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METHOD
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**Key Points:**

- We present a simplified and low-cost gas sampling system, developed for quantifying $CO_2$ fluxes in soil incubation experiments
- Respiration rates down to 0.1 µmol $CO_2$ $m^{-2}$ $s^{-1}$ were quantified, confirming the suitability of the system for low-$CO_2$ flux measurements
- A balloon was installed, mitigating 72% of pressure changes in the headspace during sampling

**Supporting Information:**

Supporting Information may be found in the online version of this article.

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# Tracing Low-$CO_2$ Fluxes in Soil Incubation and $^{13}C$ Labeling Experiments: A Simplified Gas Sampling System for Respiration and Photosynthesis Measurements

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**Abstract** Quantifying carbon dioxide ($CO_2$) fluxes between soil and atmosphere is key in understanding net ecosystem C exchange and biogeochemical C cycling in plant-soil systems. In ecosystems with low primary production and sparse vegetation, for example, dry lands or subpolar regions where C fluxes are small, measurement sensitivity is key—even so when measurements are combined with isotopic labeling. Here, we present a simplified gas sampling system developed to facilitate sampling and measurement of low soil $CO_2$ fluxes as well as in situ $^{13}CO_2$ labeling in the same setup. The capacity of the system was tested in a set of feature tests along with gas measurements of dryland soil-biocrust systems. The system's sensitivity to capture minor changes in $CO_2$ concentration was confirmed in respiration and photosynthesis measurements of soil-biocrust systems, where fluxes down to 0.1 µmol $CO_2$ $m^{-2}$ $s^{-1}$ were quantified. A balloon, implemented to counterbalance underpressure build-up during gas withdrawal, mitigated 72% of pressure differences at sampling. The overall system volume was reduced to a minimum to limit contamination caused by residual air, and the design enabled one-step flushing and evacuation of system compartments and gas sample bags, successfully ruling out cross-contamination between samples. Ultimately, this system offers a flexible and accessible solution for $CO_2$ measurements that can be applied not only on arid soils with low biological activity and turnover rates, but also on plant-soil systems. The modifications enabled larger, and thereby more representative, sample volumes to be collected while limiting incubation, contamination, and pressure effects on the intact soil system.

**Plain Language Summary** Measurements of carbon dioxide ($CO_2$) fluxes between soil and atmosphere are crucial to understand the terrestrial carbon cycle. In dry regions, these fluxes are rather small as both the release of $CO_2$ via microbial respiration and uptake of $CO_2$ via photosynthesis is limited by lack of water and sparse vegetation cover. We have developed a simplified $CO_2$ gas sampling setup suitable for incubation experiments of soils with low biological activity and turnover rates, for example, soils from dry lands or subpolar regions, using only cost-effective, easily available and replaceable system compartments. We tested system parameters in a set of feature tests and could rule out leakage and cross-contamination. We further confirmed the suitability of the system for capturing small $CO_2$ fluxes in photosynthesis and respiration measurements of soils with biocrusts. A balloon was installed to counterbalance pressure build-up during sampling, allowing for larger gas volumes to be collected, which resulted in a mitigation of 72% of the underpressure build-up. This system offers a straightforward and accessible solution for $CO_2$ measurements, with features limiting contamination and pressure effects on the research sample.

## 1. Introduction

Quantifying $CO_2$ fluxes of soils or combined plant-soil systems and the atmosphere is key for understanding fundamental biogeochemical processes involved in the cycling and fate of carbon (C). Yet, our understanding of the magnitude of, and controlling factors on, net ecosystem $CO_2$ exchange is limited—particularly so in arid, semiarid and dry subhumid areas, commonly known as dry lands (Scholes, 2020; Warner et al., 2019). The lack of representability of these systems in $CO_2$ flux and respiration studies compared to other biomes is ambiguous as

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dry lands store >25% of the global soil organic C stocks and are, thus, of great significance in the global C cycle (Reynolds et al., 2007; Safriel et al., 2005).

