Soil Carbon Sequestration using Lawn Ground Covers

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Abstract

Climate change is a global threat to life on Earth, and as such, action must be taken to prevent the increase of atmospheric CO_2 concentrations. In the process of reaching net- zero and net-negative emissions, carbon sequestration will play a pivotal role in offsetting emissions by transferring carbon from the atmosphere to the terrestrial biosphere. Within the field of carbon sequestration, storing carbon in soil via land management practices is a promising endeavor. Further, lawn use practices in developed areas pose a potential way to increase carbon storage in soil. The goal of this experiment was to investigate ground-covers commonly used in lawns to determine their abilities to stabilize carbon in soil as a step towards evaluating the feasibility of storing carbon in lawn soil.

An experiment was designed to grow Kentucky bluegrass and white clover in relatively controlled conditions and to measure the change in soil organic carbon (SOC) over time for each plant. SOC was measured using a destructive loss-on-ignition (LOI) method which involved weighing samples before and after being ignited in a furnace.

The experiment was found to have numerous significant sources of error due to complexities that were not accounted for in the design and from fundamental limitations that arose due to the short time scale and lack of resources available to run the experiment. As a result, the experiment could not conclude whether one of the plants was more effective for stabilizing carbon than the other. There were some trends that suggested that SOC decreased in the soil over time, which may have been a result of some of the possible mechanisms of carbon stabilization such as leaching, erosion, or microbial respiration, though there is a significant likelihood that the trends were due to confounding or random error.

Introduction

Climate Change

Climate change is a global phenomenon wherein the environment is changing, largely due to human activity. The primary symptom of climate change is global warming, which is a steady increase in global temperatures, which models indicate will lead to a plethora of disastrous effects if substantial measures are not taken to address it. The primary mechanism of global warming, and thus climate change, is the greenhouse effect, where carbon-based compounds, colloquially referred to as greenhouse gasses or just as carbon, build up in the atmosphere and trap heat from leaving Earth into outer space, resulting in a net increase in heat from the sun over time (Wilcox, J; Kolosz, B.; Freeman, J., 2021).

While the main concern about these carbon-based compounds is regarding their concentration in the atmosphere, these compounds exist in three main bodies that are continuously in balance with each other: the atmosphere, the terrestrial biosphere, and the shallow ocean. The terrestrial biosphere includes all life on land, which is mainly vegetation, as well as the soil at the surface of the land. The exchange of carbon between these stocks has a high throughput, though without any net change; the oceans release and take up 330 Gt CO_2/yr, and vegetation photosynthesizes and respires 440 Gt CO_2/yr. However, human activity results in unbalanced emissions, as humans bring more carbon into the total stocks from outside sources, such as from underground oil deposits. Human emissions amount to 40 Gt CO_2/yr; while these emissions are initially released to the atmosphere, half of them are eventually taken up by the ocean and land to balance it out (Wilcox, J; Kolosz, B.; Freeman, J., 2021).

Of the options for addressing global climate change, methods related to controlling atmospheric carbon are the least radical. There are two main ways to combat the yearly increase in atmospheric carbon: decreasing human emissions, and removing carbon from the atmosphere. In order to achieve net-zero emissions, and especially net-negative emissions, methods of removing carbon from the atmosphere must be used. Unfortunately, given the atmosphere is in balance with the ocean and land, by removing carbon from the atmosphere, the other stocks will balance out some of the removal by releasing more into the atmosphere (Wilcox, J; Kolosz, B.; Freeman, J., 2021).

Sequestration

A major method of removing carbon from the atmosphere is using plants to sequester carbon. This process works on the basis that plants fix atmospheric CO_2 into glucose and build it into biopolymers. On its own, this process does not lead to long-term storage of carbon; carbon is returned to the atmosphere during decomposition (Chapin III, F. S. et al, 2006). For photosynthesis to lead to long-term storage past the death of the plant, the organic matter created by the plant must be stabilized in the soil (Adamczyk, B. et al., 2020). The storing of carbon in soil can be viewed as a balance of inputs and outputs that ultimately may lead to net accumulation if the inputs are large enough (Chapin III, F. S. et al, 2006).

Carbon that enters the soil ultimately derives from carbon fixation by plants undergoing photosynthesis, which is then stored in plant parts that make up organic matter. The source of organic matter is mainly litter, which is dead plant matter. This litter may originate above-ground from falling leaves or decaying plants, or it may originate below-ground from the roots of dying plants. The organic matter also may originate from living plants as a result of the plant's interactions with its surroundings: this may be from root exudates, which are chemicals that plants release from their roots to interact with surrounding microbes in the rhizosphere, or from plant secondary metabolites, such as tannins, that may be released into the soil by the plant or via leaching (Adamczyk, B. et al., 2020).

The stabilization of the organic matter in soil was once thought to be due to the formation of highly recalcitrant substances that make up something called humus, though this idea has been contested recently, as humus is thought to actually just be an unnatural byproduct of using an extraction method with a high pH to analyze soil. More recent ideas about carbon stabilization in soil center around the idea that decomposition of organic matter is a continuous process, and that stabilization results from slowing down decomposition (Lehmann, J., 2015). This concept is best understood under the premise of the microbial carbon pump (MCP), wherein carbon enters the soil through one of the mechanisms described before, and then microbes either respire the carbon back into the atmosphere or they use it to grow their own biomass and convert it into other chemicals (Adamczyk, B. et al., 2020). The microbes then can form aggregates which protect them from erosion and from other microbes, and when the microbes die and form necromass, the carbon will be stabilized in the aggregates (Moukanni, N. et al., 2022; Wilpiszeski, R. L., 2019). Some compounds formed by the microbes, as well as some that originated from the plants, may also be stabilized by forming stable complexes with minerals in the soil, protecting them against decomposition. This stability still is not permanent, as the carbon will eventually be destabilized and removed. As such, soil still needs a consistent input of new organic matter to keep its carbon storage high, and thus, for it to have a lasting impact on offsetting carbon emissions (Adamczyk, B. et al., 2020).

Different plants may contribute differently to the microbial carbon pump based on which type of organic matter they can produce most effectively. Root litter has been found to be far more effective for the formation of aggregates than aboveground litter, especially in favor of fungi, and certain root exudates have been found to form stable complexes with minerals and also to be highly beneficial for the formation of aggregates (Moukanni, N. et al., 2022; Panchal, P. et al, 2022). Microbes prefer more labile compounds, as this improves their carbon use efficiency (CUE), which is a measure of the ratio between the amount of carbon respired and the amount of carbon used to reproduce. Labile compounds also have more direct pathways towards forming stable complexes (Moukanni, N. et al., 2022).

In addition to respiration, carbon can also leave the soil through lateral transport via water leaching soluble carbon, turning it into dissolved organic carbon (DOC) (Tank, S. et al., 2018). It is also possible for water to transport carbon in a particulate form, which the different species of carbon leading to different outcomes. This carbon may ultimately be carried into a nearby body of water, may that be a lake or ocean, or it may be stabilized elsewhere along the way, or simply emitted back into the atmosphere (Tank, S. et al., 2018).

Carbon sequestration and stabilization can be artificially enhanced through land management practices that seek to optimize the amount of sequestration that occurs. Common practices used to increase sequestration are cover cropping and no-till farming. These practices are mainly incorporated into farming, but they can also be applied to unestablished land, as some ideas have been developed around turning land into grazing grasslands to optimize sequestration (Wilcox, J; Kolosz, B.; Freeman, J., 2021). These practices are complicated by how bringing in as much carbon as possible is not the only criteria necessary for success. Soil fertility, erosion, pH, and biodiversity are also important factors that play into which practices are chosen (Moukanni, N. et al., 2022). Additionally, in the context of offsetting greenhouse gas emissions, the carbon cost of implementing the strategies is also important, as practices must bring a net removal of carbon from the atmosphere in order to actually be of any use.

Lawn Management

A common land use practice among residential areas in affluent nations is to grow lawns composed of short, walkable plants, such as grasses. This practice is often a large, unnecessary sink of resources such as water and fertilizer, as these plants are not well suited to the environment they are placed in. But the deep rooted ramifications of lawn-competitiveness in suburban areas has led to a status quo that leaves little ground for loosening control of surrounding landscapes and allowing them to return to a more natural state. It is also unclear whether these lawn practices are having a positive or negative effect on carbon sequestration, and whether the soil could be better used for mitigating climate change. In the context of sequestration, this brings into question whether there are lawn care practices that could act as a carbon sink while decreasing resource consumption, while still leading to lawns that would be convenient enough to appeal to the average land owner. I chose to investigate how the use of common lawn plants in soil may be affecting carbon storage within the soil over the long term.

Methods

Growth Design

For the bluegrass pots, seeds were sprinkled onto the soil at around 50-100 seeds per square inch. For the clover pots, around 30 seeds were placed one-by-one with relatively even spacing between them. After seeds were placed, they were raked into the soil by hand to a depth of 1/4 inch.

Both the bluegrass and clover have high needs for direct sunlight. The grow light was hooked up to a power controller that would activate the grow light from 6:30 a.m. to 6:30 p.m., granting 12 hours of direct sunlight per day.

Both the bluegrass and clover have water needs of a 1 inch depth per week. The plants were watered 1/5th of an inch each weekday by hand through use of a watering can. This was done by measuring out the total amount of water needed for all the plants into the gardening can, and then spreading it evenly across the 8 pots.

