TITLE

Efficient Carbon Dioxide and Methane Flux Monitoring in Soil Microcosms Using an Automated Chamber with a Cartesian Robot

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STATEMENT

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DATA AVAILABILITY

Data used in the study and supplementary information is available for download on Zenodo at https://doi.org/10.5281/zenodo.13826352. Code used to process and analyse data are archived in the Github repository at https://github.com/loyalhow/automated_chamber.

AUTHOR’S CONTRIBUTIONS

H.L. and Z.C. conceived the project idea; H.L. and Y-N.Z. developed an early prototype under the supervision of Z.C. and R.G.; Z-Y.L. and H.L. modified and refined the design; H.L. constructed the final prototype with input from Y-N.Z.; S-Y.Z. and B-D.C. tested the system and collected data in the laboratory; H.L. wrote the manuscript, which was reviewed by Z.C. and R.G. All authors contributed critically to the drafts and gave final approval for publication.

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Abstract:

1.Accurate measurement of carbon dioxide (CO_2) and methane (CH_4) emissions from soil is crucial for understanding carbon dynamics and developing strategies to mitigate climate change. Traditional chamber techniques and soil incubation experiments each have limitations that hinder their efficiency and accuracy. This study aims to overcome these challenges by integrating automated technologies into controlled soil incubation experiments.

2.We present an automated closed dynamic chamber system, operated by a Cartesian robot, applied for the first time to controlled soil incubation. This system enables precise and efficient monitoring of CO_2 and CH_4 fluxes, with validation conducted through repeated chamber measurements across multiple soil types incubated in open microcosms. The method was optimized to achieve reliable measurements within a 90-second duration, significantly shorter than that of conventional approaches.

3.The method demonstrated robust performance, showing high repeatability and reliability in gas flux measurements. The shortened and optimal 90-second measurement cycle enhanced experimental throughput without compromising accuracy. Two microcosm experiments, using leaf litter and plastic films, further highlighted the system's ability to capture dynamic changes in greenhouse gas emissions under different environmental treatments.

4.This novel approach significantly advances gas flux measurement by improving accuracy, experimental throughput, and efficiency in laboratory-based studies. The shortened measurement duration of 90 seconds offers a substantial improvement over traditional methods, making this system a valuable tool for broader applications in greenhouse gas research and ecosystem carbon dynamics studies.

ABSTRACT

1. Accurate measurement of carbon dioxide (CO_2) and methane (CH_4) emissions from soil is crucial for understanding carbon dynamics and developing strategies to mitigate climate change. Traditional chamber techniques and soil incubation experiments each have limitations that hinder their efficiency and accuracy. This study aims to overcome these challenges by integrating automated technologies into controlled soil incubation experiments.

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DATA/CODE FOR PEER REVIEW

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KEYWORDS

automated chamber, gas flux measurement, soil incubation, microcosm experiment, carbon dioxide, methane, Cartesian robot

1 INTRODUCTION

Carbon dioxide and methane emissions from soil are key components of the global carbon cycle (Brevik, 2012). Accurate measurement of these gas fluxes is critical for understanding ecosystem carbon dynamics, particularly in the context of climate change (Andrews, et al., 2014; Wangari, et al., 2022). Although various techniques have been developed over the past decades to monitor soil respiration and methane emissions in both laboratory and field settings (Bond-Lamberty, et al., 2024; Oertel, et al., 2016), each method has limitations and cannot fully address research questions alone (Maier, et al., 2020; Yu, et al., 2015). Optimizing current methods and developing new approaches are vital for improving the accuracy of terrestrial carbon models (Phillips, et al., 2016). These innovations provide more precise data on the global carbon cycle, supporting effective climate change mitigation strategies and sustainable management of agricultural and forest ecosystems (Maier, et al., 2020).

Soil incubation experiments have a long history as a key method for studying greenhouse gas emissions, offering the advantage of tightly controlled environmental conditions such as temperature, moisture, and nutrient levels, allowing for causal analysis of specific factors

(Berglund & Berglund, 2011; Hossain, et al., 2017; Schaufler, et al., 2010). These experiments also enable the investigation of short-term dynamics over days, weeks, or even months, with relatively simple and repeatable procedures that facilitate data collection and analysis (Hodgkins, et al., 2015; Treat, et al., 2015). Moreover, they provide valuable insights into the mechanisms underlying CO_2 and CH_4 emission from soil, such as microbial community structure and enzyme activity (Wu, Jia, Wang, Chang, & Startsev, 2013). However, incubation experiments have limitations, including significant differences from natural environments, such as the absence of plant roots, litter, and soil fauna, which can limit the applicability of results to real ecosystems. Additionally, they are constrained by small spatial scales and are less capable of evaluating long-term processes like vegetation succession and soil carbon turnover. High equipment and material costs further restrict the scale and number of experiments. Recent advancements aim to improve incubation techniques through the development of more advanced instrumentation and experimental designs that better mimic natural conditions, often in combination with other field-based techniques such as chamber methods to enhance the understanding of soil greenhouse gas emissions (Oertel, et al., 2016).

