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METHODS ARTICLE OPEN ACCESS

BLOSOM: A Plant Growth Facility Optimised for Continuous ^{13}C Labelling and Measurement of Soil Organic Matter Dynamics

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ABSTRACT

Changes in soil carbon (C) stocks are largely driven by rhizosphere processes forming new soil organic matter (SOM) or stimulating SOM decomposition by rhizosphere priming effects (RPEs). Quantifying these changes is challenging and requires high spatial sampling densities or plant–soil experiments with highly distinct C isotopic signatures for plants and soils. Current methods for quantifying new SOM formation and RPEs rely on low labelling intensities, which introduces high levels of uncertainty. Here, we describe the design and operation of an experimental laboratory facility—BLOSOM (Botanical Labelling Observatory for Soil Organic Matter)—optimised for continuous ^{13}C labelling of plants at high labelling intensities (> 500‰) to quantify new SOM formation and RPEs in temperature-controlled soils from 216 experimental units. Throughout a > 6-month experimental period, independent control of soil and air temperature was achieved across diurnal cycles averaging at 5.24°C ± 0.05°C and 21.4°C ± 1.2°C, respectively. BLOSOM can maintain stable CO_2 concentrations and $\delta^{13}C$ isotopic composition within 5% of setpoints (CO_2: 440 ppm, $\delta^{13}C$: 515‰) across a > 6-month period. This high-precision control on atmospheric enrichment enables the detection of new SOM formation with a total uncertainty of ±39% to ±3% for a theoretical range of 0.5%–10% new SOM formation, respectively. BLOSOM has the potential improve quantification and mechanistic understanding of new SOM formation and RPEs across many different combinations of plants, soils and simulated climatic conditions to mimic a wide range of ecosystems and climate scenarios.

1 | Introduction

There is increasing urgency to understand the drivers and magnitude of soil carbon (C) feedbacks to climate change (Bradford et al. 2016) from both natural and managed ecosystems. Identifying practices promoting soil C sequestration whilst robustly quantifying changes and turnover of soil C are becoming more important, with large investments in increasing soil organic carbon (SOC) stocks as a nature-based solution for climate change mitigation and the rise of the voluntary carbon market (Oldfield et al. 2022).

Changes in SOC stocks hinge on the balance between the accumulation or depletion of soil organic matter (SOM), which is determined by the interactions of plant C deposition, storage and soil decomposition processes. Despite the continual deposition of plant C inputs into soil, the rates and efficiency of SOM formation are relatively low (Castellano et al. 2015), making changes in SOM stocks hard to detect, particularly against a background of large, pre-existing stocks. Therefore, in order to accurately quantify changes in SOC stocks, high sampling density in numerous paired plots with a large magnitude of SOC stock change are required (Bradford et al. 2023).

Summary

- New facility designed to investigate soil organic matter decomposition and formation.
- A strong ^{13}C label enables quantification of priming and soil organic matter formation.
- Low uncertainty on soil organic matter formation requires strong (> 500‰) labelling.
- Independent control on air and soil temperature can mimic a range of climatic conditions.

Alternatively, stable isotopic tracing experiments using ^{13}C labelling of plants, can be deployed to track C inputs into discrete SOM pools (Villarino et al. 2021). Due to the many steps and biochemical processes in the multiple pathways from atmosphere through plant biomass, rhizodeposition, microbial biosynthesis and organo–mineral chemical binding, a high-intensity ^{13}C label (large difference between plant and soil $\delta^{13}C$) is required to accurately distinguish newly formed SOM from the existing SOM in soils.

Plant C inputs and subsequent rhizosphere processes can also lead to a loss of SOC by stimulating the decomposition of SOM—known as the rhizosphere priming effect (RPE). This is a key rhizosphere process influencing soil C stock fluctuations and arises from changes in SOM decomposition caused by plant root activity supplying soil microorganisms with energy-rich compounds in the form of rhizodeposition and litter (Fontaine et al. 2007; Kuzyakov 2002).

Estimating RPEs in plant–soil systems requires the comparison of SOM-derived CO_2 efflux from soils with and without live roots. The CO_2 efflux from rooted soil will be a mixture of plant-derived CO_2 (root respiration and microbial decomposition) and SOM-derived CO_2 (microbial respiration from SOM decomposition) (Kuzyakov 2002). To reliably distinguish between plant-derived and SOM-derived CO_2 and quantify new SOM formation, high labelling intensities must be used to differentiate the origin of C (plant or soil). Continuous labelling of plants using ^{13}C-depleted fossil-derived CO_2 has been successfully deployed in multiple experimental systems (Carrillo et al. 2014; Cheng and Dijkstra 2007; Cros et al. 2019), however, the labelling intensities of such experiments are low (−50‰ $\delta^{13}C$) and whilst this may be adequate to estimate RPEs, these low labelling intensities result in high levels of uncertainty when estimating new SOM formation.

Quantifying RPEs is particularly important in northern permafrost regions—one of the largest and most vulnerable stores of SOC globally (Koven et al. 2015; Tarnocai et al. 2009). Permafrost thaw and active-layer deepening coupled with greater plant productivity and increased rooting depths may increase rates of C input into previously frozen soils thus stimulating the decomposition of SOM. Recent modelling work indicates that RPEs could increase soil C loss from northern permafrost regions by ~40 Pg soil C by 2100 (Keuper et al. 2020). To date, research into the priming of permafrost soils has been carried out using soil-only systems (no plants) with labile substrate additions (Jia et al. 2017; Wild et al. 2016), which may miss the chemical, temporal and spatial complexity of live plant-delivered root exudates (Huo, Luo, and Cheng 2017) and lack a key sink of nutrients, namely plant biomass. Overall, controls on RPEs in permafrost soils remain poorly understood.

To improve mechanistic understanding of the processes controlling SOC stocks and turnover in a range of ecological contexts the following experimental challenges must be overcome:

1. Continuous, strong and diurnally stable ^{13}C labelling of plants to robustly quantify new SOM formation and RPEs (*C1*).
2. Independent control of soil and air temperatures to facilitate experimental conditions which simulate a range of atmospheric and soil climates (this is particularly relevant in the context of northern permafrost soils) (*C2*).

To help address these challenges we describe an experimental facility: BLOSOM (Botanical Labelling Observatory for Soil Organic Matter), optimised for continuous, high-intensity ^{13}C labelling of plants grown under precise environmental control, which is essential to accurately quantify new SOM formation and RPEs in different ecological contexts. The current experiment running in BLOSOM involves growing *Agrostis capillaris* in bespoke mesososms in a $^{13}CO_2$-enriched environment, rooting into soils collected from near-surface permafrost and the overlying active layer. Although the results of this experiment are not presented or discussed here, it serves to illustrate the capacities of BLOSOM to run plant–soil experiments in cold-region soils and its potential for future studies on soils from other regions.

