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Title: Temperature Response of Soil Organic Matter Decomposition Rates: Construction and Applications of a Temperature Gradient Block

Authors and Affiliations:

AUTHORS AND AFFILIATIONS:

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**
- 3. Filming location:** Will the filming need to take place in multiple locations? **No**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 20

Number of Shots: 49

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Karen Morán-Rivera:** My research examines how warming alters soil organic matter decomposition, aiming to define temperature responses and improve climate–carbon feedback predictions.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What are the most recent developments in your field of research?~~

- 1.2. **Gabriel Moinet:** Recent studies reveal broad soil temperature responses, but oversimplified metrics limit insight; integrating data with improved methods enhances mechanistic prediction.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What advantage does your protocol offer compared to other techniques?~~

- 1.3. **Mathilde Hagens:** Our protocol uses an advanced temperature gradient block that measures soil respiration at 22 temperatures, enabling detailed, diverse response curves for SOM decomposition rates.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What new scientific questions have your results paved the way for?~~

- 1.4. **Mathilde Hagens:** This method improves mechanistic understanding of temperature effects on SOM decomposition, revealing roles of soil properties and land management.
 - 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Preparing, Incubating, and Measuring Soil Samples Using a Temperature Gradient Block

Demonstrator: Karen Morán-Rivera and Rick Hendriksen

- 2.1. To begin, place the soil sample in a metal tin [1] and mix it thoroughly until homogeneous [2].
 - 2.1.1. WIDE: Talent placing a soil sample into a metal tin on the bench.
 - 2.1.2. Talent thoroughly mixing the soil inside the metal tin.
- 2.2. Distribute the mixed soil into 22 pre-labelled 60-milliliter glass vials [1]. Using weighing paper, add about 3 grams of soil into each 60-milliliter vial [2].
 - 2.2.1. Talent distributing the mixed soil into multiple pre-labeled 60 milliliter glass vials.
 - 2.2.2. Talent weighing soil on weighing paper and transferring 3 grams into each vial.
- 2.3. With a pipette, adjust the soil in each vial to the desired water-holding capacity based on the funnel method [1]. After adjusting the water content to the target percentage water-holding capacity, record the exact weight of each vial [2].
 - 2.3.1. Talent pipetting liquid into soil inside a vial to adjust water-holding capacity.
 - 2.3.2. Talent weighing each vial and recording the weight.
- 2.4. Cap each vial using cotton wool to allow ambient air exchange [1]. Pre-incubate the vials in a temperature-controlled laboratory set to 20 degrees Celsius for 10 days [2].
 - 2.4.1. Talent placing cotton wool into the opening of each vial.
 - 2.4.2. Talent placing the vials on a shelf for pre-incubation.
- 2.5. To begin preparation for loading, adjust the water-holding capacity 1 hour before starting the incubation [1]. Check the heater control to ensure it has reached the desired temperature 10 minutes before placing samples in the block [2].
 - 2.5.1. Talent adjusting moisture content of the soil samples.
 - 2.5.2. Talent checking the heater control display.
- 2.6. Then turn on the ambient conductor located in the laboratory [1]. Connect the flushing system [2] and connect the gas analyzer to the computer [3].
 - 2.6.1. Talent switching on the ambient conductor.
 - 2.6.2. Talent connecting tubing to set up the flushing system.

