Study the effect of xyloglucan treatment on soil stability and biochar aging

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Statement of Affirmation

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2025

Abstract

This thesis looks at how bio amendments like glucose, xyloglucan, and biochar affect carbon dynamics, carbon stability, and carbon aggregation of the soil needed to maintain agricultural fertility as well as for promoting sustainable agriculture. Two research papers have been incorporated in the research that look at specific bioamendments' effects on the soil.

The first study investigates the contribution of glucose and xyloglucan to enhancing soil aggregation and stability. It assesses the monosaccharide glucose against the polysaccharide xyloglucan, positing that while glucose would rapidly elevate CO_2 emissions, xyloglucan would facilitate more stable and long-lasting soil aggregation. Two soil types, L2.1 and REC, received amendments with varying amounts of glucose, xyloglucan, and a control. Throughout a 31-day incubation, CO_2 flux and soil particle sizes were recorded. The findings revealed that the glucose treatment increased CO_2 emissions, particularly within the initial two days, due to glucose's labile properties, which encourage rapid microbial metabolism. In contrast, the more complex xyloglucan elicited slower CO_2 emissions while promoting improved long-term soil aggregation. The cumulative CO_2 emissions from the glucose treatments were significantly higher than those from xyloglucan, indicating a more immediate microbial reaction to glucose.

The second study investigates the effects of glucose and xyloglucan, with and without biochar, on soil organic carbon (SOC) priming and CO_2 emissions. The hypothesis was that glucose would produce a swift priming effect while xyloglucan would offer a more stable, gradual effect. Biochar was expected to improve SOC stabilization and priming. Two soil types, CKA and L2.1, were treated with glucose, xyloglucan, and biochar, with CO_2 flux tracked over a 35-day incubation. Isotope ratio mass spectrometry (IRMS) was employed to analyze the $\delta 13C$ signature of CO_2 emissions, facilitating an evaluation of SOC priming. The results indicated that glucose and biochar created the most significant priming effect, rapidly enhancing microbial activity and SOC degradation. Specifically, the cumulative priming effect from glucose plus biochar was nearly double that of the xyloglucan treatments. The fact that biochar had no discernible effect on CO_2 emissions from glucose suggests that when there are plenty of carbon sources, such as glucose, available, its influence on microbial turnover is less pronounced.

The findings demonstrate the distinct functions of xyloglucan and glucose in soil carbon dynamics. While xyloglucan promotes a more slow but continuous priming impact, which helps with long-term soil stability, glucose causes a quick and noticeable priming effect that increases microbial activity and SOC breakdown. It was clear that biochar helped stabilize SOC, particularly when combined with glucose. However, in the presence of easily accessible carbon sources, it seems to have no effect on microbial turnover.

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1. Introduction

1.1 Importance of soil organic matter for aggregation and sustainable agriculture

By helping to build and stabilize soil aggregates, soil organic matter (SOM) helps to prevent soil erosion (Zhao et al., 2018; Halder et al., 2024). Global food security may be threatened by the loss of SOM brought on by climate change and inappropriate agricultural land use, which might result in soil degradation, lower crop yields, and poor food quality (Galloway et al., 2017). Therefore, addressing soil structure protection is critical for promoting sustainable agriculture, especially for counteracting the adverse effects of climate change (Galloway et al., 2017; Tang et al., 2022)

Soil organic carbon (SOC) composes about 58% of SOM and is central to sustaining soil quality and functionality (Stockmann et al., 2013). The imbalance between carbon release into the atmosphere and carbon uptake by other compartments increases atmospheric CO_2 concentrations at a rate of 4.1×10^9 tons of carbon per year (IPCC, 2007). Retaining carbon in stable forms in the soil is essential to mitigate this imbalance. The stability of the different forms of SOC in soil may control aggregation, erosion, and CO_2 exchange between soil and atmosphere. Therefore, understanding how SOC forms affect aggregates' formation and stability is of interest to enhancing the sustainability and productivity of cropping systems while preventing CO_2 release from soil to atmosphere to combat climate change (Rochette et al., 1999; Fang et al., 2018; Fu et al., 2022; Kopittke et al., 2022).

1.2 Formation and stabilization of soil aggregates

The binding of soil particles (clay, silt, and sand) to create bigger aggregates with organic and inorganic materials and the fundamental units of soil structure is known as soil aggregation (Garland et al., 2024; Sarker et al., 2022). Microaggregates (less than 250 μm) and macroaggregates (more than 250 μm) are the two sizes of aggregates. Persistent agents, such as humic substances, and transient agents, such as roots, microbial products, and root exudates, are responsible for the creation and stabilization of aggregates (Totsche et al., 2017; Sarker et al., 2022). Transient agents such polysaccharides originating from plants and microorganisms, roots, and fungal hyphae bind microaggregates into macroaggregates after they have previously been created by permanent binding agents like humic substances (Totsche et al., 2017; Morris et al., 2019; Sarker et al., 2022). Soil is improved by these aggregates.

One important measure of soil fertility and quality is aggregate stability, which is the ability of these structures to withstand breaking down under outside pressures (Siebers et al., 2023; Abiven et al., 2009). A number of variables, including soil texture, clay mineralogy, pH, redox potential, mechanical stress, and SOM quantity and composition, affect how stable soil aggregates are. Thus, agricultural practices such as organic amendments can alter the dynamics of soil aggregates (Abiven et al., 2009; Totsche et al., 2017). Macroaggregates are more sensitive to management practices and environmental stresses, exhibiting faster turnover rates. In contrast, microaggregates are stabilized by persistent binding agents such as humic substances and can withstand slaking in water and resist intense mechanical and physicochemical stresses, persisting in soils for decades (Totsche et al., 2017; Tag et al., 2022). This difference in stability is due to the hierarchical nature of soil aggregation explained earlier (Totsche et al., 2017; Morris et al., 2019; Sarker et al., 2022).

Wet sieving is enhanced by sophisticated methods for evaluating particle size and stability, such as laser diffraction particle analysis, which offers high-resolution assessments of particle size distribution. This technique involves dispersing particles in a liquid and analyzing light scattering. The volume-based median diameter (Dv50) and other median particle sizes can be precisely calculated thanks to the scattering patterns. This method provides a thorough understanding of aggregation dynamics and is especially useful for monitoring the stability of soil aggregates under mechanical energy or chemical treatments (Mikutta et al., 2023). The stability of microaggregates, which are necessary building blocks for the creation of bigger aggregates, may be crucially revealed by this technique (Siebers et al., 2023).

1.3 Role of organic amendments on aggregate formation and stability

1.3.1 Polysaccharides and glucose

Polysaccharides, including glucose, are critical in soil aggregation as these compounds act as "bridges" between soil particles or form gel-like substances that glue particles into aggregates (Tisdall & Oades, 1982; Haynes & Beare, 1997; Sarker et al., 2022), even in challenging soil conditions (Martin, 1971; Oades, 1984). Sugars, the most prevalent organic substances in the biosphere, are the main source of carbon and energy for soil microbes and act as monomers of polysaccharides. (Fatemeh Rakhsh & Golchin, 2018; Dowd et al., 2001). Adding glucose to soil can lead to a rapid but transient increase in soil aggregation driven by microbial activity and the formation of macroaggregates (Sarker et al., 2022; Li et al., 2020; Gunina & Kuzyakov, 2015).

Xyloglucan, a naturally occurring root exudate, is unique among polysaccharides because of how well it promotes soil aggregation. (Galloway et al., 2018). Xyloglucan is found in soil samples and plant growth media, has been shown to increase the proportion of larger soil aggregates and improve soil structure by enhancing water retention and particle adhesion (Galloway et al., 2018;

Rillig et al., 2017; Read et al., 2003; Bacic et al., 1986). Studies utilizing Scanning Electron Microscopy (SEM) have shown that xyloglucan facilitates the adhesion of smaller soil particles to larger aggregates, creating stable structures vital for nutrient and water management in soils (Tang et al., 2022).

Polysaccharides decompose over time, leaving behind a more stable fraction that continues to contribute to soil aggregation. This stable polysaccharide fraction is essential for maintaining soil structure over extended periods, reducing soil degradation, and preventing erosion (Martin, 1971). Xyloglucan may persist longer in soil than simpler sugars, providing prolonged benefits for soil aggregation and structural stability (Traoré et al., 2000).

1.3.2 Biochar

Biochar is carbon-rich material intentionally produced via low-oxygen pyrolysis of plant and/or animal-derived materials to be applied to soil as soil ameliorant and stable SOC form (Luo et al., 2011; Keith et al., 2015; Fang et al., 2015). Biochar supports land reclamation by restoring degraded soils and expanding arable land without clearing new areas (Anawar et al., 2015). Beyond its agricultural benefits, biochar fosters a circular economy by turning agricultural waste into a valuable resource for soil amelioration while sequestering carbon and reducing reliance on energy-intensive fertilizers (Lehmann et al., 2003; Biederman & Harpole, 2013; Tilman et al., 2011; Smith, 2016).

Biochar advances waste management by recycling organic residues and developing agricultural by-products into value-added resources for soil amendment (Woolf et al., 2010). Notably, the cascading use of organic materials, such as Miscanthus straw, has gained attention in the circular economy (Kraska et al., 2018). Biochar from miscanthus improves soil microbial activity, water retention, and structure; it benefits poor soils (Basso et al., 2013). These advancements lessen the demand for irrigation and artificial fertilizers, lowering expenses and their harmful environmental effects (Jeffery et al., 2011).

Biochar may promote soil aggregation by enhancing cation exchange capacity and microbial activity, with effects that can persist for medium to long-term periods (Du et al., 2016; Luo, Zang et al., 2017). Also, biochar affects soil aggregation as it interacts with native SOM, influencing its decomposition rates and nutrient cycling, thereby causing these compounds to persist as particle binders. Although biochar provides the potential for long-term SOC storage, research on how it interacts with native SOM is still ongoing (Kalu et al., 2024).

1.4 Role of organic amendments on soil carbon Balance

Biochar, sugars, and root exudates play crucial roles in influencing SOC dynamics and balance. Steinbeiss et al. (2009) demonstrated the impact of biochar amendments on soil carbon sequestration and microbial activity during a 4-month incubation. Two biochar types, derived from glucose (carbon-dense) and yeast (nitrogen-rich), were applied to forest and arable soils. Using ^{13}C

isotope labeling, the authors observed that glucose-derived biochar supported bacterial activity, while yeast-derived biochar stimulated fungal activity. Biochar enhanced SOC storage more effectively in arable soils, illustrating its contribution as a stable carbon source in agriculture.

Similarly, Luo et al. (2017) studied the effects of biochar on SOC mineralization, focusing on the priming effects induced by labile substrates such as sucrose and Miscanthus. In a 28-day incubation experiment with ^{13}C isotope signature ($\delta^{13}C$) analysis of CO_2 ($\delta^{13}C$-CO_2) respired from the soil, the authors reported that high-temperature biochar (700°C) caused a significant increase of SOC mineralization, emphasizing the complex interactions between organic amendments and SOM dynamics. Keiluweit et al. (2015) showed that root exudates such as oxalic acid disrupted mineral-organic bonds, enhancing SOC mineralization, while glucose had a more moderate effect. These findings illustrate the importance of exudate composition in driving SOC dynamics. Accordingly, Schweizer et al. (1999) found that microbial activity alters the $\delta^{13}C$-CO_2 respired from soil and the $\delta^{13}C$ of remaining SOM.

1.5 Priming effect of root exudates and biochar on SOC

The priming effect (PE) refers to the change in SOC mineralization following organic carbon amendment to soil (Nottingham et al., 2009). The sources of organic carbon amendment can be fresh organic materials such as plant litter, root exudates, or stable carbon materials like biochar. A positive PE occurs when these exogenous organic amendments stimulate or reduce soil microbial activity, thereby accelerating or decelerating the decomposition of native SOC (Zhou et al., 2022; Kalu et al., 2024).

As roots release labile organic compounds, microorganisms rapidly utilize sugars for energy and cell production. This high microbial activity in the rhizosphere, driven by sugars, effectively triggers priming effects, highlighting their ecological role in SOC cycling (Pausch et al., 2013). Biochar, when combined with organic matter, can also interact to influence decomposition rates. Adding easily degradable substrates or labile organic matter in biochar-amended soils often accelerates biochar decay. Nonetheless, the first few days after substrate administration are usually when this effect is most noticeable (Ameloot, 2013). The dynamic function that substrate inputs have in controlling soil microbial activity and carbon cycling is highlighted by these interactions.

Among the components exuded by roots, carbohydrates are the most abundant, including monosaccharides such as glucose, fructose, sucrose, and polysaccharides (Gunina & Kuzyakov, 2015; Ma et al., 2022). These compounds are easily decomposable, providing an immediate energy source for microorganisms and fueling microbial activity in the rhizosphere. Pausch et al. (2013) hypothesized that the primary ecological function of root-released sugars is to maintain high microbial activity and trigger priming effects on SOC. This is supported by studies such as Keiluweit et al. (2015), which showed that oxalic acid and glucose, common root exudates, disrupt

organo-mineral bonds, releasing otherwise protected SOC for microbial decomposition. Similarly, Zhou et al. (2022) demonstrated that glucose and oxalic acid destabilize SOC through microbial respiration, even when firmly bound to mineral surfaces. Increased sugar concentrations in soil solutions activate dormant microbial communities, enhancing enzyme production and accelerating SOM decomposition. This releases essential nutrients, including nitrogen, phosphorus, and sulfur (Gunina & Kuzyakov, 2015). However, as Haichar et al. (2014) noted, the impact of root exudates varies based on their composition, with low-molecular-weight compounds such as sugars and organic acids inducing more pronounced priming effects.

Biochar amendment to soil has been shown to trigger positive or negative PE of SOC depending on the type of biochar, soil conditions, and the presence of labile substrates (Kuzyakov et al., 2000; Luo et al., 2011; Keith et al., 2015; Luo, Zang, et al., 2017). Ameloot (2013) highlighted that adding labile organic substrates can accelerate biochar decay, affecting biochar stability in soil and its PE of SOC. Biochars produced at lower temperatures promote positive PE, while high-temperature biochars lead to minor or negative PE of SOC (Luo et al., 2011; Zimmerman et al., 2011). For example, Keith et al. (2015) noted that wood- and sugar cane bagasse-derived biochars (rich in lignin) tend to stabilize SOC (negative PE). In contrast, grass-derived biochars (cellulose-rich) promoted positive PE of SOC.

Several mechanisms explain biochar-induced PE of SOC, including co-metabolism, where labile biochar fractions enhance microbial activity and SOC mineralization (positive PE), whereas more stable biochar fractions may interact with SOC, reducing its accessibility to microbial decomposers, resulting in negative PE (Keith et al., 2015; Fang et al., 2015). Additionally, biochar in the rhizosphere undergoes accelerated decomposition due to increased microbial activity stimulated by continuous root exudates and rhizodeposits (Ameloot, 2013), affecting biochar stability and associated mechanisms leading to negative or positive PE of SOC.

1-6 Objectives and hypotheses

This study is divided into two complementary parts.

Study 1: **The role of polysaccharides in soil aggregation and stability**

The study first compares the stability of polysaccharides (xyloglucan) and monosaccharides (glucose) amendments at two different concentrations (50 and 500 µg soil-1). The study aims to demonstrate the effect of carbohydrate structure (mono and polysaccharide) and concentration on soil CO_2 emissions. Secondly, it explores the role of polysaccharides (xyloglucan) and monosaccharides (glucose) in soil aggregate formation and stabilization. It is anticipated that glucose amendments, because of their simpler structure, will cause an initial spike in CO_2 emissions during the early stages of incubation. In contrast, with their more complex structure,

xyloglucan amendments are expected to produce a delayed response in CO_2 emissions. Furthermore, as more stable carbon sources and effective binding agents, polysaccharides will improve soil stability by promoting aggregation.

Study 2: **δ13C signature of CO2 respired from soils amended with glucose, xyloglucan, and biochar**

In the second experiment, which investigates the effects of artificial root exudates on SOC aging and the widespread use of biochar in soil amendments, the research aims to explore whether the combination of biochar and root exudates produces a distinct SOC priming effect. By utilizing Miscanthus-derived biochar from tomato farming systems, this research aims to contribute to sustainable soil management practices and address critical gaps in understanding carbon cycling and soil stability.

In this study, glucose amendments are anticipated to abruptly add to the SOC priming effect for a short period. However, xyloglucan, a polysaccharide with a more stable carbon source, has a lower PE effect than glucose.

2- Material and methods

2-1 Biochar and soil characterization:

The biochar utilized in this investigation was manufactured at the Thermo Processes and Emission Control in Waste Management and Recycling Teaching and Research Unit (TEER) at RWTH Aachen University (Germany). In a muffle furnace, the feedstock—untreated Miscanthus x giganteus—was pyrolyzed for ten minutes at 600 °C in a nitrogen atmosphere. The primary attributes of the biochar are displayed in Table 1.

Table 1 Summary of miscanthus biochar properties.

C_{fix}	Ash	Volatiles	H	N	S	O	$\delta^{13}C$	pH	WHC
(wf-%)	(wf-%)	(wf-%)	(wf-%)	(wf-%)	(wf-%)	(wf-%)		$CaCl_2$	g 100 g^{-1}
82.30	10.14	7.51	1.72	0.26	0.07	13.65	–13.66	8.96	399

In this study, three different soils were used, which were selected to comprise a range of soil pH (4.6-6.5), texture, and organic carbon content (Table 2). The soils consisted of two Luvisols collected at 0-30 cm layer. One was sampled at the agricultural research station of the University of Bonn at Campus Klein-Altendorf (hereinafter referred to as CKA) (50.37° N, 6.59° E) the other

(REC) was sampled at the post-lignite mining recultivation area (50.89° N, 6.34° E). The third soil was a standard LUFA soil named L2.1, purchased (air-dried and sieved to 2 mm) from the Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA, Speyer, Germany).

The samples were first air-dried and then sieved (2 mm). Soils were kept at room temperature until the beginning of the incubation experiments. Table 2 presents the physicochemical properties of soils used in this incubation experiment.

Table 2 Physiochemical properties of soils used in the study[1].

Soil	pH	Sand	Silt	Clay	BD	TOC	TIC	WHC	NH_4^+	NO_3	P
	$CaCl_2$	(%)	(%)	(%)	gcm^{-3}	(%)	(%)	$g100g^{-1}$	$mgkg^{-1}$	$mgkg^{-1}$	$mgkg^{-1}$
CKA	6,50	8,00	77,00	15,00	1,30	1,00	0,00	40,00	0,24	70,90	30,60
REC	7,40	5,00	78,00	17,00	1,50	0,30	1,60	44,00	1,12	21,18	5,34
L2.1	4,60	87,00	8,00	4,00	1,30	0,60	0,00	28,00	0,63	29,70	3,30

2-2 Glucose and xyloglucan characterization:

The D-(+)-Glucose Monohydrate used in this study was purchased from *Sigma-Aldrich (Merck)*[2], with a CAS number of 14431-43-7. It is ≥99.5% pure and has a molecular weight of 198.17 g/mol. The glucose was used in its crystalline form and dissolved in deionized water before being applied to the experiment. The $\delta^{13}C$ of Glucose is
The xyloglucan (Tamarind) used in this study was derived from tamarind seeds purchased from *Megazyme*[3] Xyloglucan's CAS number is 37294-28-3, and its purity exceeds 95%. A 1% solution has a viscosity of 142 centistokes (cSt). Its monosaccharide composition is as follows: 34% xylose, 45% glucose, 17% galactose, 2% arabinose, and 2% other sugars.

The $\delta^{13}C$ values for glucose and xyloglucan are (-11.13) and (-26.00).

[1] BD = bulk density; TOC= total organic carbon; TIC= total inorganic carbon; WHC= water holding capacity; NH^+_4 = ammonium ion; NO_3 = nitrate ion; P= phosphate

[2] https://www.sigmaaldrich.com/DE/de/product/sial/49159

[3] https://www.megazyme.com/xyloglucan-tamarind

2-3 Study 1 - The role of glucose and polysaccharides in soil particles aggregation and stability

2-3-1 Experimental treatments and incubation

In this study, soils REC and L2.1 were used, and five treatments were elaborated, as described below:

1. **Control:** Soil only
2. **Soil+G50:** Soil with + 50 µg g $^{-1}$ soil glucose
3. **Soil+G500:** Soil + 500 µg g $^{-1}$ soil glucose
4. **Soil+P50:** Soil + 50 µg g $^{-1}$ soil polysaccharides
5. **Soil+P500:** Soil + 500 µg g $^{-1}$ soil polysaccharides

These treatments were prepared in a single batch (about 420 g) and pre-incubated for 72 hours at 23 °C. Afterward, 3 g of dry soil was transferred to 22 ml gas chromatograph vials. The treatments were incubated for 31 days at 23 °C, and the water content was maintained at 60% of the water holding capacity by periodically dripping deionized water into the vial. Given CO2 flux measurements, four replicates were prepared in quadruplicates for each treatment.

2-3-2 CO2 flux measurements from incubated samples

On days 1, 2, 4, 5, 8, 11, 15, 19, 25, and 31 of incubation, the CO2 flux of the samples was measured using a gas chromatograph (GC-ECD/FID, Clarus 580, PerkinElmer, Waltham, USA, MA). For that, the four vials of a single replicate (quadruplicates) were closed at different times: 1h, 4h, 7h and 10h before measurement to allow the flux calculation according to the following equation:

$$\textit{Equation 1} \quad F = \frac{\frac{\Delta C}{\Delta T} \times V \times M}{m} \times 1000 \times 1000$$

in which F denotes the flux of CO2, ΔC/ΔT signifies the alteration in CO2 in parts per million (ppm) adjusted for the gas sample and air temperature (273,15 K and 20.8 °C, respectively). V denotes the headspace volume in liters, M represents the molar mass of C in CO2, and m denotes the quantity of soil in grams of dry weight.