Current methods for determining greenhouse gas fluxes during soil incubation experiments have several limitations. Typically, soil samples incubated in vials or open microcosms are sealed for measurement periods ranging from 15 min to tens of hours. The gas accumulated in the headspace is then sampled at discrete times and analyzed using gas chromatography coupled with specific detectors. However, these samplings and post-sampling analysis are time-consuming. Moreover, the prolonged sealing may introduce interferences, such as altered gas concentration gradients that affect microbial activity and gas exchange processes. Consequently, these methods may fail to capture the rapid dynamics of greenhouse gas emissions, leading to results that deviate from natural soil systems. An automated novel method could significantly enhance laboratory studies by providing continuous, real-time monitoring with minimal disturbance to the system. Automated chamber methods have been employed in field studies for a long time, demonstrating several advantages over manual chambers despite challenges related to high cost and complexity. Integrating automated chamber technology into soil incubation experiments presents a promising solution to overcome the current methodological limitations and achieve more accurate and representative measurements.

In this study, we present a novel application of an automated closed dynamic chamber system, operated by a Cartesian robot, for use in controlled soil incubation experiments. This is the first time the automated chamber method, previously used mainly in field studies, has been integrated into microcosm experiments for precise and efficient monitoring of CO_2 and CH_4 fluxes. In this work, we validated the method's robustness and repeatability through repeated chamber measurements across different soil types incubated in open microcosms. Additionally, we explored the potential to shorten the measurement duration, aiming to enhance experimental efficiency and throughput. Two examples of microcosm experiments, involving treatments with leaf litter and plastic films, demonstrate the applicability of this method in laboratory studies.

2 METHODS

2.1 Design of the Cartesian robot, chamber, and microcosms

The gas flux measurements were conducted using an automated system that incorporated a Cartesian robot (Figure 1a), a closed dynamic chamber (Figure 1b) and open microcosms (Figure 1c). For real-time detection of CO_2, CH_4, and H_2O concentrations, the system was equipped with the

LI-7810 trace gas analyzer (LI-COR, Lincoln, NE, USA), which provided continuous online monitoring of gas concentrations. This setup was designed to allow precise, repeatable, and efficient measurements of gas fluxes from soil incubation experiments.

The Cartesian robot forms the core of the automated gas flux measurement system, designed for precise movement of the chamber. It is mounted on an aluminum profile frame, which provides stability and serves as a stand, creating space to position the microcosms beneath the robot. The robot operates within a working range of 600 mm in X, 500 mm in Y, and 150 mm in Z. Its motion is programmed and controlled by a 4-axis motion controller, ensuring accurate and automated movement during the experiment.

The closed dynamic chamber is used for real-time gas flux measurements and is securely mounted on the Cartesian robot system via a mounting plate. Constructed from aluminum, the chamber is designed to provide an airtight seal with minimal gas adsorption during operation. Two PTFE gas tubes connect the chamber to the LI-7810 trace gas analyzer (LI-COR, Lincoln, NE, USA), functioning as the gas inlet and outlet. Additionally, two solenoid valves are controlled by the 4-axis motion controller to regulate pressure balance between the inside and outside of the chamber when sealing or opening the microcosms.

The open microcosms were constructed using PVC square pipes with dimensions of 100 mm x 100 mm x 120 mm, which held the soil or sediment samples. The top of each microcosm featured a PVC water seal, which was filled with pure water prior to measurements. During the gas flux measurements, the closed dynamic chamber was positioned into the water seal, creating a temporary gas-tight headspace for accurate gas flux analysis. This setup ensured that ambient air did not interfere with the gas emissions measured from the incubated soils.

