources of organic matter, such as compost, crop residues or green manures. Techniques such as cover cropping and diversified crop rotations can provide additional support for the microbial community.

Moderate BIOTREX Score (260,000 - 320,000; facing microbial biodiversity and activity issues)

Your soil sits in a middle ground of health, signalling room for improvement. If regenerative farming is already part of your routine, continue the work. If not, start by limiting synthetic fertilisers and pesticides. Incorporate organic amendments, cover crops and crop rotation into your practices to increase microbial health and ward off plant diseases.

Low BIOTREX Score (200,000 - 260,000; low microbial biodiversity and activity)

This type of soil, often found in intensive agriculture, is at risk of further degradation. The solution is to revive microbial life. Start by reducing chemical inputs and soil disturbance, using crop rotations, planting cover crops and integrating livestock where possible. These actions will stimulate microbial activity and help control disease.

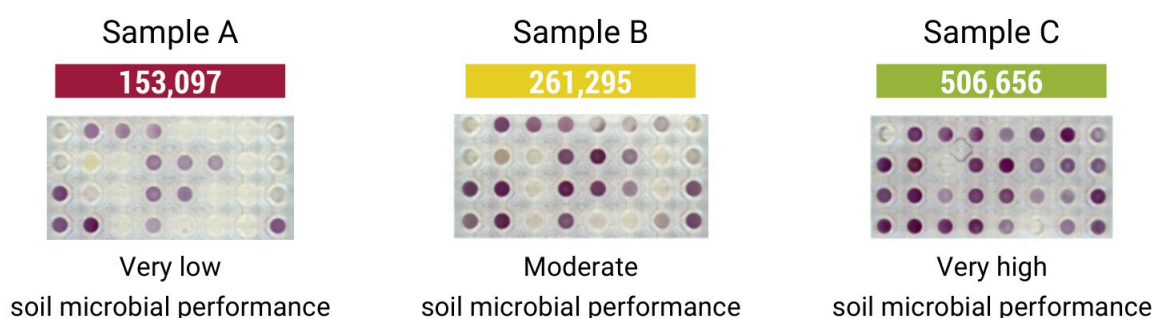
Very low BIOTREX Score (140,000 - 200,000; severe loss of microbial biodiversity and activity)

This soil condition presents a challenging scenario that requires long-term regenerative approaches. Prioritise soil cover to reduce erosion and replenish organic matter. Use diversified crop rotations, cover crops and add organic matter such as compost. Remember that patience is key, as substantial improvements will take time.

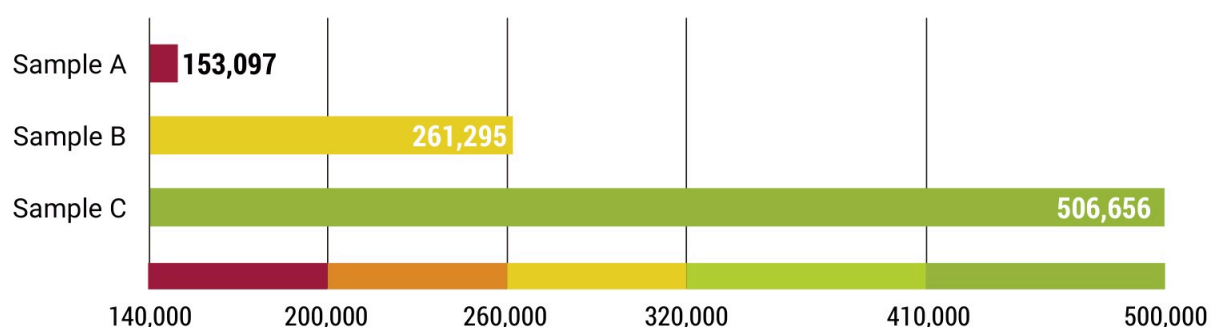
The scale is universal for all agricultural soils. About 97% of all the soils we test fall in between the highest and lowest points of the scale. It is designed to make it easy to compare very different soils. Although different plots of land may differ geographically, physically or chemically - the microbial processes are somewhat universal. For example, we can say with high degree of certainty that a field with a significantly higher BIOTREX score will have shorter decomposition times for crop residues, regardless of other parameters.

