

## **Determining Carbon Fractions in Composts of Differing Chemical Compositions with Hot Water Extraction**

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### **ABSTRACT**

The amount of labile versus persistent carbon present in compost indicates how much carbon can be sequestered with its application to soil. Labile carbon is carbon that is more readily available, making it an important resource for plants and microbes. More persistent carbon, is the carbon that is more stable, it is more difficult for microbes to access and more likely to stay stored in soil. Compost is decayed organic material used as an organic fertilizer, for plant growth and soil health, and contains labile carbon. I determined the pH, and total carbon and nitrogen of Hi-Test Compost, Nicasio Compost, and VermiCompost. Hot water extraction of peat material, similar to compost, is a fractionation method to determine labile soil organic material in organic soils. The method I used is optimal in terms of yield of organic carbon extracted, without altering the chemical structure of the organic matter or having a low extract efficiency. With hot water extraction I determined the amount of labile and more stable carbon present in the three compost varieties. Through this study I explored the effect of a compost's chemical composition on labile carbon content. I determined that with a more ideal C:N ratio for microbial diet, Hi-Test and Nicasio had the largest C:N ratio and largest amounts of labile carbon, 62.79% and 49.41% more labile carbon than VermiCompost, respectively.

### **KEYWORDS**

Labile Carbon, Hot Water Extraction, Compost, Persistent Carbon, Nitrogen, Sequestration, Carbon Storage

## INTRODUCTION

Soils play a vital role in the functioning of terrestrial ecosystems. Achieving the goal of climate change mitigation requires employment of negative emission technologies. Often overlooked is soil's capacity to mitigate greenhouse gas emissions (Paustain et al. 2016). Sequestering organic carbon in soil has the potential to remove between 0.79 and 1.54 Gt C yr<sup>-1</sup> from the atmosphere (Amelung et al. 2020). For soils to be healthy, they require soil organic material (SOM) as it retains water, soil nutrients, and supports soil structure (Jackson et al 2017, Martínez-Blanco et al. 2013). To enhance resilience to climate change and mitigate its effects, it is crucial to maintain or increase SOM content (Kutos et al. 2023). Organic matter (OM) improves soil characteristics such as soil structure by enhancing aggregation and stability (Diacono and Montemurro 2010, Martínez-Blanco et al. 2013, Li and Evanlyo 2013, Wang et al. 2022). The stability of soil aggregates contributes to soil health by preventing surface sealing, improving water infiltration, water holding capacity (Martínez-Blanco et al. 2013, ROU 2007), promoting soil C storage, and providing habitat for soil biology (Wang et al. 2022).

Compost application to soil has been studied for its success in improving soil health (Li and Evanlyo 2013, Ryals and Silver 2013, Wang et al. 2022). Compost applications increase SOM. SOM amendments can enhance soil C storage through the promotion of soil microbial activity, improving soil aggregate stability, and the introduction of C (Wang et al. 2022, Liang et al., 2017, Mpeketula and Snapp, 2019, Smith, 2016). The nutrients provided via compost application support vegetation growth and increases C captured through photosynthesis (Ryals et al. 2015). The amount of organic carbon within soils is crucial for soil fertility and its potential to sequester carbon, providing a valuable tool in the fight against climate change (Baldock et al. 2021).

Compost applications have been proposed as a strategy to mitigate climate change through the enhancement of soil organic carbon (SOC) (Kutos et al. 2023, Ryals et al. 2015). Compost applications increase SOC across many agroecological contexts (Amelung et al. 2020, Kutos et al. 2023, Li and Evanlyo 2013). Compost application to soils has been proven to have the potential to reduce atmospheric CO<sub>2</sub> due to its ability to promote plant productivity (Ryals et al. 2013). Increases in SOC are dependent on many factors such as environment, soil type, management practices, and the quality and composition of composts (Wang et al. 2022).