2-3-3 Soil particle size and stability after incubation

After incubation, the particle size and stability were analyzed in a laser diffraction particle analyzer (LA-950, Horiba, Kyōto, Japan). The particle size range of analysis spans from a few nm to 5 mm. To ensure four replicates for each treatment, equal amounts of the replicate quadruplicates were combined. These replicates were placed into a plastic cylinder containing 20 ml of deionized water and gently stirred manually for homogenization. An aliquot of this suspension was injected into

the analyzer, and by continuous stirring, the particles circulated across the flow cell where light at two wavelengths (650 nm and 405 nm) was applied to measure the median particle size. Results are given after processing scattered light data through a software algorithm utilizing the Mie theory (Eshel et al., 2004). Results are given as a volume-based median diameter (Dv50) in µm, with the diameter of half of detected particles above this value and the diameter of another half below this value (Siebers et al., 2023).

The particle stability assessment considers that the stirrer's mechanical force in the circulation system breaks down soil aggregates, leading to changes in the median particle size over 40 minutes of continuous analysis (35 sequential measurements) (Siebers et al., 2023). Particle stability is assessed by comparing the differences in median particle size across the 35 measurements to the initial measurement. The first 25 measurements were taken in 40-second intervals, whereas the last 10 were taken in 1-minute intervals. The changes in Dv50 along these sequential measurements assess the particles' stability.

2-3-4 Statistical analysis

The data in this study are time series, which presents significant complexity for statistical analysis. AI tools specialized in statistical analysis from the DataCamp platform were employed to identify the most suitable model and perform necessary adjustments. Subsequently, the suggested models and adjustments were implemented using the RStudio_(2024.04.02) application. Numerous calculations and graphs were also performed using Microsoft Excel.

2-3-4-1 Glucose and xyloglucan stability

Various comparisons were made to answer the research questions, and suitable statistical tests were chosen based on the comparison and data characteristics.

The Paired Wilcoxon (Wilcoxon signed-rank) test was used to compare the CO2 concentrations of (G50 and G500) and (P50 and P500) and the values of each treatment to the control.

The Wilcoxon signed-rank test is a non-parametric statistical test used to compare two related samples or repeated measurements on the same subjects (Shi et al., 2023). It is beneficial when data are not normally distributed, sample sizes are small, or the measurement scale is ordinal(Sheskin, 2020).

The test determines whether the median difference between paired observations differs significantly from zero. In this comparison, both daily and cumulative concentrations were studied. P-values less than 0.05 were considered significant differences (Rosner et al., 2006).

2-3-4-2 Soil particle size and soil stability

The purpose of the statistical study was to ascertain whether xyloglucan and glucose treatments improved soil stability. As food supplies for microbial populations, it was thought that xyloglucan and glucose would be binding agents to promote soil aggregation. This was anticipated to

strengthen the soil particles' resistance to crushing during analysis. We tested this by looking at the rate at which soil particles were reduced and determining whether the different treatments differed significantly.

A generalized additive model (GAM) was fitted to each soil type (REC and L2.1). To test this, we examined the rate of soil particle reduction and assessed whether there were significant differences among the various treatments.

For each soil type (REC and L2.1), a generalized additive model (GAM) was fitted.

2-3-4-2-1 The objective of the model

The goal of the model is to comprehend and forecast how particle size varies over time under different treatments. It considers aspects while recording and modifying sounds and effects. This technique enables us to understand particle size behavior more accurately.

2-3-4-2-2 GAM model structure

Using a statistical model to predict particle sizes accounts for various influencing factors such as time, treatment, soil type, and replication, which raw data may not adequately represent. Raw measurements often include noise, errors, and confounding effects that obscure the underlying relationships between the predictors and the response variable (Wood, 2024). By fitting a model like a Generalized Additive Model (GAM), the predictions smooth out these inconsistencies and help isolate the effects of key factors, such as time and treatment, ensuring more reliable and consistent results. Furthermore, the model addresses non-linear relationships between predictors and the response variable that might be overlooked in the raw data (Wood, 2024). The model provides generalizable estimates, allowing for more accurate comparisons across treatments and capturing subtle differences in how treatments influence particle size over time, thereby enhancing the interpretation of reduction rates.

The gam() function fits a Generalized Additive Model (GAM). The model predicts Particle_median using a smooth term for measurment_time and Treatment_Replicate as a factor. This allows us to model the non-linear effect of time while accounting for the treatment-replicate combinations. The model structure is shown in Figure 1. Libraries such as “mgcv” with the gamm() function to fit the model, "dplyr” as part of the tidyverse, and “ggplot2” for creating plots were among the libraries used in this section.

```
model_gam_clean <- gam(Particle_median ~ s(measurment_time, k = 10) +
Treatment_Replicate, data = treatment_soil_data_clean)
```

Figure 1 GAM model structure

- Response Variable (Particle_median):

This is the dependent or response variable that you want to model. In this case, it is the median particle size at different time points.

- Predictors (Independent Variables):
    - s(measurment_time, k = 10):
        - measurment_time is the predictor variable representing the time the measurements were taken. The s() function specifies a smooth term for this variable, meaning we do not assume a linear relationship between measurment_time and Particle_median. Instead, we let the model learn the non-linear relationship between time and particle size.
        - The basic dimension, or the number of knots required to match the smooth term, is specified by k = 10. Although a greater number gives the model more flexibility, it may also make overfitting more likely.
    - Treatment_Replicate:

      This categorical predictor accounts for the interaction between treatment and replicate. It is included as a factor (or categorical variable), meaning the model will fit a different intercept for each combination of treatment and replicate. Using this variable, you can capture the effects of different treatments and replicates on the particle size.

- Predict Initial and Final Particle Sizes

In this step, the model is used to predict the particle sizes at time = 0 (the initial time) and time 20 minutes (the final time). These predictions are obtained by using the fitted Generalized Additive Model (GAM).

```
initial_size_clean <- predict(model_gam_clean, newdata =
data.frame(measurment_time = 0, Treatment_Replicate =
treatment_soil_data_clean$Treatment_Replicate))
final_size_clean <- predict(model_gam_clean, newdata =
data.frame(measurment_time = 20, Treatment_Replicate =
treatment_soil_data_clean$Treatment_Replicate))
```

Figure 2 Predict Initial and Final Particle Sizes

- initial_size_clean: This gives the predicted particle size at time=0. The model predicts the particles' starting size based on the treatment and replicate values.
- final_size_clean: This gives the predicted particle size at time = 20 minutes. The model estimates the final particle size after 20 minutes using the same factors (treatment and replicate).

2-3-4-3 The Percentage Reduction calculation

The percentage reduction is calculated by comparing the initial and final predicted particle size values. It represents the relative change in particle size as a percentage of the initial size.

The formula for percentage reduction is:

Equation 2 $$\text{Percentage Reduction} = \text{Initial Size} \frac{(\text{Initial Size} - \text{Final Size})}{} \times 100$$

Where:

- *Initial Size is the predicted particle size at time= 0.*
- *Final Size is the predicted particle size at time=20 minutes.*
- *Percentage Reduction represents the relative decrease in particle size over time.*

2-3-4-4 Residuals

Residuals, the differences between observed values (y_i) and predicted values ($\hat{y}_i$), are key to evaluating the performance of statistical models (Raschke, 2013). They help assess model fit, with smaller residuals indicating a better fit, and can identify systematic patterns or deviations from assumptions like normality, homoscedasticity, and independence (Lasfar & Gergely Tóth, 2024). Residual analysis also aids in detecting outliers and comparing models, with smaller residuals generally favoring one model over another (Son, 2004).

2-3-4-5 Outliers removal

We applied a custom function to remove extreme residual outliers that identify and filter out residuals lying beyond a defined threshold of standard deviations from the mean. Specifically, we considered outliers residuals more significant than 3 standard deviations above or below the mean residual and excluded them from the dataset (Dastjerdy et al., 2023).

Outlier removal helps ensure that the model is not disproportionately influenced by extreme data points, thereby improving the model's robustness by focusing on most of the data that follows the expected pattern. The cleaned dataset, excluding these extreme residuals, was then used for further analysis (Kwak & Kim, 2017).

2-3-4-6 Model evaluation

Model evaluation is critical in assessing the performance and reliability of the Generalized Additive Mixed Model (GAMM) used in this study. Several libraries like "*mgcv*," "*dply*r", "*ggplot2*", "*caret,*" and "*nlme*" were utilized in this analysis. The assessment uses a variety of metrics and visuals to check that the model effectively represents the underlying patterns in the data and generalizes well to new data.

2-3-4-6-1. Root Mean Squared Error (RMSE)

One popular statistic for regression models is the Root Mean Squared Error, which assesses model accuracy by calculating the average magnitude of prediction errors (Hodson, 2022).

$$\textit{Equation 3} \quad RMSE = 1n_i = 1n(y_i - \hat{y}_i)^2$$

Where:

- y_i = *the observed value (actual value) for the iii-th observation.*
- $\hat{Y}_i$ = *the predicted value for the iii-th observation (from the model).*
- n = *the total number of observations (data points).*

Lower RMSE indicates better model performance, meaning the predictions are closer to the actual values(Chai & Draxler, 2014).

2-3-4-6-2 Akaike Information Criterion (AIC)

AIC measures the relative quality of a statistical model for a given dataset. It penalizes the number of parameters to balance the goodness of fit and model complexity (Wahyu Triyoso, 2024).

$$\textit{Equation 4} \quad AIC = 2k - 2ln(L)^2$$

Where:

- k=*number of estimated parameters in the model*
- L= *maximum likelihood of the model*

Lower AIC indicates a better model, suggesting a good fit with fewer parameters (Bozdogan, 1987).

2-3-4-6-3 Bayesian Information Criterion (BIC)

BIC is similar to AIC but imposes a more substantial penalty for models with more parameters. It is helpful for model comparison, especially when the sample size is large (Cavanaugh, 2016).

Equation 5 $BIC=kln(n)-2ln(L^)$

Where:

- *k = the number of parameters estimated by the model*
- *n = the sample size (number of observations)*
- *L^= is the maximized value of the likelihood function for the mode*

Lower BIC: Indicates a better model, as it suggests a good fit with fewer parameters (Vrieze, 2012).

2-3-4-6-4 Residuals vs. Measurement Time Plot

This plot helps to identify any systematic patterns in the residuals. If the residuals show a pattern (e.g., an increasing or decreasing trend), it may indicate that the model has not fully captured the relationship between measurement time and the response variable, or that other underlying factors are influencing the data (Yu, 2021).

2-3-4-6-5 Autocorrelation (ACF) of Residuals

The autocorrelation function (ACF) evaluates the correlation between residuals at various time points or observations. When residuals are autocorrelated, it indicates a time-dependent structure that the model has not captured (Parlak et al., 2023). A high level of autocorrelation in the residuals implies that the model has not wholly captured the time-dependent structure of the data, implying possible model misspecification (F.Dormann et al., 2007).

2-3-4-6-6 Partial Autocorrelation (PACF) of Residuals

The Partial Autocorrelation Function (PACF) of residuals is a statistical tool used in time series analysis to measure the correlation between a variable and its lagged values while controlling for the effects of intervening lags. When applied to model residuals, it helps assess whether any remaining patterns exist in the data that the model has not addressed captured (Yakubu & Saputra, 2022).

2-3-4-6-7 Residuals vs. Fitted Values (Predicted vs. Actual) Plot:

The residuals vs. fitted values plot helps identify whether the model is underfitting or overfitting the data. If the model fits well, the residuals should be randomly scattered around zero. Any patterns in the plot may indicate model misspecification (Spiegelhalter et al., 2002).

2-3-4-2 CO2 flux measurements

Various comparisons were made to answer the research questions, and suitable statistical tests were chosen based on the comparison and data characteristics.

The Paired Wilcoxon (Wilcoxon signed-rank) test was used to compare the CO2 concentrations of (G50 and G500) and (P50 and P500) and the values of each treatment to the control.

The Wilcoxon signed-rank test is a non-parametric statistical test used to compare two related samples or repeated measurements on the same subjects (Jesussek, 2025). It is beneficial when data are not normally distributed, sample sizes are small, or the measurement scale is ordinal (Sheskin, 2020).

The test determines whether the median difference between paired observations differs significantly from zero. In this comparison, both daily and cumulative concentrations were studied. P-values smaller than 0.05 were accepted as significant differences (Rosner et al., 2006).

2-3-4-3 soil particle size and stability

The statistical study sought to ascertain if xyloglucan and glucose treatments had a beneficial effect on soil stability. As food supplies for microbial populations, it was thought that xyloglucan and glucose would serve as binding agents to promote soil aggregation. This, in turn, was expected to increase the resilience of soil particles against crushing during analysis. To test this, we examined the rate of soil particle reduction and assessed whether there were significant differences among the various treatments.

For each soil type (REC and L2.1), a generalized additive mixed model (GAMM) was fitted using 10-fold cross-validation.

2-4 Study 2: $\delta^{13}C$ signature of CO_2 respired from soils amended with glucose, xyloglucan, and biochar

2-4-1 Experimental treatments and incubation

This study used soils CKA and L2.1, and six treatments were elaborated, as described below. The soil CKA was used instead of REC because of the presence of high concentrations of lignite in REC soil. The existence of lignite could act as a new carbon soil and, as a result, complicate the CO_2 fraction calculation. Treatments in this study are as bellow:

1-Control: Soil only

2- BC: Soil + 10 Mg ha-1 biochar

3- Glucose: Soil + 500 µg g -1 soil glucose

4- Polysac: Soil + 500 µg g -1 soil polysaccharides

5- Glucose + BC: Soil + 500 µg g -1 soil glucose + 10 Mg ha-1 biochar

6- Polysac + BC: Soil +500 µg g -1 soil polysaccharides + 10 Mg ha-1 biochar

These treatments were prepared in larger amounts (about 420 g) and pre-incubated for 72 hours at 23 °C. Thereafter, the equivalent to 3 g of dry soil was transferred to 22 ml gas chromatograph vials (for CO2 flux measurements) and the equivalent to 1 g was transferred to 12 ml vials for $\delta^{13}C$-CO_2 measurements. The two sets of samples were incubated in parallel for 31 (CO2 flux vials) or 33 days ($\delta^{13}C$-CO_2 vials) at 23 °C, and the water content of the samples was maintained at 60% of the water holding capacity by periodically dripping deionized water into the vial. For CO2 flux measurements, the vials were prepared considering four replicates (and their quadruplicates), as explained in Study 1. For $\delta^{13}C$-CO_2 measurements, four replicates were prepared.

2-4-2 CO2 flux measurements from incubated samples

The CO2 flux of the samples was measured on 31 days of incubation following the same procedures and calculations described in Study 1.

2-4-3 Isotope-ratio mass spectrometry analysis of incubated samples for δ13C-CO2 determination

The δ13C-CO2 of the incubated samples was measured on days 3, 6, 10, 13, 17, 20, 25, and 33 of incubation using a Gas Bench coupled to an isotope-ratio mass spectrometer (IRMS, Delta Plux XP, Thermo Fisher Scientific). The vials containing the samples had to be closed 48 hours before

the measurement to allow the determination of the δ13C of the respired CO2. Table 3 exhibits the correspondence between days of measurements and days of incubation.

Table 3 Schedule for IRMS analysis.

Time of vial sealing (day of incubation)	Time of IRMS measurement (day of incubation)	Day of incubation representation
1st	3rd	(1-3td)
4th	6th	(4-6th)
8th	10th	(8-10th)
11th	13th	(11-13th)
15th	17th	(15-17th)
18th	20th	(18-20th)
23rd	25th	(23rd-25th)
31st	33rd	(31st-33rd)

2-4-3-1 Partitioning of CO_2 flux

From two soils (L2.1 and CKA), the Partitioning of CO_2 flux could only be calculated for L2.1 soil. This limitation was due to the close value of δ13C-TOC for CKA soil (-27.2), compared to δ13C for xyloglucan (-26).

The partitioning of the CO_2 flux in the Glucose ($F_{Soil\ in\ Glucose}$), BC ($F_{Soil\ in\ BC}$), and Polysac ($F_{Soil\ in\ Polysac}$) treatments were calculated for each δ^{13}C-CO2 day according to Maestrini et al. (2014), as described in the following equation:

Equation 6 $f = 1 - (\delta 13C_{treatment} - \delta 13C_{amendment})/(\delta 13C_{control} - \delta 13C_{amendment})$

where:

- *f = fraction of CO2 flux derived from the organic carbon amendment (glucose, polysaccharide, biochar)*
- $\delta^{13}C_{treatment}$ *= δ^{13}C in the CO_2 respired from the treatment*
- $\delta^{13}C_{amendment}$ *= δ^{13}C of the organic amendment (glucose = –11.13, polysaccharide =*

–26.00, biochar = –13.66)

- *$\delta^{13}C_{control}$ = $\delta^{13}C$ in the CO_2 respired from the control treatment.*

For "Glucose + BC "and "Polysac+ BC" treatments, the contribution of soil CO_2 emission ($F_{soil\ in\ G+BC}$) and ($F_{soil\ in\ P+BC}$) was calculated using Equations 7.

Equation 7

$$F_{soil\ in\ G+BC}\ OR\ F_{soil\ in\ P+BC} = \frac{(co_2 Flux\ in\ control * (1 + (average\ PE\ in\ "soil + BC\ tretment")\ (average\ PE\ in\ "soil + Glucose\ or\ polysac.\ treatment"))}{ug\ of\ co_2 - Carbon\ in\ "Glucose+ BC"\ OR\ "Polysac + BC\ "treatment}$$

For "Glucose + BC "and "Polysac+ BC", the contribution of biochar CO_2 emission (F_{BC}) and glucose(F_G) or polysaccharide ($F_{Polysac}$) CO_2 emissions were calculated using equations 8 and 9

Equation 8

$$F_{BC} = \frac{(F_{soil\ in\ G+BC}\ and\ F_{soil\ in\ P+BC} * (average\ biochar\ in\ "BC\ tretment")}{average\ F_{Soil\ in\ BC}}$$

Equation 9

$$F_G\ or\ F_{Polysac} = \frac{(F_{soil\ in\ G+BC}\ and\ F_{soil\ in\ P+BC} * (average\ of\ glucose\ or\ polysacharide\ in\ "Glucose\ or\ BC\ tretment")}{averageF_{Soil\ in\ Glucose}\ OR\ averageF_{Soil\ in\ polysac}}$$

2-4-3-2 Priming Effect calculation using δ13C

The PE of biochar, glucose, and polysaccharides on SOC in the BC, Glucose, and Polysac Treatments were calculated according to Maestrini et al. (2014), as follows:

Equation 10

$$PE\% = \frac{((\text{f soil in the treatment} * \text{CO2} - \text{C µg in the treatment}^4) - co_2 Flux\ in\ control\ )}{co_2 Flux\ in\ control}$$

[4] C µg in the treatment = µg carbon molcule in each treatment

The PE in the complete treatments ("Glucose + BC "and "Polysac+ BC".) was calculated using equation 11.

Equation 11

$$\text{PE in "Glucose } + \text{ BC "or "Polysac} + \text{ BC" tretments } = 1 - ((F_{soil\ in\ G+BC}\ OR\ F_{soil\ in\ P+BC}) + \text{glucose}(F_G) \text{ or polysaccharide } (F_{Polysac}) + F_{BC})$$

2-4-4 statistical analysis

2-4-1 CO_2 emissions in root exudate treatment experiment

The Wilcoxon rank-sum test (Mann-Whitney U) was used to compare the CO_2 concentration fractions of each treatment across incubation days, assessing both the control and treatments against each other to determine whether significant differences existed. The test was performed using the Wilcox.test() function with paired = FALSE, as the comparisons were between independent treatments during each incubation interval. Libraries such as "tidyverse," "readxl" and "dplyr" were used in this analysis. The null hypothesis (H_0) posits that the distributions of CO_2 concentrations are the same between the two treatments, while the alternative hypothesis (H_1) indicates a significant difference. A p-value threshold of 0.05 was applied; if $p < 0.05$, the null hypothesis was rejected, indicating a statistically significant difference between treatments (Rosner et al., 2006).

2-4-2 Root exudate treatments and SOC priming effect

The Wilcoxon rank-sum test (Mann-Whitney U) was employed to compare two groups and assess whether their distributions differ significantly. The test was executed using the Wilcox.test() function with paired = FALSE, as the comparisons involved independent treatments within each incubation interval. Libraries such as "tidyverse," "readxl" and "dplyr" were employed in this analysis. The null hypothesis (H_0) posits that the distributions of the SOC priming effect are identical between the two treatments, while the alternative hypothesis (H_1) implies a significant difference. A p-value threshold of 0.05 was applied; if $p < 0.05$, the null hypothesis was dismissed, indicating a statistically significant difference between treatments (Rosner et al., 2006).