Soil microbial performance for different soil samples

(Plate photos after 48 hours)



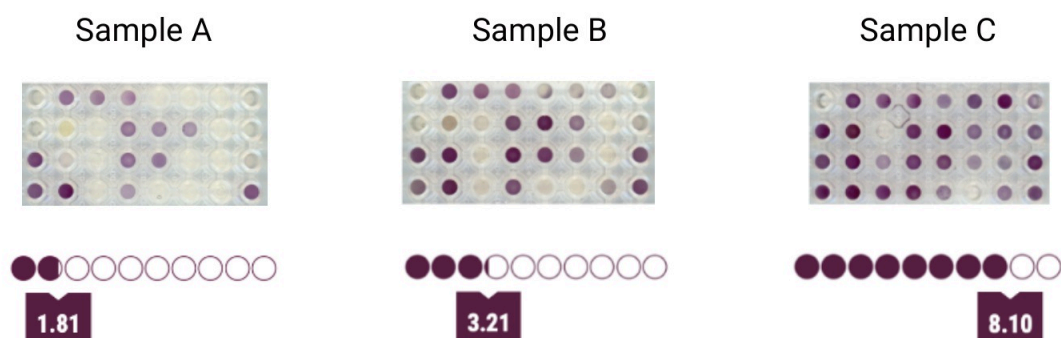
Soil microbial performance graph



4. Eco-physiological diversity

The eco-physiological diversity index measures the evenness of substrate utilization among the 31 different substrates. It is derived from a well-known tool - the Shannon index. The higher the number, the more evenly the substrates are consumed by the microbiome. The maximum index is 10, but this is not possible in natural soil because it is a perfect utilization of all substrates. If a substrate is not utilized, it does not contribute to the index, so sterile soil will have an index of 0. The general scientific consensus is that the greater the diversity, the more stable, resilient and healthy the microbiome.

Eco-physiological diversity for different soil samples



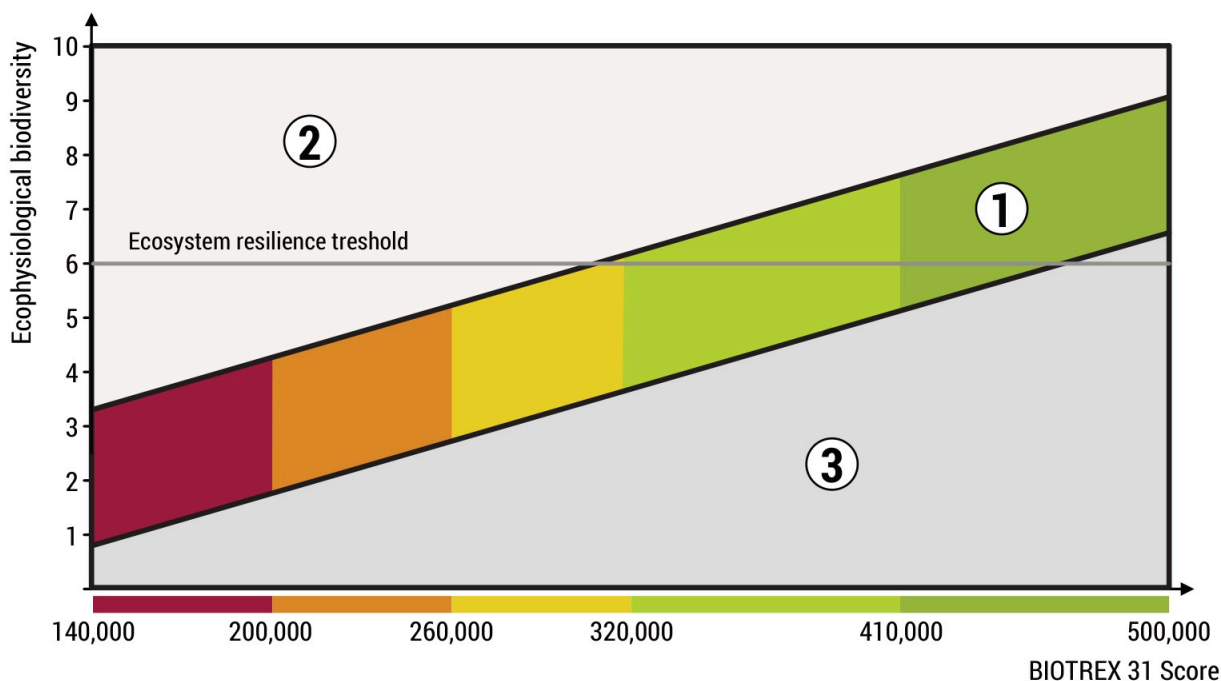
The current state of knowledge is that a more robust representation of various processes in the soil is connected to healthier, more resilient soil that is less prone to carbon content loss and climate pressure. Thus, biodiversity that is measured on the basis of the ability of the microbiome as a whole to contribute to the body of the processes that take place in the soil is likely to be connected to soil health and resilience.