Consideration of how the composition of applied composts affects its ability to increase stored soil carbon is required. The carbon fractions in composts vary among types due to differences in their chemical compositions. Compost varies in what they are made of. Recycled yard trimmings, manures, food waste, and biochar are common components of compost. The ratios these components are in is one of the main factors that differentiates compost varieties from one another. These differences in their chemical compositions is what accounts for the differences in C:N and carbon fractions of each compost variety.

Labile carbon in soil is carbon that is more accessible for use in processes such as microbial decomposition and plant growth. The increase in soil C indirectly benefits plants through the promotion of soil health, which in turn increases other nutrients such as N, P or K, which plants do utilize. Specifically, labile carbon content of compost is related to its chemical composition because composition contributes to determining what processes occur and at what rate they occur in soils. Water-soluble OM represents the most active fraction of compost chemically and biologically. It reflects the biochemical changes of OM (Said-Pullicino 2007). Microbes decompose OM, and in the process, mineralize present nutrients. Carbon is sequestered through this process, and labile carbon content is reduced. From the initial to the final stages of composting, there is a significant decrease in water-extractable organic C (Said-Pullicino 2007). Microbes have used the available C and so it is now stored in the soil. For example, composting decreased (from 29 to 9%) the proportion of the plant residue labile pool and increased the residence time of both labile and more stable pools (Lerch et al. 2019). These fractions tell us about how much carbon is available to be stored, used in processes, and respired back out into our atmosphere.

The amount of labile versus persistent carbon present in compost indicates how much carbon can be sequestered in soil with its application. It is for this reason it is important to understand the carbon fractions of compost varieties. In this study I explored the effect of a compost's chemical composition on labile and persistent carbon content and if a compost's C:N ratio is correlated to its carbon fractions. The purpose of this study was to isolate more stable and more labile carbon fractions in different compost types using the hot water extraction method (HWE) (Haddix et al. 2016, Heller and Weiß 2015).

## METHODS

### Compost Collection and Types

The three varieties of compost evaluated in this study are Nicasio, Hi-Test, and VermiCompost. Nicasio and Hi-Test come from West Marin Compost, Nicasio, CA. Nicasio compost is made from recycled yard trimmings, dairy manure, and horse manure. The Hi-Test compost is the same as Nicasio but includes manure from species like goats and chicken that produce high-nitrogen manure. The Nicasio blend contains 54.5% green material, 18.18% cow manure-separated solids, and 27.27% horse bedding manure (Jeffery Creque from West Marin Compost). The Hi-Test compost can come in two mixes, 1( 56.25% green material, 6.25% cow manure-separated solids, 18.75% horse bedding manure, and 18.75% goat bedding material, or 2( 56.25% green material, 21.875% cow corral manure, and 21.875% horse bedding manure (Jeffery Creque from West Marin Compost). Both compost varieties were initially wetdown by the West Marin Compost facility with dairy waste water, before they are handled in turned windrows in accordance with the National Organic Program, EPA, and CDFA specifications.

VermiCompost comes from Jepson Prairie Organics, Vacaville, CA. VermiCompost is produced from worm castings of Red Wiggler worms fed recovered yard trimmings and food scraps.

Yocelyn Villa collected three varieties of compost in 5 gallon buckets from random parts of the compost piles with a shovel and brought the compost samples back to the Silver Lab at UC Berkeley. The three different composts were tested by members of the Silver Lab for pH, gravimetric water content, and total carbon and nitrogen.

### pH

Members of the Silver Lab recorded the pH of the composts by adding 1 ml of deionized water to a 1g sample, vortexing it for one minute, leaving the sample for 10 minutes uncapped, inserting a pH probe in the last 2-3 minutes, and using the probe to recording measurement value. Each compost was tested for gravimetric water content (mass of water per mass of dry

soil) by weighing the samples in a tin, drying the tins in an oven overnight at 65°C, and measuring the dry weight and tin afterward.