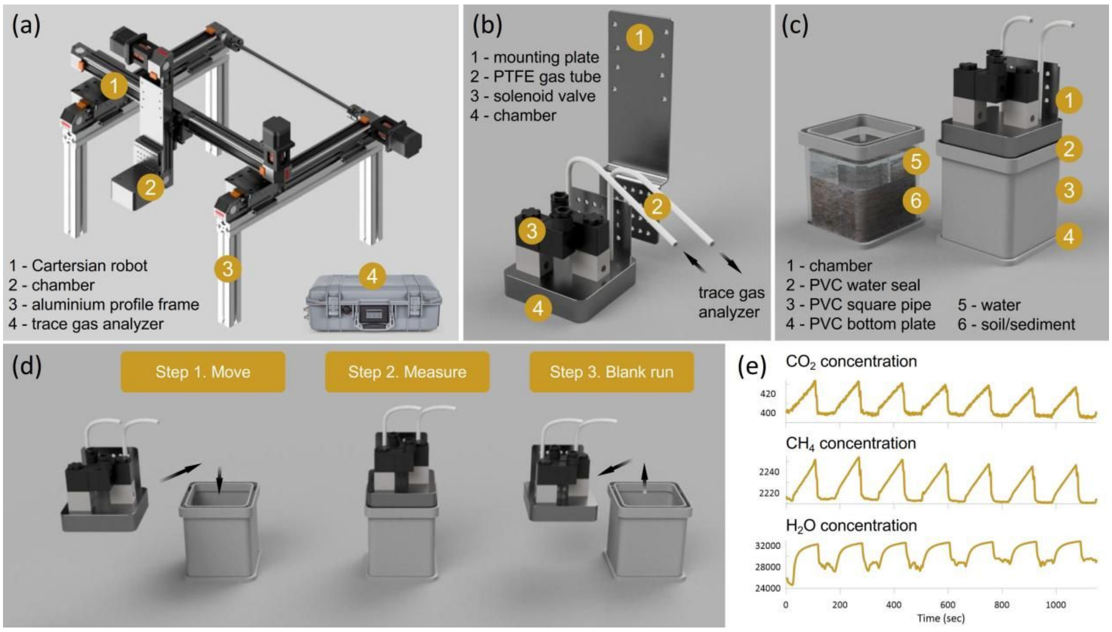

FIGURE 1 | Schematic representation of the automated chamber system for gas flux measurements. (a) Overview of the Cartesian robot system equipped with a measurement chamber. (b) Close-up of the closed dynamic chamber system. (c) Setup of the open microcosm during incubation (left) and the gas flux measurement configuration (right). (d) Representation of the three-step operational cycle of a single gas flux measurement. (e) Example of real-time CO_2, CH_4, and H_2O concentration data measured using the closed dynamic chamber automated by the Cartesian robot.

2.2 The three-step measurement cycle

Each measurement cycle comprises three distinct steps. In the first step, the Cartesian robot positions the closed dynamic chamber directly above the selected microcosm and lowers it into the water seal. During this process, the solenoid valves are opened to balance the internal and external pressure, minimizing any disturbances. Once the chamber is sealed, the solenoid valves are closed, initiating the second step—a 90-s measurement period during which real-time concentrations of CO_2, CH_4, and H_2O are continuously recorded. In the final step, the solenoid valves are reopened, allowing the chamber to be lifted and moved away from the microcosm. A blank run of 60 to 75 s is then performed to account for background levels of ambient air and to clear the system before the next measurement. This cycle is repeated until all microcosms have been processed.

2.3 Data analysis and software

Raw data were processed using Python to calculate gas fluxes for CO_2, CH_4, and H_2O. These raw concentration data files were first merged with relevant metadata to associate each dataset with the respective experimental conditions. Gas fluxes were calculated by determining the slope of the gas concentration over time during the sealed chamber measurement period. Specifically, the rate of change in gas concentration was obtained through linear regression analysis. The resulting slope was then used to compute the gas flux based on the chamber volume, the surface area of the microcosms, and the ambient temperature and pressure. The processed data and calculated fluxes were saved to CSV files for further analysis and interpretation. The Python code used for data processing is available via a Github repository https://github.com/loyalhow/automated_chamber.

3 RESULTS

3.1 Validation of gas flux measurements

The raw concentration data of CO_2, CH_4, and H_2O from 12 repeated measurements across 7 soil microcosms, with each measurement lasting 90 s, are presented in Figure 2a, 2c, and 2e. The CO_2 concentration data (Figure 2a) display periodic peaks, which are consistent across the repeated measurements but vary in magnitude between different microcosms, indicating differences in background concentrations. A similar trend is observed for CH_4 (Figure 2c), where the peak patterns remain consistent across repeated measurements for each microcosm, while the background levels differ between microcosms. In contrast, the peak shape of water vapor (Figure 2e) differs significantly from CO_2 and CH_4. The sharp ascending front of the water vapor peak is due to the high flux intensity, followed by a slowing and flattening of the concentration increase as the chamber's headspace becomes saturated. Despite these differences in peak shapes and background concentrations between microcosms, the consistency in peak patterns across repeated measurements suggests steady-state fluxes for CO_2 and CH_4 within each microcosm. These results validate the method's robustness in capturing stable gas fluxes over multiple measurement cycles.

