Examining Solvita Soil Tests in Soils of the Southeast United States

By

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Abstract

Nitrogen (N) an essential nutrient for healthy crop growth. Nitrogen fertilizer may be over applied because standard soil tests do not account for nitrogen mineralized by soil microorganisms throughout the growing season. Quantification of potentially mineralizable soil N by multiweek incubations is time-consuming, and highly variable with soil-type and other soil conditions. Other methods for measuring N mineralization such as CO_2 base trap titrations, or analysis via gas chromatography are also not fit for high volume use. The Solvita soil tests are a commercially available alternative that require only a 24-hour incubation period and minimal soil, where evolved CO_2 or labile amino soil nitrogen is directly correlated to the quantity of N mineralized. However, the Solvita tests have not been widely examined in some soils and cropping systems, especially in the southern United States. The objective of this project was to conduct incubations, titrations, and gas analysis on sets of soils from: 1) fields throughout the state of Alabama managed under a variety of cropping systems, and 2) the Cullars Rotation at Auburn University, and to compare N mineralization results from those measures to that predicted via Solvita testing. In two separate studies initial Solvita data was collected, and subsequent titration or gas chromatograph data was collected to assay the utility of the Solvita method to predict inorganic N release from mineralization. Correlation between gas chromatography, base trap titration, Solvita methods, and inorganic nitrogen varied from study to study. However, the Solvita methods were consistently well-correlated to inorganic nitrogen content at the end of the incubation period.

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Literature Review

Importance of Nitrogen

Nitrogen (N) is an essential macronutrient for all agronomic crops due to its structural role in many vital plant molecules. Photosynthetic pigments chlorophyll A and chlorophyll B require N as part of their central framework (Conant et al., 1931a; Conant et al., 1931b). Nitrogen is also a key component of amino acids and nucleic acids which form proteins and DNA, respectively. With plenty of N, plants will have a healthy root to shoot ratio, adequate cell turgor, and fruits and seeds rich in protein (Maathuis, 2009). Without adequate nitrogen, row crops will produce reduced yield of a lower quality (Boquet et al., 2009; Moreira et al., 2015). Because nitrogen is highly mobile in plants, older leaves show more severe symptoms of a N deficiency than newer ones. Leaves will turn a yellow color and show stunted growth.

There are two broad classes of N found in soil: organic and inorganic. Organic nitrogen accounts for 97-98% of total soil nitrogen (Bremner, 1949). Organic nitrogen is incorporated into the soil through decomposing plant and animal material and broken down by soil microorganisms during mineralization to release inorganic nitrogen, available for plant uptake and use as nitrate (NO_3^-) and ammonium (NH_4^+). This conversion of organic N to plant-available inorganic N occurs through the process of mineralization and will be discussed further below.

Mineralization

Mineralization is composed of three steps: aminization, ammonification, and nitrification. During aminization, heterotrophic organisms digest organic matter to initiate nitrogen release as amines. Amines are further decomposed in the next step, ammonification, by a biologically diverse range of microbes to generate ammonium. The next step is nitrification. While

nitrification is often treated as a singular process, there are actually two distinct steps. First, ammonium-oxidizing bacteria convert ammonium to nitrite, and next nitrite-oxidizing bacteria convert nitrite to nitrate. Ammonium-oxidation, the rate limiting step of nitrification, is performed by members of *Nitrosomonas* (Gee et al., 1990). Most ammonium oxidation ceases at a 5.2 – 5.6 pH range (Molina, 1985). Ammonium oxidation peaks at approximately day 20 of the lag phase. The lag phase is the period of soil bacterial cycling after the initial exponential population burst in which the population remains constant. The second part of nitrification, the conversion of nitrite to nitrate, is performed by members of *Nitrobacter*. Until the mid-1990s, these organisms were the only ones thought to participate in the nitrifying process (Jetten et al., 2009). Examination of wastewater treatment systems in the 1990s yielded significant insights into the diversity of nitrifying organisms. According to Hiorns et al. (1995) *Nitrosospira* also have ammonium-oxidizing capabilities. *Nitrospira* have nitrite-oxidizing capabilities but play a much larger role in wastewater treatment systems than in agricultural systems (Burrel et al., 1998; Juretschko et al., 1998). Like *Nitrosospira* and *Nitrospira*, anaerobic ammonium oxidation (anammox) bacteria were discovered in wastewater (Mulder et al., 1995).

Plants can absorb three kinds of nitrogen: nitrate, ammonium, and amines (Daniel-Vedele et al., 1998). Nitrate is the most common form of inorganic nitrogen both found in soil and taken up by plants. Nitrate is highly mobile in the soil because of its negative charge and can easily be leached out of the soil profile, making it irretrievable by plant roots. In conventional agricultural systems, between 4 and 107 kg N ha^{-1} is lost to leaching each year (Di and Cameron, 2002). The quantity of nitrate leached is influenced by soil texture, management practices, and environmental conditions. As soils become coarser, the risk of nitrate leaching increases (Hergert, 1986; Peng et al., 2015). Irrigation increases nitrate leaching. When compared to

nonirrigated corn systems, irrigated corn systems saw an increased nitrate loss of 29 to 112 kg ha^{-1} per year (Timmons and Dylla, 1981). The greater the moisture content of the soil, the greater the risk of leaching freshly mineralized nitrate. For this reason, nitrogen testing is particularly difficult in hot, humid areas like Alabama.

Factors Influencing Mineralization

Nitrogen mineralization in a soil fluctuates with moisture, soil temperature, soil texture, level of soil disturbance, and organismal interaction, and crop (Stewart, 1959). Soil moisture and temperature are often studied together because of their interactive effects (Zak et al., 1999). The greater the moisture content of the soil, the greater the mineralization rate, until saturation is reached (Miller and Johnson, 1964). Rahman et al., (2013) found that a soil moisture content between 75% and 100% of field capacity produced the greatest mineralization rate. Optimum soil temperature for nitrogen mineralization was 25 degrees Celsius (Knoepp and Swank, 2002). When the moisture content of a soil fell below 40% water holding capacity, high temperature compensated for moisture, and increased the mineralization rate (Guntiñas et al., 2012).

In other work, mineralization rate decreased with increased depth (Cassman and Munns, 1980). This was because the upper layers of a soil profile contained the most organic matter, and had a greater temperature. According to Hadas et al. (1986), the upper 60 centimeters of the soil profile was responsible for 66-77% of mineralization, or 120-330 mg N kg^{-1} over 32 weeks. This experiment was performed on Israeli soils with a range of climates, parent materials, and management systems.

Soils with a high clay content had a lower nitrogen mineralization rate than soils with a sandier texture (Cassity-Duffey et al., 2020). Protein molecules form complexes with clay

minerals such as kaolinite, bentonite, and smectite (Esterman et al., 1959; Harter and Stotzky, 1971). These bonds between the clay and protein restrict access and delay digestion by microbes. (Cassity-Duffey et al., 2020). To gain access to the bound protein, microbes must break down the bonds using specialized enzymes.

Management and crop rotation can be modified to enhance nitrogen mineralization. When soil is tilled, aggregates break apart and mineralization rate increases, but this effect is brief (Wyngaard et al., 2016). When studied over the entire crop season, no-till systems have consistently shown higher rates of mineralization, than that observed in conventional till systems (Muruganandam et al., 2009). In an experiment studying the mineralization rate of corn-soybean rotations, soil from corn planted in soybean residue was found to have a higher mineralization rate than that from soybean planted in corn residue (Fernandez et al., 2017). This was because soybean residue had a low C:N ratio, while corn had a high C:N ratio.

The ratio of mineralizable carbon to nitrogen (C:N) in the soil influences the rate of nitrogen mineralization. While carbon is typically abundant in the soil, nitrogen is not. When the C:N ratio falls below 12:1, mineralization occurs, and microbial populations dramatically rise (Agarwal et al., 1971). Microbes will continue to mineralize until free nitrogen is depleted, and the carbon to nitrogen ratio returns to 12:1 or higher. At that time, microbial populations diminish until there is another addition of nitrogen. Depending on the factors above, 1-3% of native mineralizable nitrogen in a soil can be converted to inorganic N each year (Keeney and Bremner, 1966).

Methods of Quantifying and Predicting Potentially Available Mineralizable Nitrogen

In-situ Tissue Sampling

The more available nitrogen in the soil, the more plants will take up (Cerrato and Blackmer, 1991). This inorganic nitrogen can be introduced to the system via mineralization or fertilization. While plants take up nitrogen in proportion with soil nitrogen, they also continue to absorb nitrogen past their requirement (Qiu et al., 2015). Over the course of the growing season, plants are repeatedly sampled and analyzed for nitrogen content, which is potentially correlated to soil nitrogen content. Leaf, stalk, and petiole N content have all been investigated to determine which is most accurate for prediction of future crop N use and yield (Moulin et al., 2012; Rauschkolb et al., 1974; Iversen et al., 1985). Tissue samples collected from potato petioles showed increased nitrogen content as nitrogen fertilization rate increased (Meyer and Marcum, 1998). However, the same study showed a significant interaction between irrigation rate and fertilization rate. Plants took up less nitrogen at lower rates of irrigation, meaning petiole N content was not a pure reflection of soil N content, and was often affected by plant water content.

In some cases, yield did not increase with elevated fertilization rate. For example, plots receiving 45 kg N $acre^{-1}$ had a yield of 17.6 kg $acre^{-1}$ while plots receiving 181 kg N $acre^{-1}$ had a yield of 17.5 kg $acre^{-1}$ (Meyer and Marcum, 1998). Analyzation of cranberry leaf N content at variable fertilization rates yielded similar results (Davenport and Provost, 2008). Leaf N content increased with fertilizer rate, but yield did not. Because of this phenomenon, studies have been conducted to generate nitrogen yield response curves for various crops that indicate the fertilization rate and leaf N content at which yield ceases to increase (Vos, 2009; Cerrato and Blackmer, 1991; Schmidt et al., 2002).

Despite some correlations between leaf N content and soil N content, in-situ studies have been deemed unusable for commercial practices due to their high expense and tendency to inflate nitrogen mineralization rate (Fox and Piekielek, 1984). To function as an accurate tool, a study must be designed for every field tested, and that study carried out for several years before it is calibrated as a true predictor. The labor and time this requires creates great expense. For these reasons, in-field sampling methods are often poor predictors for N fertilizer recommendations.

Soil Extracts for Nitrogen

Since the early 1900s, extractions have been used to quantify soil nitrate and ammonium content. Many extractants have been examined. Soil extraction with barium hydroxide greatly over estimated nitrogen in acid and slightly acid soils (Jenkinson, 1968). Other alkaline extractions using NaOH and $NaHCO_3$ + $NaCO_3$ also overestimated potentially available nitrogen (Prasad, 1965).

Today, the common soil extracts for N are potassium chloride (KCl), calcium chloride ($CaCl_2$), and water. While these methods can accurately measure soil N at a single moment in time, all one-time chemical extractions cannot account for the biotic element of mineralization (Curtin et al., 2006). For water, KCl, and $CaCl_2$ extractions, small samples of soil are shaken with a solution of each extractant (2M KCl and 0.01M $CaCl_2$) (Dou et al., 2000). In Chen et al. (2005), KCl extracted 39% soluble organic N, and 2.3% of total soil N. In the same study, water extracted 43% soluble organic N, and 0.7% total soil N. Other work confirmed KCl was a better extractor of total N than water (Inselsbacher, 2014). However, the two methods extracted similar amounts of inorganic N for a set of soils collected in Sweden (Inselsbacher, 2014). Potassium chloride extractable inorganic N was highly correlated to total soil inorganic N ($r = 0.91$) (Barret et al., 2002). Extractions with $CaCl_2$ have successfully been used to discern soil inorganic N

content distinctions in fields under different crop rotations (Barłóg et al., 2017). Potentially mineralizable nitrogen and soil microbial biomass are closely correlated to $CaCl_2$-extractable organic N (Szabó et al., 2014). Both $CaCl_2$ and KCl extractions were able to detect changes in inorganic N content with increasing depth into the soil profile (Dou et al., 2000). According to the same study, KCl overestimated NH_4^+ concentration by up to eight-fold compared to $CaCl_2$.

Gianello and Bremner (1988) proposed a method of chemical measurement that did not involve tedious extractions or time-consuming incubation. Instead, soil samples were steam distilled with a phosphate borate buffer for 8 minutes. Titration of the distillate provided a quantification of the nitrogen that might be mineralized during the growing season. Results were moderately correlated with methods for traditional, long-term disturbed soil incubation. However, like chemical extraction, this test was unable to account for the effects of climate and management, two of the greatest factors that affect agricultural soils.

Incubation

Incubation is the process of estimating a soil's nitrogen mineralization capacity by sampling soils under standardized conditions to eliminate mineralization variability based on environment. This allows a focus on the soil's native ability to mineralize N. Depending on the time frame of the experiment, anywhere from 5g to upwards of 200g of dry soil is rewetted to approximately 80% of field capacity and placed into a container (Maynard et al., 1983). Incubation may last from 5 days to 40-weeks. Each week soils are either leached or extracted to remove nitrate and ammonium from the soil, and concentrations of each determined.

Incubating disturbed soils artificially inflated mineralization rate (Rice et al., 1987). When soils were sieved or crushed, soil aggregates were broken apart. The nitrogen that was

protected within the aggregates was exposed, allowing it to be mineralized (Moberg et al., 2013). When both undisturbed and disturbed soils were incubated at room temperature (21.1°C), net mineralization increased by as much as 238% in disturbed soils with a sandy texture (Stenger et al., 1994). Thus, the intrinsic nitrogen mineralization capacity of the soil was overestimated. While incubation will always require some level of disturbance, incubating undisturbed soil cores gave more accurate results than that from disturbed soil incubation (Moberg et al., 2013).

While one-time extractions are not an accurate method to predict potentially available nitrogen, weekly extractions in combination with long-term incubations have shown excellent results (Wingeyer et al., 2015). A nitrogen release curve was developed by plotting time against nitrogen quantity, as a tool to predict supplemental nitrogen requirement over time. Studies like those of Fox and Piekielek (1984) performed repeated chemical extractions in combination with week-long incubations of disturbed soils. They had success in determining the peak time of release but overestimated the quantity of N released. They found there was no universal extractant that worked well on soils with different soil characteristics, microbial populations, and environmental conditions.

Mineralization during the incubation period can also be determined by measuring carbon dioxide production via titrations. This method was employed successfully by Haney et al. (2008a). Microbe-produced carbon dioxide was captured by a potassium hydroxide base trap and titrated at 1, 3, 7, 14, 21, and 28 days after incubation start (Haney et al., 2008a; Franzlubbers et al., 1996). The base trap was back-titrated with hydrochloric acid to attain the concentration of CO_2 originally in the trap (Anderson, 1982). Wennman and Kätterer (2006) used a similar procedure to measure the effects of temperature and moisture on the breakdown rate of sewage sludge. This study found that moisture greatly influenced the results from the procedure, proving

how important standard incubation temperatures and moisture levels are to producing reliable results.

As an alternative to titration, gas chromatography (GC) or infrared gas analysis (IRGA) can be used to directly measure carbon dioxide in the headspace of the incubation jar. First, rewetted soil samples are sealed in airtight jars. After an acclimation period in which CO_2 production is abnormally high due to disturbance, gas samples are taken at regular intervals through needles inserted into the septa of the incubation jars. This small hole allows for controlled amounts of air to be removed from the jar headspace. The gas analyzer provides CO_2 concentrations based on the samples provided. The greater the concentration of CO_2 in the jar, the greater the nitrogen mineralization occurred. Both of these gas analysis methods yield reliable results in approximately 24 hours (Bustamante and Hartz, 2016). The work of Haney et al. (2008a) found IRGA to be well correlated to other methods of CO_2 measurement ($r=0.79$), and that when compared to titration, gas analysis underestimated CO_2 production. However, this method was not suited to high-volume lab use because of the high cost, tediousness, and need for high density testing.

Autoclaved-citrate Extractable Soil Protein

Research has explored using soil protein as a quick indicator of potentially available nitrogen. Just as carbon mineralization rate is correlated to nitrogen mineralization rate, soil autoclaved-citrate extractable (ACE) protein, sometimes called Bradford reactive soil protein, is correlated to nitrogen mineralization capacity (Honeycutt et al., 1988; Halvorson and Gonzalez, 2006). The test was able to determine differences in N mineralization due to management, land use, and crop. In the ACE protocol, sieved, dried soil was combined with sodium citrate, shaken, and autoclaved. The resulting slurry was centrifuged and combined with Pierce BCA protein

reagent to cause color development. Samples of this mixture were measured using a spectrophotometer (Caudle et al., 2020). Compared with long term incubation, ACE was inexpensive, suitable for high volume laboratory use, and required limited special equipment (Hurisso et al., 2018). However, Geisseler et al., (2019) found that because of protein denaturing caused by the autoclaving process and overestimation of potentially available nitrogen, measurement of total nitrogen was a better indicator of potentially available nitrogen. That study also found the ACE protocol was most accurate on soils with a high native nitrogen content.

Illinois Soil Nitrogen Test (ISNT)

The Illinois Soil Nitrogen Test was first developed and described by Khan et. al in 2001 to predict mineralization under corn fields. Dried soil was treated with NaOH and heated to release ammonium and amino-sugar N as ammonia gas. The gas was captured and quantified via titration. The entire process took 6-7 hours as opposed to incubation, which took weeks. Performing the ISNT on 10 samples per field gave a representative average of the native mineralization output of the soil (Ruffo et al., 2005). The Illinois Soil Nitrogen Test is not affected by same year fertilization, making it a qualified tool to estimate native nitrogen mineralization release. However, soil samples must be taken from the upper six meters of the soil profile to account for the root length of corn plants.

One of the issues with measuring N mineralization rate and output is the variability based on environmental conditions. The Illinois Soil Nitrogen Test (ISNT) is an area-specific test designed to measure amino sugar nitrogen and ammonium nitrogen in cooler, dryer environments to predict inorganic nitrogen output from a soil (Osterhaus et al., 2008). With proper sampling technique, the Illinois Soil Nitrogen Test has shown success in predicting N mineralization in corn cropping systems. However, the high geographic calibration is both a

great asset and a strongly limiting factor. The ISNT has low applicability to other crops and areas of the country, disqualifying it for use in the southeastern United States.

