

Plant Bioassay Testing Soil Quality Following Carrion Insect Activity

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Abstract

The decomposition of animal carrion, particularly small animals that are used as a breeding resource for carrion insects, is little studied but is likely to be a major component of nutrient cycling in many ecosystems. This project focuses on how carrion insects affect small vertebrate carrion decomposition and what nutrients are put back into the soil. My research question is: How nutritious does soil become after carrion insect activity, and how can this be observed? Using three different types of carrion insects (two species of beetles and the common flies found here at RMBL), and carrion sources I plan to collect impacted soil and allow small vertebrate carrion decomposition and carrion insect activity to occur on the soil. I aim to compare how carcass visitation and consumption by three different types of carrion insects affects soil nutrients and will measure this nutrient addition through a plant growth bioassay. The findings may be applied to techniques for restoring impacted soils.

Introduction

Carrion is an essential source of nutrients in any ecosystem, providing many forms of life much needed nitrogen, phosphorus, calcium, and many micronutrients (Benbow et al., 2016). Carrion decomposition that occurs in one location creates nutrient hotspots- “islands of fertility”- that can remain active in the soil years after the death of the organism (Danell and Brathen, 2002). The impact large vertebrate carrion has on soil and various animals from different trophic levels has been well-studied (see Barton, 2016), but there are still knowledge gaps of what is cycled through ecosystems and how various invertebrates, especially insects that breed on small animals carcasses,

aid the cycling process (Barton et al. 2013). Another important aspect of carrion is how it can impact ecosystems, providing food sources not only for vertebrate scavengers but for invertebrates, both expected insects (i.e. flies) and unexpected insects (i.e. honey bees) (van Klink et al., 2020). The consumption of carrion also impact plant communities, with the leakage of body fluids transporting nutrients to the soil (van Klink, 2020), allowing plants to take advantage of the carrion.

Insects play an important role in the decomposition of large and small deceased vertebrate organisms by aiding decomposition (van Klink, 2020), yet it is poorly understood which insects benefit the most from carrion sources and how the nutrients are moved by insects (Ilardi et al., 2021). It is also poorly understood what effects carrion sources have on its ecosystem past obligate scavengers and how many trophic pathways carrion sources can impact (Klink et al. 2020). Of particular interest is the link between carrion decomposition and nutrient uptake by plants. As mentioned in Barton (2013), the knowledge of obligate carrion insects is poorly understood, and the nutrient content of their main food source, primarily small rodent carcasses, is poorly understood as well.

There are different ways to determine the nutrient levels and plant nutrient uptake in soils, one being a bioassay. A bioassay is conducted by using an organism to determine what chemicals or nutrients are present. For example, a growing plant can be used to determine if there are chemicals present that affect growth by measuring root and leaf growth, mass, and comparing plants grown in the soil in question with known soils (Neal, 2021). A bioassay can also be used to determine what nutrients are available to aid plant growth, by comparing plants thought to be impacted, low nutrient areas

with plants with access to more nutrients. A method to compare plants' access to nutrients is by determining the root weight fraction (RWF), where the dry root weight is divided by the overall dry plant weight (Crawley, 1986). A high RWF value indicates poor soil, as it means that the plant needed to grow more roots to find more nutrients. A low RWF value indicates access to good nutrients, as plants allocated less growth to roots due to access to nutrients soil. It is important to understand the impact of nutrients that originate from carrion sources due to how understudied plant responses is to carrion (Klink), especially small carrion sources like rodents.

Methods

Study organisms

The following insects were included in my study: *Nicrophorus investigator* and *Nicrophorus defodiens* (Subfamily Nicrophorinae: burying beetle), *Thanatophilus lapponicus* (Sub-family Silphinae, and the common species of blowflies or bottleflies (Family Calliphoridae, blue and green bottleflies). In order to obtain the beetles chicken can traps were set up around Gothic, two by the Gothic Research Center and 8 by Ore House and Pelton cabin. Due to late emergence the insects were not set into their container until 20 *N. investigators* (10 females, 10 males) were collected and placed in breeding pairs due to them being the last specimen to emerge. Containers with *N. investigator* and *N. defodien* will have one breeding pair in each container while *T. lapponica* will have two breeding pairs in each container, as in the wild *Nicophorus* beetles defend their carcass from other insects while *T. lapponica* breeds alongside others in its species and other insects. The carcass source was a 18-23 gram *Peromyscus maniculatus* (Deer Mouse) salvaged from RMBL facilities.