The slopes and R^2 values (correlation coefficient calculated using X) of the linear fitting curves for repeated measurements of CO_2 and CH_4 fluxes across 7 soil microcosms are shown in Figure 2 (subplots 2b and 2d), respectively. The slopes (S1-S7) and R^2 values (R1-R7) indicate the rate of change in gas concentrations and the goodness of fit for the linear regression models. Both CO_2 and CH_4 measurements display low relative standard deviations in the variation rates of gas concentration, and the regression quality is high, with R^2 values consistently greater than 0.90, and often exceeding 0.95. As these R^2 values are close to 1 they demonstrate excellent fitting accuracy, highlighting the reliability of the method for capturing flux dynamics. There is only one exception, where the CH_4 flux is extremely low (near zero for S6), leading to a lower R^2 value. This result suggests that the method is statistically robust and capable of producing highly repeatable and accurate flux measurements, even across varied microcosms, with minimal deviation in the observed flux rates.

The repeatability of the gas flux measurements from soil incubation microcosms was further assessed using the Intraclass Correlation Coefficient (ICC). The dataset comprised 7 soil incubation microcosms, with 12 repeated measurements per microcosm. ICC values were calculated to evaluate the consistency of the measurements across repeated trials. The results demonstrated excellent reliability, with ICC values for single raters (ICC1 and ICC2) of 0.956, indicating strong agreement between individual measurements. Furthermore, when considering the average of the repeated measurements (ICC1k, ICC2k, ICC3k), the ICC values exceeded 0.996, suggesting near-perfect consistency and repeatability of the method. The narrow confidence intervals ([0.89, 0.99] for individual measurements and [0.99, 1.00] for averaged measurements) further confirm the robustness and reliability of the measurement protocol. These findings indicate that the method provides highly repeatable results, whether using individual or averaged measurements, supporting its validity for gas flux quantification in soil incubation experiments.

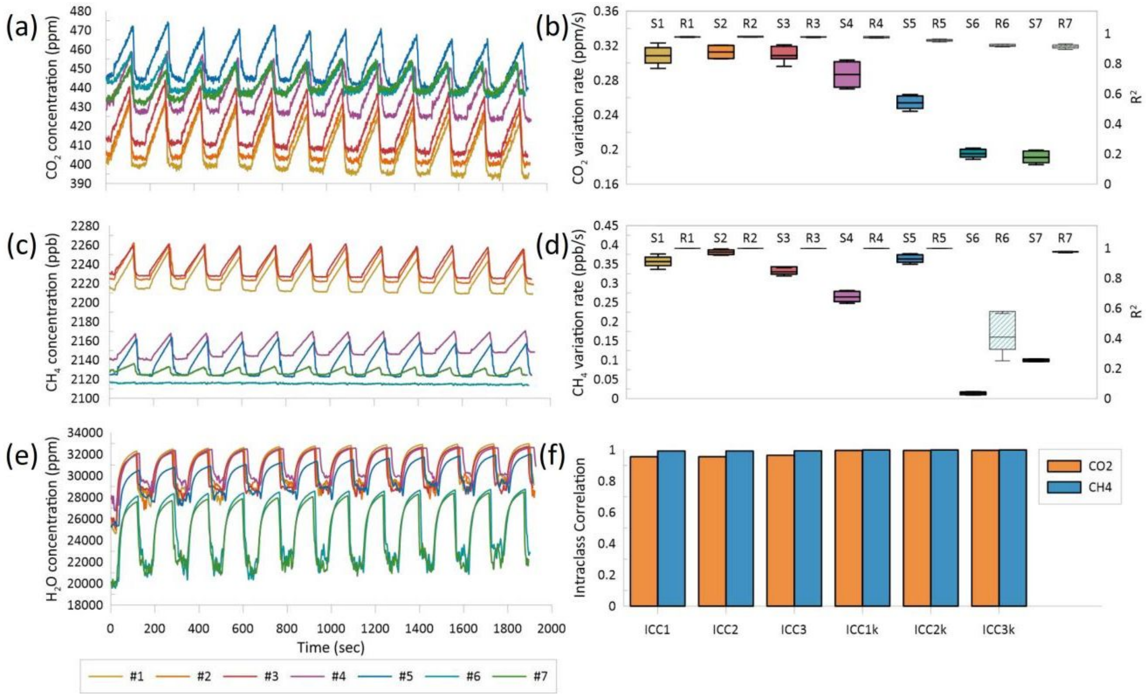

FIGURE 2 | The raw concentration data and validation results for the flux measurements of carbon dioxide, methane and water vapor over 12 repeated measurements across 7 microcosms. Plots represent (a) CO_2 (ppm) (c) CH_4 (ppb) and (e) H_2O (ppm) concentrations over time. Each colored line represents an individual microcosm (#1–#7), with measurements taken repeatedly over time. (b) Boxplots of CO_2 flux variation rates (slopes, S1-S8) and their corresponding R^2 values (R1-R8), illustrating the quality of linear regression fits. (d) Boxplots of CH_4 flux variation rates (slopes, S1-S8) and their corresponding R^2 values (R1-R8), showing the fitting quality for CH_4 measurements. (f) Intraclass correlation coefficients (ICC) for repeated measurements of CO_2 and CH_4 fluxes, demonstrating the reliability and repeatability of the developed method.

