



Innovative measurement techniques for assessment of microbial activity respiration of PRIMAGRO in field conditions (17-706)

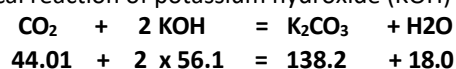
Introduction:

Soil health is the continued capacity of the soil to function as a vital agroecosystem. Soil health constraints beyond nutrient limitations and excesses currently limit agroecosystem productivity and sustainability, resilience to drought and extreme rainfall, and progress in soil and water conservation. AgroLiquid's goal is to help growers find economical solutions to optimize yield, strengthen soil biology, and provide application efficiency while creating robust, biologically- active healthy soils. Therefore, AgroLiquid formulated PRIMAGO™ line combine carbon, nutrients, and microbes to improve organic carbon and nutrients release.

During the summer of 2017, our research focused on testing PRIMAGO™'s proficiency in field conditions at the North Central Research Station (NCRS) to determine the effectiveness of PRIMAGRO line. Microbial respiration is an important indicator of microbial activity, that the level of increased microbial activity is an indicator of nutrients contained in the organic matter being converted to forms available to crops (e.g., phosphate as PO_4^{3-} , nitrate-nitrogen as NO_3^- , and sulfate as SO_4^{2-}).

Material and methods:

Following the method of CO_2 capture developed by Cornell University, we assembled a simple tool with a chemical method called a sealed PVC chamber of alkali trap respirometer, to measure the amount of soil respiration by trapping of carbon dioxide (CO_2) released as a waste product of microbes respiration. The tool tested in the Lab. under controlled conditions during the wintertime in NCRS and efficiently used during the summertime in our applied research fields. The measurement is based on the chemical reaction of potassium hydroxide (KOH) and carbon dioxide (CO_2) as following:



From the above equation, a 56.11 molar mass (g/mol) of KOH reacts with 44 molar mass of CO_2 to produce 138.2 molar mass of K_2CO_3 and release 18.01 molar mass of H_2O . As known in chemistry, the reaction is similar to an acid-base neutralization reaction, which is driven by favorable energy change. KOH is a strong base (alkali) and CO_2 is an acidic oxide. Therefore, the potassium hydroxide and carbon dioxide readily react to form salt and water.

The core of reaction application is that electrical conductivity (EC) of alkali (KOH) decreases as much as CO_2 captured, due to K_2CO_3 formation and release of water. Therefore, 20 ml of 0.5 M of KOH poured in a glass jar, hanged in the PVC chamber and left for 2, 3, or 4 days based on the weather conditions. The electrical conductivity (EC) of KOH were measured (in μS) to reference the values before the installation in the field (Figure 1).

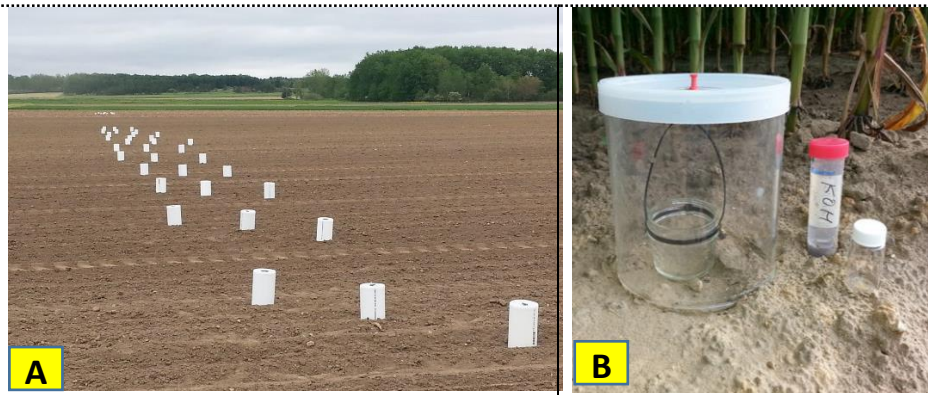
To validate the microbial content of PRIMAGRO in field conditions, and assessment the CO_2 release due to the microbial activity respiration. The experiment conducted under rainfed conditions with 5 treatments, as follows:

1. Treatment 1: a) Pro-Germinator + b) Kalibrate + c) Micro 500 and d) High NRG-N (at side dressing)
2. Treatment 2: a) PRIMAGRO P + b) PRIMAGRO K + c) Micro 500, and d) PRIMAGRO N (at side dressing)
3. Treatment 3: a) PRIMAGRO P + b) Kalibrate + c) Micro 500 and d) High NRG-N (at side dressing)
4. Treatment 4: a) Pro-Germinator + b) PRIMAGRO K + c) Micro 500 and d) High NRG-N (at side dressing)
5. Pro-Germinator + Kalibrate + Micro 500 and PRIMAGRO N (at side dressing)

Three replications, i.e., three PVC chambers were installed in each treatment of the study. Rates of application compromised 4 + 6 + 0.5 gal/A for a, b, and c consequently, and 51 gal/A High NRG-N (at side dressing) and 51 gal/A for PRIMAGRO N (at side dressing)

Corn planted at 30" between rows and at 6.6" within-row, and side-dressed on June 20, 2017.