For the first time we introduced the *Ecosystem resilience threshold* which we believe can be a valuable tool for agronomists and farmers looking to assess the resilience of their soils. A high score on this index indicates a soil microbiome that is able to perform multiple processes and use different substrates equally, a critical factor in maintaining soil functionality in the face of challenges such as climate change and other stressors. This threshold can serve as a "target" for agronomists and farmers to strive for in their soil management practices. Scores above this threshold can signal a robust and resilient soil ecosystem that is conducive to healthy crop growth and productivity. On the other hand, scores below this threshold may indicate the need for further intervention, such as soil amendments or changes in cropping practices, to especially improve soil microbiome biodiversity.

5. BIOTREX Score and expected biodiversity

The BIOTREX index is a measure of both the activity and biodiversity of the microbiome. It is worthwhile to interpret it with the addition of the eco-physiological biodiversity index. For each BIOTREX score, there is an expected range of biodiversity scores. If the soil has a higher biodiversity score, this means that the overall performance of the microbiome is more due to high biodiversity, but the activity may be lower than expected. In this case, the microbes are consuming a wide variety of substances, but at a slow rate. Conversely, lower than expected biodiversity means that most of the microbial performance comes from activity. This means that the microbes are fast at metabolism, but may lack the ability to digest some substances. You can find your set of scores on the chart below - this may help you to make targeted changes to your farming practices.

Expected biodiversity graph



① Expected biodiversity

If the eco-physiological value of biodiversity is within the expected range, improvements in soil microbial performance must be achieved through a balance of activity-promoting and biodiversity-inducing interventions. Most soils will tend towards this balance, so you can take this as a good sign of stability. Thoughtful changes in practices should result in an improvement in the BIOTREX score while remaining within the expected range of biodiversity.

② Unexpectedly high biodiversity

Although the biodiversity of soil microorganisms is high, additional interventions may be needed to increase their activity. Consider adding more organic matter to the soil, which will provide additional nutrients and energy for microorganisms. Also, minimize soil disturbance, such as intensive tillage, which can inhibit microbial activity.

