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Root Biomass and Other Soil Properties Affecting the CO2 Flush from Laboratory Dried and Rewetted Soils

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ROOT BIOMASS AND OTHER SOIL PROPERTIES AFFECTING THE CO_2 FLUSH FROM LABORATORY DRIED AND REWETTED SOILS

By

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An Abstract of the Thesis Presented

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Soil that has been dried and rewetted has been observed to release a 'burst' or 'flush' of carbon dioxide (CO_2) upon rewetting. This CO_2 flush has been proposed as an indicator of soil health. This may be a valuable indicator of soil health, however the CO_2 flush has yet to be fully evaluated. Roots and root exudates influence the soil in a variety of ways that may impact the CO_2 flush, such as increasing aggregation, organic carbon (C), and microbial biomass. We conducted both field and greenhouse experiments to elucidate the relationship of root biomass to the CO_2 flush. The field experiment was conducted with barley grown at Rogers Farm, Old Town, ME in 2017 (two sampling times) and 2018 (three sampling times). Three greenhouse experiments were conducted in the Roger Clapp Greenhouse. In Experiment 1, barley was grown for 4, 6, or 8 weeks; in Experiment 2, barley, corn, crimson clover, soybean, and ryegrass were grown for 4 weeks; and in Experiment 3, corn and barley were grown for 5 weeks at 4 levels of nitrogen. All had unplanted controls. We measured root biomass, microbial biomass carbon (MBC), dissolved organic carbon (DOC), and the amount of CO_2-C released during 72 hours after rewetting dried soil. Roots were quantified by wet sieving, rinsing, and drying. MBC was

determined by the difference between microwaved and non-microwaved samples, and DOC was extracted by water. For both, C was quantified with a Shimadzu TOC-V_{CPH}. For the CO_2 flush, dried soil was rewetted in sealable jars containing a septum, and the CO_2 in the headspace was quantified using an infrared gas analyzer. We found that planted soil had a larger CO_2 flush than bare or unplanted soil, but the effect was not large. Root biomass did not consistently correlate with the CO_2 flush. In unfertilized soils, the CO_2 flush was not influenced by plant species, but in fertilized soils, the CO_2 flush was significantly different between corn and barley. We found strong correlations between DOC and the CO_2 flush, and inconsistent correlations between MBC and the CO_2 flush. Because the CO_2 flush was not strongly influenced by collection time or plant species, the CO_2 flush may be a robust soil health indicator among different crops and sampling times. Our findings of a strong correlation between the CO_2 flush and DOC suggest that DOC could be explored as an indicator of soil health across a range of soils and regions.

DEDICATION

I would like to dedicate this thesis to my husband Scott for his love, devotion, and support throughout this adventure and for all the other adventures we have had, are having, and will experience.

To my parents Sandy and Jesse for showing me love and support and letting me try and fail and try and succeed.

To my naturally gifted brother Thomas who helped me develop a healthy sense of competition, and a sense of humor.

To my current pet Chestnut, who reminds me to take walks and enjoy the little things.

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LIST OF ABBREVIATIONS

Symbol	Meaning
C	Carbon
CO_2	Carbon dioxide
CRD	Completely randomize design
DOC	Dissolved organic carbon
GS	Growth Stage
MAFES	Maine Agricultural Forest and Experiment Station
MBC	Microbial biomass carbon
N	Nitrogen
NH_4	Ammonium
NO_3	Nitrate
NRCS	Natural Resource Conservation Service
O_2	Oxygen
ODE	Oven dried equivalent
SOC	Soluble organic carbon
TOC	Total organic carbon
WFPS	Water filled pore space

CHAPTER 1

INTRODUCTION

Soil science is an increasingly interdisciplinary field, important on all scales from the microscopic to the biosphere level (Ferris et al. 2010). Soils support terrestrial plant ecosystems and are the foundation for agricultural and forest productivity. In order to ensure the integrity of natural ecosystems and the continued use of human-altered land without loss of soil function and productivity, there is a need to develop practical indicators for soil health. Such indicators will allow monitoring of soils in order to ensure sustainable use. Soil health implies an ecological approach to looking at soil, encompassing such parameters and concepts as microbial life in the soil, ability for the soil to withstand stress, and nutrient cycling (van Bruggen and Semenov 2000). Comprehensive soil health testing generally involves the determination of a suite of biological, chemical and physical parameters or properties. One proposed indicator of biological soil health is the amount of CO_2 release that occurs after soil drying and rewetting under laboratory conditions. However more research is required to fully explore the factors that influence the CO_2 flush and how it relates to soil health (Franzluebbers 2016). This study attempts to identify important factors influencing the magnitude of the CO_2 flush. A biological soil health indicator would ideally respond to significant changes in soil health and not to transient or short-term changes that might occur over the course of a growing season. There is currently little work in published literature on what soil factors, either stable or transient, affect the CO_2 flush. Our focus was on providing a better understanding of the CO_2 flush in relation to root growth. These experiments help quantify the CO_2 flush as a biological soil health indicator.

1.1 Literature Review

Soil testing has a long history as a management tool, and soil tests for agricultural fields generally include measurements of soil organic matter; pH; macronutrients like N, P, and K; and many micronutrients. Traditional soil tests have not included any measure of soil biological activity. There is a progression towards evaluating soils on a more ecological scale. Soil health is

a broad term that encompasses chemical, physical, and biological properties. Soil health focuses on soil as part of an ecosystem promoting nutrient cycling and supporting microbial biomass and the microbial community's ability to be resilient in the times of disturbances and stress. These disturbances could include physical disturbances, such as tilling, and naturally-occurring stresses, such as a prolonged drought (van Bruggen and Semenov 2000). Understanding soil health is essential for many aspects of current agricultural land management as well as the maintenance and enhancement of many other ecosystems that are influenced by human activity (Morrow et al. 2016).

Franzluebbers et al. (2000) advocated the development of a method for quantifying potential soil biological activity. They evaluated the CO_2 flush following drying and rewetting as an indicator of the variability in soil biomass and C and N mineralization. They then accounted for mean annual temperature and precipitation to improve the quality of the indicator. They argued for using the CO_2 flush as an indicator for soil microbial health because it met many of the goals for a practical method. It is patterned after natural occurrences in most soils. The process has relatively simple steps with minimal equipment or procedural requirements, and it is rapid to implement. It also characterizes key properties of soil organic matter, with output changing according to amendments and manipulations of the microbial biomass. Another argument for use of the CO_2 flush as an indicator rather than exact measurements of individualized criteria is that it simplifies the information presented to land managers to give direction for future actions (Franzluebbers et al. 2000). Additional information, e.g. about root biomass effects on the CO_2 flush, will improve our understanding of the CO_2 flush as a biological indicator.

Others, including commercial laboratories, have advocated using the CO_2 flush as a rapid and practical means of assessing soil respiration and soil biological activity. Solvita® 'CO_2 burst' testing, from Woods End Laboratories in Mt Vernon Maine, provides an affordable and easy way of measuring C mineralized during the CO_2 flush (the amount of CO_2 released in a 24 hour or 72 hour period from laboratory dried and rewetted soils). This company is utilizing the CO_2 flush as

a potential indicator for biological activity within the soil to establish one part of measuring the overall soil health. Solvita® testing provides land managers with a number related to microbial activity; however, how to interpret that number is not always clear. The amount of CO_2 flush that indicates a biologically active and healthy soil is not fully understood across an array of diverse soils (Franzluebbers 2016).

The CO_2 flush, i.e. the rapid increase and then decline in CO_2 release rate upon rewetting dried soil, was first observed and described by Birch and Friend (1956) and is sometimes referred to as the 'Birch effect'; it has been investigated in a variety of studies since (Fraser et al. 2016). The microbial influence on the CO_2 flush has been demonstrated by Fraser et al. (2016). Fraser et al. (2016) investigated whether the CO_2 flush was caused by abiotic pathways, biochemical pathways, or biological pathways. An abiotic pathway is a strictly chemical reaction that does not require living things, e.g. calcium carbonate dissolution. Biochemical pathways involve extracellular enzymes existing in the soil that are not bound by a cell membrane. Biological pathways are cell bound processes, basically respiration from living organisms. Fraser et al. (2016) found the abiotic pathway was not a significant contributor to the CO_2 flush compared to both biological and biochemical pathways in the soils they examined. Although the microbial population was a significant contributor, they could not distinguish whether the biochemical pathways were more significant than the biological. This study showed that microbial activity was an important cause of the CO_2 flush in the laboratory and, presumably, the field (Fraser et al. 2016).

The CO_2 flush is thought, in part, to be due to the increase in microbial substrates caused by the accumulation of the contents of lysed cells desiccated during the drying phase and also to the increase in microbial substrate because of the exposure of previously protected organic matter with aggregate breakdown (Yu et al. 2014). The reason for the decline in CO_2 release rate after an initial increase may be related to the depletion of labile substrates or to an increase in predation of the microbial biomass (Anderson 2011). Others have examined the buildup of osmolytes and

other organic material in rapidly air-dried soils. Warren (2016) found that extracellular osmolytes and depolymerized soluble C was higher in soils after drying and rewetting, which increases the potential substrate for remaining microbes.

The CO_2 flush also occurs in natural environment when soils undergo drying. Drying and rewetting events are common in soils, particularly in deserts and semi-arid environments. In natural environments if the water potential drops below a threshold of 100 to 1000 kPa that is likely to lead to a CO_2 flush, or elevated soil respiration, following a precipitation event (Lado-Monserrat et al. 2014). Much of what we know about factors influencing the CO_2 flush comes from field and laboratory studies of unmanaged, non-agricultural soils. While investigating CO_2 flux on semiarid perennial steppe in Spain, Rey et al. (2017) found that across different ground covers characteristics of the region there was a significant increase in the CO_2 release for 24 hours after rainfall events. Other studies have indicated that the CO_2 flush could occur seasonally. For four different land uses (cropland, jujube orchard, shrubland and grassland) on the Loess Plateau in China, Sun et al. (2018) noticed when volumetric water content reached less than 3 percent and soils were rewetted by an extreme (>40mm) or moderate (10 mm) precipitation event there was an increase in soil respiration for 24 hours to four days, followed by a decline in soil respiration. Although the flush refers specifically to drying and rewetting events, soil may release increased CO_2 pulses in tropical environments, where moisture is often not a limiting factor. This is due to soluble organic C leaching through the soils during the rainy season (Cleveland et al. 2007). Cleveland et al. (2007) experimented with tropical soils by adding a leachate from native leaf litter which increased the microbial populations and also caused a significant increase in CO_2 respiration (Cleveland et al. 2007). These studies demonstrate that a pulse of CO_2 occurs in many different environments over a range of climates in response to changes in environmental factors.

Agricultural practices, such as tillage, can also cause a rapid release of CO_2 from soils. Tillage is an important technique used on many conventional and organic farms to suppress weeds and incorporate nutrients, but it can lead to detrimental side effects like loss of soil

aggregates and the formation of a plow pan. Ellert and Janzen (1999) looked at the effects of tilling a previously untilled field on the CO_2 release. They found that tilling the soils did cause an increase in the CO_2 release initially. After 24 hours, the tilled field was back to the same CO_2 respiration as the surrounding area. They found the increase was likely due to previously trapped CO_2 being released to the atmosphere from the soil (Ellert and Janzen 1999). There is also evidence that increased soil respiration with tillage is partly attributable to increased O_2 incorporation into the soil (Fiedler et al. 2015).

Suitable microbial substrates may also be trapped in soil aggregates and unavailable to soil microbes prior to a drying and rewetting event. Adu and Oades (1978) used ^{14}C starch to elucidate the question of whether aggregates release previously unavailable C to the soil after aggregate disruption. After confirming that they were able to create soil aggregates with ^{14}C starch trapped within, Adu and Oades (1978) used physical mechanisms (sieving), and drying and rewetting of the soil as methods to destroy aggregates. They found that physical disruption of aggregates by sieving led to a higher respiration of $^{14}CO_2$ in comparison to their control, confirming that the breakdown of aggregation lead to previously unavailable ^{14}C being respired. They also noted that disturbed macroaggregates released more C than disturbed microaggregates. After the soil was dried and rewetted, they found there was a larger $^{14}CO_2$ release than that due to sieving, which indicated previously unavailable starch being utilized after the rewetting event (Adu and Oades 1978). Denef et al. (2001) also investigated aggregate disruption upon drying and rewetting. In soil samples that were air dried and rewetted to field capacity, there was a reduction in amount of aggregation and size of soil aggregates compared to the control which was maintained at field capacity (Denef et al. 2001). In the treated samples Denef et al. (2001) noted an increase in microaggregates compared to the control, which was likely the result of macroaggregate breakdown during the drying and rewetting process. These studies demonstrate that rewetting may decrease aggregation, which increases previously unavailable C substrate, and may potentially increase the CO_2 flush.

Drying and rewetting events may increase rates of microbial growth and lead to increased microbial populations. When investigating soils and sediment from a transect of Barnett Creek in Pilbara region of Western Australia, McIntyre et al. (2009) found rapid microbial growth, with a 3-fold increase in the first 24 hours after previously dried soils were rewetted. Drying before a rewetting cycle tends to cause a significant increase microbial growth after a lag period (Meisner et al. 2017). Meisner et al. (2017) found that when soils that were dried to approximately 3 % water holding capacity and rewetted to field capacity, there was an approximate 23 hour lag in growth. However the maximum growth rate was 5 times higher than the soils that were maintained at field capacity with no drying (Meisner et al. 2017).

There have been a few studies that have linked the CO_2 flush to the microbial biomass C (MBC) in the soil. A study that collected soils before row crops or forage were planted from several different sites (Alberta, British Columbia, Georgia, Maine and Texas) found that 86 % of the 72 hour CO_2 flush could be explained by the MBC using linear regression (Franzluebbers et al. 2000). A more recent Franzluebbers et al. (2018b) study, utilizing 47 corn sites from North Carolina and Virginia found that MBC explained 64 % of the 72 hour CO_2 flush. These studies suggest that the amount of microbial biomass is a strong contributor to the CO_2 flush and that it is an indicator of the biological activity of the soil. The trend of a relationship between MBC and the CO_2 flush was also noticed in tall fescue pastures that were occasionally grazed. This study examined 57 fields in Georgia, North Carolina, Virginia, and West Virginia, and found that MBC correlated with the 72 hour CO_2 flush (r value 0.83) (Franzluebbers et al. 2018a). These studies demonstrate that across several different soil types, and two land uses (row crops and pasture), the MBC has a strong relationship to the CO_2 flush.

Both soil characteristics and how soils are processed in the laboratory may influence the CO_2 flush. Some of the parameters that have been investigated are sieve size, soil depth, soil texture, wetting method, change in moisture, and extent of soil drying. Franzluebbers and Haney (2018) found no apparent effect of sieve size on the CO_2 flush. There was an influence of soil

texture on CO_2 flush, however it was mostly due to the influence of texture on the rewetting method. When wetted bottom up through capillary action, coarser-textured soil had a smaller CO_2 flush than finer-textured soil, which was likely due to having a higher water filled pore space (WFPS) than finer-textured soil (Franzluebbers and Haney 2018). Top down rewetting methods ensured similar WFPS across soil textures. Soil sample depth had a significant influence on the flush. Soils collected from the top 10 cm of soil had a higher 24 hour CO_2 flush than soils collected from 10 to 20 cm, however that effect no longer occurred with the 72 hour CO_2 flush (Franzluebbers and Haney 2018). Lado-Monserrat et al. (2014) found the change in moisture in both field and laboratory experiments had a strongly positive correlation to the CO_2 flush i.e. the greater the change in moisture the larger the flush (Lado-Monserrat et al. 2014). This corresponds with Guo et al. (2014) who examined the extent of drying before rewetting on the CO_2 flush of soils in a laboratory experiment. They found that the CO_2 flush was greater when soils were more extensively dried (Guo et al. 2014). Knowing how testing parameters influence the flush can allow for comparisons across studies and consistency among commercial laboratories.

A few studies have correlated different C fractions to the amount of CO_2 release from dried and rewetted soil. The rate of CO_2 release may be limited by the amount of C in the soil instead of the MBC population (Wang et al. 2003). One study examined the laboratory respiration rate of dried and rewetted soil after a 7 day incubation period and used various extractants to quantify total organic C (TOC), water-soluble organic C, $KMnO_4$-oxidisable C, and K_2SO_4 extractable C (Wang et al. 2003). They found that all forms of extractable C significantly correlated, and K_2SO_4-C had the strongest correlation, with the release of CO_2. They concluded that a linear model predicting CO_2 release from dried and rewetted soil would be significantly improved if both MBC and K_2SO_4-C were measured and not just MBC (Wang et al. 2003). Franzluebbers et al. (2018b) investigated 47 corn sites and ran regressions for TOC and particulate organic C (POC) with the CO_2 flush. They found that TOC had the lowest r^2 value and that POC had a higher r^2 value (69 % of the flush explained by POC) (Franzluebbers et al.

2018b). In the tall fescue pasture experiment by Franzluebbers et al. (2018a) they found significant correlations between the CO_2 flush and TOC and POC (r values of 0.73 and 0.43, respectively).

Nitrogen is an important agricultural nutrient, and how N affects the microbial community may have consequences for the CO_2 flush. Geisseler et al. (2016) looked at the effects of added N on microbial biomass in both permanent grassland and agricultural fields. They found a decrease in microbial biomass in the grassland but an increase in microbial biomass in the agricultural field due to added N. In the permanent grassland, N decreased the diversity of the plant life, which led to the decrease in microbial biomass. The increase in the microbial biomass in the agricultural field was likely due to the increase in root biomass in the soil which provided more niches and substrates for microbial organisms. This study is important because it demonstrates how a commonly performed agricultural task, adding N, might change the soil health depending on land use (Geisseler et al. 2016). Zhu et al. (2016) investigated the effect of different levels of N (0, 10, 30, 50, 80 and 160 mg N L^{-1} soil) on microbial populations in the corn rhizosphere. There was a trend for an increase in microbial abundance as N increased with 160 mg N L^{-1} being the greatest, and significantly different from 30 mg N L^{-1} and lower rates (Zhu et al. 2016). One of the reasons for the increase in microbial growth could be the effects of N on root exudation. They examined the amount and types of root exudates released at three different levels of N (30, 80, and 160 mg N L^{-1} soil) and found that there was a general increase of exudation with increased N with sugar, and sugar alcohol, exudates being the greatest at N160 (Zhu et al. 2016). The tendency for N to increase both root exudation and the microbial population in agricultural soils, may lead to an increase in the CO_2 flush.

Roots influence the microbial community immediately surrounding them in the soil. Since the microbial community and its extracellular enzymes significantly affect the CO_2 flush (Fraser et al. 2016), it is possible that the root microbial community could affect the amount of CO_2 flush. Each plant species selects for a unique microbial community possibly with a unique

root exudate profile (Berg and Smalla 2009). Roots may attract r-strategist microorganisms with light weight C exudates (Shi et al. 2015). Having a higher amount of r-strategist in the soil could lead to a larger initial flush because r-strategist tend to rapidly use simpler C sources. Baumert et al. (2018) found that high root exudation led to an increase in microbial populations and significant changes in the ratio of fungi to bacteria, because fungal growth was significantly increased. This shift in the microbial community could impact the CO_2 flush because the fungi increased soil aggregation (Baumert et al. 2018), which may sequester more C to be available to surviving microorganisms during the CO_2 flush. Shifts in microbial communities may influence the CO_2 flush, but this has not been studied.

1.2 Study Objectives

Our field and greenhouse experiments focused on the influence of the presence, or lack, of root biomass on the magnitude of the CO_2 flush in order to improve interpretation of the CO_2 flush parameter as a soil health indicator. Additionally, our study investigated added mineral N as well as various crop species. Examining different crop species, and the effects of added N, was intended, in part, to increase the range in root amount among treatments in our experiments and in order to examine correlations between quantity of roots and the CO_2 flush. We also investigated the root biomass influence on two factors that are known to play a part in the CO_2 flush, MBC and dissolved organic C (DOC). We expected DOC might reflect root exudation or, at least, the amount of readily available microbial substrate.

We hypothesized that the CO_2 flush would be greater in soils with roots than soils without roots. We also expected that with increased root biomass there would be an increase in the CO_2 flush that correlated with root amount. We expected that root biomass would increase with soil N level, possibly leading to more root exudates, and to a corresponding increase in the CO_2 flush. We expected that any observed increases in the CO_2 flush due to experimental conditions would be highly correlated with changes in the MBC and DOC that are associated with the presence of roots.

CHAPTER 2

MATERIALS AND METHODS

2.1 Field Experiment

The Field Experiment was located at Rogers Farm in 2017, and in 2018. Rogers Farm is a Maine Agricultural and Forest Experimental Station (MAFES) facility located in Old Town, ME at 44º 56' 24" N and 68º 42' 0" W. The soil is a Nicholville with a soil texture of loam in 2017, and fine sandy loam in 2018. In 2017 the field was fertilized with 67 kg NH_4NO_3 ha^{-1}, 90 kg P_2O_5 ha^{-1} (triple super phosphate) spread by hand and incorporated with Perfecta harrow. In 2018 the field was fertilized with a 10-10-10 fertilizer from Northeast Agriculture Sales Inc., comprised of urea, potassium nitrate, diammonium phosphate, and potash, at a rate of 616 kg ha^{-1}. The 2018 Field Experiment was treated with an herbicide, MCPA Amine 4 on June 8, 2018. The rate of the active ingredient, dimethylamine salt of 2-methyl-4-chlorophenoxyacetic acid, was 0.05 ml m^{-2}. Barley (*Hordeum vulgare* 'Newdale') was planted May 25, 2017, and on May 12, 2018 with a target planting density of 350 plants m^{-2}.