Pot setup

Eight concrete pots were placed in a 2-by-4 arrangement up against each other. The pots were square and their sides were straight-vertical, having dimensions 9” long by 9” wide by 13” tall. Each pot had a drain hole at the center of the bottom that was slightly raised up from the surface below to allow water to escape. The pots were laid out on a tray that would collect drained water into a sink drain. At the bottom of the inside of each pot, there was a 1-inch deep layer of pea-sized river gravel to enhance draining. A layer of mesh was placed below the gravel to prevent it from leaving through the drain hole. Another layer of mesh was placed on top of the gravel to separate it from the soil. Soil was filled in above the gravel and mesh up to about half-an inch below the top of the pot. The pots had a small lip at the top that constrained the surface area at the top to be slightly below the area of the soil throughout the majority of the depth of the pot.

A thin, 3-foot long grow light was hung about 8 inches above the pots. It was positioned at the center between the 2 rows of 4 pots. The light was attached using adjustable chains, though the height was not adjusted during the experiment. The light was plugged into a power controller that allowed the lighting to be set to a timer.

Preparation of 2:1 Loam/Potting mix soil

The loam was collected from an outdoor site, used to store other types of soil, from the side of a hill that was seemingly dug into previously. The loam was sifted through by hand to pick out any large debris, including leaves, sticks, rocks, mulch, insects, and worms.

To form the 2:1 loam and potting mix mixture, two 5-gallon buckets of equal proportions were used to measure out soil: one bucket was filled to a certain height with loam, and the other was filled to approximately half the height of the first with potting mix. The two buckets were then poured into an empty trash bag and mixed together by hand for around 15 minutes.

The 2:1 loam/potting mix soil is also referred to just as loam mix in the discussion.

Trial design

The experiment primarily sought to investigate the differences in sequestration between different plant species. The experiment focuses on two plants, representing common lawn-covers: Kentucky bluegrass and white clover. In achieving results that would give the most similarity to an in situ application, the factor of whether the grass was to be trimmed was considered, as grass used in lawns is typically mowed frequently to prevent it from growing too high. As a result of difficulties obtaining soil that would accurately represent natural soil, the type of soil used was also a defining factor considered. As such, 8 pots were used: 4 used a commercial potting mix, and 4 used a mixture of loamy soil obtained from the ground and potting mix in a 2:1 ratio. The potting mix used was Fafard Professional Potting Mix, which was composed mainly of organic material, such as peat moss. For each set of 4 pots, 2 grew bluegrass, 1 grew clover, and 1 acted as a control with no seeds planted on it. Of the two bluegrass pots, 1 was trimmed when the grass reached a height above 3 inches, and the other was not trimmed. Additionally, the carbon content of the potting mix and loam mix were measured on their own, separate from the trials.

Collecting and preparing soil samples

A 1-foot long, 1.5 inch diameter pipe was used to collect soil samples from the pots. One end of the pipe was sharpened inwards like a cone for ease of pushing into the soil. The pipe was driven into the soil by pushing and twisting. Using a hammer to drive the pipe into the soil was found to be impractical, as it deformed the shape of the pipe. The soil was emptied out of the pipe by pushing it out using a rod with a slightly lower diameter than the pipe. The pipe was inserted into the same location of soil twice to ensure that all of the soil from the area was collected.

Samples were collected once every two weeks, starting two weeks after the seeds were planted. Soil samples were taken from the quadrants of the pots. Originally, all pots would have their quadrants sampled in the order of top left, bottom left, top right, bottom right, but this was modified given that there would only be time to collect three sets of samples. Instead, the last two sets of samples were simplified into the last sample being taken from whichever of the remaining two quadrants was closer to the grow light.

When emptying the pipes, the soil was laid out onto a tray in a way that roughly retained the organization of the soil by depth. The cylinder of soil was separated roughly into three sections corresponding to the top, middle, and bottom of the soil sample. Each of these sections was then transferred into a separate crucible.

LOI procedure

The loss-on-ignition (LOI) procedure used was a combination of a few procedures (Nelson, D. W. et al., 1996; Hoogsteen, M. J. J. et al., 2015).

1. Crucibles were cleaned and then weighed. Then soil samples were placed in the crucibles, and air-dried for a week.
2. Air-dried samples were ground with a pestle and mortar, and then sieved to 0.425 mm. The literature calls for sieving to 0.4 mm, but a sieve with this size was not available. The sieved samples were then weighed.
3. The samples were heated in a drying oven at 100 ℃ for 24 hours, air-cooled for around 5-10 minutes, and then weighed.
4. The samples were heated in a muffle furnace at 550 ℃ for 3 hours, air-cooled for around 5-10 minutes, and then weighed.

The methods called for the use of desiccators for cooling samples after use of the oven and furnace, but they were not used due to the impracticality of transferring samples into and out of them.

The LOI was calculated as the difference between the sample's mass before and after being ignited in the muffle furnace. This value was then used in equation 1 to calculate the soil organic carbon

(SOC) percentage of the sample, where a_T is the carbon content of soil organic matter, b_T is the clay correction factor, C is the percent soil clay content, and the suffix T refers to the ignition temperature.

$$SOC = a_T \cdot \left(LOI_T - b_T \cdot C\right) \qquad (1)$$

Soil Compaction Test

A test was run to estimate the amount of compaction that resulted from collecting soil samples with the pipe. The 1.5 inch diameter pipe was pushed into the control potting mix pot, and then emptied into a container of known weight, using the same procedure for collecting samples as described before. The sample was weighed, and it represented the compacted soil. Then, with the pipe held with the palm of one hand covering the bottom opening, soil from the control potting soil pot was sprinkled into the pipe to the height at which the soil would fill up to during the collection procedure if it was not compacted. The pipe was shaken lightly to make sure there were no air-holes when filling it this way. The pipe was then emptied into another container of known weight.

Soil Texture Analysis

A test was run to estimate the composition of the loam in terms of the relative amounts of silt, sand, and clay, based on a method for soil texture analysis (Jeffers, 2018). First, a large mason jar was filled around 1/3 full with some loam that was set aside previously. Then the jar was filled up to around 80% with water, and 1 tablespoon of powdered dishwashing detergent was added. The jar was capped and then shaken vigorously for about a minute. The jar was then left on a level surface for 1 week. After this period, the size of the three distinct sections in the soil were measured digitally, and a loam soil texture diagram was used to determine the composition.

pH Test

A test was run on the 2:1 loam/potting soil mix to determine whether it had a suitable pH based on the method (Richards, L. A., 1954). A jar was filled about half-full with soil. DI water was added to the soil until it reached the state of a saturated paste. If too much water was added, more soil was added, and then water was added in smaller increments. The saturated paste was then allowed to sit on a level surface for an hour. After the hour, a pH probe was used to measure the pH of the paste, wherein the probe was moved all around the sample to get the average pH across all parts of the sample.

Results and Discussion

Soil composition

The ratio of sand, silt, and clay in the loam was analyzed by measuring the relative length of the three separated sections in the jar from the soil texture analysis. The relative lengths in terms of arbitrary units are shown in Figure 1. The sand's length was multiplied by a correction factor of 0.9 to account for the curved shape of the jar's bottom overestimating the amount of sand. As such, the loam was determined to be 18.6% sand, 67.7% silt, and 13.7% clay. Additionally, the LOI experiment found that the loam had around 36% organic matter, which is far higher than was expected for the loam. This is indicative that the loam obtained may have already had significant decomposition of organic matter in the environment it was taken from, given it was extracted from the side of a hill that seemed to be dug out, indicating the loam may have been taken from what was at one point subsoil. This indicates it may already have had a significant amount of decomposing matter present, which may not be desirable for the application of trying to sequester more carbon in the soil, as discussed in Errors 63 and 66.

The pH test on the loam found it to have a pH of around 6.8, which is reasonable for growing bluegrass and clover, given they prefer a pH of around 6, as evidenced by that the plants were able to grow in the loam successfully.

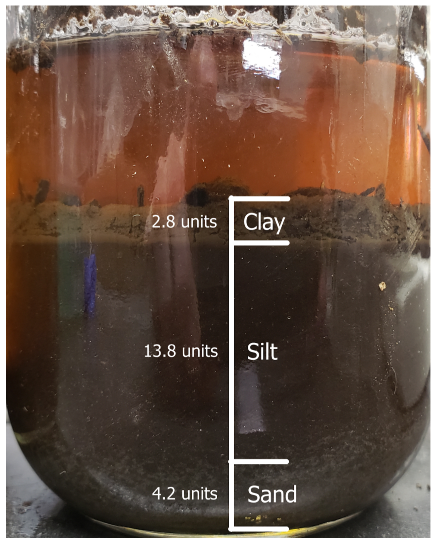

Figure 1: Loam soil separated into sand, lint, and clay, with boundaries marked digitally and measured with arbitrary units.

Experimental Discussion

Experimental Design

Kentucky bluegrass is known to have high water and fertilizer needs, and is colloquially considered an unsustainable option. In comparison, white clover is considered to have lower needs, and is lauded for its nitrogen fixing capabilities. Bluegrass and clover were chosen as a means of putting two somewhat controversial plants up against each other. Various bits of advice about growing the two plants in pots were gained from talking with gardeners and with the WPI horticulturist. This is where the growth design choices originated from, as they would allow for favorable growth conditions. A significant diversion was that fertilizer was recommended against for this experiment, as it would not be beneficial for the early development of the plants, though if the experiment were over a longer term it would be beneficial to investigate.