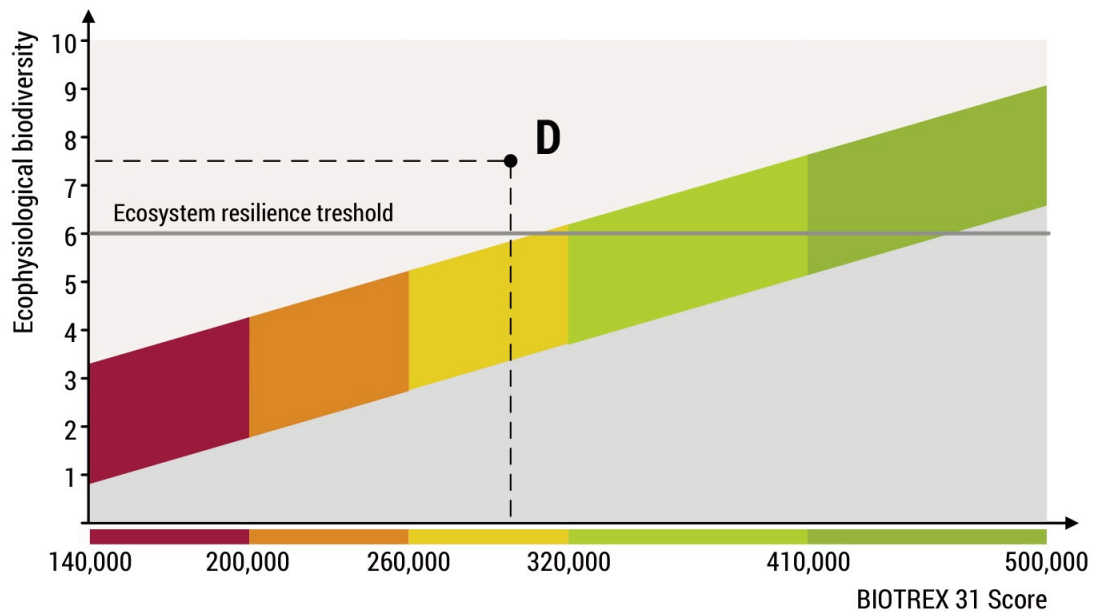
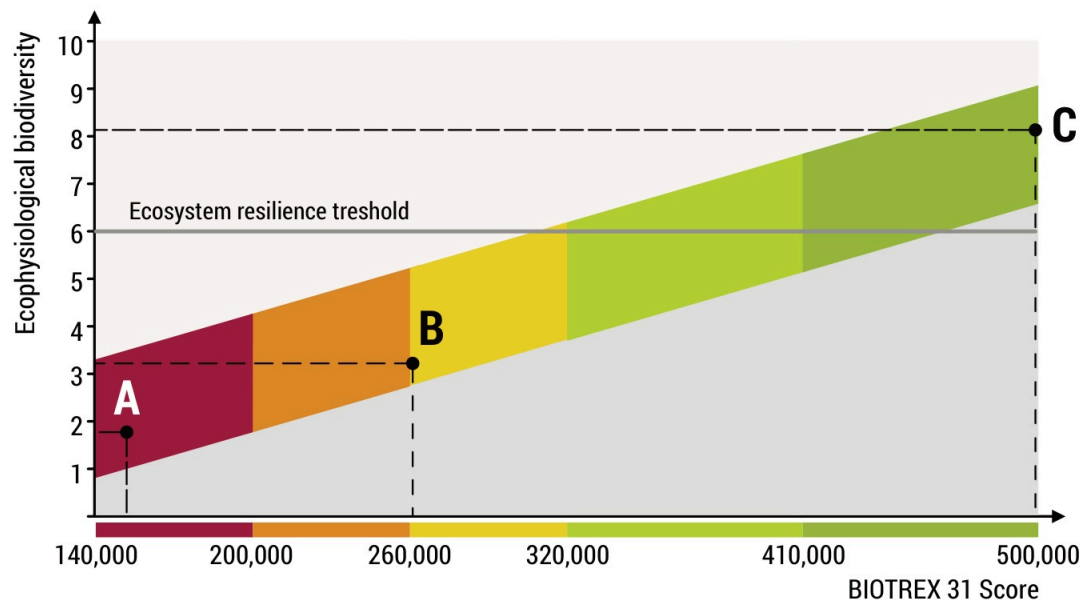
③ Unexpectedly low biodiversity

You can improve the biodiversity of microorganisms in the soil by introducing different sources of organic matter, such as compost, plant residues or green manure. Different organic materials may support different groups of microorganisms. Therefore, using diversified crop rotations and cover crops will help improve soil microbial diversity.

Expected biodiversity for different soil samples

	BIOTREX 31 Score	Eco-physiological diversity	Expected diversity
Sample A	153,097	1,81	expected
Sample B	261,295	3,21	expected
Sample C	506,656	8,10	expected
Sample D	306,377	7,52	higher

Of the samples shown, only sample D is outside the expected range of biodiversity. Since its biodiversity is higher than expected, we can see that in this case we should use methods to improve microbial activity. This is a kind of shortcut - we know that microbes can digest many different substances, so we just need to get them to do it faster! Samples A, B, and C all fall within the expected range of biodiversity - so we need to work on both activity and biodiversity. However, these actions will also be different. Sample A is at risk of degradation because the microbial life is really struggling. In this case, for example, adding large amounts of fresh organic matter won't work - it won't decompose. In sample C, which has a very high BIOTREX index, we need to be careful to provide enough organic matter for the thriving microbial ecosystem. Every field is different!



Remember, that soil biodiversity and activity are strongly correlated. When one grows, the other does too. Based on the BIOTREX index and eco-physiological biodiversity index you can, however, choose the best methods.