In 2017 Field Experiment (layout Figure A1), treatments were a factorial combination of two factors each with two levels: roots (presence vs. absence), and sampling time (mid vs. late season). The experimental design was a randomized complete block design (RCBD) with 5 replicates, for a total of 20 plots. The mid-season sampling took place on July 11 and the late season sampling on August 7, 2017. The Zadok growth stage of barley was determined to be 49 and 85 (soft dough), respectively, at the sampling times (Zadoks et al., 1974). The plots were buffered from the edge of the field by 1 to 4 m of barley. Plots were 3 m by 1.8 m with a buffer strip of about 0.7 m between them. The 2018 Field Experiment (layout Figure A2) treatments were a factorial combination of two factors with two levels and three levels: roots (presence vs. absence), and sampling time (early vs. mid-season vs. post-harvest). The experimental design was a RCBD with 5 replicates, for a total of 30 plots. In 2018 the early season sampling was June 20, the mid-season sampling was on July 9, and the post-harvest sampling was August 24, two weeks

after harvest. In addition, in 2018 there was an initial sampling on May 17. This was before herbicide application and 5 days after the field was planted, but before coleoptile emerged through the soil surface. This data was not included in the statistical analysis; however, it is referenced in the results. The Zadok growth stage (GS) of the barley was 32 at the first sampling date and 49 at the second sampling date; harvest occurred around GS85 (soft dough) (Zadoks et al., 1974). The 2018 plots were 1.25 m by 1.25 to 1.5 m. There were shorter plot sizes for some due to tire ruts from the herbicide applications. There was a minimum buffer of 0.5 m between each plot. In both years the bare plots were maintained by hand weeding throughout the season. In 2017 the plots were weeded about once every week. In 2018 the plots were weeded approximately every 2 weeks. For both seasons, hand weeding occurred more frequently in the beginning of the season and less frequently as the season progressed.

Sampling for soil characterization took place June 6, 2017. Twelve 2 cm cores were collected from each block and mixed in a large plastic bag. Three blocks from the stored dried soil from the May 17, 2018 sampling were subsampled on November 21 2018 and A subsample was submitted to the MAFES laboratory for a standard soil test (Hoskins, 1997).

At each sampling time, soil temperature was measured at two location for each plot with a digital thermometer at a soil depth of 15 cm approximately 0.25 m in from the east and west end of the plot in 2017 and the north and south end of the plot in 2018. Roots were sampled using an 8 cm bucket auger to a depth of 20 cm by the following procedure. The middle row of the plots was located by counting the rows in, from the south side of each plot to the sixth row in 2017, and from the west side to the fourth row in 2018. For both years the samples were taken from either side of the center of the plots. Two bucket augers of soil were extracted from either just north, or just south of center in 2017, and from east or west of the center in 2018; for both years one auger of soil was taken from the opposite side of center from the first two. For each plot these samples were combined in a large sealable plastic bag and placed in a cooler immediately after collection. In 2017 both planted and bare plots were sampled for roots. In 2018, only planted plots were

sampled. Once transported back to the laboratory the samples were stored in a refrigerator for 2-3 days.

After the auger cores were collected, ten 2-cm diameter core samples were collected by the following procedure. A meter square grid, with a total of 25 squares (20 cm by 20 cm) within it, was placed with the center square lined up with the center of the plot. R's base function "sample" was used to pre-determine which ten squares would be sampled. Each core was collected to a depth of 20 cm. The cores for each plot were combined in a large plastic bag and immediately placed in cooler for transport back to the laboratory.

Roots were extracted from bucket auger samples using a procedure similar to Rivas et al. (2014). Samples were removed from the refrigerator and gently mixed by inverting the bag and stirring with gloved hand. Approximately 1100 ±110 g of field moist soil was added to clean 13.25 L plastic buckets filled half way with tap water. The soil/ water mixture was gently stirred and then allowed to soak for 4-7 hours in 2017, and 2-4 hours in 2018. The roots were then extracted using a combination of sieving and forceps. Clearly-visible roots floating on top were removed. A 500 μm sieve was placed on a holder that allowed for rinse water to be caught and the soil/water mixture poured through the sieve. Roots remaining on the sieve were extracted using forceps. The soil was gently rinsed away from the roots. The above procedure was repeated with 150 μm and 75 μm sieves using the rinse water that passed through the larger sieves. Once all roots were collected from the sieve, the sieve was rinsed, and the rinse water was sieved a second time to collect remaining roots. Extracted roots were rinsed in a clean beaker of water and dabbed on a paper towel to remove some moisture. These roots were then placed in a previously weighed tin. The roots were dried for 24 hours at approximately 50º C, and were returned to the oven and reweighed after an additional 3 hours to insure they had reached a consistent weight.

The small diameter cores were sieved moist through a 2 mm sieve and mixed the same day that they were collected from the field. Approximately 400 g of field moist soil was dried at 40º C for a minimum of 24 hours. Oven dried soil was stored for 3-6 days in plastic bags in the

dark before being analyzed. The remaining 2 mm sieved soil was placed in plastic bags and refrigerated. To determine percent moisture (Pw) 12 ±2 g of field moist soil was dried at 106º C overnight, and reweighed at 24 hours to ensure that consistent weight was reached.

The CO_2 flush was determined by two methods (LI COR and Solvita®) after rewetting oven dried soil (40º C). CO_2 was analyzed using a LI-COR Li-7000 CO_2/H_2O Analyzer. To create a standard curve a 5 ml syringe was used to inject 1, 2, 3, 4, and 5 ml of a 2000 ppmv CO_2 standard (2 replicate injections). The ideal gas law was used to convert the volume-based standard concentration to a mass-based concentration (Equation 1). The mass-based concentration of CO_2-C was multiplied by the volume injected to determine amounts injected. The standard curve represents the relationship between mass of CO_2 –C and instrument counts.

$$Cm = \frac{(Cv * M * P)}{(R * T)} \quad (1)$$

Equation 1: Ideal gas law. Cm is the mass-bass concentration, Cv is the volume-based standard concentration is the µmol of C, M is the molecular weight of C (12 µg µmol^{-1}), P is the atmospheric pressure (1 atm), R is the universal gas constant (0.08206 L atm K-1 mole-1), T is the temperature in Kelvin

The volume of water required to reach 50% WFPS was calculating using Equation 2 by adding 40 g of 40º C dried soil to a 50 ml plastic graduated cylinder, tapping the cylinder on the counter to settle, and recording the volume.

$$WFPS = \left(Total\ Soil\ Volume - \left(\frac{Total\ Soil\ Weight}{Particle\ Density(2.65\ g\ cm^{-3})}\right)\right) * Desired\ \% \quad (2)$$

Equation 2: water filled pore space

Soil (40g) was added to clean 0.479 L Mason jars with predrilled lids containing Swagelock fittings holding a septa. The volume of water to reach 50% WFPS was determined for

each sample (Field Experiment n=10, Greenhouse Experiment 1 n=8, Greenhouse Experiment 2 and 3 n =12), and the appropriate mean volume was added to each individual sample for that experiment.

The soil in the Mason jars was rewetted by hand pipetting. Immediately after the last drop of water was added the jars were sealed. A 5 ml syringe was used to extract 1 ml of headspace gas from the jar and injected into the LI-COR. Injections were done in duplicate. The headspace gas was replaced by 2 ml of room air. Room air was gathered near an air conditioner with the fan function on. After room air was added back to the mason jars, they were placed in an incubator set at 25º C. There were 4-5 minutes between each rewetting to allow for sampling time. After the initial LI-COR reading, additional readings were taken at 1, 3, 5, 8, 12, 24, 48, and 72 hours. The jars were flushed after the readings at 12, 24, and 48 hours, by opening them in front of the fan and rotating the jar back and forth. After flushing the jars, there was an additional LI-COR reading, and the 2 ml of air extracted from the jars again replaced by 2 ml of room air.

To calculate the rates of CO_2-C release at each sampling time, the headspace volume of the jars was calculated. For each sampling date the average volume of the soil was found, including soil pore space, and was subtracted from the total volume of the jars. The LI-COR readings were then converted to rates (μg CO_2-C g^{-1} of soil hr^{-1}) by multiplying the change in μg CO_2-C between the intervals, by the average headspace volume of the jars. That number was then divided by the dry weight of soil (40g) and by the interval time.

The total mass of μg CO_2-C produced was calculated for two time periods, 24 and 72 hours. This was done by summing the amount of CO_2 produced during 2 intervals (0-12 and 12-24 hr.) for the 24 hour production, and 4 intervals (0-12, 12-24, 24-48, and 48-72 hr.) for the 72 hour production.

The commercial Solvita® test was carried out following the directions, with one slight modification. The amount of water added to the dried soil was the average 50% WFPS for all samples rather than adding individualized amounts to each sample. These jars were placed in an

incubator set at 25º C for 24 hours. The colorimetric Solvita® paddles were read with an electronic reader.

MBC was determined by the microwave method as described in Islam and Weil (1998) for both field moist and oven dried soil. The amount of water necessary to reach 80% WFPS was determined by placing 15 g of dried soil in a plastic 50 ml graduated cylinder and using Equation 2. Soil (15 g oven dried equivalent [ODE]) and the water needed to bring it to 80% WFPS was added to 50 ml plastic centrifuge tubes. For the samples to be microwaved, centrifuge tube tops had holes drilled for venting. Microwaved and non-microwaved samples were run in duplicate.

The microwaved samples were exposed in batches of eight to 400 J g^{-1} of soil in three pulses of 24 seconds. The microwave was a SHARP household microwave oven and supplied 660 J s^{-1}. The sample temperature was checked between microwave bursts to ensure the samples did not exceed 88º C.

The microwaved and non-microwaved samples were extracted by adding 30 ml of 0.01 M K_2SO_4 to the 50 ml centrifuge tubes. Caps that had holes in them were replaced with regular caps. The samples were shaken horizontally at 180 rpm for 1 hour, then centrifuged for 30 minutes at 3300 rpm. The supernatant was filtered through a 0.45 µm pore size filter. After all samples were filtered, they were analyzed with a Shimadzu TOC-V_{CPH} for dissolved C. The MBC was estimated by subtracting the non-microwaved sample C from microwaved sample C. Islam and Weil (1998) determined that the microwave method correlated strongly with the chloroform fumigations (r^2=0.908).

The DOC was obtained, for field moist and oven dried soil, by weighing out 15 g ODE soil into centrifuge tubes and adding 30 mL deionized water to oven dried soil and an amount of water to bring the total to 30 mL for field moist soil. Tubes were sealed and shaken on a table shaker horizontally for 1 hour at 180 rpm. The supernatant was filtered through 0.4-0.45 µm filter paper under vacuum assistance and analyzed using the Shimadzu TOC-$V_{CPH.}$

2.2 Greenhouse Experiments

Soil collection for greenhouse experiments occurred on November 14, 2017. The soil was collected over the entirety of the field that was used in 2017. A shovel was used to extract the soil from 20 cm squares to a depth of approximately 20 cm. The soil was placed in a lined 5 gallon bucket, transported to a tarp, and mixed by coning and quartering i.e. quartering it into the four corners of the tarp, and then pulling the soil back in towards the center. This was repeated 8 times in the field. The soil was then transported to the laboratory. Over the next 3 days 400 kg of soil was sieved (4 mm); debris and rocks were discarded. The sieved soil was placed in clean plastic lined 113.5 L bins. The bins were stored with secured lids and tarps around them in an unheated shed. A subsample of the stored soil was collected on April 18, 2018 and submitted to the MAFES laboratory for standard soil testing (Hoskins, 1997).

Prior to use in each greenhouse experiment, an appropriate estimated amount of soil, sampled equally from each storage bin, was transported to the laboratory in 5-gallon buckets. The soil was coned and quartered 10 times the day prior to, and stored at room temperature until, use. Four 10 ± 2 g samples of soil were dried overnight at 106ºC to calculate the Pw. A starting Pw of 0.26 was used for all experiments.

This soil was used in three experiments that took place in Roger Clapp Greenhouse, house 2. Before starting the greenhouse experiments a grid was constructed on the bench. It was setup that widthwise used numbering (1-4) and lengthwise used lettering (A-U). This was done to establish completely randomized design (CRD) for pot location. Experiment 1 treatments were a factorial combination of two factors, one with two levels and one with three levels: roots (presence vs. absence), and number of weeks (4 vs. 6 vs. 8 weeks). The first factor was barley (‘Pinnacle’) or unplanted. There were 4 replications, and the 24 pots were placed on the greenhouse bench using CRD on grid sections corresponding to numbering 1-4, and lettering A-F. This experiment was started on February 13, 2018 and soil was collected on March 13, March 27, and April 10. Experiment 2 had 6 treatments, unplanted, barley (‘Pinnacle’), corn (*Zea mays)*,

crimson clover (*Trifolium incarnatum)*, soybean (*Glycine max)*, or ryegrass (*Lolium multiflorum)*. Experiment 2 was started on March 5, 2018 and harvested on April 18. The location on the bench for experiment 2 was numbering 1-4, and lettering P-U. The pots were placed based on CRD with 4 replications of each treatment. There was a total of 24 pots for Experiment 2. Experiment 3 treatments were a factorial combination of two factors, one with three levels and one with four levels: plant species (barley ('Pinnacle') vs. corn vs unplanted), and N level (0 vs. 1 vs. 2 vs. 3). The N levels correspond to different amounts of added N (0, 0.015, 0.030, or 0.060 g kg^{-1} soil, respectively). This experiment took place after experiment 2 finished in the greenhouse and was started on May 2, 2018 and soil was collected on June 5. There were 4 replications for a total of 48 pots. CRD was used to place the pots on the grid at numbering 1-4 and lettering J-U.

The pots were cylindrical in shape and approximately 1.5 L in volume. The bottom of the pots contained 8 evenly spaced 1 cm diameter drain holes arranged in a circle. The pots for the greenhouse experiments were rinsed and then soaked in bleach water for a minimum of 3 hours prior to use. Soaked pots were then rinsed four times and allowed to air dry. A piece of gardening fabric was cut to fit the bottom and placed within each pot to prevent soil from falling out of the drainage holes.

Experiment 1 and 2 had 1100g ODE of soil added to the pots with no added fertilizer. Experiment 3 had additional P (0.132 g of K_2HPO_4 per pot) and N (0, 0.0429, 0.0857, or 0.1714 g NH_4NO_3 per pot). Fertilizer treatments were added to individual plastic bags each containing 1000 g of ODE soil. The plastic bags were inverted twenty times to mix and then the soil was added to the appropriate pot.

For all experiments, eight pregerminated seeds were added to the planted pots by using a wooden dowel to create a 5 cm hole. A seed was placed into each hole using forceps, and soil was gently brushed over it. DI water was used to bring the soil in the pots up to 0.26 Pw, and the total weight of the pots was recorded. Pots in Experiment 3 had 45 g of small diameter plastic balls added to the surface after the plants had emerged to decrease surface evaporation. Pots were

watered approximately daily. A watering can was used with modified spout to help reduce the impact of the water on the soil surface. At each watering time the pots were placed on a scale within the greenhouse and were watered with DI water until the weight was approximately equal to the starting weight, or the modified starting weight based on plant growth (see next paragraph).

For each experiment 4 extra pots of each treatment, except unplanted, were prepared and as described above. This was done to account for the weight added to pots due to plant growth. Periodically an extra treatment for each type was sampled for plant growth weight. The plants were removed, and soil was rinsed from the roots. Excess water was dabbed off with paper towels and the plants were weighed. The corresponding treatment type had the weight of the plants added to the amount of DI water that that treatment was going to receive.

The experiments were rotated within their grid locations. They were rotated weekly following a pattern to make sure they experienced similar greenhouse conditions i.e. wind (fan), light, and heat. To check for variability of surface heat on the pots 16 ibuttons were placed on a representative sample of the pots. There was no significant variation in surface temperature.

At harvest, pots were lightly squeezed and then the entire contents were dumped into a separate clean bin. The roots were gently shaken from the soil. The gardening fabric was cut from the roots, and the roots that were entwined in the gardening fabric were gently scraped out using a razor. The roots were washed in the sink, with a bin below to catch falling root debris. After the roots were washed they were dabbed with paper towels and then cut from the stem of the plant. Both stem and the roots were weighed separately. The stems and roots were left to air dry for the first 3 days, and then finished off in the oven at 55^{o} C for a minimum of 24 hours. Then the dry weight of the stems and roots were recorded.

Soils from the pots were analyzed for CO_2 release (LI-COR), MBC, and DOC as described previously. For greenhouse experiment 1 both the microwaved and non-microwaved, moist and oven dried samples for week 4 were accidentally moistened with 20 % more water than calculated. To compensate for this, extra samples were run during week 6 at 20% more water, and

the average percent change compared to the correctly-moistened samples from week 6 was used to correct the readings from week 4. This correction factor increased the week 4 readings by 16%.

In addition to the above laboratory procedures, moist soil from Experiment 3 was submitted to the MAFES laboratory for NH_4 and NO_3 analysis. This was done by weighing out 4 grams of ODE soil into 50 ml centrifuge tubes. They were extracted by MAFES personnel with 40 mL of 1N KCl and analyzed colorimetrically by flow injection analysis using a O.I. Alpkem A/E ion analyzer.

2.3 Statistical Analysis

R studio was used for statistical analysis. Normality and equal variance were checked for all data sets using Shapiro-Wilk test and Levene's test, respectively. If the data met these standards, then they were analyzed. Protected least significant difference test (LSD) was used for mean separation. Data that did not meet the assumptions are listed in Table 1, along with the correction used before it was analyzed. Some data did not meet one of the assumptions and in this case a logarithmic base ten transformation was used. The means from the protected LSD test for the log logarithmic base ten results were transformed back to the same format as the original data by raising 10 to the power of the mean.

Manly permutations were used in instances when data would not meet the assumptions above, and there were no suitable transformations for the data, i.e. all transformations tried resulted in one of the assumptions being false. Manly permutations randomize the results over the entire data set for a set number of times. In this case 1000 permutations were randomly generated in R. These permutations result in F values that can then be used in analysis of variance. For data sets that had blocking, the randomization of the results was constrained within the appropriate block and not over the entire data set. Bonferroni adjustment was used for separation of means in these cases because it is resistant to issues caused by unequal variance (William Halteman, personal communication).

The relationship between CO_2-C release in 24 hours (LI-COR) and different factors was examined with Pearson Chi Squared testing. The factors examined were dried root biomass, dried soil DOC, moist soil MBC, and, in Experiment 3, the amount of NO_3^- remaining in moist soil. For all correlations involving dried root biomass samples without plant growth in them were excluded from the Pearson's Chi Square testing. In 2018 post-harvest data was eliminated because in 2017 we did not have post-harvest data.

Table 1: Data that did not meet the assumptions for ANOVA testing and underwent a correction.

Experiment	Data	Correction
Field Experiment:		
2017	Moist MBC	Manly
	Moist DOC	Manly
2018	Moist MBC	Log_{10}
	CO_2 Flush	Manly
	Dried MBC	Manly
Greenhouse Experiment:		
1	Dried MBC	Log_{10}
	Moist DOC	Manly
2	CO_2 Flush "Unplanted" included	Manly
	CO_2 Flush "Unplanted" excluded	Manly
3	Moist MBC	Log_{10}
	NO_3 remaining "planted only"	Log_{10}
	Moist DOC	Manly
	Dried DOC	Manly
	NO_3 remaining	Manly

CHAPTER 3

RESULTS

3.1 Field Experiments

Table 2 shows the soil characteristics for each year. There was a sight difference in percent sand between 2017 and 21018 (Table 2). Table 3 shows soil temperature and moisture at the time of soil collection. In 2017 the planted plots were much drier than the bare plots, and August was the driest sampling date. In 2018 the Pw was similar for both treatments and closer to equal for collection times. This is likely a product of the weather. In 2018 some of the collections were delayed because of rain; this led to the collection times occurring shortly after rain events. In 2017 collection times were not rain delayed.

In 2017 the bare plots contained no measurable root mass (data not shown); based on these results bare plots in 2018 were not sampled. Root biomass recovered from the planted plots was variable from sample to sample with coefficients of variation from 15 to 56. The biomass increased somewhat with barley growth stage, and by two weeks post-harvest had clearly decreased (Table 3).

Figures 1 and 2 show rates of CO_2 release for 72 hours after rewetting dried soil for the 2017 and 2018 field seasons, respectively. In 2017 rates of CO_2 release were high initially, and then dropped off. At hour 12 the rates increased again slightly, and then they began to decrease. In 2018 the initial rate was not as high as in 2017. The rate decreased at the 3 hour mark but the decrease was not as large as in 2017. In 2018 two of the post-harvest replicate samples had CO_2 release rates that were double the rates of the other three replicates beginning after the 8 hour sampling and for all following times.

Figure 3 shows the amount of CO_2 released in a 24-hour period after rewetting dried soil. Roots significantly increased the amount of CO_2 released in both years (p-values <0.001 both years, Table A.1, Table A.2). There was no significant effect of collection date and no significant interaction between the two factors in either year. In 2018 there was an initial measurement of

CO_2 before the herbicide application and after the planting date. This soil released 52.5 µg CO_2-C g^{-1} soil in 24 hours, and its release rate pattern (Figure A.3) more closely resembled the 2017 pattern (Figure 1). There was a significant correlation between the 24 CO_2 flush and the 72 hours CO_2 flush for both years (Figure 4). Including the post-harvest data in 2018, the slope suggests that the 72 hour release is nearly three times the 24 hour CO_2 release (Figure 4B). However, if the post-harvest data is excluded from the 2018 data, then 2017 and 2018 have similar slopes (1.99 and 1.96) (Figure 4A, 4C)), suggesting the 72 hour release is about twice the 24 hour release.

Table 2. Field Experiment, soil characteristics 2017 and 2018, Rogers Farm Old Town, ME.