### **Total Carbon and Nitrogen**

Each variety of compost was tested for total carbon and nitrogen at intake and after HWE. I prepared the compost for testing by pouring liquid nitrogen on the compost and then grinding with a mortar and pestle until as much OM as possible was powderized. A few grams of compost were ground, enough to pull from to make samples. For the intake sample, four mg of compost were weighed into tin capsules and analyzed on the CE Elantech elemental analyzer. For the HWE samples, 3 milligrams were added to each tin. The CE Elantech elemental analyzer was used to determine the %C and %N of the labile and persistent material. C and N concentrations were calculated and the intake samples were corrected for moisture.

### **Hot Water Extraction**

To isolate labile metabolic organic material, I used HWE (Figure 1) which leaves behind more stable structural organic material and mobilizes the labile carbon into the solution. To prepare compost for extraction, I covered the OM in liquid nitrogen and finely ground it using a mortar and pestle. I dried the material at 60°C in a drying oven for about 48 hours. To rehydrate the material for extraction I added two grams of dried material to 50ml of deionized water and left it to stir on a stirrer plate for 30 minutes at 60°C (Nkhili et al. 2012). This mixture of rehydrated organic material was then poured through a nylon mesh filter system and the solution was poured into its own tin, frozen, and then freeze-dried (Figure 2). I washed the residue remaining on the filter with deionized water into its own tin and oven dried it at 60°C for approximately 48 hours. Both components weights were recorded and run on the elemental analyzer for total C and N (Haddix et al. 2016). This process provided measurements on the amount of labile C is in each compost tested. I repeated this to create 3 replicates of each compost type, for a total of 9 samples.



**Fig 1. Laboratory techniques.** (a) Hot Water Extraction of Nicasio sample C. Solution at the bottom of the vacuum filtering flask contains the labile carbon and the residue on the filter is the more persistent carbon. (b) Freeze dryer with HWE samples in the chamber. The freeze dryer allows for removal of water, leaving behind residue for later analysis, without applying heat.

## Data Analysis

I used analysis of variation (ANOVA) to evaluate the differences in persistent carbon content, labile carbon content, and C:N ratios of the compost types. ANOVA allowed me to determine if there were significant differences among the compost types for these variables. Subsequently, I conducted a post-hoc analysis using Tukey's Honestly Significant Difference test to identify specific pairwise differences between compost types. This allowed for a comprehensive understanding of which compost types significantly differed from each other in terms of persistent carbon, labile carbon, and C:N ratios. All statistical analyses were performed using R-4.3.3.