Figure 1. Sealed PVC chambers of alkali trap respirometer as distributed in the entire treatments after planting the corn in NCRS (17-706) (A), and the prototype tested in the Lab. (B)



Results and discussion.

Measurements of CO₂ capture started on May 11, 2017, on May 17, 2017 straight after planting and continued up to July 20th, 2017. In each process of measurement, 20 ml of 0.5 KOH liquid added in the glass jars in each sealed PVC chamber, left in for the desired period of measurement. After that, the liquid poured back in vials and sent to the Lab., and EC measured to report the changes in EC due to the K₂CO₃ and water formation. The differences in EC presented the quantity of CO₂ captured in field conditions.

Following the measurements of EC in all the studied treatments, the data summarized and calculated per day of EC changes. Later, values of EC measured (in ppm or in μS) converted to (mS/cm), then converted to the decreasing of values of formulated K₂CO₃ molar weight, integrated with the E.C of standard 0.5 M of K₂CO₃ (in mS/cm), converted to molar weight, and calculated as actual molar weight of captured (CO₂) in ppm. Accordingly, we were able to standardize an equation to calculate the CO₂ released by soil microbial activity respiration in actual quantities, as follows:

$$y = 2E-05x^2 + 0.0045x + 0.0035$$

where:

y= actual loss of CO₂,

x= Mass concentration of K₂CO₃ (%) vs. solution molarity Molar concentration (in: c/mol L⁻¹).

Knowing that the carbon dioxide (CO₂) levels in the atmospheric air is around 400 ppm. Therefore, the measured values of CO₂ release are shown in Figure 3 in actual values.

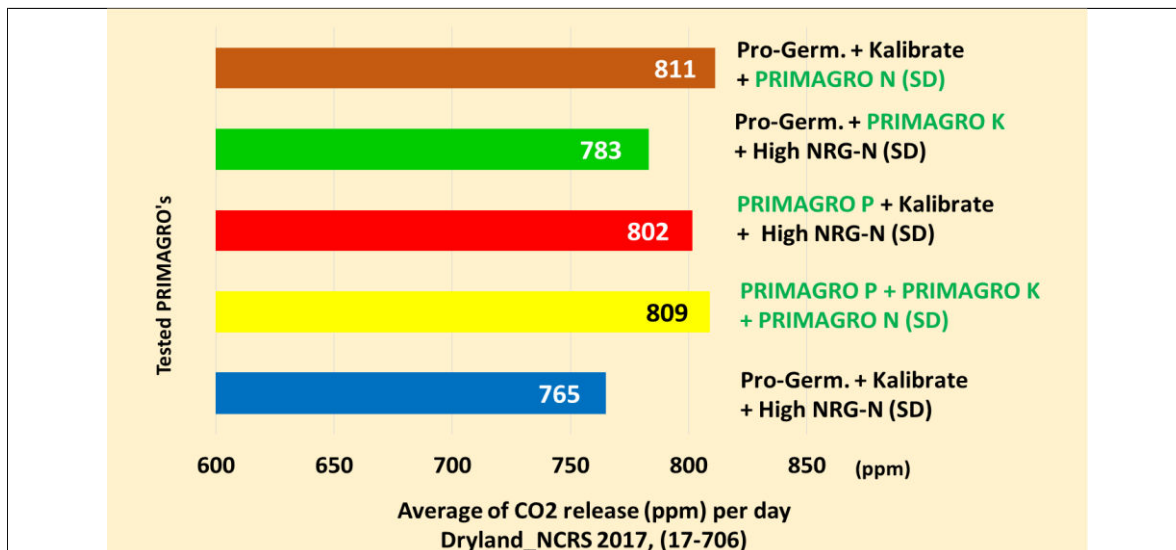


Figure 3. Measured the values of carbon dioxide (CO₂) released from soil following fertilizer application (17-706)

Figure 3, shows an active respiration function of bacteria (Genus: *Bacillus subtilis*, *Bacillus methylotrophic*) in PRIMAGRO P, K, and N resulted by higher values of CO₂ release, compared with the component in treatment 1 (Pro-Germ. + Kalibrate

+ Micro 500). That released values of CO₂ were higher (783, 802, 809 ppm) for treatments 4, 3, and 2 consequently compared with treatment 1 (765 ppm). However, the value of side dressing of PRIMAGRO N, in June 20th, 2017.