	2017 N= 5		**2018 N= 3**	
	Mean	**Standard Deviation**	**Mean**	**Standard Deviation**
Sand (%)	46	1.1	59	6.8
Silt (%)	44	1.1	30	4.0
pH	6	0.05	6	0.1
P (lb/A)	6.02	0.26	5.83	1.7
K (lb/A)	219	18.3	211	60.0
Mg (lb/A)	350	56.9	109	17.6
Ca (lb/A)	1490	159	1398	500
OM (LOI, %)	4	0.2	3.7	0.2
NO_3-N (ppm)	35.6	3.51	7.3	2.5
NH4+ - N (ppm)	5.4	3.13	13.3	4.7
Total C (Leco, %)	2.07	0.036	1.87	0.032
Total N (Leco, %)	0.192	0.005	0.18	0.003

Table 3. Soil temperature and moisture (Pw), Rogers Farm, Old Town ME.
n=5, mean(SD)

2017						2018					
Date	**Barley growth stage (Zadok)**	**Treatment**	**Pw**	**Temp (C)**	**Dry Root Biomass (g kg^{-1} soil)**	**Date**	**Barley growth stage (Zadok)**	**Treatment**	**Pw**	**Temp (C)**	**Dry Root Biomass (g kg^{-1} soil)**
						6/20	GS32	Bare	0.23(0.003)	20.4(0.8)	-
								Planted	0.22(0.007)	20.0(0.93)	0.4(0.21)b
7/11	GS49	Bare	0.23(0.007)	20.3(0.09)	-	7/9	GS49	Bare	0.22(0.011)	22.7(1.01)	-
		Planted	0.18(0.022)	19.3(0.14)	0.6(0.09)			Planted	0.22(0.013)	20.0(0.44)	0.8(0.24)a
8/7	GS85	Bare	0.20(0.013)	16.7(0.22)	-	8/24	PH*	Bare	0.21(0.006)	17.3(0.22)	-
		Planted	0.10(0.015)	16.3(0.18)	0.7(0.29)			Planted	0.23(0.007)	17.6(0.2)	0.3(0.09)b

*PH (post-harvest) indicates collection was 2 weeks after harvest, plants stage 85 at harvest
lower case indicate significant difference, $p<0.05$

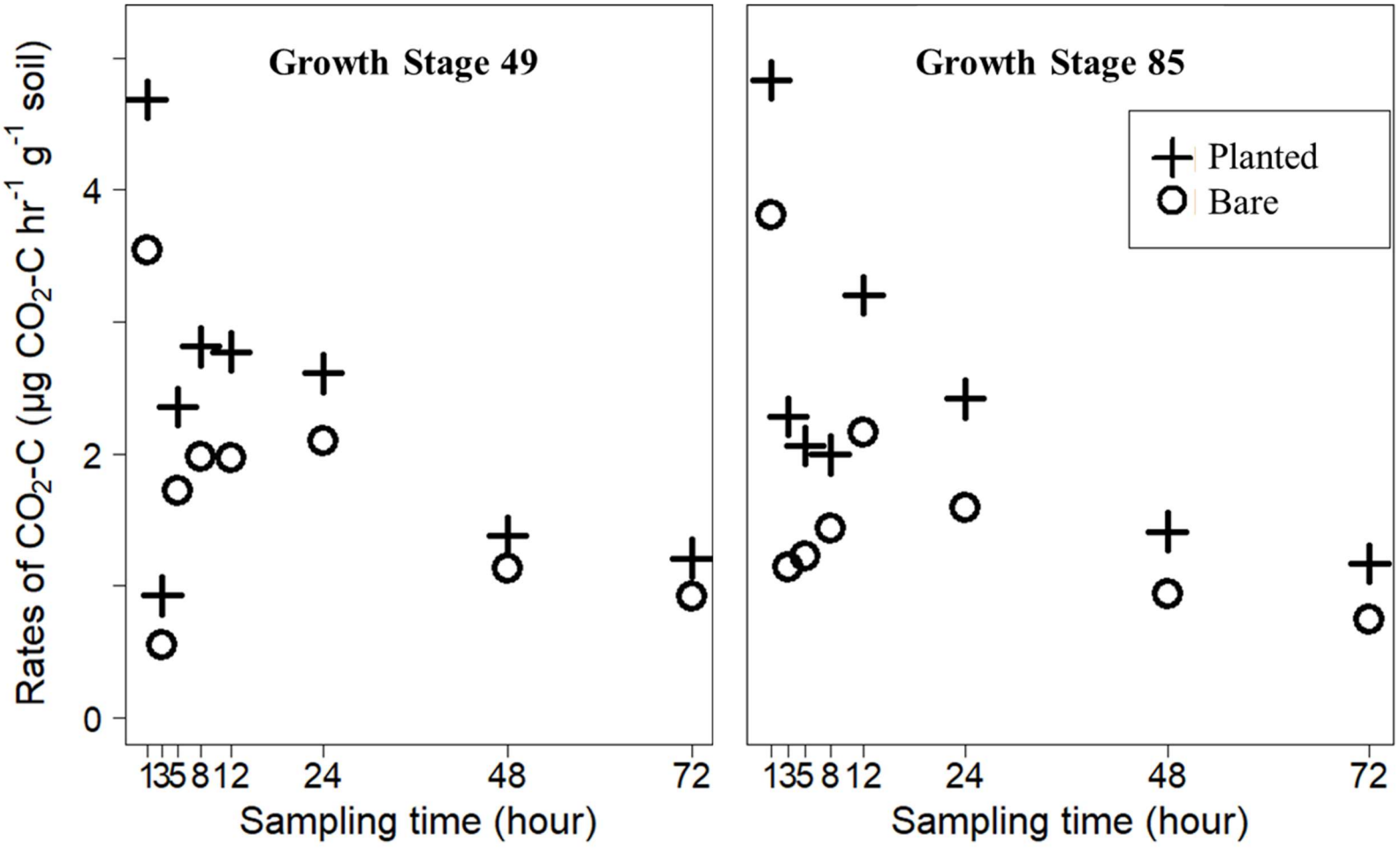


Figure 1. CO_2 release rates (LI-COR), dried and rewetted soil from Rogers Farm, Old Town ME, 2017.

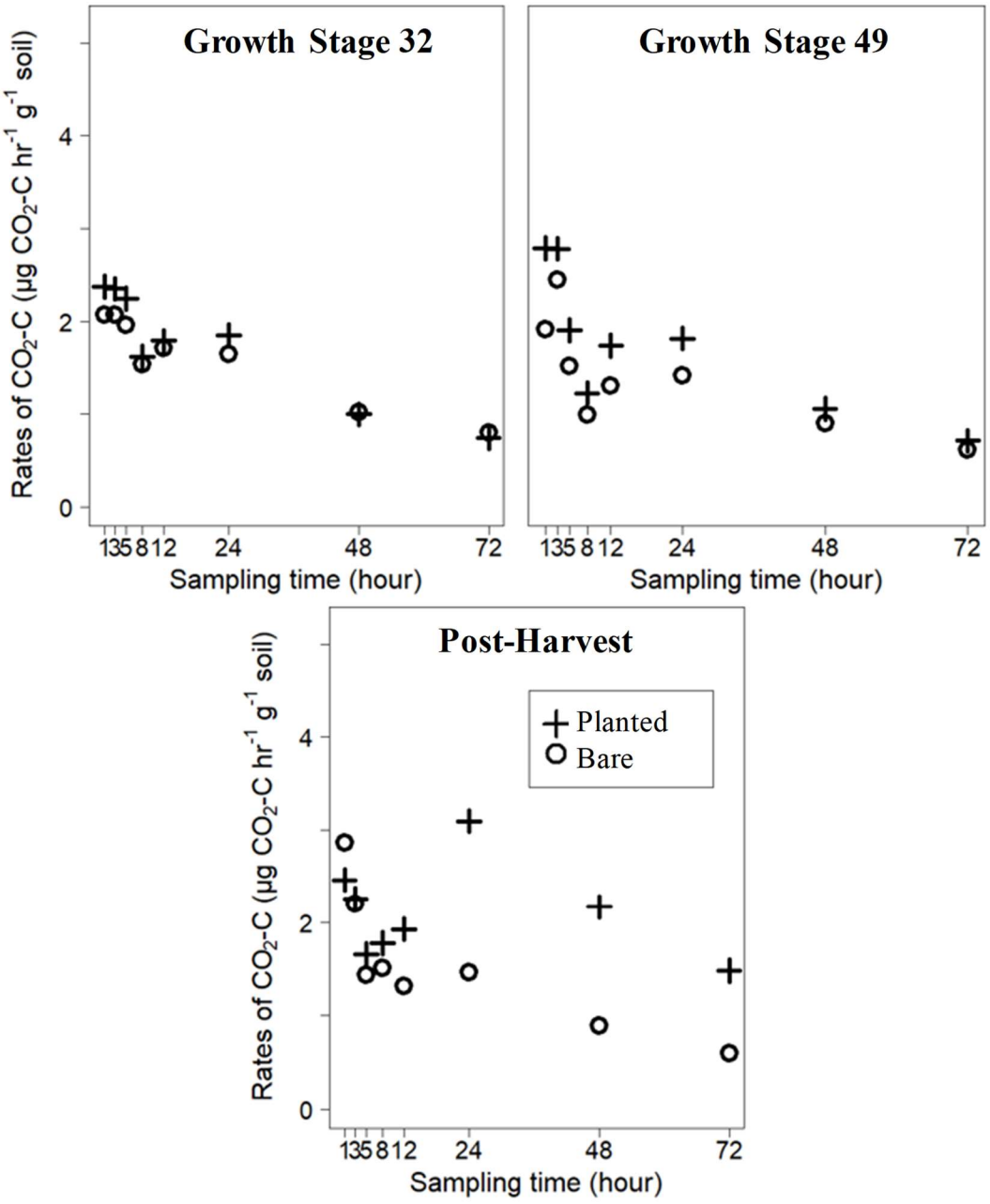
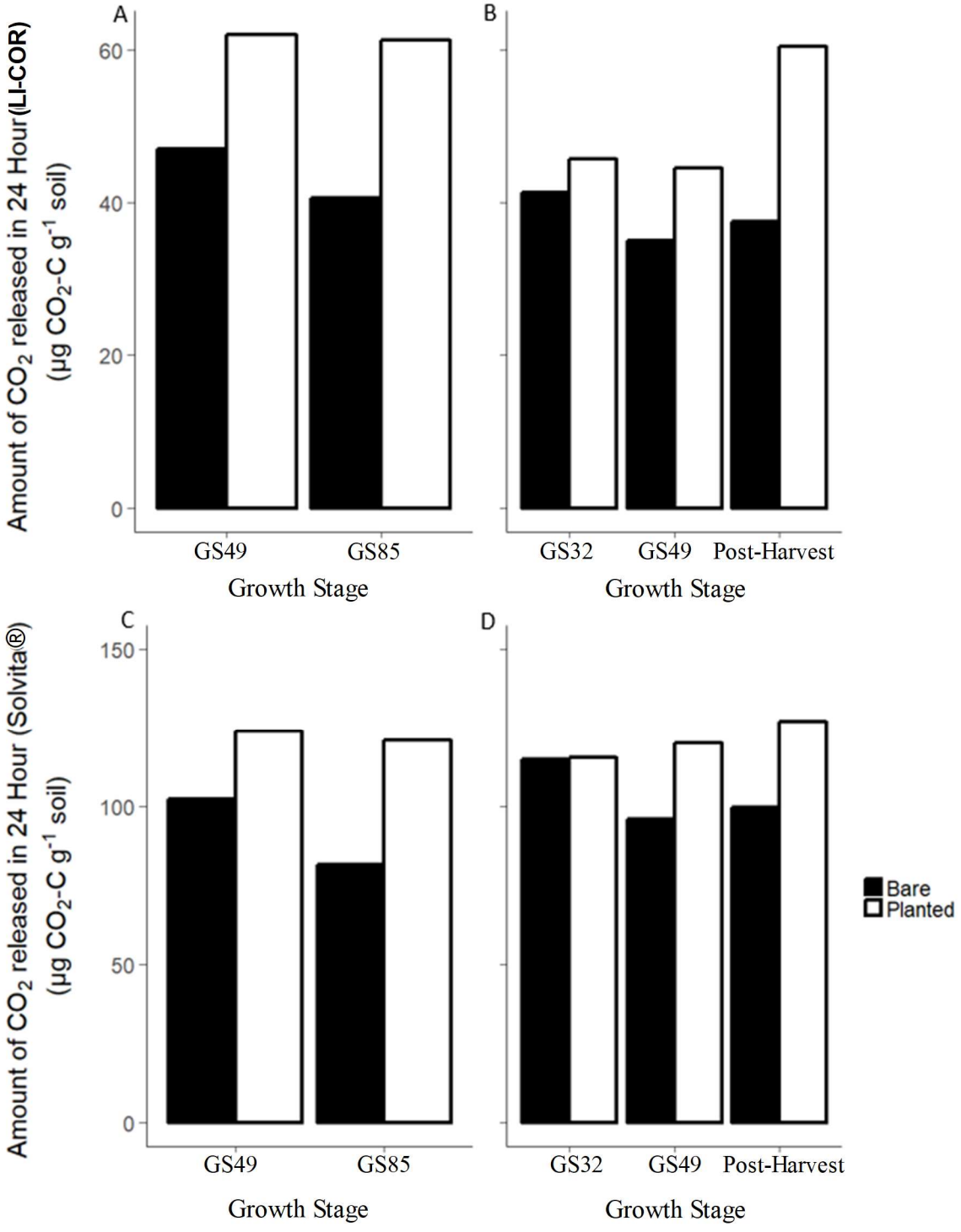
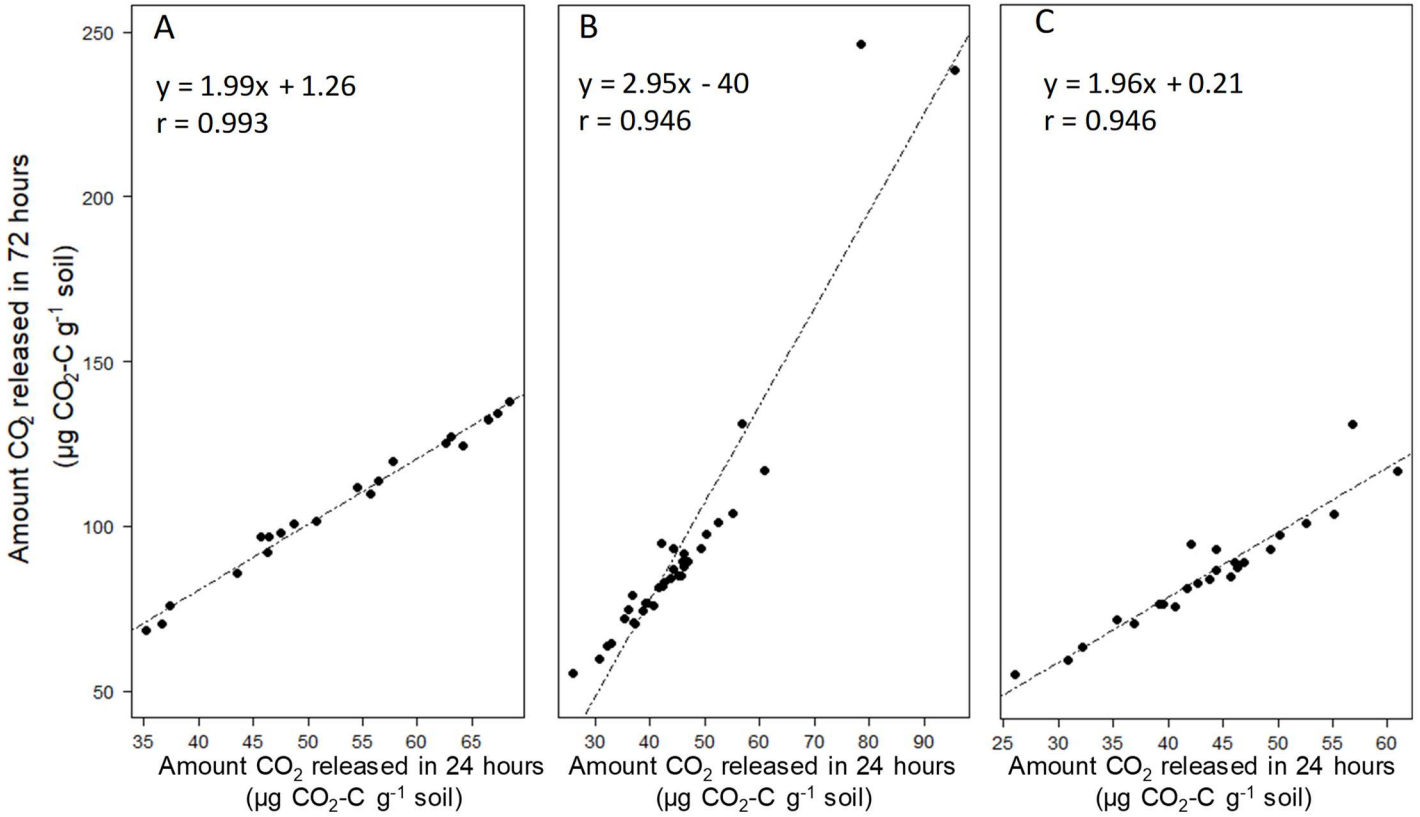


Figure 2. CO_2 release rates (LI-COR), dried and rewetted soil from Rogers Farm, Old Town ME, 2018.



(A) 2017 LI-COR (B) 2018 LI-COR (C) 2017 Solvita® (D) 2018 Solvita®

Figure 3. Amount of CO_2 released in 24 hours from dried and rewetted soil from Rogers Farm, Old Town ME.



(A) Field Experiment 2017 (B) Field Experiment 2018 including post-harvest data (C) Field Experiment 2018 excluding post harvest data.

Figure 4. Correlations between amount of CO_2 released in 24 hours and amount released in 72 hours (LI-COR) from rewetted soil from Rogers Farm, Old Town ME

The results from the commercially available Solvita® test also showed a significant effect of roots for both years, with $P<0.01$ (Figure 3, Table A.3, Table A.4). The Solvita® shows the same significant results in that there is no significant effect of barley growth stage (collection time), and no significant interaction effect. However, the values from the Solvita® test were nearly double those from the LI-COR readings for the 24 hour CO_2 flush.

In 2017 plots with roots had significantly higher values of both field-moist soil MBC ($p<0.01$, Table A.5) and dried and rewetted soil MBC ($p<0.05$, Table A.6) (Table 4). There was no significant treatment effect based on barley growth stage (collection time) and no significant interaction effect. In 2018 moist and rewetted MBC showed no significant treatment effects (Table A.7, Table A.8).

In 2017 there were significant effects of roots and barley growth stage on the level of DOC in the field-moist soil, and the interaction between the factors was also significant (Table 4). GS49 and GS85 were significantly different from each other in moist soil DOC. However, only roots had a significant effect on the DOC released from dried soil (Table 4). In 2018, there were significant effects of roots and barley growth stage on the level of DOC in both the field-moist soil and the rewetted soil, but no significant interaction between the factors. GS32 and GS49 were similar to each other, but they were significantly different from the post-harvest samples in the moist soil DOC. In the rewetted soil DOC the only significant difference in means was between the GS32 and the post-harvest sample (Table 4).

Figure 5 shows correlations between the amount of CO_2-C released in 24 hours (LI-COR) and dried root biomass, rewetted soil DOC, and moist soil MBC. There was no significant relationship between the amount of roots and the amount of CO_2 released in 24 hours (p-values 0.656 (2017), 0.751 (2018)). There was a significant relationship between CO_2 released in 24 hours and the amount of DOC in the rewetted soil for both years (p-values < 0.001). In 2017 there was a significant correlation between the amount of CO_2 released in 24 hours and MBC (p-value

<0.001), but in 2018 there was no significant correlation with MBC (p-value = 0.1154). The r values for CO_2 and DOC from rewetted soil were the highest of any factor tested for both years.

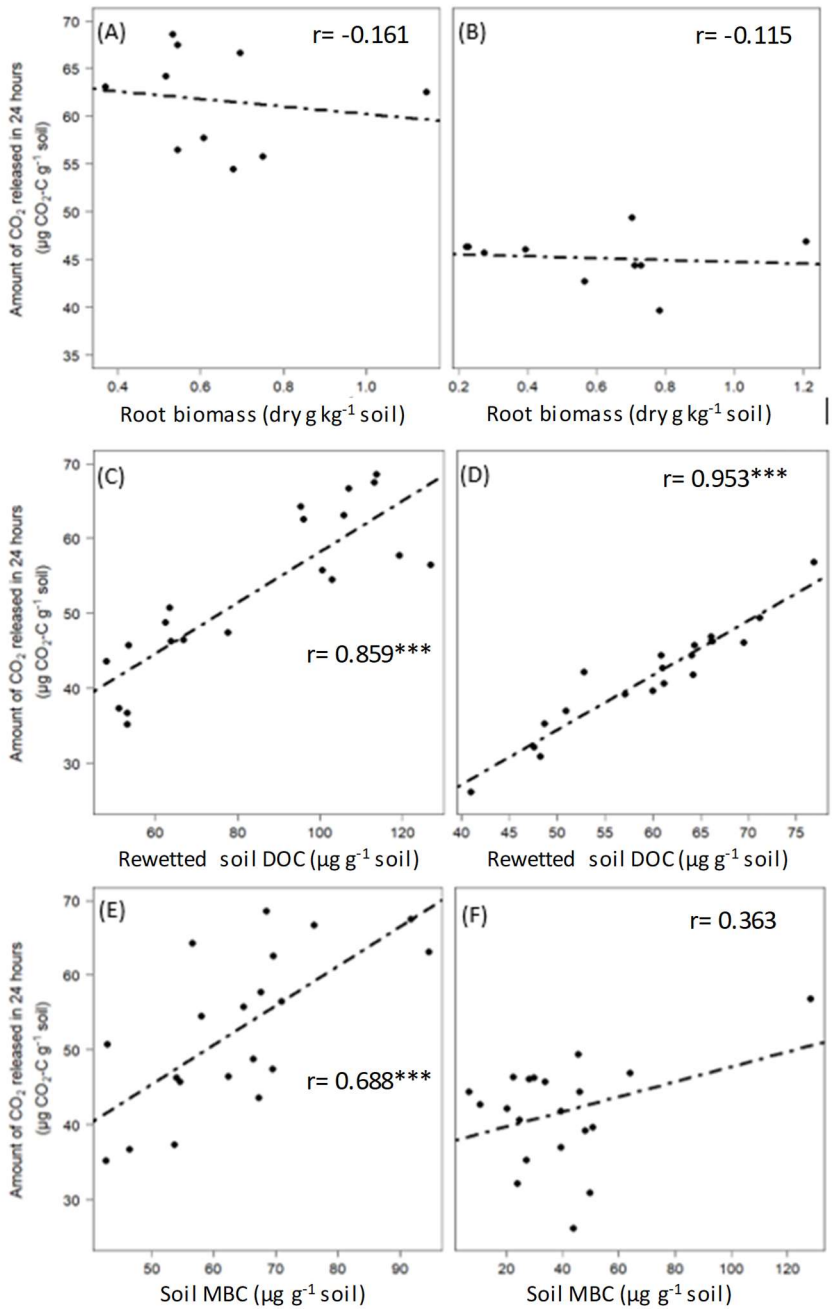
Table 5 examines the relationship between total CO_2-C released in 24 hours and 72 hours and the DOC released from dried soil. The amount of C respired in 24 hours was generally 60-70 % of the DOC released from dried soil and the amount respired in 3 days was greater than the dried soil DOC.