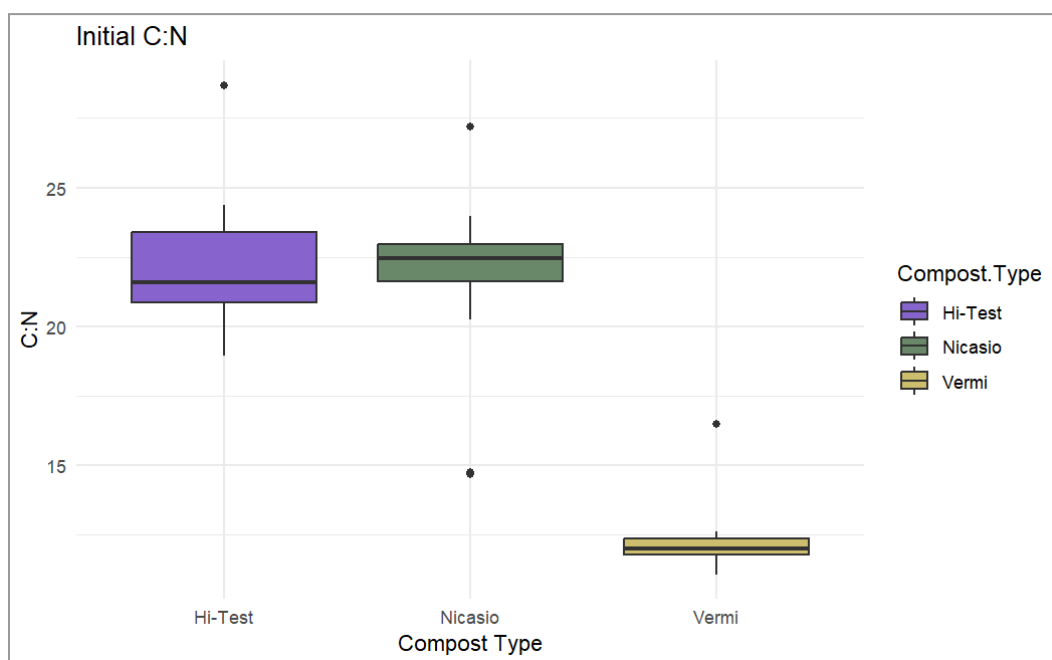
## RESULTS

### Compost Characteristics Summary

The C:N ratios of the compost varieties varied across all three, with Hi-Test having the largest C:N ratio at intake (Table 1). VermiCompost was found to be significantly different from Hi-Test,  $p < 0.0$ , and Nicasio,  $p < 0.0$ , for intake C:N.

**Table 1. pH, total carbon and nitrogen, and C:N ratio of each compost variety at intake.** Results calculated from elemental analyzer results.

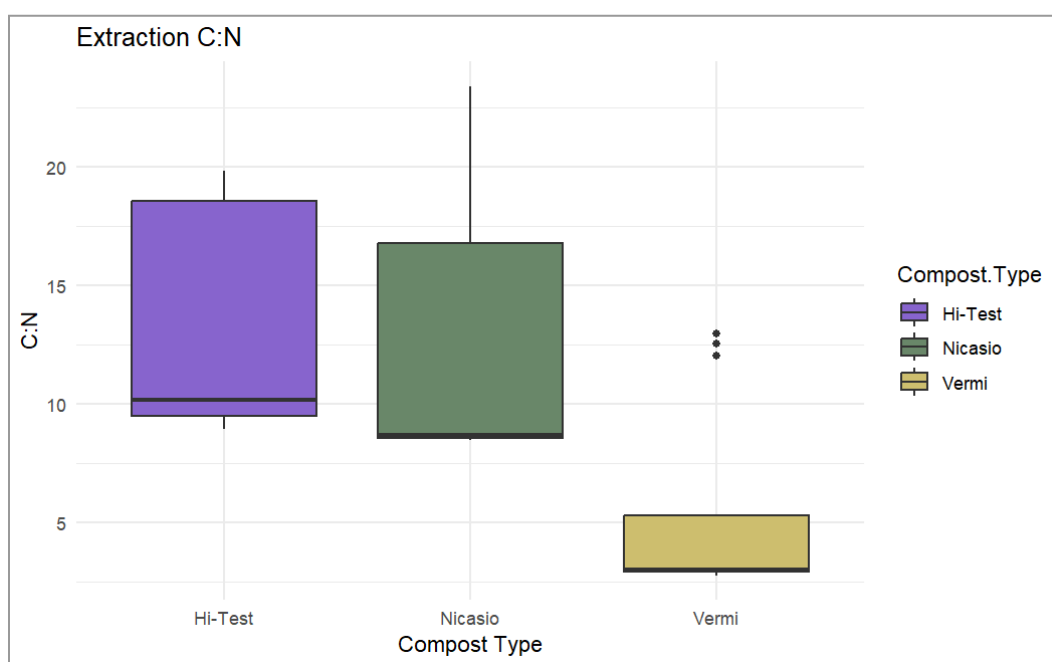
Compost Variety	pH	Total C (%)	Total N (%)	C:N
Hi-Test	$8.28 \pm 0.01$	$17.161 \pm 0.561$	$0.767 \pm 0.013$	$22.448 \pm 0.856$
Nicasio	$7.54 \pm 0.01$	$24.697 \pm 0.822$	$1.174 \pm 0.093$	$21.894 \pm 0.632$
VermiCompost	$7.23 \pm 0.01$	$12.805 \pm 0.479$	$1.034 \pm 0.016$	$12.402 \pm 0.474$



**Fig 2. A comparison of compost initial C:N ratio.** The C:N ratio of each compost type at after collection, before HWE.