3.2 Effect of measurement duration on accuracy

The measurements of CO_2 and CH_4 concentrations over different durations were conducted to assess the effect of measurement duration on the accuracy of flux determinations (Figure 3a, 3b). The slopes of the linear fitting curves, which act as proxies for gas fluxes, were plotted as a function of measurement duration, showing how flux estimates stabilize over time (Figure 3c, 3d). At shorter durations (10–30 s), both CO_2 and CH_4 slopes showed significant variability, especially for methane, where some microcosms exhibited near-zero or even negative values. For example, microcosms #2 and #3 for CH_4 had notably inconsistent slopes at these shorter durations. However, after 90 s, the slopes for both gases stabilized, indicating reliable flux measurements. The R^2 values of the linear fitting curves were also plotted to assess the quality of the regressions across different durations (Figure 3e, 3f). The R^2 values steadily increased with duration for both gases, with values surpassing 0.90 at 90 s for most microcosms and exceeding 0.95 in many cases, further confirming the accuracy of flux estimates. By 90 s, the CO_2 slopes for all microcosms had converged to consistent values (around 0.19–0.22 ppm/s), and the CH_4 slopes had stabilized as well (around 0.15–0.22 ppb/s), reflecting reliable flux rates. These results explore the potential for shortening measurement cycles to increase experimental throughput. The combination of high R^2 values and stable slopes at 90 s indicates that this duration is sufficient for reliable gas flux measurements. This duration is significantly shorter than those required by other methods, allowing for faster data acquisition without compromising measurement reliability.

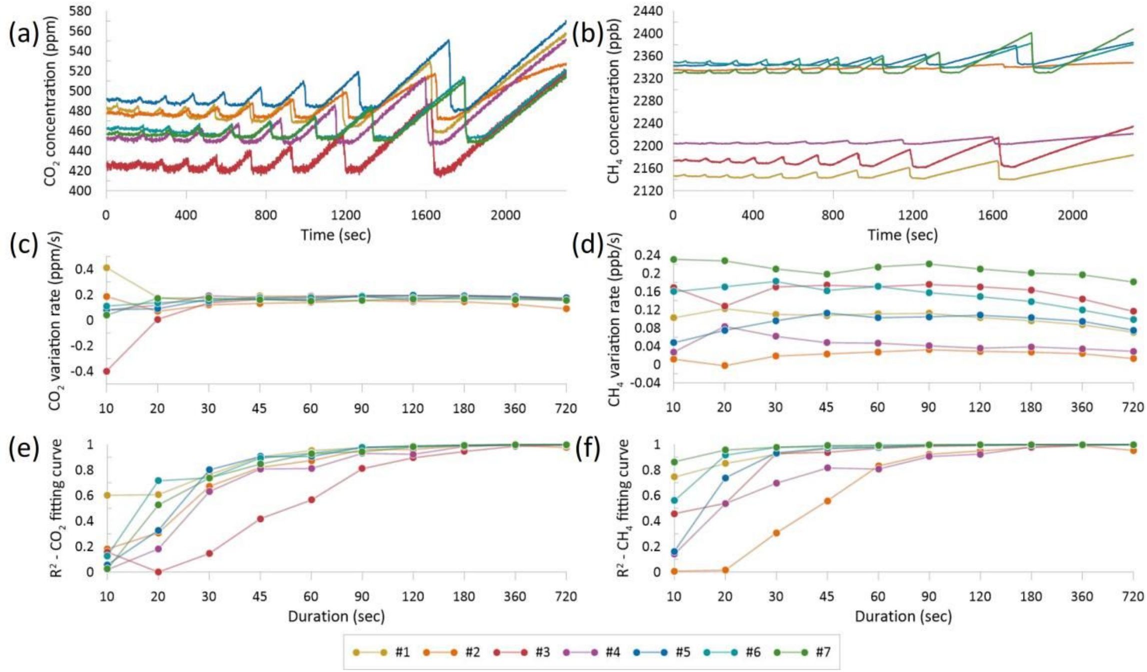

FIGURE 3 | Validation of CO₂ and CH₄ flux measurements over different measurement durations across seven microcosms. (a) CO₂ concentration (ppm) and (b) CH₄ concentration (ppb) as a function of time, with different durations (10, 20, 30, 45, 60, 90, 120, 180, 360, and 720 s) shown for each microcosm (#1–#7). (c) CO₂ flux variation rates (ppm/s) and (d) CH₄ flux variation rates (ppb/s) as a function of measurement time, illustrating how the slope of the flux curve stabilizes with longer durations. (e) *R*² values for the CO₂ flux fitting curves, and (f) *R*² values for the CH₄ flux fitting curves, plotted against different measurement duration, showing improved fitting accuracy with increased measurement time.