3- Results and discussion

3-1 Study 1 - The Role of glucose and polysaccharides in soil particles aggregation and stability

3-1-1 Glucose and xyloglucan stability

3-1-1-1 Comparative analysis of daily CO_2 concentration for selected treatments

The effects of glucose and polysaccharide treatments, each applied at concentrations of 50 and 500 ug g-1 of soil, were evaluated regarding their impact on CO_2 fluxes. Specifically, the daily and cumulative CO_2 flux values for two equivalent concentrations of sugar (G50 vs. P50 and G500 vs. P500) were statistically compared to assess the influence of glucose and xyloglucan on CO_2 emissions.

Figures 3 and 4 compare the average daily Soil+G50 vs Soil+P50 CO_2 fluxes in both soil types. The Y-axis shows CO_2 concentration in $\mu g\ g^{-1}$soil day^{-1}, and the X-axis shows the incubation days. In L2.1 soil, the peak value for Soil+G50 CO_2 fluxes (8.84 $\mu g\ g^{-1}$ soil day^{-1}) appears on the first day. In contrast, the peak value for Soil+P50 CO_2 fluxes (5.84 $\mu g\ g^{-1}$ soil day^{-1}) is recorded on the second day of incubation, which remains lower than the glucose concentration. Statistical results (Table 4) indicate almost no significant difference between these two treatments on the other days of incubation.

For REC soil, the same as L2.1, the significant values primarily occur during the first and second incubation days (Table 4). On the first day of incubation, the Soil+G50 CO_2 concentration (5.87 $\mu g\ g^{-1}$ soil day^{-1}) is the dominant value, while the Soil+P50 CO_2 concentration follows in second place with 4.27 $\mu g\ g^{-1}$ soil day^{-1}which is very close to the Control.

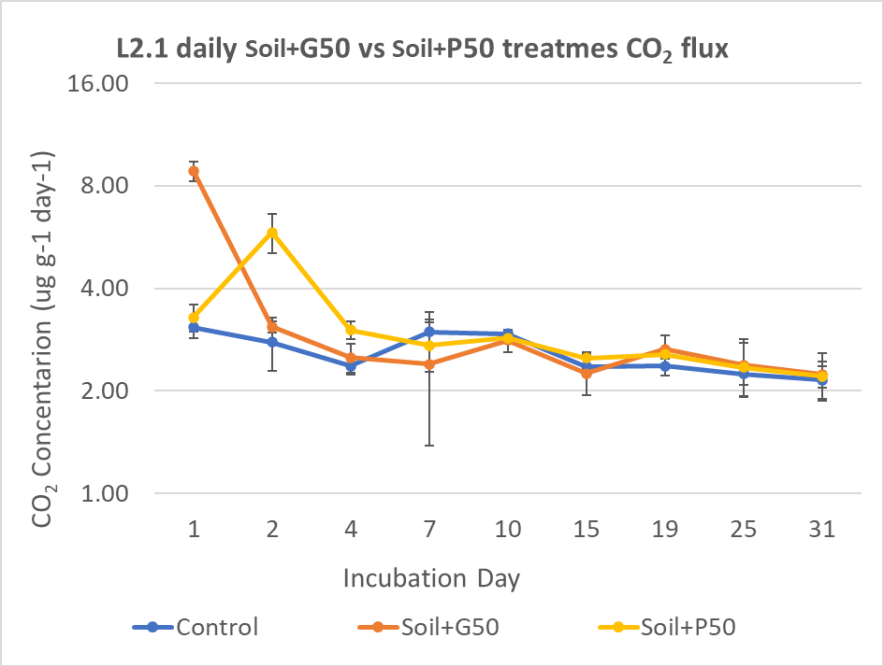

Figure 3 Distribution of daily CO2 concentration over time for Soil+G50 versus Soil+P50 treatments in L2.1 soil.

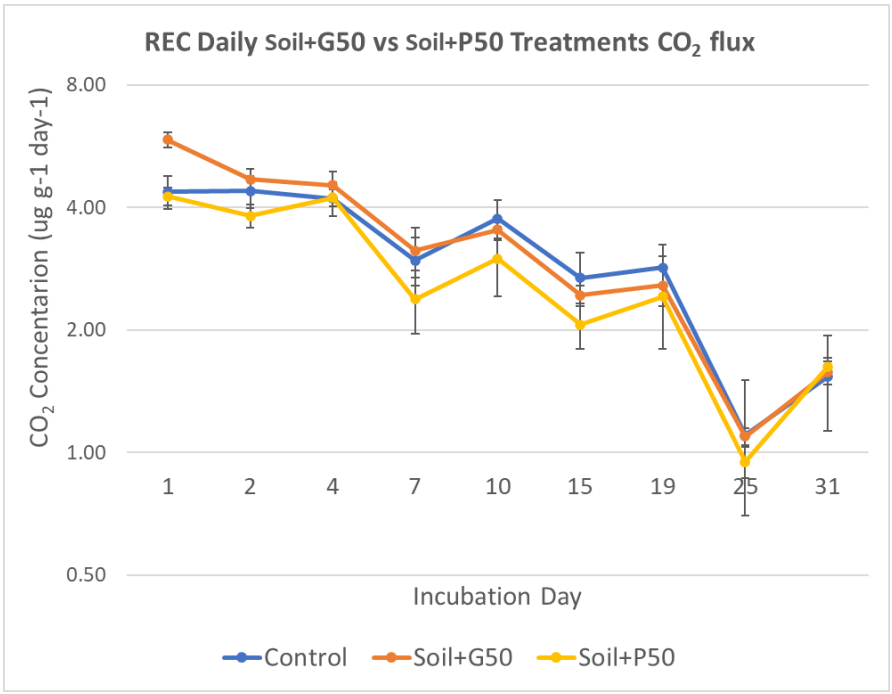

Figure 4 Distribution of daily CO2 concentrations over time for Soil+G50 versus Soil+P50 treatments in REC Soil.

Figures 5 and 6 illustrate the average daily CO_2 flux (µg g^{-1} day^{-1}) for Soil+G500 compared to Soil+P500. According to this graph, for both soil types, on the first and second days of incubation, the highest average CO_2 concentrations (23 and 30.17 µg g^{-1} day^{-1} for L2.1; 45 and 8.80 µg g^{-1} day^{-1} for REC) are associated with the Soil+G500 treatment. The Soil+P500 treatment, similar to the 50 µg g^{-1} xyloglucan concentration, shows significantly lower fluxes (3.67 and 8.24 µg g^{-1} day^{-1} for L2.1; 4.34 and 6.34 µg g^{-1} $day^{-1}$for REC), peaking on the second day of incubation. Based on the statistical results (Table 5), the most significant values for both L2.1 and REC soil are only on the first and the second day of incubation (except for days 7 and 10 in L2.1), and the other incubation days do not significantly change CO_2 concentration.

The pick for CO_2 daily flux for polysaccharide treatments occurs on the second day of incubation, measuring 8.23 µg g^{-1} day^{-1} for L2.1 soil and 6.34 µg g^{-1} day^{-1} for REC soil.

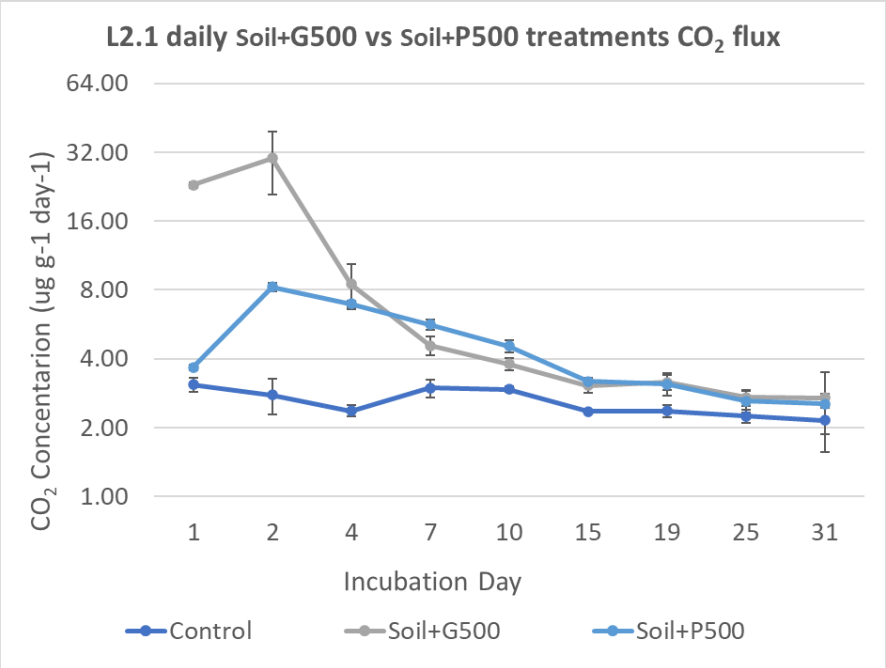

Figure 5 Distribution of daily CO2 concentration over time for Soil+G50 versus Soil+P50 treatments in L2.1 soil

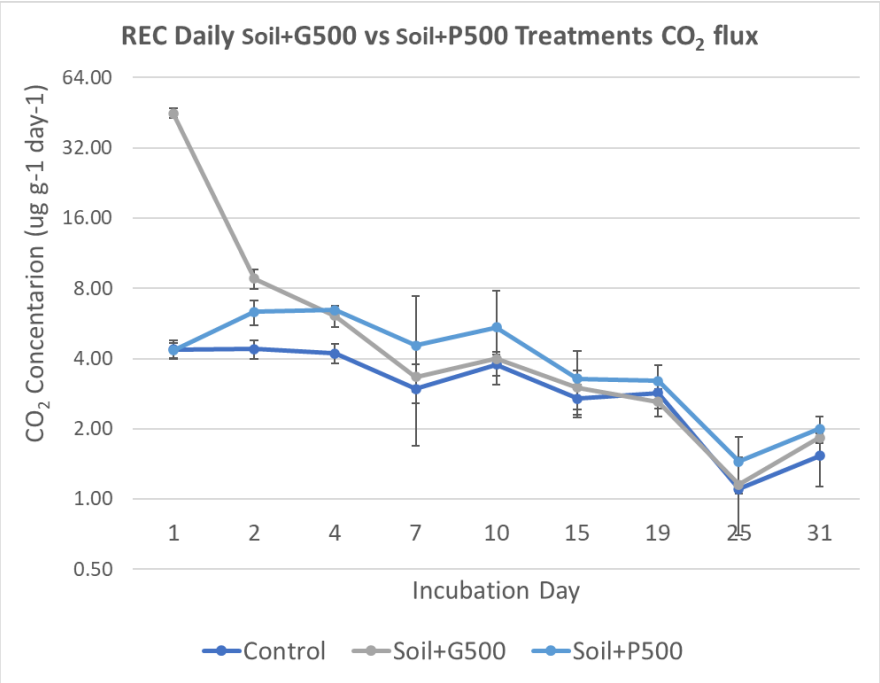

Figure 6 Distribution of daily CO2 concentrations over time for Soil+G50 versus Soil+P50 treatments in REC Soil.

Table 4 Results of the Wilcoxon test comparing Soil+G50 and Soil+P50 treatments for each incubation day.

Incubation day	P_value	Significantcy	Soil type	Treatment Comparison
1	0.03	Yes	L2.1	Soil+G50 vs. Soil+P50
2	0.03	Yes	L2.1	Soil+G50 vs. Soil+P50
4	0.06	No	L2.1	Soil+G50 vs. Soil+P50
7	1.00	No	L2.1	Soil+G50 vs. Soil+P50
10	0.88	No	L2.1	Soil+G50 vs. Soil+P50
15	0.19	No	L2.1	Soil+G50 vs. Soil+P50
19	0.88	No	L2.1	Soil+G50 vs. Soil+P50
25	0.89	No	L2.1	Soil+G50 vs. Soil+P50
31	0.89	No	L2.1	Soil+G50 vs. Soil+P50
1	0.03	Yes	REC	Soil+G50 vs. Soil+P50
2	0.03	Yes	REC	Soil+G50 vs. Soil+P50
4	0.31	No	REC	Soil+G50 vs. Soil+P50
7	0.06	No	REC	Soil+G50 vs. Soil+P50
10	0.31	No	REC	Soil+G50 vs. Soil+P50
15	0.06	No	REC	Soil+G50 vs. Soil+P50
19	0.67	No	REC	Soil+G50 vs. Soil+P50
25	0.04	Yes	REC	Soil+G50 vs. Soil+P50
31	0.67	No	REC	Soil+G50 vs. Soil+P50

Table 5 Results of the Wilcoxon test comparing Soil+G500 and Soil+P500 treatments for each incubation day.

Soil type	Incubation Day	P_value	Significancy	Compared treatments
L2.1	1	0.03	Yes	Soil+G500 vs Soil+P500
L2.1	2	0.03	Yes	Soil+G500 vs Soil+P500
L2.1	4	0.34	No	Soil+G500 vs Soil+P500
L2.1	7	0.03	Yes	Soil+G500 vs Soil+P500
L2.1	10	0.03	Yes	Soil+G500 vs Soil+P500
L2.1	15	0.49	No	Soil+G500 vs Soil+P500
L2.1	19	1.00	No	Soil+G500 vs Soil+P500
L2.1	25	0.69	No	Soil+G500 vs Soil+P500
L2.1	31	0.89	No	Soil+G500 vs Soil+P500
REC	1	0.03	Yes	Soil+G500 vs Soil+P500
REC	2	0.03	Yes	Soil+G500 vs Soil+P500
REC	4	0.69	No	Soil+G500 vs Soil+P500
REC	7	0.49	No	Soil+G500 vs Soil+P500
REC	10	0.34	No	Soil+G500 vs Soil+P500
REC	15	0.69	No	Soil+G500 vs Soil+P500
REC	19	0.20	No	Soil+G500 vs Soil+P500
REC	25	0.34	No	Soil+G500 vs Soil+P500
REC	31	0.49	No	Soil+G500 vs Soil+P500

3-1-1-2 Comparative analysis of cumulative CO_2 concentration for selected treatments

Another research question was to compare the cumulative CO_2 fluxes emitted from glucose and polysaccharide treatments to determine which treatment emits a higher total CO_2. As shown in Figure 7, the cumulative fluxes for both Soil+P50 and Soil+G50 treatments in L2.1 are very close in value (86.82µg g^{-1} day^{-1} and 84.09 µg g^{-1} day^{-1}), and they only exhibit a significant difference on the first and second days of incubation (Table 6) and continue to be substantial until the seventh day in REC soil. Interestingly, the cumulative flux of polysaccharides for L2.1 is slightly higher than that of glucose.

Figure 8 compares the cumulative fluxes of Soil+P50 and Soil+G50 for REC soil. Like L2.1, the Soil+P50 and Soil+G50 treatments show close trends and values compared to the control. However, the dominant flux here belongs to Soil+G50 (85.21 µg g^{-1} day^{-1}), with very close values to the Control (85.29 µg g^{-1} day^{-1}). The Soil+P50 CO_2 concentrations (73.57 µg g^{-1} day^{-1}) are even lower than the control.

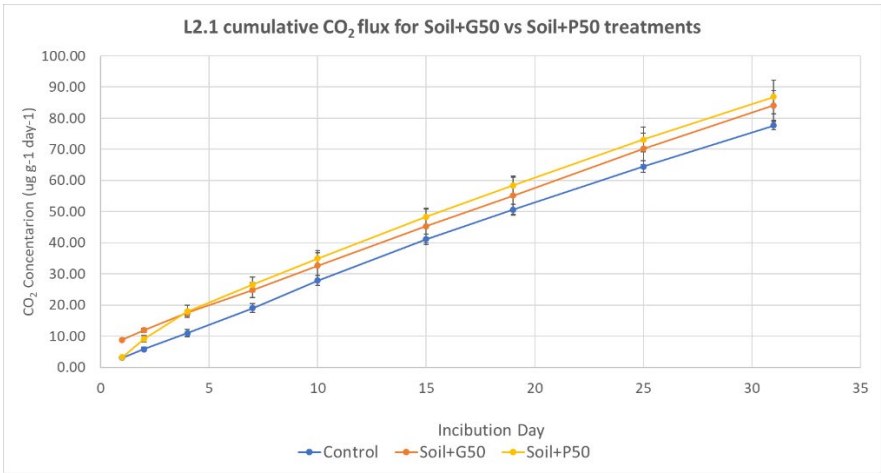

Figure 7 Distribution of cumulative CO2 concentration for Soil+G50 vs Soil+P50 Treatments over time in L2.1 soil.

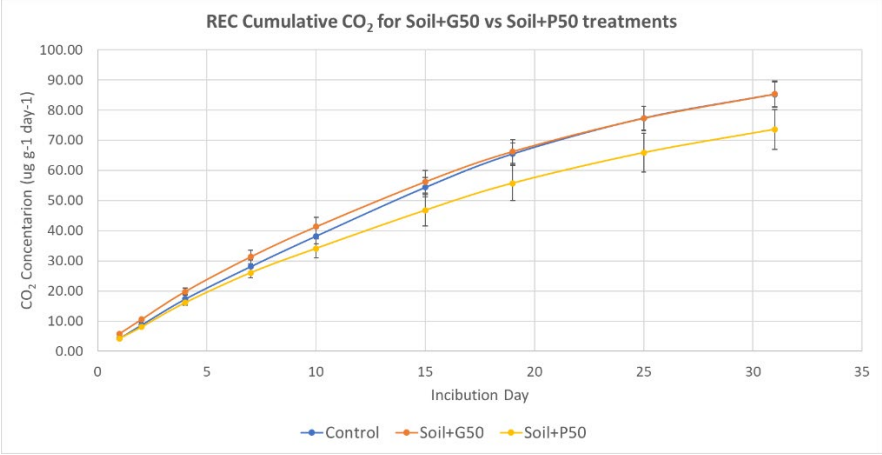

Figure 8 Distribution of cumulative CO2 concentration for Soil+G50 vs Soil+P50 Treatments over time in REC soil.

Figures 9 and 10 illustrate the Cumulative CO_2 Concentration distribution for the Soil+G500 and Soil+P500 treatments for L2.1 and REC soils. Based on the data represented for both soils, Soil+G500 has a higher CO_2 concentration (187.87 µg g^{-1} day^{-1} and 142.85 µg g^{-1} day^{-1}) than the Soil+P500 treatment (179.82 µg g^{-1} day^{-1} and 114.03 µg g^{-1} day^{-1}) for L2.1 and REC soils, respectively. However, values of cumulative Polysaccharide and glucose fluxes are close in L2.1 soil.

Statistical analysis (Table 7) indicates that Significant comparisons for Soil+G500 and Soil+P500 treatments extend to the fourth day in L2.1 soil and the fifteenth day in REC soil.

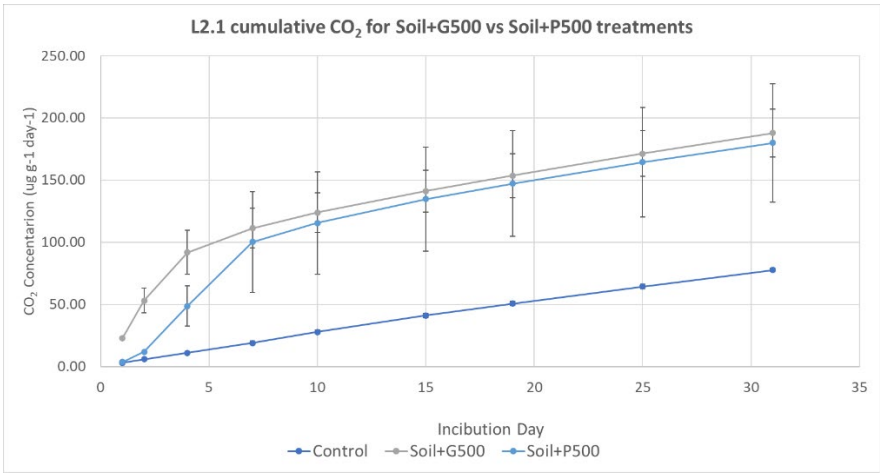

Figure 9 L2.1 distribution of cumulative CO2 concentration for Soil+G500 vs Soil+P500 treatments over time.

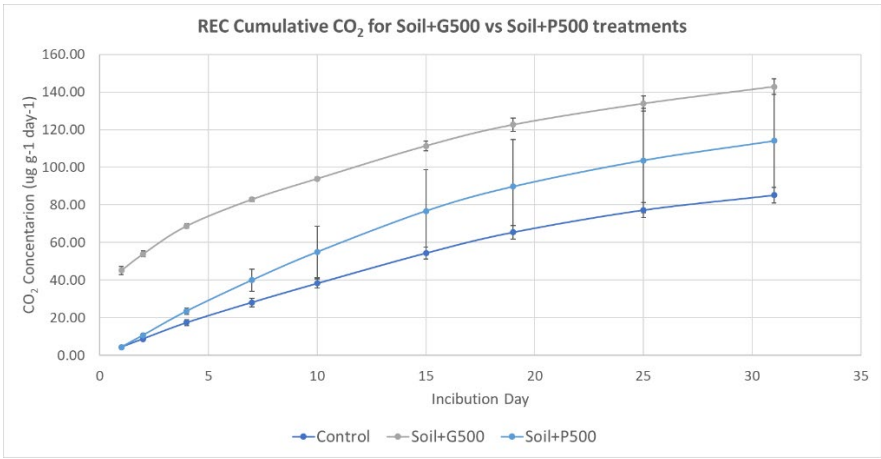

Figure 10 REC distribution of cumulative CO2 concentration for Soil+G500 vs Soil+P500 treatments over time.

