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Determining the Influence of Soil Microbial Biomass Size on Soil Organic Matter Priming and Plant Residue Decomposition

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TITLE:

Determining the Influence of Soil Microbial Biomass Size on Soil Organic Matter Priming and Plant Residue Decomposition

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KEYWORDS:

litter decomposition, microbial biomass carbon, priming effect, respiration rate, soil microbial biomass carbon, soil organic carbon, soil organic matter mineralization

SUMMARY:

This protocol describes a method to determine the influence of ryegrass residue addition on soil organic matter mineralization (i.e., priming effect) as well as explore the changes in soil microbial biomass size induced by soil organic matter priming, which involves artificially changing the size of microbial biomass.

ABSTRACT:

Soil microbial biomass is of great importance for soil organic matter (SOM) and residue decomposition. The effects of soil microbial biomass size (MBS) on SOM mineralization are still unclear, especially regarding mineralization in the presence of fresh plant residue input, known as the priming effect (PE). A traditional approach to determining this influence is the collection of soils with contrasting MBS, determination of the SOM mineralization rate, and further exploration of the relationships between MBS and SOM mineralization. In this protocol, the initial MBS is artificially changed in a preliminary experiment. Afterwards, the response of SOM priming with plant residue applications is estimated. Also presented is a detailed protocol for changing the initial size of microbial biomass as well as the determination of residue decomposition and SOM priming. The protocol includes six main steps: sample preparation, determination of optimal

glucose level to increase MBS, preincubation experiment, confirmation of MBS changes, incubation experiment, and determination of PE and residue decomposition. The advantage of this methodology is that the relationships between the initial size of soil microbial biomass and SOM priming and plant residue decomposition are easily tested by altering microbial biomass artificially in a preincubation setting. This avoids potential confounding influence on the relationships by other factors, such as various soil nutrients or textures of different soils used in the traditional method.

INTRODUCTION:

Despite accounting for a small fraction of soil organic matter (SOM)[1], microbial biomass plays a critical role in SOM and plant residue decomposition, and it significantly affects SOM dynamics and pools. Previous studies have shown that microbial communities are of great importance in the process of SOM and residue decomposition[2,3,4]. However, the influence of soil microbial biomass size (MBS) on residue decomposition, especially SOM mineralization as affected by residue addition (the priming effect, [PE]), is still unclear. For example, similar SOM mineralization rates as affected by the supply of fresh plant residue were observed among soils with different soil MBS[5]. A lack of relationship between soil MBS and SOM priming has also been reported[6,7]. These inconsistent observations are likely results of many other confounded factors, such as soil carbon and nutrient contents from the different soils that are selected[8]. In these studies, it is difficult to regulate the size of soil microbial biomass to test its influence on SOM mineralization. Similar difficulties have occurred in testing the effects of soil MBS on other soil processes.

This protocol describes a method to alter the initial size of soil microbial biomass via preliminary incubation and further test how changes in soil MBS influence residue decomposition and SOM mineralization in the presence of exogenous residue. The protocol includes six main steps: sample preparation, determination of optimal glucose level to increase MBS, preincubation experiment, confirmation of MBS changes, incubation experiment, and determination of PE and residue decomposition. The step-by-step procedure is partly modified from previous publications[9,10].

PROTOCOL:

1. Sample preparation

1.1. Randomly collect five samples to a depth of 20 cm from arable soil (Mollic Haploxeralf) and form a composite sample.

1.2. Remove visible plant residue and sieve soils through a 2 mm sieve.

1.3. Store soil samples at 4 °C for the incubation experiment.

2. Determination of optimal glucose level

2.1. Determine soil water-holding capacity (WHC) as described by Rey et al.[11].

2.2. Place soils (~20 g dry weight) in specimen cups (120 mL), mix with different concentrations of glucose solutions (0, 80, 160, 240, 320, 400, 800, 1,600, and 3,200 µg glucose-C/g), and move cups into 500 mL Mason jars (**Table of Materials**).