Soil source and decomposition treatments:

Soil was collected near the Visitor Center due to it being impacted by human activity, such as renovation and human traffic.

These are the treatments for the soil and carcass decomposition samples, replicated 10 times.

Soil only	Soil + Carcass	Soil + Carcass + Body Fluids	Soil +carcass +flesh flies	Soil + Carcass+ <i>T.lapponica</i>	Soil + Carcass + <i>N. investigator</i>	Soil + Carcass+ <i>N. defodan</i>
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The first soil treatment will had no manipulation to it and placed in a container similar to the other containers. The 2nd treatment is soil with just a carrion source (one dead *Peromyscus*, 18-23 g) laid on top of soil and allowed to decompose without the presence of any carrion insect, but it will have access to air and sunlight (two of the containers became unusable due to small flies species getting in and caused more decomposition). The 3rd treatment aimed to replicate the possible effects of *T. lapponica* observed last year. Based on the paper by van Kink (2020), a good amount of nutrients is transferred into the soil via body fluids. So, body fluids were released from the mouse

to see the impact it would have, without any insects. The 4th treatment is a carrion source with the addition of flies, with a lid with holes that will allow flies to come and go. The 5th treatment included two breeding pairs of *T. lapponica*. The 6th and 7th treatments included *Nicrophorus investigator* and *N. defodiens*, and each container had one breeding pair. After two weeks the *Nicrophorus* beetles, the insects that took the longest to raise their brood, completed raising their brood. After that soil was mixed around and collected the second part of my project began; the second bioassay.

Soil fertility bio-assay (plant growth):

Two bioassays were conducted this year, the first used fertilizer to detect the differences of root mass and architecture based on nutrient access and second used to test the fertility of the soil that had the different carrion treatments. There were 5 manipulations: 0% of fertilizer added to the soil, 25% of fertilizer ratio added to the soil, 50% of fertilizer ratio added to the soil, and 100% of fertilizer ratio added to the soil. Before any fertilizer was added to the soil oat seedlings were soaked for two days and plated, being watered with distilled water, enough to keep soil moist without dripping out of the pots, preventing the leaching the nutrients. When they sprouted, fertilizer treatments was added. Once two weeks have passed, the plants were harvested, root architecture will be noted by taking pictures, and the root weight fraction was determined after drying.

After the insects completed their reproduction (adults have left, larvae have completed development and moved to pre-pupal stage), the larvae were temporarily removed and the soil mixed. Two soil samples will be taken from each replicate

treatment for a total of ~120 pots. The pre-pupae and larvae will be returned to the container to complete pupation. Each soil plug received one soaked oat seed. The plants will receive daily waterings of distilled water.

Results

Labeling

Note that the numbers and letters given to the treatments in the carrion bioassay does not indicate something special with the container but to keep track of the breeding pairs and if a container needed to be pulled due to invasion or another reason that would impact the experiment.

Pulled Data

Data was pulled from treatments that were impacted over the course of the insects breeding. Two containers from the Soil + Carrion treatment were removed due to flies invading the containers, essentially turning the treatment into a Soil + Carrion + Flies. Other pulled data was due to seeds not growing after being planted.

Fertilizer Bioassay

One-way ANOVAS were ran on plant mass and root weight fraction. The analysis of the weight (g) of the plants indicates that the high fertilizer treatment (50% and 100%) grew more than the plants in the no and low fertilizer treatment (0% and 25%). While there was a difference, there was no significant difference. As for the analysis of the root weight fraction, there was significance between the data of the different treatments, but not for the expected treatments. The samples treated with 25% strength

fertilizer had the highest RWF value, indicating it had the poorest soil, while the control had a low RWF value, indicating it had good soil.