## Hot Water Extraction

The Hi-Test compost had the highest total carbon content and the highest labile carbon content of the three composts after the hot water extraction (Table 2). VermiCompost was significantly different from Hi-Test,  $p < 0.0$ , and Nicasio,  $p < 0.0$ . There is approximately 86.043% and 83.003% less total carbon in the VermiCompost compared to Hi-Test and Nicasio, respectively.



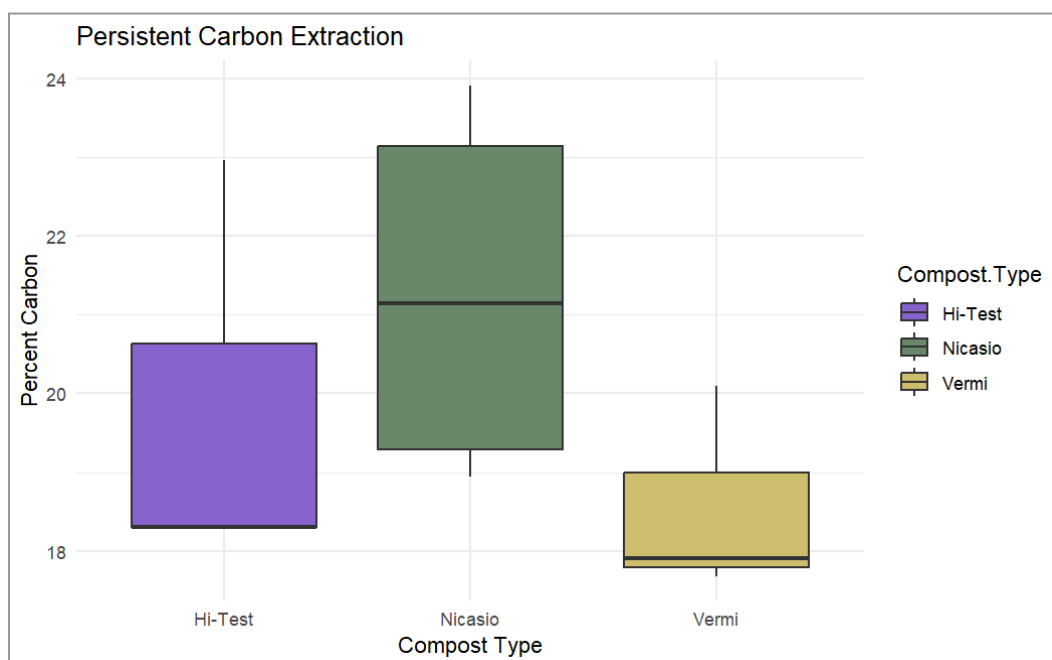
**Fig 3. A comparison of compost HWE C:N ratio.** The C:N ratio of each compost type after HWE.

Persistent carbon fractions did not significantly,  $p > 0.05$ , vary between compost varieties. However, significant differences were found between compost types in labile carbon content. VermiCompost was significantly different from Hi-Tests,  $p < 0.0$ , and Nicasio,  $p < 0.0$ . There is averagely 62.79% and 49.41% more labile carbon in the Hi-Test and Nicasio composts, respectively, compared to VermiCompost.