2 | Methods

2.1 | Growth Chamber Construction

The BLOSOM chamber at the University of Exeter, UK, is built in two halves, a top and a bottom (Figure 1). Both halves are timber-framed, double-skin structures with cavity walls to aid insulation from laboratory temperatures. The bottom half of the chamber is designed to be a large water bath to facilitate soil temperature control (see Section 2.4 below). This section is made from 50 × 75 mm Canadian Lumber Standard (CLS) timber and 18 mm plywood sheets filled with 150 mm thick Loft Roll glass mineral wool insulation (Knauf, Kent, UK). The timber structure is made watertight internally using Cure It (G&B Northwest Ltd., Wigan, UK) fibre glass and resin. The water bath at the base of the chamber is 40 cm deep and ~1800 L in volume.

The top half of the chamber is made from 50 × 75 mm CLS timber, 18 mm OSB structural board and clad with 5 mm plywood sheets. The horizontal roof section is filled with 150 mm glass mineral wool insulation, but the vertical sides are not to enable tubes to pass through them (see Section 2.3 below).

The top half is fitted with six hatch doors to enable 'reach-in' access. The doors are made of 5 mm PVC sheet and 5 mm plywood sheet encasing 150 mm glass mineral wool insulation. Two doors

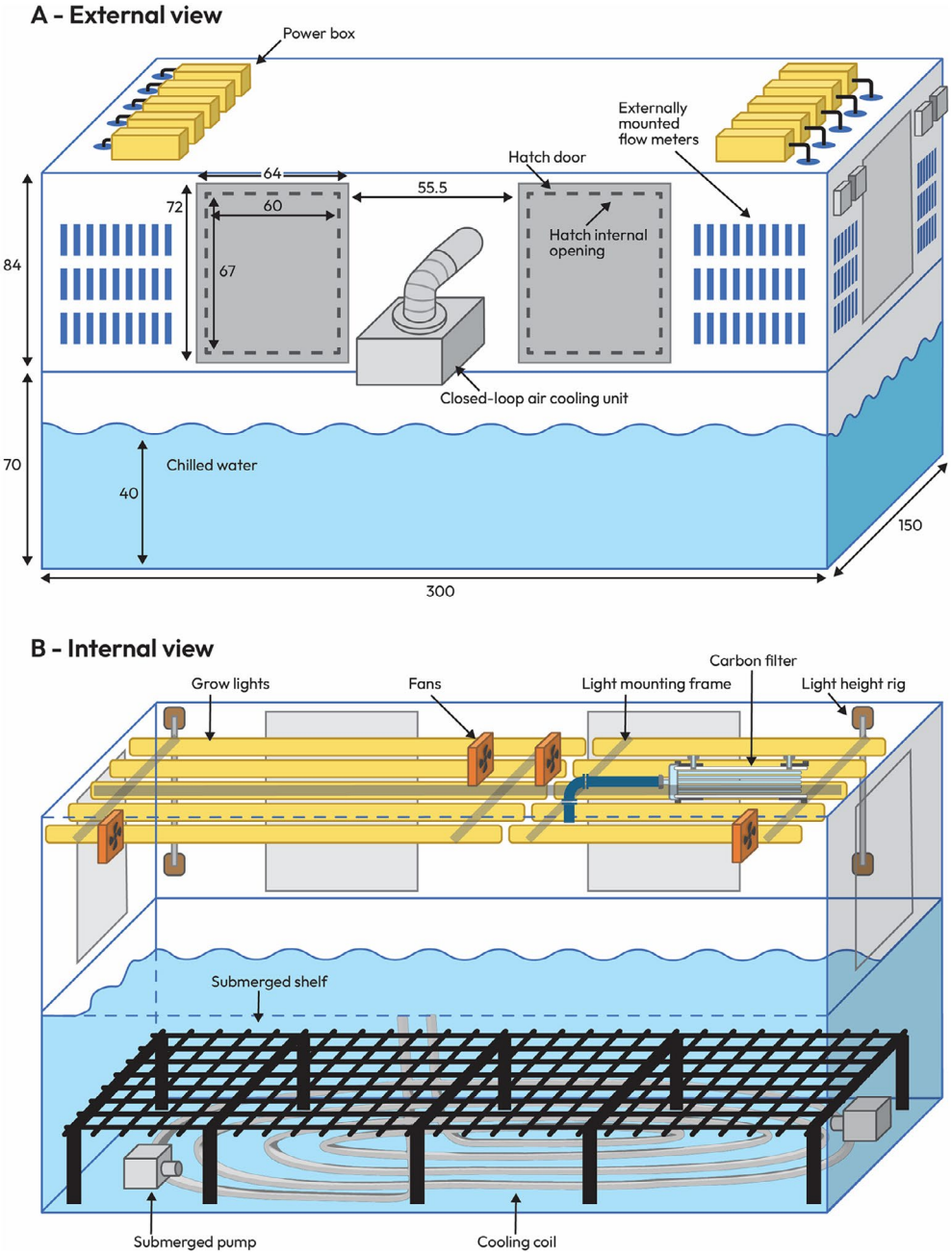

FIGURE 1 | Diagram of the external (A) and internal (B) views of the BLOSOM chamber. Units are cm. (A) Externally mounted flow meters control the respiration system (Figure 2). Power boxes on chamber roof (A) regulate internal grow lights (B). (B) The submerged shelf supports plant–soil experimental units and the light height rig allows for adjustable height of grow lights above plants.

are fitted with clear Perspex windows to allow visual monitoring of the internal environment without opening. The doors' closing mechanism uses an overlapping crush seal with a compressible foam layer to maintain airtightness of the chamber when closed. The whole chamber is finished internally and externally with high gloss white paint (Ronseal, UK).

We estimate that the total cost of materials used for the construction of BLOSOM and the equipment required for gas mixing (see Section 2.2), respiration system (see Section 2.3), temperature control (see Section 2.4) and lighting (see Section 2.6) is approximately £70,000. This estimate excludes personnel time.

2.2 | Gas Mixing and Flow Control

To achieve continuous labelling at a constant level of enrichment in BLOSOM, three gases are mixed and controlled by mass flow controllers (MFC) (Bronkhorst UK Ltd., Newmarket, UK).