3-1-1-3 Soil differences and their potential impact on CO_2 fluxes

The soil's physiochemical properties (Table 2) in this study are crucial in influencing the observed CO_2 fluxes and microbial activity (Moitinho et al., 2021). These properties, including pH, sand/silt/clay composition, bulk density (BD), total organic carbon (TOC), water holding capacity (WHC), and nutrient contents (NH_4^+, NO_3^-, P), differ significantly between the two soils (REC and L2.1), which may explain the variations in the observed CO_2 flux patterns during the incubation. Both daily and cumulative

While L2.1 soil has a higher sand content (87%) and lower water-holding capacity (WHC), which suggests reduced microbial activity due to drier conditions, the increased TOC may compensate for this drawback. Soil texture affects water-holding capacity and microbial habitats (Moitinho et al., 2021). The sandier texture of L2.1 might enhance oxygen diffusion, supporting aerobic

respiration and allowing for more efficient carbon breakdown, resulting in higher CO_2 fluxes (Yang et al., 2018).

Table 6 Results of the Wilcoxon test comparing L2.1 cumulative values of Soil+G50 vs Soil+P50 treatments for each incubation day.

Soil type	Incubation day	p_value	Significancy	Compared treatments
L2.1	1	0.03	Yes	Soil+G50 vs Soil+P50
L2.1	2	0.03	Yes	Soil+G50 vs Soil+P50
L2.1	4	0.69	No	Soil+G50 vs Soil+P50
L2.1	7	0.49	No	Soil+G50 vs Soil+P50
L2.1	10	0.49	No	Soil+G50 vs Soil+P50
L2.1	15	0.49	No	Soil+G50 vs Soil+P50
L2.1	19	0.49	No	Soil+G50 vs Soil+P50
L2.1	25	0.34	No	Soil+G50 vs Soil+P50
L2.1	31	0.49	No	Soil+G50 vs Soil+P50
REC	1	0.03	Yes	Soil+G50 vs Soil+P50
REC	2	0.03	Yes	Soil+G50 vs Soil+P50
REC	4	0.03	Yes	Soil+G50 vs Soil+P50
REC	7	0.03	Yes	Soil+G50 vs Soil+P50
REC	10	0.06	No	Soil+G50 vs Soil+P50
REC	15	0.06	No	Soil+G50 vs Soil+P50
REC	19	0.06	No	Soil+G50 vs Soil+P50
REC	25	0.06	No	Soil+G50 vs Soil+P50
REC	31	0.06	No	Soil+G50 vs Soil+P50

Total Organic Carbon (TOC) is significantly higher in L2.1 soil (0.6%) than in REC soil (0.3%), suggesting that L2.1 soil has more organic material available for microbial degradation and respiration. This higher organic content in REC soil likely contributed to the higher CO_2 fluxes observed in the study (Ashik Rubaiyat et al., 2023).

Although L2.1 soil has a lower water holding capacity (WHC) of 28% compared to REC soil's 44%, it still exhibits higher CO_2 emissions during the incubation period. This could be due to a higher organic carbon content (TOC) and soil texture in L2.1 soil, which can influence microbial activity despite lower moisture retention (Tang, Yang et al., 2022). The sandier texture of L2.1 soil, which allows for better oxygen diffusion, could create more favorable conditions for aerobic microbial respiration and enhance CO_2 fluxes, even without high moisture (Yang et al., 2018). The microbial community in L2.1 soil might be better adapted to more aerobic conditions, where microbial degradation of organic material remains efficient despite lower moisture availability (Keiluweit et al., 2017). Thus, the higher CO_2 fluxes in L2.1 soil could be attributed to its higher organic carbon content, soil texture, and microbial adaptation to the soil environment rather than solely to moisture availability.

Table 7 Results of the Wilcoxon test comparing REC Cumulative values of Soil+G500 vs Soil+P500 treatments for each incubation day.

Soil type	Incubation day	P_value	Significancy	Compared treatments
L2.1	1	0.03	Yes	Soil+G500 vs Soil+P500
L2.1	2	0.03	Yes	Soil+G500 vs Soil+P500
L2.1	4	0.03	Yes	Soil+G500 vs Soil+P500
L2.1	7	0.34	No	Soil+G500 vs Soil+P500
L2.1	10	0.34	No	Soil+G500 vs Soil+P500
L2.1	15	0.34	No	Soil+G500 vs Soil+P500
L2.1	19	0.34	No	Soil+G500 vs Soil+P500
L2.1	25	0.49	No	Soil+G500 vs Soil+P500
L2.1	31	0.49	No	Soil+G500 vs Soil+P500
REC	1	0.03	Yes	Soil+G500 vs Soil+P500
REC	2	0.03	Yes	Soil+G500 vs Soil+P500
REC	4	0.03	Yes	Soil+G500 vs Soil+P500
REC	7	0.03	Yes	Soil+G500 vs Soil+P500
REC	10	0.03	Yes	Soil+G500 vs Soil+P500
REC	15	0.03	Yes	Soil+G500 vs Soil+P500
REC	19	0.20	No	Soil+G500 vs Soil+P500
REC	25	0.34	No	Soil+G500 vs Soil+P500
REC	31	0.34	No	Soil+G500 vs Soil+P500

Nutrient availability can strongly influence microbial activity and, thus, CO_2 flux (Pihlblad et al., 2023). Although REC soil has higher nutrient content and better moisture retention, these factors alone did not outweigh the impact of higher organic carbon in L2.1 soil. The difference in microbial communities and their adaptation to the soil environment may also affect the observed CO_2 emissions.

The pH of the two soils significantly influences microbial activity and, consequently, CO_2 flux (Ashik Rubaiyat et al., 2023). While pH plays a vital role in microbial activity, other factors like soil texture, bulk density, and the presence of nutrients (e.g., nitrogen and phosphorus) are equally important. L2.1 soil, despite its acidic pH, may offer other favorable conditions for microbial activity, such as higher porosity and better oxygen availability, which can lead to increased CO_2 emissions.

3-1-1-4 Implications for CO_2 fluxes and treatment effects

The results in the 5.1.1 and 5.1.2 sections show significant differences in CO_2 concentrations between glucose and polysaccharide treatments (xyloglucan) across different concentrations (50 µg g^{-1} and 500 µg g^{-1}). In particular, glucose treatments exhibited notably higher CO_2 fluxes, both daily and cumulative, compared to polysaccharides.

3-1-1-4-1 Glucose vs. polysaccharide CO_2 emissions

Glucose fractionss consistently led to higher CO_2 concentrations than polysaccharide fractions, with a notable peak observed in L2.1 and REC soils during incubation's first and second days. The initial high emissions associated with glucose can be attributed to its labile nature (Gunina & Kuzyakov, 2015; Demoling et al., 2007). Soil microbes rapidly metabolize glucose, producing swift microbial respiration and CO_2 (Shimizu et al., 2015). This aligns with previous studies indicating that glucose is a powerful carbon source for soil microorganisms (Kuzyakov, 2006).
Conversely, the polysaccharide treatment (xyloglucan) displayed lower CO_2 fluxes. This is likely due to polysaccharides' more complex and recalcitrant nature, which require a longer time for microbial degradation (Gunina & Kuzyakov, 2015; Ravachol et al., 2016). As larger and more chemically complex molecules, polysaccharides are not as readily available to soil microbes as glucose, which accounts for the slower and lower CO_2 emissions throughout the incubation period (Saha et al., 2023; Stubbusch et al., 2024).

3-1-1-4-2 Trends over time and cumulative CO_2 emissions

Both glucose and polysaccharide treatments exhibited a decrease in CO_2 emissions over time, which is expected as the initial surge of microbial activity from the labile carbon source (glucose) subsides(Gunina & Kuzyakov, 2015; Demoling et al., 2007). In L2.1 soil, glucose emissions remained higher than those from polysaccharide treatments throughout the entire incubation period, although peak CO_2 concentrations occurred early in the process. Similarly, in REC soil, the highest CO_2 fluxes for glucose treatments were observed on the first and second days, after which the CO_2 concentrations declined.

The cumulative CO_2 emissions (Figures 7 and 9) further support these trends, with glucose treatments producing significantly higher cumulative CO_2 fluxes than polysaccharide treatments. Notably, in L2.1 soil, the cumulative fluxes for glucose and polysaccharide treatments were relatively close, with glucose slightly surpassing polysaccharides. In REC soil, the cumulative CO_2 flux for glucose treatments remained higher, while polysaccharide treatments displayed relatively stable, lower emissions over time.

3-1-1-4-3 Statistical analysis

The statistical analysis, particularly the Wilcoxon test results, demonstrated that the differences in CO_2 emissions between glucose and polysaccharide treatments were statistically significant on the first and second days of incubation for both L2.1 and REC soils. This suggests that the availability of glucose as a carbon source induces a rapid microbial response, which is not as pronounced with the polysaccharide treatment (Gunina & Kuzyakov, 2015; Sinsabaugh et al., 2013). However, as the incubation period progressed, the differences between the treatments became less significant,

reflecting the depletion of the labile carbon sources and a return to the decomposition of more recalcitrant soil organic carbon (Blagodatskaya & Kuzyakov, 2008; Lehmann & Kleber, 2015).

3-1-1-5 Assessment of daily and cumulative CO_2 concentration in comparison to the control

Another crucial aspect of the study involved comparing the treatments with the control group to assess their soil stability. As illustrated in Figures 11 and 12, the highest CO2 flux was recorded during the initial days of incubation for the Soil+G500 treatment. Although polysaccharides typically peak later than glucose, they never attain the flux levels of glucose.

The statistical analysis revealed that nearly all treatment fluxes (daily and cumulative) significantly differed from the control values (Table 8). There are some exceptions in this regard. For L2.1 and REC soil, on the first day of incubation, the Soil+P50 treatment shows insignificant values. In REC, the Soil+P500 treatment also exhibits similar behavior in CO_2 flux. Since polysaccharides have a more complex structure, their consumption by microorganisms will take longer than that of glucose; hence, this result is expected (Ravachol et al., 2016). The Soil+G50 treatment from day 15 onward is insignificant, which can be explained by the low concentration of glucose and the possibility of completely consuming glucose by microorganisms.

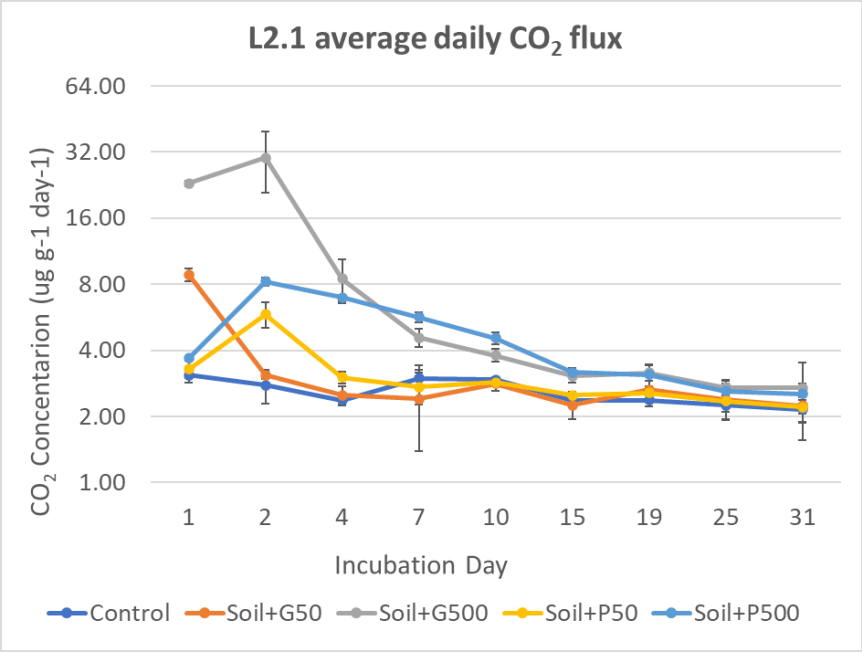

Figure 11 Distribution of daily CO2 concentration for all treatments over time in L2.1 Soil.

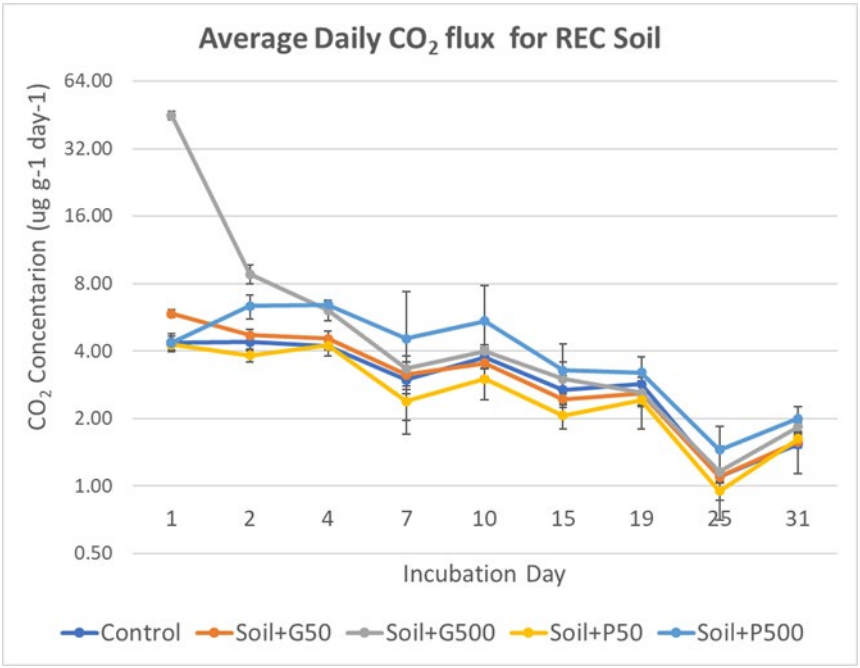

Figure 12 Distribution of daily CO2 concentration for all treatments over time in L2.1 Soil.

Table 8 Results of the Wilcoxon test comparing all treatments vs control for each incubation day.

Soil type	incubation day	treatment	P value	significant	Soil type	incubation day	treatment	P value	significant
L2.1	1	Soil+G50	3.05E-05	Yes	REC	1	Soil+G50	3.05E-05	Yes
L2.1	1	Soil+G500	3.05E-05	Yes	REC	1	Soil+G500	3.05E-05	Yes
L2.1	1	Soil+P50	0.0591	No	REC	1	Soil+P50	0.3	No
L2.1	1	Soil+P500	0.0005	Yes	REC	1	Soil+P500	0.98	No
L2.1	2	Soil+G50	0.0005	Yes	REC	2	Soil+G50	3.05E-05	Yes
L2.1	2	Soil+G500	3.05E-05	Yes	REC	2	Soil+G500	3.05E-05	Yes
L2.1	2	Soil+P50	3.05E-05	Yes	REC	2	Soil+P50	0.0017	Yes
L2.1	2	Soil+P500	3.05E-05	Yes	REC	2	Soil+P500	6.10E-05	Yes
L2.1	4	Soil+G50	0.0005	Yes	REC	4	Soil+G50	0.0002	Yes
L2.1	4	Soil+G500	3.05E-05	Yes	REC	4	Soil+G500	3.05E-05	Yes
L2.1	4	Soil+P50	0.0005	Yes	REC	4	Soil+P50	0.0076	Yes
L2.1	4	Soil+P500	3.05E-05	Yes	REC	4	Soil+P500	3.05E-05	Yes
L2.1	7	Soil+G50	3.05E-05	Yes	REC	7	Soil+G50	0.0010	Yes
L2.1	7	Soil+G500	3.05E-05	Yes	REC	7	Soil+G500	3.05E-05	Yes
L2.1	7	Soil+P50	3.05E-05	Yes	REC	7	Soil+P50	0.0063	Yes
L2.1	7	Soil+P500	3.05E-05	Yes	REC	7	Soil+P500	3.05E-05	Yes
L2.1	10	Soil+G50	0.001	Yes	REC	10	Soil+G50	0.0052	Yes
L2.1	10	Soil+G500	3.05E-05	Yes	REC	10	Soil+G500	3.05E-05	Yes
L2.1	10	Soil+P50	3.05E-05	Yes	REC	10	Soil+P50	0.0008	Yes
L2.1	10	Soil+P500	3.05E-05	Yes	REC	10	Soil+P500	3.05E-05	Yes
L2.1	15	Soil+G50	0.006	Yes	REC	15	Soil+G50	0.13	No
L2.1	15	Soil+G500	3.05E-05	Yes	REC	15	Soil+G500	3.05E-05	Yes
L2.1	15	Soil+P50	3.05E-05	Yes	REC	15	Soil+P50	0.0002	Yes
L2.1	15	Soil+P500	3.05E-05	Yes	REC	15	Soil+P500	6.10E-05	Yes
L2.1	19	Soil+G50	0.008	Yes	REC	19	Soil+G50	0.50	No
L2.1	19	Soil+G500	3.05E-05	Yes	REC	19	Soil+G500	3.05E-05	Yes
L2.1	19	Soil+P50	3.05E-05	Yes	REC	19	Soil+P50	6.10E-05	Yes
L2.1	19	Soil+P500	3.05E-05	Yes	REC	19	Soil+P500	6.10E-05	Yes
L2.1	25	Soil+G50	0.0002	Yes	REC	25	Soil+G50	0.98	No
L2.1	25	Soil+G500	3.05E-05	Yes	REC	25	Soil+G500	3.05E-05	Yes
L2.1	25	Soil+P50	3.05E-05	Yes	REC	25	Soil+P50	3.05E-05	Yes
L2.1	25	Soil+P500	3.05E-05	Yes	REC	25	Soil+P500	0.0002	Yes
L2.1	31	Soil+G50	9.16E-05	Yes	REC	31	Soil+G50	0.9799	No
L2.1	31	Soil+G500	3.05E-05	Yes	REC	31	Soil+G500	3.05E-05	Yes
L2.1	31	Soil+P50	6.10E-05	Yes	REC	31	Soil+P50	3.05E-05	Yes
L2.1	31	Soil+P500	3.05E-05	Yes	REC	31	Soil+P500	9.16E-05	Yes

The following graphs, illustrated in Figure 13 and 14, depict the differences between each treatment and the control for each incubation day. As shown in the figure, on days 1, 2, and 4, the Soil+G500 treatment exhibits the most significant value. Although CO_2 fluxes have decreased notably from day 7 onward, the Soil+P500 treatment displays the most dominant values. From day 19, these treatments (Soil+G500 and Soil+P500) slightly differ. Finally, on the last day of incubation, the Soil+P500 treatment exhibits the highest difference compared to the control. An intriguing point is that even on the final day of incubation, the L2.1 Soil+G500 treatment still shows a significant difference from the control, with Soil+P500 ranking second. A substantial question remains: Which treatment produces the highest cumulative CO_2 emissions?

Comparing the cumulative values shown in Figures 13 and 14 indicates that the CO2 fluxes of Soil+G500 for both soils (142.85 in REC and 187.78 in L2.1) are generally more significant than those of Soil+P500 (114.03 in REC and 179.82 in L2.1). However, the difference between soil+G500 and soil+P500 is slight in L2.1 soil. Polysaccharides are more stable in soil, allowing them to persist longer (Stubbusch et al., 2024).

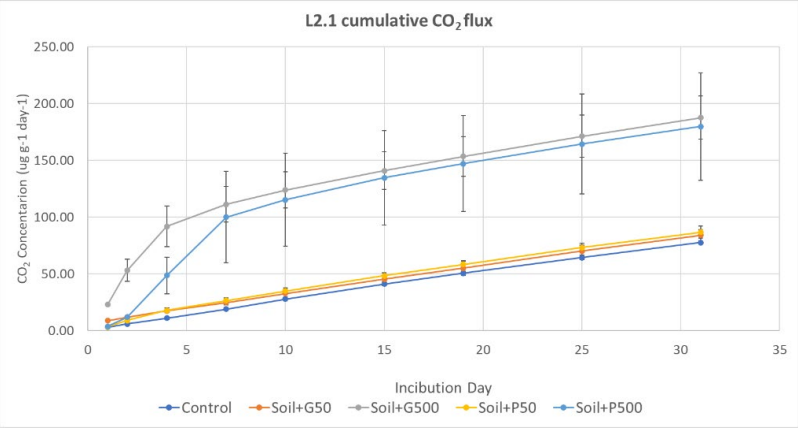

Figure 13 L2.1 Cumulative CO2 concentration.

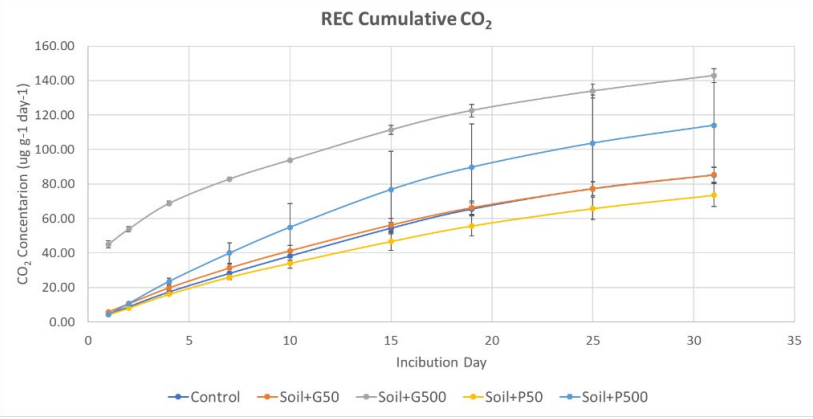

Figure 14 REC Cumulative CO2 concentration.