3.3 Demonstration with leaf litter and plastic film treatment

The flux variations of CO₂ and CH₄ were monitored over two-week incubation periods for two distinct experimental setups: the addition of dried leaf litter and the application of different plastic films. In the leaf litter experiment (Figure 4a, 4b), CO_2 fluxes varied significantly between the treatments. The uncovered leaf litter (LU) resulted in the highest CO_2 emissions, peaking at 0.32 ppm/s around day 2 before gradually declining, while the treatment with leaf litter covered by a thin soil layer (LC) showed consistently lower but still elevated CO_2 fluxes, stabilizing around 0.18 ppm/s. The control group (NL) with no leaf litter maintained low, stable fluxes near zero throughout the experiment. CH_4 fluxes displayed a distinct trend, with the LC treatment showing a significant peak in emissions on day 4, reaching 1.2 ppb/s before declining rapidly. In contrast, both the LU and NL treatments exhibited negligible CH_4 fluxes.

In the plastic film experiment (Figure 4c, 4d), the addition of plastic films had a distinct impact on CO_2 fluxes. After the plastic film treatments at day 0, CO_2 fluxes declined by more than 50% in the first two days for all treatment groups—ST025 (0.025-mm-thick starch-based bioplastic), PE055 (0.055-mm-thick polyethylene), and PE090 (0.090-mm-thick polyethylene)—compared to control group (CTR). This was followed by a gradual recovery, with the CO_2 fluxes narrowing the gap between the CTR and the treated groups, but remaining consistently lower for the plastic-treated microcosms throughout the remainder of the incubation. In contrast, the control group showed a steady increase in CO_2 emissions during the same period, with fluxes remaining higher than the treatment groups post-treatment. CH_4 fluxes showed a pronounced response to the plastic films, with PE090 demonstrating the highest emissions, exceeding 5 ppb/s by day 7. The PE055 and ST025 treatments also showed elevated CH_4 fluxes, though to a lesser extent, while the control group exhibited minimal CH_4 emissions throughout the incubation.

Together, these experiments highlight the applicability of the flux measurement method across different treatment conditions and experimental setups. The method successfully captured both CO_2

and CH_4 flux variations in response to different types of organic matter and plastic films, offering a reliable means to monitor gas flux dynamics over time. The ability to detect subtle differences between treatments demonstrates the robustness of the method and its potential for broader applications in greenhouse gas flux studies, both in controlled experiments and potentially in field settings. By allowing for repeated measurements with high temporal resolution, this method provides a valuable tool for researchers investigating soil-atmosphere gas exchange under various environmental and experimental conditions.