- 2.6.3. Talent connecting the gas analyzer to the computer.
- 2.7. To begin collecting blanks, connect the flushing line to the injection system of the gas analyzer [1] and record the carbon dioxide concentration in parts per million directly from the flushing line [2].
- 2.7.1. Talent connecting the flushing line to the gas analyzer injection system.
- 2.7.2. SCREEN: [69785_2.7.2.mp4](#). 00:02-00:20
- 2.8. Place five 60-milliliter glass vials in an empty holder [1]. Insert one flushing line into each vial [2]. A minute later, remove the flushing line and cap each vial [3]. Measure the carbon dioxide inside the vials after 4 hours [4].
- 2.8.1. Talent placing empty vials into a holder.
- 2.8.2. Talent inserting flushing lines into each vial.
- 2.8.3. Talent removing flushing lines and capping the vials.
- 2.8.4. SCREEN: [69785_2.8.4.mp4](#) 00:04-00:17, 00:31-00:50
AUTHOR'S NOTE: Highlight the time on top of the screen:
16:03:00 Start remark
16:03:10 Insert needle
16:03:30 Take needle out
16:03:45 End REMARK
- 2.9. Next, open the temperature gradient block [1] and place the computer and flushing system on top of the block lid [2]. Place the sample boxes on a movable table [3].
- 2.9.1. Talent opening the temperature gradient block.
- 2.9.2. Talent positioning the computer and flushing system on the block lid.
- 2.9.3. Talent placing sample boxes on a movable table.
- 2.10. Prepare a spreadsheet to record the closing time of each vial and simultaneously visualize the time displayed on the gas analyzer [1].
- 2.10.1. SCREEN: [69785_2.10.1 and 2.10.2.mp4](#). 00:02-00:30
- 2.11. Start loading samples into the temperature gradient block in batches of eight [1]. Place uncapped samples inside the block starting from the cooler end [2-TXT].
- 2.11.1. Talent loading the first batch of uncapped vials into the block.
Videographer's Note: 2.11.1 also includes one shot of the positioning of the 16 vials with the tubes and taps
- 2.11.2. Shot showing placement beginning from the cooler end of the block. **TXT: First 8 samples positioned at A1, B1, C1, D1, A2, B2, C2, D2**

2.12. Insert the flushing line into the eight vials [1]. Remove the flushing line after a minute [2] and immediately cap each vial [3]. Record the exact closing time in the spreadsheet [4].

2.12.1. Talent inserting flushing lines into eight vials.

2.12.2. Talent removing flushing lines .

2.12.3. Shot of vials being capped.

2.12.4. SCREEN: [69785_2.12.4.mp4](#). 00:04-00:13, 01:23-01:28

2.13. While the first batch is flushing, load the next batch of samples into the block [1] and insert the flushing line into this next batch [2].

2.13.1. Talent loading the next batch of samples into the block.

2.13.2. Talent placing flushing lines into the newly loaded vials.

2.14. Repeat the loading and flushing procedure until the temperature gradient block is fully loaded [1]. Close the temperature gradient block and wait 3.5 hours from the moment the last vial was closed [2].

2.14.1. Shot of the fully loaded block.

2.14.2. Talent closing the block lid.

2.15. To measure the carbon dioxide, turn on the flushing system [1] and place the gas analyzer onto the movable table [2]. After 3.5 hours of incubation, begin measuring samples starting with those at the highest temperatures [3].

2.15.1. Talent switching on the flushing system.

2.15.2. Talent positioning the gas analyzer on the movable table.

2.15.3. Talent selecting the first high-temperature samples for measurement.

2.16. Connect the injection needle of the analyzer to a flushing line between samples [1]. In the gas analyzer software, create a **UNIQUE** and **SIMPLE** remark before each measurement using the **REMARK** function [2].

2.16.1. Talent connecting the injection needle to the flushing line.

2.16.2. SCREEN: 69785_2.16.2_and_2.18.1_and_2.18.4_and_2.20.3.mp4 00:07-00:11

2.17. Insert the analyzer needle into a 60-milliliter sample vial for 20 seconds [1], then remove the needle from the vial [2].

2.17.1. Talent inserting the needle into the vial.

2.17.2. Talent removing the needle after the measurement period.

2.18. Start the **REMARK** at the zeroth second[1], insert the needle at 10th second[2], then remove the needle at the 30th second [3], and stop the remark at the 45th second [4-

TXT].

2.18.1. SCREEN: 69785_2.16.2_and_2.18.1_and_2.18.4_and_2.20.3.mp4. 00:25-00:30

2.18.2. Talent inserting the needle at hh:mm:10.

2.18.3. Talent removing the needle at hh:mm:30.

2.18.4. SCREEN: 69785_2.16.2_and_2.18.1_and_2.18.4_and_2.20.3.mp4 01:06-01:13

.TXT: Repeat measurement for all samples and blanks

2.19. Replace the 0.45-micrometer filter connected to the injection needle every 16 samples [1].

2.19.1. Talent removing and replacing the filter on the injection needle.

2.20. To unload samples, remove each vial one by one from the temperature gradient block [1] and place it into a box [2] while continuing carbon dioxide measurements [3].