Carrion Bioassay (1st Harvest Group)

As predicted based on last year's data, *Thanaphilus lapponica* had the lowest root weight fraction value out of all of the treatments and was significantly lower than the control (plain soil). While the other treatments had lower root weight fraction values, there was no significance and high variance between the groups. A second group of plant samples was allowed to grow for an extra five days, in order to see if the seed nutrients was what caused the variance and the seeds from the first harvest were not given enough time to use up the seed nutrients. A soil test was conducted for this bioassay, which showed the carrion treatments put in more phosphorus and potassium than the control.

Carrion Bioassay (2nd Harvest Group)

Harvesting of the second group was delayed by five days to see the impact of time on a bioassay. Similar to the fertilizer bioassay, the RWF values was not what was expected. The soil only treatment did not have the highest RWF value, and the values were different compared to the first harvest group. However, the wet shoot, length, and total lengths were collected and analyzed, and showed that carrion did impact plant growth. The soil only treatment grew the least, while obvious differences were seen between the carrion insects. The values of the carrion insects changed as well, with flies still being one of the lowest RWF values and saw the highest plant growth, but *N. investigator* also had the other lowest RWF value and high plant growth. The sprouting

of a second leaf was also noted, with the carrion treated plants growing more leaves than the control.

Discussion

Bioassays are an effective way to determine the presence of nutrients in the soil, and the experiment conducted this year expanded on the significance of using such a test. As mentioned, body fluids transport nutrients to the soil when the carcass is consumed. However, this can differ based on who is consuming the carcass, as seen with the different behaviors of the insects used in the study. While the *Nicrophorus* beetles bury the carrion, they do not tear into the carcass like flies and *Thantaphilos lapponica*, which I wanted to compare. And as expected, *lapponica* affected plant growth, but so did the flies, which was not expected. The soil collection was also different for this year, with it being collected from an area near the visitor center rather than behind the GRC.

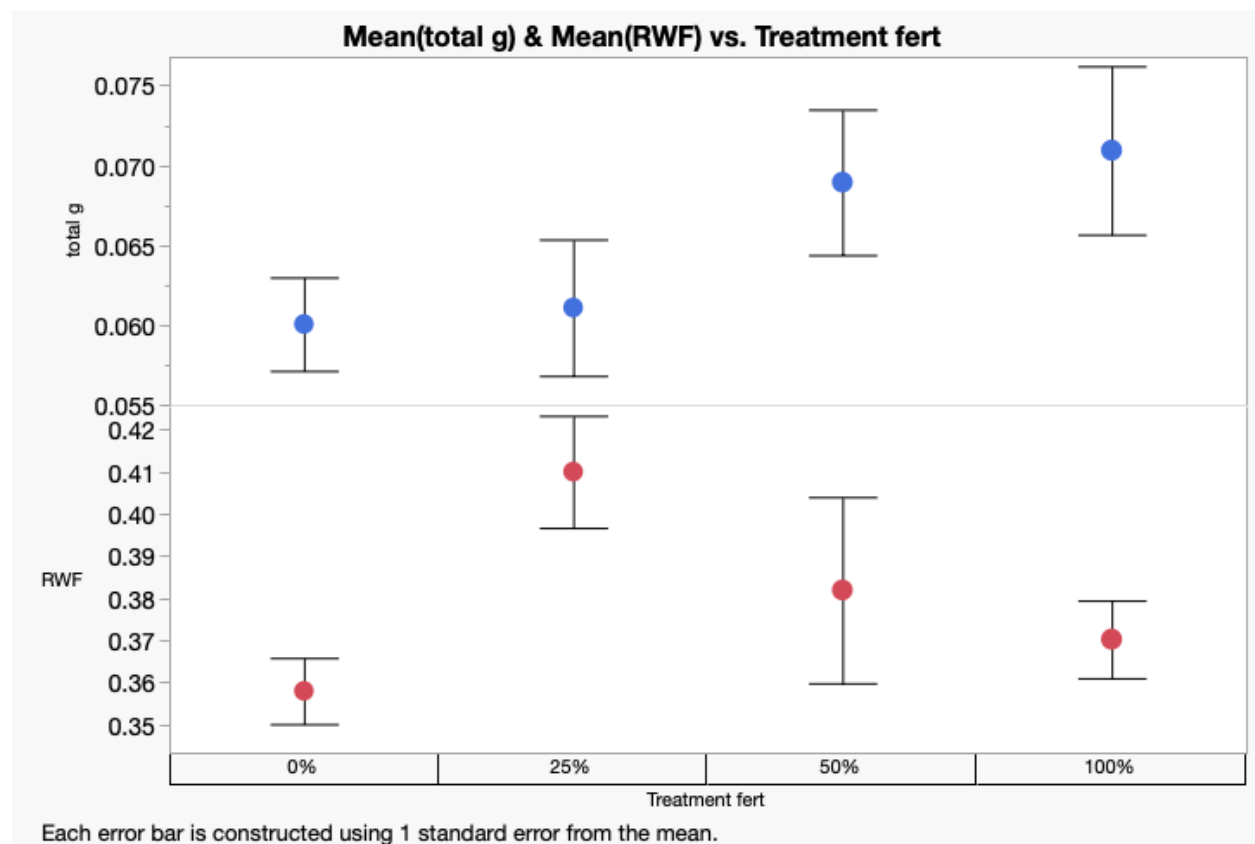
The analyses on the fertilizer bioassay indicated differences in nutrient quality that was not expected. While the samples from the fertilized treatments (25%, 50%, and 100%) produced results that were expected, the no fertilizer treatment produced a low RWF value as well. This could be due to accidentally giving the control a dosage of fertilizer or the watering schedule was not enough for the fertilizer treatments. Another issue that was realized was that the fertilizer was for gardening, which would put focus on growing the shoot rather than the whole plant. Another thing that would be better to do is to treat the soil with the fertilizer before the planting of the seeds to provide the plant the nutrients.