Solvita

Drs. Brinton and Droffner began developing the suite of Solvita soil health tests to reduce the cost of evaluating soil respiration in 1994. Their original goal was to measure carbon cycling via measuring CO_2 evolution. Today, Solvita has a range of tests to measure biological activity of soils, stored organic nitrogen, carbon cycling, aggregate stability, maturity of composted products, and fungal respiration and spoilage of grains. These tools are often used as a measure of soil health standards. Each of these tests utilize easy-to-read color changing probes to indicate CO_2 produced (http://solvita.com/soillabtest).

During the development process, Brinton and Droffner linked CO_2 evolution to nitrogen availability. From this conclusion, three Solvita tests were developed: the Basal CO_2 test, the CO_2-Burst test, and the Labile Amino-Nitrogen (SLAN) test. Each test is a brief incubation lasting no more than 24 hours. Tests are designed to be used under normal conditions in any area of the world. These tests are not crop specific.

The Field CO_2 test (also called the Basal Respiration test) and the CO_2-Burst test are two methods of measuring CO_2 production. Both are used to indicate soil microbial activity and use a 24-hour incubation in a sealed jar with a color changing, gel-imbedded probe to measure CO_2 produced by microorganisms during the incubation period (Brinton, 2019a; Brinton 2019c). The quantity of CO_2 produced is directly correlated to the quantity of potentially mineralizable nitrogen (Rogers et al., 2019). The tests are capable of measuring CO_2 concentration in the jar air space between 0-3%. The Field CO_2 test is a simpler version of the laboratory CO_2-Burst test. In

this protocol, a fresh, roughly sieved (4mm) 40g soil sample is incubated with little to no prior processing. Results indicate the basal respiration rate under standardized conditions. In comparison, the CO_2-Burst lab test requires the samples be passed through a 2mm sieve and dried to 1-3% moisture. When soils are rewetted, microorganisms produce a burst of CO_2 that is quantified by the probe color transition. However, the vigorousness of sieving and degree of rewetting an influence the reliability of results (Brinton, 2020). For example, if soils are crushed prior to sieving, CO_2 production will be artificially inflated (Brinton, 2019a). Wetting soil past 70% WHC causes a decline in microbial activity (Rahman et al., 2013). Probe color can be read by the naked eye or by a digital color reader. When compared to the gas chromatography method commonly used to quantify CO_2 concentration in a headspace, results were highly correlated (McGowen et al., 2018).

While the Basal Respiration and CO_2-Burst tests measure evolved CO_2 as a means of estimating potentially available nitrogen, the SLAN test is comparable to the ISNT. Both tests use NaOH to liberate PON from soil (Brinton 2019b; Moore et al, 2019a). The SLAN test is a measure of the labile amino nitrogen that could be mineralized in the soil profile, making it a chemical, and not biological test.

The accuracy of these tests has been studied in cool, turfed environments that experience less leaching and volatilization than in the humid southern United States (Moore et al., 2019a; Moore et al, 2019b; Moore et al., 2019c; Moore et al., 2019d). These studies found that Solvita CO_2-Burst results were highly variable, causing low correlations between Solvita results and individual indicators of turf quality. Increased soil activity did not correlate with improved turfgrass quality. However, overall quality responded positively to CO_2-Burst results. A similar

result was observed between quality and Solvita SLAN results, but correlations were higher between indicators.

Solvita respiration tests have been shown to be well correlated to the standard methods of base-trap titration, gas analysis, and anaerobic incubation for measuring CO_2 output and mineralization rate (Haney et al., 2008a; Alvarez-Campos and Evanylo, 2019). Solvita CO_2-Burst is well correlated to both Total N ($r = 0.66$) and N mineralized over a 28-day incubation period ($r = 0.91$) (Alverez-Campos and Evanylo, 2019; Haney et al., 2008b). However, one study found that Solvita CO_2-Burst was poorly correlated to inorganic N content ($r = 0.18$) (Alverez-Campos and Evanylo, 2019). This was likely due to the transient nature of inorganic N.

As of now, most work determining the efficacy of the Solvita soil testing system has been performed in the northern United States. Before Solvita can be adopted for country wide use, the accuracy of the tests must be evaluated in warm, humid climates. The humid, sandy soils of the southeastern United States exhibit high nitrate loss, further complicating the issue of measuring nitrogen mineralization. Solvita must be both compared against the traditional methods of incubation and in-field studies and tested to see how precisely the system differentiates between paired fields under different management. The goal of this study was to compare inorganic N extraction results to Solvita CO_2-Burst, Solvita SLAN, Solvita Basal Respiration, gas chromatography, and base trap titration results to determine which method best predicts inorganic N evolution over the incubation period.

Materials and Methods

Two different studies were conducted, each repeated in time. Specific details will follow, but general information is supplied here. The first study was the "Alabama Wide Study" in which 13 soils were selected from a wide range of soil types and cropping systems. The second study was the "Cullars Rotation Study" which utilized soils from 4 distinct cover crop/fertilizer treatments within the 107-year old Cullars Rotation. In both studies the Solvita methods were compared to CO_2 evolution via gas chromatography, base trap titration, and 2M KCl extractable NO_3-N and NH_4-N. Specific methods follow.

Alabama Wide Study

Soils for the Alabama Wide Study were chosen to reflect a range of management strategies and soil physical properties. The goal of this study was to measure the burst of CO_2 produced after soil rewetting and determine the efficacy of this measurement in predicting nitrogen mineralization. Thirteen soils were selected in total and collected in spring of 2021. Soils were sampled to a depth of 15 centimeters, passed through a 2mm sieve, dried at room temperature (21.1°C), and stored in a cold room at 5°C until ready for testing. A sample of each soil was analyzed for background soil analysis. Collection location, background characteristics, and treatment information for each soil can be found in Table 1.

Four methods were used to evaluate these soils for differences in nitrogen mineralization capacity: 1) Solvita CO_2-Burst (Brinton, 2019a), 2) Solvita Soil Labile Amino Nitrogen (SLAN) (Brinton, 2019b), 3) gas chromatography (Thompson, 1977), and 4) base trap titration (Haney et al., 2008a). The Solvita methods were rapid tests that yielded results within 24-hours (Brinton, 2019a; Brinton, 2019b). The gas chromatography and base trap titration methods were the

traditional methods for evaluating soil activity (Haney et al., 2008a). Results from these methods were compared to inorganic nitrogen extraction data taken throughout the 7-day incubation period to determine which method best correlated with measured inorganic nitrogen evolution. Details of each method are explained in detail below. This study was conducted twice in its entirety, creating two Runs of data for each method.

Solvita CO_2-Burst

Solvita CO_2-Burst incubations were performed for each of the thirteen soils following the Solvita CO_2-Burst protocol provided by Woodsend Laboratories (Brinton, 2019a). There were four replicates of each soil. Instructions were followed exactly, as deviations can affect results (Franzlubbers, 2020). Each incubation was prepared as follows. A 30cc sample of soil was measured into a clean 50mL beaker and evenly rewet with 9mL of deionized water. A Solvita color-changing CO_2 detection probe was inserted into the soil-filled beaker. The soil-filled beaker was sealed inside an air-tight 475mL Solvita Standard Jar. The jar was left to incubate undisturbed at a constant temperature of 21.1°C for the 24-hour incubation period indicated in the CO_2-Burst protocol (Brinton, 2019a). At the end of the incubation period, the jar was unsealed and the color-changing probe removed. The probe color was read using the Digital Color Reader (DCR) colorimeter on the CO_2-Burst setting (Brinton, 2019a). The DCR illuminated the color probe and measured the resulting wavelengths of light-emission (Brinton, 2019d). The color of the probe was used to calculate the CO_2 concentration in the jar headspace. The CO_2 concentration and color for each probe was recorded (Brinton, 2019a). This experiment was completed twice in its entirety.

Solvita Soil Labile Amino Nitrogen

Solvita SLAN incubations were performed for each of the thirteen soils following the Solvita SLAN protocol provided by Woodsend Laboratories (Brinton, 2019b). There were four replicates of each soil. Each incubation was prepared as follows. A 4g sample of soil was measured into a clean 50mL beaker and treated with 10mL of 2N NaOH. A Solvita color-changing NH_3 detection probe was immediately inserted into the beaker. The beaker was sealed inside an air-tight 250mL Solvita SLAN Jar. The jar was left to incubate undisturbed at a constant temperature of 21.1°C for 24-hours. At the end of the incubation period, the jar was unsealed and the color-changing probe removed. The probe color was read using the Digital Color Reader (DCR) colorimeter on the SLAN setting (Brinton, 2019b). The DCR illuminated the color probe and measured the resulting wavelengths of light-emission (Brinton, 2019d). The color of the probe was used to calculate the mass of NH_3 in the soil sample. The mass of NH_3 in mg and color for each probe was recorded (Brinton, 2019b). This experiment was completed twice in its entirety.

Gas Chromatography

Gas chromatography incubations were performed for each of the thirteen soils, with four replications of each soil. To ensure Solvita CO_2-Burst and gas chromatography respiration results were directly comparable, each soil sample was measured and moistened according to the Solvita CO_2-Burst section above. The 30cc soil sample was sealed in an air-tight 950mL mason jar with a rubber septum installed in the lid. Additionally, there were four control jars with no soil inside. Soil was incubated undisturbed at 21.1°C. At 1 day, 3 days, and 7 days after the start of the incubation, 10mL air samples were extracted from the jars by inserting a syringe through the rubber septum. Air samples were expelled into evacuated, air-tight 5mL vials and stored until

analysis on a Shimadzu GC-14 gas chromatograph (Shimadzu Corporation, 1 Nishinokyo Kuwabara-cho, Nakagyo-ku, Kyoto 604-8511, Japan) (Thompson, 1977). The average CO_2 concentration in the empty jars was subtracted from the CO_2 concentration of the soil-filled jars to account for atmospheric CO_2. Volumetric concentration results from the gas chromatography analysis were adjusted to account for the difference in headspace volume between Solvita Standard Jars and mason jars. Lastly, the adjusted volumetric concentrations were converted to mass concentrations (A. Gamble- personal communication). This experiment was completed twice in its entirety.

Base Trap Titration

Base trap titration incubations were performed for each of the thirteen soils. There were four replications of each soil. To ensure Solvita CO_2-Burst and base trap titration respiration results were directly comparable, each 30cc soil sample was measured and moistened according to the Solvita CO_2-Burst procedure, as given previously. The soil sample was sealed in an air-tight 950mL mason jar along with a base trap containing 10mL of 1M KOH. Four control jars were assembled with no soil, only base traps. Soil was incubated undisturbed at 21.1°C. At 1 day, 3 days, and 7 days after the start of the incubation, the base trap was removed and back-titrated with 1N HCl to determine the amount of CO_2 absorbed by the base trap (Haney et al., 2008a). On days 1 and 3, the base trap was replaced and the soil continued to incubate until the end of the experimental period. The amount of CO_2 absorbed by the base trap between base trap replacements was recorded and used to calculate total evolved CO_2 at all three titration times. The average CO_2 concentration in the empty jars was subtracted from the CO_2 concentration of the soil-filled jars to account for atmospheric CO_2. This method was derived from Haney et al., 2008a. This experiment was completed twice in its entirety.

Inorganic Nitrogen Extraction

Inorganic nitrogen (NO_3-N and NH_4-N) extraction from the soil samples was conducted for each of the thirteen soils, with four replicates of each. To ensure respiration results and inorganic N results were directly comparable, each 30cc soil sample was measured and moistened according to the Solvita CO_2-Burst section, above. The soil sample was sealed in an air-tight 950mL mason jar and left to incubate undisturbed at 21.1°C. At 1 day, 3 days, and 7 days after the start of incubation 5g of each incubated soil was removed, placed in a 30mL scintillation vial, and extracted with 25mL of 2M KCl (Mulvaney, 1996). Vials were sealed and shaken for 1 hour. After removal from the automatic shaker, vials were allowed to settle before pouring the contents through a funnel lined with 40ug filter paper into a 20mL storage vial (Mulvaney, 1996). Extracts were analyzed for NO_3-N and NH_4-N concentration analysis on a μQuant microplate spectrophotometer (BioTek Instruments, Inc, P.O. Box 998, Highland Park, Winooski, Vermont 05404-0998 USA) (Sims et al., 1995). This experiment was completed twice in its entirety.

Cullars Rotation Study

Soils from the Cullars Rotation at Auburn University were chosen because plots have received the same treatments since 1914, creating a pronounced disparity in potential nitrogen mineralization capacity. Four distinct cover crop/fertilization treatments were selected (Table 2), with plots selected so that 3 “pseudo replicates” of each of the four cropping/fertilization treatments were evaluated. Thus, three plots (reps) of each of the 4 crop/fertilization treatments were sampled. Soils were sampled in the spring of 2020 to a depth of 15 centimeters, sieved, and stored in a sealed cold room at 5°C until ready for testing. Selected plots, background characteristics, and treatment information for each soil can be found in Table 2.

Three methods were used to evaluate these soils for differences in nitrogen mineralization capacity: 1) Solvita Basal Respiration (Brinton, 2019c), 2) gas chromatography (Thompson, 1977), and 3) base trap titration (Haney et al., 2008a). The Solvita method was a rapid test that yielded results within 24-hours. The gas chromatography and base trap titration methods were the traditional method for evaluating soil activity (Haney et al., 2008a). Results from these methods were compared to inorganic nitrogen extraction data taken throughout the 28-day incubation period to determine which method best correlated with measured inorganic nitrogen evolution. Details of each method are explained in detail below. This study was conducted twice in its entirety, creating two Runs of data for each method.

Solvita Basal Respiration

Solvita Basal Respiration incubations were performed for each of the four sampled treatments following the Solvita Basal Respiration protocol provided by Woodsend Laboratories (Brinton, 2019b). There were four replicates of each soil. Each incubation was prepared as follows. A 40g sample of moist soil was measured into an air-tight 475mL Solvita Standard Jar. A Solvita color-changing CO_2 detection probe was inserted into the soil and the jar sealed. The jar was left to incubate undisturbed at a constant temperature of 21.1°C for 24-hours (Brinton, 2019c). At the end of the incubation period, the jar was unsealed and the color-changing probe removed. The probe color was read using the Digital Color Reader (DCR) colorimeter on the CO_2-Burst setting (Brinton, 2019a). The DCR illuminated the color probe and measured the resulting wavelengths of light-emission (Brinton, 2019d). The color of the probe was used to calculate the CO_2 concentration in the jar headspace. The CO_2 concentration and color for each probe was recorded (Brinton, 2019a). This experiment was completed twice in its entirety.

Gas Chromatography

Gas chromatography incubations were performed for each of the four sampled treatments. There were four replicates of each soil. To ensure Solvita Basal Respiration and gas chromatography respiration results were directly comparable, each soil sample was measured according to the Solvita Basal Respiration section above. The 40g soil sample was sealed in an air-tight 950mL mason jar with a rubber septum installed in the lid. Additionally, four control jars were assembled with no soil inside. Soil was incubated undisturbed at 21.1°C. At 1 day, 7 days and 28 days after the start of incubation, 10mL air samples were expelled into evacuated, air-tight 5mL vials and stored until ready to be run on a Shimadzu GC-14 gas chromatograph (Shimadzu Corporation, 1 Nishinokyo Kuwabara-cho, Nakagyo-ku, Kyoto 604-8511, Japan) (Thompson, 1977). The average CO_2 concentration in the empty jars was subtracted from the CO_2 concentration of the soil-filled jars to account for atmospheric CO_2. Volumetric concentration results from the gas chromatography analysis were adjusted to account for the difference in headspace volume between Solvita Standard Jars and mason jars. Lastly, the adjusted volumetric concentrations were converted to mass concentrations (A. Gamble- personal communication). This experiment was completed twice in its entirety.

Base Trap Titration

Base trap titration incubations were performed for each of the four sampled treatments, with four replications of each. To ensure Solvita Basal Respiration and base trap titration respiration results were directly comparable, each 40g soil sample was measured according to the Solvita Basal Respiration section above. The soil sample was sealed an air-tight 950mL mason jar along with a base trap containing 10mL of 1M KOH. Additionally, four control jars were assembled with no soil, only base traps. Soil incubated undisturbed at 21.1°C. At 1 day, 7 days,

and 28 days after the start of the incubation, the base trap was removed and back-titrated with 1N HCl to determine the amount of CO_2 absorbed by the base trap (Haney et al., 2008a). On days 1 and 7, the base trap was replaced and the soil continued to incubate until the end of the experimental period. The amount of CO_2 absorbed by the base trap between base trap replacements was recorded and used to calculate total evolved CO_2 on all three titration times (Haney et al., 2008a). The average CO_2 concentration in the empty jars was subtracted from the CO_2 concentration of the soil-filled jars to account for atmospheric CO_2. This experiment was completed twice in its entirety.

Inorganic Nitrogen Extraction

Inorganic nitrogen extraction incubations were also completed for each of the four sampled treatments, with four replications of each. To ensure respiration results and inorganic N results were directly comparable, each soil sample was measured and moistened according to the Solvita CO_2-Burst section above. The soil sample was sealed in an air-tight 950mL mason jar and left to incubate undisturbed at 21.1°C. At 1 day, 7 days, and 28 days after the start of incubation 5g of each incubated soil was removed, placed in a 30mL scintillation vial, and extracted with 25mL of 2M KCl (Mulvaney, 1996). Vials were sealed and shaken for 1 hour. After removal from the automatic shaker, vials were allowed to settle before pouring the contents through a funnel lined with 40ug filter paper into a 20mL storage vial (Mulvaney, 1996). Extracts were analyzed for NO_3-N and NH_4-N concentration analysis on a μQuant microplate spectrophotometer (BioTek Instruments, Inc, P.O. Box 998, Highland Park, Winooski, Vermont 05404-0998 USA) (Sims et al., 1995). This experiment was completed twice in its entirety.

All statistical analysis for both studies was performed using SAS® on Demand for Academics. First, the PROC ANOVA statement was used to generate sample means and p-

values for the relationships between measurement methods. Duncan's multiple range test was used to group statistically different results for each sample or treatment. The statement was modified to evaluate result means based on the presence or absence of a treatment factor (cover crop, tillage, etc.). Interaction between treatment factors was evaluated at this time. Correlations were generated using the PROC CORR statement.