2.3. Adjust soil moisture to 60% of WHC by adding deionized (DI) water using a syringe equipped with a fine-tipped needle (**Table of Materials**). Close all jars with air-tight lids containing a septum for gas sampling.

2.4. Collect soil gas samples with a 20 mL gas-tight syringe (**Table of Materials**) from the lid of each Mason jar and store in 12 mL preevacuated vacuum bottles (**Table of Materials**) immediately after closing the jars. Incubate the jars in the dark at 22 °C.

2.5. Sample gases again after 2 h.

2.6. Determine the CO_2 concentration in gas samples with a gas chromatograph equipped with a thermal conductivity detector (**Table of Materials**).

2.7. Calculate microbial respiration rate (µ mol/h, Rs) as the rate of CO_2 production in 2 h according to the following equation:

$$Rs = (CO_{2,\,2h} - CO_{2,\,initial})/2$$

where $CO_{2,\,2h}$ and $CO_{2,\,initial}$ are the total CO_2 production (µ mol) from soils after a 2 h incubation and at the beginning of the incubation.

2.8. Identify the optimal amount of glucose added for a given soil (here, 240 µg C/g and 1,600 µg C/g soil for the crop and grass soils, respectively), defined as the minimum amount of glucose that induces the maximal respiration rate.

3. Preincubation experiment

3.1. Incubate bulk soils (~20 g dry weight) with glucose solution (240 µg C/g and 1,600 µg C/g soil for the crop and grass soils, respectively) in Mason jars (500 mL) in the dark at 50% of WHC at 22 °C for 7 days to increase soil MBS.

3.2. Simultaneously with glucose addition, add a mixture of mineral salts to ensure no nitrogen, phosphorus, and potassium limitation during the preincubation. Add nitrogen in the form of ammonium sulfate (15 mg/mL) to achieve a glucose-C/ammonium sulfate-N ratio of 10. Add phosphorus and potassium in the forms of two phosphate salts (here, K_2HPO_4 and KH_2PO_4) in respective ratios so that pH changes are less than 0.1 units after the mixture addition[12].

3.3. Incubate soils without glucose and mineral salts additions in parallel at 50% of WHC at 22 °C

for 7 days as control soils without the alteration in soil MBS.

3.4. Maintain soil moisture at 50% of WHC by regularly weighing the Mason jars and adding DI water to compensate for moisture loss.

4. Confirmation of changes in microbial biomass size

4.1. At the end of the preincubation, take a subsample (5 g) of the preincubated soils for analyses of soil microbial biomass carbon (MBC) and soil microbial community structure and composition.

4.2. Determine soil MBC using fumigation-extraction[13,14]. Analyze microbial community structure and composition using real-time qPCR and high-throughput sequencing methods[15]. Alternatively, analyze the phospholipid fatty acids (PLFA) following the method described by Buyer and Sasser[16].

4.3. Confirm the increase in soil microbial biomass with glucose preincubation based on soil MBC by comparing with soils without glucose preincubation.

4.4. Confirm negligible shifts in the microbial community structure and composition based on the high-throughput sequencing analysis or PLFA analysis after the preincubation. Perform the principal component analysis using statistical software (**Table of Materials**).

5. Incubation experiment

5.1. Mix the glucose preincubated soils (~20 g dry weight) with or without ^{13}C-labelled ryegrass residue powder (<2 mm, 2.1 mg C/g dry soil) in specimen cups (120 mL) using a spatula. Place the cups into Mason jars (500 mL).

NOTE: Here, the residue has an organic carbon of 420.0 g/kg, total nitrogen of 10.1 g/kg, and ^{13}C (atom %) of 1.70.

5.2. Adjust soil moisture to 60% of WHC by weighing the Mason jars and adding DI water using a syringe equipped with a fine-tipped needle.

5.3. Add 2 mL of DI water to each jar and close all jars with air-tight lids. Incubate them in the dark at 22 °C.

5.4. Set up three jars without soil and residue in the same way to determine the background CO_2 concentration and ^{13}C natural abundance.