Table 4. MBC and DOC for both moist and rewetted soil from Rogers Farm, Old Town ME

	2017				2018			
	MBC		DOC		MBC		DOC	
Treatment	**Moist (µg C g^{-1} soil)**	**Rewetted (µg C g^{-1} soil)**	**Moist (µg C g^{-1} soil)**	**Rewetted (µg C g^{-1} soil)**	**Moist (µg C g^{-1} soil)**	**Rewetted (µg C g^{-1} soil)**	**Moist (µg C g^{-1} soil)**	**Rewetted (µg C g^{-1} soil)**
Bare-GS32	-	-	-	-	38.2	35.2	16.1	59.7
Planted-GS32	-	-	-	-	21.2	24.3	19.4	66.1
Bare-GS49	58.7	52.3	16.3c	58.9	38.4	30.9	17.0	50.0
Planted-GS49	66.1	59.8	45.4a	109	37.4	44.4	19.6	66.9
Bare-GS85	53.3	38.6	15.0c	59.9	-	-	-	-
Planted-GS85	77.8	59.9	35.9b	107	-	-	-	-
Bare-PH	-	-	-	-	30.3	29.3	20.3	48.5
Planted-PH	-	-	-	-	26.5	28.0	23.7	57.2
Bare	56.0b	45.5b	15.7b	59.4b	35.4	31.8	17.8b	52.8b
Planted	71.9a	59.8a	40.7a	108a	27.6	32.2	20.9a	62.4a
GS32	-	-	-	-	28.5	38.1	17.7b	62.9a
GS49	62.4	56.1	30.9a	84.1	37.9	28.5	18.3b	56.9ab
GS85	65.5	49.2	25.4b	83.5	-	-	-	-
PH	-	-	-	-	28.3	29.4	22.0a	52.8b
$P_{Planted/Bare\ X\ Growth\ Stage}$	0.498	0.311	0.048*	0.704	0.505	0.535	0.948	0.505
$P_{Planted/Bare}$	0.003 **	0.048 *	<0.001***	<0.001***	0.238	0.960	0.01 **	0.002**
$P_{Growth\ Stage}$	0.598	0.315	0.014*	0.879	0.427	0.613	0.008 **	0.018 *

*Significant at $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$

lower case letters indicate significant differences between values of interaction, plant/bare, and/or plant stage. If no letters there was no significant difference for that factor or interaction.



*Significant at $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$
(A) roots 2017 (B) roots 2018 (C) DOC from dried and rewetted soil 2017 (D) DOC from dried and rewetted soil 2018 (E) MCB from moist soil 2017 (F) MCB from moist soil 2018.

Figure 5. Correlations between the amount of CO_2 released (LI-COR) in 24 hours from rewetted soil from Rogers Farm, Old Town ME.

Table 5. Comparison of rewetted soil DOC and amount of CO_2 released in 24 hours and 72 hours from dried and rewetted soil from Rogers Farm, Old Town ME.

	2017		2018	
	CO_2 respired (24 hrs) compared to DOC from rewetted soil (%)	**CO_2 respired (72 hrs) compared to DOC from rewetted soil (%)**	**CO_2 respired (24 hrs) compared to DOC from rewetted soil (%)**	**CO_2 respired (72 hrs) compared to DOC from rewetted soil (%)**
Bare-GS32	-	-	69	142
Planted-GS32	-	-	69	132
Bare-GS49	79.8	163	70	143
Planted-GS49	56.8	114	70	136
Bare-GS85	67.8	135	-	-
Planted-GS85	57.1	115	-	-
Bare-PH	-	-	77.3	151
Planted-PH	-	-	106	259

3.2 Greenhouse Experiments

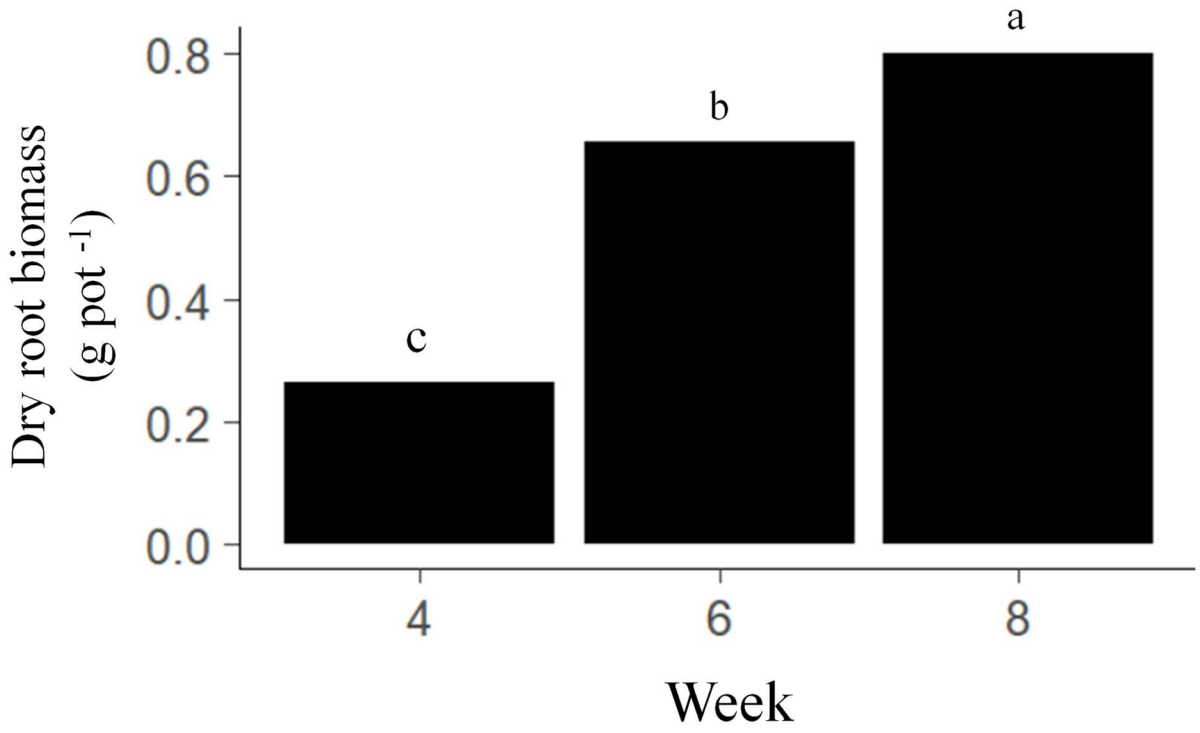
3.2.1 Experiment 1

In Experiment 1 there was a significant increase in barley root biomass for each collection week (Figure 6). The CO_2-C release for Experiment 1 (Figure 7) had a similar pattern to the 2017 Field Experiment (Figure 1). There was a large initial release, and then the rate decreased, and increased again at hour 8. After that the rates declined again and appeared to be starting to level off.

There was both a significant week effect, and a significant root effect on the CO_2 flush, but there was no significant interaction between the two factors (Table 6). Week four was significantly different from weeks six and eight, however weeks six and eight were not significantly different from each other (Table 6). There was a significant correlation between the 24 CO_2 flush and the 72 hour CO_2 flush with a slope of 1.93 (Figure 8). The 72 hour CO_2 flush was about double the 24 hour CO_2 flush, which is similar to what was observed in the Field Experiment.

There was a significant root effect and week effect on the moist soil MBC and a significant interaction between the two factors (Table 6). For the rewetted soil MBC there was a significant root effect and week effect but no significant interaction between the two factors (Table 5). There was a significant root effect, but no significant effect based on weeks and no significant interaction between the two factors for both moist and rewetted soil DOC (Table 5). Table 6 also shows the CO_2 respired in 24 and 72 hours as compared to rewetted soil DOC as a percent. In 24 hours the amount of C respired was about 50% of the DOC from the rewetted soil, and in the 72 hours the amount of C respired was approximately equal to the DOC released from the rewetted soil (Table 6). The respired C percent in Experiment 1 was less than what was found in the Field Experiment for both 24 and 72 hours CO_2 release.

Figure 9 shows correlations between the amount of CO_2-C released and dried root biomass, rewetted soil DOC, and moist soil MBC. Each variable was significantly correlated with the amount of CO_2-C released in 24 hours (p-values 0.012 (roots), 0.004 (DOC), 0.039 (MBC)).



lower case letters indicate significant differences, $p<0.05$

Figure 6. Greenhouse Experiment 1, dry root biomass.

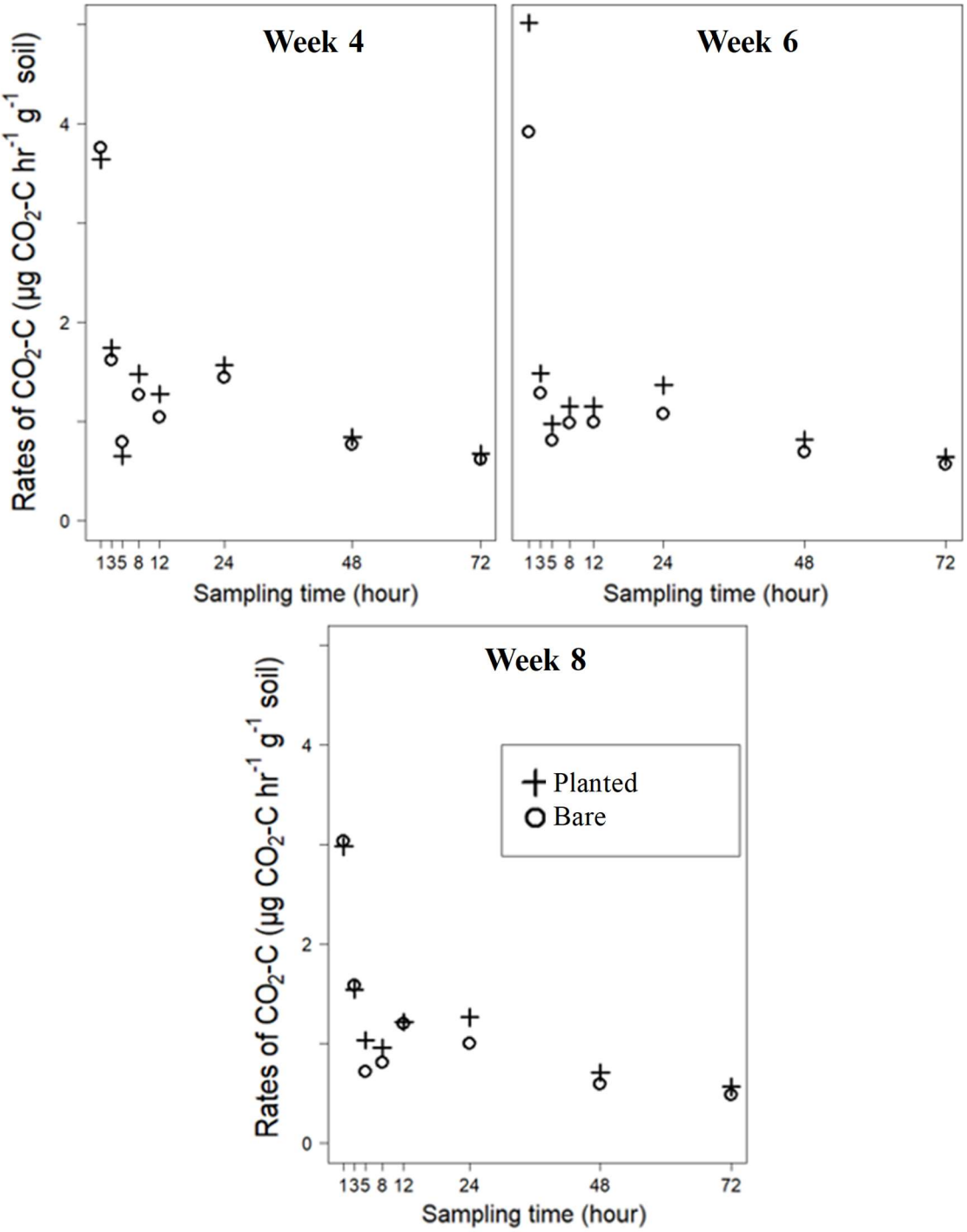


Figure 7. Greenhouse Experiment 1, CO_2 release rate (LI-COR) for dried and rewetted soil.

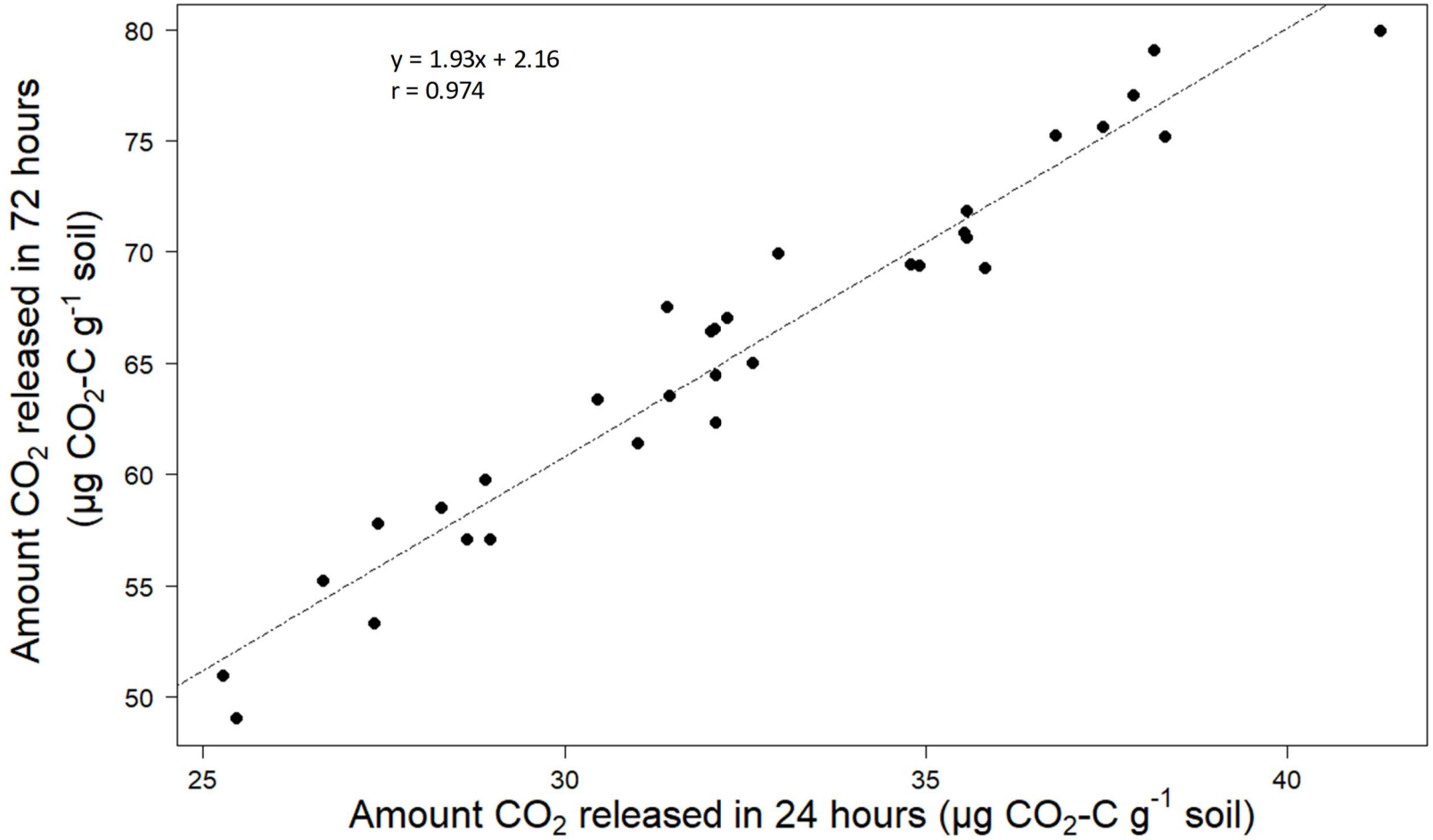


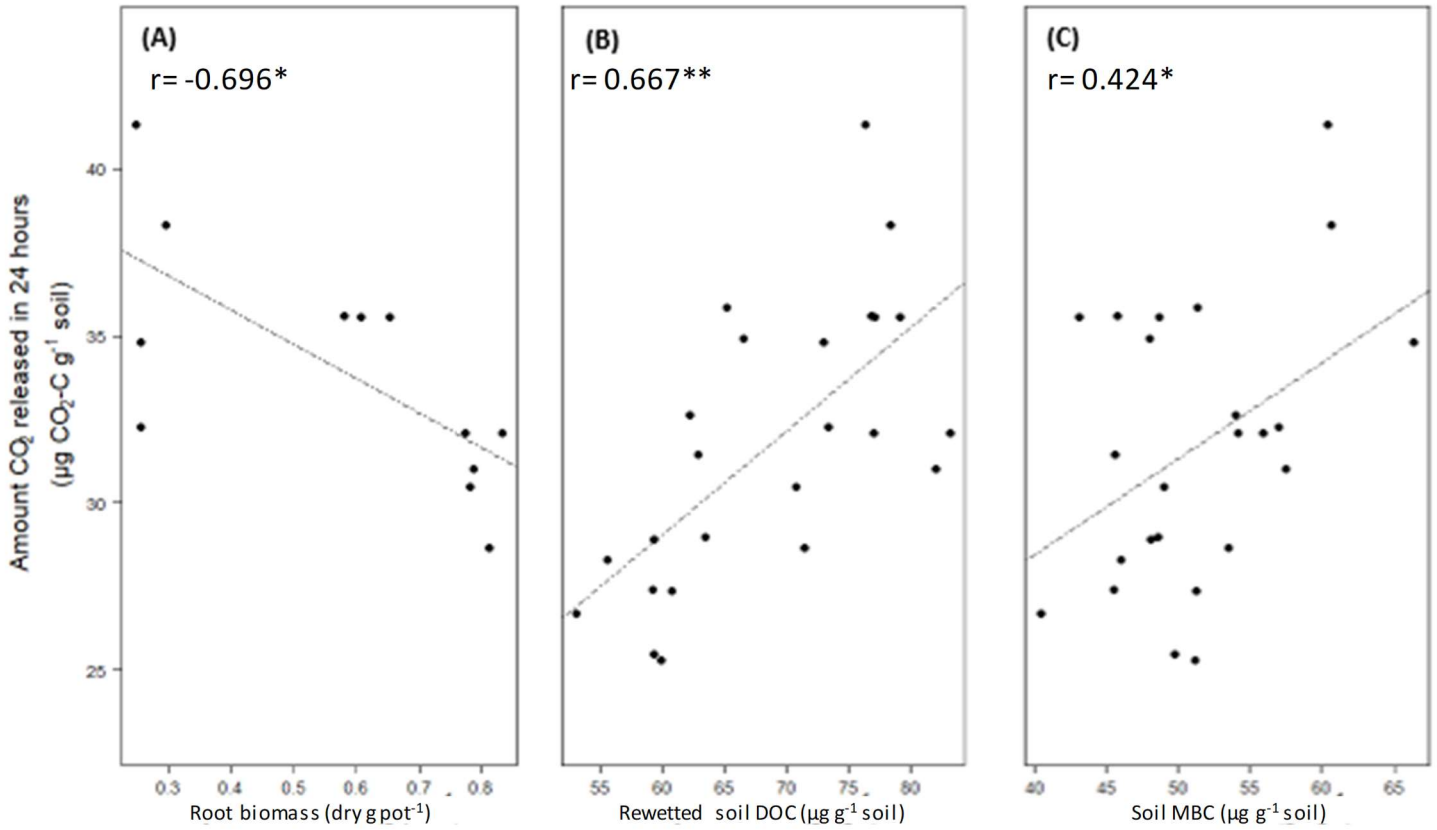
Figure 8. Greenhouse Experiment 1, correlation between amount of CO_2 release in 24 hours and amount of CO_2 released in 72 hours (LI-COR) from rewetted soil.

Table 6. Greenhouse Experiment 1, amount of CO_2 released in 24 hours (LI-COR) from rewetted soils. MBC and DOC, for both moist and rewetted soil, and comparison of rewetted soil DOC .

Treatment	24 hour flush	MBC		DOC		CO_2 respired (24 hrs) compared to DOC from rewetted soil (%)	CO_2 respired (72 hrs) compared to DOC from rewetted soil (%)
	(CO_2-C µg g^{-1} soil)	Moist (µg C g^{-1} soil)	Rewetted (µg C g^{-1} soil)	Moist (µg C g^{-1} soil)	Rewetted (µg C g^{-1} soil)		
Unplanted-4 weeks	33.7	49.7c	39.9	18.8	64.2	53	104
Planted-4weeks	36.7	61.1a	47.3	24.4	75.2	49	97
Unplanted-6 weeks	27.8	45.0d	36.6	19.4	56.8	49	102
Planted-6 weeks	34.3	46.6cd	37.3	25.4	75.9	45	91
Unplanted-8 weeks	26.8	50.2c	33.8	18.6	60.9	44	86
Planted-8 weeks	31.0	55.3b	42.1	31.2	78.4	40	78
4 weeks	35.2a	55.4a	43.4a	21.6	69.7		
6 weeks	31.1b	45.8b	36.9b	22.4	66.4		
8 weeks	28.9b	52.7a	37.7b	24.9	69.6		
Unplanted	29.4b	48.3b	36.7b	19.0b	60.6b		
Planted	34.0a	54.3a	42.0a	27.0a	76.5a		
$P_{(Un)Planted\ X\ Week}$	0.341	0.013 *	0.16	0.263	0.055		
P_{Week}	< 0.001 ***	< 0.001 ***	0.01 *	0.387	0.095		
$P_{(Un)Planted}$	< 0.001 ***	< 0.001 ***	0.004 **	<0.001***	<0.001***		

*Significant at P < 0.05, ** P < 0.01, ***P < 0.001

lower case letters indicate significant differences between values of interaction, week, and/or (un)planted. If no letters there was no significant difference for that factor or interaction.