As for the carrion bioassay, the experiment this year gave more information on what we can gain from bioassays. While the soil only treatment did not give a high RWF value, the overall plant growth was stunted, while the other carrion treatments saw much more plant growth. While sometimes the RWF value does show significance, looking at the overall plant growth is important to understand the overall impact carrion nutrients have on plants. For this experiment the harvesting was split into two different groups, a early harvest and a late harvest. This was done to see how time affects the growth and viability of nutrients. In order to further understand the impacts of time the same soil used in the project were placed in Dr. Rosemary Smith's overwinter pits and a new bioassay will be conducted on the soil next year to see the nutrient availability.

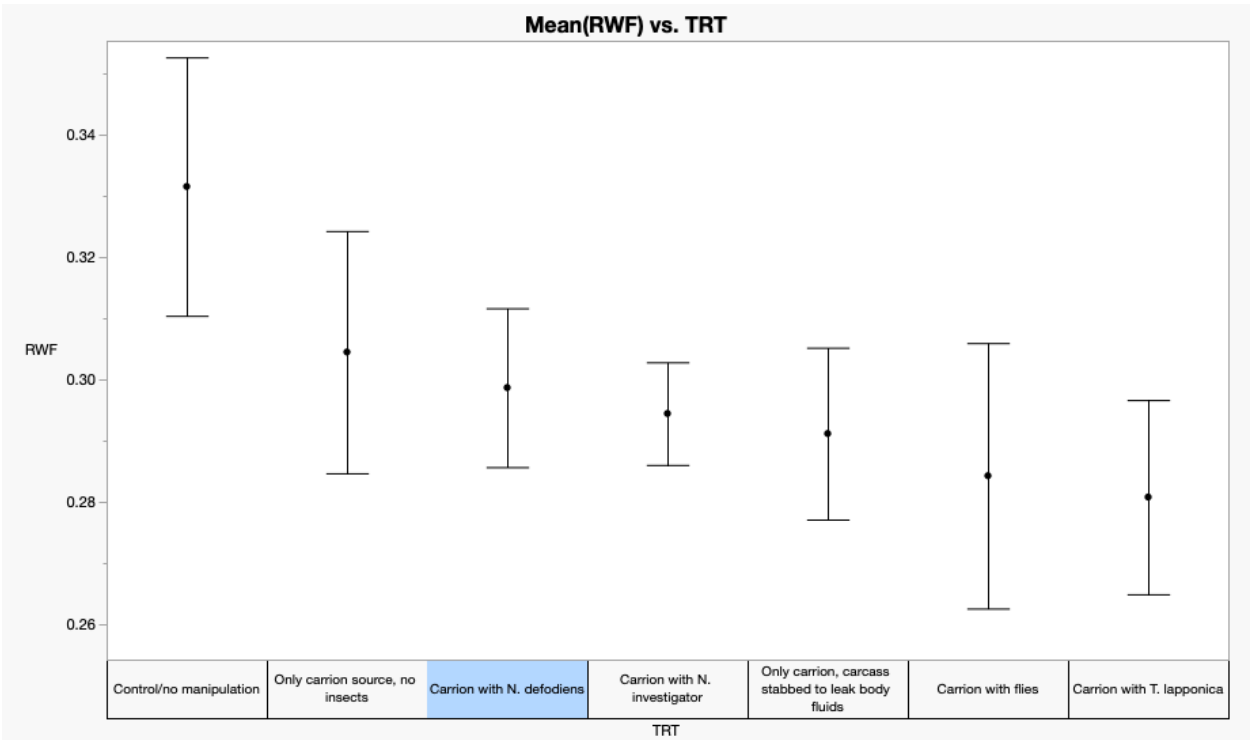
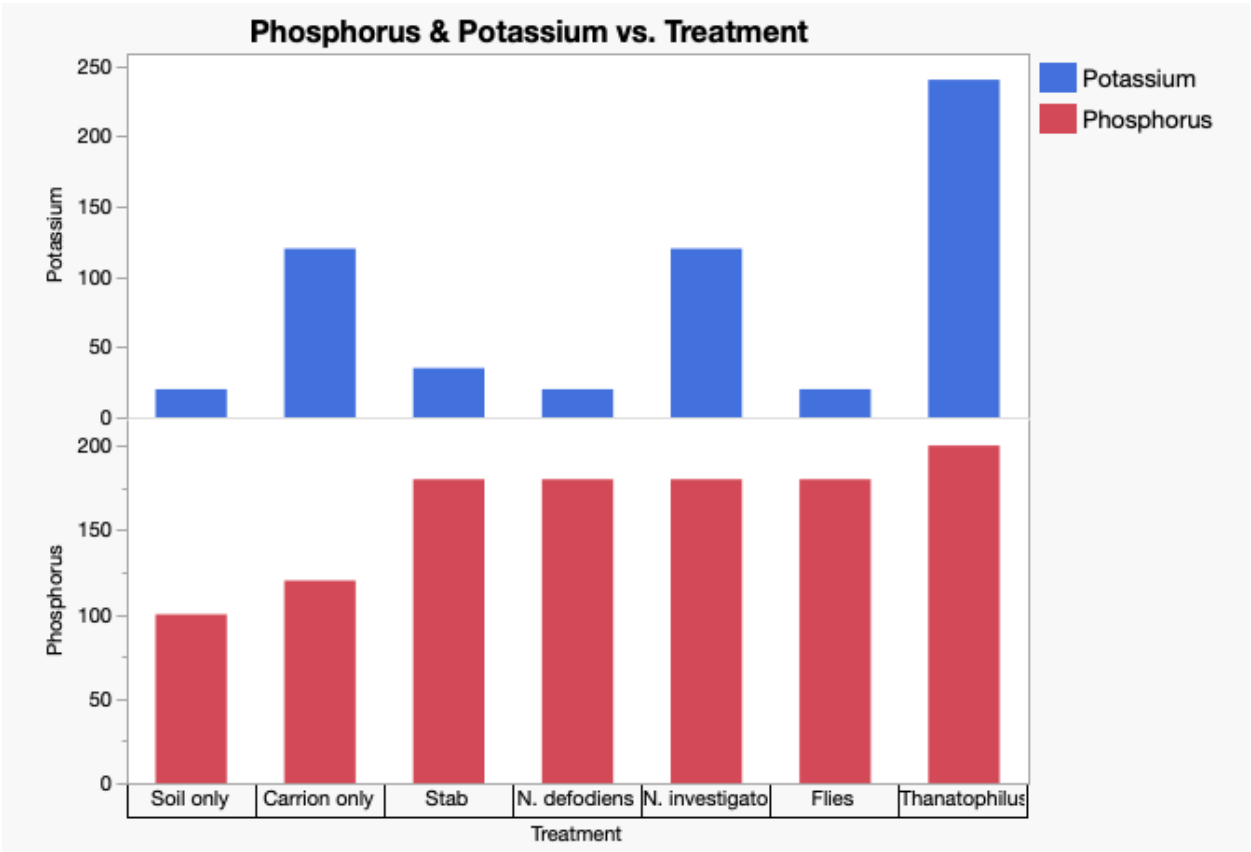
Understanding how carrion impacts plants is important, but so is understanding how plants respond to nutrient spots and how long these spots last. While the impacts of nutrient spots of larger vertebrates is studied, the same cannot be said for smaller carrion sources, and for how long they provide nutrients. Conducting this bioassay will be able to see how long the nutrients stay in the soil, and if they will be similar to the data collected this year.