Results and Discussion

Alabama Soil Set

Data from the Alabama soil set was analyzed with Run as a treatment variable, for all collected data, to see if differences existed due to Run. For most datasets 'Run' was significant, and so results will be discussed by Run. Although the same collected soils were used for both runs, at some point the cold storage room failed, and soils were allowed to warm for a period of at least two weeks. This failure likely accounted for the differences between Runs. Additionally, although mg kg^{-1} is considered the 'most correct' unit of measurement, this thesis will use parts per million (ppm), as that is also accepted in scientific literature, and is the unit most often used to report Solvita data results. The relationship between mg kg^{-1} and ppm is 1:1.

Mineralized Inorganic Nitrogen

Two molar (2M) KCl extractable inorganic N (NO_3-N + NH_4-N, hereafter called 'N') for each Run as affected by treatment is shown in Tables 3 and 4. In Run 1, after 7 days of incubation, differences in N due to treatment were after variable, but there were some consistent responses. For example, if soils could be paired by the presence or absence of a winter cover crop (the WG, OR, HF, and N sites, NC vs. C) the majority of the time the soil with a cover crop had significantly more mineralizable N than the one without (Table 3). When averaged over soils that had been cropped with a winter cover inorganic N averaged 80.2 mg kg^{-1}, compared to an average of 51.2 mg kg^{-1} for inorganic N in soils that had never received a cover crop (Table 3). Previous work has also shown that the addition of various cover crops increased soil microbial activity and N mineralization (Chahal and Van Eerd, 2020; Holmes et al., 2019; Larkin, 2020). According to these studies, both species and species diversity played a role in the effectiveness of a cover crop in stimulating N mineralization. For example, a mix of 8 cover crop

species increased pepper yield and soil inorganic N content more than winter rye (*Secale cereale*)/hairy vetch (*Vica villosa*) or mustard (*Brassica juncea*) green manure alone (Larkin, 2020). However, when a variety of mixes of cover crops were compared to leguminous monoculture cover crops, the leguminous cover crop increased soil inorganic N content more than the mixes (Holmes et al., 2019). Field pea monoculture cover crop increased soil inorganic N content by 21.8% compared to a cover crop mixture with field pea and by 13.6% compared to a cover crop mixture with spring wheat.

In this study, cover crop had the greatest effect on inorganic-N content at the end of the incubation period, when compared to tillage or fertilization. Some differences in N due to tillage were also apparent, and soils with higher N were often from sites in which conservation-tillage had been practiced. In conservation-till soils inorganic N ranged from 58.0 to 122.3 mg N kg^{-1}, while in soils that had been tilled the range was 30.4 to 95.9 mg kg^{-1} (Table 3). Despite the fact that conservation tillage systems generally had higher inorganic N contents at the end of the incubation, the difference in inorganic N content between conservation and conventional tillage systems was not significant (Table 5).

The range in inorganic N due to tillage is also likely an artifact of the different types of tillage that were practiced. The degree of tillage was often not known in the fields from which soil was collected, so it is challenging to discuss inorganic N as a function of tillage (eg. Chisel versus no tillage, etc.). However, differences in soil inorganic N due to tillage have been well documented, with tillage practices that left the most surface residue increasing soil nitrate-N, with less of an effect on ammonium-N (Eghball et al., 1994). In our work, a decrease in inorganic N-content caused by tillage was found to be significant (Table 5). For example, the paired HF(NC) and HF(C) treatments showed a significant difference in inorganic N content at

the end of the incubation period (Table 3). The HF(NC) treatment, which included tillage, had an inorganic N content of 95.9 mg N kg^{-1} while the HF(C) treatment, which did not include tillage, had an inorganic N content of 122.3 mg N kg^{-1} (Table 3). It is challenging to make explicit statements about the effects of tillage when the no tillage treatments were almost always confounded by also having a cover crop. Still, in almost all cases tilled soil with no cover had lower inorganic N content. While the EVS and TU treatments were not paired, they exemplify the significant effect tillage has on inorganic N content. While the EVS treatment included tillage, the TU treatment did not (Table 3). The EVS treatment featured yearly cropping and harvest while the TU treatment featured turfgrass monoculture and year-round cover. The EVS treatment had an inorganic N content of 37.4 mg N kg^{-1} while the TU treatment had an inorganic N content of 65.0 mg N kg^{-1} (Table 3). While tillage certainly affected this, cropping history also had a profound effect.

Effects of fertilization were less obvious, with soils that had a history of fertilization having 7-day N contents of 30.4 to 122.3 mg kg^{-1}, and those that had not been fertilized having N contents of 58.0 to 65.0 mg kg^{-1} (Table 3). Average inorganic N over all soils that were not fertilized was 61.5 mg kg^{-1}, compared to 65.2 mg kg^{-1} for soils that were fertilized, an insignificant increase in inorganic N-content caused by fertilization (Table 5). The ephemeral nature of fertilizer N in the humid soils of the southeastern United States accounts for this lack of significance and is one reason that soil testing is not a standard for accounting for inorganic N in southern soil tests (Aparicio et al., 2008).

Similar results in inorganic N were also measured in Run 2 (Table 4). After 7 days of incubation, differences in N were highly variable, with the same treatment effects on inorganic N as observed in Run 1. In this Run average inorganic-N in soils that had cover crops (at 7 days)

was 67.6 mg g^{-1}, while average inorganic-N in soils that did not have cover crops was 46.6 mg g^{-1} (Table 6). The difference in inorganic N content was significant for Run 2 (Table 6). As in Run 1, cover crop had the greatest effect on inorganic N-content at the end of the incubation period, and the increase in inorganic-N content caused by cover crop was found to be significant. For example, the paired OR(NC) and OR(C) were identical except for the presence of a cover crop (Table 4). In this case, cover crop increased the inorganic N content of the soil from 36.7 to 54.1 mg N kg^{-1} (Table 4). However, both tillage and cover crop failed to cause a significant difference in inorganic N content for the paired N(C) and N(NC) treatments (Table 4). Many of the plots in this study had received the same treatment for over five years. For the N(C) and N(NC) treatments, it was unclear how long the treatments described in Table 4 had been applied. If the treatments had been applied for only a few years, differences in inorganic N content would be less apparent.

Generally, soils that received tillage had lower inorganic-N content than those that did not, with an average of 50.0 mg kg^{-1} in soils that did not receive tillage versus 70.5 mg kg^{-1} in soils that did (Table 6). In no-till soils N ranged from 44.9 to 126.8 ug g^{-1}, while soils that had been tilled the range was 25.7 to 99.3 ug g^{-1} (Table 4). The decrease in inorganic-N content caused by tillage was significant (Table 6). However, as for Run 1, no tillage treatments were almost always cofounded by also having a cover crop, making it difficult to say with absolute certainty which factor is responsible for the difference in inorganic N content. For example, treatment HF(NC) featured conventional tillage and no cover crop while treatment HF(C) featured conservation tillage and a cover crop (Table 4). These factors decreased the inorganic N content of the soil from 126.8 to 99.3 mg N kg^{-1} (Table 4). While the EVS and TU treatments were not paired, they exemplify the impact tillage has on soil inorganic N content. As in Run 1,

the EVS treatment featured yearly cropping of *Zea mays* while the TU treatment featured year-round grass mixture cover. The EVS treatment, which included tillage, had an inorganic N content of 34.1 mg N kg^{-1} at the end of the incubation period (Table 4). The TU treatment, which did not include tillage, had an inorganic N content of 44.9 mg N kg^{-1} at the end of the incubation period (Table 4). Both tillage and cover cropping affected these results.

As in Run 1, fertilization had a lesser effect on inorganic-N content. Soils with a history of fertilization had 7-day N contents from 25.7 to 126.8 ug g^{-1}, while soils with no history of fertilization had 7-day N contents from 44.9 to 51.7 ug g^{-1} (Table 4). The increase in inorganic N-content caused by fertilization was not found to be significant. The lack of differences due to fertilization are likely a function of the more temporal nature of soil fertility, as compared to increased N with years of cover cropping. Six years of cover cropping increased labile carbon and N, when compared to those without cover crops (Chahal and Van Eerd, 2020). However, the quality (C:N) of the cover crop had a strong effect on the amount of inorganic-N mineralized in the soil. The lower the C:N ratio of the cover crop residue, the greater the mineralization rate (Constantin et al., 2011). Long term cover cropping with low C:N ratio crops caused the greatest increase in pre sidedress nitrate when compared to other cover crops (Kuo and Jellum, 2000). Contrary to the results from this study, high fertilization increased the inorganic N content of the soil more than the integration of a cover crop over a two-year period despite the use of leguminous cover crops (Sainju et al., 2000). In comparison, many of the soils in this study had cover crops much longer than two years, giving the cover crop residue time to build up in the soil and increase mineralization.

As in this study, tillage has previously been found to significantly decrease N mineralization rate in soils over the long term (both an 8-year and 22-year period) (Kandeler et

al., 1999; Balota et al., 2004). These are long-term processes, as previous work often indicates that both cover cropping and tillage are more likely to have detectable effects when measured over a period of years rather than a period of months or weeks. In other work, only cover crop, not tillage, was found to have a significant effect on nitrogen mineralization in silage corn fields (Sainju and Singh, 2001). In that study, however, soil was only treated with tillage and cover for a 2-year period. The cover crop was a legume (*Vicia villosa*) which has already been shown to have a strong affect on soil inorganic N content (Larkin, 2020).

Relationships Between N and Secondary Methods of Measurement

Gas Chromatography Measurements of CO_2 Emission

Carbon dioxide evolution has long been a standard technique for measuring microbial activity as a function of N mineralization (Gilmour et al., 1985; Sheremata and Hawari, 2000). Thus, this was used as our 'standard technique', to compare to the rapid methods, which will be discussed later. Seven-day CO_2 emission data due to soil was variable, in both Runs (Tables 3 and 4). For example, soils from the Headland location (HF) had high rates of emitted CO_2 (compared to other soils), regardless of cover crop in Run 1, but in Run 2 that soil with long-term cover cropping emitted significantly more CO_2, when compared to the same soil without cover crops (Tables 3 and 4). Studies examining the effects of cover crop on microbial communities found that soil from plots with cover crops produced more CO_2 than soil from plots not treated with cover crops (Drost et al., 2020; Christiansen et al., 2015). The same study found CO_2 production was especially high in plots treated with legume or legume-mixture cover crops, as in our OR treatments. While CO_2 production was not significantly different between OR(NC) and OR(C) for Run 1, in Run 2 the OR(NC) soil produced only 31.4 mg CO_2-C kg^{-1} soil while the OR(C) treatment produced 91.4 mg CO_2-C kg^{-1} soil. This difference was significant.

In an effort to better examine differences due to underlying soil factors (tillage, fertilization, cover crops) a basic analysis of variance was performed to create means. These were "pseudo treatments" as soils were collected from different fields with varying tillage, cover crop, and fertilization regimes. So, this does provide a rough idea of "treatment" effects (Tables 5 and 6). Tables 3 and 4 are also available to show individual soil effects. Since there was no significant two- or three-way interaction between tillage, fertilization or cover crop main effects are shown in Tables 5 and 6. This helps to more clearly show differences due to those soil factors.

Only cover crop significantly affected gas chromatography results across both runs (Tables 5 and 6). Previous research indicated vetch (*Vicia villosa*) and barley (*Hordeum vulgare*) cover crops significantly increased CO_2 production measured by gas chromatography compared to fallow fields (Sanz-Cobena et al., 2014). Increased CO_2 output may be due in part to disturbing the soil to plant cover crops and incorporate residues. A study examining the effects of tillage and cover crop on CO_2 emissions in Portuguese vineyards found fields that received no tillage and resident vegetation as a cover crop produced less CO_2 than plots treated with tillage and no cover crop (Marques et al., 2018). Decreased CO_2 output in the field that received cover crop could be due to decreased soil disturbance. However, this difference was not statistically significant and it was impossible to separate the effects of tillage and cover crop on CO_2 production.

Carbon dioxide evolution, as measured by gas chromatography, significantly changed from day to day of measurement. Carbon dioxide production during the first 24 hours of incubation accounted for approximately half of the CO_2 produced over the entire 7-day incubation period. This agrees with the general concept of the Solvita CO_2-Burst test. In both

Runs there was a significant correlation between total inorganic N and CO_2 as measured by gas chromatography at days 1, 3 and 7 (Tables 7 and 8). The relationship was stronger at the 7-day reading, with significant r values of 0.86 and 0.70 for Run 1 and Run 2, respectively for the correlation between emitted CO_2 and total inorganic N (Tables 7 and 8).

The relationship between gas chromatography CO_2 concentration on day 7 and total inorganic N is plotted in Figures 1 and 2. Despite the strong correlations between CO_2 evolution and inorganic N evolution, the relationships were not significant for Run 1 or Run 2, a result of variability in the data. Previous research found that high mineralization rate corresponded to high CO_2 output over a 42-day incubation period (Shaaban et al., 2014). That same study found that CO_2 production was better correlated to NH_4-N concentration (0.561) than NO_3-N concentration (-0.090). Total N and evolved CO_2 had a direct relationship in a study which compared the CO_2 outputs from 8 soil samples taken in Iowa (Burford and Bremner, 1971). The Glencoe sample, which had the highest total N content, also had the highest CO_2 output as measured by a gas chromatograph.

While few studies have directly compared soil inorganic N content and CO_2 evolution, many have compared N fertilization rate and CO_2 evolution. As stated previously, fertilization did not significantly increase gas chromatography results from this study. This agrees with previous research, which showed fertilization either had no effect or a decreasing effect on CO_2 output over a short-term period (less than 4 years) (Sainju et al., 2010; Gagnon et al., 2016; Ni et al., 2012). A study utilizing soils from eastern Montana showed that a N fertilization rate of 80 kg N ha^{-1} caused no significant increase in CO_2 flux compared no N fertilization (Sainju et al., 2010). For soils selected from the province of Quebec in Canada, N fertilization with either $(NH_4)_2SO_4$ or KNO_3 significantly decreased CO_2 output in five out of nine soils (Gagnon et al.,

2016). Fertilizer solutions were injected into test soils, not incorporated, which decreased soil disturbance, limiting the ability of disturbance to confound N fertilization effects. In both planted (*Zea mays*) and unplanted fields N fertilization decreased CO_2 emission (Ni et al., 2012). However, the decrease was only significant for the unplanted plots.

Rapid Solvita® Measurements

The Solvita SLAN method had a better correlation to mineralizable N than that observed when the Solvita CO_2 burst method was used (Tables 7 and 8). Soil Labile Amino Nitrogen (SLAN) was strongly correlated to inorganic-N content on day 7 for both Runs ($r = 0.86$ for Run 1 and $r = 0.75$ for Run 2) (Tables 7 and 8). These relationships were significant for both Runs. In comparison, relationships between inorganic N and the CO_2-Burst method had lower r values of 0.68 and 0.69 for Runs 1 and 2 respectively, but they were still significant (Tables 7 and 8). Unlike the inorganic N data, the CO_2-Burst method was unable to detect differences when a cover crop was present (Table 5 and 6). In comparison, the SLAN method did detect such differences. Previous work has shown soil amino nitrogen content is significantly increased by cover crop and fertilization (Campbell et al., 1991). Soil Labile Amino Nitrogen is a chemical test that measures the amount of labile nitrogen available in the soil to be mineralized. Since it does not measure the biological activity of the soil, it is less affected by soil processing and incubation temperature. The SLAN protocol issued by Woodsend Laboratory indicates SLAN is less affected by temperature than CO_2-Burst (Brinton, 2019b). Several papers have indicated the sensitivity of the Solvita respiration tests to sample preparation and incubation (Franzlubbers, 2020). For example, crushing instead of sieving soil can inflate mineralization as can incubation at temperatures above typical soil temperatures (Brinton, 2020).

Total organic N has been shown to be an accurate predictor of nitrogen mineralization (Wang et al., 2001; Groot and Houba, 1995). Since labile amino nitrogen is a subset of total nitrogen, it is a strong indicator of potential inorganic N availability (Bremner, 1950; Campbell et al., 1991; Moore et al., 2019a). In other work, Solvita SLAN had a stronger, more significant relationship to a variety of yield quality measurements than that from Solvita CO_2-Burst (Guillard et al., 2014). For example, SLAN and mean NDVI had a correlation coefficient of 0.73 while CO_2-Burst and NDVI had a correlation coefficient of 0.34 (Guillard et al., 2014).

Base Trap Measurements of Evolved CO_2

For both Runs, the base trap titration method was the least accurate method for predicting N content at the end of the incubation period. Base trap titration was unable to detect the significant effect of cover crop on inorganic N content in both Runs (Tables 5 and 6). In Run 1, base trap titration measured a CO_2 content of 159.5 mg CO_2-C kg^{-1} soil for soils without cover crop and 205.9 mg CO_2-C kg^{-1} soil for soils with a cover crop, the opposite of the expected relationship (Table 5) (Nilahyane et al., 2020). A similar trend was observed in Run 2 (Table 6). Likewise, base trap titration results were unaffected by tillage, despite the fact that tillage significantly affected inorganic N content for both Runs (Tables 5 and 6). The HF soil exemplify the inability of base trap titration to detect the effects of cover crop and tillage as treatment factors. While conservation tillage and *Secale cereale*/*Trifolium pratense* mix cover crop in that HF soil significantly increased inorganic N content in both Runs, CO_2 evolution measured by base trap titration was not significantly increased (Tables 3 and 4).

Thus, CO_2 evolution as measured via base titration was not strongly correlated to total N at 1, 3 or 7 days after the start of the experiment (Tables 7 and 8). In Run 1 the relationship between base trap evolved CO_2 and total N had r values of 0.35 to 0.57, and in Run 2 those

values were 0.26 to 0.15 (Tables 7 and 8). While this study found the base trap titration method highly unreliable, many studies have used it to accurately discern nitrogen mineralization differences between soils (Wood et al., 1990; Burke et al., 1989). However, those studies measured over a 30-day incubation period, as opposed to the 7-day incubation used in this study. A study with a 28-day incubation period also found a strong correlation ($r = 0.87$) between base trap titration CO_2 and mineralized N (Haney et al., 2008b). Differences in accuracy between this study and previous work may be due to a difference in measurement period or accuracy of titration equipment. Measurement period is most likely not to blame, as similar problems were observed in the Cullars Rotation Study, which had a 28-day observation period.