*Significant at $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$
(A) roots (B) dry soil DOC (C) moist soil MBC

Figure 9. Greenhouse Experiment 1, correlations between the amount of CO_2 released (LI-COR) in 24 hours from rewetted soil .

3.2.2 Experiment 2

Figure 10 shows the differences in root growth for different plant species. The root biomass was significantly different for soybean, corn, and clover. Barley and ryegrass were not not significantly different from each other, but were significantly different than the other plant species (Figure 10).

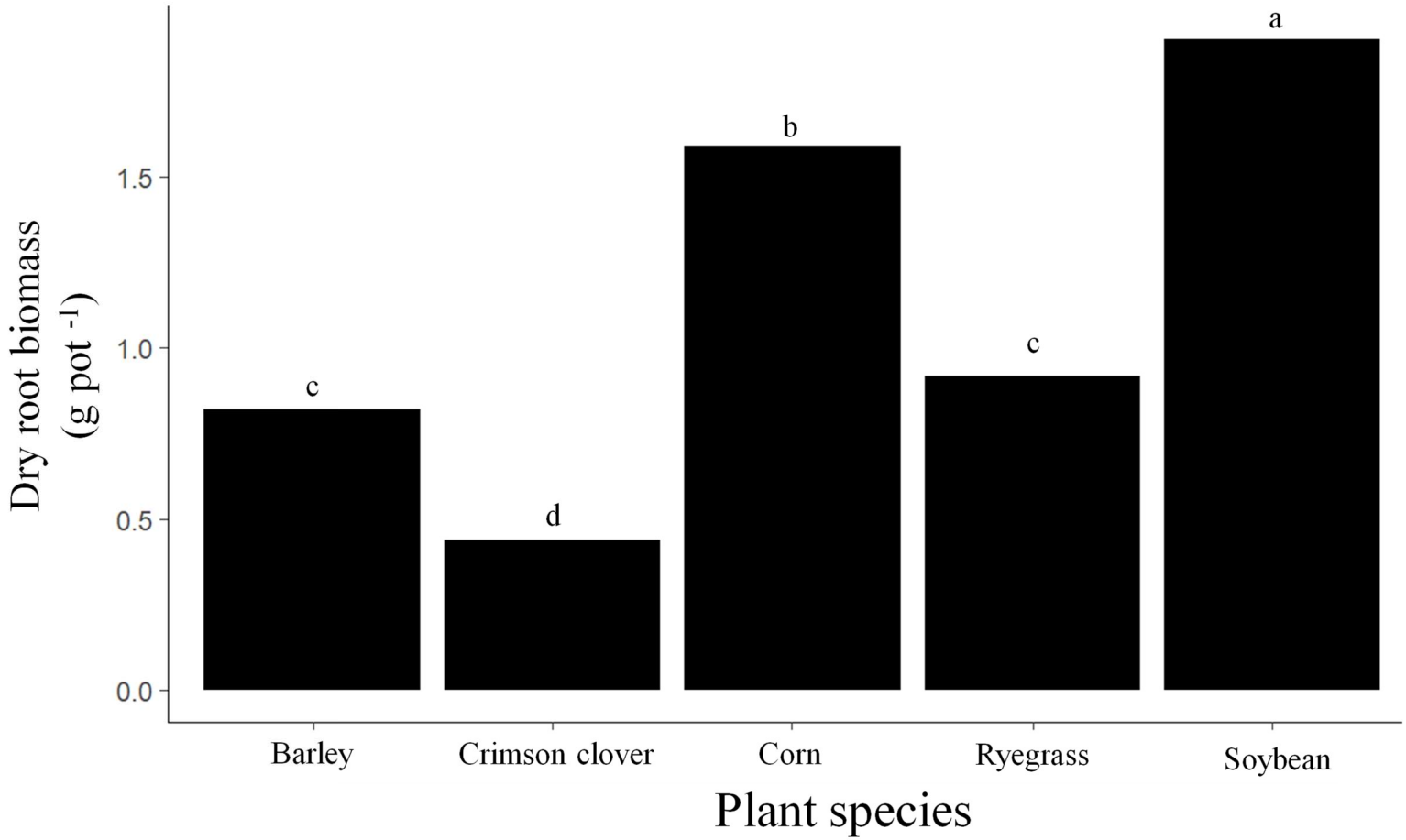
The rate pattern for the CO_2 release over the 72-hour period (Figure 11) resembled Experiment 1 (Figure 7), and the 2017 Field Experiment (Figure 1). The unplanted soil had a lower CO_2-C release rate than the planted soil, especially after hour 3, and by hour 72 the rates of the planted treatments and unplanted soil became more equal.

There was a plant species effect on the CO_2 flush with the unplanted soil included in the analysis (p value = 0.017, Table A.18) (Figure 12). The only significant difference between the unplanted soil and the planted soil was between the unplanted soil and corn. However, corn was not significantly different from any of the other planted treatments. When the unplanted treatment was removed from the data set, there was no significant difference among plant species. There was a significant correlation between the 24 CO_2 flush and the 72 hours CO_2 flush (Figure 13). Similar to Experiment 1 (Figure 8), and the Field Experiment (Figure 4(A, C)), the 72 hours CO_2 flush is twice as much as the 24 hours CO_2 flush (Figure 13).

There was no significant treatment effect for moist or rewetted soil MBC (Table 6). There was a significant treatment effect for both moist and rewetted soil DOC. The unplanted treatment was significantly different from all plant species in both moist and rewetted soil DOC. In moist DOC corn, barley, and ryegrass were similar to each other but were significantly different from soybean and clover, which were not significantly different from each other. However, in the rewetted soil DOC there were no significant differences among the plant species (Table 7).

Figure 14 shows correlations between the amount of CO_2-C released and dried root biomass, rewetted soil DOC, and moist soil MBC. There was no relationship between the amount

CO_2-C released and dry roots, or MBC (P-values 0.169, 0.939). There was a significant relationship between the amount of CO_2-C released and rewetted DOC (p-value <0.001). Table 7 shows the CO_2 respired in 24 and 72 hours as compared to dried soil DOC as a percent. In 24 hours the amount of C respired about 45 % of the DOC released from the rewetted soil, and in 72 hours the amount of C respired ranged from 85-94 % of the DOC from dried soil (Table 7).



lower case letters represent mean separation for roots

Figure 10. Greenhouse Experiment 2, dry root biomass..

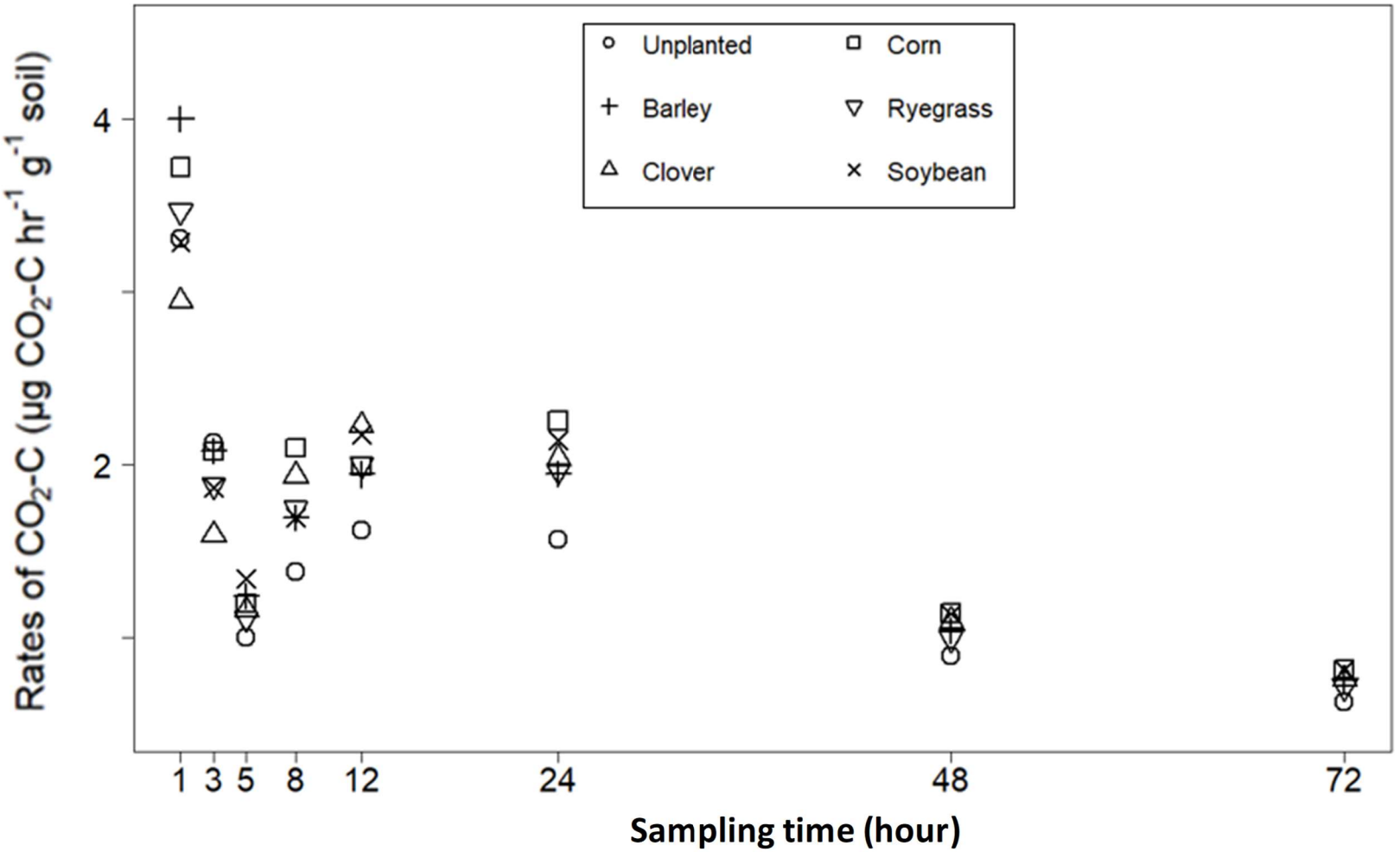


Figure 11. Greenhouse Experiment 2, CO_2 release rate (LI-COR) for rewetted soil.

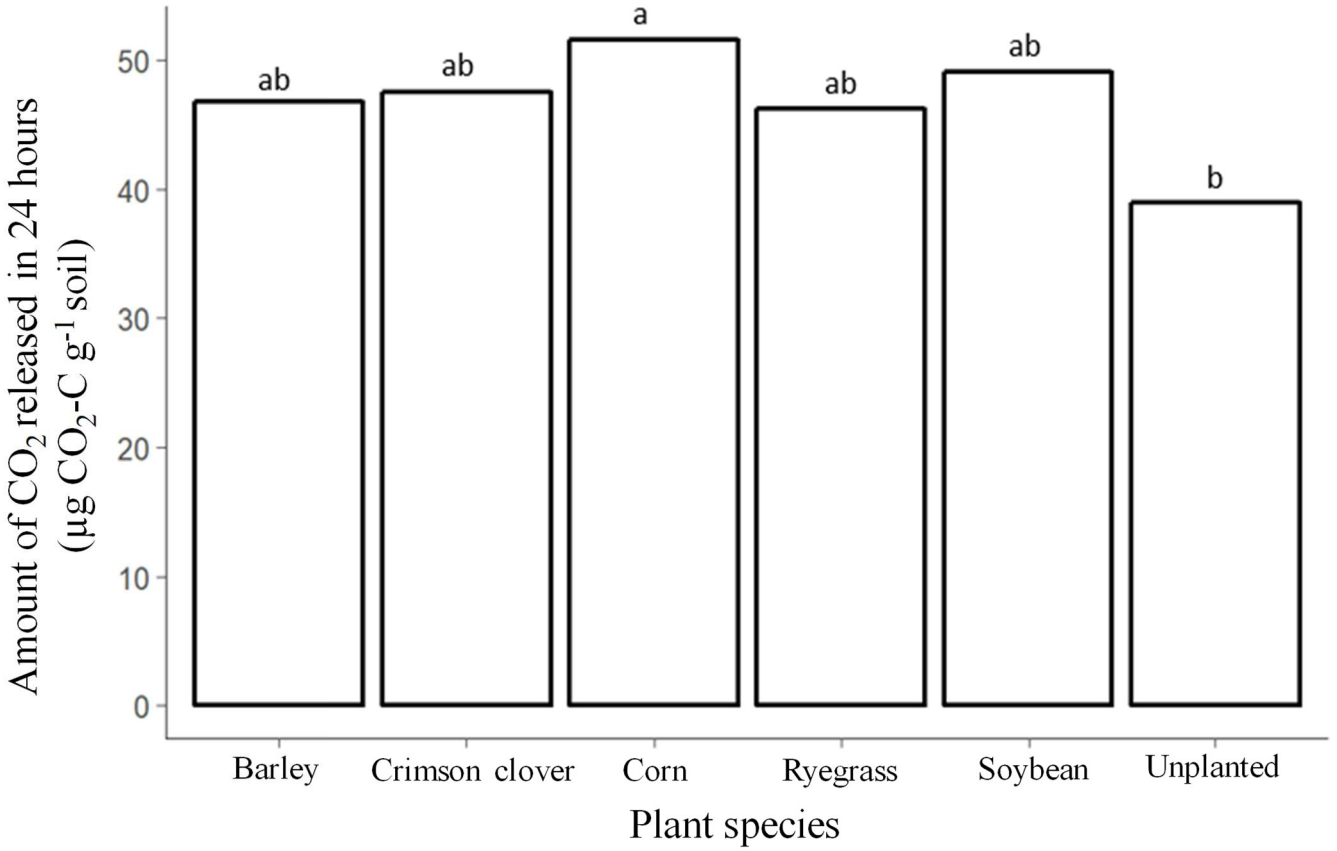


Figure 12. Greenhouse Experiment 2, amount of CO_2 released in 24 hours (LI-COR) from rewetted soil.

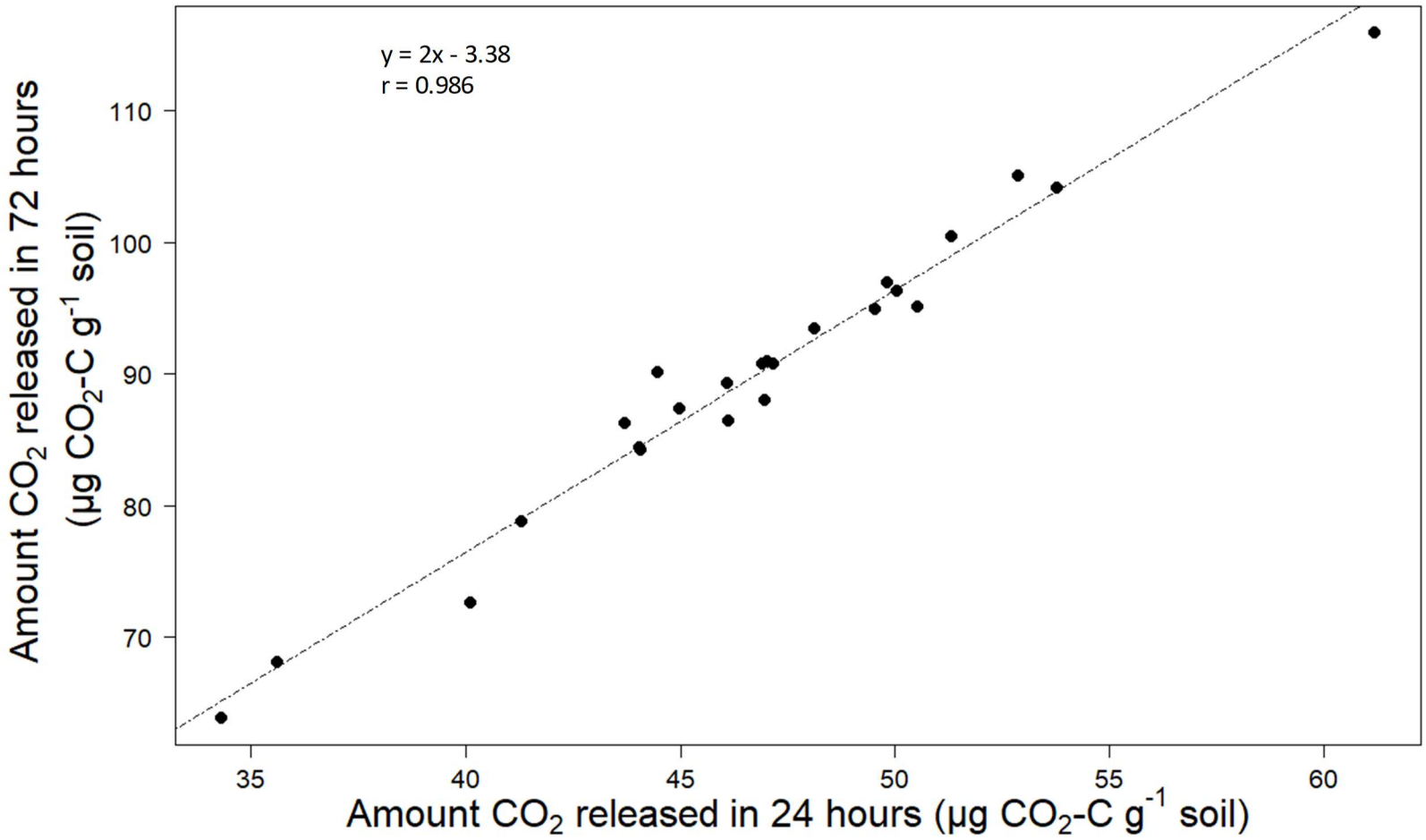
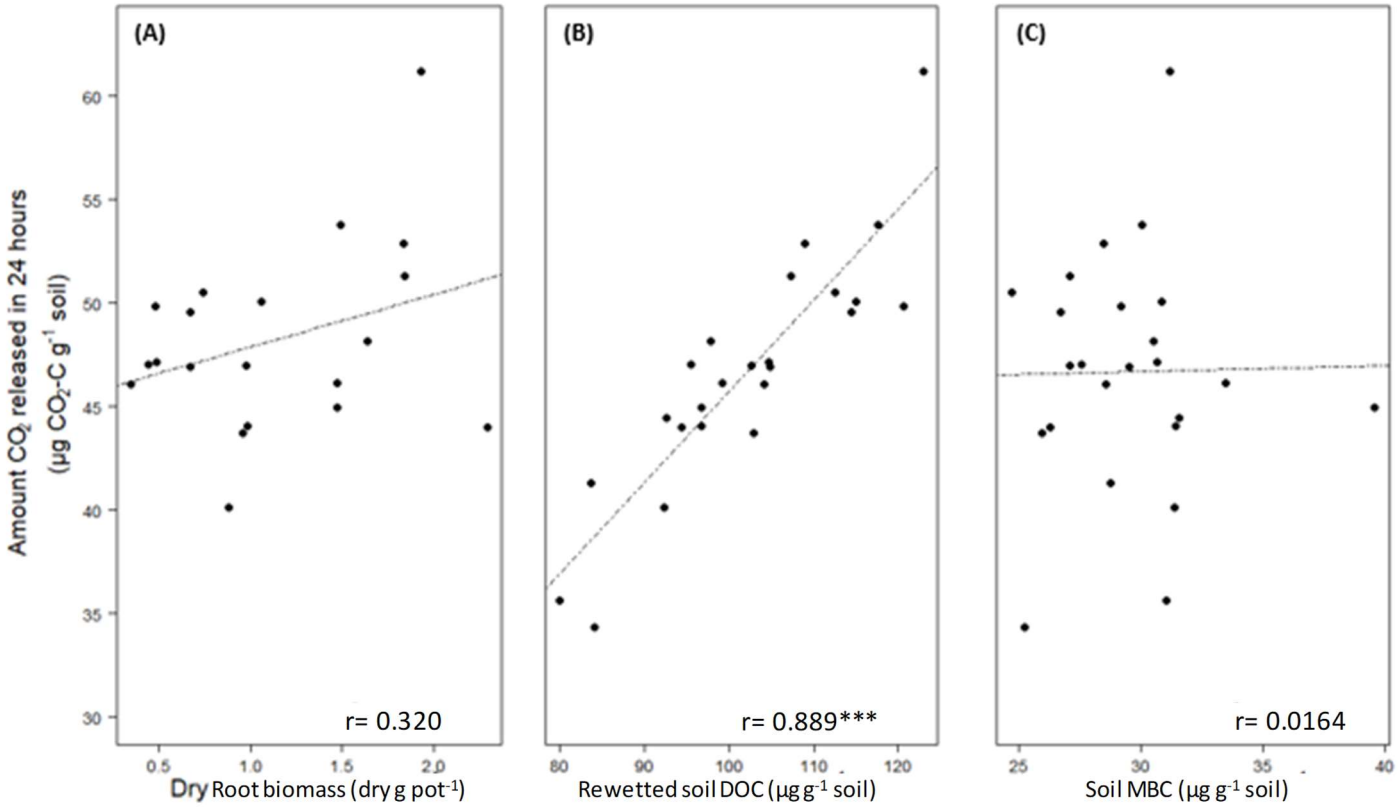


Figure 13. Greenhouse Experiment 2, correlation between amount of CO_2 release in 24 hours and amount of CO_2 released in 72 hours (LI-COR) from rewetted soil.