The experiment was designed to control only as much as was deemed necessary about the design to allow the plants to grow in fairly natural conditions. The largest design choice in doing this was the decision to grow the plants in pots rather than grow them outside in a natural environment. Given the experiment was mostly performed over the winter, the latter would not have been feasible, but using pots and growing the plants indoors shielded a majority of the interactions that plants and soil have with their natural surroundings. This in turn also allowed more control in what the plants were subjected to for the purposes of measuring the effects of specific processes.

Potting mix was not the first choice when designing the experiment; the use of potting mix was entirely due to the time of year in which the experiment was performed, as garden centers and nurseries have very limited supplies during the winter months, and don't offer most of their usual supplies until April. There was no loam for sale anywhere near campus, nor was there local bluegrass or clover seeds. The time of year also made it difficult to obtain loam from the ground, as oftentimes the ground was frozen or had snow covering it.

Although the potting mix was not a good fit for the experiment, as reasoned in Errors 63-65, it was still included in the experiment as a back-up in case the loam mix was not able to grow, as potting mix is far more suitable to in-pot use than loam. Information gained from gardeners seemed to suggest that using ground soil for in-pot use can be detrimental to plant growth due to poor drainage and lack of access to air. To address this, the WPI horticulturalist recommended mixing in some peat moss to improve both of these issues, and to use gravel at the base of the pots to improve drainage. Having implemented these improvements, it was still unclear whether this would be enough to allow the plants to grow in the pots, so the trials were designed to rely on each of them equally.

LOI Method

The loss on ignition method (LOI) was chosen over other methods of measuring soil content due to its simplicity and its improved safety over wet oxidation methods which require the use of unsafe chemicals, such as dichromate. It works on the basis that organic matter will combust when heated at high temperatures, and the amount of mass lost from the sample during this combustion can be attributed to the loss of organic matter in the sample, thus giving the composition. This is only a simplification though, as there are other factors that can control the amount of mass loss. For this experiment, the LOI method was not performed in a way that would minimize the error involved with it.

The mass of samples used is highly impactful to the error associated with LOI. Sample sizes above 20 g have been found to yield a minimal standard deviation, but the samples used were in the range of 1 to 5 g. Hoogsteen et al. found that standard deviation decreases logarithmically with sample size; for silt loam soils, the standard deviation is significantly higher for masses around 1 g than for above 2.5 g, and the same can be said for masses around 2.5 g compared to 5 g (Hoogsteen, M. J. J., 2015). As such, there is likely to be considerable uncertainty for all the data collected, but the uncertainty will be far more significant for the samples of smaller size.

Additionally, the position of samples in a muffle furnace can influence the mass loss; samples close to the door were suspected to have less mass loss, and Hoogsteen et al. found that turning the tray of samples halfway through igniting led to significantly lower standard deviation by minimizing the effects of positioning (Hoogsteen, M. J. J., 2015). Tray turning was not used for this experiment, which would further increase the uncertainty, though the degree to which is uncertain, especially given the positions of the crucibles in the furnace were not recorded. Tray turning was not used because it was impractical given that the samples were not actually on a tray, as there was not one available; rearranging the samples would have been time consuming and challenging, as it would introduce a high likelihood that samples would accidentally be tipped over.

Hoogsteen et al. considered soils with LOI around 3%, whereas this experiment resulted in LOI values ranging from 30-60%, so it is unclear how well the standard deviations obtained by Hoogsteen et al. apply to these data (Hoogsteen, M. J. J., 2015). Thus, the extent of the error of the LOI method is not fully understood for these data.

Another confounding factor for mass loss is the clay content of the soil. At temperatures of 550 °C or above, structural water loss from clay minerals can occur (Hoogsteen, M. J. J., 2015). As such, calculations of the SOC from the LOI need a clay correction factor based on the temperature of ignition, which is accounted for in equation 1. Given the clay content of the soil was found to be 12%, that value was used for C, and the clay correction factor, b_T, is around 0.075 at an ignition temperature of 550 °C. Equation 1 also calls for a correction factor for the carbon content of the ignited matter, a_T, which is around 0.58 at 550 °C. This value for the correction factor may not be entirely applicable, as the correction factor has been found to vary significantly with the type of organic matter that is being studied (Pribyl, 2010). As such, it's possible that the correction factor differs greatly between the potting soil and the loam, and that neither set of soil is well represented by the 0.58 correction factor. However, the value of 0.58 was used anyway to give a rough approximation of the scale, though it likely significantly lowers the accuracy of the comparison of scales of carbon composition between the potting mix, the loam, and other studies of soil.

The calculation of SOC reported in some literature (Nelson, D. W.,1996) used the total soil, which is the mass of soil before oven drying to remove moisture, as the basis to compare loss on ignition to. As such, the amount of moisture remaining when the total soil was measured would influence the calculation of SOC, as the LOI occurs after both drying and igniting. This was problematic for the data in this experiment, as some trials were air-dried significantly less than others, meaning that some samples had total soil measurements with significantly higher moisture content. As such, instead of using the total soil, the sample mass after drying was used, as this would mitigate the impact of the inconsistent drying.

Error

Quantitative Error

There were numerous aspects of the procedure that introduced error to the measurements of the sample mass. Many of these arose from opting for convenience due to a limited amount of time and assistance. The aspects contributing to quantitative error are those that are expected to offset the measured values from the true values, though the degree to which these would affect the uncertainty is unknown for most cases, due to difficulty in measuring their effects. Although, many sources of error may have resulted in uncertainty in the same direction for all the samples, thus canceling out the effects of error when viewing values relative to each other.

The grinding and sieving process introduced some error. The sieve and the pestle and mortar were not washed between samples, but were only wiped down with a paper towel, which may have led to some contamination between samples (Error 1). This may have been furthered given that the paper towel was not replaced after each use, but was replaced after every 3-4 samples. The amount of small particles that remained on surfaces and that would be likely to dislodge from the surfaces of the equipment seemed very low, so this would likely add around 0.2% uncertainty to the LOI. Using the sieve and the pestle and mortar led to some of the smaller particles becoming air-borne, which may have led to some mass loss of the samples, disproportionately affecting smaller particles (Error 2). Smaller particles also tended to be harder to remove from the sieve pan and the mortar, meaning they were disproportionately transferred less to the samples in the crucibles. Depending on the organic content of the smaller particles, this may have altered the average carbon content of the samples. This could reasonably have added around 0.5% uncertainty to the LOI.

Similarly to these two issues, for each sample, the crucible used to store the sample before sieving was also used to hold the sieved sample; the crucible was wiped with a paper towel in between, but not washed (Error 3). Given many of the crucibles had severe staining from previous use, it was difficult to identify whether they were fully cleaned. This could have led to some larger particles remaining in the sample after sieving, which may not have ignited properly with the LOI method. Also, given the improper cleaning, some smaller particles may have clung to the sides or bottom of the crucibles and may not have been cleaned off (Error 4). The degree to which these additional particles affected the measurements would be based on if they fell off between measurements or if they were ignited and had a different composition than the sample, as otherwise the mass difference would be accounted for. Both the unsieved particles and the peripheral particles would not likely amount to more than 0.5% uncertainty in the LOI. In a similar manner, crucibles were not always handled with tongs, which may have resulted in fingerprints or dust from bricks or soil getting on the sides of crucibles (Error 5). This may have been added between measurements, which could have affected the measurement of the change in mass. This likely amounted to significantly less than 0.2% uncertainty for the LOI.

The grinding process with the pestle and mortar may not have been performed to completion for all the samples due to obstructions from larger debris; pebbles, twigs, and clumps of grass made using the pestle more difficult, and this may have led to incomplete grinding of some parts of the sample (Error 6). This uneven grinding would have disproportionately affected particles that were more resilient to the grinding, which may have altered the overall composition of the sample to differ from the soil source. After sieving, there seemed to be a significant amount of particles that were just barely too large to fit through the sieve, and it is uncertain whether they would have fit through if they were ground more directly in the absence of other obstructing debris. Additionally, larger debris may have obstructed the sieving process by making it harder for particles that were just barely small enough to fit to pass through the sieve. If the particles in the soil could have been ground to fit through the sieve but were limited by the debris, then this likely could have introduced upwards of 5% uncertainty in the LOI, given the particles too large to fit through were likely of a significantly different composition than those that fit through the sieve. Also, the potting mix had significantly more debris than the loam mix, and the samples at the top of grass pots had significant interference from tangled clumps of grass, which would lead to differences in grinding completion between strata (Error 7).

Another possible small source of error associated with the sieving was that the sieved samples tended to be clumped into separate groups based on particle size, and that when transferring the sample over to the crucible, the sample would be composed of layers of phases, rather than a well-mixed heterogeneous mixture (Error 8). This may have worsened the stirring up of smaller particles mentioned in Error 2, and also may have led to some issues with igniting if different amounts of heat were applied to the different phases. This likely contributed below 1% uncertainty to the LOI.

