

What is ACE Protein?

Autoclave-citrate extractable (ACE) protein is an indicator of protein and protein-like substances in the soil. These sources of organic nitrogen (N) can be converted into plant-available forms relatively quickly. The ACE protein test is a good indicator of soil health because it responds readily to changes in management practices.

Importance of soil protein

- Soil protein is the largest fraction of organic N in soil. It represents the type of N in living and decaying microbes, plants, and animals. Proteins are degraded to amino acids that are further decomposed to release ammonium (NH_4^+), a form of N that is readily available to plants and soil organisms throughout the growing season.
- Soil protein improves soil aggregation, which improves microbial habitat, water retention and infiltration, nutrient cycling and fertility, and aeration.
- Higher soil protein levels are associated with higher yields across different crops and management systems.

What affects soil protein?

- **Organic matter:** Organic matter contains soil protein; therefore, as organic matter increases, there is typically an increase in soil protein.
- **Soil biodiversity:** Soil microscopic organisms, including bacteria, fungi, and microinvertebrate grazers, can improve ecosystem functions such as nutrient cycling and decomposition.
- **Soil texture:** Coarse, sandy soils tend to have greater leaching, while clay soils often have greater runoff, both of which can decrease soil organic matter content and therefore protein and N. Loamy, silty soils tend to have the greatest organic matter and protein content.
- **Climate:** In moist, warm climates, organic matter decomposes faster, which may reduce soil protein content. Prolonged soil moisture, however, will slow decomposition.

Basic protocol for ACE protein index

- Air-dried homogenized soil, sized < 2 mm, is weighed into glass tubes. Sodium citrate buffer is added, and the tubes are shaken to aid aggregate dispersal.
- The soil solution is autoclaved (high temperature) to aid in the dispersal of proteins into the solution.
- A small aliquot of soil slurry is centrifuged to settle soil particles and clarify the solution. The clarified extract is transferred to a microplate, and a standard colorimetric protein quantification assay is performed.

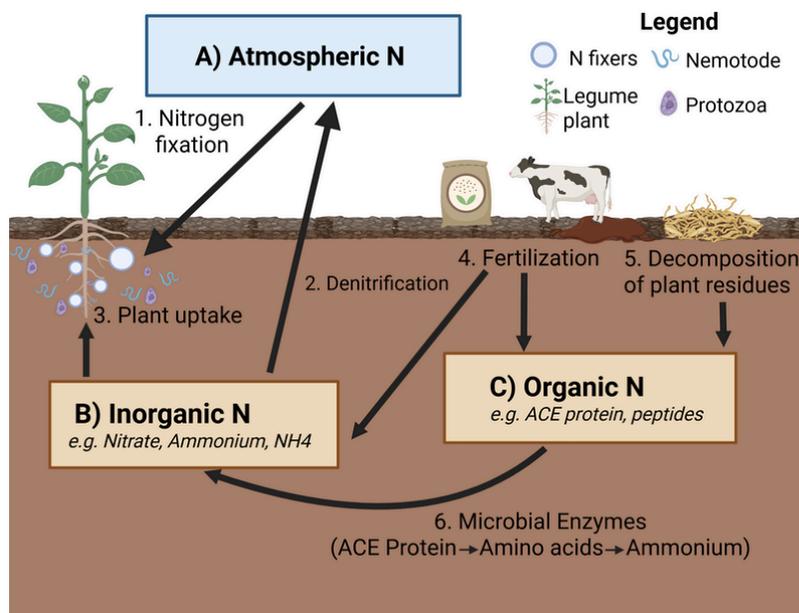


Figure 1: Pools of soil nitrogen (N): A) Atmospheric N: Dinitrogen (N_2) gas; B) Inorganic N: Ions that are available for plants (nitrate and ammonium); C) Organic N: N bound within carbon-based structures from decaying organic matter in the soil (e.g., proteins, amino acids, peptides, amino sugars). **N transformations:** 1) *N fixation*: Atmospheric N_2 gas is converted to ammonium, mostly through industrial processes creating fertilizers (such as urea or anhydrous ammonia) or naturally by N-fixing microbes in the root nodules of legumes; 2) *Denitrification* is the opposite: microbes turn soil N into gaseous forms that return to the atmosphere. 3) *Plant uptake*: plant roots take up the N ions in water solution. 4) *Fertilization*: Applying N in the organic (compost or animal manures) or inorganic (nitrate and ammonium) forms that are available to plants. 5) *Decomposition*: Plant, animal, and microbial residues break down into smaller and smaller pieces that are converted into organic matter. Bacteria, fungi, and microinvertebrates do this. 6) Microbes produce enzymes that convert organic N into plant-available, inorganic forms. Created with BioRender.com