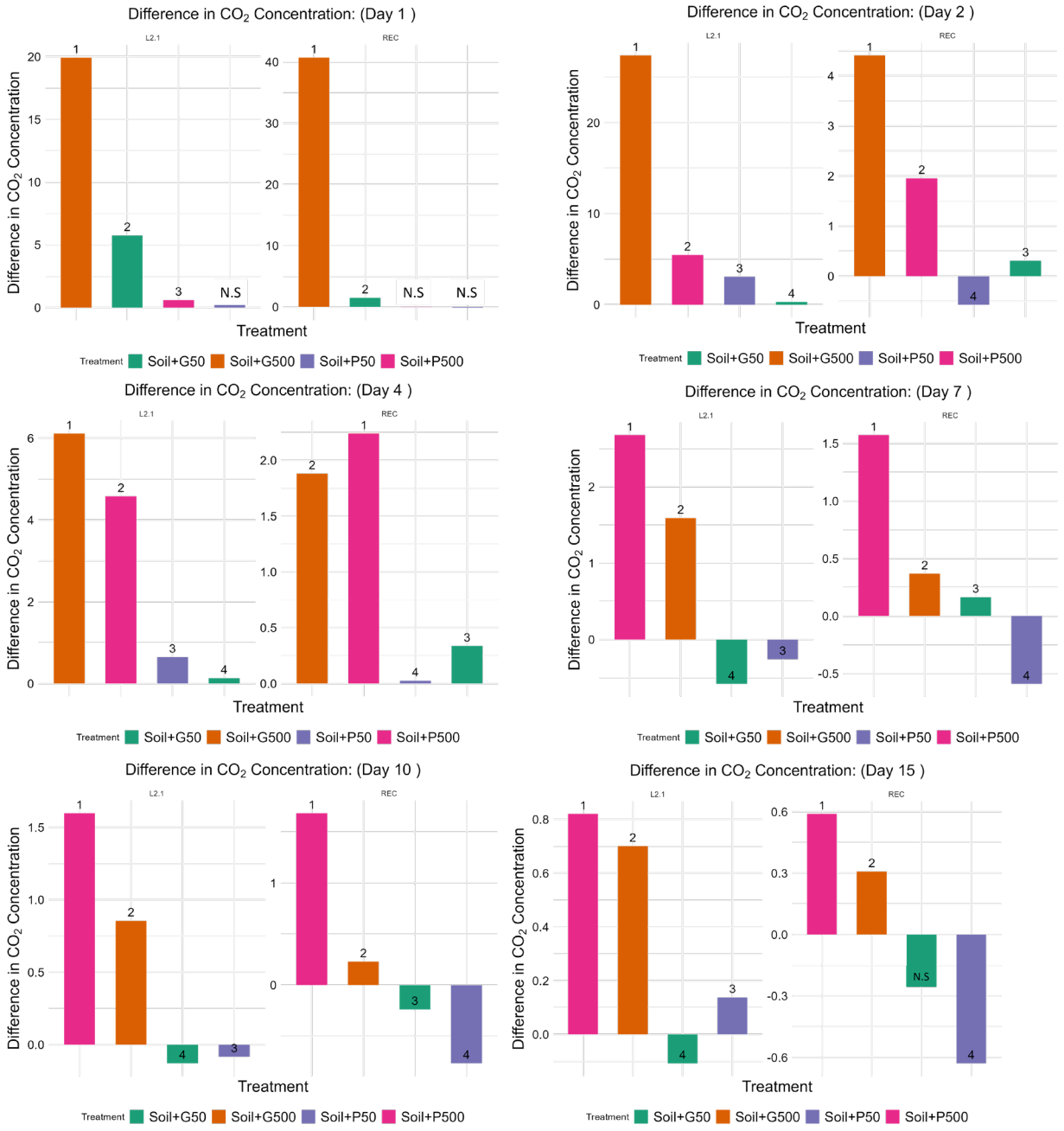

Figure 15 Daily CO_2 Concentration difference in comparison to the control. number 1 shows the most enormous difference, and as the numbers increase, the difference becomes smaller. "N.S" here means the difference with control has not been significant. (day1-15)

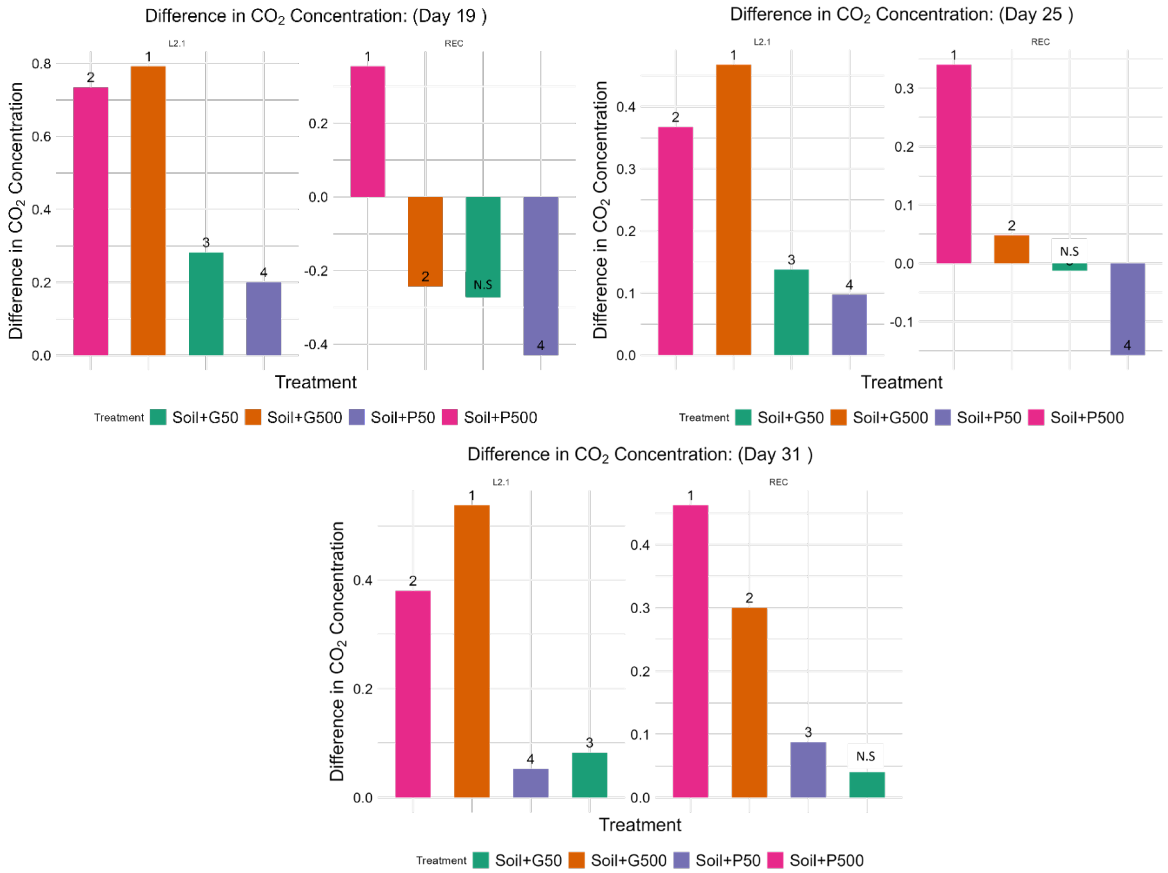

Figure 16 Daily CO_2 Concentration difference in comparison to the Control. number 1 shows the most enormous difference, and as the numbers increase, the difference becomes smaller. "N.S" here means the difference with control has not been significant.(day 19-31)

3-1-2 The Role of polysaccharides in soil aggregation and Stability

Figure 17 illustrates average median particle size changes over 10 minutes for various treatments applied to the L2.1 soil type. The x-axis represents the measurement time (minutes), while the y-axis shows the average median particle size (µm). The plot displays five treatments and one control, each represented by a unique color.

The horizontal dashed line at 20 µm indicates the boundary between small macroaggregates (<20 µm) and large microaggregates (20–250 µm). The text annotations further clarify the distinction between these categories, emphasizing the effect of treatments on changes in particle size. Soil+G500 and Soil+P50 generally exhibit smaller median sizes and mostly fall into the small macroaggregates category.

According to this graph, the L2.1 soil type shows a distinct downward trend in particle size over time, with the effects of particular treatments being more noticeable. This implies that the

treatments have an impact on the aggregation process and soil structure, which may have an impact on the physical traits and attributes of the soil.

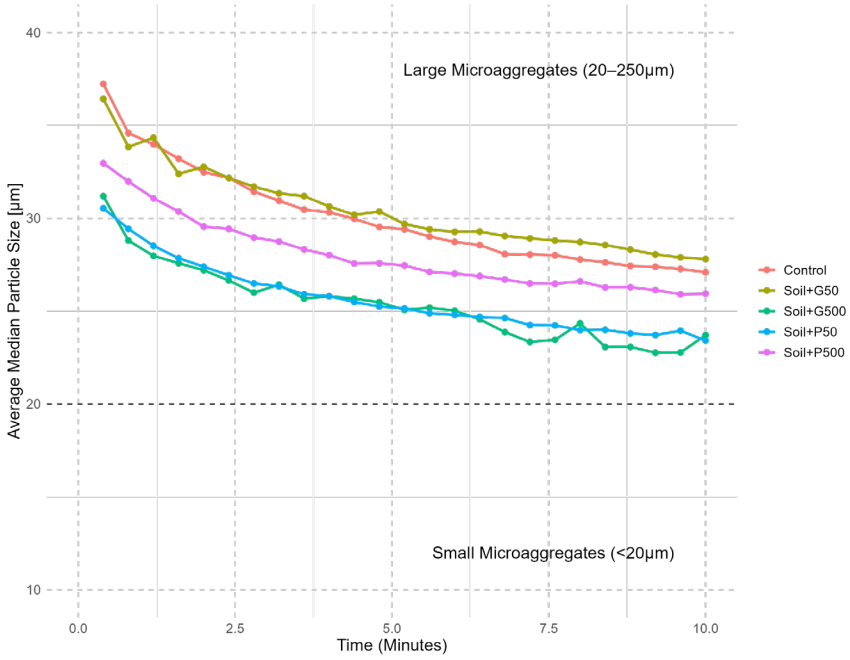

Figure 17 Soil particle size in time for L2.1 soil.

The REC soil type graph (Figure 18) illustrates the changes in particle size over 10 minutes for four different treatments. All treatments consistently decrease particle size; they even reach the area of small macroaggregates. The Soil+P500 treatment shows the largest median size, with particle sizes approaching 20 µm but remaining above it for most of the observation period.

The comparison between the L2.1 and REC soil types reveals distinct differences in the behavior of particle size over time under the same treatments. In both soil types, the particle size decreases over time for all treatments, but the rate of decrease and the final particle size show some variation between the two soils.

For the L2.1 soil type, treatments result in a more pronounced and rapid particle size reduction (from 30.53 to 37.22 and 23.41 to 27.80) for different treatments. The particle size was reduced by around 10 µm by the end of the 10-minute observation period. In contrast, the REC soil type displays a slightly slower particle size reduction rate (starting from 20.69 to 29.62 and ending from 21.80 to 18.29) around 8µm.

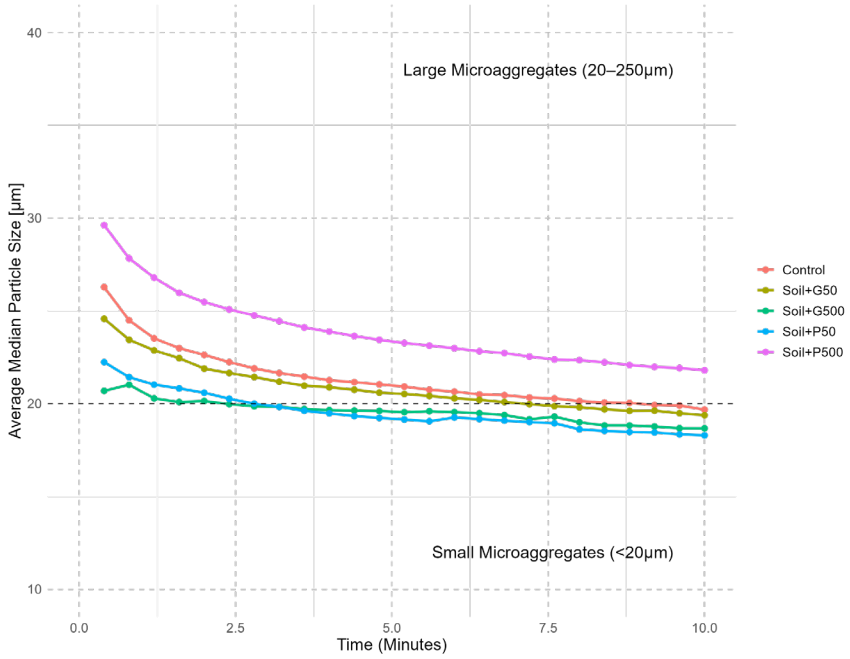

Figure 18 Soil particle size in time for REC soil.

We examined the GAM model's results (Table 9 and Figure 19) to determine whether any specific treatment significantly affects the rate of soil particle reduction (soil stability). Based on these results, the Control reduction rates in REC and L2.1 soil are 31.45% and 36.18%, respectively. Generally, L2.1 soil experiences a higher rate of size reduction than REC. In REC soil, the reduction rate varies between 19.1% and 34.7%, all lower than the control, with the smallest reduction rate occurring in the Soil+G500 treatment. According to our theory, the smallest reduction rate in soil indicates the most effective treatment for soil stability. Interestingly, Soil+P500 exhibits a more significant reduction rate than the control, suggesting this treatment adversely affects soil particle stability. In L2.1 soil, this rate changes from 30.18% to 32.59%, showing more minor fluctuations compared to L2.1, with the most stable particles belonging to Soil+P500 and Soil+P50, the latter having a slight difference that places it in second. Soil+P500 is the most stable treatment in one soil type but the most unstable in another. What could be the possible reasons for this behavior?

Table 9 Results of the GAM model for the soil particle reduction rate (%) across each treatment and control for two soil types.

Treatments	Soil Type	Reduction Rate	AIC	BIC	RMSE
Control	REC	31.45	310.48	347.58	0.45
Soil+G50	REC	28.15	183.79	220.78	0.35
Soil+G500	REC	19.01	294.70	318.42	0.61
Soil+P50	REC	23.50	267.91	298.18	0.53
Soil+P500	REC	34.07	188.77	228.41	0.33
Control	L2.1	36.18	101.02	141.50	0.29
Soil+G50	L2.1	30.42	477.16	505.03	0.88
Soil+G500	L2.1	31.84	346.33	384.12	0.61
Soil+P50	L2.1	32.59	243.09	279.24	0.50
Soil+P500	L2.1	30.18	267.34	302.64	0.51

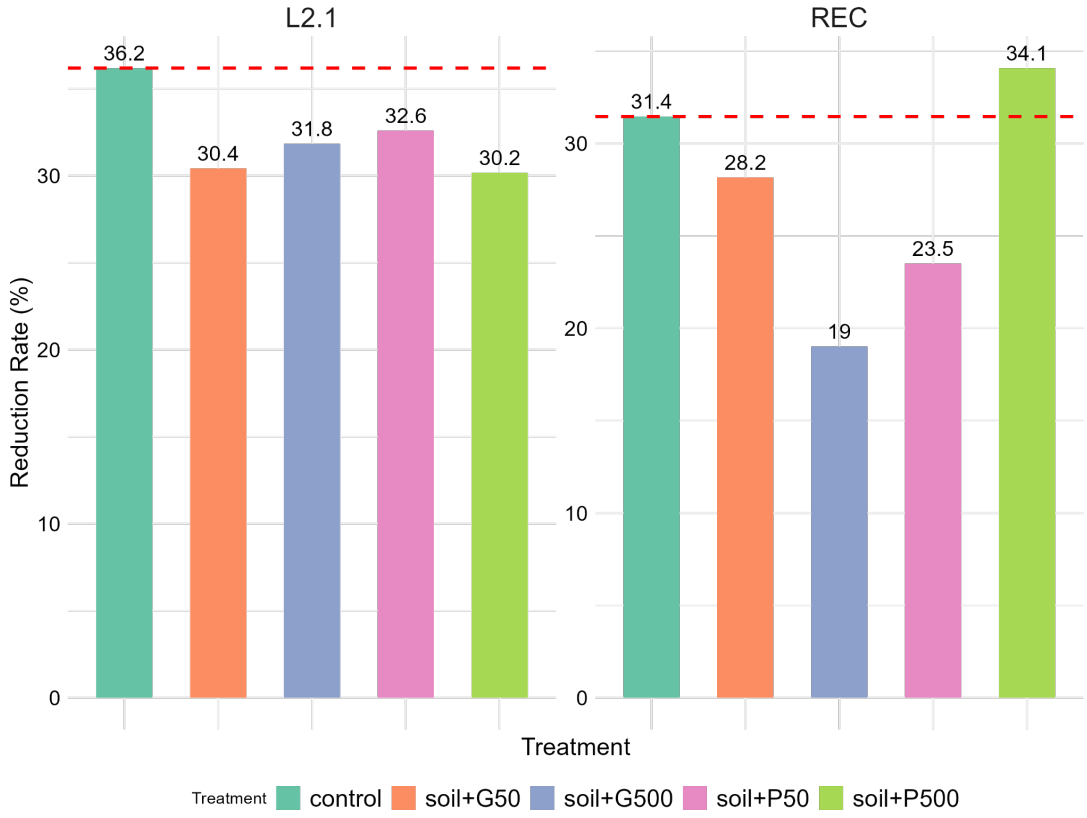

Figure 19 Reduction rate of each treatment in two soil types.

3-1-2-1 Implications for the role of polysaccharides in soil aggregation and stability

A similar study that measured soil particle sizes before incubation, such as Sader (2024), investigated L2.1 and REC soils with xyloglucan amendment. Her findings indicate that soils amended with xyloglucan have larger aggregates than the control. This observation may also apply to glucose treatment.

Since soil aggregation is a dynamic process, the formation and disintegration of aggregates occur over time and aggregate size can change during incubation(Rillig et al., 2017). The addition of

readily available carbon sources like glucose can stimulate microbial activity (Sarker et al., 2022; Li et al., 2020; Gunina & Kuzyakov, 2015), which could, in turn, affect aggregate stability and size over time. Furthermore, incubation conditions, such as wetting and drying cycles, can impact aggregate stability (Evans, 1954). We also know that polysaccharides, especially xyloglucan, act as binding agents to promote aggregation (Cania et al., 2020; Galloway et al., 2018; Read et al., 2003; Bacic et al., 1986). All this argument suggests that as sugar sources remain in the soil longer, they improve soil aggregation and stability (Traoré et al., 2000). As a result, after incubation, treatments that still contain sugar sources like polysaccharide amendments may yield better aggregation.

Another important point is the structural difference between L2.1 and REC soil, which are mainly sandy and silty soils, and the nature of laser diffraction soil particle analysis. As L2.1 particles are bigger, they settle down faster during the soil particle analysis compared to REC soil. As a result, they show a faster median soil size reduction, especially when we consider the raw data (Figures 17 and 18). Rec soil, on the other hand, has smaller soil particles, so it shows a lower soil size reduction rate. But when we look at the result of the GAM model (figure 19), we see that L2.1 is more stable, and this is REC that shows more fluctuations in the reduction rate. Furthermore, we should consider that the outcome of laser diffraction analysis results from a random collision of laser beams with a particle, whether small or large, at a specific time. It also depends on the feeding liquid and may show different fluctuations if we feed the machine with another liquid from the same sample. Finally, the soil particle size median resulting from this method may not be accurate enough to study the trend of soil reduction sizes.

In conclusion, evaluating amendment impacts on soil aggregation and stability is best accomplished by assessing them during the preincubation stage. This guarantees that the soil has not been deprived of more labile sugar sources and has had sufficient time for microbial activation (Kerner et al., 2023). Also, some other methods that examine soil particles in the solid phase, like Scanning electron microscopy (SEM), could be beneficial in identifying aggregate sizes for soil with larger particles (Amelung et al., 2023).

3-1-2-2-1 Soil Texture and Physicochemical Properties

L2.1 soil has higher total organic carbon (TOC) content (0.6%) compared to REC soil (0.3%). The higher TOC in L2.1 soil likely enhances microbial activity, which contributes to the stabilization and aggregation of soil particles, promoting the formation of more stable soil aggregates (da Silva et al., 2022). In contrast, despite having a higher moisture content and better nutrient retention, REC soil showed more fluctuation in particle size reduction. This could indicate that while moisture retention helps maintain soil structure, it might not be as effective in promoting stable aggregation compared to the higher organic carbon content of L2.1 soil (Six et al., 2004); this could later be better explained when we compare different concentrations of sugar treatments and their effect of soil particles reduction.

3-1-2-2-1 Impact of Glucose and Polysaccharides

Glucose treatments (Soil+G50 and Soil+G500) generally exhibited lower reduction rates, likely due to the easily degradable organic material that promotes microbial activity (Gunina & Kuzyakov, 2015; Demoling et al., 2007). However, the polysaccharide treatments (Soil+P50 and Soil+P500) had varying effects. For REC soil, Soil+P500 in particular led to a more notable

decrease in particle size, whereas Soil+P50 had less noticeable impacts. These results imply that the chemical nature of the polysaccharides and the soil's capacity to hold them for microbial digestion determine their impact and permanence. This ability is visible in REC soil when Soil+G500 has the lowest reduction rate, as it is more available for microbial processing and in a more significant concentration than Soil+G50.