A significant amount of error was accrued in the process of weighing samples. In theory, the digital mass balances used could measure up to 4 decimal places, meaning they would have an uncertainty of 0.0001 g (Error 9). However, some of the balances were in heavily ventilated rooms, causing the values to fluctuate by up to 0.002 g in either direction (Error 10). This only affected mass measurements of the crucible and the sample after sieving, as a different mass balance in a less windy environment was used for the other measurements, such as the sample mass before and after igniting. Additionally, the balances would randomly accrue some deviation from zero, possibly due to a build-up of small debris, which was not always immediately noticed and then zeroed; the balances were zeroed once every few measurements, or whenever they seemed to be off, rather than before every measurement (Error 11). The balance was not zeroed between measurements at all for the first set of measurements, but more zeroing was implemented past this point. The lack of zeroing accounted for an uncertainty of up to 0.001 g in the negative direction, as this likely led to overestimation. Additionally, crucibles were not always allowed to cool to room temperature before measuring, but rather until they could be handled safely with a bare hand, which may have influenced measurements by a few mechanisms, such as upwards air currents, and thermal expansion of the balance (Error 12). This may have introduced upwards of 0.001 g uncertainty for the samples measurements before and after ignition, though the effects were not particularly visible.

Obtaining the tare weight of the crucibles may also have had some error, as the crucibles were not dried in an oven before weighing (Error 13). As such, there may have been a small amount of moisture on the crucibles before they were weighed, which would overestimate their weight slightly. This likely contributed at most 0.001-0.002 g of uncertainty in the negative direction, as this likely led to overestimation.

Another issue arose from the method of cooling the crucibles; after removing the samples from the drying oven and the muffle furnace, the samples were cooled by leaving them out on a brick, rather than placing them in a desiccator (Error 14). As such, the samples may have regained some moisture after being heated and before being weighed for the 5-10 minutes they were laid out, which may have created uncertainty in the negative direction for those individual measurements due to overestimation. Given the value used in the results is taken from the difference between the two measurements affected, the uncertainty may cancel out if the moisture gain was similar both after being in the drying oven and after igniting in the muffle furnace. Given that the ignited samples had less mass than the dried samples, the ignited samples likely had less moisture gain than the dried samples, which may have thus led to slight overestimation of the difference between the values. The difference in moisture gain between the two measurements was likely at most 0.001 g.

Objective Design Error

A few aspects of the design did not inherently add uncertainty, but they could increase the likelihood of human error. The first of these is that the system used for keeping track of crucibles was not ideal, as the crucibles could not be effectively labeled, as most labels would burn off when ignited in the muffle furnace (Error 15). Codes and blemishes were used to distinguish the crucibles, which was prone to mix ups, as some crucibles had the same codes due to how they were manufactured. The difficulty of reading the labels also slowed down the efficiency in the lab. The second aspect was that the procedure for collecting samples with the pipe initially involved hammering the pipe, but this ultimately led to the shape of the pipe's end to be distorted, preventing the pushing-rod from fitting in the pipe (Error 16). This required the pipe to be switched out, and the procedure was changed to instead twist the pipe to dig it into the soil.

There were also some design flaws that may have introduced error into the experiment. A possible source of error was that the holes created in the soil by collecting samples were not filled back in with extra soil, primarily for convenience (Error 17). This may have led to inconsistent pooling of water in the pots; depending on the rates of diffusion, there may have been inconsistent water flow. When watering, if the rate of water flow from the soil to the hole is greater than the flow from the soil to other soil, then the holes may have lowered the amount of water flowing deep into the soil. Then, if the flow of water was carrying dissolved organic matter and resulting in leaching, then the holes may have been lowering the amount of leaching in deeper soil in the areas surrounding holes. This also could have led to water not flowing to all the plants' roots evenly. Additionally, the holes may have allowed more air-flow to the roots of surrounding plants, possibly increasing their growth. Given that the targeted locations for sampling has some distance between each other, the effects of the holes may not have been too significant, but this cannot be made certain without further testing.

Another major design flaw was that only one thin grow light was used, and it was positioned between the two rows of pots (Error 18). This led to fairly uneven growth across the area of each pot, as plants tended to grow better when they were closer to the pot. Additionally, the plants tended to grow towards the pot, leading them to grow at an angle and lean over to the middle. This uneven growth led to disparity between the growth across the quadrants, which brings in a confounding factor to the change in carbon content over time. Instead, an array of grow lights covering the entire area of the pots evenly should have been used.

There was some error associated with the sample collection procedure, though the extent of it could not be estimated accurately. The largest issue with the method was compaction of the soil: when pushing the pipe into the soil, the soil would get compacted in the pipe, making it unclear whether all of the soil from the targeted area was making its way into the pipe, or if some of it was instead just being pushed to the sides (Error 19). The compaction also made it difficult to get all the soil into the pipe in one go, meaning it had to be done in multiple steps, further making it difficult to tell if all the soil was collected. As such, it is unclear whether the soil collected represented the full depth of soil in the pot, or if it was falling short of this. I ran a short experiment to evaluate the degree of compaction for the soil, as described in the methods, and it found that the compacted soil had 80% of the uncompacted for the potting mix, and 75% of the uncompacted for the 2:1 loam/potting mix, as shown in Table 1. This means that the sample collection procedure was in fact not collecting all the soil from the targeted area, though it does not indicate whether this means that the soil at the bottom of the pot was not collected, or if it means that sample size was smaller in an even way across the entire depth of the soil.

Table 1: Comparison between the mass of a soil sample when collected using the pipe and the mass of a soil sample taking up the volume of the pipe uncompacted.

Soil Type	Compaction	Container Mass (g)	Container and Soil Mass (g)	Soil Mass (g)	Difference with Counterpart (%)
potting soil	compacted	10.4944	113.32	102.8256	80.19904378
	uncompacted	10.57	138.783	128.213	124.689766
loam mix	compacted	10.5487	101.448	90.8993	75.21905961
	uncompacted	10.6269	131.473	120.8461	132.9450282

There was also uncertainty with how well the bottom, middle, and top sections of the soil were separated. First, pushing soil out of the pipe using the rod was not consistent. The rod had a 1-inch diameter, while the pipe had a 1.5 inch diameter, which meant that the soil was not pushed out cleanly all at once; some soil stuck to the sides, and had to be scraped out (Error 20). This meant that some pieces of soil from the middle or bottom of the soil may have been mixed into the top, as the bits scraped out were added to the top. A similar error occurred with how, given that the soil was collected from the same spot in multiple steps due to compaction, after pulling out the pipe from the hole, some soil from the top of the hole would collapse into the hole, so subsequent steps would include a bit of soil from the top of the hole mixed with the bottom (Error 21). Also, when first inserting the pipe, it didn't always get sent straight down, but sometimes was at an angle, but then was corrected to be vertical. This may have extracted extra soil from the middle section, misrepresenting the distribution in the sample. Additionally, the pipe was not cleaned between samples, so there may have been some small contamination across samples (Error 22).

There was also error associated with transferring the collected samples into the crucibles. When a sample was emptied from the pipe, it was laid out on a tray, and then was loosely sectioned off into the top, middle, and bottom with a spatula (Error 23). There were plenty of loose particles that could have made their way into the wrong section, so the sectioning was fairly imprecise, and may have blurred between the three sections at their borders. This was amplified by how 90% or more of the soil collected from the pipe was used as samples in the crucibles to maximize the sample size; this meant there were no gaps between the sections, making the borders more blurred (Error 24). This also highlights how the top, middle, and bottom samples each represent an average composition across a third of the sample, rather than the composition at three specific depths.

Subjective Design Error

There were many aspects of the design that may have limited its ability to accurately obtain some data in a controlled way, but doing so would have also limited the realism of the experiment, possibly limiting its applicability. The design direction for the experiment often favored emulating soil in a natural environment rather than eliminating all possible confounding factors in order to measure any specific metric. Some aspects that could have been more controlled were allowed to remain uncontrolled as a result. Additionally, there were some instances in which additional control was added where it would have made more sense not to. In general, the effects of these design aspects are not feasible to estimate accurately due to the unpredictable effects they may have had.

One major debatable design error was that the seeds were not planted in a very controlled way. For the bluegrass seeds, the amount of seeds was not measured out by mass for each pot, but instead the seeds were sprinkled in until there appeared to be the right density of seeds (Error 25). This meant that the number of seeds may not have been consistent across all the pots. For the clover seeds, almost double the recommended number of seeds were used in order to err on the side of caution, which may have led to crowding of plants and competition for resources (Error 26). Additionally, the placement of seeds was not done mechanically; seeds were sprinkled wherever there appeared to not be enough seeds (Error 27). Some patches may have had too many or too few seeds within a single pot. Also, raking the seeds into the soil was done using fingers rather than an actual rake, and was done mostly randomly, so it is unclear whether it was done consistently across all pots (Error 28). These aspects could introduce confounding factors, as the amount of growth may have been influenced by over- or under-seeding. However, for lawn applications, seeding cannot always be expected to be done in an exact manner.

Similarly, watering of the plants was not done in a very controlled way. In the procedure, the total amount of water to be used was measured in an approximately quantitative way, but the distribution of the water was done by hand, and was prone to human error (Error 29). Watering also cannot be expected to be administered evenly in a real-life application, even if performed mechanically. Additionally, watering was not done every day; the plants were watered only on weekdays (Error 30). This may have limited the plants' potential rate of growth, though it also could have helped to prevent overwatering. Plants in a real-life environment are not expected to receive water every day, as it does not rain every day, and watering too frequently can lead to rotting and mold. Also, the plants were seeded mainly on a Thursday or Friday, meaning the seed did not receive consistent watering after planting, which may have stunted the growth of some seeds (Error 31). Although, this likely does not explain the observation that the lower growth of the second and third set of grass seeds planted as described in Error 43, as the third set was planted on a Monday, and it still had similar growth to those planted in the second set.