Compressed air is controlled by MFC LOW-ΔP-FLOW F-202EV at 40 L min^{-1}. The compressed air is low in CO_2 (10–30 ppm) due to alumina desiccant used in the air compression process. Unlabelled CO_2 supplied from industrial grade CO_2 (99.8% pure, BOC, UK) is controlled by MFC EL-FLOW Select F-201CV, ranging from 5 to 150 mL min^{-1} depending on plant productivity and diurnal cycles (see Section 2.6

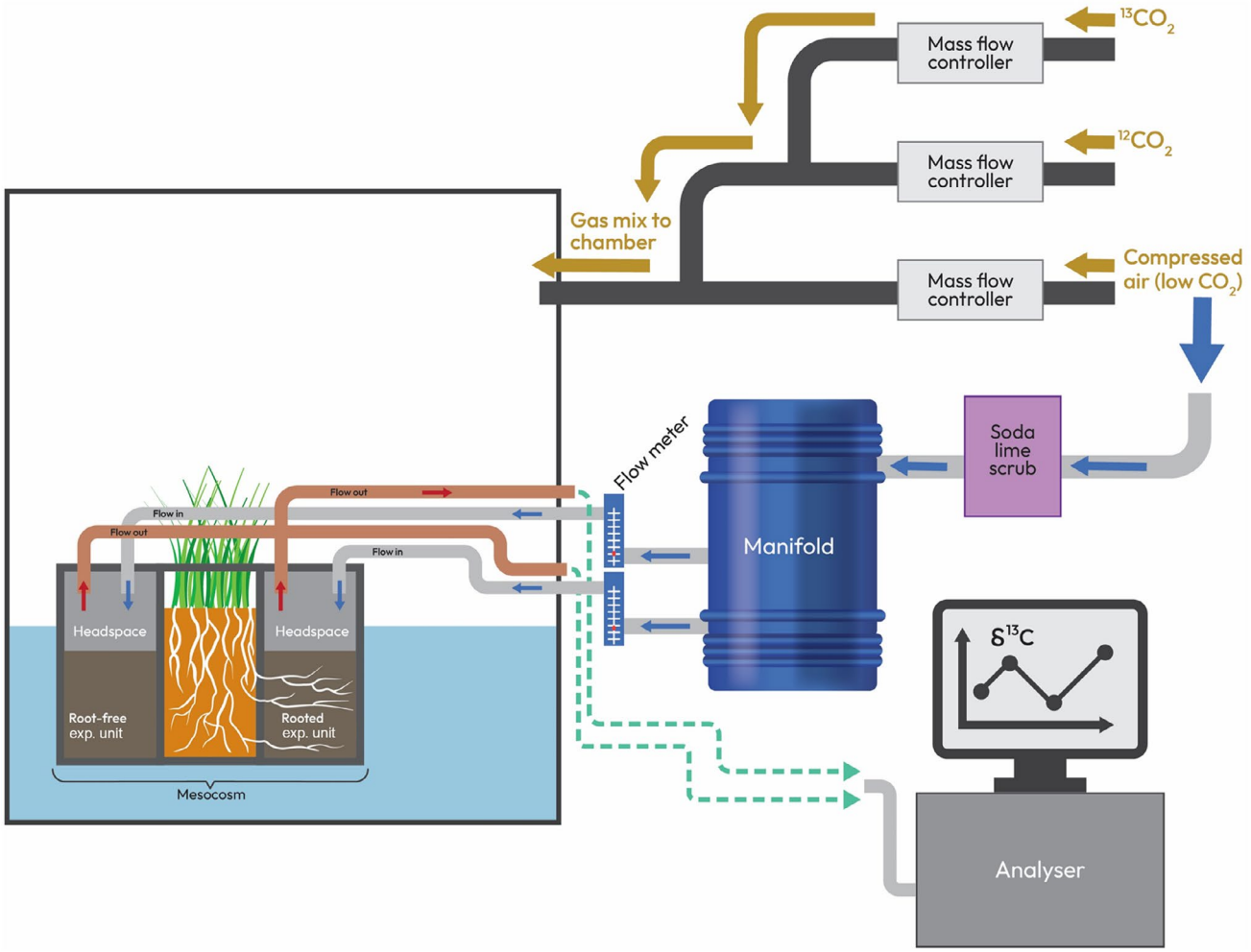

FIGURE 2 | Diagram of BLOSOM gas flows. The gases are sequentially mixed and piped into the chamber (dark grey, brown arrows). The push-and-pull through respiration system flows CO_2-free air in (light grey, blue arrows) through the soda lime scrub, manifold and into headspaces, then air flows back out (red and green arrows) to the isotopic analyser with respired CO_2 from the soil, including ^{13}C originally fixed by the plants.

below). Labelled CO_2 is supplied from 99% $^{13}CO_2$ (< 2% ^{18}O) (CK isotopes Ltd., UK) controlled by MFC EL-FLOW Select F-200CV, ranging from 0.001 to 0.6 mL min^{-1} depending on plant productivity and diurnal cycles. The three gases are sequentially mixed so that the $^{13}CO_2$ flows into the unlabelled $^{12}CO_2$ stream, which in turn flows into the compressed air stream and then into the BLOSOM chamber (Figure 2). The flow of each gas is varied depending on photosynthetic uptake throughout the > 6-month long experiment.

The controlled ratios of the gases result in ~440 ppm CO_2 and ~515‰ $\delta^{13}C$ (1.66–1.69 atom %) within BLOSOM and are maintained consistently throughout the diurnal cycle. Consistent levels of CO_2 concentration and $\delta^{13}C$ enrichment within BLOSOM are maintained by varying the flow rates of unlabelled CO_2 and $^{13}CO_2$ to match the diurnal cycles of the grow lights (see Section 2.6 below).

2.3 | Respiration System

BLOSOM has the capacity to measure fluxes via a push-and-pull-through system for 216 individual soil filled experimental units, allowing for experiments with high replication and complex experimental design. A total of 216 individually controlled lines of PVC tubing runs low CO_2 compressed air (further reduced to 0 ppm using a soda lime scrub upstream of the manifold) to 216 analogue flow meters (model RMA-1-SSV SCFH air 0.25–0.4, Dwyer Instruments) mounted externally (Figure 1). Each controls the flow of compressed air at ~20 mL min^{-1} in 216 lines running through the BLOSOM chamber wall into each of the 216 individual experimental units. Each experimental unit has an inlet for flow into the headspace and an outlet for flow out of the headspace (Figure 2).