To understand the dynamic function of PRIMAGRO N, P, and K, soil solution was suctioned by micro-lysimeters (type: 10 RHIZON SMS. See detailed study on the microlysimerts method of measurement in 2015 NCRS annual report). Three active sampling procedure were taken and samples were analyzed by Hany Lab., as part of the soil health analyses package. Figure 4, shows the values (June 24th, 2017) of water extractable carbon and nitrogen (ppm) released in soil water as impacted by active carbon and nitrogen cycling in soils at 6" depth. The Progermiantor + Kalibrate (treatment 1) showed a balanced ratio of (C:N = 0.8 /1.0%) while this ratio imbalanced to (0.49:1.0, 0.43:1.0, and 0.39:1.0 %) in treatment 3, 2, and 4 consequently (Figure 4).

The analyses showing lower values of decomposed carbon used by bacteria to build their bodies and higher nitrogen values released in the soil solution making more nitrogen available for plants uptake, a process speaks about an active immobilization-mineralization cycle by soil bacteria function at 6" in soil depth.

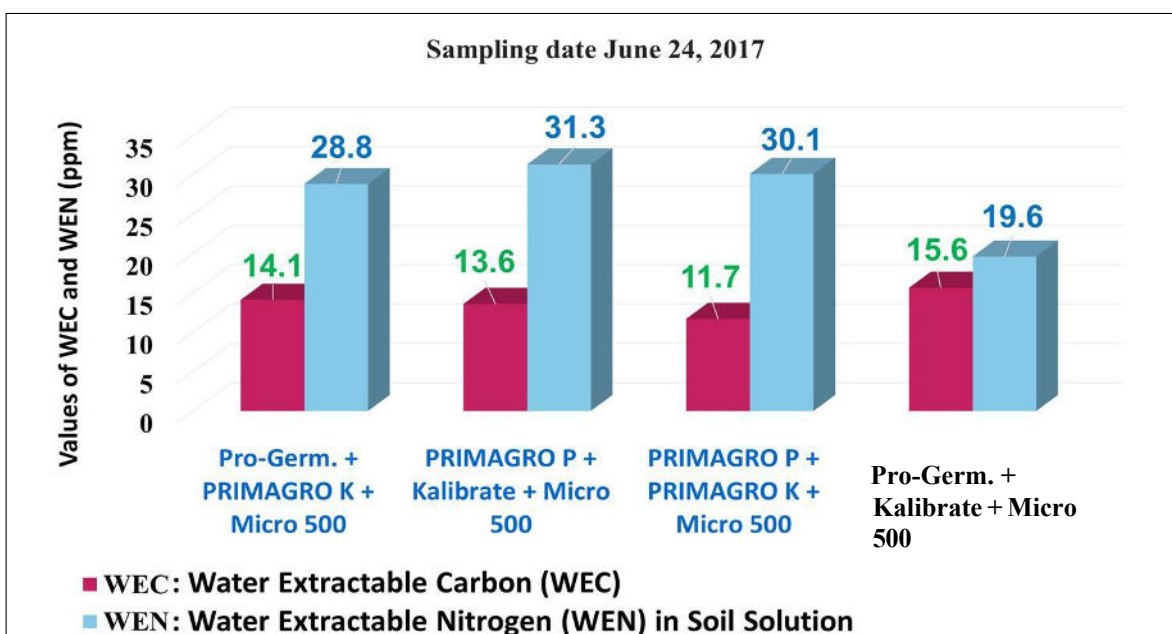


Figure 4. Values of water extractable carbon and nitrogen (ppm) releases in soil water as impacted by active carbon and nitrogen cycling processes in soils at 6" depth

Conclusion:

- The method of CO₂ capture developed by Cornell University and our simplified tool "the sealed PVC chamber of alkali trap respirometer, proved to be an innovative method valid instrument to capture the CO₂ released by microbial activity.
- PRIMAGRO line released higher values of CO₂ related to active soil respiration process compared to other non-microbial line (treatment 1: Pro-Germinator + b) Kalibrate)
- Application of PRIMAGRO line at side dressing reported higher aver values of CO₂ release and indicating the validity of application of PRIMAGRO line in the mid of summer where temperature, soil moisture, and soil rhizosphere are favorable for soil microbes to function
- Water-soluble carbon and nitrogen showed an active process of C and N flows in soil and promoting the concept of soil carbon and nitrogen turnover in soil promoted by microbial immobilization/ mineralization.