Relationships Between Each Measurement Method

Overall, the laboratory procedure of gas chromatography was best for measuring CO_2 evolution from the various soils. This method was laborious, and used highly technical equipment, which required time and training for accuracy and precision. Since the Solvita methods did show promise for their ability to accurately measure N mineralization (over the 7 day period), the next step was to see how well data collected from the Solvita methods (SLAN and CO_2-Burst) were correlated with gas chromatography (at this point viewed as the 'lab standard').

Figure 3 illustrates the relationship between gas chromatography readings and the CO_2-Burst measurements for the means of all 13 soils used in the Alabama study, at one day after the incubations were initiated. In both Runs of the one-day measurements the relationship between CO_2-Burst and gas chromatography was not significant, but the methods were strongly correlated with r^2 values of 0.64 and 0.78, respectively. Other work found a similarly high, yet insignificant, r^2 value when comparing gas chromatography to Solvita CO_2-Burst over a 24-hour

period ($r^2 = 0.79$) (McGowen et al., 2018). Figure 4 illustrates the same relationship, but at the 7-day data collections. Correlation between gas chromatography and Solvita CO_2-Burst results remained strong ($r^2 = 0.77$ and 0.76 for Runs 1 and 2, respectively) and the relationship was statistically significant ($p < 0.01$) for both Runs. As with our work, other studies demonstrated Solvita CO_2-Burst measurements often had higher respiration values than those measured via gas chromatography (Doran et al., 1997; Warren et al., 2019). Similar results were observed with the 7-day data (Figure 4).

Figure 5 illustrates the correlation between the Solvita SLAN method and gas chromatograph readings at the 7-day data collections, for Run 1 and Run 2. To lessen the number of Figures Day 1 data is not shown. Both runs had a strong positive correlation between the Solvita SLAN and gas chromatography methods, 0.91 and 0.84, respectively (Figure 5). Both SLAN and gas chromatography have been established as accurate methods of estimating nitrogen evolution, but no previous literature examined the relationship between SLAN and gas chromatography (McGowen et al., 2018; Moore et al., 2019a; Moore et al., 2019c).

Figures 6 and 7 illustrate the unpredictable relationship between base trap titration and the gas chromatography and Solvita CO_2-Burst methods of measuring CO_2 evolution. Shown as an example is the Day 1 data for Run 2, which illustrates the lack of a relationship between base trap titration versus gas chromatography (Figure 6). Not only was titration poorly correlated to gas chromatography, but the two methods had inverse relationship. The base trap titration method required titration by hand, which is particularly vulnerable to human error. Multiple individuals in the laboratory were responsible for titrating samples. While these conditions could skew results, it is unlikely that human error is responsible for the large-scale inaccuracy of the base trap titration method. Inaccuracy is more likely due to the short measurement period.

However, a study that evaluated soil CO_2 evolution over a 24-hour period found that CO_2 measured by gas chromatography and base trap titration were not significantly different (Sherrod et al., 2012).

For Run 1, Day 1 base trap titration and Solvita CO_2-Burst were significantly related with a strong correlation ($r = 0.73$) (Table 7). A study comparing CO_2 output over a 24-hour period found a similar strong significant relationship between base trap titration and Solvita CO_2-Burst ($r = 0.91$) (Haney et al., 2008a). In contrast, Day 1 base trap titration and Solvita CO_2-Burst had a moderate negative correlation in Run 2 of the experiment (Table 8). No other study found a negative relationship between the two methods. As discussed previously, CO_2 evolution differences between Run 1 and Run 2 of the experiment may be due to a storage room breakdown. However, no other CO_2 measurement method was as strongly affected by Run (Tables 7 and 8). Little research has been published comparing Solvita CO_2-Burst and base trap titration methods.

The Cullars Rotation Data Set

As with the Alabama Wide data set, two experimental Runs were performed with the Cullars study soils. When 'Run' was included in the analysis of variance there were often differences in measured variables due to Run, and so for this thesis data will be shown and discussed as separated by Run. Such differences are likely due to the same issue as with the Alabama-wide soil set, as all were stored in the same cold room that failed.

Mineralized Inorganic Nitrogen

Extractable N, Solvita Basal Respiration, base trap titration, and gas chromatography results for each Run as affected by cover crop and fertilization are shown in Tables 9 and 10.

There was no significant interaction between cover crop and nitrogen fertilization. At the end of the incubation period for Run 1, significant differences in final inorganic N content were due to long-term inclusion of N fertilizer, not cover crop. While cover crop increased inorganic N content, the effect was not significant (Table 9). Plots receiving N had an average inorganic N content of 21.4 ug g^{-1} as compared to plots that did not receive N, which had an average inorganic N content of 17.3 ug g^{-1} (at the end of the 28-day incubation period) (Table 9). Previous research has shown nitrogen fertilization has a variable effect on mineralization rate. In agreement with our work, some studies have shown N fertilization increased mineralization rate (Zhang et al., 2012; Gurlevik et al., 2004). In a study examining the effect of five nitrogen fertilizer rates on inorganic N content, N fertilizer increased soil inorganic N content in the months following fertilization (Zhang et al., 2012). However, soil inorganic N content dramatically decreased two months after fertilization. A study examining the effect of N fertilizer on fallow and vegetated plots found that applying N fertilizer to plots with vegetation increased N mineralization over a three-year period (Gurlevik et al., 2004). However, other studies have shown greater mineralization rates in fields not treated with nitrogen fertilizer (Carpenter-Boggs et al., 2000). Plots receiving no N fertilizer had an average increase in net N mineralization of 16 kg N ha^{-1} compared to low N fertilization (8 kg ha^{-1}) and high N fertilization (16 kg ha^{-1}) plots (Carpenter-Boggs et al., 2000).

Both cover crop and N fertilization significantly increased inorganic N content in Run 2 (Table 10). Cover crop increased inorganic N content from 26.3 mg N kg^{-1} soil to 31.1 mg N kg^{-1} soil at the end of the 28-day incubation period. Previous work indicates cover crops consistently increase soil nitrogen content (Chahal and Van Eerd, 2020; Larkin, 2020). Leguminous cover crops are particularly useful for cycling nitrogen into the soil, which improves

soil quality over time (Holmes et al., 2019). A study comparing rye (*Secale cereale*) and crimson clover cover (*Trifolium incarnatum*) crops found that crimson clover consistently increased N mineralization rates over rye throughout a 150-day test period (Schomberg and Endale, 2004). Previous research indicates a leguminous cover crop, like the one planted yearly at the Cullars Rotation, out performs species mixtures in increasing soil inorganic N content (Holmes et al., 2019).

Relationships Between N and Secondary Methods of Measurement

Gas Chromatography Measurements of CO_2 Emission

As in the Alabama Wide Study, carbon dioxide evolution as measured by gas chromatography was the standard technique for comparing results from the base trap titration and Solvita methods, which will be discussed later. Two types of gas chromatography statistics were reported: Day 1 and Basal. The day 1 gas chromatography statistic represents the concentration of CO_2 in the headspace of the incubation jar at the end of the first full day of incubation. The basal gas chromatography statistic is a measure of basal respiration calculated by subtracting CO_2 concentration on Day 7 from CO_2 concentration on Day 28 of incubation (Haney et al., 2008a).

There was no significant interaction between cover crop and nitrogen fertilization for these data. For both Runs, neither N fertilization nor cover crop had a significant or consistent effect on the Day 1 and Basal gas chromatography CO_2 measurements (Tables 9 and 10). This is contrary to previous work, which indicates cover crop caused a significant increase in CO_2 production (Christiansen et al., 2015). A single-year study evaluating the CO_2 output of a field planted in a hairy vetch (*Vicia villosa*) and fall rye (*Secale cereale*) mixture cover crop versus an identical field with no cover crop found the field with cover produced significantly more CO_2

over a 3-week measurement period (Christiansen et al., 2015). Other work confirms cover crop increases CO_2 output during the growing season (Drost et al., 2020; Kallenbach et al., 2010). Given that plots from the Cullars Rotation study have received consistent cover crop treatments for over 100 years, CO_2 production differences should have been evident. According to previous research, the effect of N fertilization on CO_2 output is variable (Sainju et al., 2010; Gagnon et al., 2016; Ni et al., 2012). As in our work, N fertilization was shown to have no significant effect on CO_2 output (Sainju et al., 2010).

Correlation between gas chromatography measurements and inorganic N varied with Run. In Run 1, both Day 1 and Basal gas chromatography measurements were significantly correlated to N on day 28 (Table 11). Day 1 CO_2 concentration and inorganic N were strongly correlated and had a r value of 0.90, while Basal CO_2 concentration and inorganic N were moderately negatively correlated and had an r value of -0.670 (Table 11). According to the basal method, as CO_2 production increases, N content decreases; this is the opposite of the expected relationship between soil activity and nitrogen mineralization (Shaaban et al., 2014). The Day 1 and Basal measurements assess CO_2 production during two completely separate time periods. The difference in measurement periods could be responsible for the difference in accuracy between the Day 1 and Basal CO_2 respiration measurements. However, CO_2 production throughout the incubation period should still be correlated to N mineralization (Shaaban et al., 2014). A study examining the relationship between gas chromatography measured CO_2 production and N mineralization found that the two factors shared a direct relationship during the entire 42-day test period (Shaaban et al., 2014). Given the Day 1 and Basal measurements were collected from the same soil samples, stored in the same location, and analyzed on the same gas chromatograph, the intrinsic difference in correlation must be due to the difference in

measurement period. The relationship between gas chromatography CO_2 and N is plotted in Figure 8.

A similar relationship was found between basal CO_2 production and N in Run 2 where the correlation coefficient r was -0.19 (Table 12). Unlike Run 1, CO_2 content on Day 1 and inorganic N on day 28 were also negatively correlated, with an r value of -0.48 (Table 12). Although the relationships between the gas chromatography measurements and N content were opposite from expected, both were significant.

Solvita Basal Respiration

Solvita Basal Respiration was the method best correlated to N content on Day 28 for both Runs. Solvita Basal Respiration was significantly correlated to N content on Day 28 for both Runs ($r = 0.909$ for Run 1 and $r = 0.949$ for Run 2) (Tables 11 and 12). Previous literature has confirmed a strong correlation between Solvita respiration and nitrogen mineralization (Haney et al., 2015; Haney et al., 2008b). A study comparing the CO_2 output and N mineralization of 257 soil samples found an r^2 value of 0.82 between the two methods (Haney et al., 2015). The relationship between Solvita Basal Respiration and N content is displayed in Figure 9. For both Runs, nitrogen fertilization significantly impacted N content on day 28, but not basal respiration as measured by Solvita (Tables 9 and 10). In Run 2, Solvita Basal Respiration was able to detect the influence of cover crop on N content (Table 10). The presence of a cover crop significantly increased Basal Respiration from 3.2 to 4.9 ppm (Table 10).

Base Trap Measurements of Evolved CO_2

Two types of base trap titration statistics were reported: Day 1 and Basal. The Day 1 base trap titration statistic represents the amount of CO_2 captured in the base trap during the first full

day of incubation. The Basal base trap titration statistic is a measure of basal respiration calculated by subtracting CO_2 evolved during the first 7 days of incubation from all CO_2 evolved during the 28-day incubation period (Haney et al., 2008a). In both Runs the titration statistics were positively correlated to N content on day 28, but these relationships were only significant for Run 1 (Table 11). For this Run, Day 1 titration was better correlated to N than Basal titration ($r = 0.59$ for day 1, $r = 0.45$ for basal) (Table 11). Despite this, our work found that titration was better suited to comparing soil activities than providing exact CO_2 output. Previous literature also indicated the usefulness of the titration method in detecting mineralization differences between soils (Wood et al., 1990; Burke et al., 1989). In our work, some individual titrations yielded negative CO_2 evolution values, which should not be possible given the method protocol. Differences in CO_2 are reported in parts per million (ppm). Titration with a 50mL burette may not have been specific enough to detect such precise differences in CO_2 production. Minute over application of HCl during titration can artificially create large differences in CO_2 production, accounting for the negative respiration results. For this reason, the plot of Run 1 base trap titration values versus N content on day 28 is not shown.

For both Runs, no significant relationship existed between cover crop and both base trap titration statistics (Tables 9 and 10). There was also no significant relationship between N fertilization and base trap titration statistics (Table 9 and 10). Presence of a cover crop and nitrogen fertilizer generally increased CO_2 output measured by this method, but it was not significant. As discussed above, the base trap titration method might not have been precise enough to detect differences between treatments. For example, cover crop increased measured CO_2 on day 1 for Run 1 from 5.5 to 11.0 ppm and nitrogen fertilizer increased measured basal CO_2 for Run 2 from 35.6 to 43.9 ppm (Tables 9 and 10).

Relationships Between Each Measurement Method

While gas chromatography was considered the standard method of measuring CO_2 evolution, only Run 1 Day 1 respiration was suitable for predicting nitrogen mineralization (r = 0.90) (Table 11). Additionally, Day 1 CO_2 concentration measured by gas chromatography was strongly correlated to Solvita Basal Respiration for Run 1 (r = 0.91) (Table 11). For both Runs, Day 1 CO_2 was more similar in scale to Solvita Basal Respiration than Basal respiration measured by gas chromatography. The relationship between gas chromatography respiration and Solvita Basal Respiration is shown in Figure 10. The generally poor quality of the data does not justify the laborious and expensive process of taking air samples for analysis on the gas chromatograph. Basal gas chromatography measurements consistently overestimated respiration compared to Solvita Basal Respiration (Tables 9 and 10). This is likely due to the fact that basal respiration as defined by the gas chromatography protocol (adapted from Haney et al., 2008a) is inherently different from basal respiration defined by Solvita. While the disparity in magnitude is understandable due to the difference in measurement period, the negative correlation is not (r = -0.51 for Run 1). Basal respiration defined by Haney et al., 2008a was found to have a strong positive correlation (r = 0.91) despite the difference in magnitude (Table 10) (Haney et al., 2008a). A possible reason for the difference in results between Haney et al., 2008a and our work is the use of infrared gas analysis versus gas chromatography analysis. In addition to the difference equipment, Haney et al., 2008a took air samples once an hour for 24 hours for comparison with the Solvita method. In our study, no air samples were taken between the 0- and 24-hour samples.

The relationship between Day 1 base trap titration and Day 1 gas chromatography was insignificant for both Runs. In Run 1, the two measurements shared a strong, positive correlation,

while in Run 2 the two measurements shared a strong, negative correlation (Tables 11 and 12). The relationship between Day 1 base trap titration and Day 1 gas chromatography in Run 2 is plotted in Figure 11. Given the base trap titration and gas chromatography methods measured CO_2 evolution over an identical period of time, results should have been numerically comparable, as in Run 1 (Table 9). Instead, Run 2 results show that high gas chromatography CO_2 corresponded to low base trap titration CO_2 (Figure 11). While little research has been conducted comparing the two methods, existing work indicated no significant difference in CO_2 measured by gas chromatography versus that measured by base trap titration during a 1-day period for 24 soils collected in Colorado (Sherrod et al., 2012). For example, the average gas chromatography CO_2 output for all soils on day 1 was 115 μg CO_2-C g^{-1} soil compared to 97 ug CO_2-C g^{-1} soil measured by base trap titration. This is contrary to our work which found Day 1 base trap titration and Day 1 gas chromatography results to be significantly different (Figure 11).

The relationship between Basal respiration base trap titration and Basal respiration gas chromatography was also insignificant for both runs. Correlation between methods was low but positive for both Runs ($r = 0.22$ and $r = 0.31$ for Runs 1 and 2, respectively) (Tables 11 and 12). The relationship between Basal respiration base trap titration and Basal respiration gas chromatography is plotted in Figure 11, which shows high gas chromatography values correspond to high base trap titration values. While both methods overestimate CO_2 production compared to Solvita, their results are numerically comparable and similar in scale (Tables 9 and 10). No previous research has been done comparing base trap titration and gas chromatography measured CO_2 production during the basal period defined by Haney et al., 2008a.

Solvita Basal Respiration and Day 1 base trap titration were strongly correlated across both Runs, but this relationship was only significant in Run 1 ($r = 0.70$) (Tables 11 and 12). The

relationship between these two methods for Run 1 is plotted in Figure 12. As discussed previously, the base trap titration method is susceptible to underestimation of soil respiration, causing the negative respiration values shown in Figure 12. While no other studies measured negative CO_2 production, one did find a strong correlation ($r = 0.91$) between Day 1 titration and Solvita measured CO_2 over a 24-hour period (Haney et al., 2008a). While the relationship between Solvita Basal Respiration and Basal base trap titration was significant for both runs, the correlation was stronger for Run 1 ($r = 0.73$) than for Run 2 ($r = 0.38$) (Tables 11 and 12). The relationship between the two methods for Run 1 is plotted in Figure 12. A previous study found a similar relationship between Solvita respiration and Basal Respiration measured by titration ($r^2 = 0.82$) (Haney et al., 2008a). Unlike the Day 1 base trap titration measurement, the Basal Respiration measurement did not yield any negative respiration results (Figure 12). However, basal respiration measured by titration overestimated CO_2 production compared to Solvita Basal Respiration for both Runs (Tables 9 and 10). The difference in measurement period between the basal respiration methods is responsible for the difference in magnitude of the CO_2 production results. While the Solvita test only measured respiration over a 24-hour period, the base trap titration method measured CO_2 production on days 7-28 of the incubation. Little other research has been done comparing base trap titration and Solvita Basal Respiration.