Table 7. Greenhouse Experiment 2, MBC and DOC for both dried and moist soil, and comparison of rewetted soil DOC and amount of CO_2 released in 24 hours and 72 hours

Plant species	MBC		DOC		CO_2 respired (24 hrs) compared to DOC from rewetted soil (%)	CO_2 respired (72 hrs) compared to DOC from rewetted soil (%)
	Moist (µg C g^{-1} soil)	Rewetted (µg C g^{-1} soil)	Moist (µg C g^{-1} soil)	Rewetted (µg C g^{-1} soil)		
Unplanted	29.1	21.0	18.9c	84.9b	46	88
Barley	27.3	25.2	28.7a	106a	44	85
Clover	29.0	24.5	23.7b	106a	45	87
Corn	33.6	27.7	27.8a	109a	47	90
Ryegrass	29.6	29.6	28.0a	104a	44	84
Soybean	28.1	21.6	24.8b	102a	48	94
P value	0.0644	0.0992	< 0.001***	0.0116 *		

*Significant at P < 0.05, ** P < 0.01, ***P < 0.001
lower case letters indicate significant differences between values by columns



*Significant at $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$
(A) roots (B) rewetted soil DOC (C) moist soil MBC.

Figure 14. Greenhouse Experiment 2, correlations between amount of CO_2 released (LI-COR) in 24 hours.

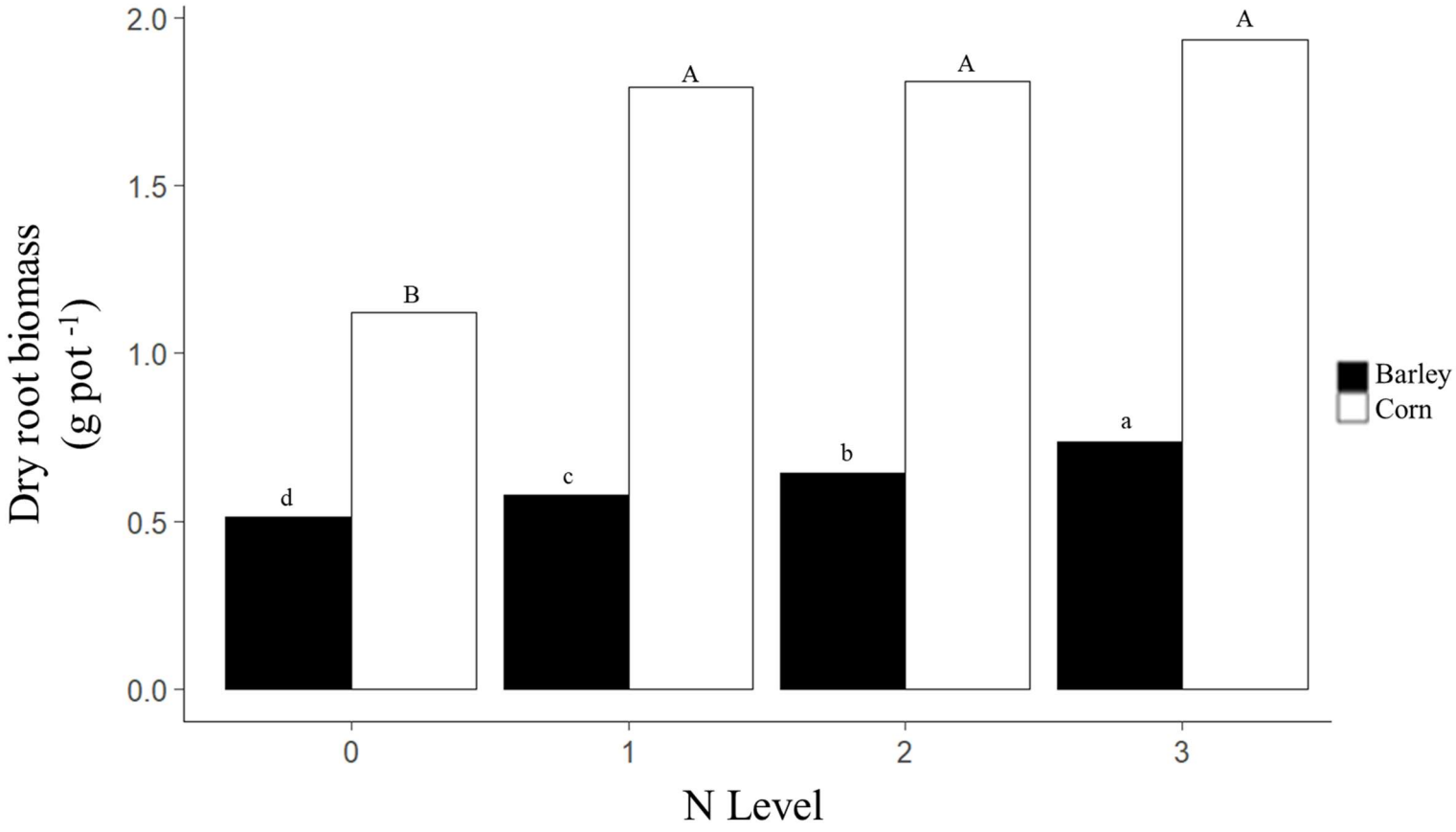
3.2.3 Experiment 3

Figure 15 shows the root growth for barley and corn at each N level. Corn produced more biomass than barley. Figure 15 shows the roots for corn increased from 1.1g to 1.8g at N level 1, and was N level 0 was significantly different from all other levels. N level 1 through 3 were not significantly different from each other for corn. However, for barley each increase in N level corresponds to a significant increase in the amount of root growth.

The CO_2-C release rates were similar to other experiments. The rate at hour 1 was greater than the other rates. After an hour there was a reduction in the rates, and at 8 hours the rates increased slightly. After 24 hours, the rates decreased and appeared to start leveling off at 48 and 72 hours (Figure 16).

There was a significant treatment effect of plants on the CO_2 flush, but no significant effect of N level on the CO_2 flush (Figure 17). With more N there was a decrease in CO_2-C flush in unplanted soils, however when plants were removed from the analysis of N level and CO_2 flush there was no significant effect ($p = 0.056$) (Table A.24). At N level 1 there was no significant difference between corn and barley. There was a significant interaction between factors ($p = 0.038$) on the CO_2 flush (Figure 17). There was significant correlation between the 24 CO_2 flush and the 72 hours CO_2 flush (Figure 18). The slope is only 1.76, and the 72 hours CO_2 flush is less than twice the 24 hours CO_2 flush (Figure 18); this was different from the Field Experiment (Figure 4), and Experiment 1, and 2 (Figure 7, 13).

There was a significant plant species effect on moist soil MBC (Table A.25), with corn, barley and unplanted all significantly different from each other (Table 8). There was no significant effect of N level and no significant interaction. There were no significant treatment effects on rewetted MBC (Table 8, Table A.26).



lower case letters indicate significant differences for barley, $p<0.05$
upper case letters indicate significant differences corn, $p<0.05$

Figure 15. Greenhouse Experiment 3, dry root biomass

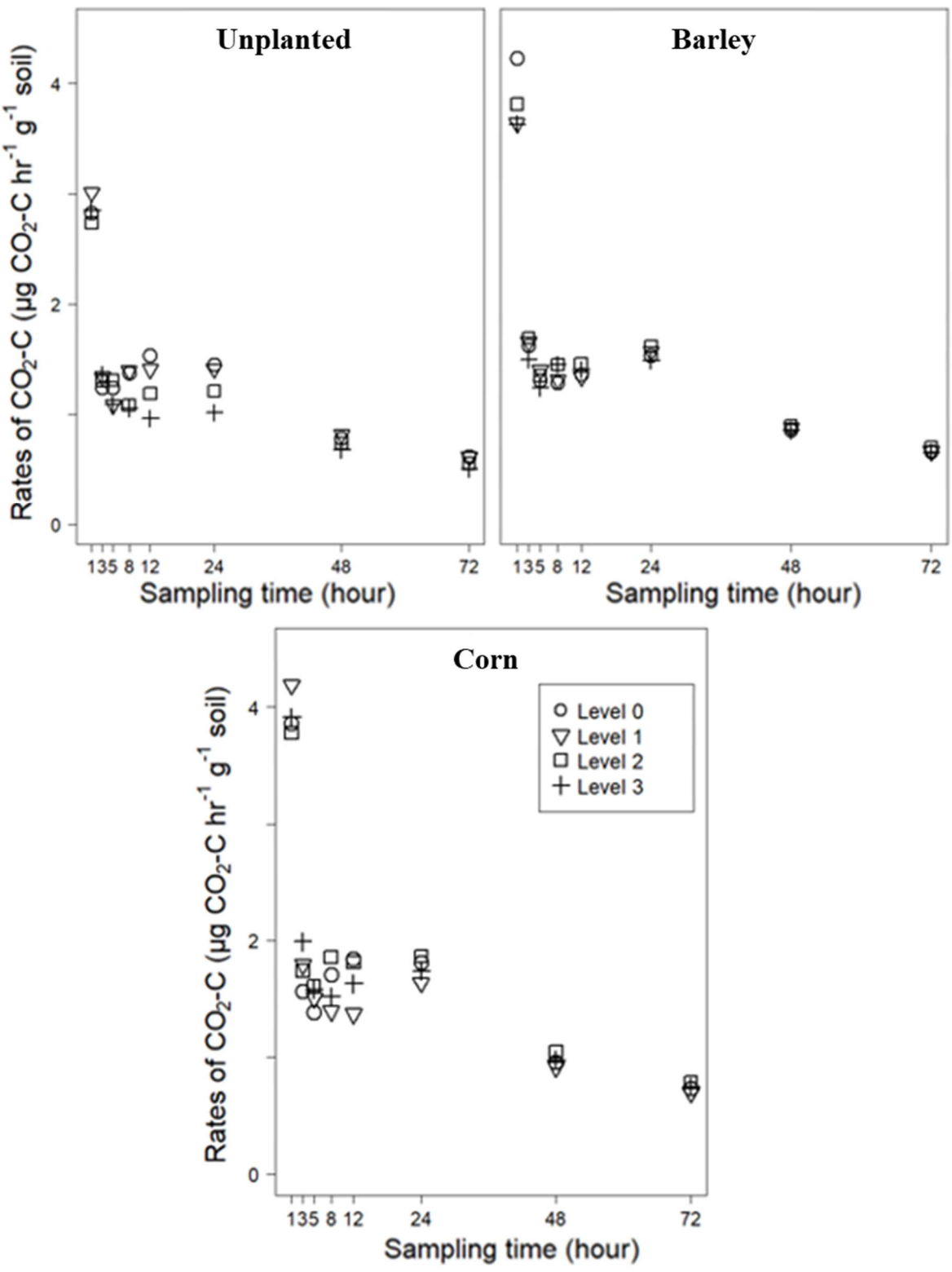
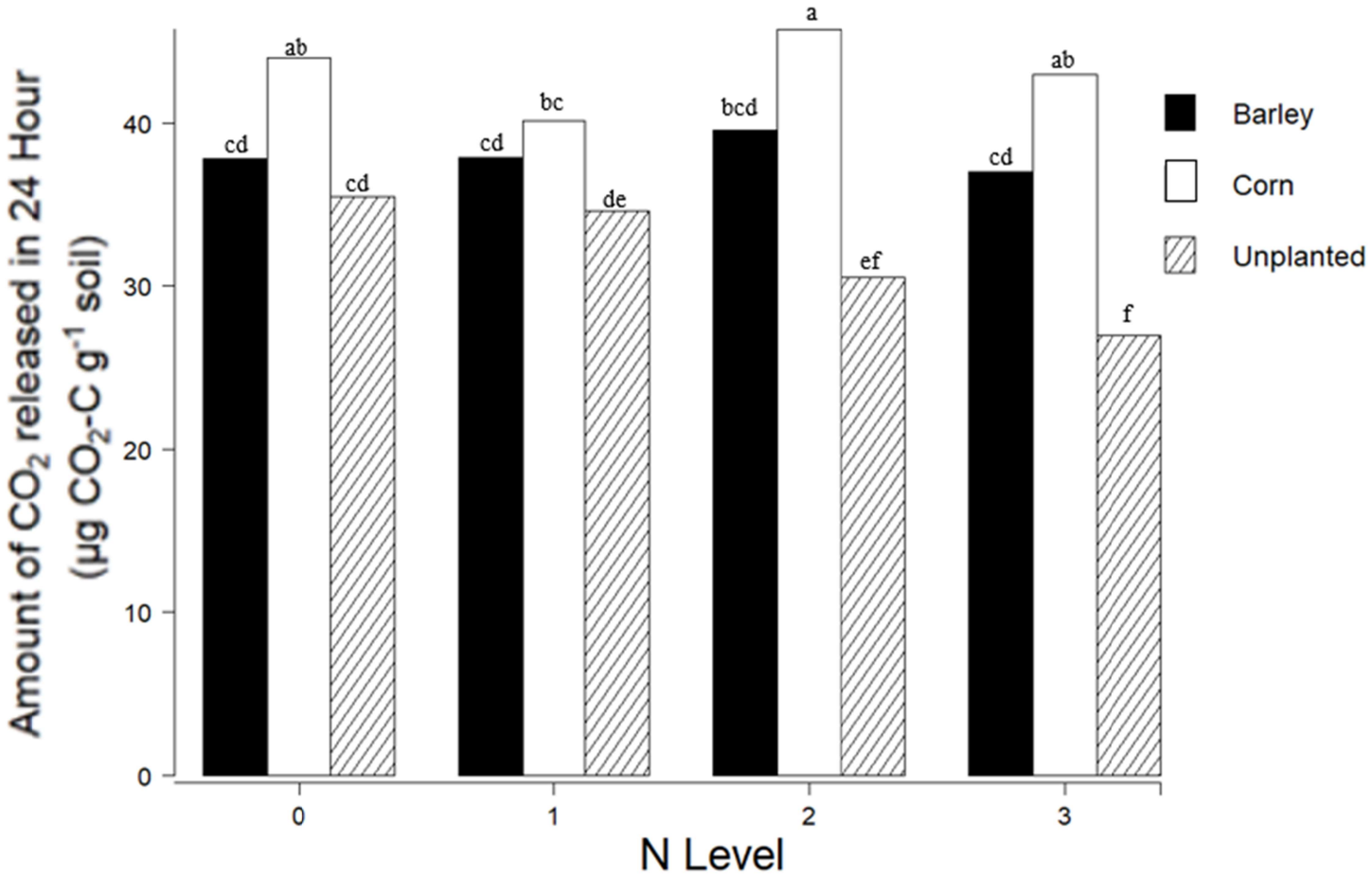


Figure 16. Greenhouse Experiment 3, CO_2 release rate (LI-COR) for rewetted soil



lower case letters indicate significant differences, $p<0.05$

Figure 17. Greenhouse Experiment 3, amount of CO_2 released in 24 hours (LI-COR) from rewetted soil.

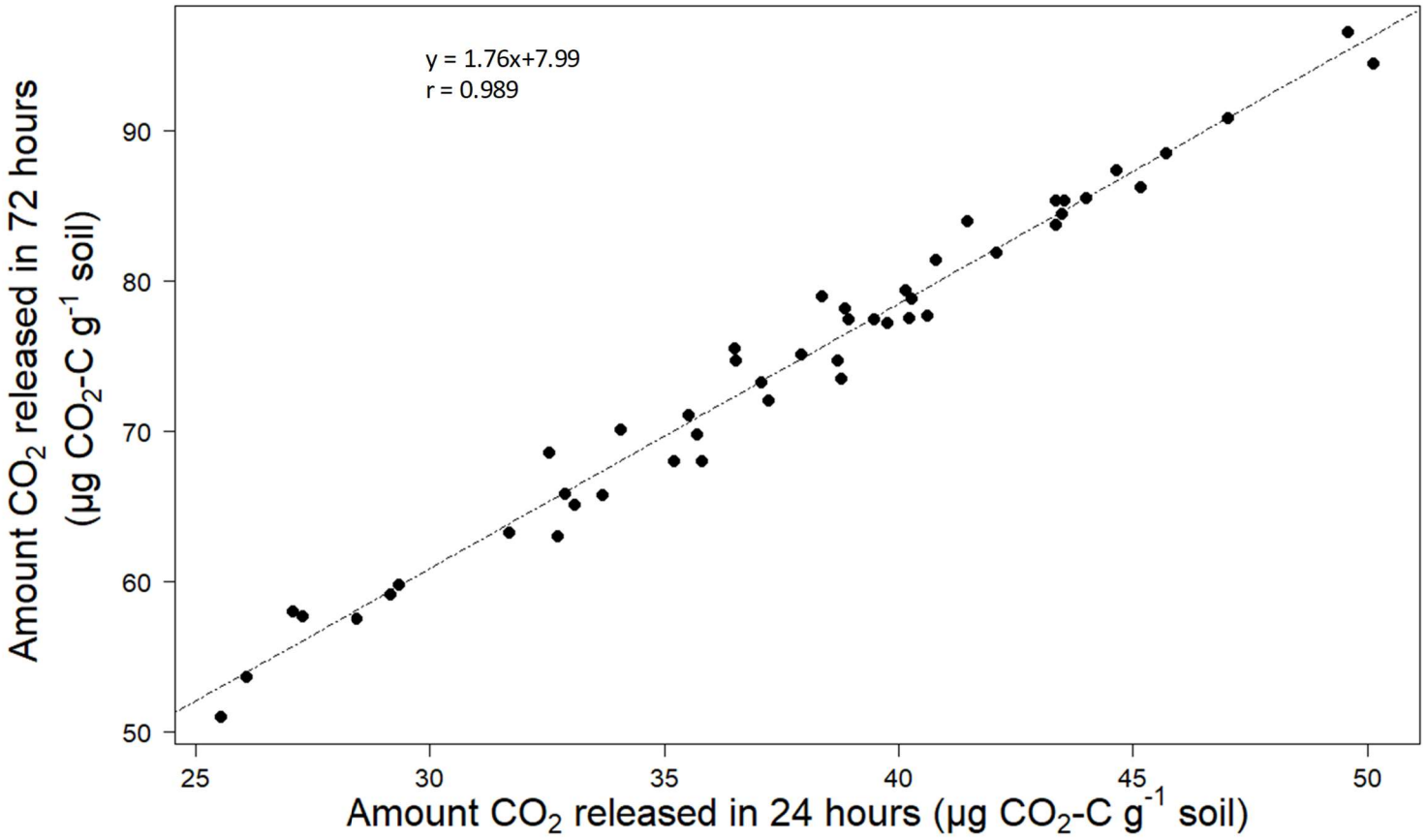


Figure 18. Greenhouse Experiment 3, correlation between amount of CO_2 released in 24 hours and amount of CO_2 released in 72 hours (LI-COR) for rewetted soil.

Table 8. Greenhouse Experiment 3, MBC and DOC for both moist and rewetted soil.