ACE protein index vs. inorganic N

Total N in the soil includes inorganic N (ammonium and nitrate) and organic N (Fig.1). While inorganic N is immediately available to plants, it dynamically changes with weather and plant uptake. The ACE protein index represents part of the organic N pool that may become available to plants throughout the growing season as microbes degrade the protein. Therefore, soil nitrate testing is best for managing fertilizer for the year. ACE protein, meanwhile, responds to long-term field management while maintaining sensitivity to track differences throughout the season and between management practices.

Understanding scoring

Greater soil protein is an indicator of organic N that is readily mineralizable into plant-available forms.

ACE protein values are reported in grams of extracted protein per kilogram of dry soil (g kg^{-1} or ppm). Higher ACE protein values are favorable and indicate a larger pool of readily convertible organic N for plant growth.

Samples submitted to SHREC will be used to inform interpretation of ACE measurements based on management practices, geography, and soil type. SHREC aims to have a Vermont-specific scoring system (high, intermediate, and low values) in place as soon as an adequate quantity of representative soils have been processed.

How to improve soil protein content

Practices such as those listed below increase organic residue and support microorganisms that can increase levels of soil protein.

- **Add and accumulate organic matter**, especially N-rich organic matter (e.g., manure, plant-residues, seed-meals) to support soil organisms responsible for making N plant available and to provide a source of protein they can convert to inorganic N.
- **Decrease soil disturbance**: Soil disturbance such as tillage or compaction can decrease organic matter and protein content, as well as harm soil organisms responsible for mineralization.
- **Increase crop diversity**: Incorporate crop diversity through cover crops, rotations, and/or intercropping.
- **Continuous living cover**: Living roots in the soil can preserve organic matter, feed microbes that make protein, and reduce soil protein and N loss.
- **Control erosion** to reduce runoff of soil protein and available N by implementing the practices above.

Legend

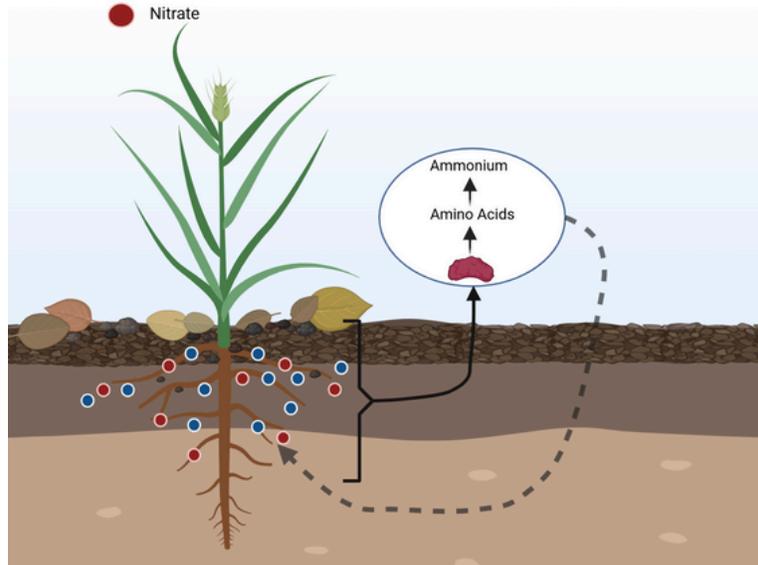
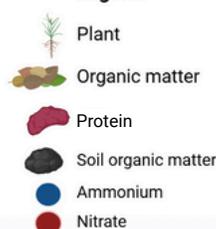


Figure 2: The transformation of organic N for plant uptake. Proteins in decaying organic matter are decomposed into their amino acid building blocks. Amino acids are further broken down into their components, which include NH_4^+ . NH_4^+ is a form that is available for plant uptake. Inspired by Sprunger and Martin (2023). Created with BioRender.com.

Sources

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