The loam collected from the ground was not very controlled. Given it was taken directly from the environment, rather than store-bought, it was not sterile, and was filled with assorted debris, including what seemed to be plastic (Error 32). There were also multiple worms found in the loam, some of which made their way into the pots and went undetected until they were found in samples. Given these characteristics are natural to the environment, they serve to make the experiment more realistic in terms of emulating the environment. Although, much of the debris in the loam, such as rocks, sticks, leaves, worms, and plastic, was picked out when preparing it, and it is unclear whether doing so was beneficial to the experiment (Error 33). It is unclear whether this debris may be beneficial or harmful to the growth of the plants, and whether leaving it in would have added to the realism.

Also, the mixing of the 2:1 loam/potting mix soil was not done in a very controlled way; the volumes that were measured out were only approximate, and they could have been measured out more exactly so that the composition of the mix could be better understood (Error 34). The mixing was also done by hand, and could easily have been nonuniform in terms of the mix of loam and potting mix. It's also possible that the loam and potting mix on their own were fairly nonuniform, as they each are solid heterogeneous mixtures, and given soil's tendency to clump into aggregates, there is no guarantee that different samples of the mixtures should have the same composition (Error 35). In a similar manner, the potting soil could have varied slightly in composition across bags based on how it was manufactured (Error 36). This all limits the ability to compare the compositions of pots directly to each other, as well as possibly even the compositions of different areas of pots to each other, though the compositions cannot be expected to be identical across different parts of the soil in a real environment.

Unexpected Results which Contributed to Error or Design Changes

There were a few small adjustments to the design based on unexpected issues that could have been done differently.

Once the pots were filled with potting mix and loam mix, after they were watered for a few days, the soil compacted to be about an inch shorter than it was initially (Error 37). As a result, the actual depth of the soil ended up being slightly lower than it was designed to be, likely putting it around 11 inches. Differences in how much soil was placed in the pots also led the first set of pots, including the potting mix non-trimmed grass, clover, and control, to be about half an inch deeper than the rest of the pots. This slightly altered the range of depth that the experiment could look at, and led to differences in depth across samples, furthering the need to use a unitless depth coordinate.

There was some unexpected growth in the loam mix pots and in some of the potting mix pots. The loam mix pots had multiple types of weeds, including a grass and a plant with spiky leaves, which likely originated from seeds that were in the loam when it was collected (Error 38). Most weeds were plucked out, though some weeds that were far away from the remaining sampling quadrants were left intact. Additionally, the potting soil control and clover had some very short green stuff growing on the surface of the soil, which was likely some sort of algae, which most likely was growing due to overwatering (Error 39). These each could have had their own effects on the carbon content of the soil, which could have been a confounding factor for measuring the impacts of the plants at the focus of the experiment.

In trying to plant the right amount of potting soil clover seeds, the first attempt involved placing far too many seeds. The second attempt was performed on the control pot, yet it too had too many seeds. Afterwards, both the clover pot and the control pot had the top few inches of potting mix removed and replaced with fresh potting mix. The third attempt at placing clover seeds was acceptable. However, despite removing the top few inches of potting mix, a few clover plants ended up sprouting in the control (Error 40). They were removed as soon as they were spotted. Given the plants were still freshly sprouting when they were removed, they likely did not impact the soil to any measurable degree. The unintended sprouts are also reasonably realistic, as the loam mix also had random weeds that sprouted in them.

When plants were first sprouting, there was some debris on the surface of the soil that was directly obstructing growth. While it may have been more realistic to allow the plants to struggle with the growth, any large debris was moved out of the way to ensure that it would not prevent plants from growing to make the most of the small sample size (Error 41). Additionally, some clover sprouts had some small debris stuck on top of them that was preventing the leaves from branching out (Error 42). This debris was also removed to err on the side of caution, as the number of clover plants was not very high, so losing a few of them could have significantly impacted the results.

There was a very large difference in the amount of growth between the non-trimmed potting mix grass and the other three grass pots; the non-trimmed potting mix grass grew tall and dense, whereas the other three grew much shorter and more sparse (Error 43). This is most likely because the non-trimmed potting mix grass was planted immediately after opening the bag of seeds, whereas the other pots were planted over a week after the bag was opened; some of the seeds may have incurred damage or died due to improper storage after the bag was opened. As there was such a large disparity in the height of the grasses, the grow-light was not raised over the course of the experiment, as doing so would give an advantage to plants that had already grown taller (Error 44). Additionally, the trimming of the trimmed grass pots was affected by the stunted growth of the grass, as both of the trimmed pots had this decrease in growth (Error 45). The grass seemed somewhat trampled, as it wasn't growing straight up, and was sort of tangled together, likely because of a lack of support from surrounding grass and from the force of the watering pushing the grass down (Error 46). The combination of the shorter growth height and the tangling meant that the grass took longer than expected to reach a trimmable height, and the tangling made it difficult to cut the grass to a consistent length. The grass was not trimmed until week 5.

When sieving the samples for bluegrass pots, the top samples had some bits of grass included in the samples, some of which ended up passing through the sieve (Error 47). This may have artificially increased the LOI for these samples, as this directly added organic matter into the samples. This grass likely composed less than 1% of the matter in the sample though, and there is no visible trend of the tops of grass samples having higher compositions during the later weeks, so it is likely that this had a minimal effect.

Inconsistencies in Procedures, and Missing or Altered Data

There were some bits of error that only affected specific data points or had different effects across different parts of the data. First, there were a few accidental mistakes when collecting data. For sample 15, the weight before drying in the oven was accidentally not measured, but this ultimately did not matter, as this value was no longer needed due to a change in the calculations (Error 48). For the week 4 loam mix grass untrimmed middle sample, the crucible was accidentally tipped over before its post-ignition mass could be weighed, leading to missing data (Error 49). For the week 6 potting soil samples, 4 samples got flipped over before ignition, with one of them, the control middle, losing the data point entirely (Error 50). The other three samples still had some mass leftover, so their data was still usable, those being the control bottom and top, and the clover bottom.

For the first set of samples, including all the week 2 samples as well as initial loam samples and 2:1 loam/potting mix samples, there was initially tape and other gunk on the outside of the crucibles. Whatever gunk that was easy to remove was cleaned off, but some could not be peeled off with bare hands. The tape and other gunk was found to burn off of the crucibles during igniting in the muffle furnace, which would lead to an overestimation of mass loss (Error 51). 9 of the samples were ignited in the muffle furnace without having the tape manually removed, while the rest of the samples had the tape removed using a razor before they were ignited for the first time. Those 9 samples were the potting mix clover and the initial loam and 2:1 loam/potting mix samples. As such, those samples were significantly affected by the mass loss of the tape and gunk, which could not be measured directly after the fact. To estimate the additional mass loss, 6 other samples were analyzed to see the mass difference of the crucible before and after removing the tape, as well as after being ignited after removing the tape, as shown in the first 6 rows of Table 2. The tape difference was found to be as much as nearly 0.07 g, which is very significant given the loss on ignition was on the order of 0.5-0.9 g. Given the amount of tape and gunk on each crucible varied significantly, it is unclear which of the 9 samples were most affected.

It is possible that there was additional mass loss than just from scraping off the tape as well. This is indicated by the difference in mass between the samples after tape removal and at the beginning of week 4. This also carried over into viewing some other crucibles that were used over the course of the experiment that were not weighed before having their tape removed, as shown in the last 6 samples in Table 2. Most samples had a mass loss below 0-0.02 g, indicating that some residual gunk could have contributed to additional mass loss. Although, a select few had unusually large mass loss, particularly 47-M and 30-O, and 23-J 2 to a lesser extent. The 30-O crucible, which for week 2 held the potting mix trimmed grass top sample, is by no means an outlier among the surrounding data, which makes it seem as though this crucible may have been mixed up with another crucible past week 2; there were multiple crucibles labeled 30-O, and it's possible that it was swapped. This may also have been the case for 47-M, which held the potting mix control middle sample; while this sample was a high outlier, the mass difference of over 0.4 g between the crucible during week 2 and week 4 would have resulted in a far greater outlier if that mass loss were to have occurred during the week 2 ignition. As an aside, it is worth noting that the differences between the week 4 and week 6 measurements may serve as insight into how much error there was in measuring sample mass, if no other confounding effects were influencing the variability.

Table 2: Change in crucible mass before and after tape loss, as well as at the beginning of each sampling day.