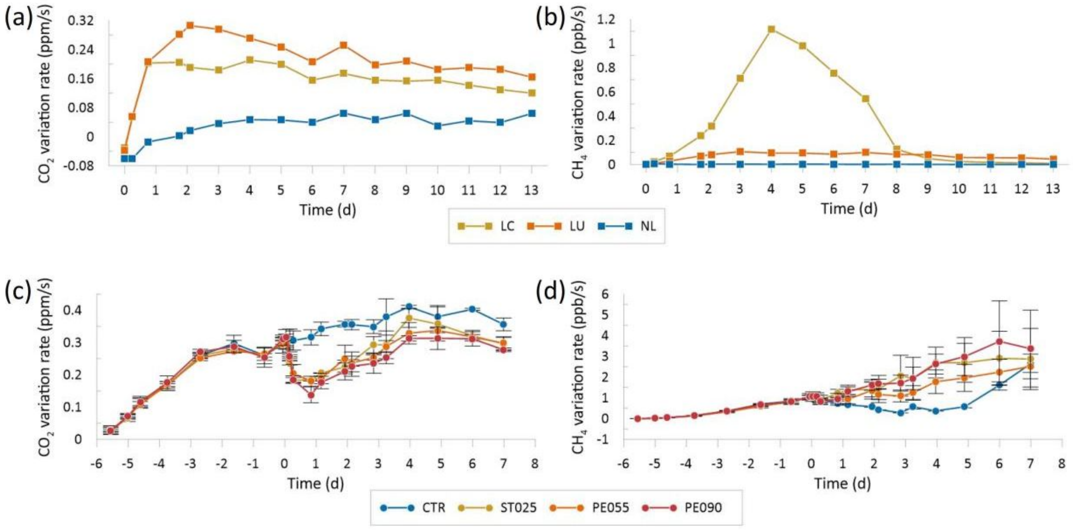

FIGURE 4 | Application of the flux measurement method in two different microcosm experiments. (a) CO_2 variation rate (ppm/s) and (b) CH_4 variation rate (ppb/s) over a two-week incubation for leaf litter treatments. NL represents the control group with no leaf litter, LC represents leaf litter covered by a thin soil layer, and LU represents uncovered leaf litter. (c) CO_2 variation rate (ppm/s) and (d) CH_4 variation rate (ppb/s) over a two-week incubation for plastic film treatments. CTR represents the control group with no plastic film, ST025 represents the treatment with 0.025-mm-thick starch-based bioplastic, PE055 represents the treatment with 0.055-mm-thick polyethylene, and PE090 represents the treatment with 0.090-mm-thick polyethylene.

4 DISCUSSION

The findings presented in this study underscore the efficacy and reliability of the automated chamber method for measuring greenhouse gas fluxes from soil ecosystems. This method, controlled and operated by a Cartesian robot system, represents a significant advancement in the automation of gas flux measurements. The integration of an automated closed dynamic chamber system, which has been traditionally used in field applications, with controlled soil incubation experiments is introduced here for the first time. The Cartesian robot system automates the entire measurement process, enabling precise, repeatable and thus robust measurements while minimizing manual intervention and reducing potential human error. The robustness and repeatability of this method are evident in the consistent peak patterns observed across repeated measurements for CO_2 and CH_4, despite variations in background concentrations between different microcosms. This consistency not only validates the method's capacity to capture stable gas fluxes but also demonstrates its potential applicability across a wide range of environmental conditions and soil types.

A key advancement highlighted in this study is the shortened measurement duration, made possible by the automation provided by the Cartesian robot system. With this precise robotic control, measurement cycles can be as short as 90 s, representing a significant improvement in gas flux measurement techniques . which in other systems typically take 10-15 min per cycle. Importantly, this reduction in measurement time does not compromise the accuracy of the flux estimates, as evidenced by the high R^2 values and stable slopes obtained for both CO_2 and CH_4 at this duration. The ability to collect reliable data in a fraction of the time typically required by conventional methods has profound implications for increasing experimental throughput and minimizing disturbance to the ecosystems under study.

The excellent fitting accuracy, with R^2 values consistently greater than 0.90 and frequently exceeding 0.950, further reinforces the reliability of the automated chamber method. Additionally, the ICC values exceeding 0.996 for averaged measurements confirm the method's near-perfect consistency and repeatability. These findings suggest that the automated chamber method, operated via a Cartesian robot system, is a robust tool for capturing gas flux dynamics with minimal deviation, even across diverse microcosms.

The applicability of this method for studying the mechanisms underlying greenhouse gas emissions from various ecosystems is highly promising. The demonstration using leaf litter and plastic film treatments illustrates the method's ability to detect subtle differences in gas fluxes in response to environmental changes. The variability in CO_2 and CH_4 emissions observed between treatments underscores the sensitivity of the method and its capacity to track flux dynamics over time, making it particularly useful for studies investigating the impacts of both natural and anthropogenic factors on gas emissions.

However, this method does have limitations. One notable limitation is its current inability to measure nitrous oxide (N_2O), another potent greenhouse gas, due to the current instrumentation. Since N_2O is an important component of soil-atmosphere gas exchange, particularly in agricultural systems, future improvements should focus on implementing detectors capable of measuring N_2O and other relevant gases. Expanding the system's detection capabilities would significantly broaden the scope of the Cartesian robotic system and enhance its applicability across a wider range of ecosystems and research questions. Additionally, while the system performs well in controlled lab environments, the complexity of natural ecosystems, such as soil heterogeneity and fluctuating field conditions, may present challenges in field applications that require further exploration and adaptation of the system.

5 CONCLUSIONS

In conclusion, the automated chamber method presented here represents a significant advancement in the measurement of greenhouse gas fluxes in soil incubation experiments. The integration of a Cartesian robot system has shortened measurement cycles, increased throughput, and maintained high accuracy and repeatability, making this method an invaluable tool for both laboratory and field studies. The potential for broader applications in greenhouse gas flux studies is clear, and future research can build upon these findings to further explore the underlying mechanisms of gas emissions in diverse ecosystems. Improvements, such as the inclusion of N_2O detection and adaptations for more complex field conditions, will further strengthen the utility of this method in ecology and environmental sciences.