5.5. Take soil gas samples from the jars with a gas-tight syringe (20 mL) and store in preevacuated vacuum bottles (12 mL) immediately after closing the jars.

5.6. Sample gases again after 24 h to determine the rate of CO_2 production during the past 24 h and ^{13}C value in CO_2.

5.7. Adjust soil moisture at 60% of WHC by weighing the Mason jars and adding DI water. Ventilate the jars for 10 min with CO_2-free air, close, and incubate at 22 °C in the dark.

5.8. Repeat steps 5.5–5.7 once per day during the first week, then once per week during the next 3 weeks.

5.9. Analyze CO_2 concentration in gas samples with a gas chromatograph system equipped with a thermal conductivity detector. Analyze $^{13}CO_2$ using an online gas preparation and introduction system interfaced to an isotope ratio mass spectrometer (**Table of Materials**).

6. Calculation of priming effect and residue decomposition

6.1. Calculate the fraction ($f_{residue}$) of CO_2 production from residue using the following equation:

$$f_{residue} = (^{13}CO_2\ atom\%_{residue} - {}^{13}CO_2\ atom\%_{control})/(^{13}C\ atom\%_{residue} - {}^{13}C\ atom\%_{soil})$$

where $^{13}CO_2$ atom%$_{residue}$ and $^{13}CO_2$ atom%$_{control}$ are the ^{13}C contents of mixed CO_2 produced from the soil amended with and without residue, respectively; ^{13}C atom%$_{residue}$ and ^{13}C atom%$_{soil}$ are the ^{13}C signatures of the added residue and soils, respectively.

6.2. Again, calculate CO_2 production derived from residue ($CO_{2,\ residue}$) and native SOM ($CO_{2,\ SOM}$) as follows:

$$CO_{2,\ residue} = CO_{2,\ total} \times f_{residue}$$
$$CO_{2,\ SOM} = CO_{2,\ total} - CO_{2,\ residue}$$

where $CO_{2,\ total}$ is the total CO_2 production from soils amended with residue.

6.3. Calculate the PE of residue addition on native SOM mineralization according to the following equation:

$$PE = CO_{2,\ SOM} - CO_{2,\ control}$$

where $CO_{2,\ SOM}$ and $CO_{2,\ control}$ are the CO_2 production derived from native SOM and from the control soils without residue, respectively.

6.4. Calculate the percentage ($p_{residue}$) of residue decomposed based on the following equation:

$$p_{residue} = CO_{2,\ residue}\text{-}C/C_{residue} \times 100\%$$

where $CO_{2,\ residue}$-C is the accumulative CO_2 production (CO_2-C) derived from residue, and $C_{residue}$ is the organic carbon in the added residue.

REPRESENTATIVE RESULTS:

A critical step of the protocol is to determine the optimal concentrations of glucose used to promote microbial growth while not causing a great shift of soil microbial community structure and composition. An example of glucose level determination used to increase soil MBS has been shown in a previous study[10]. Two soils with a 23-year history of crops and grass cover with contrasting soil organic carbon content were sampled and used in the study. The CO_2 production from crop and grass soils was observed **(Figure 1)** under the addition of a gradient of glucose solution. With increasing glucose addition, no statistically significant increase in CO_2 production was observed in soils with glucose applications higher than 240 μg C/g and 1,600 μg C/g for the crop and grass soils, respectively. According to the method described in section 2, glucose amounts of 240 μg C/g and 1,600 μg C/g soil were selected to promote microbial growth for crop and grass soils, respectively, based on their respiration responses.