Crucible ID	Mass Before Tape Removal (g)	Mass After Tape Removal (g)	Mass at Week 4 (g)	Mass at Week 6 (g)
20-K	17.846	17.8427	17.8172	17.8168
47-M	18.2333	18.1646	17.7402	17.7443
32-L	18.4141	18.3695	18.3428	18.344
8-L	16.649	16.6364	16.621	16.6225
23-J 1	18.057	18.0606	18.0445	18.0447
30-O	17.401	17.3976	16.8745	16.8738
23-J 2	-	17.4278	17.3697	17.3593
23-J 3	-	17.8385	17.8423	17.8322
10-H	-	16.3678	16.3558	16.3565
no num red	-	14.3278	14.3206	14.3218
no dark text	-	18.2052	18.1965	18.1969
champion	-	14.6371	14.6206	14.6204

There were issues with the sample size differing significantly between samples. The data collected systematically resulted in the potting soil having lower sample sizes than the loam mix (Error 52). This is for two reasons: first, the potting soil had a lower density than the loam while still having the same volume, leading to lower mass, and second, the potting soil had more large debris, causing it to have higher mass loss after sieving. Given how the LOI method results in higher standard deviation for lower mass, this means that the potting mix would have had significantly higher uncertainty than the loam mix. Additionally, the sample size of the samples increased over each week of the experiment (Error 53). The week 4 samples had higher sample size than the week 2 samples because the procedure changed from using a 1-inch diameter pipe to a 1.5-inch diameter pipe for week 4 and onward. The week 6 samples had a higher sample size than the week 4 samples because more effort was put in to make sure as much soil from the targeted areas was collected, and the collected soil was compressed into the crucibles to fit more mass. As such, the uncertainty associated with the LOI method would have decreased significantly over time. Another inconsistency regarding the LOI method was that tray turning was used only for the 9 samples that still had tape on them during ignition as mentioned before (Error 54). However, given the large uncertainty of the mass loss from the tape for these same samples, the benefits of tray turning were negligible.

As mentioned in Error 53, the pipe shape was changed after week 2; in week 2, a pipe with 1-inch diameter was used, while in weeks 4 and 6, a pipe with 1.5-inch diameter was used (Error 55). The 1-inch diameter pipe likely had better success in separating the top, middle, and bottom of the samples, as the pushing rod fit better in the 1-inch diameter pipe, given the rod had a diameter of 1 inch, so there was less residual soil that needed to be scraped out, as described in Error 20. The 1-inch pipe needed to be swapped out because the rod stopped being able to fit in it, making it harder to push out the samples. The rod stopped fitting in the middle of finishing collecting the week 2 samples, and for the last few samples, the soil had to be scraped out using a spatula, which led to far worse separation (Error 56). The samples affected were the loam mix grass trimmed and the loam mix control. As such, the week 2 samples had more clear separation between the bottom, middle, and top than the other weeks except for the exceptions mentioned.

There was some inconsistency in the order in which the quadrants of the pots were sampled between the potting mix pots and the loam mix pots. As described in the methods, for both sets of samples, the top left was sampled in week 2, and the bottom left was sampled in week 4. However, this was not mirrored across the grow light; given that the plants were found to grow more when closer to the grow light, and given that the grow light was in the middle between the two pots, there was likely to be differences in growth across the four quadrants (Error 57). As such, for the loam, the bottom left is closer to the grow light, whereas for the potting mix, the top left is closer to the grow light. As such, if the degree of plant growth had a large impact on the carbon content in the soil, then this could have led to a minor confounding difference between the week 2 and week 4 measurements for the two soil types.

The final set of samples collected, including the week 6 loam mix pots as well as the week 6 potting soil trimmed grass, was not fully air-dried before sieving and drying in the oven (Error 58). This meant that the sieving process was different for this set of samples than the others; sieving and grinding may have been less effective for this set, as moisture leads to clumping. The clumping also meant that the different particle sizes of soil were not separated as much as the more air-dried sets of samples though, which may have minimized some of the error associated with Error 8. Most notably, the higher moisture meant that the total soil measurement included far more water mass, and that the drying oven removed far more water than the other sets of data, meaning that it could not be compared to the rest of the data using those metrics.

There were some inconsistencies with the loam collection and loam mix preparation that could have been more controlled. The loam was not all collected from the exact same spot and at the same time, and was mixed across multiple batches, all of which could have had slightly different compositions (Error 59). The loam was collected in two samples on two different days, as the first amount of loam was not enough to fill all the pots, and were both taken from the same general area. It was then mixed across three batches with the potting soil to form the 2:1 loam/potting mix soil as described in the methods. The first batch contained loam from the first sample, and was used to fill the trimmed grass and clover, and filled the bottom half of the control. The second batch contained loam from the first sample, and was used to fill the top half of the control. The third batch contained loam from the second sample, and was used to fill the non-trimmed grass. The loam from the second sample was used for the composition analysis.

As another consequence of the three separate batches, the non-trimmed grass was planted three days later than the rest of the loam plants, but was sampled on the same days as them. As a result, the non-trimmed grass was always three days behind the other plants in growth (Error 60). Additionally, due to the timing of the shipping of some pots, as well as the timing of obtaining the loam, the growth of the plants, and thus the sampling of the pots, was split into two groups that would each be sampled on alternating weeks; the first group contained the potting mix non-trimmed grass, clover, and control, while the second group contained the potting mix trimmed-grass and all the loam mix pots (Error 61). As such, any environmental changes that may have occurred, such as temperature, atmospheric carbon, or watering fluctuations, would have affected the two sets of plants at different times in their growth.

As mentioned in Error 17, the holes created by sampling soil from the pots were not filled back in. The first set of samples that were taken, including the week 2 potting mix non-trimmed grass, clover, and control, had the soil filled back in with potting mix after collecting, but no other samples did (Error 62). As such, the week 4 samples of these pots would not have been affected by the presence of a hole. Although, the potting mix that was placed in the hole may have had a different composition, as it would not have been subject to leaching, and this also could have affected the surrounding soil if there was lateral transport of dissolved organic matter, though this was likely not the case. This also highlights that the effects of the holes could not have affected any of the week 2 samples, as there were no holes to start out.

Fundamental Design Issues

Some aspects of the experiment's design limited the potential applicability of the results, regardless of experimental error.

A major issue with the experiment was the use of potting mix as a soil. Potting mix contains mainly organic matter, including compost, bark, and sphagnum peat moss. In having so much organic material, the carbon contributed by the plants in the experiment would be significantly hidden by the carbon already present in the soil (Error 63). All of the organic matter would serve as a carbon substrate for microbes, meaning that if any changes in carbon content occurred, it would likely be due to the decomposition of the potting mix, rather than any contributions of the plants. However, this issue is also countered by how potting mix, given it is designed for potting use and not in-ground use, is sterile, meaning that it would not have any microbes to begin with (Error 64). As such, no decomposition could occur, which means the entire stabilization process with microbial carbon pump could not occur. Even if there were microbes, the potting soil is also likely missing many of the minerals that are integral to forming stable complexes and occluding carbon from erosion and decomposition (Error 65). And, while the loam was an improvement given it was not sterile and it likely had necessary minerals, its high organic content, as well as the organic matter added by mixing it with potting mix, leads to the same issue of the current SOC taking precedence over the plants' contributions (Error 66). These issues severely limit the experiment's ability to view the stabilization of carbon in the soil.

Another major issue was that the soil was only monitored over 6 weeks of plant growth. The first issue with this is that the plants did not live through their entire lifecycle over this duration, meaning the impacts of the later stages of the plants, such as the flowering and seed-bearing, were not seen (Error 67). More significantly, this means that the plants, besides the trimmed grass, did not contribute any litter to the soil, and litter is considered the primary source of soil organic matter (Error 68) (Adamczyk, B., 2020). There was only time to trim the grass once, so the impacts of the trimming could not be analyzed. Most importantly of all, since the timescale was so short, it was impossible to view stabilization, as it is inherently a process that occurs over the long-term, with certain stabilization mechanisms having turnover rates from decades to centuries in length (Error 69).

Even if the experiment did have a long enough time scale to view stabilization, the LOI method still would have limited the ability to view stabilization and carbon composition. Given the LOI method only measures organic matter content, it treats all organic matter equally, regardless of what form it is in (Error 70). As such, it can only measure stability by viewing the carbon content over time. However, given the LOI method is destructive of the soil, it also is not very effective at this; any given bit of soil can only be measured once, so using it to view change over time requires sampling from a general location having nearly uniform conditions over a long period of time, which introduces significant error given the land is not likely to be uniform in practice (Error 71).

The experiment also was not designed with some complexities of carbon stabilization in mind, though these are not as relevant given the limitations mentioned previously. Microbial carbon use efficiency is affected significantly by temperature, as it decreases with increasing temperature, meaning that more of the organic matter is respired as CO_2 at higher temperatures (Frey, S. D. et al., 2013). The temperature of the soil was not considered, as the pots were simply placed in a room that may have had significant fluctuations in temperature that were not recorded (Error 72). Another complexity is that storage of carbon in soil has two main distinctions of SOC for the purposes of stabilizing carbon through land management practices: mineral associated organic matter (MAOM), and particulate organic matter (POM). This distinction is important because MAOM has an upper limit to the amount that can be stored in soil, whereas POM does not seem to have a limit to the amount which can be stored. As such, different strategies are used based on how much MAOM content is already in the soil (Moukanni, N., 2022). This experiment did not measure the MAOM content in the loam to account for this (Error 73).

The experiment also suffered due to the use of pots rather than performing the experiment in the ground, as this cuts off the ability to view a few processes. Given the pot was only 1 foot deep, the experiment could only emulate topsoil, not subsoil (Error 74). Subsoil has different mechanisms for stabilization than topsoil given it receives less organic matter and it is more shielded from environmental impacts. The short depth also limited the ability to view lateral transport, as it was simplified into water draining out of the bottom of the pot.