Graphs and Data

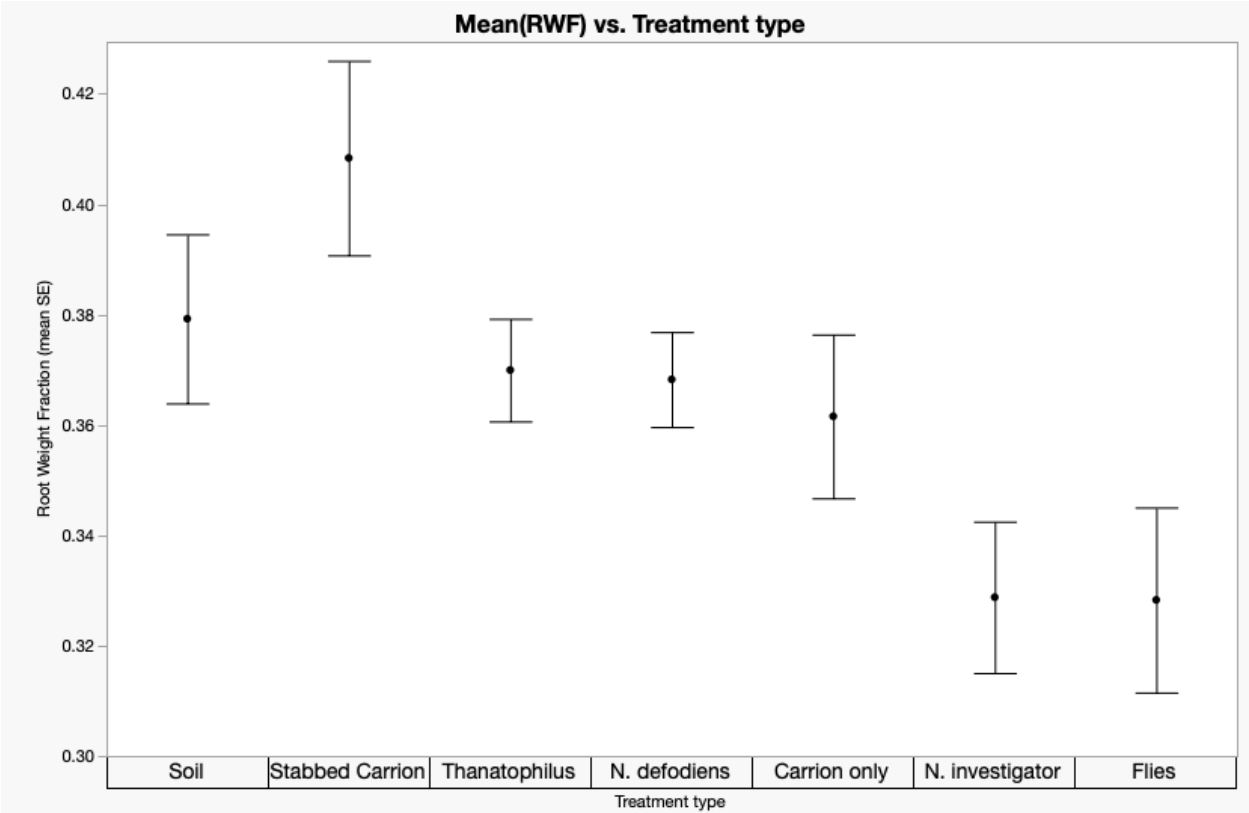
Data from fertilizer bioassay



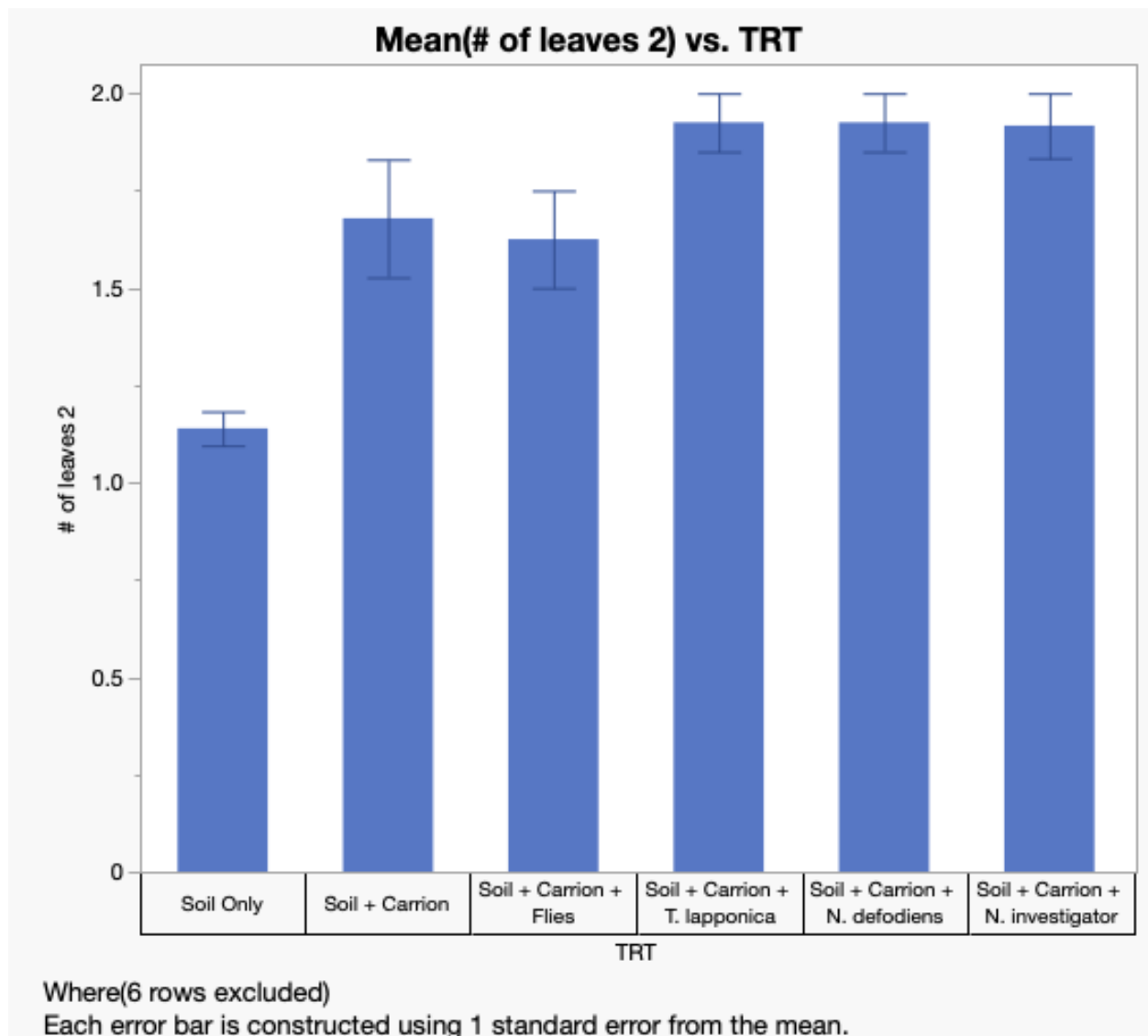
Data from the first harvest group