6. Substrates utilisation

The plate used in BIOTREX 31 consists of 32 wells, each containing a different set of substances. We call it 31 because the 32nd well has no carbon source, only water. After 48 hours of incubation, the purple color in the wells indicates whether and how much metabolism has occurred in each well. This is the basis of the BIOTREX Index. We provide the photo of the plate for those curious to learn more about the microbiome of their soil. The causal relationships between events in the field and the rate of digestion of a particular substrate are quite complicated, so this type of data is really hard to interpret. However, we encourage you to take a deep dive. There are more than 500 scientific publications on the eco-physiological profiles of different soils tested with this plate design, so the body of knowledge is vast and growing. In the future, we hope to see targeted biostimulants, microbial deficiency diagnostics, and plant-specific profiles, but this is an industry-wide effort for the near future of soil regeneration.

Substrates utilisation pattern (after 48 hours)

Reaction intensity:

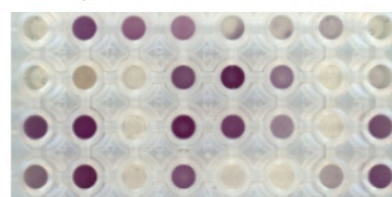
-  No - very low
-  Low
-  Moderate
-  High

Substrates groups:

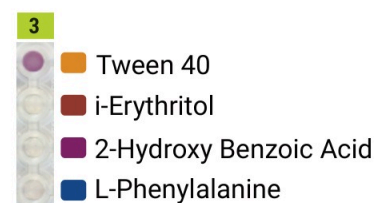
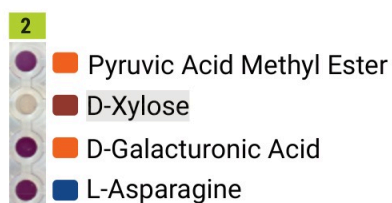
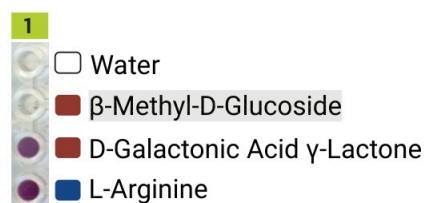
(sources of carbon, nitrogen, and phosphorous)

- | | |
|--|--|
|  Carbohydrates |  Amino Acids |
|  Carboxylic Acids |  Amines and Amides |
|  Polymers |  Phenolic Compounds |

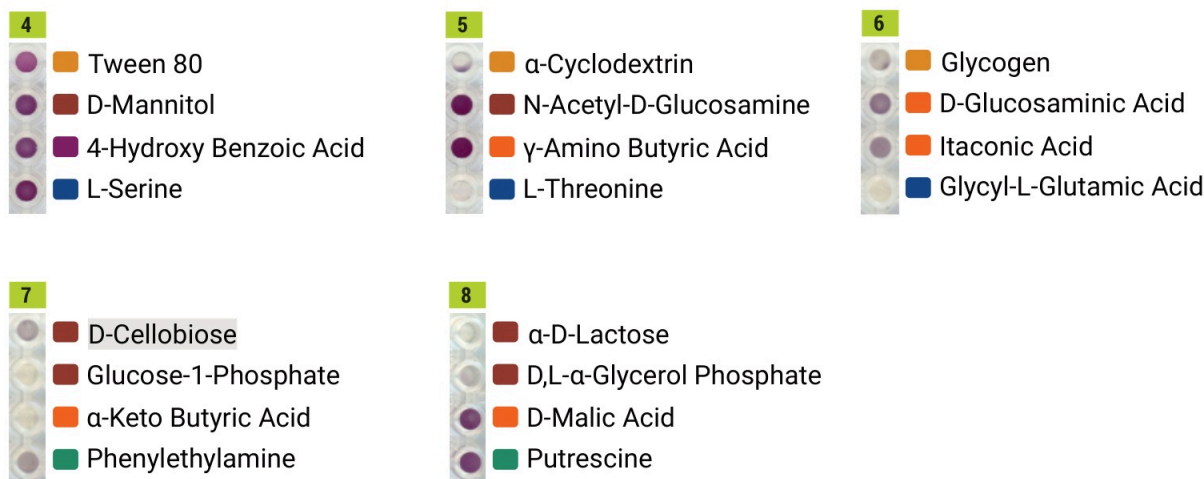
Sample B



column number 1 2 3 4 5 6 7 8



 Substrates for selected enzymes related to plant residues decomposition: **Cellobiohydrolases, β-Glucosidases, Xylanases**



7. Recommendations

Leveraging our extensive understanding of agriculture, regenerative practices, soil microbiology and microbial biodiversity, and armed with data from over a thousand different soil samples, we've been able to construct a dynamic set of practices tailored to your specific BIOTREX score and the eco-physiological biodiversity dynamics of your soil.