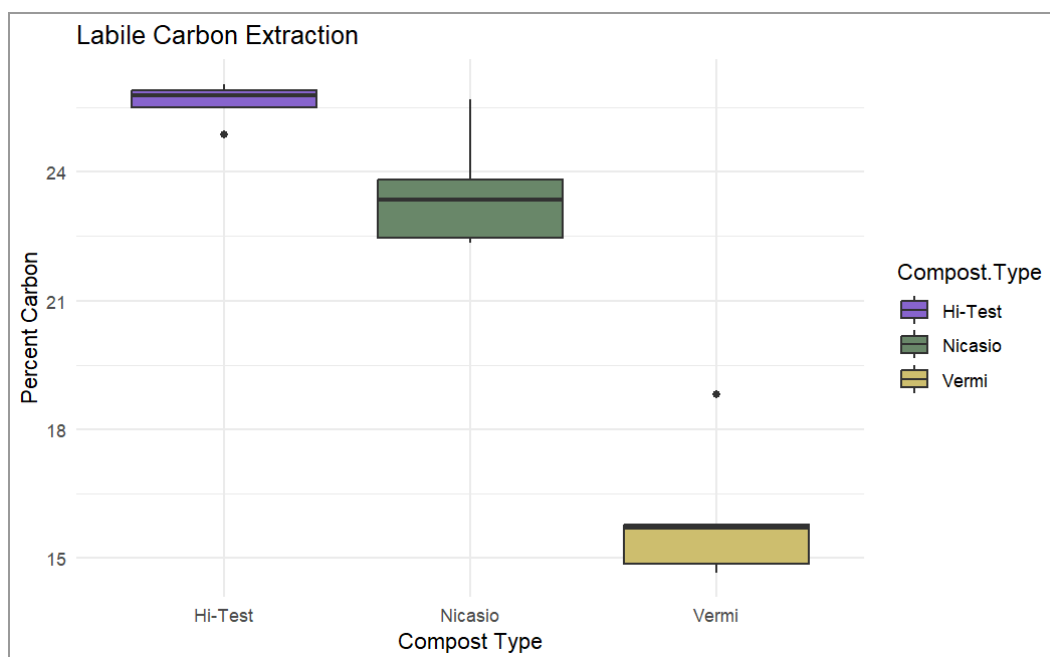


**Table 2. Extraction C:N, percentage C and N, percent labile carbon, and percent persistent carbon of each compost variety.** Results calculated from elemental analyzer results.

Compost Variety	C:N of original compost	Sum of both fractions C:N after HWE	Sum of both fractions C (%) after HWE	Sum of both fractions N (%) after HWE	Labile Carbon (%)	Persistent Carbon (%)
Hi-Test	22.448 ± 0.856	13.593 ± 1.922	23.146 ± 1.3114	1.986 ± 0.339	25.615 ± 0.261	19.854 ± 1.555
Nicasio	21.894 ± 0.632	12.451 ± 1.721	22.768 ± 0.566	2.175 ± 0.239	23.509 ± 0.436	21.285 ± 1.239
VermiCompost	12.402 ± 0.474	5.340 ± 1.253	12.441 ± 0.506	4.377 ± 0.512	15.735 ± 0.412	18.559 ± 0.772



**Fig 4. A comparison of compost persistent carbon.** The persistent carbon content of each compost type after HWE.



**Fig 5. A comparison of compost labile carbon.** The labile carbon content of each compost type after HWE.

## DISCUSSION

Carbon fractions of compost inform us of how much carbon is available to be stored, used in processes, and respired back into our atmosphere. These carbon fractions are related to the compost's chemical composition. Findings show that the C:N ratio of a compost is an indicator of its labile carbon content.

### C:N Ratios

Due to their composition, Hi-Test and Nicasio were predicted to have the more ideal C:N ratio. Results from the initial compost samples revealed that Hi-Test compost had the largest C:N ratio of the three compost varieties. Only VermiCompost was significantly different in C:N from Hi-Test and Nicasio, however despite Hi-Test having a slightly larger C:N, it was not actually significantly different in comparison to Nicasio. C:N ratio is considered one of the key factors affecting composting process and quality (Azim et al. 2018). The ideal C:N ratio of compost includes enough nitrogen for microbial activity, but not an excessive amount. In lower ratios,

nitrogen is supplied in excess and lost through leaching or volatilization as ammonia (Azim et al. 2018, Gao et al. 2010). In higher ratios, there is insufficient nitrogen for optimal growth of microbes. With more ideal C:N ratios for microbial diet, nearing 24:1, the Hi-Test and Nicasio composts have nitrogen levels for microbial turnover to occur more, leading to more labile carbon in the soil than VermiCompost.