This push-and-pull through respiration system enables the flux of CO_2 to be derived from the flow rate of air into each experimental unit and the CO_2 concentration of the outcoming air. Air flow, CO_2 concentration and isotopic composition are measured externally. During measurements each outlet line is connected to a Picarro G2201-i isotopic analyser (Picarro Inc. Santa Clara, CA) for 3–4 min until CO_2 is stable. During this time, the inlet line is connected to a digital flow meter LOW-ΔP-FLOW F-101D (Bronkhorst UK Ltd., Newmarket, UK) to accurately record the flow into the headspace at the time of measurement. Drift from

the set point was found to be limited based on continuous monitoring of some lines.

The flux of unlabelled CO_2 from each soil was calculated by the following equation:

$$\text{Unlabelled } CO_2 = \left(\left(\frac{\delta^{13}C_{chamber} - \delta^{13}C_{ExU}}{\delta^{13}C_{chamber} - \delta^{13}C_{air}} \right) \times CO_{2ExU} \right) \times Flow_{air} \quad (1)$$

where chamber is the BLOSOM chamber environment, ExU is the experimental unit headspace and air is the incoming air flowing into the headspace.

2.4 | Soil and Air Temperature Control

The temperature of the BLOSOM chamber air and experimental unit soil are controlled separately with cooling power provided by a common 4 kW FL4003 recirculating cooler (Julabo UK Ltd.) running a 50:50 water:glycol mix.

Soil temperature is maintained at 5°C by partly submerging (15 cm water height) mesocosms within the water bath (Figures 1 and 2). The water bath temperature is maintained at 5°C by a submerged 40 m long, 16 mm ø flexible metal cooling coil circulating a water: glycol mix (50:50) between the cooling coil in the chamber water bath and the Julabo cooler. Two submerged 3000 L h^{-1} pond pumps (AllPondSolutions Ltd., UK) at opposite ends of the water bath continuously mix the water to ensure even water temperature (Figure 1).

Air temperature is maintained at 20°C–22°C by a bespoke closed-loop air conditioning system (Cambridge HOK, UK). The system draws warm air from the top of the chamber, passes it over a coil cooled by the Julabo cooler and circulates the cooled air back into the BLOSOM chamber at plant height.

Air and water bath temperature is measured and logged using waterproof thermistors connected to a PicoLog (Pico Technology Ltd., UK) ADC-16 analogue to digital converter logging every 10 min. To compare the water bath temperature and the mesocosm soil temperature TinyTag TGP-4204 temperature data loggers were deployed in three mesocosms for 10 days. During this time, the water bath temperature was 5.28°C ± 0.04°C (mean ± SD) and the soil temperature was 5.23°C ± 0.04°C (mean ± SD). Therefore, we conclude that measured water bath temperature is representative of soil temperature and will be referred to as such hence forth.

2.5 | Chamber Air Mixing

Air circulation and mixing of the gases within the BLOSOM chamber is achieved by four axial fans (Figure 1), 24 V dc, 68 $m^3 h^{-1}$, 80 × 80 × 25 mm (RS Components Ltd., UK). Additional air movement and purification are achieved using a rapid air movement carbon filter fitted to a 199 $m^3 h^{-1}$ in-line fan (London Grow Ltd., UK).

2.6 | Plant Growth Lighting and Maintenance

Grow lighting within the BLOSOM chamber is achieved by five BX120c2 and five BX180c2 LED strip lights with output wavelengths of 380–780 nm (Valoya Ltd., Helsinki, Finland). Each bank of lights is mounted end to end to cover the growth area. This arrangement was designed and modelled by Valoya Ltd. to achieve a maximum light intensity of 500–700 μmol $m^{-2} s^{-1}$ across 95%–97% of the growth area. Externally mounted LED drivers are fitted with resistance dimmers controlled independently to achieve 250–300 μmol $m^{-2} s^{-1}$ across the growth area as this was determined optimal through extensive trials.

The lights are run on an inverted diurnal cycle with a 16 h photoperiod programmed and controlled by a digital light switch timer (mydome, Switched On Products Ltd.). The diurnal cycle is inverted, that is, grow light are on during the night (17:30–09:30) and off during the day (09:30–17:30), to allow plant maintenance and flux measurements when plants in the chamber are not photosynthetically active.

For the current experiment in BLOSOM *Agrostis capillaris* is grown in bespoke mesocosms, with rooted and root free experimental units. *Agrostis capillaris* was used as it combines a distribution that includes high northern latitudes with high germination rates from seed and an ability to establish and grow well in growth chamber studies. This was deemed the best combination for geographical relevance and experimental application. Routine surface watering according to plant demand (100–500 mL) is carried out twice weekly throughout the duration of the experiment. Once fully mature, shoots were clipped once per month or when shoots reached 50 cm height or more for the duration of the experiment.

2.7 | Soils Used in the Current BLOSOM Experiment

The field area selected was a transect in northwest Canada from Tuktoyaktuk on the Arctic Ocean coast through the East Channel region of the Mackenzie Delta near Inuvik, NT, to the Klondike Goldfields of interior Yukon Territory. The transect crosses from continuous permafrost at Tuktoyaktuk and in the Mackenzie Delta to extensive discontinuous permafrost in the Klondike. Samples were collected in August 2019.

Four types of permafrost soil profiles were sampled: (1) non-lake orthels, (2) drained thermokarst-lake sediments, (3) turbels and (4) yedoma. The rationale was threefold: first, to compare examples from the main types of permafrost soils (Gelisols) that is, Turbel, Orthel; second, to compare soils developed on epigenetic permafost with syngenetic permafrost represented by yedoma deposits; and third, to compare soils developed in drained thermokarst-lake basins (an integral part of lowland, ice-rich permafrost terrain, which often preferentially accumulate soil organic carbon) with non-lake profiles.

All collected soil samples where frozen upon return to the field station and shipped frozen to the University of Exeter where