To understand these recommendations, it's important to understand the implications of the BIOTREX score. This metric serves as a marker of your soil's microbial performance - the higher the score, the more active and diverse your soil microbiome is. However, this also indicates an increased rate of organic matter decomposition and a greater need for nutrients to support this active microbiota. Therefore, your choice of management practices should be strategically balanced to maintain this microbial diversity and activity while meeting its nutritional needs.

Your farming practices, derived from both traditional agricultural knowledge and the BIOTREX score, will serve as a roadmap for creating a sustainable and resilient farming system. While a high BIOTREX score indicates faster decomposition, it also indicates increased nutritional needs of the microbiome. This calls for regenerative practices such as cover cropping, organic amendments, and reduced tillage, which can increase soil organic matter and, in turn, meet the nutritional needs of a thriving soil microbiome.

The following recommendations are tailored to provide the most effective results for your unique soil parameters. They represent the latest knowledge in regenerative agriculture and soil microbiology to promote soil health and farm productivity.

Please note that these recommendations are not static. As our understanding of soil science deepens with each research and development project, we continually refine these guidelines to reflect the most current, evidence-based practices. We remain committed to translating research into actionable knowledge that can improve the health and productivity of your soil.

Group 1

Practices to improve biodiversity

Crop rotation

Alternating crops in a specific sequence helps break disease cycles, encourages diverse microbial populations, and improves overall soil health.

Composting

Adding compost to the soil introduces beneficial microbes and organic matter, supporting a thriving soil food web.

Cover cropping

Planting a diverse range of cover crops helps increase soil biodiversity by providing different root structures, exudates, and organic matter for various soil organisms.

Mulching

Applying organic mulch to the soil surface provides habitat and food sources for various soil organisms, fostering biodiversity.

Hedgerows and windbreaks

Planting native vegetation around fields creates wildlife corridors and encourages beneficial organisms to thrive.

Inoculation

Introducing beneficial microorganisms through inoculants or compost tea can stimulate soil biodiversity and nutrient cycling.

Biological products

With proper care and good choice, you can introduce additional, strong microorganisms to your ecosystem.

Avoiding overuse of pesticides, herbicides, fungicides

Reducing the use of various chemical protective agents might help preserve soil biodiversity by preventing harm to soil microorganisms.

Group 2

Practices to improve microbial activity

Balanced organic fertilisation

Providing essential nutrients in the right proportions enhances soil activity and promotes nutrient cycling.

Reducing synthetic fertilisation

If it is possible, reduction of synthetic fertilisation can improve soil microbial activity by making the microbiome "work" harder to get their share of nutrients. This might seem counterintuitive, but is proven quite effective in boosting microbial performance.

Conservation tillage

Minimizing soil disturbance through reduced or no-till practices preserves the habitat and structure for soil organisms.

Crop residue management

Incorporating crop residues into the soil increases organic matter, stimulating microbial decomposition.

Soil structure improvement

Enhancing soil structure with amendments like gypsum or organic matter improves air and water movement, benefiting soil organisms.

Soil moisture management

Maintaining optimal soil moisture levels supports microbial activity and nutrient availability.

Aeration

Loosening compacted soil through aeration improves oxygen availability, benefiting soil microbes and root health.

Biostimulants

Applying algae, aminoacids, biochar, or other stimulating products can boost soil activity.

Humic acids

Though quite expensive, humic acids are a great source of readily available carbon for the soil microbes, boosting activity quickly and effectively.

Group 3

Practices to improve both activity and biodiversity

Crop diversity

Planting diverse crop species in the same area encourages a wide range of soil organisms to thrive, boosting both biodiversity and activity.

Cover cropping with legumes

Utilizing leguminous cover crops fixes nitrogen in the soil, benefiting both biodiversity and soil activity.

Integrated Pest Management (IPM)

Employing IPM strategies reduces the reliance on chemical pesticides, preserving soil biodiversity, and enhancing activity.

Managed grazing

Properly managed grazing by livestock can improve soil health, increase biodiversity, and enhance nutrient cycling.