Interestingly, while glucose treatments (Soil+G50 and Soil+G500) resulted in higher cumulative CO_2 fluxes, polysaccharides (Soil+P50 and Soil+P500) showed a more lasting impact. This suggests that whereas polysaccharides may gradually enhance soil structure, glucose's effects on soil aggregation may only last a short while. These results are in line with earlier research on soil stabilization (Sarker et al., 2022), which highlights the role polysaccharides play as short-term binding agents that support soil aggregate stabilization over the long run.

However, polysaccharides such as xyloglucan (Soil+P50) have a distinct role due to their complex structure and slower rates of breakdown. The effects on soil aggregation are therefore more gradual and persistent. This is especially evident in the Soil+P500 treatment, where polysaccharides seemed to stabilize soil aggregates, particularly in L2.1 soil, as indicated by the more minor fluctuations in particle size reduction in this treatment compared to glucose treatments.

The study concludes that although while L2.1 soil retains less moisture, its increased organic carbon content provides more substantial particle size reductions and improved soil aggregation stability. On the other hand, REC soil showed more notable variations in particle size decrease due to its increased moisture content, suggesting more variability in soil stability. considering both soils reacted differently to the treatments highlights the need of taking the soil's characteristics into account when choosing additives to improve its stability and structure.

The study's findings highlight how crucial it is to comprehend how different soil types and additives affect soil aggregation and particle size stability. The findings suggest that although glucose and polysaccharides can significantly impact particle size and CO_2 fluxes, the response highly depends on the soil's inherent properties, such as total organic carbon, texture, and moisture content. Future studies should concentrate on understanding the microbial mechanisms driving these changes to optimize soil management practices for enhancing soil health and sustainability.

3-1-2-1 Implications for the role of polysaccharides in soil aggregation and stability

3-1-2-1-1 Effects of Sugar treatments and incubation process

Aggregates develop and disintegrate throughout time, making soil aggregation a dynamic process (Rillig et al., 2017). Aggregate stability may also be impacted by incubation parameters including wetting and drying cycles (Evans, 1954). Adding readily available carbon sources, like glucose, stimulates microbial activity (Sarker et al., 2022; Li et al., 2020; Gunina & Kuzyakov, 2015), affecting aggregate stability and size over time. Polysaccharides, especially xyloglucan, are binding agents that promote aggregation (Cania et al., 2020; Galloway et al., 2018; Read et al., 2003; Bacic et al., 1986). This indicates that as sugar sources persist in the soil, they enhance soil aggregation and stability (Traoré et al., 2000). Recent studies have demonstrated that amendments with

polysaccharides, particularly xyloglucan, can significantly influence soil aggregate size and stability. For instance, Sader (2024)[5] observed larger aggregates in xyloglucan-amended soils compared to controls, a finding that may also apply to glucose treatments. Consequently, treatments that retain sugar sources, such as polysaccharide amendments, may lead to improved aggregation after incubation.

Glucose treatments (Soil+G50 and Soil+G500) generally exhibited lower reduction rates, likely due to the easily degradable organic material stimulating microbial activity (Gunina & Kuzyakov, 2015; Demoling et al., 2007). Polysaccharide treatments (Soil+P50 and Soil+P500) had varying effects, with Soil+P500 resulting in a more significant reduction in particle size for REC soil. These findings suggest that the persistence and impact of polysaccharides vary based on their chemical structure and the soil's capacity to retain them for microbial processing. While glucose treatments resulted in higher cumulative CO_2 fluxes, polysaccharides demonstrated a more lasting impact on soil structure. This indicates that glucose's effects on soil aggregation may be temporary, whereas polysaccharides could sustainably enhance soil structure over time, corroborating previous studies on soil stabilization (Sarker et al., 2022).

3-1-2-1-2 Soil texture, physicochemical properties, and analysis considerations

The texture differences between L2.1 (sandy) and REC (silty) soils influence their behavior during laser diffraction soil particle analysis. L2.1 larger particles settle more quickly during the analysis, demonstrating a faster median soil size reduction in the raw data (Figures 17 and 18). In contrast, with its smaller particles, REC soil exhibits a slower soil size reduction rate. However, the GAM model results (Figure 19) indicate that L2.1 is more stable, while REC experiences more significant fluctuations in the reduction rate.
L2.1 soil's higher total organic carbon (TOC) content (0.6%) compared to REC soil (0.3%) likely enhances microbial activity, contributing to the stabilization and aggregation of soil particles (da Silva et al., 2022).

3-1-2-1-3 Recommendations for future research

It is advised to conduct evaluation at the preincubation stage in order to compare the effects of amendments on soil stability and aggregation. According to Kerner et al. (2023), this method guarantees enough time for microbial activation without exhausting labile sugar sources. Additionally, methods that examine soil particles in the solid phase, such as Scanning Electron Microscopy (SEM), could be beneficial in identifying aggregate sizes for soils with larger particles (Amelung et al., 2023).

[5] ”Sader M, Schrey SD, unpublished data”

when we compare L2.1 and REC soils react to different treatments, the study emphasizes how crucial it is to take soil characteristics into account when choosing additions to improve soil stability and structure. The results highlight the intricate interactions among soil types, amendments, and how they affect particle size stability and soil aggregation. Future research should focus on understanding the microbial mechanisms driving these changes to optimize soil management practices for enhancing soil health and sustainability.

3-1-2-2 model validation

The GAM model's performance is assessed using several evaluation metrics: the AIC (Akaike Information Criterion), the BIC (Bayesian Information Criterion), and the RMSE (Root Mean Squared Error) (Table 10).

3-1-2-2-1 AIC, BIC and RMSE

Generally, smaller AIC and BIC values suggest a better fit for the model (Vrieze, 2012; Bozdogan, 1987). Comparing the AIC and BIC values for the treatments and soil types demonstrates that the Soil+G50 and Soil+G500 treatments in L2.1 soil, have higher values for both AIC and BIC, suggesting that they are the least suitable models for the treatments.
The RMSE value indicates the average size of the prediction errors. A lower RMSE signifies better model predictions (Chai & Draxler, 2014). For example, Control in L2.1 and Soil+P500 in REC soil have the lowest RMSE (0.29 and 0.33), indicating that they fit the observed data more accurately than others. Treatments like soil+G500 (0.60) exhibit a higher prediction error.

3-1-2-2-2 Residual analysis

The residuals analysis evaluates whether the model has captured all the patterns in the data and whether there are any systematic errors.

A. Residuals vs. Measurement Time Plot

Figure 20 shows the residuals (differences between observed and predicted values) versus the fitted values (predicted values from the model) for two soil types, L2.1 and REC. The dots represent different treatments, with each color representing a different treatment.
Ideally, the residuals should be randomly scattered around zero without any noticeable pattern, which indicates that the model has captured most of the underlying data structure and that no systematic errors are present (Kutner et al., 2004). For both soil types, the residuals are evenly distributed, indicating that the model has successfully fitted the data. The model has effectively taken into consideration the primary patterns in the data if the

residuals show no notable trends or curved forms (Hair et al., 2022). This is further supported by the plot's smoothness, which shows no signs of directional drift.

While there is a general scattering of points, the residuals for some treatments in REC soil show slight groupings, especially around specific values on the x-axis. This might suggest some unexplained variability in the data the model has not fully captured (Gujarati et al., 2012). Observing patterns in the residuals, such as the blue line not being entirely horizontal (indicating slight curvature), could signify a non-linear relationship that the model has not thoroughly addressed (Fox, 2015). While this may not be a compelling case in this plot, some residuals still show slight curvature, indicating potential challenges in accommodating certain complexities within the data.

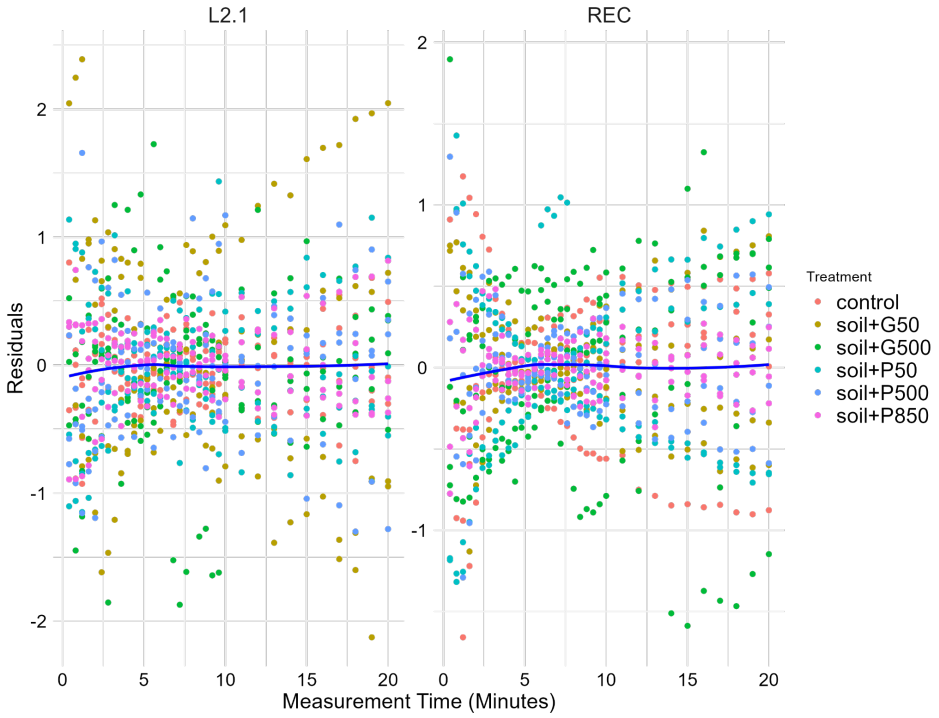

Figure 20 Residuals vs. measurement time by soil types.

B. Residuals vs. Fitted Values Plot

In Figure 21, we observe the relationship between the fitted values (predicted by the model) on the x-axis and the residuals (the differences between observed and predicted values) on the y-axis. Each point represents a different measurement for a specific treatment, with different colors indicating different treatments.

The points are randomly scattered around zero without revealing any significant patterns, a positive sign that the model does not experience major misspecification. Random scatter indicates no

systematic error in the model and has successfully captured the underlying data patterns (Kutner et al., 2004). Neither the L2.1 nor the REC plots display clear, non-random patterns in the residuals, which implies that the model has sufficiently captured the relationship between the predictors and the response variable. The red line fitted through the points represents a trend or relationship identified by the model. The smoother curve implies that the GAM model, which employs smooth functions, effectively addresses the data's non-linearity well.

The L2.1 residuals have a slight U-shaped curvature (around the middle of the graph). This suggests a possible nonlinearity that the model could not fully capture, implying that the relationship might need additional terms or a different modeling approach (e.g., adding a polynomial term or further smoothing) (Fox, 2015).

The REC plot shows a clustering of points, particularly toward the lower fitted values. These clusters suggest that certain factors may influence these observations, which the model has not fully captured. These residual clusters also indicate that the model might have overlooked some underlying patterns specific to the REC soil type (Gujarati et al., 2012).

There are signs of non-linearity (in L2.1) and clustering in the residuals (in REC), suggesting that the model may benefit from additional refinements or the inclusion of more complex terms. The increasing variability in residuals with higher fitted values indicates that the model does not effectively capture the full range of data performance (Hair et al., 2022).

A. ACF (Autocorrelation Function) and PACF (Partial Autocorrelation Function) of Residuals

The ACF (Autocorrelation Function) and PACF (Partial Autocorrelation Function) of residuals (Figure 22) are used to determine if your model's residuals are time-dependent (Box et al., 2016). In ideal model validation, residuals should be independent and exhibit no autocorrelation, indicating that the model has captured all patterns in the data (Shumway & Stoffer, 2017).

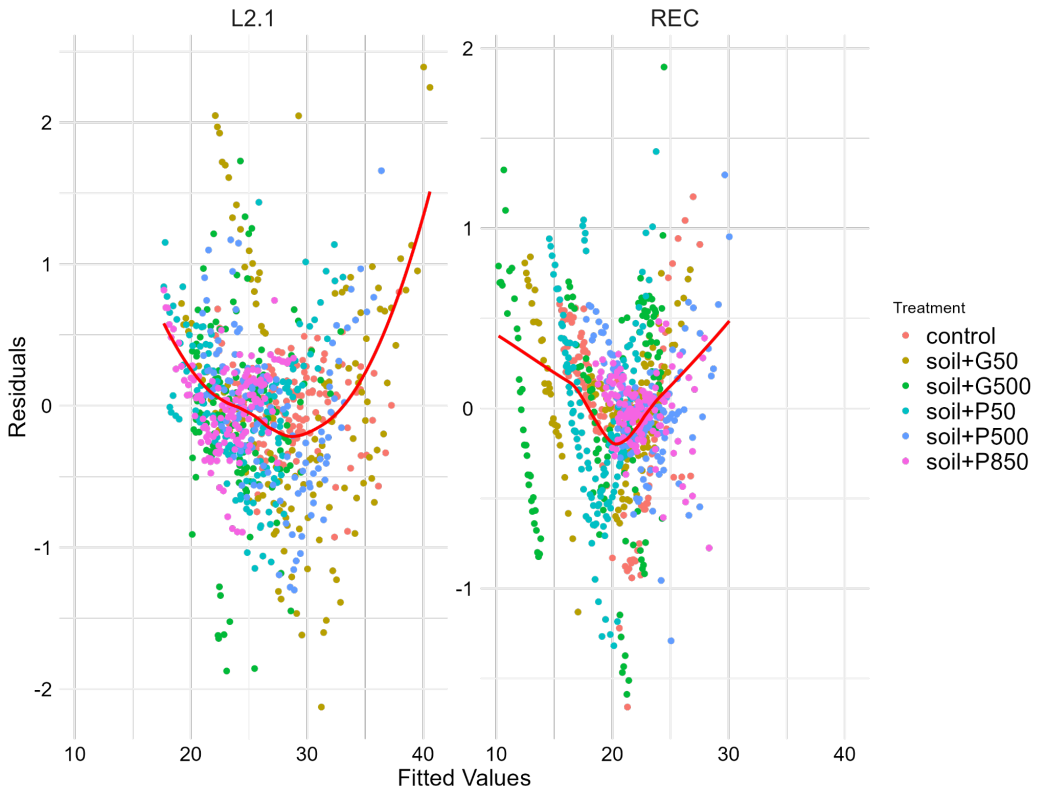

Figure 21 Residuals vs. fitted values by soil types.

- ACF

In an ideal scenario, we want to see that the residuals have no autocorrelation in the ACF graph, meaning that the model missed no systematic pattern (Box et al., 2016). In this plot, the bars for the lags gradually decay to zero without showing any sustained spikes. The values approach zero and stay close to zero across most lags.

There are few or no spikes after lag 0. If the residuals quickly decay towards zero after the first few lags, the model fits the data well and has captured the relevant temporal structure (Shumway & Stoffer, 2017). This is observed in the graph, as the values rapidly decrease after lag 0. The lack of significant autocorrelation suggests that the model has appropriately captured the underlying structure of the data.

- PACF

In an ideal scenario, the PACF plot would show no significant correlations at lags beyond the first few lags, indicating that the model has captured the necessary time dependencies (Box et al., 2016). The PACF plot suggests that most bars are near zero, which is a good sign. The values do not show significant spikes beyond lag 0, which indicates that no additional time-dependent structure is left in the residuals. The model seems well-fitted as there are no significant spikes or patterns in the PACF, and residuals quickly drop to zero after lag 0.

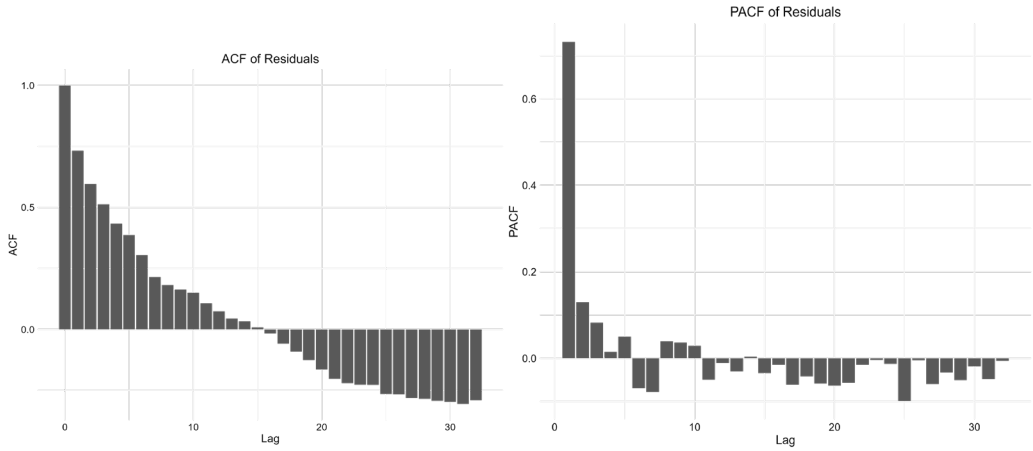

Figure 22 ACF and PACF of residuals.

3-2 Study 2: δ13C signature of CO2 respired from soils amended with glucose, xyloglucan, and biochar

3-2-1 CO_2 emissions in root exudate fractions

The primary concern regarding CO_2 emission concentrations from GC analysis, as highlighted in the "Role of Polysaccharides in Soil Aggregation and Stability" experiment, is that the measured CO_2 reflects a combination of emissions from different soil sources, including soil, glucose, and polysaccharides (Wu et al., 2024; Ray et al., 2020). Furthermore, in the second study, biochar, another key contributor to CO_2 emissions, is added to the potential sources. However, in many studies, biochar amendments have resulted in the reduction of CO_2 emissions, especially in more extended incubation periods (Yang et al., 2020; Deng et al., 2024; Wang et al., 2020). Consequently, when we report the CO_2 concentration for a particular treatment, we indicate the total emissions from all sources. Additional methods are necessary to distinguish the contribution of each carbon source. We can accurately quantify the CO_2 emitted from each carbon source by employing ^{13}C isotopic calculations based on CO_2 concentrations analyzed via GC and the ^{13}C isotopic values for each treatment determined through IRMS analysis (Kuzyakov, 2006). This method lets us directly compare carbon emissions from each source (Fry, 2019). The question is whether the varied use of biochar amendment for soil improvement, particularly for agricultural purposes, will influence the effect of root exudate amendments (specifically glucose and polysaccharide amendments). Do soils amended with root exudates behave differently with or without biochar on carbon emissions and, more generally, the priming effect of soil organic carbon?

Figure 23 illustrates the daily concentration of CO_2 generated from Glucose, Glucose + Biochar, Polysaccharide (xyloglucan), and Polysaccharide + Biochar fractions in L2.1 soil. The x-axis

shows the incubation days, while the y-axis represents the concentration of grams of carbon released from CO_2 in micrograms per gram of soil.

Figure 17 shows that on the second day of incubation, the emissions from Glucose and Glucose + Biochar fractions (31.87 and 30.97µg g^{-1}soil) are considerably higher than the values for Polysaccharide and Polysaccharide + Biochar (10.84 and 10.97 µg g^{-1}soil). As incubation continues, the concentration of released carbon decreases, reaching values of 0.1, 0.09, 0.24, and 0.18 on the last day, corresponding to Glucose, Glucose + Biochar, Polysaccharide, and Polysaccharide + Biochar fractions.

Regarding the research question, both Glucose and Glucose + Biochar and Polysaccharide and Polysaccharide + Biochar fractions exhibit the same behavior regarding carbon emissions, indicating that biochar does not influence this behavior.

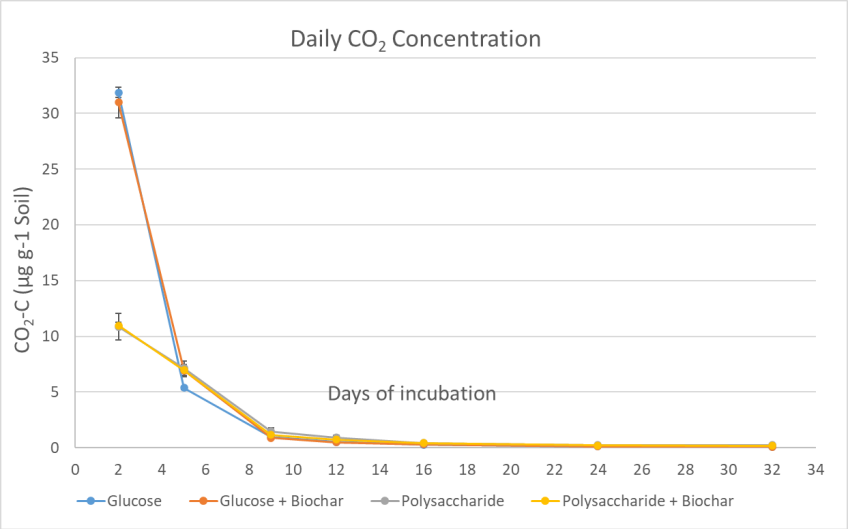

Figure 23 Dynamic of Glucose and Polysaccharide composition with or without Biochar.