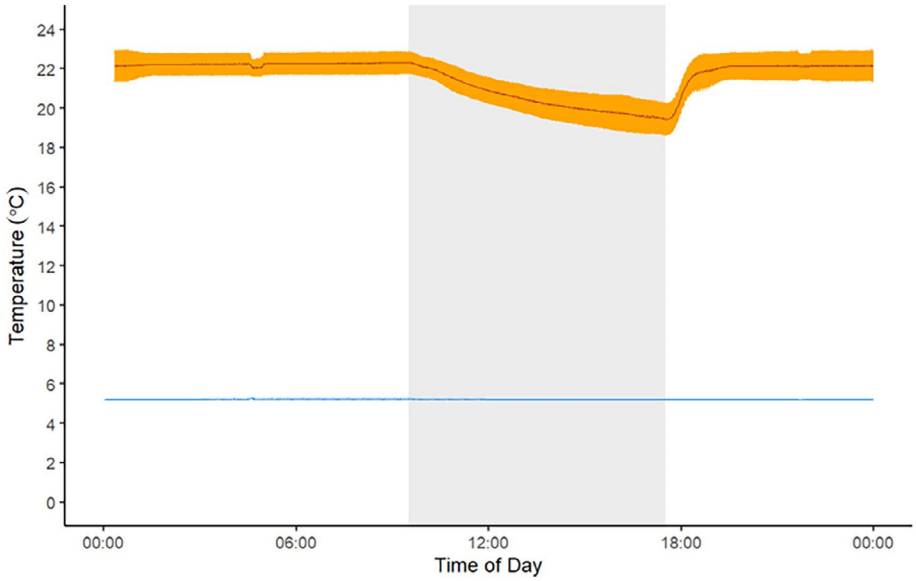

FIGURE 3 | Two-minute rolling average for diurnal temperatures of air (red with orange standard deviation ribbon) and soil (dark blue with light blue standard deviation ribbon) recorded between June 2023 and January 2024. Grey area indicates daily period of lights-off between 09:30 and 17:30. Days when BLOSOM chamber was opened for maintenance were excluded from this dataset.

they remained in frozen storage until processed for the beginning of the experiment.

2.8 | Statistical Analyses

All statistical analyses were performed using R version 4.3.0 (2023-04-21 ucrt). The 'rollapply' function from the 'zoo' package (Zeileis and Grothendieck 2005) in R was used to compute rolling summaries of time-series temperature and CO_2 concentration and isotopic data.

Uncertainty of new SOM formation estimates assuming a ±0.5‰ instrument analytical error was derived for different levels of new SOM formation (%) using the following equation:

$$\text{Uncertainty}(\%) = 100 - \left(\frac{\left(\frac{\delta^{13}C_{\text{sample}} - \delta^{13}C_{\text{SOM}}}{\delta^{13}C_{\text{New SOM}} - \delta^{13}C_{\text{SOM}}} \right)}{\left(\frac{\text{New SOM formation}(\%)}{100} \right)} \right) \times 100 \quad (2)$$

where sample is a soil sample from a continuous ^{13}C labelling experiment, SOM is the pre-existing soil organic matter in that sample and New SOM is the new soil organic matter in that sample. The same equation was used to derive additional uncertainty associated with ±0.5‰ error on $\delta^{13}C$ of pre-existing SOM and ±5‰ error on $\delta^{13}C$ of new SOM. $\delta^{13}C_{\text{New SOM}}$ depends on the labelling intensity, modelled for running BLOSOM with high (500‰), low (100‰) and fossil CO_2 (−50‰) labelling intensities.

3 | Results

3.1 | Environmental Conditions

BLOSOM mean air temperature was 21.4°C ± 1.2°C and ranged from 17.8°C to 23.5°C across diurnal cycles (Figure 3). The average air temperature during the lights-on period between 17:30 and 09:30 was 22.0°C ± 0.9°C (mean ± SD) and during the lights-off period between 09:30 and 17:30 was 20.4°C ± 1.3°C (mean ± SD).

BLOSOM mean soil temperature was 5.24°C ± 0.05°C and ranged from 5.1°C to 5.5°C across diurnal cycles (Figure 3). The average soil temperature during the lights-on period was 5.23°C ± 0.05°C (mean ± SD) and during lights-off was 5.22°C ± 0.05°C (mean ± SD).

3.2 | Growth Chamber Atmosphere Stability Over Time

The CO_2 concentration in the BLOSOM chamber ranged from 360.39 to 525.92 ppm across diurnal cycles (Figure 4). The average CO_2 concentration during the lights-on period was 455.20 ± 36.34 ppm (mean ± SD) and during the lights-off period was 450.79 ± 33.39 ppm. The CO_2 concentration was within 5% of the ~440 ppm set point for 83.6% of the average diurnal cycle and within 10% of the set point for 100% of the average diurnal cycle.

The $\delta^{13}C$ isotopic composition ranged from 410.76‰ to 579.93‰ across diurnal cycles (Figure 5). The average $\delta^{13}C$ isotopic composition during the lights-on period was 506.11‰ ± 26.95‰ (mean ± SD) and during the lights-off period was 506.33‰ ± 29.01‰. The $\delta^{13}C$ isotopic composition was within 5% of the 515‰ set point for 98.1% of the average diurnal cycle and within 10% of the setpoint for 100% of the average diurnal cycle.

The greatest variation in both CO_2 concentrations and $\delta^{13}C$ isotopic composition across diurnal cycles occurred in the time immediately following the grow lights switching on or off. This effect was greatest in the $\delta^{13}C$, resulting in a small spike in $\delta^{13}C$ after the grow lights went off and a small and brief dip in $\delta^{13}C$ after they came on.

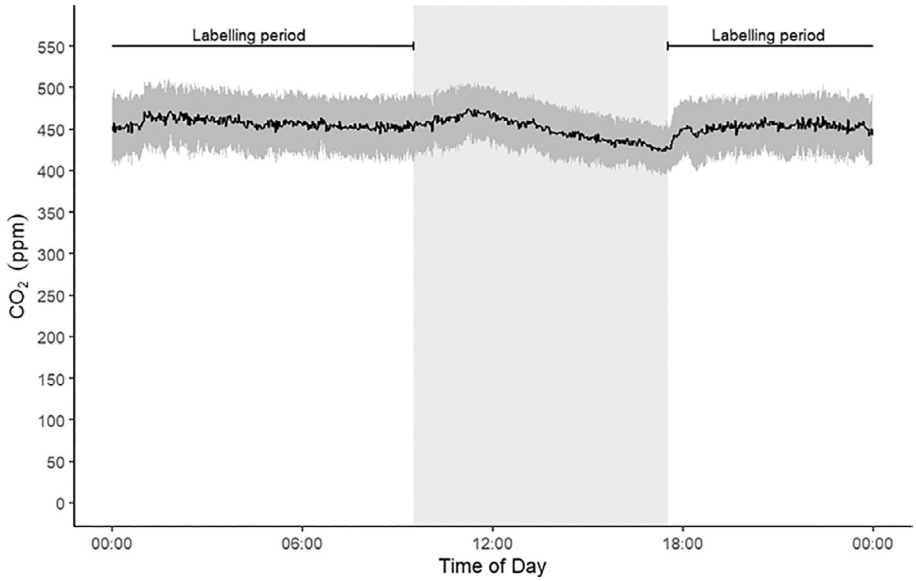

FIGURE 4 | BLOSOM diurnal CO_2 concentrations with a 2 min rolling average (black) and standard deviation ribbon (dark grey) recorded between June 2023 and January 2024. Light grey area indicates daily period of lights off between 09:30 and 17:30. Days when BLOSOM chamber was opened for maintenance were excluded from this dataset.