REFERENCES

Andrews, A.E., Kofler, J.D., Trudeau, M.E., Williams, J.C., Neff, D.H., Masarie, K.A., et al. (2014). CO2, CO, and CH4 measurements from tall towers in the NOAA Earth System Research Laboratory's Global Greenhouse Gas Reference Network: instrumentation, uncertainty analysis, and recommendations for future high-accuracy greenhouse gas monitoring efforts. *Atmospheric Measurement Techniques, 7*(2), 647-687, doi:10.5194/amt-7-647-2014.

Berglund, Ö., & Berglund, K. (2011). Influence of water table level and soil properties on emissions of greenhouse gases from cultivated peat soil. *43*(5), 923-931, doi:10.1016/j.soilbio.2011.01.002.

Bond-Lamberty, B., Ballantyne, A., Berryman, E., Fluet-Chouinard, E., Jian, J., Morris, K.A., et al. (2024). Twenty Years of Progress, Challenges, and Opportunities in Measuring and Understanding Soil Respiration. *Journal of Geophysical Research: Biogeosciences, 129*(2), doi:10.1029/2023jg007637.

Brevik, E.C. (2012). Soils and Climate Change: Gas Fluxes and Soil Processes. *Soil Horizons, 53*(4), doi:10.2136/sh12-04-0012.

Gagnon, S., L'Hérault, E., Lemay, M., & Allard, M. (2016). New low-cost automated system of closed chambers to measure greenhouse gas emissions from the tundra. *Agricultural and Forest Meteorology, 228-229*, 29-41, doi:10.1016/j.agrformet.2016.06.012.

Hodgkins, S.B., Chanton, J.P., Langford, L.C., McCalley, C.K., Saleska, S.R., Rich, V.I., et al. (2015). Soil incubations reproduce field methane dynamics in a subarctic wetland. *Biogeochemistry, 126*(1-2), 241-249, doi:10.1007/s10533-015-0142-z.

Hossain, M.B., Rahman, M.M., Biswas, J.C., Miah, M.M.U., Akhter, S., Maniruzzaman, M., et al. (2017). Carbon mineralization and carbon dioxide emission from organic matter added soil under different temperature regimes. *6*(4), 311-319, doi:10.1007/s40093-017-0179-1.

Maier, M., Gartiser, V., Schengel, A., & Lang, V. (2020). Long Term Soil Gas Monitoring as Tool to Understand Soil Processes. *Applied Sciences, 10*(23), doi:10.3390/app10238653.

Oertel, C., Matschullat, J., Zurba, K., Zimmermann, F., & Erasmi, S. (2016). Greenhouse gas emissions from soils—A review. *Geochemistry, 76*(3), 327-352, doi:10.1016/j.chemer.2016.04.002.

Phillips, C.L., Bond-Lamberty, B., Desai, A.R., Lavoie, M., Risk, D., Tang, J., et al. (2016). The value of soil respiration measurements for interpreting and modeling terrestrial carbon cycling. *Plant and Soil, 413*(1-2), 1-25, doi:10.1007/s11104-016-3084-x.

Saleska, S.R., Harte, J., & Torn, M.S. (2008). The effect of experimental ecosystem warming on CO_2 fluxes in a montane meadow. *Global Change Biology, 5*(2), 125-141, doi:10.1046/j.1365-2486.1999.00216.x.

Schaufler, G., Kitzler, B., Schindlbacher, A., Skiba, U., Sutton, M.A., & Zechmeister-Boltenstern, S. (2010). Greenhouse gas emissions from European soils under different land use: effects of soil moisture and temperature. 683-696, doi:10.1111/j.1365-2389.2010.01277.x.

Treat, C.C., Natali, S.M., Ernakovich, J., Iversen, C.M., Lupascu, M., McGuire, A.D., et al. (2015). A pan-Arctic synthesis of CH(4) and CO(2) production from anoxic soil incubations. *Glob Chang Biol, 21*(7), 2787-2803, doi:10.1111/gcb.12875.

Wangari, E.G., Mwanake, R.M., Kraus, D., Werner, C., Gettel, G.M., Kiese, R., et al. (2022). Number of Chamber Measurement Locations for Accurate Quantification of Landscape-Scale Greenhouse Gas Fluxes: Importance of Land Use, Seasonality, and Greenhouse Gas Type. *Journal of Geophysical Research: Biogeosciences, 127*(9), doi:10.1029/2022jg006901.

Wu, F., Jia, Z., Wang, S., Chang, S.X., & Startsev, A. (2013). Contrasting effects of wheat straw and its biochar on greenhouse gas emissions and enzyme activities in a Chernozemic soil. *49*(5), 555-565, doi:10.1007/s00374-012-0745-7.

Yu, K., Hiscox, A., & DeLaune, R.D. (2015). Greenhouse Gas Emission by Static Chamber and Eddy Flux Methods *Methods in Biogeochemistry of Wetlands* (pp. 427-437).