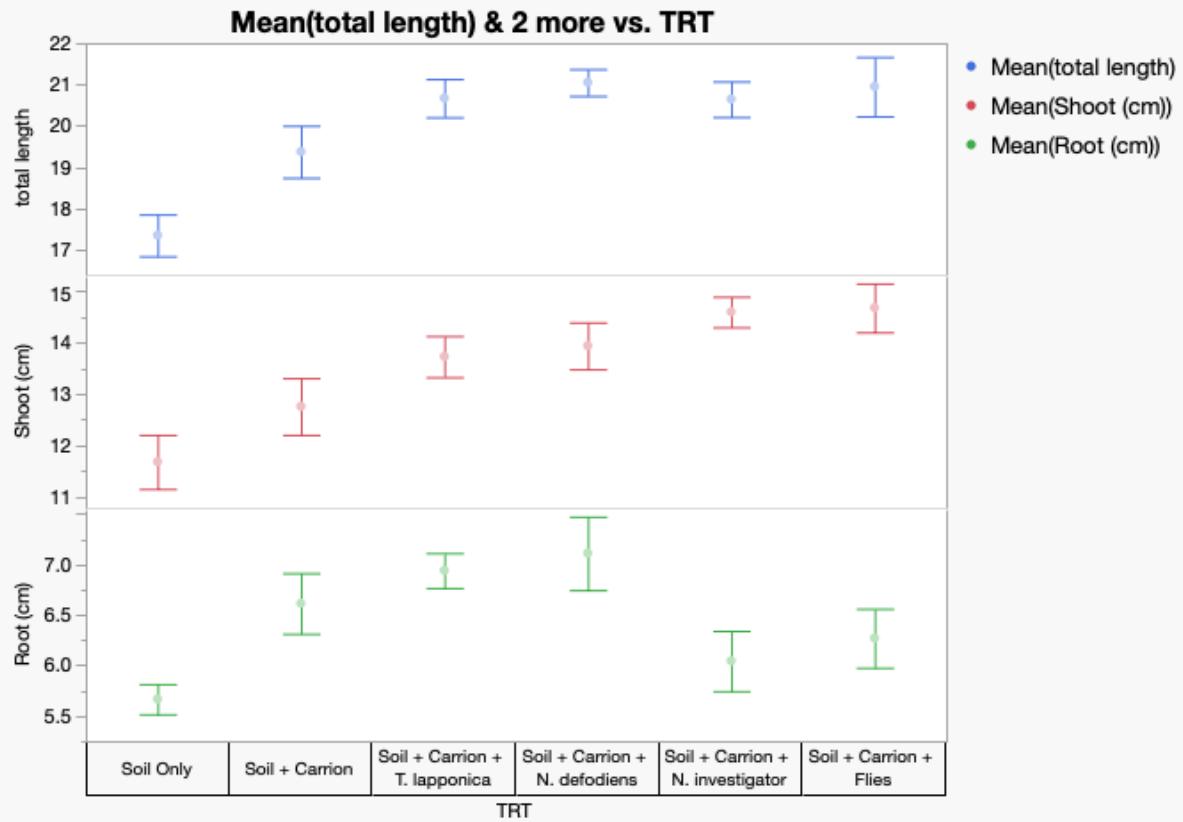


Each error bar is constructed using 1 standard error from the mean.



Data from the second bioassay\





Where(6 rows excluded)

Each error bar is constructed using 1 standard error from the mean.

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