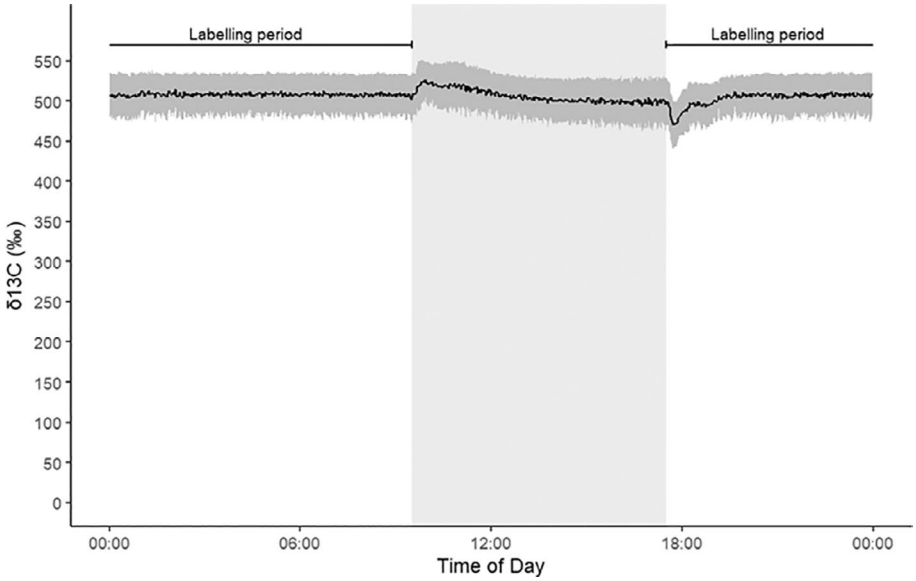

FIGURE 5 | BLOSOM diurnal $\delta^{13}C$ concentrations with a 2 min rolling average (black) and standard deviation ribbon (dark grey) recorded between June 2023 and January 2024. Light grey area indicates daily period of lights off between 09:30 and 17:30. Days when BLOSOM chamber was opened for maintenance were excluded from this dataset.

3.3 | Uncertainty of New SOM Formation Estimates

The uncertainty of new SOM formation estimates depends on the $\delta^{13}C$ labelling intensity at which plant–soil experiments are run (Figure 6). To illustrate this, the total additive error associated with (i) analytical error of ±0.5‰, (ii) pre-existing SOM $\delta^{13}C$ uncertainty of ±0.5‰ and (iii) new SOM $\delta^{13}C$ uncertainty of ±5‰ was modelled. The total additive error ranges from ±1108, ±163 and ±39% at 0.5% new SOM formation to ±65, ±11 and ±3% at 10% new SOM formation at −50‰, 100‰ and 500‰, respectively. This illustrates a 'worst-case scenario' by assuming all errors are additive, which they may not be, and could in some cases cancel each other out. Furthermore, these derivations do not take replication into account and uncertainty may vary from the levels assumed here.

3.4 | Measurements of Soil $\delta^{13}C$ and CO_2 Fluxes

Measurement of soil $\delta^{13}C$ and CO_2 fluxes was carried out monthly and consisted of manually changing the intake line connected to a Picarro isotopic analyser between each of the 216 outlet lines connected to headspaces (Figure 2). For each measurement the Picarro was connected to the outlet line for 3–4 min until $\delta^{13}C$ and CO_2 concentrations were stable. Figure 7A shows a representative period of measurements between 12:30 and 14:30 on 23rd August 2023. For each

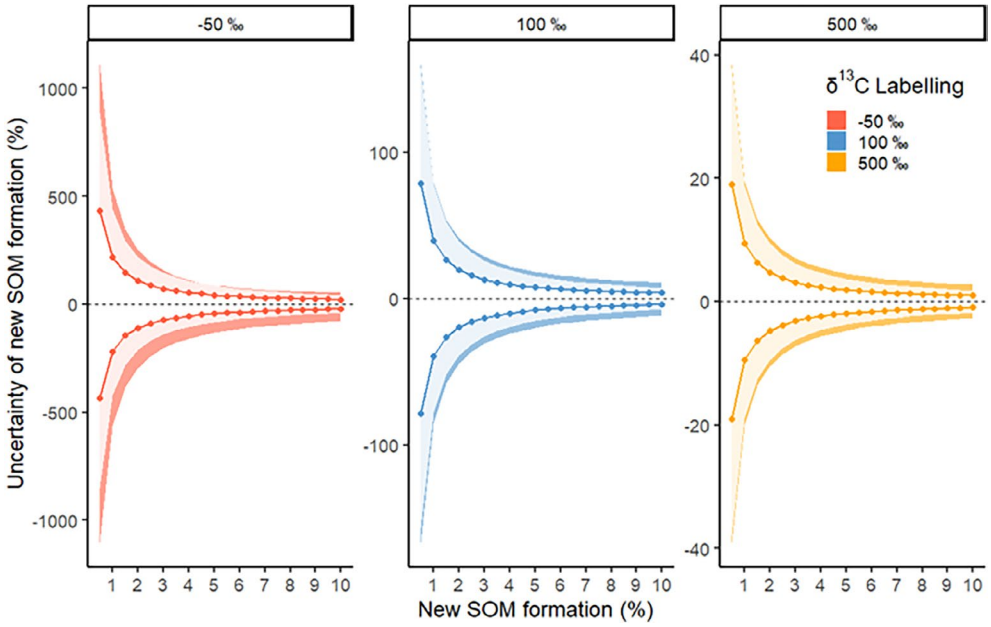

FIGURE 6 | Estimated uncertainty of new SOM formation derived for 0.5%–10% new SOM formation at three different labelling intensities for un-replicated samples. Uncertainty of new SOM formation was derived from Equation (2) above. Points indicate uncertainty associated with ±0.5‰ analytical error, light shading indicates additional error associated with ±0.5‰ error on $\delta^{13}C$ of pre-existing SOM and dark shading indicates additional error associated with ±5‰ error on $\delta^{13}C$ of new SOM. Note *y*-axis scale differs for each labelling intensity.

experimental unit (Figure 7B) the average $\delta^{13}C$ and CO_2 concentration in the headspace over the stable measurement period was taken and applied to Equation (1) with the chamber background $\delta^{13}C$ and CO_2 concentrations as well as the flow and $\delta^{13}C$ of compressed air into the headspace at the time of measurement.