2.20.1. Talent removing a vial from the block.

2.20.2. Talent placing the vial into a storage box.

Videographer's Note: 2.20.1-2.20.2 are shot together

2.20.3. SCREEN: 69785_2.16.2_and_2.18.1_and_2.18.4_and_2.20.3.mp4. 01:26-01:40

Results

3. Results

- 3.1. Most of the 8 temperature sensors reached their target temperature within 1 hour after switching the cooling system and heater on, and remained stable after an initial overshoot during the incubation period [1].
 - 3.1.1. LAB MEDIA: Figure 13A. *Video editor: Please trace all lines from start point showing the initial curving of the lines and then lines becoming flat*
- 3.2. After stabilization, the temperatures recorded by the 8 sensors showed a perfectly linear distribution along the length of the aluminum block [1].
 - 3.2.1. LAB MEDIA: Figure 13B. *Video editor: Highlight the straight line formed by the colored dots across the distance axis.*
- 3.3. Soil samples placed inside the stabilized block reached the target temperatures recorded by the sensors within approximately 12 minutes [1].
 - 3.3.1. LAB MEDIA: Figure 14. *Video editor: Please trace all lines from start point showing the initial curving of the lines and then lines becoming flat*
- 3.4. After 12 minutes of incubation, soil temperatures displayed a strong linear relationship with the sensor temperatures across the block [1].
 - 3.4.1. LAB MEDIA: Figure 15. *Video editor: Highlight the alignment of the soil temperature points along the straight fitted line.*
- 3.5. With an average incubation time of 3.5 hours, higher incubation temperatures resulted in greater soil moisture loss, regardless of soil texture [1].
 - 3.5.1. LAB MEDIA: Figure 16. *Video editor: Please highlight all the red, green, blue and purple points going upward*
- 3.6. An optimal measuring time of 20 seconds was identified, allowing the carbon dioxide signal to reach equilibrium for approximately 5 seconds [1]. When the gas analyzer was used as a closed system, a pressure drop occurred [2].
 - 3.6.1. LAB MEDIA: Figure 17. *Video editor: Please highlight the green curve corresponding to Equilibrium*
 - 3.6.2. LAB MEDIA: Figure 17. *Video editor: Please highlight pressure drop*

- 3.7. Soil respiration rates measured across 22 discrete temperatures produced consistent temperature response curves with small standard deviations [1].
 - 3.7.1. LAB MEDIA: Figure 18. *Video editor: Sequentially show A and then B*
- 3.8. Soil respiration data collected with the temperature gradient block were successfully fitted using Arrhenius [1] and macromolecular rate theory models [2].
 - 3.8.1. LAB MEDIA: Figure 19 A-B. *Video editor: Highlight the red fitted curve*
 - 3.8.2. LAB MEDIA: Figure 19 C-D *Video editor: Highlight the blue fitted curve*
- 3.9. Some soil samples exhibited altered respiration patterns associated with fungal growth and were excluded from further analysis [1].
 - 3.9.1. LAB MEDIA: Figure 20. *Video editor: Highlight the irregular scatter of respiration rate points across the temperature range.*

Pronunciation Guide:

Decomposition

Pronunciation link: <https://www.merriam-webster.com/dictionary/decomposition>

IPA: /ˌdi:kəmˈpəʊzɪʃən/

Phonetic Spelling: dee·kaam·puh·zi·shuhn

Temperature

Pronunciation link: <https://www.merriam-webster.com/dictionary/temperature>

IPA: /ˈtemp(ə)rətʃər/

Phonetic Spelling: tem·pruh·cher

Gradient

Pronunciation link: <https://www.merriam-webster.com/dictionary/gradient>

IPA: /ˈɡreɪdiənt/

Phonetic Spelling: gray·dee·uhnt

Organic

Pronunciation link: <https://www.merriam-webster.com/dictionary/organic>

IPA: /ɔːrˈɡænik/

Phonetic Spelling: or·ga·nik

Respiration

Pronunciation link: <https://www.merriam-webster.com/dictionary/respiration>

IPA: /ˌrɛspəˈreɪʃən/

Phonetic Spelling: res·puh·ray·shuhn

Incubation

Pronunciation link: <https://www.merriam-webster.com/dictionary/incubation>

IPA: /ˌɪŋkjəˈbeɪʃən/

Phonetic Spelling: ing·kyuh·bay·shuhn

Homogeneous

Pronunciation link: <https://www.merriam-webster.com/dictionary/homogeneous>

IPA: /ˌhoʊməˈdʒiːniəs/

Phonetic Spelling: hoh·muh·jee·nee·uhs

Pre-incubate

Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>

IPA: /'ɪŋkjəˌbeɪt/

Phonetic Spelling: ing·kyuh·bayt

Analyzer

Pronunciation link: <https://www.merriam-webster.com/dictionary/analyzer>

IPA: /'ænəˌlaɪzər/

Phonetic Spelling: a·nuh·lye·zer

Carbon dioxide

Pronunciation link: <https://www.merriam-webster.com/dictionary/carbon%20dioxide>

IPA: /ˌkɑːrbən daɪˈɑːksaɪd/

Phonetic Spelling: kar·buhn dye·aak·syd

Aluminum

Pronunciation link: <https://www.merriam-webster.com/dictionary/aluminum>

IPA: /əˈluːmɪnəm/

Phonetic Spelling: uh·loo·muh·nuhm

Micrometer

Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>

IPA: /maɪˈkroːmɪtər/

Phonetic Spelling: my·kraa·muh·ter

Injection

Pronunciation link: <https://www.merriam-webster.com/dictionary/injection>

IPA: /ɪnˈdʒɛkʃən/

Phonetic Spelling: in·jek·shuhn

Equilibrium

Pronunciation link: <https://www.merriam-webster.com/dictionary/equilibrium>

IPA: /iːkwəˈlɪbriəm/

Phonetic Spelling: ee·kwuh·li·bree·uhm

Arrhenius

Pronunciation link: <https://www.merriam-webster.com/dictionary/Arrhenius>

IPA: /ə'reɪniəs/

Phonetic Spelling: uh·ray·nee·uhs

Macromolecular

Pronunciation link: <https://www.merriam-webster.com/dictionary/macromolecular>

IPA: /ˌmækroʊməˈleɪkjələr/

Phonetic Spelling: mak·roh·muh·lek·yuh·ler

Stabilization

Pronunciation link: <https://www.merriam-webster.com/dictionary/stabilization>

IPA: /ˌsteɪbələˈzeɪʃən/

Phonetic Spelling: stay·buh·luh·zay·shuhn

Overshoot

Pronunciation link: <https://www.merriam-webster.com/dictionary/overshoot>

IPA: /ˈoʊvərˌʃu:t/

Phonetic Spelling: oh·ver·shoot

Linear

Pronunciation link: <https://www.merriam-webster.com/dictionary/linear>

IPA: /ˈliːniər/

Phonetic Spelling: li·nee·er

Distribution

Pronunciation link: <https://www.merriam-webster.com/dictionary/distribution>

IPA: /ˌdɪstrəˈbjʊːʃən/

Phonetic Spelling: dis·truh·byoo·shuhn

Texture

Pronunciation link: <https://www.merriam-webster.com/dictionary/texture>

IPA: /ˈtɛkstʃər/

Phonetic Spelling: teks·cher

Fungal

Pronunciation link: <https://www.merriam-webster.com/dictionary/fungal>

IPA: /'fʌŋɡəl/

Phonetic Spelling: fun·guhl

Mechanistic

Pronunciation link: <https://www.merriam-webster.com/dictionary/mechanistic>

IPA: /,mekə'nɪstɪk/

Phonetic Spelling: mek·uh·nis·tik

Deviation

Pronunciation link: <https://www.merriam-webster.com/dictionary/deviation>

IPA: /,di:vi'eɪʃən/

Phonetic Spelling: dee·vee·ay·shuhn

Incubator

Pronunciation link: <https://www.merriam-webster.com/dictionary/incubator>

IPA: /'ɪŋkjəˌbeɪtər/

Phonetic Spelling: ing·kyuh·bay·ter

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