Treatment	MBC		DOC	
	Moist (µg C g^{-1} soil)	Rewetted (µg C g^{-1} soil)	Moist (µg C g^{-1} soil)	Rewetted (µg C g^{-1} soil)
Unplanted- N Level 0	26.1	13.8	16.1de	78.0
Barley- N Level 0	36.0	20.0	25.8a	91.5
Corn- N Level 0	40.5	14.6	25.8a	100
Unplanted- N Level 1	24.5	12.2	13.8ef	70.9
Barley- N Level 1	32.3	19.6	24.1ab	92.3
Corn- N Level 1	37.5	30.1	24.8a	90.3
Unplanted- N Level 2	22.4	7.5	12.6ef	62.9
Barley- N Level 2	37.2	13.6	23.7ab	88.3
Corn- N Level 2	39.4	15.1	24.4ab	100
Unplanted- N Level 3	27.8	12.9	11.4f	53.1
Barley- N Level 3	32.7	13.8	17.8cd	76.5
Corn- N Level 3	36.7	18.0	21.1bc	90.7
N Level 0	33.6	16.1	22.6a	89.8a
N Level 1	31.1	20.8	20.9b	84.5a
N Level 2	32.0	12.1	20.2b	83.9a
N Level 3	32.2	14.9	16.8c	73.4b
Unplanted	25.2c	11.6	13.5b	66.2c
Barley	34.5b	16.8	22.7a	87.2b
Corn	38.5a	19.6	24.0a	95.3a
$P_{Plant\ X\ N\text{-}Level}$	0.193	0.557	0.031 *	0.077
$P_{N\text{-}Level}$	0.512	0.172	< 0.001***	< 0.001***
P_{Plant}	< 0.001***	0.068	< 0.001***	< 0.001***

*Significant at $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$
lower case letters indicate significant differences between values of interaction, N level, plant type. If no letters there was no significant difference for that factor or interaction

Table 9. Greenhouse Experiment 3, comparison of rewetted soil DOC and amount of CO_2 released in 24 hours and 72 hours from dried and rewetted soil

Treatment	CO_2 respired (24 hrs) compared to DOC from dried soil (%)	CO_2 respired (72 hrs) compared to DOC from dried soil (%)
Unplanted- N Level 0	46	89
Barley- N Level 0	41	81
Corn- N Level 0	44	84
Unplanted- N Level 1	43	87
Barley- N Level 1	41	81
Corn- N Level 1	44	87
Unplanted- N Level 2	49	98
Barley- N Level 2	45	88
Corn- N Level 2	46	90
Unplanted- N Level 3	51	103
Barley- N Level 3	48	96
Corn- N Level 3	47	93

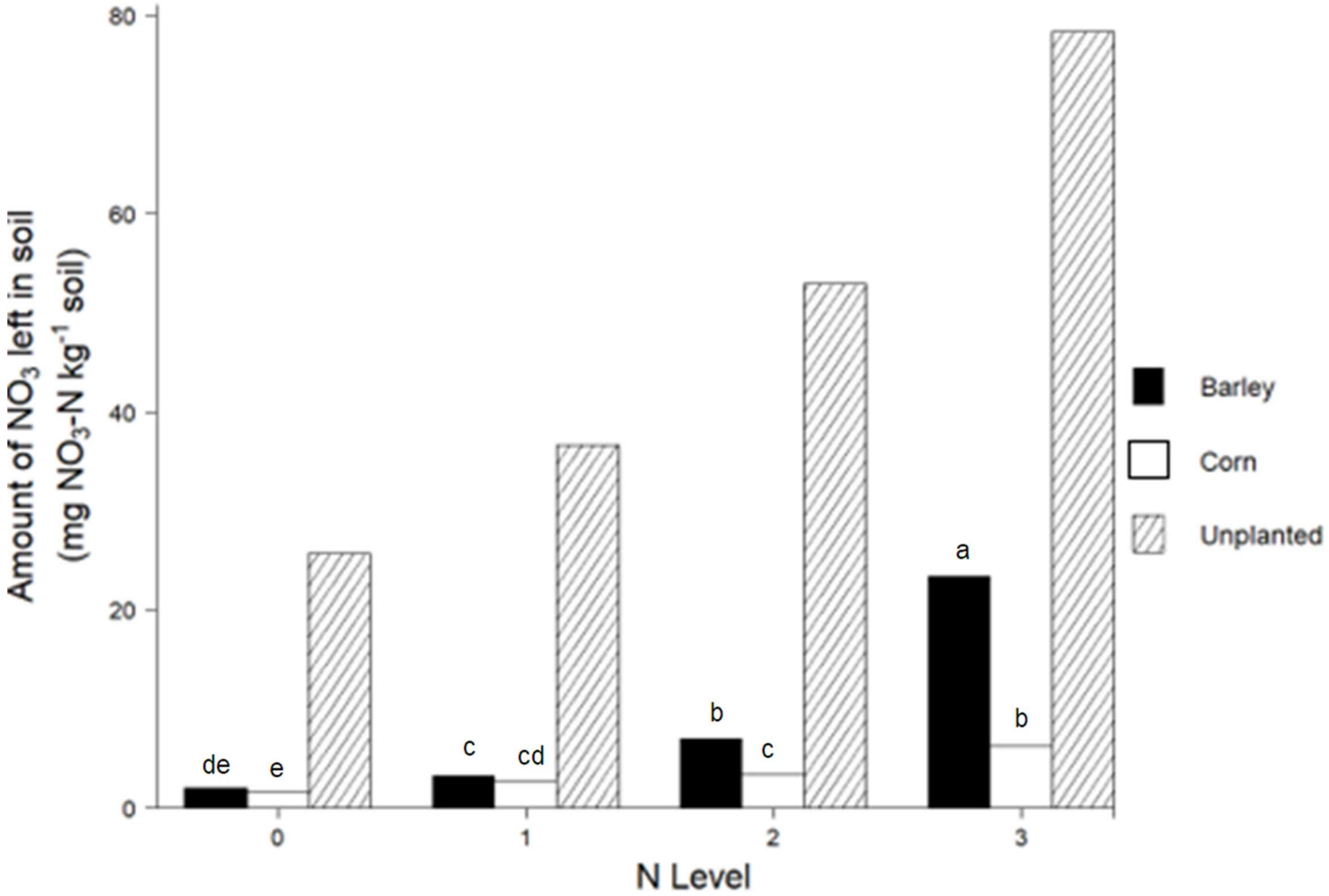
There was a significant treatment effect of both N Level and plant species on both moist and rewetted soil DOC, however there was a significant interaction between these factors only for moist DOC (Table A.27, Table A.28). There was a general trend that with increased N there was a decrease in soil DOC (Table 8). Table 8 shows that barley and corn were similar in moist DOC and the unplanted treatment was significantly different, and in the rewetted soil DOC all plant species treatments are different. The greatest amount of soil DOC was in the corn treatment and the least amount of soil DOC was in the unplanted treatment. The amounts of DOC in moist soil at N level 0 and 3 were significantly different from all other N levels, however N levels 1 and 2 were not significantly different from each other (Table 8). In rewetted soil DOC, N level 3 was significantly lower than all other N levels (Table 8).

Table 9 shows the percent of DOC that was respired in the CO_2 flush in 24 and 72 hours. The trends in percentage was similar to all other experiments. There was approximately twice as much respired DOC percent in 72 hours than in 24 hours (Table 8).

The amount of NO_3 remaining in the soil was significantly different for factors of N level, plant species and interaction (Table A.29). Unplanted treatment was excluded from the analysis

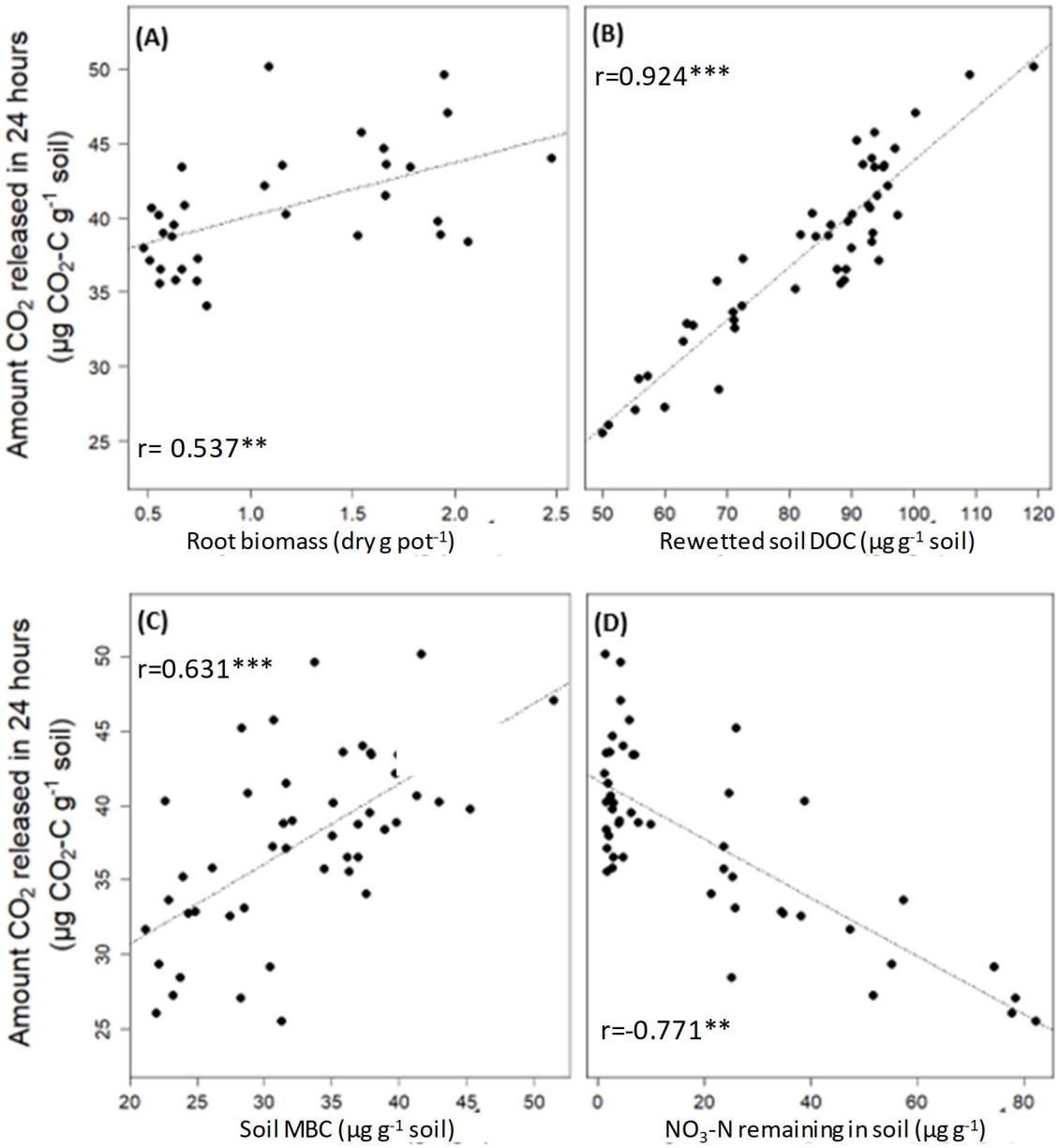
all factors remained significant (Table A.30). Figure 19 shows the amount of N remaining in the soil, and the significant differences for the interactions of factors (excluding the unplanted treatment). In general corn had less remaining NO_3 in the soil in comparison to barley, and there were significant differences at N level of 2 and 3 (Figure 19). At N levels of 2 and 3 the difference between corn and barley is likely due to corn, with available NO_3 in the soil, being highly responsive to uptake N (Bundy and Malone 1988). The soil NH_4 was very low, (data not shown) and no statistics were run on it.

Figure 20 shows correlations between the amount of CO_2-C released compared to dried root biomass, dried and rewetted soil DOC, moist soil MBC, and moist NO_3. All correlation were significant. All the p-values were less than 0.001, except for the amount of CO_2-C released and root biomass which was 0.002. The correlation between the CO_2 flush and dried soil DOC had the largest r value of 0.924.



lower case letters indicate significant differences between values (unplanted soil excluded)\

Figure 19. Greenhouse Experiment 3, amount of NO_3^- remaining in moist soil.



*Significant at $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$
(A) roots (B) rewetted soil DOC (C) moist soil MBC (D) NO_3^- remaining in moist soil.

Figure 20. Greenhouse Experiment 3, amount of CO_2 released (LI-COR) in 24 hours.

CHAPTER 4

DISCUSSION AND CONCLUSIONS

4.1 Discussion

These experiments were designed to determine if the CO_2 flush would be greater in soils with roots than soils without roots and to find if root biomass would correlate with the CO_2 flush. This is important because the CO_2 flush, which occurs naturally in soils subject to wet-dry cycles, is also being used as a relatively simple and rapid method to measure the biological activity in the soil as one parameter indicating overall soil health (Franzluebbers 2016). Our primary goal was to investigate the effects of roots on the CO_2 flush. In field and laboratory studies we compared planted conditions with bare or unplanted conditions, and quantified root biomass, DOC, and MBC, as well as the CO_2 flush. It was important to measure these edaphic factors under field moist and laboratory dried conditions to better understand the differences between field moist and dried soil characteristics. We expected both DOC and MBC to be affected by planted treatments and related to the CO_2 flush.

In general, for the CO_2 released in 24 hours after rewetting dried soil, both years of the Field Experiment and all greenhouse experiments showed a significant effect of having plants, and thus root biomass, in the soil in comparison to bare or unplanted soils. Rey et al. (2017) examined the soil respirations rates of vegetated areas, biological soil crusts and bare soils in response to rain events. Rey et al. (2017) found that the soil respiration was significantly greater in vegetated areas than bare soils, particularly in degraded grasslands. The vegetation effect was less in natural grasslands (Rey et al. 2017).

Although sampling roots from the Field Experiment proved to be difficult and there was high variation among replicates, it was clear that the planted plots contained more roots in comparison to bare plots. The plots were sampled between rows, which is representative of where soil samples may be collected during the growing season. The location of the sampling, however, may not provide a representative amount of root biomass for the entire plot. It is likely that a

sample taken directly over a plant may have provided more roots to be extracted than the location where our samples were collected. In 2017 there was an increase in root mass from the first collection time, GS49, to the second collection time, GS85. There was a larger increase in root mass in 2018 from the first collection time, GS32, to the second collection time, GS49. Steingrobe et al. (2001) showed that barley roots are constantly growing and senescing in the soil. In winter barley, after about a month of growth in the spring the growth rate and the senescing rates were similar, which resulted in a relatively constant amount of root biomass (Steingrobe et al. 2001). Our sampling likely took place during the early growth period. Of note, in our post-harvest collection in 2018, the root mass decreased by more than half compared to the prior collection time, GS49. This decrease could be because the roots were senescing, desiccated, and broken, such that they were more difficult to extract than in earlier sampling. In the greenhouse experiments, the roots were more easily extracted because they were contained within a pot, and soil was washed away from relatively intact root systems. In the Greenhouse Experiments 1 and 3, root biomass was correlated to the CO_2 flush, however in the Field Experiment root biomass was not correlated with the flush, possibly because we were unable to extract all the roots from field soil.

The rates of CO_2 release varied over the 24 hour flush period. The highest rates usually occurred during the first hour. This is in line with other studies that have examined the CO_2 released from soil after rewetting, which found the highest rates of CO_2 release within the first hour followed by a decrease, with an increase in CO_2 release around hour six, with sequential hours showing a decline through 72 hours (Fraser et al. 2016). This rate pattern is also similar to that found by Guo et al. (2014). The 2018 growing season samples had lower rates of CO_2 release during the first hour in comparison to all other experiments and the rates for the sample in May 2018. Both the MBC and the amount of DOC released from rewetted dried soils were lower in 2018 than 2017 (Table 4) and the differences were typically larger in planted soils. These differences may be partially explained by the 2018 field having a higher percentage of sand and a

lower amount of OM and total C (Table 2). The rate curve for the May 2018 sample (Figure A.3) was similar to samples taken during the field season for 2017 and the soils from the greenhouse experiments. In the May 2018 sample DOC from rewetted dried soil was higher than all bare samples in both 2017 and 2018. The MBC for the May 2018 sample was higher than the other sampling dates for 2018. This may partially explain the change in the rate pattern for the later sampling times in 2018. There was an application of a post emergent herbicide (MCPA) that took place after the May 2018 sample, and before the subsequent sampling of 2018, which did not occur in 2017. In a Chilean experiment using recommended applications of MCPA, the microbial communities became significantly different from the control with no herbicide after one day of incubation (Marileo et al. 2016). Marileo et al. (2016) found that MCPA and fertilizer (urea) resulted in the microbial community still being significantly different from the control at the end of a 15 day incubation even when approximately 97% of the herbicide had dissipated. It is possible for a shift in the microbial community to result in variations in the CO_2 rates of release curve, and this may explain the lower hour one rate for 2018 samples. However, microbial community composition was not measured during our experiment. Investigations into how rate patterns of CO_2 flush are influenced by pesticides may warrant future exploration. There have been some studies that examined impact of pesticides on basal soil respiration rate (Ahtiainen et al. 2003, Yousaf et al. 2013). Yousaf et al. (2013) found suppressed respirations in soils in associations with pesticide applications, and Ahtiainen et al. (2003) found decreased respiration associated with some pesticides but not all, suggesting that pesticide applications could affect microbial populations and potentially the flush. Currently, no studies were found that examined the impact of pesticides on the CO_2 flush.

In addition, in 2018 the overall flush was lower than in 2017, except for the post-harvest planted data which contained two high outlier data points. This could be related to differences between the fields used in 2017 versus 2018. The 2018 field had a higher percentage of sand (59%) with greater soil texture variability in comparison to the field in 2017 (46%) (Table 2).

There were also higher amounts of organic matter in 2017 than 2018 (Table 2). Other differences between the two years are that the barley yields in adjacent areas of the field were higher in 2017 (3309 kg ha^{-1}) than 2018 (3058 kg ha^{-1}) (personal communication, Brogan Tooley), however growing conditions beyond the scope of this paper could play a role in the differences in yields between the years. More robust plant growth along with higher levels of DOC and MBC in 2017 compared to 2018 could explain higher levels of CO_2 release in 2017.

Experiment 2 examined different plant species. In this experiment there was a significant difference for the CO_2 flush between planted and unplanted treatments; however, the only significant difference was between the corn treatment and the unplanted treatment. There were no significant differences among the plant species although the different species had different amounts of root biomass. Our data suggests that the presence of roots in the soil increases the flush, but the effect is independent of the plant species and amount of root biomass. However, in Experiment 3 there was a significant difference in the CO_2 flush between barley and corn at N levels 0, 2, and 3. All soils in Experiment 3 were treated with K_2HPO_4 while in Experiment 2 there was no added fertilizer. It is possible that with having more available K and P the two different plants species responded by releasing different types and amounts of root exudates. Experiment 2 had overall more DOC for barley and corn than Experiment 3, with barley showing a greater difference between experiments. A review article on P noted that lupine at low levels of P released more citrate (Hinsinger 2001), and having more P available in the soil may have reduced exudates. While DOC was not significantly different between barley and corn for Experiment 2 or Experiment 3, barley is consistently lower than corn by 3µg C g^{-1} soil in Experiment 2, and for N levels 0, 2, and 3 in Experiment 3 ranges from 8.5 to 14.2 µg C g^{-1} soil lower than corn. Also, the daylength was shorter for Experiment 2 than Experiment 3, and daylength affects all stages of growth in grain crops (Slafer and Rawson 1996). Future investigation of the CO_2 flush could examine field experiments with different crops and levels of

fertilization, to examine if trends noticed in the greenhouse experiment transfer to the field environment.

The CO_2 flush did not appear to be influenced by the collection time (growth stage) of barley for the Field Experiment. Because roots increased the CO_2 flush in comparison to bare or unplanted soil, we expected to observe a correlation between root mass and the CO_2 flush. In the Field Experiment there was no correlation between root mass and the CO_2 flush, possibly because of the difficulty in extracting roots from field samples. There was also no correlation for Experiment 2 in regards to the root mass and the CO_2 flush. There was a negative correlation between the two factors in Experiment 1. Experiment 3 had a positive correlation. Overall this indicates there was no clear relationship between root biomass and CO_2 flush.

All greenhouse experiments were conducted under natural light conditions. In Experiment 1 the average daylength was approximately 11 hours and was the shortest photoperiod among any of the experiments. Daylength affects plants growth (Slafer and Rawson 1996). This could help explain why Experiment 1 has less DOC than Experiment 2, and 3 for similar amount of time. Experiment 1 also had the lowest shoot to root ratio; this may be partially explained by the daylength. Machackova et al. (1998) found that in potatoes a 10 hour photoperiod resulted in smaller shoot to root ratio than with longer photoperiod. The shorter photoperiod may be part of the reason why the CO_2 flush, and DOC were lower in Experiment 1 in comparison to Experiment 2 and Experiment 3 (N level 0).

DOC can be influenced by plant roots because plants actively move recently photosynthesized C to microbial communities, which, when lysed by drying, add the C to the soil (Kaiser et al. 2015). Additionally, root exudates can act as a primer to increase microbial activity in the rhizosphere to actively break down soil organic matter (Haichar et al. 2014), potentially increasing the amount of soluble C. The DOC, from both field moist and rewetted dried soils, was higher in the planted treatments than the bare or unplanted treatments. DOC correlated strongly with CO_2 flush in both field and greenhouse experiments. Our results for the correlation between

the CO_2 flush and DOC are similar to those of Guo et al. (2014) who measured CO_2 released over a 120 hour period.

The CO_2 flush for our experiment was primarily focused on the 24 hour time period for the CO_2 accumulation. Other articles that refer to the CO_2 flush use a 72 hour collection period, instead of 24 hours. Our results found strong correlation between the 24 hour and 72 hour CO_2 flush with a slope typically around 2. Franzluebbers et al. (2000), examined soil from Texas and found that there was a high correlation between the 24 hour CO_2 flush and the 72 hour CO_2 flush. This provides strong evidence that the 24 hour CO_2 flush may maybe be suitable as a test for soil laboratories, and would reduce the amount of testing time. The current draft recommendations from Natural Resources Conservation Service (NRCS) recommend a 72 or 96 hour CO_2 flush as more reliable than the 24 hour flush (Soil Health Technical Note No. SH-XX (Draft), 2018). More studies should be carried out on the time of incubation for the CO_2 flush to allow for clear comparisons of results across studies that utilize either the 24 hour CO_2 flush or a longer time for CO_2 collection.

N is an important plant nutrient, and in Experiment 3 we investigated different amounts of N and crops and the influence on the CO_2 flush. In Experiment 3 we found no significant difference in the CO_2 flush considering only the N level, however in the unplanted pots there was a trend for N to decrease the CO_2 flush at each level of N. The interaction between N level and plant species on the CO_2 flush was significant. Corn has a high demand for N that may approach 2 kg N ha^{-1} day^{-1} for corn before maturity (Robertson and Vitousek 2009). Therefore, greater biomass for corn at each level of N was both expected and observed in Experiment 3. In comparison to unplanted and barley, corn had more DOC in dry soils. Zhu et al. (2016) found that increased N in maize crops led to an increased abundance in root exudates, and a corresponding increase in abundance of microbial biomass based on 16s rRNA qPCR analysis. In Experiment 3 we found that DOC declined with N level overall and was significantly lower at N level 3 in comparison to all other N levels. Increased root exudation would likely lead to higher DOC, but

that was not observed in Experiment 3. Corn did have significantly higher MBC in comparison to barley and unplanted, however unlike Zhu et al. (2016) we did not find an increase in microbial biomass (MBC) with the increase of N in corn.

In the 2017 field season, Experiment 1, and Experiment 3 there were significant correlations between CO_2 flush and MBC. However, in the 2018 field season and Experiment 2 there was no correlation between CO_2 flush and MBC. Franzluebbers et al. (2018b) investigated yield responses to N over 47 field locations growing corn and found good correlations between MBC and CO_2 flush. While our experiments suggest that MBC may be related to the CO_2 flush, the mixed results suggest a need for further investigation. While the size of the microbial population may be important in the magnitude of the CO_2 flush, the availability of a soluble C substrate is also important.

For soils from the Field Experiment we additionally ran the commercially available 'CO_2 Burst' test, Solvita®, from Woods End Laboratory in Mount Vernon, Maine. The Solvita® test showed same significance that was found using LI-COR, meaning that both tests detected a root effect, but no collection time effect. Solvita® utilizes paddles that have a colorimetric response to CO_2 in the head space of their jar. The paddles are calibrated, with their jar, to report CO_2-C as ppm, mg kg^{-1} soil, when following their procedure (Brinton 2018). We compared the Solvita® results with our results measured by LI-COR, and found the Solvita® results were in general double the LI-COR. The jars for the Solvita® we approximately 250 ml, and the ones we used with the LI-COR instrument we approximately 500 ml jars. Woods End Laboratories is now suggesting the use of larger jars (Brinton et al. 2018). This may reduce the reading of CO_2 on the Solvita® colorimetric paddle. McGowen et al. (2018) found that Solvita® results were 4 to 6 times higher than a based gas chromatograph method. Research is actively on-going on comparing different methodologies for measuring the CO_2 flush.