The graph (Figure 24) allows us to examine daily CO_2 concentrations more specifically and compare these treatment values for each incubation period. Generally, only fluxes greater than control are associated with Days 2 and 5, where we observe glucose CO_2 flux first on day 2, followed by polysaccharide CO_2 flux. With a delay on day 5, CO2 fluxes representing Glucose+Biochar, Polysacharide, and Polysacharide+Biochar fractions are placed at the first level and Glucose fraction at the second. This behavior mirrors the first study, where the highest daily CO_2 flux for L2.1 soil on the second day of incubation was observed in Soil+G500 treatment, while on the fourth day, Soil+P500 treatment surpassed it. From day 9 onward, all CO_2 concentrations are lower than the control, and fluxes containing polysaccharides typically have slightly higher numbers. The results of the Wilcoxon test (Table 10) were used to compare the CO_2 concentrations between treatments in each incubation period to determine if there was a significant difference.

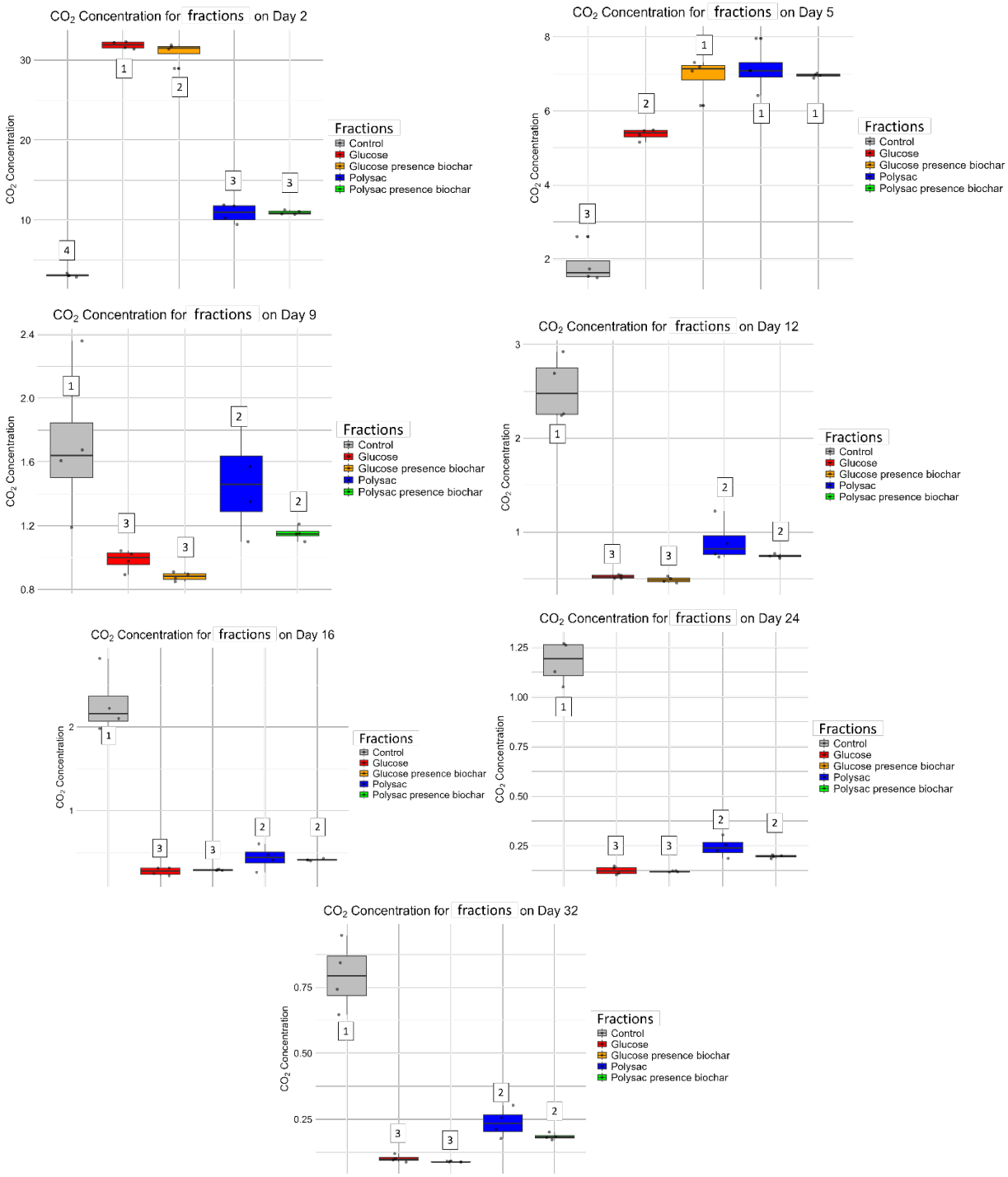

Figure 24 CO_2 concentration (µg g-1 soil) across incubation days and fractions. This ordering is based on statistical analysis; fractions with the same order number don't show significant differences. Level 1 shows the highest, and 4 shows the lowest level regarding the CO_2 concentration. fractionss. fractions: BC=Biochar; Glucose+ BC= Glucose+ Biochar; Polysac= Polysacharide; Polysac+BC= Polysacharide+ Biochar.

Table 10 Results of the Wilcoxon test comparing the CO2 concentrations of all treatments against each other for each incubation day. fractionss. fractions: BC=Biochar; Glucose+ BC= Glucose+ Biochar; Polysac= Polysacharide; Polysac+BC= Polysacharide+ Biochar.

Day of Incubation	Treatment 1	Treatment 2	P_value	Significancy	Day of Incubation	Treatment 1	Treatment 2	P_value	Significancy
2	Control	Glucose	0.03	Yes	12	Glucose	Polysaccharide	0.03	Yes
2	Control	Glucose + Biochar	0.03	Yes	12	Glucose	Polysaccharidecharide+ Biochar	0.03	Yes
2	Control	Polysaccharide	0.03	Yes	12	Glucose + Biochar	Polysaccharide	0.03	Yes
2	Control	Polysaccharidecharide+ Biochar	0.03	Yes	12	Glucose + Biochar	Polysaccharidecharide+ Biochar	0.03	Yes
2	Glucose	Glucose + Biochar	0.49	No	12	Polysaccharide	Polysaccharidecharide+ Biochar	0.34	No
2	Glucose	Polysaccharide	0.03	Yes	16	Control	Glucose	0.03	Yes
2	Glucose	Polysaccharidecharide+ Biochar	0.03	Yes	16	Control	Glucose + Biochar	0.03	Yes
2	Glucose + Biochar	Polysaccharide	0.03	Yes	16	Control	Polysaccharide	0.03	Yes
2	Glucose + Biochar	Polysaccharidecharide+ Biochar	0.03	Yes	16	Control	Polysaccharidecharide+ Biochar	0.03	Yes
2	Polysaccharide	Polysaccharidecharide+ Biochar	1.00	No	16	Glucose	Glucose + Biochar	1.00	No
5	Control	Glucose	0.03	Yes	16	Glucose	Polysaccharide	0.11	No
5	Control	Glucose + Biochar	0.03	Yes	16	Glucose	Polysaccharidecharide+ Biochar	0.03	Yes
5	Control	Polysaccharide	0.03	Yes	16	Glucose + Biochar	Polysaccharide	0.34	No
5	Control	Polysaccharidecharide+ Biochar	0.03	Yes	16	Glucose + Biochar	Polysaccharidecharide+ Biochar	0.03	Yes
5	Glucose	Glucose + Biochar	0.03	Yes	16	Polysaccharide	Polysaccharidecharide+ Biochar	0.69	No
5	Glucose	Polysaccharide	0.03	Yes	24	Control	Glucose	0.03	Yes
5	Glucose	Polysaccharidecharide+ Biochar	0.03	Yes	24	Control	Glucose + Biochar	0.03	Yes
5	Glucose + Biochar	Polysaccharide	0.89	No	24	Control	Polysaccharide	0.03	Yes
5	Glucose + Biochar	Polysaccharidecharide+ Biochar	0.34	No	24	Control	Polysaccharidecharide+ Biochar	0.03	Yes
5	Polysaccharide	Polysaccharidecharide+ Biochar	0.34	No	24	Glucose	Glucose + Biochar	1.00	No
9	Control	Glucose	0.03	Yes	24	Glucose	Polysaccharide	0.03	Yes
9	Control	Glucose + Biochar	0.03	Yes	24	Glucose	Polysaccharidecharide+ Biochar	0.03	Yes
9	Control	Polysaccharide	0.49	No	24	Glucose + Biochar	Polysaccharide	0.03	Yes
9	Control	Polysaccharidecharide+ Biochar	0.06	No	24	Glucose + Biochar	Polysaccharidecharide+ Biochar	0.03	Yes
9	Glucose	Glucose + Biochar	0.11	No	24	Polysaccharide	Polysaccharidecharide+ Biochar	0.20	No
9	Glucose	Polysaccharide	0.03	Yes	32	Control	Glucose	0.03	Yes
9	Glucose	Polysaccharidecharide+ Biochar	0.03	Yes	32	Control	Glucose + Biochar	0.03	Yes
9	Glucose + Biochar	Polysaccharide	0.03	Yes	32	Control	Polysaccharide	0.03	Yes
9	Glucose + Biochar	Polysaccharidecharide+ Biochar	0.03	Yes	32	Control	Polysaccharidecharide+ Biochar	0.03	Yes
9	Polysaccharide	Polysaccharidecharide+ Biochar	0.34	No	32	Glucose	Glucose + Biochar	0.31	No
12	Control	Glucose	0.03	Yes	32	Glucose	Polysaccharide	0.03	Yes
12	Control	Glucose + Biochar	0.03	Yes	32	Glucose	Polysaccharidecharide+ Biochar	0.03	Yes
12	Control	Polysaccharide	0.03	Yes	32	Glucose + Biochar	Polysaccharide	0.03	Yes
12	Control	Polysaccharidecharide+ Biochar	0.03	Yes	32	Glucose + Biochar	Polysaccharidecharide+ Biochar	0.03	Yes
12	Glucose	Glucose + Biochar	0.20	No	32	Polysaccharide	Polysaccharidecharide+ Biochar	0.20	No

The cumulative CO_2 values in Figure 26 show that glucose fractions with and without biochar (109.58, 106.78 µg g^{-1}soil) exhibit the most significant values. This indicates that glucose in the soil leads to the most considerable CO_2 fluxes, and biochar does not influence the behavior of glucose and polysaccharides, or more precisely, root exudates in the soil.

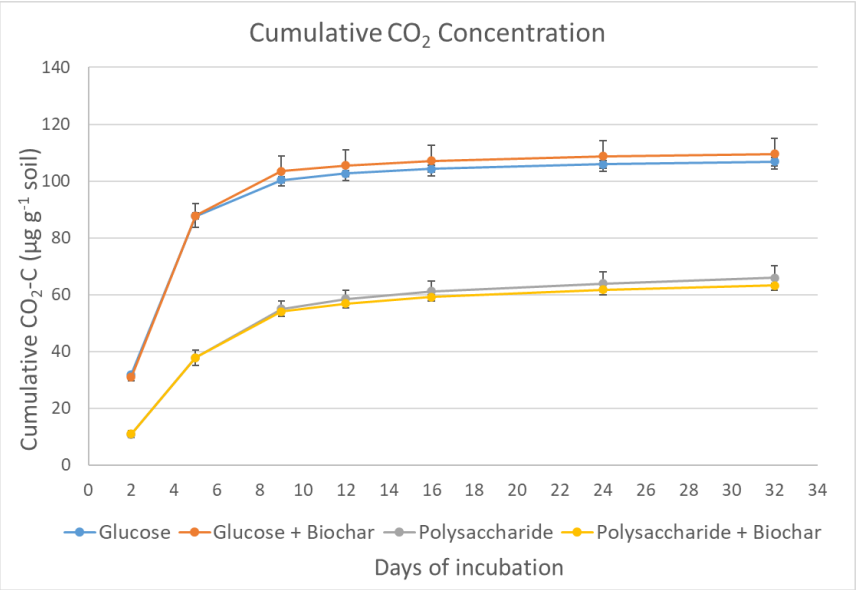

Figure 25 Cumulative glucose and polysaccharide composition with or without biochar.

3-2-1-2 Implication for Carbon emissions in root exudates treatment

The results from the two sections, 3-1-1 (Glucose and xyloglucan stability) and 3-2-1 (CO_2 emissions in root exudate treatments), show a significant difference in CO_2 emissions between glucose and xyloglucan fractions. Expressly, in terms of cumulative emissions, glucose fractions consistently release higher amounts of CO_2 and carbon compared to the same concentrations of xyloglucan, regardless of the presence of biochar. Research on soil carbon cycling and its possible effects on agricultural practices depends on a knowledge of the various mechanisms that may account for this variance in carbon emissions.

3-2-1-2-1 Differences in carbon bioavailability and microbial degradation

Glucose, a simple monosaccharide, is highly bioavailable to soil microorganisms (Gunina & Kuzyakov, 2015; Nguyen & Guckert, 2001). It provides an immediate and readily accessible carbon source, allowing microbes to rapidly metabolize it through aerobic respiration, leading to a substantial and quick release of CO_2 (Fierer et al., 2007; Sinsabaugh et al., 2013). On the other hand, xyloglucan, a polysaccharide, is a more complex organic compound that needs microbial enzymatic processing before it can be fully utilized (Shimada et al., 2024; Vieira et al., 2021). The slower breakdown of xyloglucan may lead to lower CO_2 emissions than glucose, as soil microbes metabolize it more slowly (Saha et al., 2023). The slower breakdown of xyloglucan by soil microbes (Saha et al., 2023) may result in lower CO_2 emissions than simpler carbohydrates like glucose. Although A study that directly compares the breakdown rates of xyloglucan and glucose was not found, Rui & Anderson (2016) noted that xyloglucan's role in cell wall structure and

stomatal function implies its breakdown is regulated within broader plant physiological processes, potentially leading to a more controlled release of CO_2. Research by ikbel benalaya et al. (2024) indicates that polysaccharides with a more complex structure have a slower degradation rate and CO_2 emissions than monosaccharides, which often experience a sudden spike.

3-2-1-2-2 Biochar's Role in CO_2 Emissions

The presence of biochar in both glucose and xyloglucan fractions did not significantly alter the overall pattern of CO_2 emissions, as evidenced by the similar emissions observed in both Glucose + Biochar and Polysaccharide + biochar fractions. Biochar affects soil microbial communities by providing additional surface area for microbial colonization, improving soil structure, and enhancing nutrient and water retention (Du et al., 2016; Luo, Zang, et al., 2017). However, in this study, biochar did not significantly influence CO_2 emissions from glucose or xyloglucan. This suggests that while biochar might alter soil physical properties, its effect on microbial carbon turnover and CO_2 emissions may be less significant in the presence of more readily available carbon sources like glucose (Luo et al., 2011; Whitman et al., 2014). Furthermore, it's possible that the experiment's very brief incubation time was insufficient for biochar to have any discernible impact on carbon cycling and microbial dynamics (Wang et al., 2015). Additionally, it might take some time for microorganisms to adjust to the presence of biochar and create plans for using it as a substrate. Lehmann et al. (2011) observed that the microbial colonization of biochar particles and the formation of biochar-associated microbial communities is a gradual process.

3-2-1 Root exudate fractions and SOC priming effect

Figure 27 illustrates the SOC priming effect across various fractions during incubation. The x-axis indicates the days of incubation (from 0 to 35), while the y-axis displays the priming effect on SOC in $\mu g\ C\ g^{-1}$ soil. The Glucose fraction shows a notable peak in priming effect on day 1 or 2 (just after the incubation begins), followed by a sharp decline as the incubation progresses. The glucose + Biochar fraction reflects a similar trend to glucose alone, but the magnitude of the priming effect is generally lower. The polysaccharide fractions (Polysaccharide and Polysaccharide+ Biochar) demonstrate a much smaller priming effect than glucose, with a relatively steady and low priming effect observed throughout the incubation period. As expected, biochar alone shows little to no priming effect on SOC because biochar typically influences long-term soil characteristics and microbial colonization rather than causing immediate priming effects.

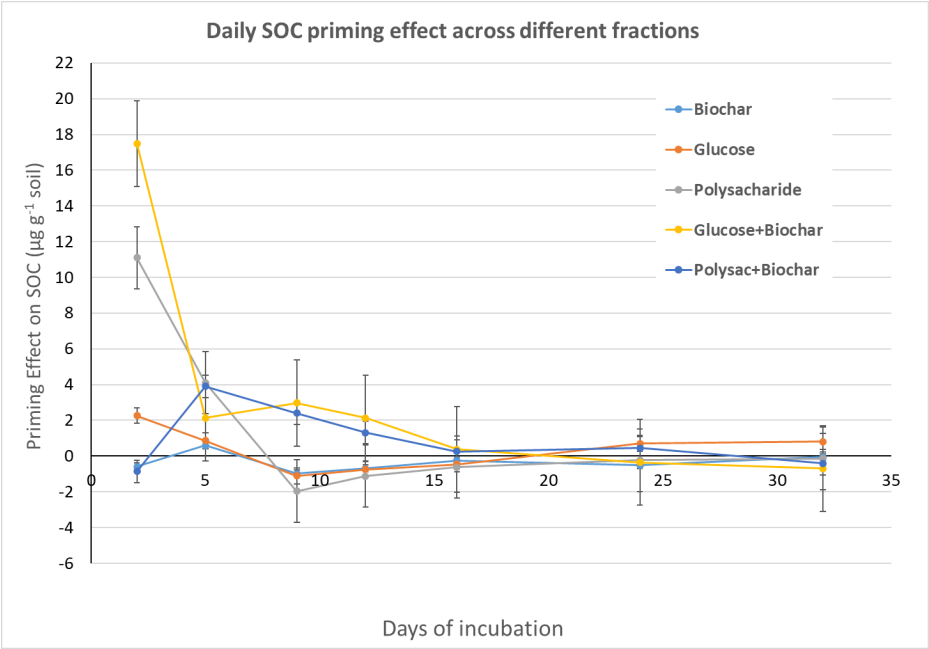

Figure 26 Soil organic carbon priming effect across different fractions.

Figure 27 enables a close examination of each incubation period. These graphs also present the pairwise Wilcoxon rank-sum test results, which were used to establish the order of significance for their PE values. Fractions indicated with the same number do not show a significant difference.

As observed in the first week of incubation, PE values are more significant. Interestingly, the Glucose + Biochar fraction has the highest PE value in almost all incubation periods. On days 4-6, the polysaccharide fractions (Polysaccharide and Polysaccharide+ Biochar) have the dominant PE value. From day 6 onward, the Polysaccharide + Biochar and Glucose+ Biochar fractions show the most significant PE values. From day 23, the Glucose treatment takes the lead for PE value; from this day forward, nearly all other treatments exhibit negative PE values. The results of the Wilcoxon test (Table 11) were used to compare the significance of CO_2 concentrations between fractions in each incubation interval.

Examining cumulative PE values (Figure 28), the Glucose+ Biochar fractions, with a value of 65.68 (µg g^{-1} soil), exhibit nearly double the PE compared to Polysaccharide+ Biochar (28.05 µg g^{-1} soil) and Polysaccharide (25.17 µg g^{-1} soil), which have close values for second and third place, respectively. The PE values for the Glucose fraction remain consistently low across incubation periods, while the Biochar fraction, except for the first week, indicates negative PE.

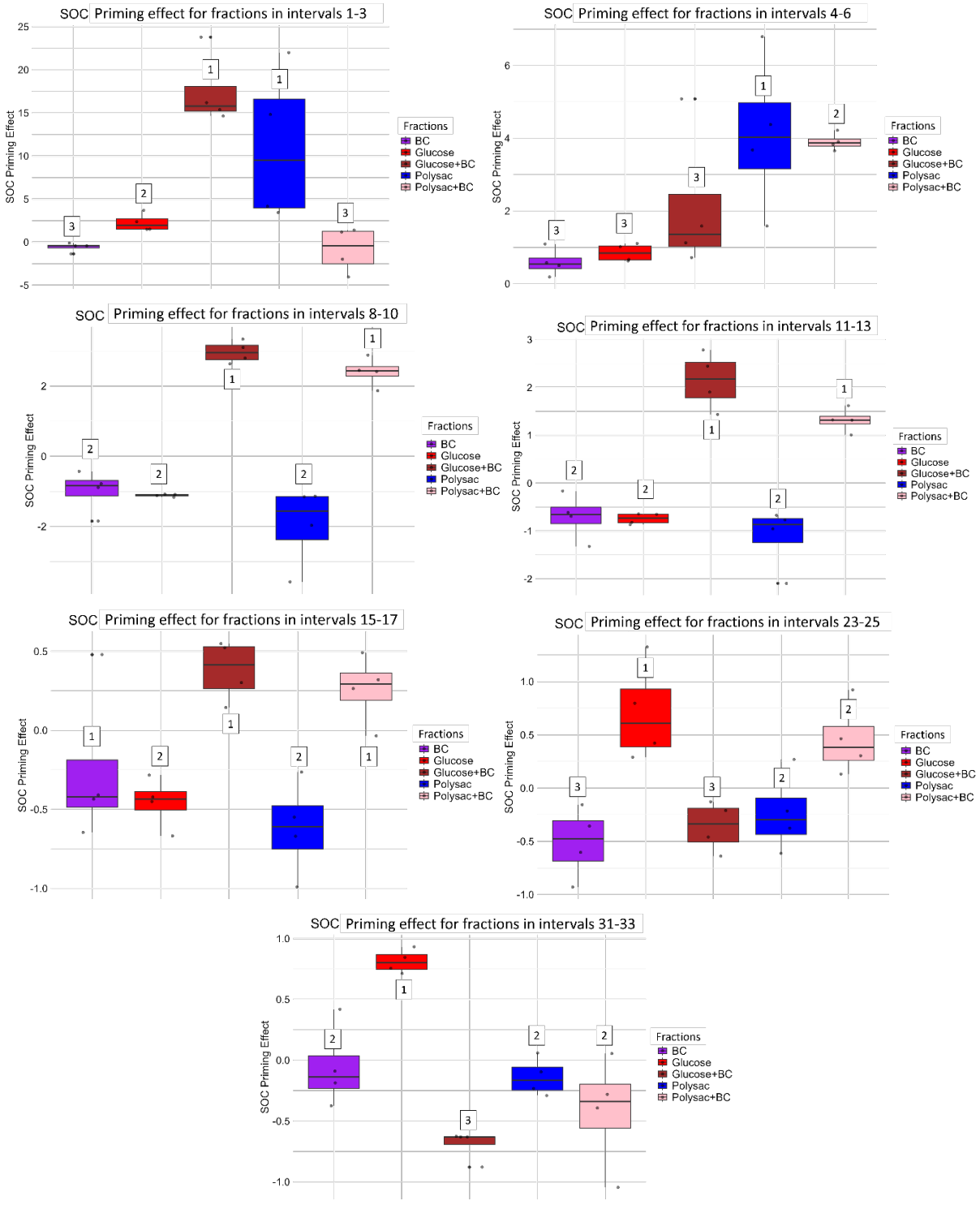

Figure 27 Priming Effect on SOC (µg g-1 soil) across incubation intervals and fractionss. fractions: BC=Biochar; Glucose+ BC= Glucose+ Biochar; Polysac= Polysacharide; Polysac+BC= Polysacharide+ Biochar.