After a 1 week glucose preincubation to increase soil MBS, it should be noted that soil microbial community structure should be tested to confirm negligible changes in soil microbial community structure and composition. At the end of preincubation, PLFA amounts of the pre-amended soils with glucose were higher in crop soil (59.9 nmol/g soil) and grass soil (288.7 nmol/g soil) in comparison to the corresponding unamended soils (50.4 nmol/g and 233.5 nmol/g soil), respectively[10]. The fungal-to-bacterial ratio did not greatly shift with glucose amendment in both crop (0.12 and 0.11 for the control and amended soils, respectively) and grass (0.09 and 0.08 for the control and amended soils, respectively) soils[10]. These results confirm the expected increase in soil MBS and no significant changes in microbial community structure, as shown by the principal component analysis on PLFAs in **Figure 2**.

FIGURE LEGENDS:

Figure 1: CO_2 production from crop (black line) and grass (red line) soils added with a gradient of glucose solution. The error bars represent standard errors.

Figure 2: Score plot of principal component analysis on phospholipid fatty acids. CS-glucose, crop soil without glucose amendment; CS+glucose, crop soil with glucose amendment; GS-glucose, grass soil without glucose amendment; and GS+glucose, grass soil with glucose amendment.

DISCUSSION:

The protocol provides a method to determine SOM priming from the supply of fresh plant residue as well as its decomposition, which has been reported in previous studies. The creative aspect provided in this protocol is that soil microbial biomass is artificially changed to explore the relationships between the size of microbial biomass and SOM priming. By using the method, only the size of soil microbial biomass is changed, maintaining other soil properties (i.e., soil nutrients, soil texture, etc.) that are similar among studied soils. In traditional studies, soils with various microbial biomass size are collected to explore the relationships between soil microbial biomass size and SOM mineralization.

In particular, Liu et al.[7] suggested that soil microbial biomass size is not strongly related to SOM priming, and that the weak relationships between them may be obscured by soil physiochemical factors among different soils from the four ecosystems. Based on the method, potential confounding influences (i.e., microbial biomass and other factors from different soils) may obscure the relationships between soil microbial biomass size and SOM mineralization or priming effect. Therefore, the advantage of this protocol is that it avoids these confounding factors that may have effects on the relationships between the initial size of soil microbial biomass and SOM priming and residue decomposition. It accomplishes this by altering microbial biomass artificially in a preincubation experiment.

To increase the size of soil microbial biomass, soils are incubated with glucose in a preincubation experiment. With an increase in the size of soil microbial biomass, soil microbial populations can be altered with glucose amendments[12]. In the glucose preincubation technique presented here, the critical step is to select the minimum amount of glucose. This ensures that the microbial community structure and composition is not greatly altered while also inducing microbial growth. Soil microbial community composition and structure have been considered determinants of SOM mineralization in the presence of labile carbon (i.e., priming effect)[17]. Furthermore, the changes in microbial community structure and composition should be tested after the glucose preincubation to confirm any alterations.

In a previous study[10], a PLFA analysis was used to show the changes in soil microbial community structure and composition. However, it is worth noting that the PLFA method is less accurate than modern techniques used for soil microbiome analysis (e.g., 16S-rRNA by qPCR) to detect changes in soil microbial community structure and composition. Therefore, it is recommended to test changes in soil microbial community using the high-throughput sequencing technique presented in this protocol.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure

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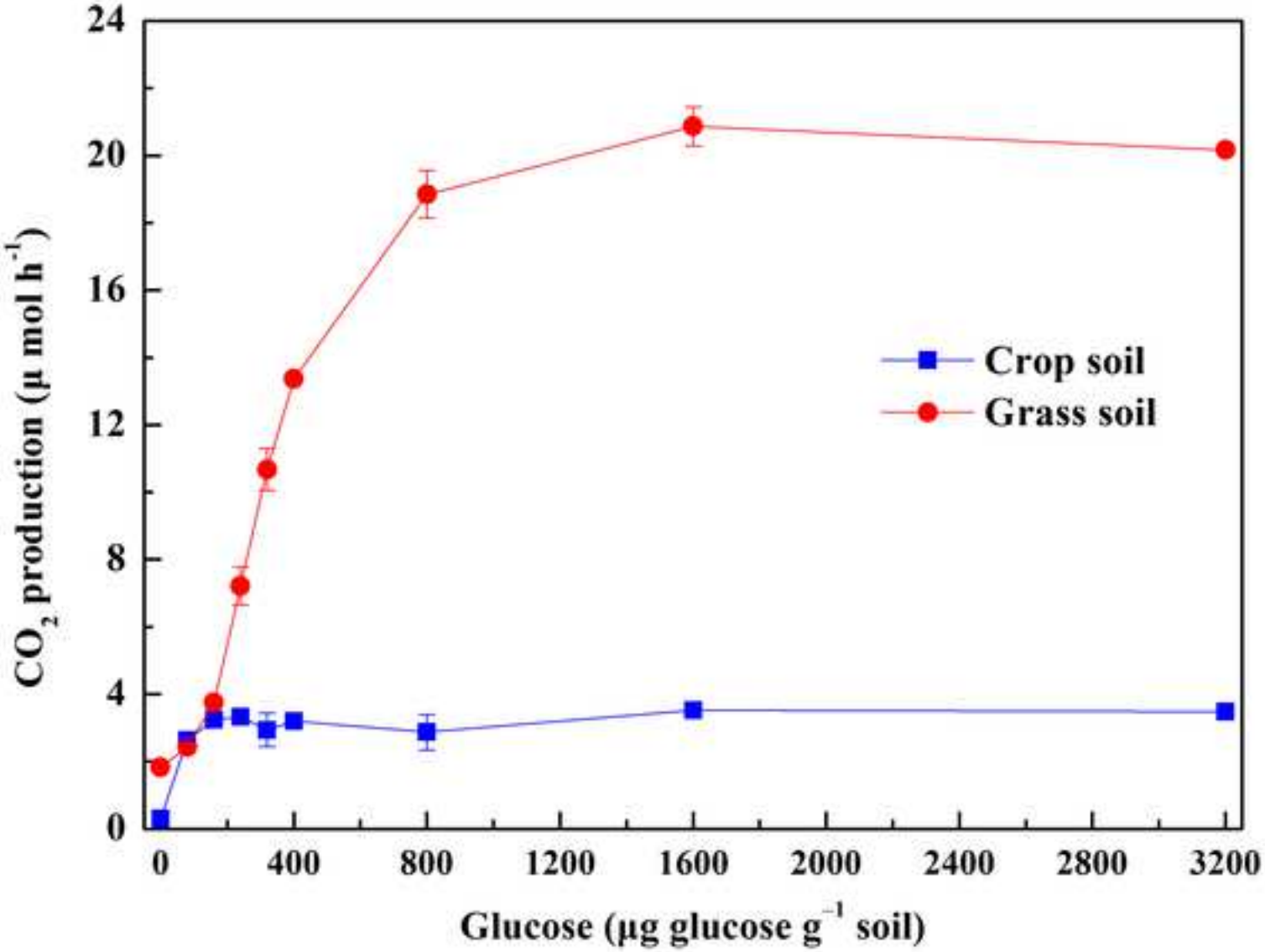