Relationship Between Solvita Tests and Inorganic N

The Solvita CO_2-Burst, SLAN, and Basal Respiration methods are well correlated to inorganic N content at the end of the incubation period across all studies and Runs (Tables 7, 8, 11, and 12). Generally, these relationships were significant. For purposes of this discussion, a summary of the relationships between the Solvita tests and inorganic N extracted during incubation for Run 1 of each study is shown in Table 13. Soil Labile Amino Nitrogen (SLAN)

was the most well correlated to Day 1 inorganic N content (r = 0.80) (Table 13). The relationship was significant. Because SLAN is a chemical test that measures current soil N content, this result was expected. Correlation between SLAN and inorganic N content increased over the course of the incubation (Table 13). Of all the Solvita methods, Basal Respiration showed the strongest correlation, r = 0.91, to inorganic N at the end of the incubation period (Table 13). This may be due to the extended inorganic N measurement period or the protocol itself, which does not artificially stimulate CO_2 production as in CO_2-Burst. The relationship between Basal Respiration and inorganic N on Day 28 was significant (Table 13). Correlation between Basal Respiration and inorganic N content increased over the course of the incubation (Table 13). On both Day 1 and Day 7 Solvita CO_2-Burst was well correlated to inorganic N content (Table 13). However, this relationship was only significant on Day 7 (Table 14). To date, no other study has compared the ability of all three of these Solvita tests to predict inorganic N content.

Conclusion

For both the Alabama Wide and Cullars Rotation Studies, the Solvita methods were very well correlated to inorganic N content throughout the incubation period. Not only was Solvita Basal Respiration the most effective Solvita method for predicting soil inorganic N content at the end of the incubation period, but it was also the best method overall. While the Solvita methods were suitable predictors of N mineralization rate, their results cannot be directly translated into N fertilizer recommendations, limiting their applicability.

Solvita CO_2-Burst was strongly correlated to the standard for measuring CO_2 evolution, gas chromatography. However, Solvita Basal Respiration and gas chromatography had a variable relationship in the Cullars Rotation Study. Of the two gas chromatography measurements, Day 1 gas chromatography showed the strongest correlation with basal respiration measured by Solvita. Both basal respiration measured by gas chromatography and base trap titration overestimated CO_2 production compared to Solvita Basal Respiration. This is due to a difference in measurement period.

The accuracy of the base trap titration method was further hindered by the vulnerability of the method to human error. In both the Alabama Wide and Cullars Rotation study base trap titration had an inconsistent relationship to gas chromatography. Due to tendency to generate negative respiration results the variable reliability of the method, base trap titration was the method most poorly suited to measuring soil activity.

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Table 1. Soil data and background characteristics for the thirteen soils selected for the Alabama Wide Study.

Identification	County	Previous Crops		Taxonomic Classification	P	K	Mg	Ca	pH	CEC	OM	Active C
		2019	2020		---------- kg ha^{-1} ----------					cmolc kg^{-1}	mg kg^{-1}	mg kg^{-1}
EVS$^{\Delta}$	Macon	*Zea mays*	*Zea mays*	Fine, smectitic, thermic Oxyaquic Hapludert	55	121	87	845	6.3	3.5	0.56	229
TU$^{\Pi}$	Lee	*Stenotaphrum secundatum*, *Axonopus affinis*, *Eremochloa ophiuroides, Cynodon dactylon*	*Stenotaphrum secundatum*, *Axonopus affinis*, *Eremochloa ophiuroides, Cynodon dactylon*	Fine-loamy, kaolinitic, thermic Typic Kanhapludult	61	86	179	583	5.5	3.3	0.95	521
WG$^{\Sigma}$(P)	Henry	Cattle on *Eremochloa ophiuroides*	Cattle on *Eremochloa ophiuroides*	Fine-loamy, kaolinitic, thermic Plinthic Kandiudult	45	246	129	578	6.0	4.3	1.21	434
WG(Ct)	Henry	*Gossypuim hirsutum* with *Vicia villosa* cover crop	*Gossypuim hirsutum* with *Vicia villosa* cover crop	Fine-loamy, kaolinitic, thermic Plinthic Kandiudult	108	80	63	1590	6.3	4.8	0.85	298
WA$^{\Psi}$	Sumter	*Gossypuim hirsutum*	*Zea mays*	Fine-silty, carbonatic, thermic Rendollic Eutrudepts	57	253	159	9995	8.0	24.0	1.73	503
PBU$^{\Omega}$	Macon	*Zea mays*	*Gossypuim hirsutum*	Fine-loamy over sandy, siliceous, semiactive, thermic Typic Hapludult	123	104	66	1951	6.6	5.5	0.66	190
OR$^{\beta}$(NC)	Lee	*Gossypuim hirsutum*	*Gossypuim hirsutum*	Loamy, kaolinitic, thermic Grossarenic Kandiudult	237	108	101	1307	6.1	5.8	1.02	410
OR(C)	Lee	*Gossypuim hirsutum* with *Vicia villosa* cover crop	*Gossypuim hirsutum* with *Vicia villosa* cover crop	Loamy, kaolinitic, thermic Grossarenic Kandiudult	286	121	356	3450	6.4	6.7	1.56	735

HF┼(NC)	Limestone	*Gossypuim hirsutum*	*Gossypuim hirsutum*	Fine-silty, mixed, active, nonacid, mesic Fluvaquentic Endoaquept	100	363	146	3252	5.7	9.0	2.34	1030
HF(C)	Limestone	*Zea mays* with *Secale cereale* and *Trifolium pratense* cover crop	*Zea mays* with *Secale cereale* and *Trifolium pratense* cover crop	Fine-silty, mixed, active, nonacid, mesic Fluvaquentic Endoaquept	76	439	136	2531	5.5	7.0	2.51	1133
N▲(C)	Shelby	*Gossypuim hirsutum* with *Vicia villosa* and cover crop	*Gossypuim hirsutum* with *Trifolium pratense* cover crop	Fine, kaolinitic, thermic Rhodic Paleudults	122	275	148	5114	6.8	15.0	1.54	889
N(NC)	Shelby	*Gossypuim hirsutum*	*Gossypuim hirsutum*	Fine, kaolinitic, thermic Rhodic Paleudults	38	266	137	3803	6.4	3.4	1.06	471
IFDC○	Colbert	*Gossypuim hirsutum* with *Trifolium pratense* cover crop	*Zea mays* with *Trifolium pratense* cover crop	Fine-silty, mixed, active, nonacid, mesic Fluvaquentic Endoaquept	583	288	254	8693	6.3	9.0	1.97	925

Δ E.V Smith Field Crops Unit, Shorter, AL
Π Turfgrass Research Unit, Auburn, AL
Σ Wiregrass Research Unit, Headland, AL
Ψ West Alabama, Webster, AL
Ω Plant Breeding Unit, Tallassee, AL
β The Old Rotation, Auburn, AL
┼ Haney Family Farm, Athens, AL
▲ Neil Family Farm, Collierville, AL
○ International Fertilizer Development Center, Muscle Shoals, AL
P: Taken from pasture used for cattle production
Ct: Taken from field used for cotton production
NC: Taken from field without a cover crop
C: Taken from a field with a cover crop
Paired samples titles include an indicator in parenthesis to detail how the paired samples are different.

Table 2. Fertilizer and cover crop treatments for selected plots in the Cullars Rotation.

Cullars Symbol♦	Fertilizer			Cover Crop	P	K	Mg	Ca	pH	CEC	Organic C
	$N^{\dagger}$	$P_2O_5^{\ddagger}$	$K_2O^{\mp}$		---------- mg kg^{-1} ----------					cmolc kg^{-1}	g kg^{-1}
A	0 kg ha^{-1} over a 3-year rotation$^{\Pi}$	224 kg ha^{-1} over a 3-year rotation	304 kg ha^{-1} over a 3-year rotation	*Trifolium pratense*	131	85	64	792	5.8	4.4	7.6
B	0 kg ha^{-1} over a 3-year rotation	224 kg ha^{-1} over a 3-year rotation	304 kg ha^{-1} over a 3-year rotation	None	120	66	70	804	6.2	3.8	6.6
1	135 kg ha^{-1} over a 3-year rotation	224 kg ha^{-1} over a 3-year rotation	304 kg ha^{-1} over a 3-year rotation	None	95	72	48	566	5.6	3.9	7.5
10	135 kg ha^{-1} over a 3-year rotation	224kg ha^{-1} over a 3-year rotation	304 kg ha^{-1} over a 3-year rotation	*Trifolium pratense*	170	55	88	1094	5.8	4.5	9.6

♦ Symbol used to indicate treatment on the Cullars Rotation map produced by Auburn University
†Ammonium nitrate source (43-0-0)
‡Superphosphate source (0-45-0)
∓Muriate of potash source (0-0-60)
Π Total fertilizer applied in a 3-year period

Table 3. Summary of differences in the inorganic nitrogen and soil respiration data at the end of the seven-day incubation period for each treatment in the Alabama Wide Study, Run 1. EVS: E. V. Smith Field Crops Unit; TU: Turf Unit; WG: Wiregrass Research Unit; WA: West Alabama; PBU: Plant Breeding Unit; OR: Old Rotation; HF: Haney Farm; N: Neil Farm; IFDC: International Fertilizer Development Center

Sample	Fertilizer (Y/N)	Tillage (Y/N)	Cover Crop (Y/N)	Inorganic N Day 7$^{\Pi}$ mg N kg^{-1} soil	CO_2 Burst$^{\sigma}$ mg CO_2-C kg^{-1} soil	SLAN$^{\otimes}$ mg NH_3-N kg^{-1} soil	Titration Day 7$^{\#}$ mg CO_2-C kg^{-1} soil	GC Day 7$^{\Omega}$ mg CO_2-C kg^{-1} soil
EVS	Y$^{\Delta}$	Y	N$^{\gamma}$	37.4 f	15.1 de	21.9 h	39.9 f	35.2 bc
TU	N	N	Y	65.0 d	10.7 efg	46.9 fg	77.7 f	53.3 bc
WG(P)	N	N	Y	58.0 d	9.4 g	58.1 f	96.0 ef	29.9 c
WG(Ct)	Y	Y	N	41.4 f	13.4 defg	35.0 gh	121.1 def	48.6 bc
WA	Y	Y	N	30.4 g	14.1 def	73.1 e	185.1 cd	54.8 bc
PBU	Y	Y	N	49.9 e	9.1 g	36.3 gh	97.7 ef	27.0 c
OR(NC)	Y	Y	N	41.8 f	10.2 fg	36.3 gh	195.2 bcd	44.5 bc
OR(C)	Y	Y	Y	80.6 c	14.9 def	141.3 c	163.6 cde	69.6 b
HF(NC)	Y	Y	N	95.9 b	28.5 c	184.4 b	202.6 bcd	103.3 a
HF(C)	Y	N	Y	122.3 a	37.2 b	180.0 b	270.2 b	121.3 a
N(C)	Y	N	Y	63.2 d	15.8 d	120.0 d	218.7 bc	49.4 bc
N(NC)	Y	Y	N	61.9 d	34.5 b	129.4 cd	275.1 b	67.3 b
IFDC	Y	Y	Y	92.7 b	55.7 a	208.8 a	409.5 a	122.1 a

Δ "Y" indicates the soil received the treatment
γ "N" indicates the soil did not receive the treatment
Π Inorganic nitrogen (NO_3 + NH_4) extracted from a 5g sample of each soil using 2M KCl on day 7 of the incubation (Mulvaney, 1996)
σ Carbon dioxide produced by the soil sample over a 24-hour incubation period according to the Solvita CO_2-Burst protocol (Brinton et al., 2019a)
$\otimes$ Labile amino nitrogen measured during the 24-hour incubation period according to the Solvita SLAN protocol (Brinton et al., 2019b)
Carbon dioxide produced by the soil sample over the 7-day incubation period according to the base trap titration protocol (Haney et al., 2008)
Ω Carbon dioxide produced by the soil sample over the 7-day incubation period according to the gas chromatography protocol (Haney et al., 2008)
P: Taken from *Eremochloa ophiuroides* pasture used for cattle production
Ct: Taken from field used for cotton production
NC: Taken from field without a cover crop
C Taken from a field with a cover crop
Means with no letter in common are significantly different at $\alpha = 0.05$. Duncan's multiple range test was used.
Paired sample titles include an indicator in parenthesis to detail how the paired samples are different.

Table 4. Summary of differences in the inorganic nitrogen and soil respiration data at the end of the seven-day incubation period for each treatment in the Alabama Wide Study, Run 2. EVS: E. V. Smith Field Crops Unit; TU: Turf Unit; WG: Wiregrass Research Unit; WA: West Alabama; PBU: Plant Breeding Unit; OR: Old Rotation; HF: Haney Farm; N: Neil Farm; IFDC: International Fertilizer Development Center

Sample	Fertilizer (Y/N)	Tillage (Y/N)	Cover Crop (Y/N)	Inorganic N Day 7[Π] mg N kg^{-1} soil	CO_2 Burst[σ] mg CO_2-C kg^{-1} soil	SLAN[⊗] mg NH_3-N kg^{-1} soil	Titration Day 7[#] mg CO_2-C kg^{-1} soil	GC Day 7[Ω] mg CO_2-C kg^{-1} soil
EVS	Y[Δ]	Y	N[γ]	34.1 de	11.7 f	88.1 bc	227.0 a	35.6 def
TU	N	N	Y	44.9 cde	19.0 ef	66.9 bc	109.8 a	74.6 cde
WG(P)	N	N	Y	51.7 cde	16.4 ef	70.6 bc	89.2 a	50.7 cdef
WG(Ct)	Y	Y	N	34.9 de	18.2 ef	41.3 c	132.8 a	52.1 cdef
WA	Y	Y	N	25.7 e	16.5 ef	73.1 bc	181.5 a	51.6 cdef
PBU	Y	Y	N	38.8 de	12.8 ef	38.8 c	279.1 a	24.7 f
OR(NC)	Y	Y	N	36.7 de	11.9 f	40.0 c	112.7 a	31.4 ef
OR(C)	Y	Y	Y	54.1 cd	40.1 bc	208.1 a	97.3 a	91.4 bc
HF(NC)	Y	Y	N	99.3 b	50.7 b	183.1 a	121.8 a	79.0 cd
HF(C)	Y	N	Y	126.8 a	41.0 bc	197.5 a	147.2 a	122.6 ab
N(C)	Y	N	Y	58.6 cd	24.7 de	119.4 b	144.4 a	45.6 def
N(NC)	Y	Y	N	56.9 cd	33.1 cd	113.1 b	97.9 a	57.0 cdef
IFDC	Y	Y	Y	69.6 c	65.9 a	208.1 a	243.6 a	137.9 a

Δ "Y" indicates the soil received the treatment
γ "N" indicates the soil did not receive the treatment
Π Inorganic nitrogen (NO_3 + NH_4) extracted from a 5g sample of each soil using 2M KCl on day 7 of the incubation (Mulvaney, 1996)
σ Carbon dioxide produced by the soil sample over a 24-hour incubation period according to the Solvita CO_2-Burst protocol (Brinton et al., 2019a)
⊗ Labile amino nitrogen measured during the 24-hour incubation period according to the Solvita SLAN protocol (Brinton et al., 2019b)
Carbon dioxide produced by the soil sample over the 7-day incubation period according to the base trap titration protocol (Haney et al., 2008)
Ω Carbon dioxide produced by the soil sample over the 7-day incubation period according to the gas chromatography protocol (Haney et al., 2008)
P: Taken from *Eremochloa ophiuroides* pasture used for cattle production
Ct: Taken from field used for cotton production
NC: Taken from field without a cover crop
C: Taken from a field with a cover crop
Means with no letter in common are significantly different at $\alpha = 0.05$. Duncan's multiple range test was used.
Paired sample titles include an indicator in parenthesis to detail how the paired samples are different.

Table 5. Means for inorganic nitrogen, Solvita CO_2-Burst, Solvita SLAN, Base Trap Titration, and Gas Chromatography grouped by management factor for Alabama Study, Run 1.

	Inorganic Day 7[Π]	CO_2-Burst[σ]	SLAN[⊗]	Titration Day 7[#]	GC Day 7[Ω]
	mg N kg^{-1} soil	mg CO_2-C kg^{-1} soil	mg NH_3-N kg^{-1} soil	mg CO_2-C kg^{-1} soil	mg CO_2-C kg^{-1} soil
Treatment Means					
Cover crop	80.3 a[┼]	23.9 NS	125.8 a	205.9 NS	74.3 a
No cover crop	51.2 b	17.8 NS	73.8 b	159.5 NS	54.4 b
Fertilizer	65.2 NS	22.7 a	106.0 a	198.1 a	67.5 a
No fertilizer	61.5 NS	10.0 b	52.5 b	86.8 b	41.6 b
Tillage	59.1 b	21.7 NS	96.3 NS	187.8 NS	63.6 NS
No tillage	77.1 a	18.2 NS	101.3 NS	165.6 NS	63.5 NS

Π Inorganic nitrogen (NO_3 + NH_4) extracted from a 5g sample of each soil using 2M KCl on day 7 of the incubation
σ Carbon dioxide produced by the soil sample over a 24-hour incubation period according to the Solvita CO_2-Burst protocol
⊗ Labile amino nitrogen measured during the 24-hour incubation period according to the Solvita SLAN protocol
Carbon dioxide produced by the soil sample over the 7-day incubation period according to the base trap titration protocol
Ω Carbon dioxide produced by the soil sample over the 7-day incubation period according to the gas chromatography protocol
┼ Within each treatment group means with the same letter are not significantly different from each other via mean's separation at an alpha of 0.05.
NS: not significant

Table 6. Means for inorganic nitrogen, Solvita CO_2-Burst, Solvita SLAN, Base Trap Titration, and Gas Chromatography grouped by management factor for Alabama Study, Run 2.