Analysis of Carbon Sequestration

Possible Mechanisms of Carbon Storage in the Experiment

Plenty of the errors eliminated possible mechanisms for carbon to be added to the soil and stabilized. Given Error 68, no litter was added to the soil by the bluegrass and clover besides a small amount of grass trimmings near the end of the experiment. Though, there was still some organic matter that could have been affected by various mechanisms in the form of the initial organic matter of the potting soil and loam, as mentioned in Errors 63 and 66. While the effects stabilization could not have been present or analyzed in the experiment given Errors 69 and 70, it is possible that two mechanisms still had an effect on the SOC: leaching of dissolved organic carbon (DOC), and respiration due to microbes. Given Error 64, respiration likely did not occur for the potting mix pots, and given Error 72, the amount of respiration is not clear from the experiment. While there was no litter, it is still possible that the plants had rhizodeposition that could have collected in the soil and then increased the organic matter content in the soil. Though, rhizodeposition takes the form of DOC, meaning it could have just as easily been leached out of the soil (Panchal, P. et al, 2022; Moukanni, N. et al, 2022).

Change in SOC over time

Figures 2 and 3 show the change in the average SOC across the entire depth of the soil for each pot over time. Figure 2 shows for the potting mix, and Figure 3 shows for the loam mix. Both graphs indicate that there was an apparent trend of the SOC decreasing over the course of the experiment, though this trend is not the same for the potting mix and the loam. The potting mix shows a trend of decreasing over the course of each week, whereas the loam mix shows a decrease from week 2 to week 4 and then an increase from week 4 to week 6. The steady decrease in SOC of the potting mix lines up with the expected mechanism in which some DOC may have been lost due to leaching, though the increase in SOC of the loam mix does not seem to line up with expected mechanisms; the only expected mechanism for an increase was a possible effect of rhizodeposition, but this appears unlikely given that the potting mix was also susceptible to having rhizodeposition, and it didn't show this trend. It may be possible that the rhizodeposition in the potting mix was leached away, whereas the minerals in the loam prevented the rhizodeposition from leaching (Panchal, P. et al, 2022; Moukanni, N. et al, 2022). Another possible explanation is that the sampling method differed from a confounding factor on the day that the week 4 loam mix samples were taken, but this is unlikely given that the potting mix trimmed grass week 4 samples were also taken on this day, and they did not exhibit this trend.

It's possible that the trends visible are entirely due to confounding or random error. The error bars on the graphs include the quantitative errors that contributed most directly to error in measurements, and thus are those which had the most effect on the trends. The errors used were Errors 9-14 and Error 51. Errors 1-8 were most likely to affect all the data in a similar way, meaning they would not likely affect the trends, so they were excluded, though they would affect the accuracy of comparing the SOC to other experiments. The week 2 potting mix samples were heavily affected by Error 51, whereas the loam mix samples were affected by it to a lesser extent. As such, it is within the uncertainty for the week 2 potting mix samples to not have had much higher SOC than the week 4 samples. Additionally, based on Error 53, the variation in LOI decreased as time went on, so it's possible that the week 2 and week 4 samples varied as they did largely due to this, though this does not explain the consistency in the trends across all the plants. The increase in SOC from week 4 to week 6 of the loam may also have been due to Error 58, which only affected those week 6 samples; it's possible that some aspect of this error was overlooked and may have caused this result.

If the trends were in fact not due to random or confounding error, then they may be indicative of each plant's ability to protect the soil from erosion and or leaching. It is noteworthy that for both the potting mix and the loam, the control pots had the largest decreases in SOC over the course of the experiment, as they would be the pots least protected from erosion. For the potting mix, the difference in SOC loss between the non-trimmed grass and the trimmed grass may be given by how the non-trimmed grass grew significantly more than the non-trimmed grass, as mentioned in Error 43, and as such, it would have done a better job at protecting against erosion, which lines up with how the non-trimmed lost significantly less SOC. Given how many sources of error there were that didn't have well-measured effects, such as many of the subjective design errors, it is possible that some of the errors are having a confounding effect that is difficult to trace, so not much confidence is placed on these observations.

One trend that is likely due to confounding factors is that the non-trimmed loam mix grass had significantly lower SOC than the rest of the loam pots. This is likely directly a result of Error 59, as the different batch of loam used for that individual pot was likely to have some variation in composition from the other pots which used a different batch.

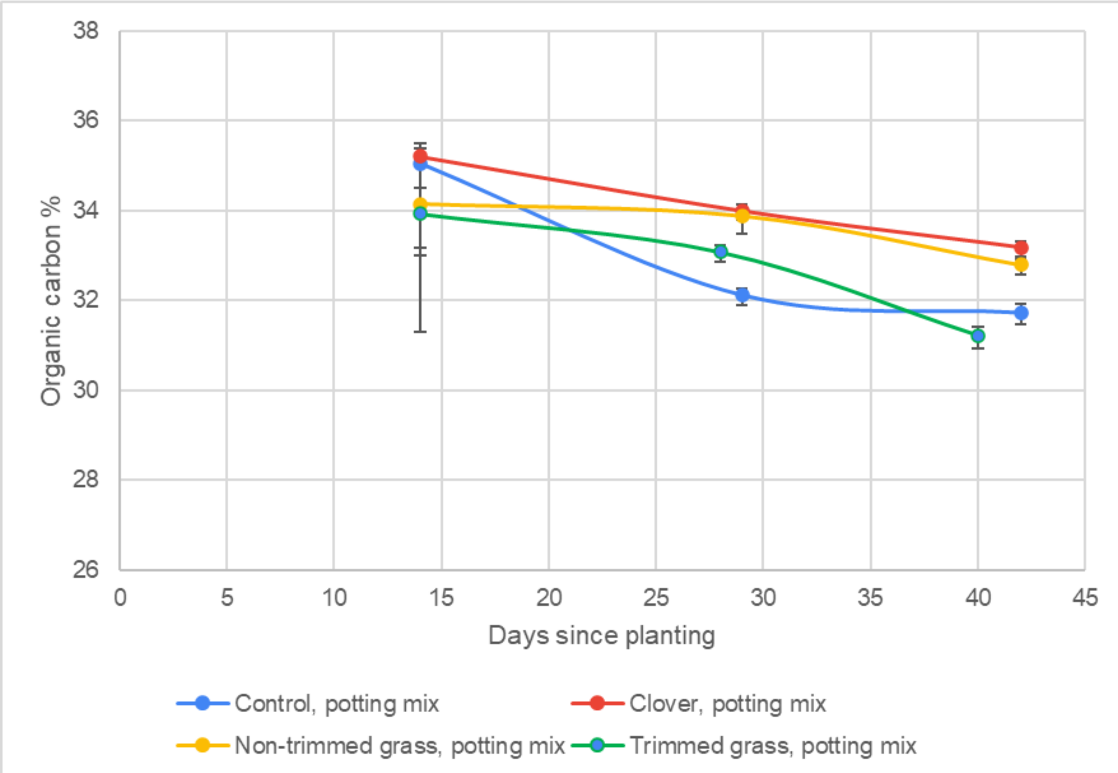

Figure 2: Change in average SOC over time for potting mix.

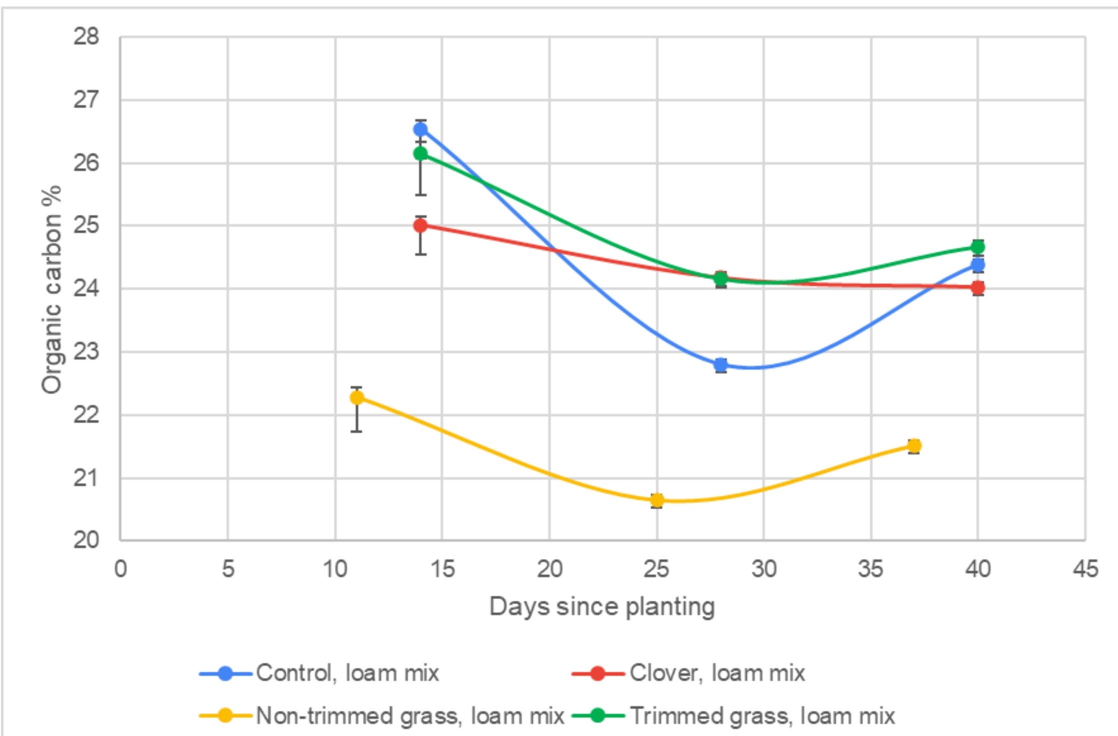

Figure 3: Change in average SOC over time for loam mix.