Figure

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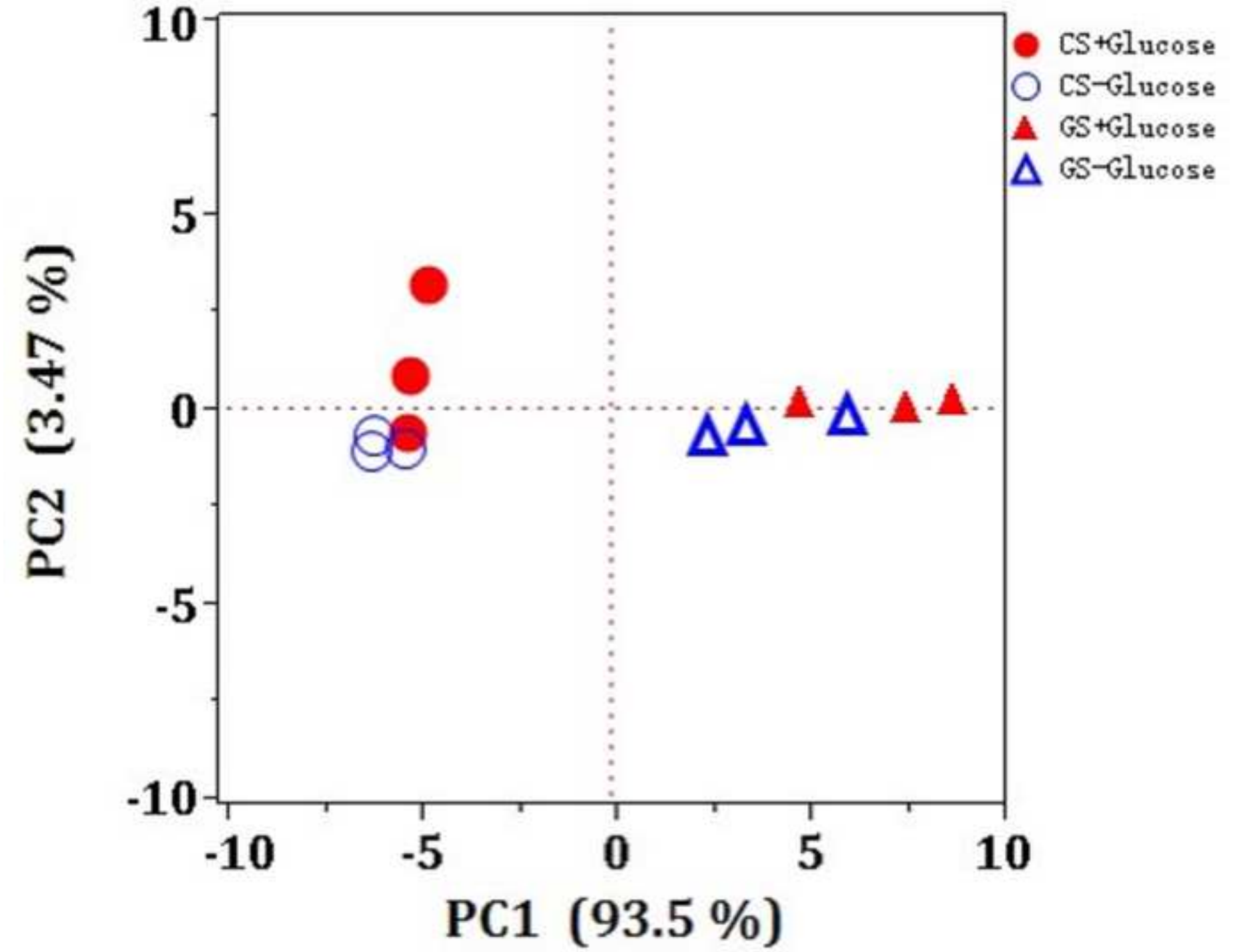

Name of Material/Equipment	Company	Catalog Number
Delta V Advantage IRAM through a GC/C-III interface	Thermo Electron Corp., Bremen, Germany	
Gas chromatograph (GC) system	Shimadzu	
Gas-tight syringe (20 mL)	Amazon.cn	B01N98U7GX
GC combustion isotope ratio mass spectrometer (GC/C-IRMS)	Thermo	
JMP 13	Jmp.com	
Mason jar	Amazon.cn	B00B80TJUI
Trace GC Ultra gas chromatograph	Thermo Electron Corp., Milan, Italy	
Vacuum bottles (Labco bottle)	Ojielabs.com	039W

Comments/Description

It can be used to add water and sample gas.

Dear Editor,

We have addressed all the editorial comments, and kept all the changes in the revised manuscript. In addition, we also provided all relevant supplies, equipment and software used.

Hope this revision matches the demand of the JoVE.

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