	Inorganic N Day 7[Π]	CO_2-Burst[σ]	SLAN[⊗]	Titration Day 7[#]	GC Day 7[Ω]
	mg N kg^{-1} soil	mg CO_2-C kg^{-1} soil	mg NH_3-N kg^{-1} soil	mg CO_2-C kg^{-1} soil	mg CO_2-C kg^{-1} soil
Treatment Means					
Cover crop	67.6 a[┼]	34.5 a	145.1 a	138.6 NS	87.1 a
No cover crop	46.6 b	22.1 b	82.5 b	164.7 NS	47.3 b
Fertilizer	57.8 NS	29.7 a	119.2 a	162.3 NS	66.3 NS
No fertilizer	48.3 NS	17.7 b	68.8 b	99.5 NS	62.6 NS
Tillage	50.0 b	29.0 NS	110.4 NS	166.0 NS	62.3NS
No tillage	70.5 a	25.3 NS	113.6 NS	122.7 NS	73.4NS

Π Inorganic nitrogen (NO_3 + NH_4) extracted from a 5g sample of each soil using 2M KCl on day 7 of the incubation
σ Carbon dioxide produced by the soil sample over a 24-hour incubation period according to the Solvita CO_2-Burst protocol
⊗ Labile amino nitrogen measured during the 24-hour incubation period according to the Solvita SLAN protocol
Carbon dioxide produced by the soil sample over the 7-day incubation period according to the base trap titration protocol
Ω Carbon dioxide produced by the soil sample over the 7-day incubation period according to the gas chromatography protocol
┼ Within each treatment group means with the same letter are not significantly different from each other via mean's separation at an alpha of 0.05.
NS: not significant

Table 7. Correlation coefficients between inorganic nitrogen measurements and various carbon dioxide evolution measurements for Run 1 of the Alabama-Wide Study.

	CO_2 Burst$^{\sigma}$	SLAN$^{\otimes}$	GC1$^{\Delta}$	GC3°	GC7$^{\Omega}$	T1$^{\exists}$	T3$^{\bullet}$	T7$^{\#}$	Nit1^{-}	Amm1$^{\Sigma}$	Nit3$^{\nabla}$	Amm3$^{\uparrow}$	Nit7$^{\blacklozenge}$	Amm7$^{\Im}$
SLAN	0.836*													
GC1	0.802	0.799*												
GC3	0.717*	0.875*	0.689*											
GC7	0.876*	0.911*	0.809*	0.908*										
T1	0.733*	0.470*	0.466*	0.332	0.598*									
T3	0.769*	0.674	0.678*	0.525*	0.755*	0.911*								
T7	0.876*	0.822*	0.716*	0.612*	0.788*	0.769*	0.886*							
Nit1	0.639	0.792*	0.648*	0.793*	0.762*	0.228*	0.364*	0.451*						
Amm1	0.758*	0.500*	0.731*	0.285*	0.551*	0.672*	0.658*	0.672*	0.246*					
Nit3	0.660	0.771*	0.781*	0.728*	0.707*	0.255*	0.395*	0.491*	0.897	0.491*				
Amm3	0.777*	0.551*	0.686*	0.363*	0.621*	0.777*	0.772*	0.750*	0.215*	0.954	0.433*			
Nit7	0.689*	0.865*	0.691*	0.834	0.863*	0.348	0.533*	0.582*	0.949*	0.271*	0.794*	0.281*		
Amm7	-0.274	-0.275*	-0.293	-0.208*	-0.173*	-0.014*	0.180*	-0.344*	-0.133	-0.053*	-0.128	-0.125*	-0.128*	
Total N$^{\Pi}$	0.677*	0.855*	0.678*	0.827*	0.858	0.349*	0.526*	0.566*	0.947*	0.270*	0.791*	0.275*	0.998*	-0.072*

σ Carbon dioxide produced by the soil sample over a 24-hour incubation period according to the Solvita CO_2-Burst protocol
⊗ Labile amino nitrogen measured during the 24-hour incubation period according to the Solvita SLAN protocol
Δ Carbon dioxide produced by the soil sample during the first day of the incubation period according to the gas chromatography protocol
° Carbon dioxide produced by the soil sample during the first three days of the incubation period according to the gas chromatography protocol
Ω Carbon dioxide produced by the soil sample over the 7-day incubation period according to the gas chromatography protocol
∃ Carbon dioxide produced by the soil sample during the first day of the incubation period according to the base trap titration protocol
• Carbon dioxide produced by the soil sample during the first three days of the incubation period according to the base trap titration protocol
Carbon dioxide produced by the soil sample over the 7-day incubation period according to the base trap titration protocol
– Nitrate (NO_3) extracted from a 5g sample of each soil using 2M KCl on the first day of the incubation
Σ Ammonium (NH_4) extracted from a 5g sample of each soil using 2M KCl on the first day of the incubation
∇ Nitrate (NO_3) extracted from a 5g sample of each soil using 2M KCl on the third day of the incubation
↑ Ammonium (NH_4) extracted from a 5g sample of each soil using 2M KCl on the third day of the incubation
♦ Nitrate (NO_3) extracted from a 5g sample of each soil using 2M KCl on the seventh day of the incubation
ℑ Ammonium (NH_4) extracted from a 5g sample of each soil using 2M KCl on the seventh day of the incubation
Π Inorganic nitrogen (NO_3 + NH_4) extracted from a 5g sample of each soil using 2M KCl on day 7 of the incubation
*Indicates a significant result at $\alpha = 0.05$. Results from ANOVA analysis using SAS.

Table 8. Correlation coefficients between inorganic nitrogen measurements and various carbon dioxide evolution measurements for Run 2 of the Alabama-Wide Study.

	CO_2 Burst$^{\sigma}$	SLAN$^{\otimes}$	GCD1$^{\Delta}$	GCD3°	GCD7$^{\Omega}$	TD1$^{\exists}$	TD3$^{\bullet}$	TD7$^{\#}$	NitD1^{-}	AmmD1$^{\Sigma}$	NitD3$^{\nabla}$	AmmD3$^{\uparrow}$	NitD7$^{\blacklozenge}$	AmmD7$^{\Im}$
SLAN	0.902*													
GC1	0.881	0.688*												
GC3	0.871*	0.820*	0.845*											
GC7	0.874*	0.843*	0.819*	0.977*										
T1	-0.498*	-0.368	-0.435*	-0.424*	-0.419									
T3	-0.385*	-0.286	-0.356*	-0.390*	0.377*	0.947								
T7	0.020*	-0.055*	0.171*	0.030*	-0.022*	0.699*	0.749							
Nit1	0.758	0.827*	0.453*	0.688*	0.779*	-0.279*	-0.204*	-0.170*						
Amm1	0.697*	0.717*	0.481*	0.779*	0.776*	-0.367*	-0.325*	0.003*	0.684*					
Nit3	0.811*	0.841*	0.494*	0.696	0.775*	-0.368*	-0.259*	-0.128*	0.957	0.797*				
Amm3	0.328*	0.171*	0.524*	0.593*	0.541*	-0.257*	-0.394*	0.064*	0.208*	0.231	0.102*			
Nit7	0.701*	0.758*	0.337*	0.600	0.674*	-0.264*	-0.167*	-0.114*	0.913	0.796*	0.961	-0.001*		
Amm7	-0.247*	-0.286*	-0.096*	0.035*	0.009*	0.038*	-0.144*	-0.264*	-0.075*	-0.224	-0.283*	0.734	-0.295*	
Total N$^{\Pi}$	0.690*	0.745*	0.335*	0.622	0.696	-0.267*	-0.191*	-0.151*	0.932*	0.791*	0.954*	0.093*	0.992*	-0.175*

σ Carbon dioxide produced by the soil sample over a 24-hour incubation period according to the Solvita CO_2-Burst protocol
⊗ Labile amino nitrogen measured during the 24-hour incubation period according to the Solvita SLAN protocol
Δ Carbon dioxide produced by the soil sample during the first day of the incubation period according to the gas chromatography protocol
° Carbon dioxide produced by the soil sample during the first three days of the incubation period according to the gas chromatography protocol
Ω Carbon dioxide produced by the soil sample over the 7-day incubation period according to the gas chromatography protocol
∃ Carbon dioxide produced by the soil sample during the first day of the incubation period according to the base trap titration protocol
• Carbon dioxide produced by the soil sample during the first three days of the incubation period according to the base trap titration protocol
Carbon dioxide produced by the soil sample over the 7-day incubation period according to the base trap titration protocol
– Nitrate (NO_3) extracted from a 5g sample of each soil using 2M KCl on the first day of the incubation
Σ Ammonium (NH_4) extracted from a 5g sample of each soil using 2M KCl on the first day of the incubation
∇ Nitrate (NO_3) extracted from a 5g sample of each soil using 2M KCl on the third day of the incubation
↑ Ammonium (NH_4) extracted from a 5g sample of each soil using 2M KCl on the third day of the incubation
♦ Nitrate (NO_3) extracted from a 5g sample of each soil using 2M KCl on the seventh day of the incubation
ℑ Ammonium (NH_4) extracted from a 5g sample of each soil using 2M KCl on the seventh day of the incubation
Π Inorganic nitrogen (NO_3 + NH_4) extracted from a 5g sample of each soil using 2M KCl on day 7 of the incubation
*Indicates a significant result at $\alpha = 0.05$. Results from ANOVA analysis using SAS.

Table 9. Effect of nitrogen and cover crop on inorganic nitrogen and method means, Cullars Rotation Study, Run 1.

	Inorganic N Day 28[Π]	Solvita Basal Respiration[σ]	Day 1 Titration[Σ]	Basal Titration[#]	Day 1 GC[Ω]	Basal GC[β]
	mg N kg^{-1} soil	---------- mg CO_2-C kg^{-1} soil ----------				
Treatment Means						
Cover crop	20.37 NS[┼]	15.63 NS	11.03 NS	67.52 NS	16.67 NS	49.50 NS
No cover crop	18.37 NS	10.73 NS	5.46 NS	50.49 NS	14.27 NS	44.59 NS
Nitrogen	21.44 a	15.43 NS	11.00 NS	53.58 NS	17.85 NS	75.22 NS
No Nitrogen	17.30 b	10.93 NS	5.49 NS	64.43 NS	13.08 NS	133.82 NS

Π Inorganic nitrogen (NO_3 + NH_4) extracted from a 5g sample of each soil using 2M KCl on day 28 of the incubation
σ Carbon dioxide produced by the soil sample over a 24-hour incubation period according to the Solvita Basal Respiration protocol
∑ Carbon dioxide produced by the soil sample during the first day of the incubation period according to the base trap titration protocol
Carbon dioxide produced by the soil sample during the first seven days of the incubation subtracted from the carbon dioxide produced by the soil sample during the entire 28-day incubation period for the base trap titration protocol
Ω Carbon dioxide produced by the soil sample during the first day of the incubation period according to the gas chromatography protocol
β Carbon dioxide produced by the soil sample during the first seven days of the incubation subtracted from the carbon dioxide produced by the soil sample during the entire 28-day incubation period for the gas chromatography protocol
Cover crop was *Trifolium incarnatum*.
Nitrogen fertilization rate was 45 kg N ha^{-1} $year^{-1}$ with ammonium nitrate (43-0-0) source.
┼Means with no letter in common were significantly different at $\alpha = 0.05$ within each treatment. Duncan's multiple range test was used. NS indicates a non-significant result.

Table 10. Effect of Nitrogen and Cover Crop on Total Nitrogen and Method means Cullars Rotation Study, Run 2.

	Inorganic N Day 28[Π]	Solvita Basal Respiration[σ]	Day 1 Titration[Σ]	Basal Titration[#]	Day 1 GC[Ω]	Basal GC[β]
	mg N kg^{-1} soil	---------- mg CO_2-C kg^{-1} soil ----------				
Treatment Means						
Cover crop	31.09 a	4.88 a	30.31 NS	41.35 NS	6.22 NS	45.30 NS
No cover crop	26.31 b	3.17 b	9.54 NS	38.16 NS	8.38 NS	74.44 NS
Nitrogen	30.81 a	4.45 NS	19.28 NS	43.87 NS	7.66 NS	71.31 NS
No Nitrogen	26.58 b	3.60 NS	20.57 NS	35.64 NS	6.94 NS	48.43 NS

Π Inorganic nitrogen (NO + NH4) extracted from a 5g sample of each soil using 2M KCl on day 28 of the incubation
σ Carbon dioxide produced by the soil sample over a 24-hour incubation period according to the Solvita Basal Respiration protocol
∑ Carbon dioxide produced by the soil sample during the first day of the incubation period according to the base trap titration protocol
Carbon dioxide produced by the soil sample during the first seven days of the incubation subtracted from the carbon dioxide produced by the soil sample during the entire 28-day incubation period for the base trap titration protocol
Ω Carbon dioxide produced by the soil sample during the first day of the incubation period according to the gas chromatography protocol
β Carbon dioxide produced by the soil sample during the first seven days of the incubation subtracted from the carbon dioxide produced by the soil sample during the entire 28-day incubation period for the gas chromatography protocol
Cover crop was *Trifolium incarnatum*.
Nitrogen fertilization rate was 45 kg N ha^{-1} $year^{-1}$ with ammonium nitrate (43-0-0) source.
┼Means with no letter in common are significantly different at $\alpha = 0.05$ within each treatment. Duncan's multiple range test was used. NS indicates a non-significant result.

Table 11. Correlation coefficients between inorganic nitrogen measurements and carbon dioxide evolution measurements for the Cullars Rotation Study, Run 1. GC: gas chromatography; T: base trap titration; Nit: nitrate; Amm: ammonium; Total N: NO_3 + NH_4 at the end of the 28-day incubation period.

	Solvita Basal$^{\sigma}$	*GC1*$^{\Delta}$	*GCB*$^{\#}$	*T1*$^{—}$	*TB*^{A}	*Nit1*$^{\blacklozenge}$	*Amm1*$^{\forall}$	*Nit7*$^{\gamma}$	*Amm7*$^{\psi}$	*Nit28*$^{\Pi}$	*Amm28*$^{\Omega}$
GC1	0.910*										
GCB	-0.509*	-0.816*									
T1	0.697*	0.877	-0.799*								
TB	0.726*	0.387*	0.217	0.199*							
Nit1	0.850	0.673*	-0.290*	0.258	0.686*						
Amm1	0.485	0.080*	0.482*	-0.207	0.910*	0.661					
Nit7	0.907	0.970*	-0.789*	0.739	0.384*	0.798	0.157				
Amm7	0.956	0.752*	-0.255*	0.470*	0.867*	0.916	0.717	0.786*			
Nit28	0.917*	0.907*	-0.663*	0.594*	0.481*	0.905*	0.322*	0.977*	0.853*		
Amm28	0.717*	0.798*	-0.742*	0.456	0.173*	0.808*	0.094*	0.914*	0.631*	0.935*	
TotalN$^{\Sigma}$	0.909*	0.904*	-0.670*	0.588*	0.466*	0.903*	0.311*	0.977*	0.844*	0.999*	0.942*

σ Carbon dioxide produced by the soil sample over a 24-hour incubation period according to the Solvita Basal Respiration protocol
– Carbon dioxide produced by the soil sample during the first day of the incubation period according to the base trap titration protocol
A Carbon dioxide produced by the soil sample during the first seven days of the incubation subtracted from the carbon dioxide produced by the soil sample during the entire 28-day incubation period for the base trap titration protocol
♦ Nitrate (NO_3) extracted from a 5g sample of each soil using 2M KCl on the first day of the incubation
γ Nitrate (NO_3) extracted from a 5g sample of each soil using 2M KCl on the seventh day of the incubation
Π Nitrate (NO_3) extracted from a 5g sample of each soil using 2M KCl on the twenty eighth day of the incubation
∀ Ammonium (NH_4) extracted from a 5g sample of each soil using 2M KCl on the first day of the incubation
ψ Ammonium (NH_4) extracted from a 5g sample of each soil using 2M KCl on the seventh day of the incubation
Ω Ammonium (NH_4) extracted from a 5g sample of each soil using 2M KCl on the twenty eighth day of the incubation
Π Inorganic nitrogen (NO_3 + NH_4) extracted from a 5g sample of each soil using 2M KCl on day 28 of the incubation
Δ Carbon dioxide produced by the soil sample during the first day of the incubation period according to the gas chromatography protocol
Carbon dioxide produced by the soil sample during the first seven days of the incubation subtracted from the carbon dioxide produced by the soil sample during the entire 28-day incubation period for the gas chromatography protocol
Σ Inorganic nitrogen (NO_3 + NH_4) extracted from a 5g sample of each soil using 2M KCl on day 28 of the incubation
*Indicates a significant result

Table 12. Correlation coefficients between inorganic nitrogen measurements and carbon dioxide evolution measurements for the Cullars Rotation Study, Run 2. GC: gas chromatography; T: base trap titration; Nit: nitrate; Amm: ammonium; Total N: NO_3 + NH_4 at the end of the 28-day incubation period.

	Solvita Basal$^{\sigma}$	*GC1*$^{\Delta}$	*GCB*$^{\#}$	*T1*$^{—}$	*TB*^{A}	*Nit1*$^{\blacklozenge}$	*Amm1*$^{\forall}$	*Nit7*$^{\gamma}$	*Amm7*$^{\psi}$	*Nit28*$^{\Pi}$	*Amm28*$^{\Omega}$
GC1	-0.641										
GCB	-0.466*	0.844*									
T1	0.796	-0.947	-0.667								
TB	0.375*	-0.240*	0.314	0.488							
Nit1	0.993*	-0.588*	-0.367*	0.774	0.462*						
Amm1	0.560*	0.245*	0.434*	0.050	0.449*	0.632*					
Nit7	0.978*	-0.539*	-0.284*	0.748	0.526*	0.996*	0.683				
Amm7	0.409*	0.342*	0.612*	-0.028	0.581*	0.502	0.968*	0.571*			
Nit28	0.942*	-0.439*	-0.150	0.680	0.592*	0.974*	0.764*	0.991*	0.674*		
Amm28	0.295*	0.891*	-0.641*	0.812	0.367*	0.261*	-0.484*	0.230	-0.471*	0.152*	
TotalN$^{\Sigma}$	0.949*	-0.484*	-0.185	0.719	0.607	0.979*	0.729*	0.994*	0.641*	0.998*	0.207*

σ Carbon dioxide produced by the soil sample over a 24-hour incubation period according to the Solvita Basal Respiration protocol
– Carbon dioxide produced by the soil sample during the first day of the incubation period according to the base trap titration protocol
A Carbon dioxide produced by the soil sample during the first seven days of the incubation subtracted from the carbon dioxide produced by the soil sample during the entire 28-day incubation period for the base trap titration protocol
♦ Nitrate (NO_3) extracted from a 5g sample of each soil using 2M KCl on the first day of the incubation
γ Nitrate (NO_3) extracted from a 5g sample of each soil using 2M KCl on the seventh day of the incubation
Π Nitrate (NO_3) extracted from a 5g sample of each soil using 2M KCl on the twenty eighth day of the incubation
∀ Ammonium (NH_4) extracted from a 5g sample of each soil using 2M KCl on the first day of the incubation
ψ Ammonium (NH_4) extracted from a 5g sample of each soil using 2M KCl on the seventh day of the incubation
Ω Ammonium (NH_4) extracted from a 5g sample of each soil using 2M KCl on the twenty eighth day of the incubation
Π Inorganic nitrogen (NO_3 + NH_4) extracted from a 5g sample of each soil using 2M KCl on day 28 of the incubation
Δ Carbon dioxide produced by the soil sample during the first day of the incubation period according to the gas chromatography protocol
Carbon dioxide produced by the soil sample during the first seven days of the incubation subtracted from the carbon dioxide produced by the soil sample during the entire 28-day incubation period for the gas chromatography protocol
Σ Inorganic nitrogen (NO_3 + NH_4) extracted from a 5g sample of each soil using 2M KCl on day 28 of the incubation
*Indicates a significant result

Table 13. Summary of the effectiveness of the Solvita methods at predicting soil inorganic nitrogen (NO_3 + NH_4) content at 1, 7, and 28 days after the start of incubation, for the Alabama Wide and Cullars Rotation Studies, Run 1.