Hi-Test compost had the largest C:N ratio at intake, suggesting that it would have the largest C:N ratio after HWE. C:N ratio variation between compost varieties remained the same after HWE. Hi-Test compost had the largest C:N prior to and after HWE of the compost varieties. Nicasio had the second largest. Only VermiCompost's C:N was significantly different from the other two varieties. HWE of peat material, similar to compost, is a fractionation method to determine labile SOM in organic soils (Heller and Weiß 2015). There are various hot water extraction methods to extract OM from soil. Some however risk altering the chemical structure of OM or have low extraction efficiency. Extraction at 60°C for 30 minutes has been found to be optimal in terms of yield of organic carbon extracted (Nkhili et al. 2012). By avoiding altering the chemical composition of the compost, initial and post HWE results for measurements such as C:N ratio can be considered for their predictive capability of labile carbon content. C:N ratio at intake of a compost sample is a good indicator of what the C:N ratio would be in comparison to other composts after HWE.

### **Persistent Carbon Content**

The persistent carbon content of the three compost varieties was not found to be significantly different between composts. Despite having different chemical compositions and different C:N ratios, the persistent carbon content was relatively the same. This suggests that persistent carbon content is not dependent on the chemical composition of the compost.

### **Labile Carbon Content**

Findings showed that having the largest C:N ratio, both before and after HWE, was an indicator of labile content. Hi-Test compost had the largest C:N ratio at intake, suggesting that it would have the largest amount of labile carbon. Hi-Test compost had the largest labile carbon

content, as expected due to it having the largest C:N ratio both before and after HWE. C:N ratio is found to be an indicator of labile carbon content.

### **Chemical Composition of Compost**

Compost composition depends on multiple factors, such as the inputs used to create it, what ratio they are in, and how the compost was made and processed. These results demonstrate how variable compost is. With only studying carbon in three compost varieties, significant variations in labile carbon content were still observed. This labile carbon content provides insight into how much carbon is accessible to microbes. In this study Hi-Test and Nicasio were found to have the most labile carbon to offer. The persistent carbon, and any labile carbon resistant to decomposition, have the potential to be sequestered in soil long term. I found that the C:N ratio of compost varieties prior to and after HWE were predictive of which compost types would contain the most labile carbon content.

### **Limitations and Future Directions**

Only 3 replicates of each compost type were created using the HWE method. To improve the standard errors and implications of the data, more replicates should be done. Composts composition is variable. It is difficult to fully break down the organic material, especially wood fragments. This contributes to the variability between samples, and thus more replicates would create a more representative dataset.

The use of three compost varieties provides an initial look into the carbon fractions of composts of varying chemical compositions, but only represents a small portion of the composts available. There would likely be greater variation in results if more compost types were included, due to the different chemical compositions that would be represented in the study.

Interactions of these composts with soils of any kind was not studied. Without this, there are no insights into how critical labile and persistent carbon content is for carbon sequestration with specific varieties of soil.

Due to the limitations of this study, it is important that this work is bolstered with results from more compost varieties. By determining the carbon fractions of most composts with

varying chemical composition, we will have an improved understanding of what characteristics of compost indicate labile and persistent carbon percentage. This work combined with studying how each compost variety interacts with soils is important to understanding how composts can be implemented for carbon sequestration purposes.

### **Broader implications and Management Implications**

Composts are applied to land for a variety of purposes, such as to improve soil health and mitigate climate change by sequestering carbon. Understanding the carbon content of compost is crucial for effective application. By identifying the average carbon fractions of compost varieties, users like land managers, farmers, ranchers, and home growers can make more informed decisions about what kind of compost is suitable for their specific needs and environment.

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