When evaluating the CO_2 flush as a potential soil health indicator it is important to remember it is only measuring one part of overall soil health, which is the biological health of the

soil. Our study provides information to improve interpretation and understanding of the CO_2 flush's usefulness as a biological indicator of soil health. We found with one agricultural crop, barley, there was no collection time effect on the CO_2 flush, which suggest seasonal robustness. Among six agricultural field crops the CO_2 flush appeared to be relatively similar, and if there was a difference between agricultural crops the magnitude of that difference was relatively small. Cropping system with dense root systems, such as pasture or turf, could have a larger effect on the CO_2 flush, although we did not investigate this. In this study we found that DOC was strongly correlated with the CO_2 flush. We suggest that DOC may be an appropriate biological heath indicator for soils. In comparison to the 24 or 72 hour CO_2 flush analytical time is greatly reduced. This leads to more rapid results. The DOC should be evaluated across a gradient of soil types, and climates before being used as a substitute for CO_2 flush because out study includes only one soil type. However, for some soil types DOC may be an appropriate part of soil health testing.

4.2 Conclusions

We found that the CO_2 flush is influenced by the presence of roots in the soil, but the effect is not large. While there is some evidence that the CO_2 flush may vary at different times over an entire year, there was no influence of collection time on the CO_2 flush during the growing season after plants were established and before harvest. There also appears to be a negligible and inconsistent plant species effect. While in our Experiment 2 there was no significant difference between crop species, in Experiment 3 there was a significant difference in corn and barley, but this may be due to how the plants responded to added nutrients in the soil. In our experiments correlations between MBC and the CO_2 flush were inconsistent. Having a readily available substrate may be more influential on the CO_2 flush than MBC alone. We found strong correlations between DOC and the CO_2 flush. DOC may be an appropriate substitute for the CO_2 flush test as a soil health biological indicator in some soils. These results should to be confirmed in other soils, among different crop species, and in different climates. Because the CO_2 flush was

not strongly influenced by collection time or plant species, the 24 hour CO_2 flush seems to be a robust soil health indicator among different crops and sampling times.

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APPENDIX

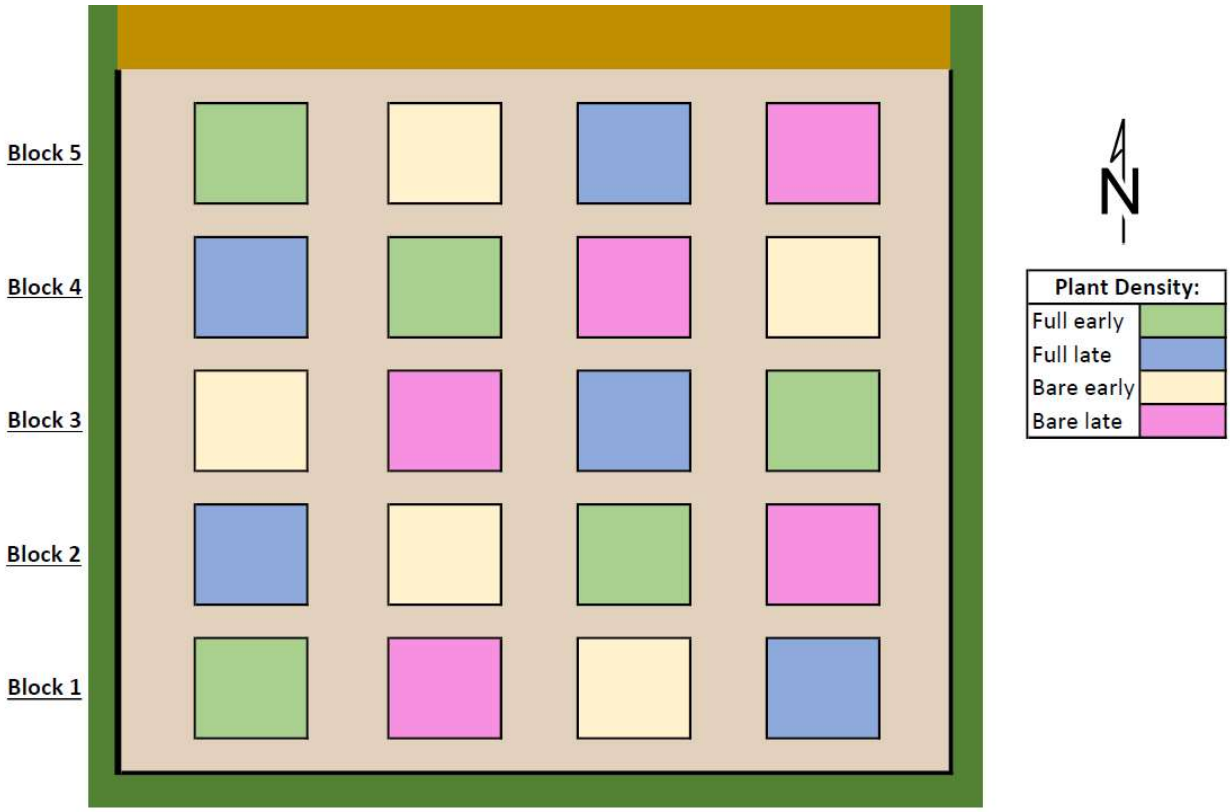

Figure A.1. Field Experiment 2017, field layout at Rogers Farm Old Town, ME.

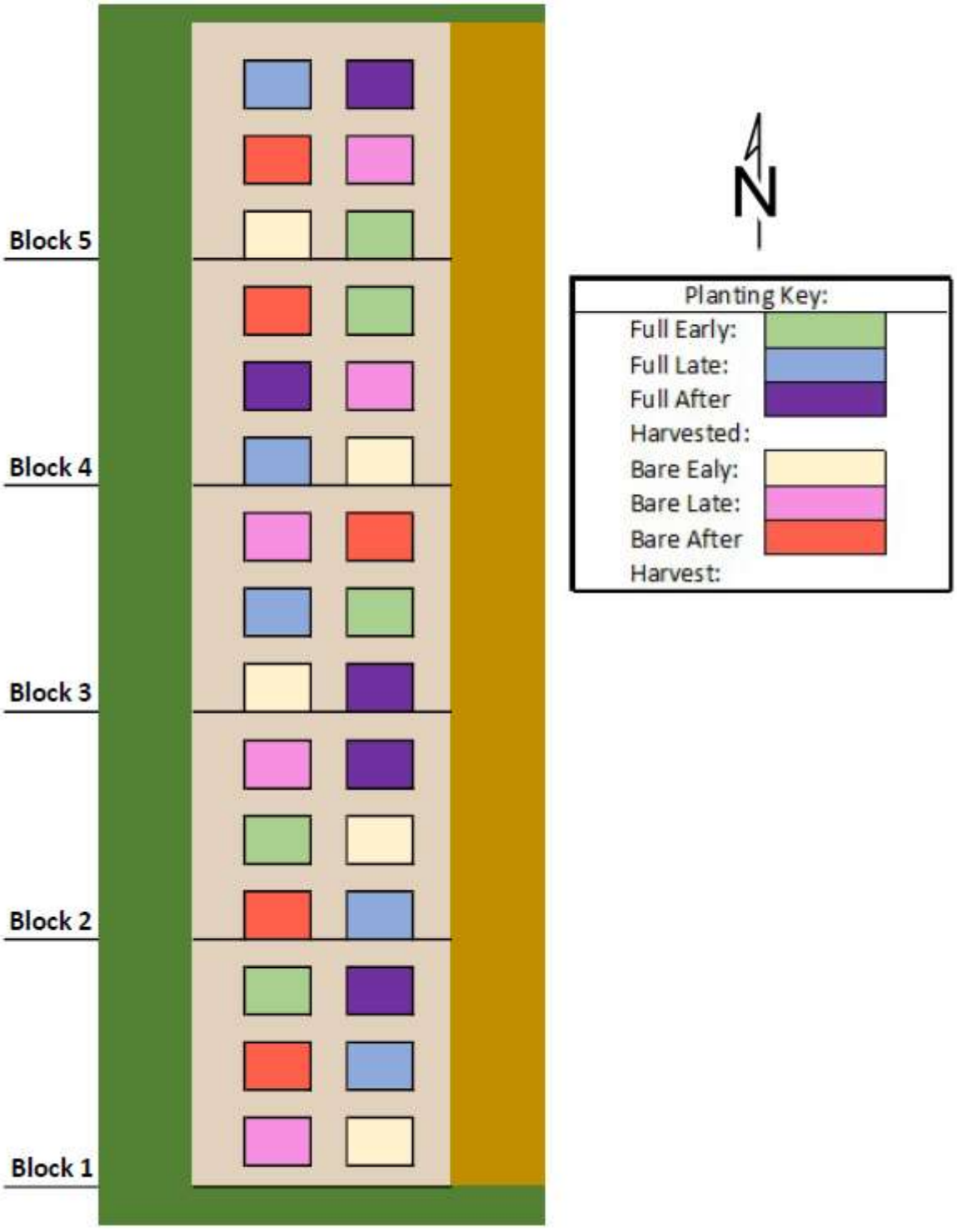

Figure A.2. Field Experiment 2018, field layout at Rogers Farm Old Town, ME.

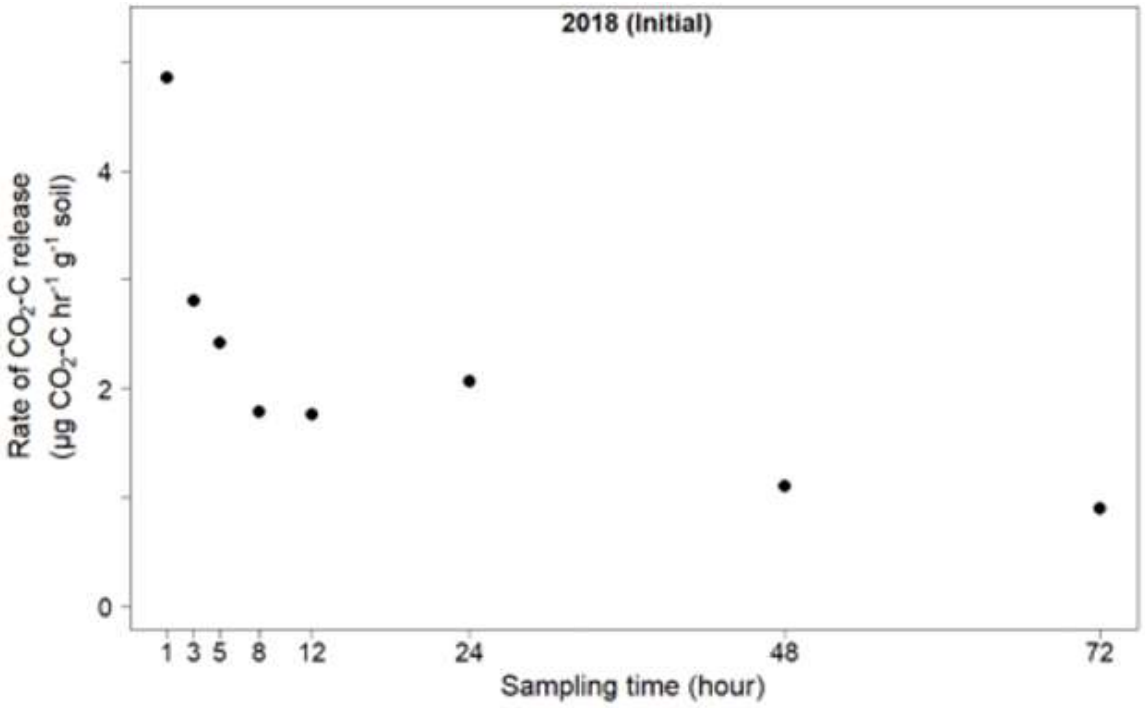

Figure A.3. Field Experiment 2018, rates of CO_2-C release from rewetted soil from May 17 prior to plant emergence, Rogers Farm Old Town, ME.

Table A.1. Field Experiment 2017 ANOVA for 24 hour CO_2 release (LI-COR).

	Degrees Freedom(DF)	Sum Squares (Sq)	Mean Sq	F value	P-Values
Roots	1	1600	1600	52.2	1.05e-5
Collection Time	1	64.7	64.7	2.11	0.17
block	4	40	10	0.326	0.86
Interaction	1	40.3	40.3	1.32	0.27
Residuals	12	368	30.7		

Table A.2. Field Experiment 2018 ANOVA for 24 hour CO_2 release (LI-COR).

	DF	Sum Sq	Mean Sq	F value	P-Values
Roots	1	1130	1130	10.4	2e-4
Collection Time	2	425	212	1.94	0.155
Block	4	985	246	2.25	0.194
Interaction	2	458	229	2.09	0.104
Residuals	20	2190	110		

Table A.3. Field Experiment 2017 ANOVA for 24 hour CO_2 release (Solvita®).

	DF	Sum Sq	Mean Sq	F value	P-Values
Roots	1	4660	4660	16.6	0.002
Collection Time	1	682	682	2.43	0.145
Block	4	1780	445	1.59	0.241
Interaction	1	393	393	1.40	0.259
Residuals	12	3360	280		

Table A.4. Field Experiment 2018 ANOVA for 24 hour CO_2 release (Solvita®).

	DF	Sum Sq	Mean Sq	F value	P-Values
Roots	1	2260	2260	12.2	0.002
Collection Time	2	283	142	0.764	0.479
Block	4	1662	415	2.24	0.101
Interaction	2	1070	535	2.89	0.079

Table A.5. Field Experiment 2017 ANOVA for moist soil MBC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Roots	1	1260	1260	9.39	0.003
Collection Time	1	48.9	48.9	0.363	0.588
Block	4	332	83.0	0.617	<2e-16
Interaction	1	366	366	2.72	0.498
Residuals	12	1610	135		

Table A.6. Field Experiment 2017 ANOVA for rewetted soil MBC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Roots	1	1030	1030	4.86	0.048
Collection Time	1	234	234	1.1	0.315
Block	4	51.5	12.9	0.061	0.992
Interaction	1	238	238	1.12	0.311
Residuals	12	2550	213		

Table A.7. Field Experiment 2018 ANOVA for moist soil MBC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Roots	1	0.087	0.087	1.48	0.238
Collection Time	2	0.105	0.052	0.888	0.427
Block	4	0.372	0.093	1.58	0.219
Interaction	2	0.083	0.042	0.707	0.505
Residuals	20	1.18	0.059		

Table A.8. Field Experiment 2017 ANOVA for rewetted soil MBC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Roots	1	1	1.4	0.004	0.959
Collection Time	2	480	240	0.631	0.614
Block	4	1210	302	0.794	1
Interaction	2	758	379	0.996	0.527
Residuals	20	7600	380		

Table A.9. Field Experiment 2017 ANOVA for moist soil DOC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Roots	1	3130	3130	230	<2e-16
Collection Time	1	148	148	10.9	0.014
Block	4	179	44.7	3.29	0.001
Interaction	1	82.0	82.0	6.03	0.048
Residuals	12	163	13.6		

Table A.10. Field Experiment 2017 ANOVA for rewetted soil DOC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Roots	1	2970	2970	151	3.76e-8
Collection Time	1	0.48	0.48	0.024	0.879
Block	4	179	44.8	2.27	0.122
Interaction	1	2.98	2.98	0.151	0.704
Residuals	12	236	19.7		

Table A.11. Field Experiment 2018 ANOVA for moist soil DOC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Roots	1	70.8	70.8	8.15	0.01
Collection Time	2	108	54.1	6.22	0.008
Block	4	35.4	8.85	1.02	0.422
Interaction	2	0.935	0.467	0.054	0.948
Residuals	20	174	8.7		

Table A.12. Field Experiment 2018 ANOVA for rewetted soil DOC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Roots	1	695	695	13.5	0.002
Collection Time	2	510	255	4.95	0.018
Block	4	311	77.9	1.51	0.237
Interaction	2	72.9	36.4	0.706	0.505
Residuals	20	1030	51.6		

Table A.13. Greenhouse Experiment 1 ANOVA for 24 hour CO_2 release (LI-COR).

	DF	Sum Sq	Mean Sq	F value	P-Values
Week	2	165	82.3	15	1.47e-4
Roots	1	124	23.6	22.5	1.61e-4
Interaction	2	12.5	6.27	1.14	0.341
Residuals	18	98.8	5.49		

Table A.14. Greenhouse Experiment 1 ANOVA for moist soil MBC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Roots	1	218	218	25.3	8.64e-5
Weeks	2	391	196	22.7	1.2e-5
Interaction	2	97.2	48.6	5.64	0.013
Residuals	18	155	8.62		

Table A.15. Greenhouse Experiment 1 ANOVA for rewetted soil MBC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Roots	1	0.021	0.021	10.6	0.004
Week	2	0.023	0.012	5.94	0.01
Interaction	2	0.008	0.004	2.03	0.16
Residuals	18	0.036	0.002		

Table A.16. Greenhouse Experiment 1 ANOVA for moist soil DOC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Root	1	386	386.	17.4	<2e-16
week	2	47.6	23.8	1.07	0.387
Interaction	2	61.5	30.7	1.38	0.263
Residuals	18	400	22.2		

Table A.17. Greenhouse Experiment 1 ANOVA for rewetted soil DOC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Roots	1	1520	1520	141	6.01e-10
Weeks	2	58	29	2.69	0.095
Interaction	2	73.5	36.8	3.41	0.055
Residuals	18	194	10.8		

Table A.18. Greenhouse Experiment 2 ANOVA for 24 hour CO_2 release (LI-COR).

	DF	Sum Sq	Mean Sq	F value	P-Values
Plant Species	5	361	72.3	3.38	0.017
Residuals	18	385	21.4		

Table A.19. Greenhouse Experiment 2 ANOVA for moist soil MBC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Plant Species	5	95.2	19	2.56	0.064
Residuals	18	134	7.44		

Table A.20. Greenhouse Experiment 2 ANOVA for rewetted soil MBC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Plant Species	5	226	45.2	2.2	0.099
Residuals	18	370	20.5		

Table A.21. Greenhouse Experiment 2 ANOVA for moist soil DOC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Plant Species	5	277	55.3	17.3	2.57e-6
Residuals	18	57.6	3.2		

Table A.22. Greenhouse Experiment 2 ANOVA for rewetted soil DOC.

	DF	Sum Sq	Mean Sq	F value	P-Values
Plant Species	5	0.03	0.006	4.1	0.012
Residuals	18	0.027	0.001		

Table A.23. Greenhouse Experiment 3 ANOVA for 24 hour CO_2 release (LI-COR).

	DF	Sum Sq	Mean Sq	F value	P-Values
N Level	3	83.7	27.9	2.32	0.091
Plant Species	2	1020	512	42.6	3.23e-10
Interaction	6	182	30.3	2.52	0.038
Residuals	36	432	12.0		

Table A.24. Greenhouse Experiment 3 CO_2 flush unplanted only.

	DF	Sum Sq	Mean Sq	F value	P-Values
N Level	3	186	62	3.34	0.056
Residuals	12	223	18.6		

Table A.25. Greenhouse Experiment 3 ANOVA for moist soil MBC.

	DF	Sum Sq	Mean Sq	F value	P-Values
N Level	3	0.007	0.002	0.781	0.512
Plant Species	2	0.294	0.147	49.9	4.2e-11
Interaction	6	0.027	0.005	1.54	0.193
Residuals	36	0.106	0.003		

Table A.26. Greenhouse Experiment 3 ANOVA for rewetted soil MBC.

	DF	Sum Sq	Mean Sq	F value	P-Values
N Level	3	476	159	1.76	0.172
Plant Species	2	523	261	2.90	0.068
Interaction	6	447	74.5	0.827	0.557
Residuals	36	3240	90		

Table A.27. Greenhouse Experiment 3 ANOVA for moist soil DOC.

	DF	Sum Sq	Mean Sq	F value	P-Values
N Level	3	214	71.3	38.9	<2e-16
Plant Species	2	1080	538	293	<2e-16
Interaction	6	29.9	5.0	2.71	0.031
Residuals	36	66.0	1.8		

Table A.28. Greenhouse Experiment 3 ANOVA for rewetted soil DOC.

	DF	Sum Sq	Mean Sq	F value	P-Values
N Level	3	1700	566	10.4	4e-5
Plant Species	2	7230	3610	66.4	<2e-16
Interaction	6	691	115	2.12	0.077
Residuals	36	1960	54		

Table A.29. Greenhouse Experiment 3 ANOVA for NO_3-N remaining in moist soil (all treatments).

	DF	Sum Sq	Mean Sq	F value	P-Values
N Level	3	4750	1580	416	<2e-16
Plant Species	2	19300	9640	2530	<2e-16
Interactions	6	2760	460	121	<2e-16
Residuals	36	137	4		

Table A.30. Greenhouse Experiment 3 ANOVA for NO_3-N remaining in moist soil (excluding unplanted).

	DF	Sum Sq	Mean Sq	F value	P-Values
N Level	3	3.12	1.04	89.7	3.5e-13
Plant Species	1	0.652	0.652	56.2	9.77e-8
Interaction	3	0.291	0.097	8.36	5.594e-4
Residuals	24	0.278	0.012		

BIOGRAPHY OF THE AUTHOR

Audrey E Laffely was born in Brunswick. Maine on September 3, 1985. She was raised in Brunswick, Maine and graduated from Brunswick High School in 2004. She attended Allegany College in Meadville, Pennsylvania from the fall of 2004 to the spring of 2005. In 2005 she transferred to Unity College in Unity, Maine. She received a bachelor's degree of science, with a double majoring in Park, Recreation and Ecotourism, and Landscape Horticulture in 2008. After she received her degree, she walked from Georgia to Maine on the Appalachian Trail in 2008. In 2009 she moved to the Chicago area, where she worked for Elk Grove Park District where she helped maintain 50 small parks. She returned to Maine in 2011 and worked at a variety of places. Audrey received her graduate certificate in GIS from the University of Maine in December 2018. Audrey is a candidate for the Master of Science degree in Plant, Soil, and Environmental Science from the University of Maine in May 2019.