	CO_2-Burst$^{\sigma}$	SLAN$^{\otimes}$	Basal Respiration$^{\blacklozenge}$
Day 1 Inorganic N$^{\Pi}$	0.66	0.80*	0.57*
Day 7 Inorganic N$^{\Sigma}$	0.67*	0.86*	0.57*
Day 28 Inorganic N$^{\Delta}$	--	--	0.91*

Π Inorganic nitrogen (NO_3 + NH_4) extracted from a 5g sample of each soil using 2M KCl on day 1 of the incubation
Σ Inorganic nitrogen (NO_3 + NH_4) extracted from a 5g sample of each soil using 2M KCl on day 7 of the incubation
Δ Inorganic nitrogen (NO_3 + NH_4) extracted from a 5g sample of each soil using 2M KCl on day 28 of the incubation
σ Carbon dioxide produced by the soil sample over a 24-hour incubation period according to the Solvita CO_2-Burst protocol
$\otimes$ Labile amino nitrogen measured during the 24-hour incubation period according to the Solvita SLAN protocol
$\blacklozenge$ Carbon dioxide produced by the soil sample over a 24-hour incubation period according to the Solvita Basal Respiration protocol
No Day 28 inorganic N measurement is available for comparison with the CO_2-Burst and SLAN methods because N mineralization incubations for the Alabama Wide Study only ran for 7 days.
* Indicates a significant result at $\alpha = 0.05$.

Figure 1. CO_2 production measured by gas chromatography compared to soil inorganic nitrogen (NO_3 + NH_4) content at the end of the 7-day incubation period for the Alabama Wide Study, Run 1.

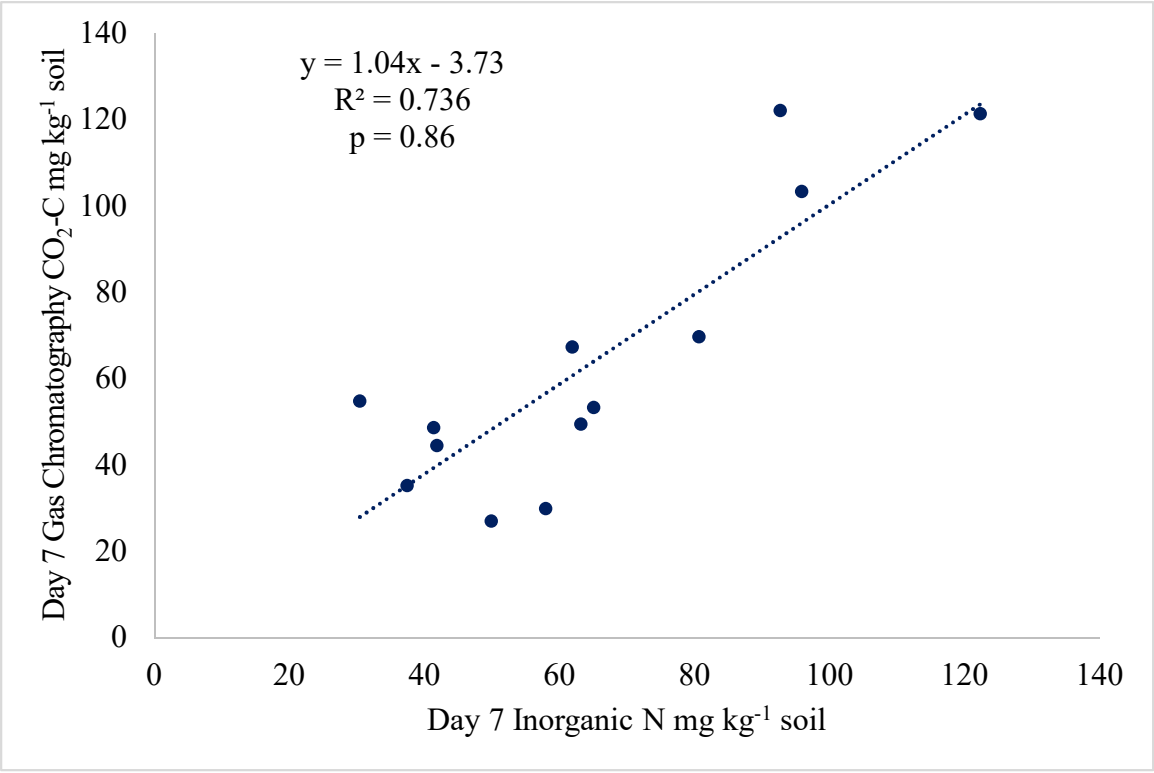

Figure 2. CO_2 production measured by gas chromatography compared to soil inorganic nitrogen (NO_3 + NH_4) content at the end of the 7-day incubation period for the Alabama Wide Study, Run 2.

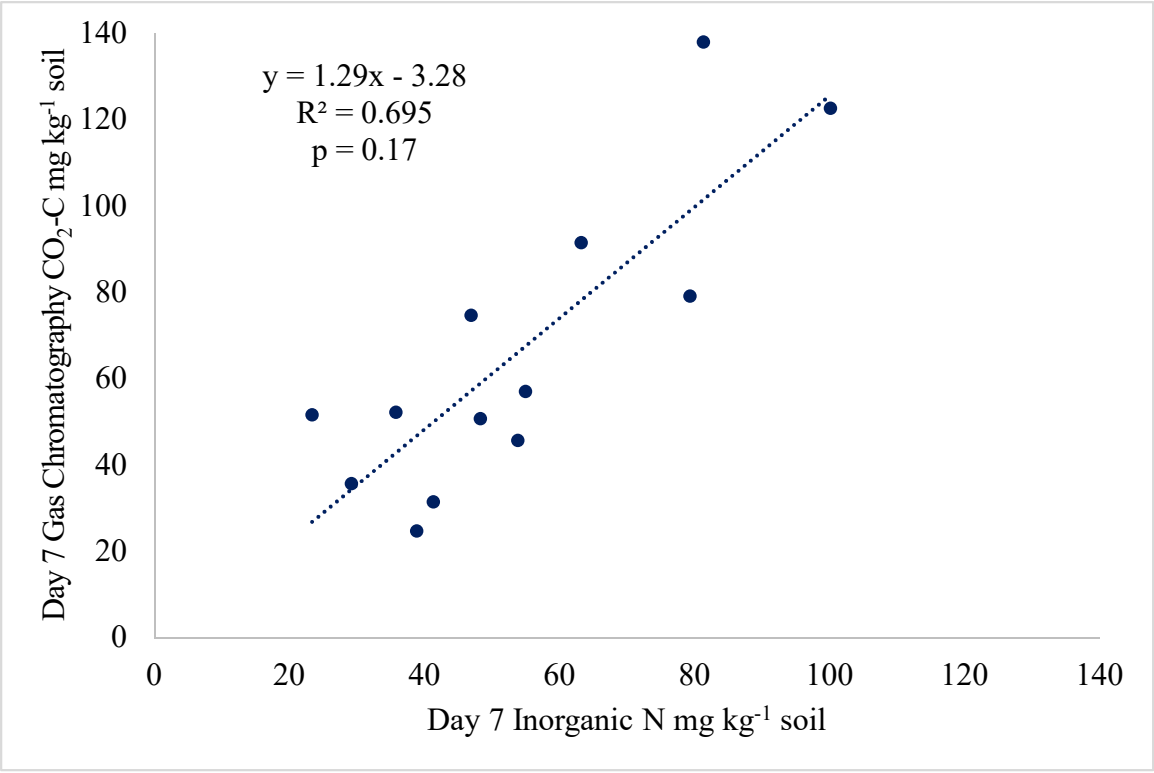

Figure 3. CO_2 production measured by Solvita CO_2-Burst compared to CO_2 production measured by gas chromatography after one day of incubation for the Alabama Wide Study, Runs 1 and 2.

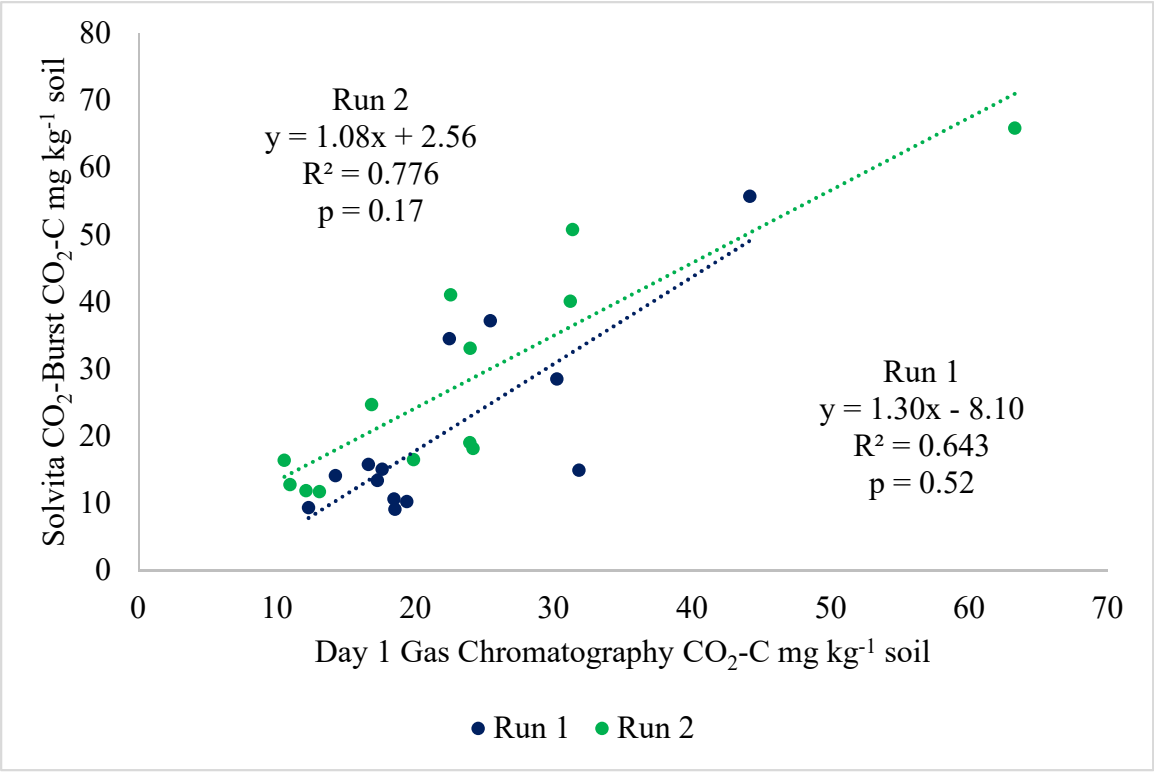

Figure 4. CO_2 production measured by Solvita CO_2-Burst compared to CO_2 production measured by gas chromatography at the end of the 7-day incubation period for the Alabama Wide Study, Runs 1 and 2.

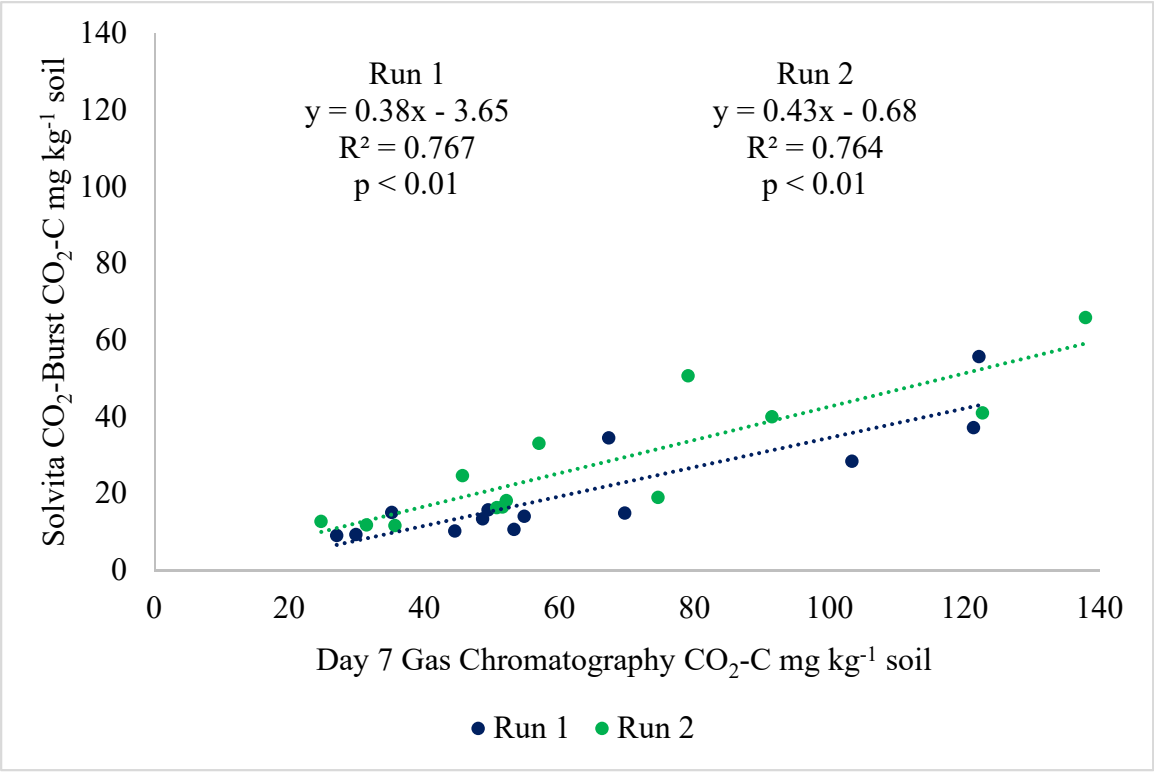

Figure 5. Labile nitrogen content measured by Solvita SLAN compared to CO_2 production measured by gas chromatography at the end of the 7-day incubation period for the Alabama Wide Study, Runs 1 and 2.

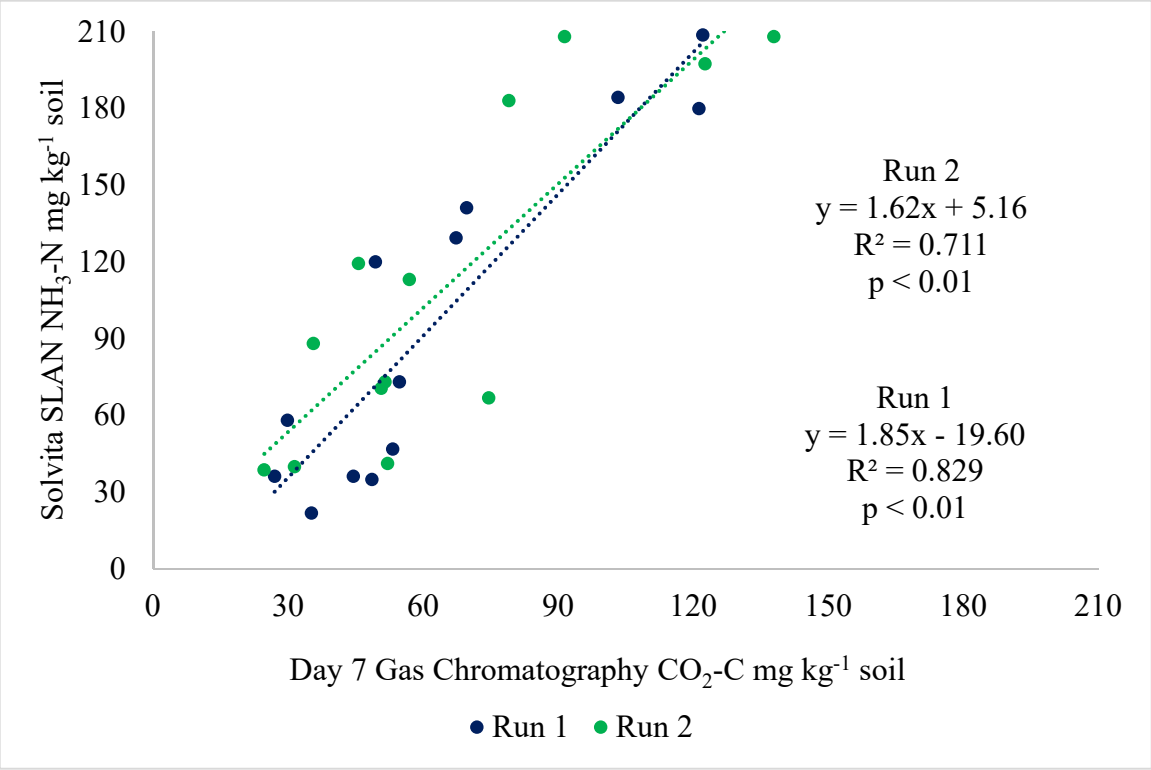

Figure 6. CO_2 production measured by base trap titration compared to CO_2 production measured by gas chromatography after one day of incubation for the Alabama Wide Study, Run 2.