Table 11 Results of the Wilcoxon test comparing the priming effect on SOC across all fractions. fractionss. fractions: BC=Biochar; Glucose+ BC= Glucose+ Biochar; Polysac= Polysacharide; Polysac+BC= Polysacharide+ Biochar.

Incubation Interval	Treatment1	Treatment2	P.value	Significancy	Incubation Interval	Treatment1	Treatment 2	P.value	Significancy
1_3	BC	Glucose	0.03	Yes	11_13	Glucose	Glucose+BC	0.03	Yes
1_3	BC	Polysac	0.03	Yes	11_13	Glucose	Polysac+BC	0.03	Yes
1_3	BC	Glucose+BC	0.03	Yes	11_13	Polysac	Glucose+BC	0.03	Yes
1_3	BC	Polysac+BC	1.00	No	11_13	Polysac	Polysac+BC	0.03	Yes
1_3	Glucose	Polysac	0.06	No	11_13	Glucose+BC	Polysac+BC	0.06	No
1_3	Glucose	Glucose+BC	0.03	Yes	15_17	BC	Glucose	0.69	No
1_3	Glucose	Polysac+BC	0.03	Yes	15_17	BC	Polysac	0.34	No
1_3	Polysac	Glucose+BC	0.34	No	15_17	BC	Glucose+BC	0.11	No
1_3	Polysac	Polysac+BC	0.03	Yes	15_17	BC	Polysac+BC	0.20	No
1_3	Glucose+BC	Polysac+BC	0.03	Yes	15_17	Glucose	Polysac	0.49	No
4_6	BC	Glucose	0.20	No	15_17	Glucose	Glucose+BC	0.03	Yes
4_6	BC	Polysac	0.03	Yes	15_17	Glucose	Polysac+BC	0.03	Yes
4_6	BC	Glucose+BC	0.06	No	15_17	Polysac	Glucose+BC	0.03	Yes
4_6	BC	Polysac+BC	0.03	Yes	15_17	Polysac	Polysac+BC	0.03	Yes
4_6	Glucose	Polysac	0.03	Yes	15_17	Glucose+BC	Polysac+BC	0.49	No
4_6	Glucose	Glucose+BC	0.11	No	23_25	BC	Glucose	0.03	Yes
4_6	Glucose	Polysac+BC	0.03	Yes	23_25	BC	Polysac	0.69	No
4_6	Polysac	Glucose+BC	0.20	No	23_25	BC	Glucose+BC	0.69	No
4_6	Polysac	Polysac+BC	0.89	No	23_25	BC	Polysac+BC	0.03	Yes
4_6	Glucose+BC	Polysac+BC	0.34	No	23_25	Glucose	Polysac	0.03	Yes
8_10	BC	Glucose	0.34	No	23_25	Glucose	Glucose+BC	0.03	Yes
8_10	BC	Polysac	0.11	No	23_25	Glucose	Polysac+BC	0.69	No
8_10	BC	Glucose+BC	0.03	Yes	23_25	Polysac	Glucose+BC	0.89	No
8_10	BC	Polysac+BC	0.03	Yes	23_25	Polysac	Polysac+BC	0.06	No
8_10	Glucose	Polysac	0.11	No	23_25	Glucose+BC	Polysac+BC	0.03	Yes
8_10	Glucose	Glucose+BC	0.03	Yes	31_33	BC	Glucose	0.03	Yes
8_10	Glucose	Polysac+BC	0.03	Yes	31_33	BC	Polysac	0.89	No
8_10	Polysac	Glucose+BC	0.03	Yes	31_33	BC	Glucose+BC	0.03	Yes
8_10	Polysac	Polysac+BC	0.03	Yes	31_33	BC	Polysac+BC	0.34	No
8_10	Glucose+BC	Polysac+BC	0.11	No	31_33	Glucose	Polysac	0.03	Yes
11_13	BC	Glucose	0.69	No	31_33	Glucose	Glucose+BC	0.03	Yes
11_13	BC	Polysac	0.34	No	31_33	Glucose	Polysac+BC	0.03	Yes
11_13	BC	Glucose+BC	0.03	Yes	31_33	Polysac	Glucose+BC	0.03	Yes
11_13	BC	Polysac+BC	0.03	Yes	31_33	Polysac	Polysac+BC	0.34	No
11_13	Glucose	Polysac	0.34	No	31_33	Glucose+BC	Polysac+BC	0.31	No

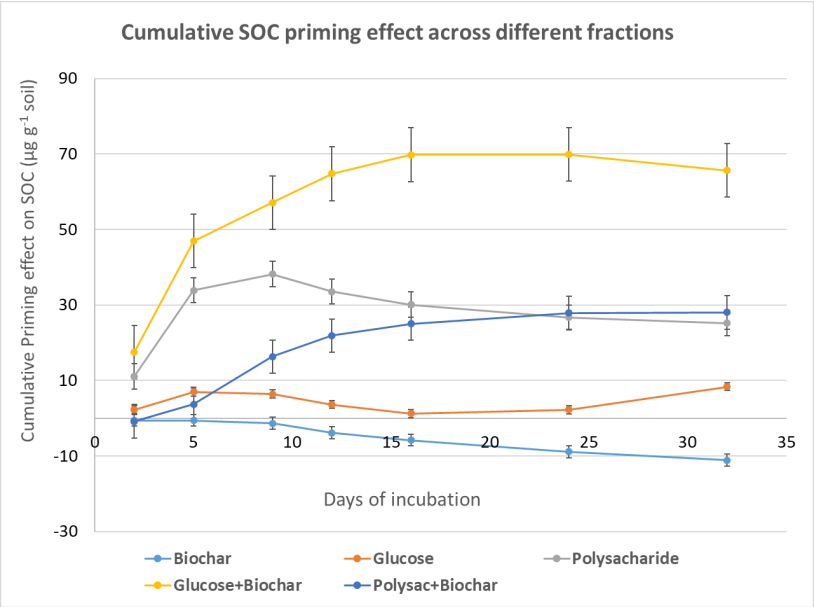

Figure 28 Cumulative Priming Effect of Soil Organic Carbon Across Different Treatments.

3-2-1-1 Implication for root exudate treatments and SOC priming effect:

The observed priming effect (PE) values throughout the incubation period reveal intriguing patterns, particularly the dominance of the Glucose + Biochar fraction in most of the incubation stages and the subsequent behavior of other fractions, such as Polysaccharide and Polysaccharide + Biochar. These patterns suggest the inherent characteristics of the carbon sources (Glucose vs. Polysaccharides) and the influence of biochar amendments.

3-2-1-1-1 The Glucose + Biochar fraction

The Glucose + Biochar fraction exhibits the highest PE values during the first week, particularly on days 1-3. This rapid increase in PE can be attributed to the highly labile nature of glucose, which is quickly metabolized by soil microbes, leading to a burst of CO_2 emissions (Gunina & Kuzyakov, 2015). Biochar likely enhances this effect by providing a stable environment for microbial communities, supporting their growth, and improving soil aeration and nutrient availability (Jeffery et al., 2011). The increased microbial activity induced by glucose and biochar's beneficial properties may facilitate enhanced breakdown of native SOC, leading to higher PE values in the early incubation period (Lehmann et al., 2011).

The synergistic effect of glucose and biochar is consistent with previous studies showing that biochar can stimulate microbial activity and improve the efficiency of carbon cycling (Kuzyakov, 2010). Biochar acts as a substrate for microbial colonization, amplifying the priming effect by enhancing glucose degradation and indirectly supporting microbial processes that involve the degradation of more recalcitrant SOC (Lehmann et al., 2011). It is worth noting that biochar can contribute to reduced SOC mineralization over longer incubation periods because of depletion of

labile SOC from initial positive priming and/or stabilization of SOC through biochar-induced organo-mineral interactions (Singh & Cowie, 2014).

3-2-1-1-2 Polysaccharide treatments leading in days 4-6

From days 4 to 6, polysaccharide fraction emerged as having the dominant PE value. Unlike glucose, as a rapidly available carbon source, xyloglucan (polysaccharide) is more complex and requires a more prolonged microbial effort to degrade (Scheller & Ulvskov, 2010). The higher priming effect observed with Polysaccharide AND Polysaccharide+ Biochar fraction during these days may indicate that microbes gradually adapt to the polysaccharide, utilizing it more efficiently over time (Luo et al., 2016; Sinsabaugh et al., 2013). Still, once the initial adaptation period is over, the microbial degradation of polysaccharides could lead to a more sustained priming effect on SOC compared to the rapid spike seen with glucose (Blagodatskaya & Kuzyakov, 2008).

Biochar may have a less pronounced effect on polysaccharide decomposition than glucose, given that polysaccharides are more complex and might not interact as efficiently with biochar's physical properties (Ameloot et al., 2013). Biochar's role in promoting microbial activity is typically more significant when dealing with easily degradable carbon sources like glucose, which supports immediate microbial activity (Lehmann et al., 2011).

3-2-1-1-3 From days 6-17, sustained priming with glucose + Biochar and Polysaccharide+ Biochar fractions

Starting from day 6 onward, the Polysaccharide + Biochar and Glucose + Biochar fraction show the most significant PE values. This shift could be explained by the fact that biochar's presence in these treatments supports the microbial community by providing a stable environment, increasing microbial diversity, and enhancing nutrient cycling (Lehmann et al., 2011; Xu et al., 2016; Gul et al., 2015). In particular, biochar can reduce the reliance of microbial communities on recalcitrant forms of carbon, providing a more favorable environment for microbial activity even when glucose is no longer readily available (Zhao et al., 2020). The result is a sustained priming effect, with continued microbial activity breaking down native SOC and producing CO_2.

3-2-1-1-4 Glucose Treatment Leading in Day 23 and Beyond

Interestingly, from day 23 onward, the Glucose treatment emerges as the leader in PE values, while many other treatments exhibit negative PE values. The behavior of the Glucose treatment can be attributed to its initial strong priming effect followed by a steady shift towards the decomposition of more recalcitrant SOC, particularly after the labile glucose carbon is exhausted. As microbes transition from glucose to more stable SOC, the priming effect can continue at lower levels, leading to a relatively consistent CO_2 emission.

The negative PE values observed for other treatments after day 23 are intriguing and could be explained by the inhibitory effects of biochar and polysaccharides (Gunina & Kuzyakov, 2015). Over time, biochar's role in enhancing microbial activity may diminish, especially in systems where microbial communities have adapted, and the available carbon is limited (LUO et al., 2014). Similarly, polysaccharides may exert a lower priming effect as they are more complex and less readily decomposed, decreasing microbial activity once the initial microbial adaptation phase has passed (Kuzyakov et al., 2009).

4- Conclusion and outlook

4-1 Study1: Impact of Glucose, Xyloglucan, and Biochar Amendments on Soil Aggregation and CO_2 Emissions

4-1-1 CO_2 fluxes and treatment effects

The CO_2 fluxes and treatment effects section findings indicated that glucose rapidly increased CO_2 emissions during the early incubation phases due to its more straightforward structure. This quick response is linked to its labile nature, which soil microorganisms metabolize swiftly (Gunina & Kuzyakov, 2015; Demoling et al., 2007). The immediate microbial reaction resulted in higher daily and cumulative CO_2 fluxes, particularly in L2.1 and REC soils. However, as the incubation continued and glucose was depleted, emissions decreased. In contrast, xyloglucan, which has a more complex and stable structure, exhibited slower and steadier CO_2 emissions over time, supporting the notion that polysaccharides serve as a more lasting carbon source (Gunina & Kuzyakov, 2015; Ravachol et al., 2016).

Statistical analysis using the Wilcoxon test showed that glucose treatments produced significantly greater CO_2 emissions than xyloglucan treatments, especially during the first two days of incubation. However, as microbial activity declined, the differences between the two treatments lessened, suggesting that glucose induces a rapid and transient microbial response compared to the more enduring microbial processes facilitated by polysaccharides (Sinsabaugh et al., 2013; Blagodatskaya & Kuzyakov, 2008).

The comparison between glucose and xyloglucan treatments at two concentrations (50 µg and 500 µg) revealed notable differences in CO_2 emissions and their impact on soil stability. The higher glucose concentration (G500) led to significantly higher CO_2 emissions and a more pronounced effect on soil stability. This can be explained by glucose's labile nature, which makes it readily available to soil microorganisms, enabling rapid microbial metabolism and an immediate increase

in CO_2 emissions (Gunina & Kuzyakov, 2015). The higher concentration of glucose likely provided an abundance of easily degradable carbon, promoting accelerated microbial activity and a stronger impact on soil stability during the early stages of incubation. Conversely, xyloglucan treatments (P500) induced more stable and gradual changes in CO_2 fluxes and soil particle size reduction. The slower degradation of xyloglucan, due to its more complex structure, likely resulted in a less immediate microbial response compared to glucose (Gunina & Kuzyakov, 2015; Saha et al., 2023). This slower breakdown process contributed to more stable CO_2 emissions and a more sustained effect on soil aggregation and stability. The cumulative CO_2 emissions from glucose were notably higher than those from polysaccharide treatments, with glucose outpacing xyloglucan in L2.1 soil. This finding further supports the idea that glucose, as a labile carbon source, induces a rapid but transient priming effect, while xyloglucan produces a more sustained, gradual effect over time. There were small differences between treatments, but the total emissions from glucose in REC soil were still high. This suggests that the soil's ability to store and absorb carbon may vary.These results highlight the differential effects of glucose and xyloglucan on microbial activity and soil stability, with glucose providing immediate but short-lived impacts, whereas xyloglucan offers more prolonged benefits for soil health.Soil type played a crucial role in these results. L2.1 soil, containing a higher total organic carbon (TOC) content (0.6%), exhibited increased microbial activity, contributing to more stable soil aggregation than REC soil, which had a lower TOC content (0.3%) and displayed more significant fluctuations in particle size reduction (da Silva et al., 2022). This implies that TOC content and soil texture are vital in determining how soils respond to amendments. L2.1 soil, with larger particles, exhibited quicker settling during laser diffraction analysis, leading to more rapid reductions in median soil size than REC soil, which showed slower reductions due to its finer particles.

4-1-2 The Role of polysaccharides in soil aggregation and Stability

This section highlights the crucial role of polysaccharides, especially xyloglucan, in improving soil aggregation and stability. Adding easily accessible carbon sources like glucose boosts microbial activity, affecting aggregates' size and stability over time (Sarker et al., 2022; Li et al., 2020; Gunina & Kuzyakov, 2015). While glucose treatments show immediate and elevated CO_2 fluxes, indicating short-term effects on soil aggregation, polysaccharides, due to their complex structures, produce more enduring changes in soil structure, suggesting long-lasting benefits for soil stability (Gunina & Kuzyakov, 2015; Sarker et al., 2022).

According to study findings, L2.1 soil, which has a higher total organic carbon (TOC) content than REC soil, shows more microbial activity and more stable aggregation, highlighting the importance of soil texture and physicochemical properties (da Silva et al., 2022). These observations highlight how important it is to consider soil characteristics when choosing additives for soil management. Future studies should delve into the microbial mechanisms behind these changes and evaluate long-

term impacts across different soil types, utilizing techniques like Scanning Electron Microscopy (SEM) better to assess the effects of amendments on soil aggregation (Amelung et al., 2023).

4-2 Study 2: The Role of Biochar, Glucose, and Xyloglucan on Soil Organic Carbon Priming

The second study examines how adding glucose, xyloglucan, and biochar affects soil organic carbon (SOC) priming, emphasizing how microbial activity and SOC dynamics change with different treatments. The findings show that glucose quickly boosts microbial activity and speeds up SOC breakdown, while xyloglucan has a slower but more lasting effect.

4-2-1 CO_2 emissions in root exudate fractions

The differences in CO_2 emissions between glucose and xyloglucan fractions arise primarily from their distinct bioavailability and microbial degradation rates. Glucose, a simple monosaccharide, is easily accessible to soil microorganisms, facilitating rapid microbial metabolism and quick CO_2 release (Gunina & Kuzyakov, 2015; Nguyen & Guckert, 2001). This results in the high and immediate CO_2 emissions observed with glucose treatments, aligning with prior studies emphasizing glucose as a labile carbon source promoting rapid microbial respiration (Fierer et al., 2007; Sinsabaugh et al., 2013). In contrast, the more complex polysaccharide xyloglucan requires enzymatic breakdown before soil microbes can completely utilize it (Shimada et al., 2024; Vieira et al., 2021). This slower decomposition leads to lower, more stable CO_2 emissions than glucose emissions as microbial activity develops gradually (Saha et al., 2023).

Biochar, recognized for improving soil structure and enhancing microbial colonization (Du et al., 2016; Luo, Zang, et al., 2017), did not significantly change CO_2 emissions when paired with glucose or xyloglucan. Although biochar supports microbial activity and boosts nutrient retention, its impact on CO_2 emissions was minimal when readily available carbon like glucose was present. This might be explained by the very brief incubation period, which could not have given biochar enough time to have a substantial impact on carbon cycling and microbial dynamics (Wang et al., 2015). Microorganisms may need additional time to adjust to biochar and utilize it as a substrate since the microbial communities associated with biochar typically develop gradually (Lehmann et al., 2011). Therefore, biochar's effect on microbial carbon turnover could be more pronounced over longer durations or in systems containing more recalcitrant carbon sources.

4-2-2 Root exudate fractions and SOC priming effect

The SOC priming effect (PE) displayed variations across treatments, mainly with glucose and xyloglucan fractions. The most pronounced priming effect was seen with the glucose fraction,

which peaked early in the incubation period (Figure 27). This swift priming effect arises from glucose's labile nature, allowing it to be rapidly metabolized by soil microbes, increasing microbial activity and CO_2 emissions (Gunina & Kuzyakov, 2015). When combined with glucose, biochar further amplified this priming effect by creating a stable environment for microbial communities, thereby supporting their growth and boosting microbial activity (Jeffery et al., 2011; Lehmann et al., 2011).

Conversely, both with and without biochar, xyloglucan treatments exhibited a more gradual and sustained priming effect. The polysaccharide fractions showed lower PE values during the early incubation stages but gradually increased over time (Blagodatskaya & Kuzyakov, 2008). This behavior is attributed to xyloglucan's complex structure, necessitating a more extended adaptation period for microbes to degrade efficiently (Scheller & Ulvskov, 2010). The effect of biochar on promoting microbial activity was less evident when paired with xyloglucan, indicating that biochar's influence is more significant when combined with easily degradable carbon sources like glucose (Lehmann et al., 2011; Ameloot et al., 2013).

As the incubation period advanced, the priming effect evolved. Glucose fractions sustained a consistent, yet lower, priming effect after day 23 (Figure 28), likely due to the depletion of labile glucose carbon. Subsequently, microbial activity shifted to the degradation of more recalcitrant SOC. In contrast, polysaccharide and biochar treatments showed negative PE values after day 23, likely reflecting the depletion of readily available carbon sources and a change in microbial dynamics. These findings suggest glucose induces a rapid priming effect that is more transient compared to the slower yet prolonged effects of polysaccharides, particularly in the presence of biochar (Gunina & Kuzyakov, 2015).

The implications of this research are vital for agricultural practices. Glucose amendments increase microbial activity and SOC degradation, which is beneficial for short-term soil management strategies. However, for long-term stability and carbon sequestration, polysaccharide-based amendments like xyloglucan may offer more lasting benefits by promoting gradual microbial activity and SOC stabilization. Combining biochar with glucose or xyloglucan may boost microbial activity and carbon cycling, although its effects are more pronounced in systems with recalcitrant

The long-term effects of biochar and polysaccharide additions on soil health under varied environmental circumstances and soil types could be investigated in future studies. More research, including extended incubation times and microbial community investigations, might help determine how biochar affects microbial adaptability and soil carbon shifts.

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