In this example mesocosm, experimental unit i contained live *A. capillaris* roots, whereas experimental unit ii was root-free. Therefore, the RPE was calculated by the absolute difference in unlabelled CO_2 flux between rooted and root-free control experimental units. In this example (Figure 7B) unlabelled CO_2 flux from the rooted experimental unit (i) was $0.036\,\mu g\ C\ g\ dw\ soil^{-1}\,h^{-1}$ and the root-free experimental unit was $0.017\,\mu g\,C\,g\,dw\,soil^{-1}\,h^{-1}$ resulting in an absolute RPE of $0.019\,\mu g\,C\,g\,dw\,soil^{-1}\,h^{-1}$.

4 | Discussion

4.1 | Measurements of Rhizosphere Priming in Temperature-Controlled Soils

BLOSOM can control soil and air temperature independently and consistently through diurnal cycles and over long periods of time (> 6 months). The ~16°C temperature difference between the air and soil demonstrates the decoupling of the subsurface thermal behaviour from surface or air temperature fluctuations. This is consistent with observations from northern permafrost regions (Hinkel et al. 2001) and illustrates the ability of BLOSOM to overcome *C2* and control soil and air temperatures independently to simulate cold-region temperature regimes. The ability of BLOSOM to independently control air and soil temperatures at levels representative of northern permafrost regions enables the unique opportunity to run long-term rhizosphere priming experiments in permafrost soils using live plants to supply the C substrates.

The current experiment was run for long periods (> 6 months) with the environmental conditions set to be uniform throughout this time, artificially extending the growing season (for high latitudes) to gain detailed mechanistic understanding of rhizosphere priming and new SOM formation. However, it would also be possible to adjust the environmental conditions over time to simulate natural seasonal variations in temperature and photoperiod.

Thus far, BLOSOM has been run to grow *A. capillaris* in thawed permafrost soils in bespoke mesocosms in a ^{13}C-enriched atmosphere. However, we consider BLOSOM highly adaptable. For example, BLOSOM could be applied to research on warmer climates with higher soil and air temperatures, but with similar soil–air temperature offsets. It could also accommodate experiments growing different plant species in different soils, with customisable isotopic composition of the atmosphere.

4.2 | Estimates of Rhizosphere Priming Relies on Endmember Certainty

BLOSOM can maintain concentrations of CO_2 and strong $\delta^{13}C$ labelling over diurnal cycles and throughout long-term experiments (Figures 4 and 5). This illustrates its ability to overcome *C1* maintaining a continuous, strong and diurnally stable ^{13}C label enabling plant–soil experiments to quantify new SOM formation and RPEs in a range of soil types.

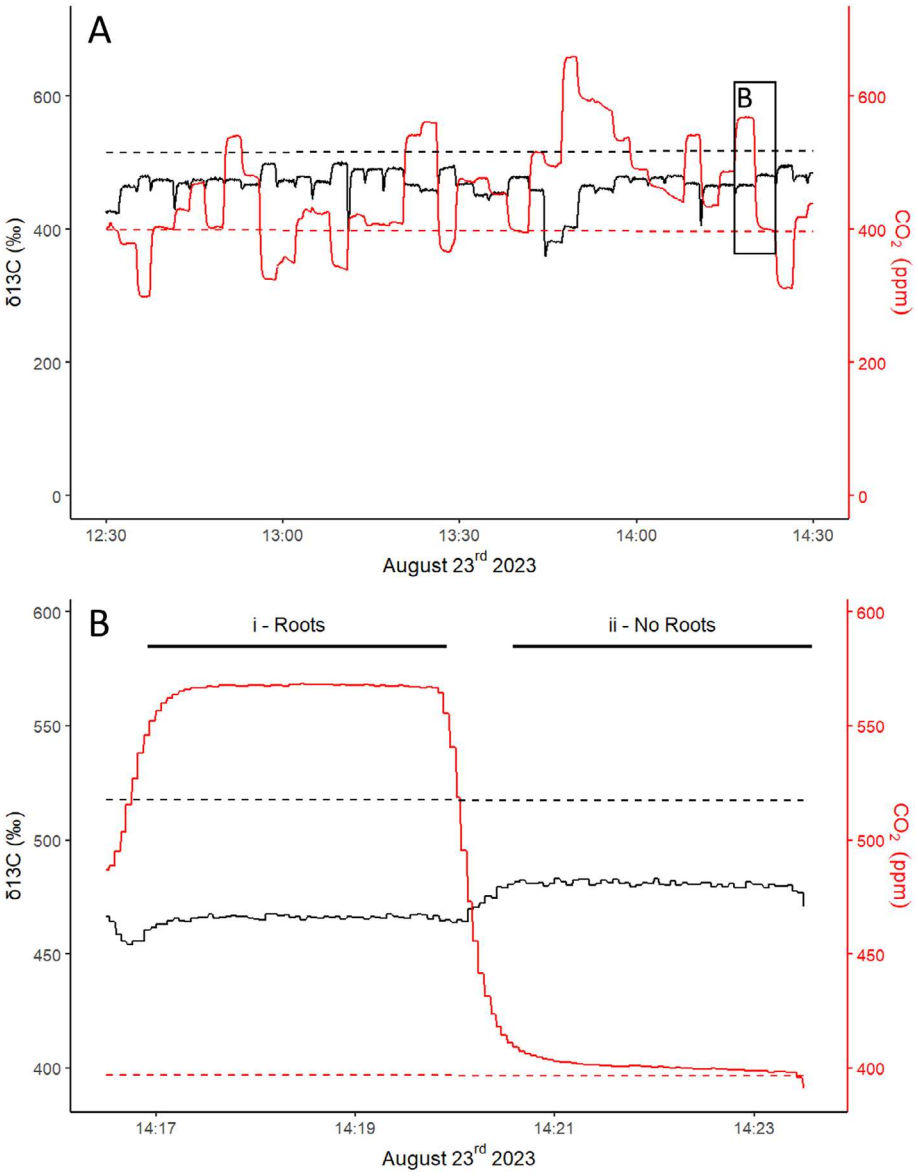

FIGURE 7 | (A) Soil $\delta^{13}C$ (‰) (solid black) and CO_2 (ppm) (solid red) measured from the mesocosm headspace using the push-and-pull through respiration system on 23rd August 2023, selected as a representative day of flux measurements. Background chamber $\delta^{13}C$ (‰) (dashed black) and CO_2 (dashed red), the atmosphere experienced by the plants, was monitored during measurements. (B) Fluxes of one mesocosm from experimental units with (i) and without (ii) *A. capillaris* roots.