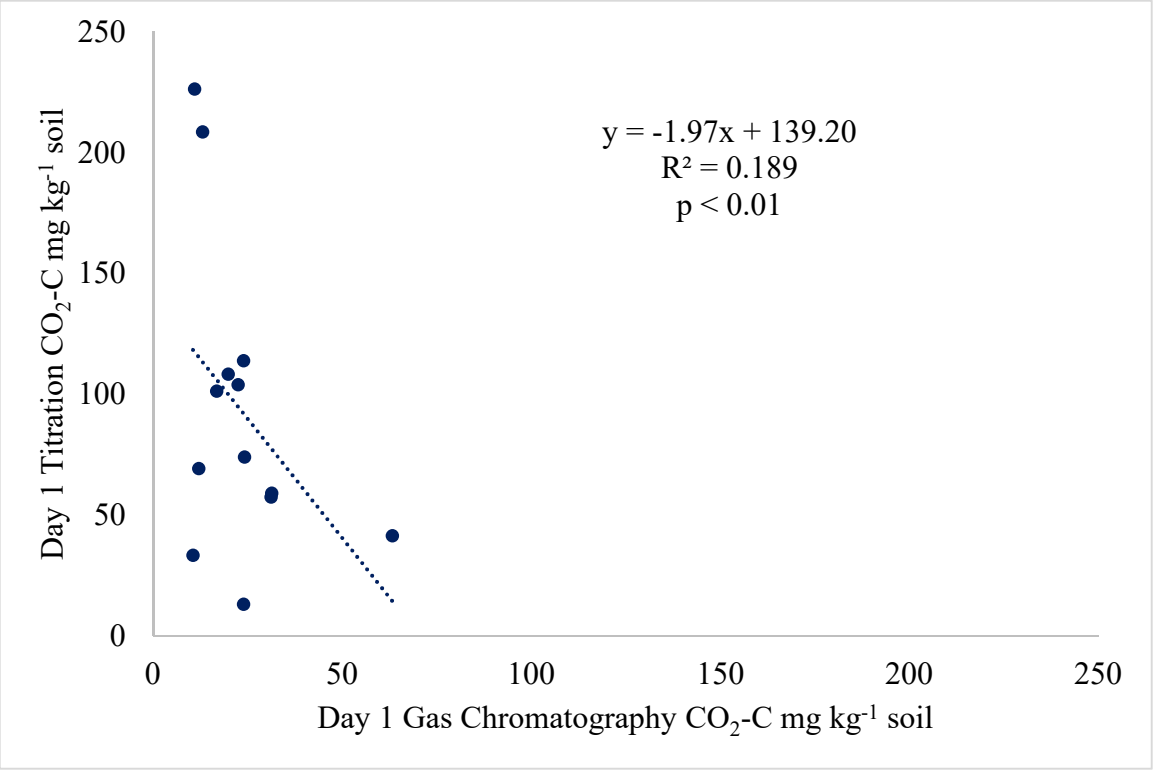

Figure 7. CO_2 production measured by Solvita CO_2-Burst compared to CO_2 production measured by base trap titration after one day of incubation for the Alabama Wide Study, Run 1.

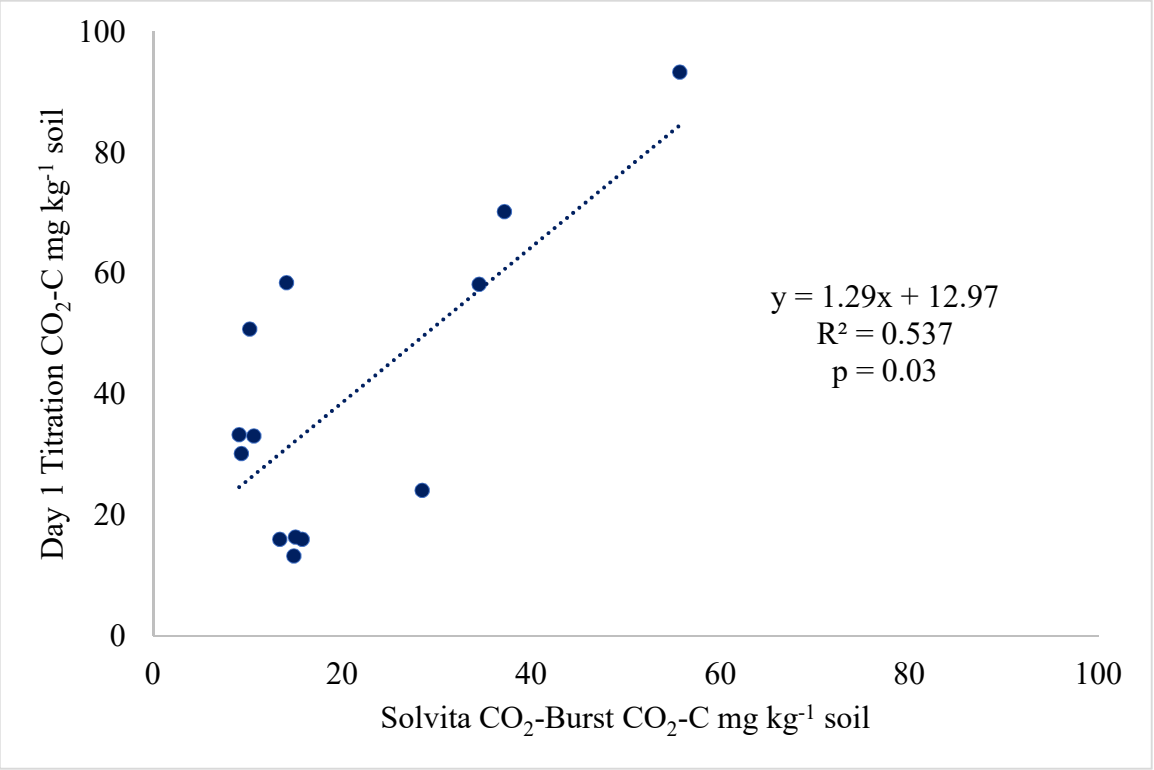

Figure 8. CO_2 production measured by gas chromatography compared to soil inorganic nitrogen (NO_3 + NH_4) content at the end of the 28-day incubation period for the Cullars Rotation Study, Run 1.

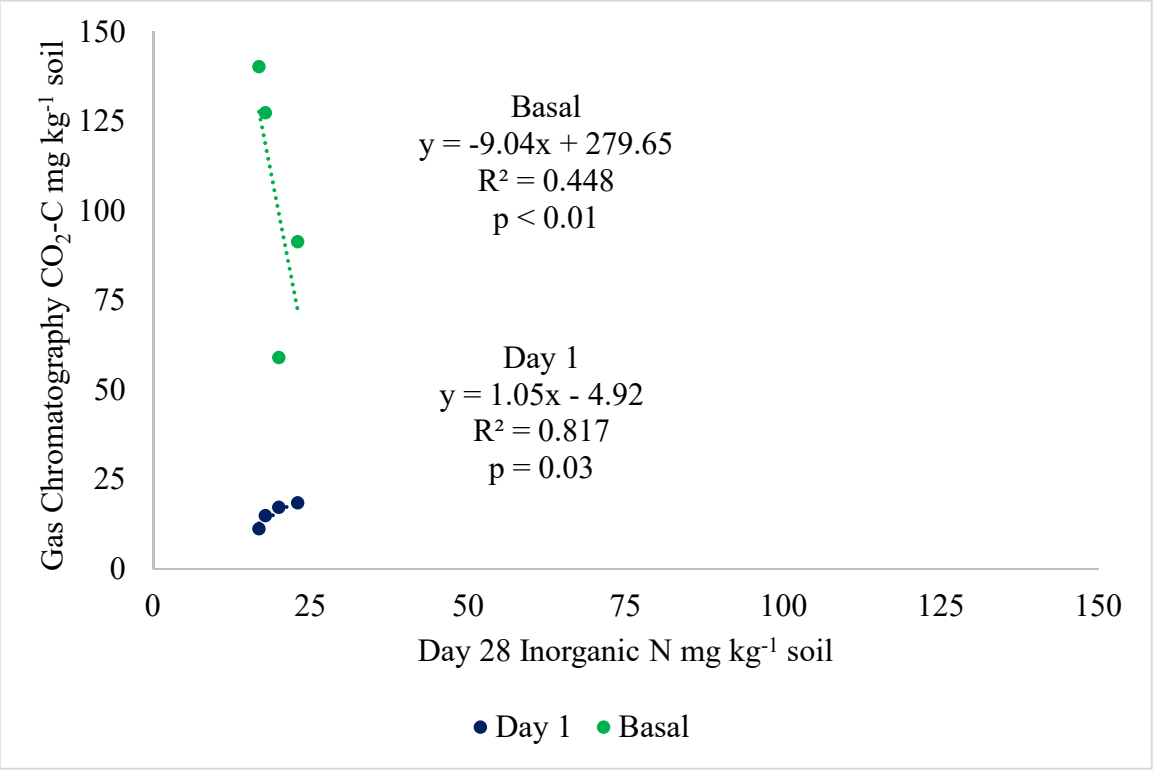

Figure 9. CO_2 production measured by Solvita Basal Respiration compared to soil inorganic nitrogen content at the end of the 28-day incubation period for the Cullars Rotation Study, Run 2.

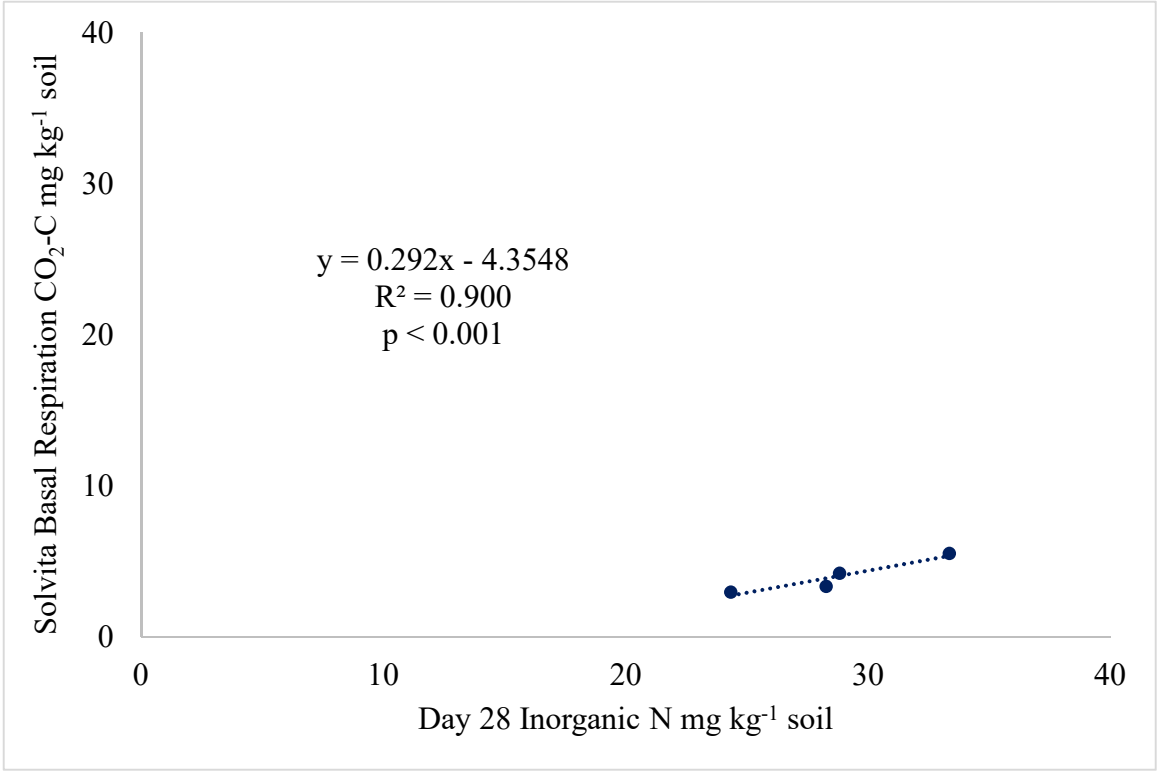

Figure 10. CO_2 production measured by Solvita Basal Respiration compared to the gas chromatography method on Day 1 and during the basal respiration period for the Cullars Rotation Study, Run 1.

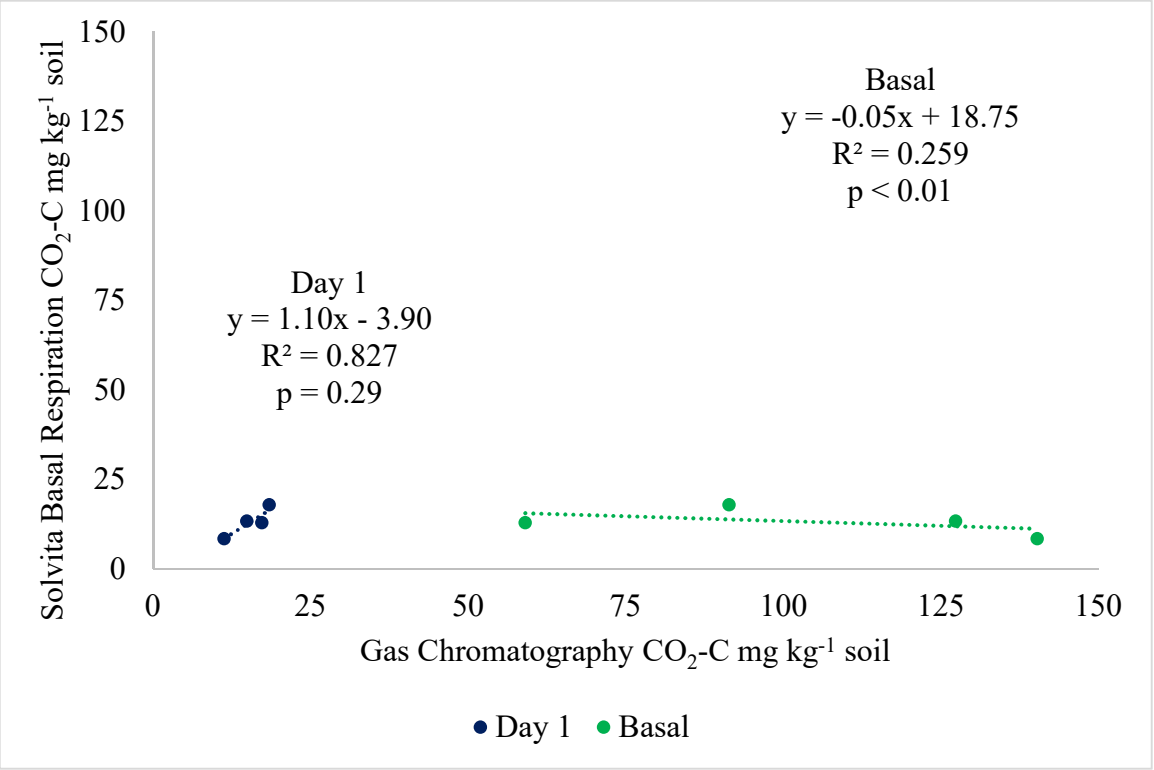

Figure 11. CO_2 production measured by base trap titration on day 1 and during the basal respiration period compared to the gas chromatography method on day 1 and during the basal respiration period for the Cullars Rotation Study, Run 2.

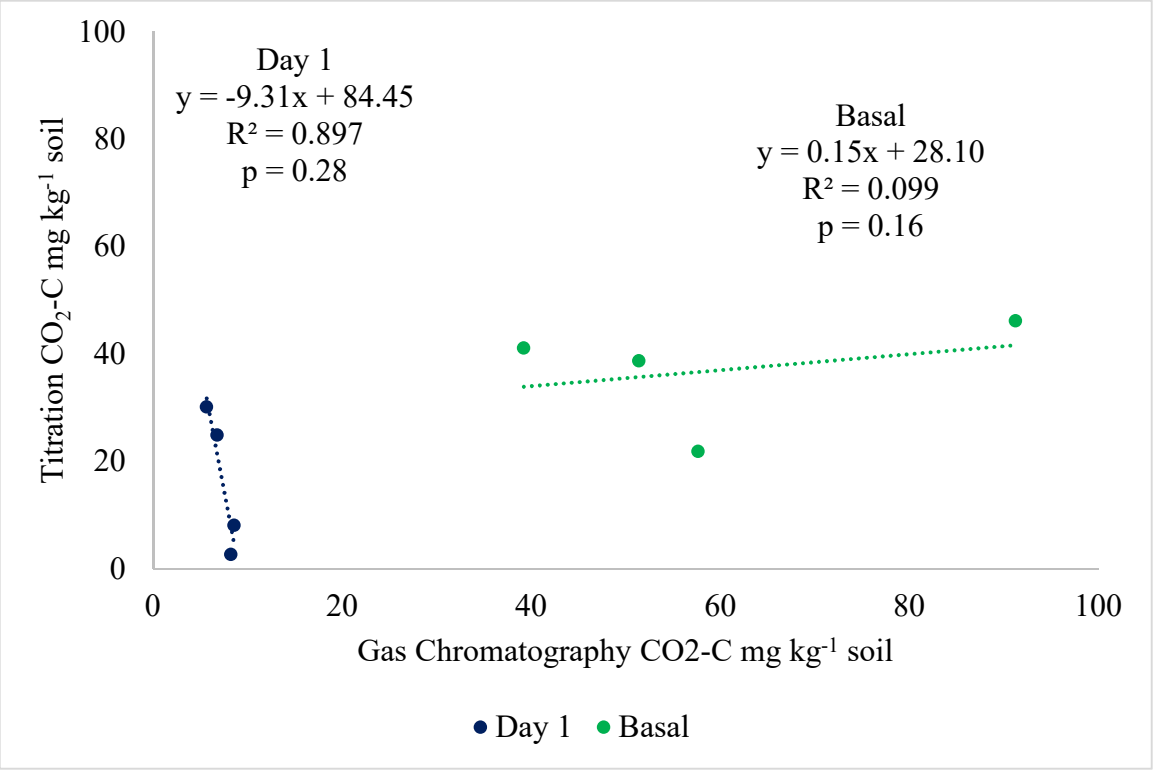

Figure 12. CO_2 production measured by Solvita Basal Respiration compared to the base trap titration method on Day 1 and during the basal respiration period for the Cullars Rotation Study, Run 1.