Change in SOC at different depths

Figures 4 and 5 show the change in SOC for each depth of soil for each pot between week 2 and week 6 of the experiment. Figure 4 shows the potting mix pots and Figure 5 shows the loam mix pots. The changes are additive percentages, as the SOC is in units of percent. The unitless depth coordinate refers to the depth along the soil that was sampled, but not necessarily over the depth of the pot, as Error 19 suggests that the entire depth of the pot was likely not represented by the samples. For the coordinate, values closer to 1 indicate being closer to the bottom of the pot, and values closer to 0 indicate being closer to the top. The coordinate values indicate the approximate average depth that the samples were intended to represent, though in reality they are subject to significant error.

The main trend visible with the graphs is that they support the previous trend that the SOC seemed to decrease over time, and these graphs particularly indicate that this was likely true across all sampled depths of the soil. There are only two points that indicated that a depth had an increase in SOC over time, though it is within the error that others also had an increase. There does not appear to be much consistency in the trends over the depth of the soil across either of the graphs. Based on the sporadic trends, one of two conclusions is likely true: either there are consistent explanatory trends in reality but they were hidden away by the error and design limitations, or there are no consistent trends in reality, regardless of how much error there is.

The sporadic trends may be due to the error in the sampling procedure for the soil affecting how well the bottom, middle, and top sections were actually sectioned off. Errors 20 through 24 are indicative that the three zones may have a lot of overlap and blending at the borders, meaning they are not likely to be very representative of the exact depths. The sporadic nature also may be due to how each bit of soil sampled was not necessarily likely to have the same composition across different parts of the pots, due to soil nonuniformity as mentioned in Errors 35, 36, and 71, as well as due to nonuniformity in growth of the plants throughout the pot, as mentioned in Errors 18 and 25. There is also a high likelihood that the high quantitative error made the measurements themselves unreliable, as evidenced by how large the error bars are for each sample. The error bars in Figures 4 and 5 are based on the same error as those in Figures 2 and 3.

It is also a possible but unlikely conclusion that the error isn’t actually very significantly affecting the trends, and that the true trends are actually as sporadic as they appear due to some mechanisms of the plants and soil. Given the trimmed and non-trimmed grass each have similar trends to each other in both Figure 2 and Figure 3, it’s possible that something about the mechanisms of each plant and soil type may be affecting the results to allow this similarity to emerge. Though it is also entirely possible, and even likely, that this is a coincidence among the chaos.

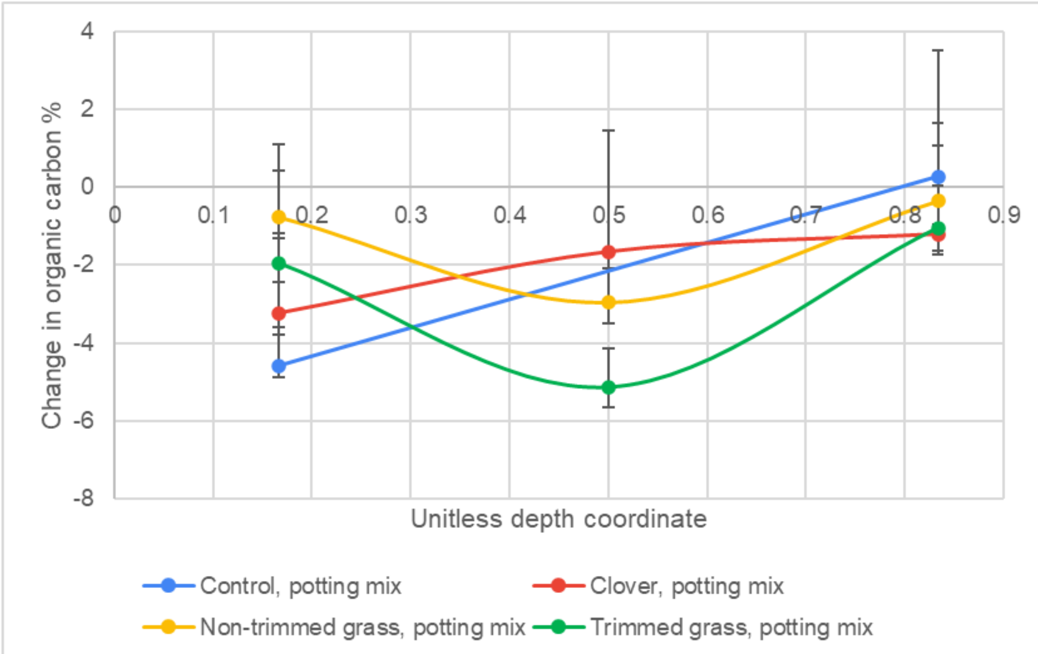

Figure 4: Change in organic matter over time for each depth of each pot for potting mix.

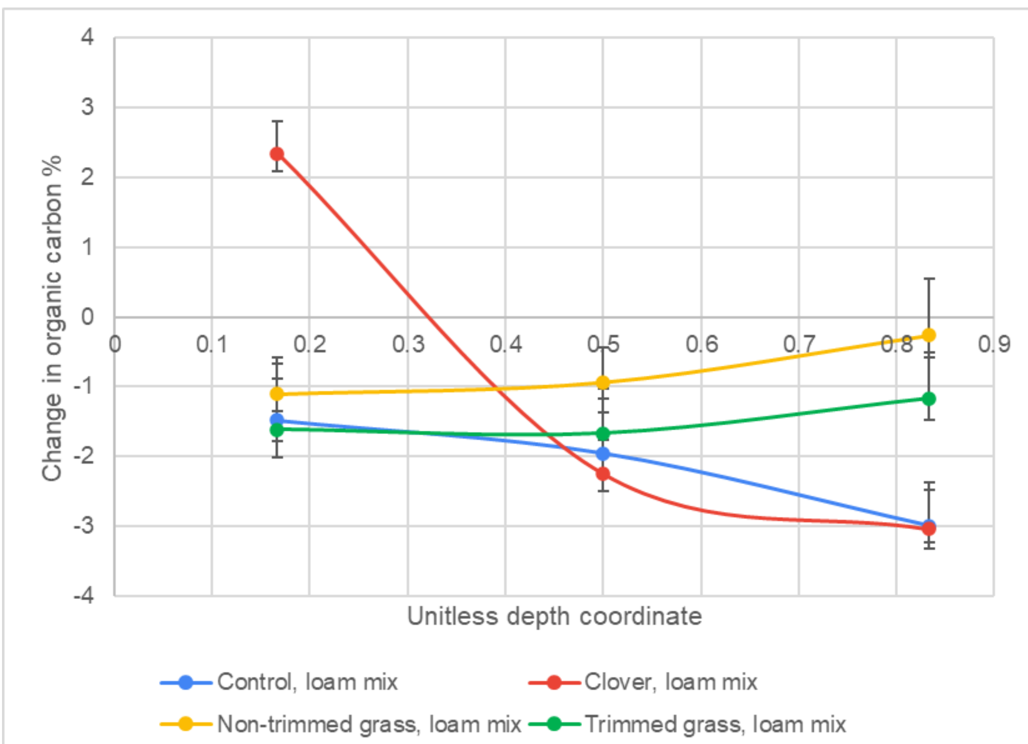

Figure 5: Change in organic matter over time for each depth of each pot for loam mix.

Conclusion

Due to the various errors and fundamental limitations of the experiment, the results of the experiment did not give much indication as to which lawn-cover is more effective for stabilizing carbon in the soil; the data was not accurate enough, and the trends were not reliable enough, to determine if there was a significant difference between how the two plants affected the soil. There may be some merit to some overall trends that the SOC may have decreased over time, possibly due to mechanisms such as leaching and microbial respiration. There may also be some merit to the trend that the amount of SOC loss over time was seemingly connected to the amount of protection to erosion that the plant growth provided.

Given the generally poor quality of the results, if the potential results of this kind of experiment are to be important, then lots of alterations to the experiment would need to be made for it to give more usable results. The most obvious changes would be to account for the objective design errors and to counteract the major limitations by increasing the time scale of the experiment and using loam soils that are more controlled and do not have a high organic content to start out with. There are also obvious changes to broaden the experiment out to view more species of plants, especially to get a better idea of whether some plants are able to stabilize carbon with more resource efficiency than others. The most significant design change needed would likely be to use a different method for measuring SOC, or to use a method that more directly measures the stability of carbon in the soil, as LOI has inherent flaws when trying to scale it up to longer durations and sample quantities, given it is a destructive method. Future experimentation should also be designed with more awareness of the complexities of soil fertility, microbial activity, and the limits of MAOM storage. There is also another layer of complexity in that using combinations of different plants for lawns may lead to better results.

Also, this experiment's approach of aiming to loosen control wherever possible to try to replicate a real environment may not have been to its benefit, as it existed in a murky combination of control and realism that prevented it from representing either extreme well. Future experimentation may benefit from sticking either to measuring trends in a controlled way or viewing the net effect of emulating the environment.

Once the capabilities of the plants are measured thoroughly, the next step to implementing the findings for offsetting carbon emissions would be to devise ways to apply the findings to lawn practices, may that involve transitioning lawns to use ground-covers that are more effective at stabilizing carbon, and performing life-cycle analyses to evaluate whether certain applications could be beneficial in the long term. Further complexity will come with convincing consumers to transition their lawns, assuming the applications prove successful.

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Appendices

See attached pdf for raw data.