The target $\delta^{13}C$ enrichment for the current experiment was ≈515‰, equivalent to ≈523‰ enrichment above ambient and ≈543‰ above soil-derived C fluxes. On average, the target levels of enrichment were achieved throughout the diurnal cycle. However, some periods at levels of $\delta^{13}C$ enrichment both above and below the target did occur (Figure 5). Despite this variation, tight control on the isotopic composition was maintained, resulting in consistent ^{13}C labelling of *A. capillaris* significantly above ambient. This enables the production and harvesting of consistently ^{13}C labelling of *A. capillaris* litter, which is a valuable resource for future experiments.

The achieved level of enrichment above ambient provided a strong plant $\delta^{13}C$ signal ($\delta^{13}C_{plant} \approx 515‰$), clearly distinguishable from the soil $\delta^{13}C$ signal ($\delta^{13}C_{soil} \approx -28‰$) (*C1*). A strong labelling intensity is critical to accurately quantify rates of new SOM formation (Figure 6). Simulations of additive uncertainty of new SOM formation across a range of theoretical levels of new SOM formation show that labelling intensities of −50‰ $\delta^{13}C$ (analogous to running plant–soil experiments with fossil-derived CO_2) cannot accurately estimate new SOM formation even at 10% new SOM, which would require extremely high rates of new SOM formation or very long experimental durations. Some of these challenges may be overcome by high levels of experimental replication. When running plant–soil experiments with strong labelling intensities of 500‰ (as BLOSOM does) the additive uncertainty of new SOM formation is much lower, even at just 0.5% new SOM. Continuous, stable high labelling intensities such as those achieved in BLOSOM are critical to accurately quantifying rates of new SOM formation in short- to medium-term experiments. Accurately quantifying rates of new SOM formation requires at least one of (a) high rates of new SOM formation, (b) high levels of replication or (c) strong labelling intensities. BLOSOM provides the latter two.

It has been demonstrated that the uncertainty of rhizosphere priming estimates depends on the strength of the plant $\delta^{13}C$ signal (Cros et al. 2019). Using a similar method to Cros et al. (2019) for quantifying the uncertainty of RPE estimations for a 1‰ deviation in $\delta^{13}C$ (+0.5‰ or −0.5‰) at the 543‰ labelling intensity achieved in BLOSOM we found an uncertainty of 0.664% and −0.665% for a +0.5‰ and −0.5‰ deviation in $\delta^{13}C$, respectively. Given that BLOSOM can run at high labelling intensities of > 500‰ with associated low uncertainties on RPE estimates, we have a high degree of confidence in the resulting data on new SOM formation and RPEs.

4.3 | Using Continuously ^{13}C-Labelled Plants Is Effective but Costly

Using continuously ^{13}C-labelled plants to deliver isotopically enriched substrates into soils has the potential to improve our understanding of the mechanisms and drivers underpinning new SOM formation and RPEs. The specific interactions between plant roots and the soil matrix can be investigated in experiments using live plants and provide understanding of analogous field processes which may be missed in experiments using manual substrate additions.

Continuously labelling plants at high levels of enrichment results in a strong plant $\delta^{13}C$ signal ($\delta^{13}C_{plant} \approx 515‰$), clearly distinguishable from the soil $\delta^{13}C$ signal ($\delta^{13}C_{soil} \approx -28‰$). This is essential for accurate detection of new SOM formation (Figure 6) and RPEs with low uncertainty (Cros et al. 2019). Therefore, future work, which aims to understand and quantify rates of new SOM formation or SOC sequestration for the benefit of climate change mitigation or carbon accounting will require continuous, high-intensity labelling of the chosen plant species as is achievable with BLOSOM. However, labelling at such high intensities comes with a high monetary cost associated with the 99 atom % $^{13}CO_2$ required. In the current experiment, 50 L of 99 atom % $^{13}CO_2$ facilitated 6 months of continuous labelling at $\delta^{13}C \approx 515‰$ costing around £8000 (GBP) at time of writing. One way to reduce the cost of experiments in BLOSOM is to run the chamber at a reduced labelling strength, for example, at ~100‰. This would reduce the labelling intensity, making new SOM formation harder to detect accurately (Figure 6) and increasing the uncertainty on RPE estimates but would reduce the amount of 99 atom % $^{13}CO_2$ and therefore monetary cost required.

Lower-cost continuous plant labelling has been achieved in several experiments using fossil-derived CO_2 (Cros et al. 2019; Pausch et al. 2013; Zhu et al. 2014). However, this approach results in a weak labelling intensity with a much-reduced difference between endmembers and greater uncertainty associated with new SOM formation and RPE estimates (Figure 6). Theoretically, BLOSOM could be run using fossil CO_2 should this provide sufficient labelling intensities for the desired experiment. There are several options to adapt the presented system to reduce the running costs, but running with pure $^{13}CO_2$ provides the greatest flexibility with regards to labelling intensities.

The ability of BLOSOM to precisely and independently control air and water temperatures as well as light intensity and photoperiod makes it highly adaptable with regards to environmental conditions and therefore suitable to run a multitude of plant–soil experiments across a wide range of ecological and seasonal contexts. For example, BLOSOM could be utilised to understand the effects of different crops or agricultural management practices on SOC dynamics and the implication of climatic extremes on SOM stability in different agricultural soils, or be deployed to investigate SOC dynamics in natural ecosystems across a wide range of soil types, temperatures, plant communities and growing season lengths under various emissions scenarios.

5 | Conclusions

We present a new experimental facility for optimised for continuous ^{13}C labelling of plants at high labelling intensities and external soil CO_2 flux measurements to quantify new SOM formation and RPEs in temperature-controlled soils. As the uncertainties of RPE and SOM formation estimates depend on the strength of the plant $\delta^{13}C$ signal, we demonstrate that continuous, stable high labelling intensities such as those achieved in BLOSOM are critical to accurately quantifying rates of new SOM formation in short- to medium-term experiments. BLOSOM is highly adaptable and has the potential improve quantification and mechanistic understanding of new SOM formation and RPEs across a range of simulated climatic conditions. An experimental system with such flexibility of environmental conditions, which delivers accurate quantification of both RPEs and new SOM formation enables significant advances in understanding SOC dynamics and can provide robust and timely evidence of which combinations of plants, soils and managements can lead to stable C sequestration and critically which cannot, critical in a changing world increasingly looking to soils for nature based solutions to climate change.

Author Contributions

The experiment was designed by N.L.F., I.P.H., G.K.P. and J.B.M. The setup of the experimental platform was done by N.L.F. and N.E. N.L.F. performed analysis. N.L.F. wrote the initial draft. All the authors contributed to improve the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.