In ecosystems with little vegetation cover, $CO_2$ measurements are connected with certain methodical challenges. These soils are characterized by relatively low microbial abundance and soil organic matter contents and, thus, exhibit lower rates of heterotrophic respiration and photosynthesis compared to more productive biomes. As vascular plant growth is restricted by the limited water availability (Sabatini et al., 2022), a substantial part of primary production and $CO_2$ fixation is instead taking place within the upper millimeters of soil, where complex microbial communities form biological soil crusts, referred to as biocrust hereafter (Elbert et al., 2012; Grote et al., 2010; Xu et al., 2022).

Measuring $CO_2$ fluxes in these systems requires sampling systems equipped to capture low-$CO_2$ fluxes, meaning that flow-through-based chamber approaches are often not suitable. Static chamber-based $CO_2$ flux measurements, on the other hand, result in a trade-off between headspace volume and gas sampling volume; the headspace volume needs to be restricted to reliably capture $CO_2$ changes during a given incubation time. At the same time, the limited headspace restricts the sampling volume that can be withdrawn without causing pressure gradients. Here, the critical issue lies in that headspace pressure changes between the chamber and surrounding air can lead to anomalous mass flows of high-$CO_2$ air-leaking from soil pore spaces, ultimately resulting in an overestimation of respiration rates (Bekku et al., 1995). Bekku et al. (1995) therefore propose that up to 0.2% of the headspace volume of a static chamber should be sampled to avoid these artifacts. When sampling only a fraction of the headspace to avoid pressure changes, this raises the question of whether this accurately represents the true headspace mean. Further, in the case of smaller chamber setups adopted for low-$CO_2$ soil systems, gas samples in that volume range would not provide enough sample for isotopic analyses in common Isotope Ratio Infrared Spectrometer (IRIS) and Cavity ring-down spectroscopy (CRDS) systems.

With these challenges in regard, we have developed a gas labeling/sampling system, aiming to fulfill the following requirements: (a) the system should be sensitive to capture small $CO_2$ fluxes—e.g., the restricted heterotrophic respiration and photosynthetic C fixation expected in dryland or other low-$CO_2$ systems. (b) The system should be built in a way to minimize undesirable pressure changes within the headspace volume during sampling, while still accommodating large enough sample volumes for analytical replication. (c) The system should be versatile and enable respiration and photosynthesis measurements as well as in situ isotopic pulse labeling using the same equipment. (d) The system should be constructed in a way to rule out cross-contamination between samples and measurements, excluding risks when handling highly labeled and natural abundance samples alternately using the same equipment. (e) The system should be easy and straightforward to use, allowing for repeated gas samplings within short time frames. (f) Finally, the system should be accessible, only requiring cost-effective and easily available system compartments, with a modular design where all system compartments can be replaced during maintenance.

## 2. Material and Methods

A range of tests were conducted to evaluate the performance of the developed system and its different system features, for example, airtightness and pressure changes during sampling (described in Section 2.3). We further verified the suitability of the system to quantify $CO_2$ fluxes in a set of net ecosystem exchange (NEE) measurements of soil-biocrust systems (Section 2.4).

### 2.1. Layout and Design of the Labeling/Gas Sampling System

In brief, the system consisted of a 1,200 mL incubation box equipped with a gas inlet (Ø8 mm) from which flush air was led in, a balloon valve (Ø8 mm), a labeling inlet, and a gas outlet (Ø6 mm) from which the sample air was collected (Figure 1). Gas tubes connecting the incubation box and sample bags were equipped with check valves and three-way valves to ensure one-directional gas flow as well as evacuation (Figure 1b) and sampling (Figure 1c) in one step, without having to reconnect or move any compartments. A detailed description of all system compartments, as well as step-by-step instructions of the operational steps, is found in supplements (Figures S1–S3 in Supporting Information S1).

### 2.2. $^{13}C$ Labeling

The system was equipped for in situ $^{13}C$ pulse labeling. A silicon septum gas fitting (Ø8 × 4 mm) was installed from which acid could be added into a beaker containing $Na^{13}CO_3$ (99 atom% $^{13}C$, Sigma-Aldrich Chemie

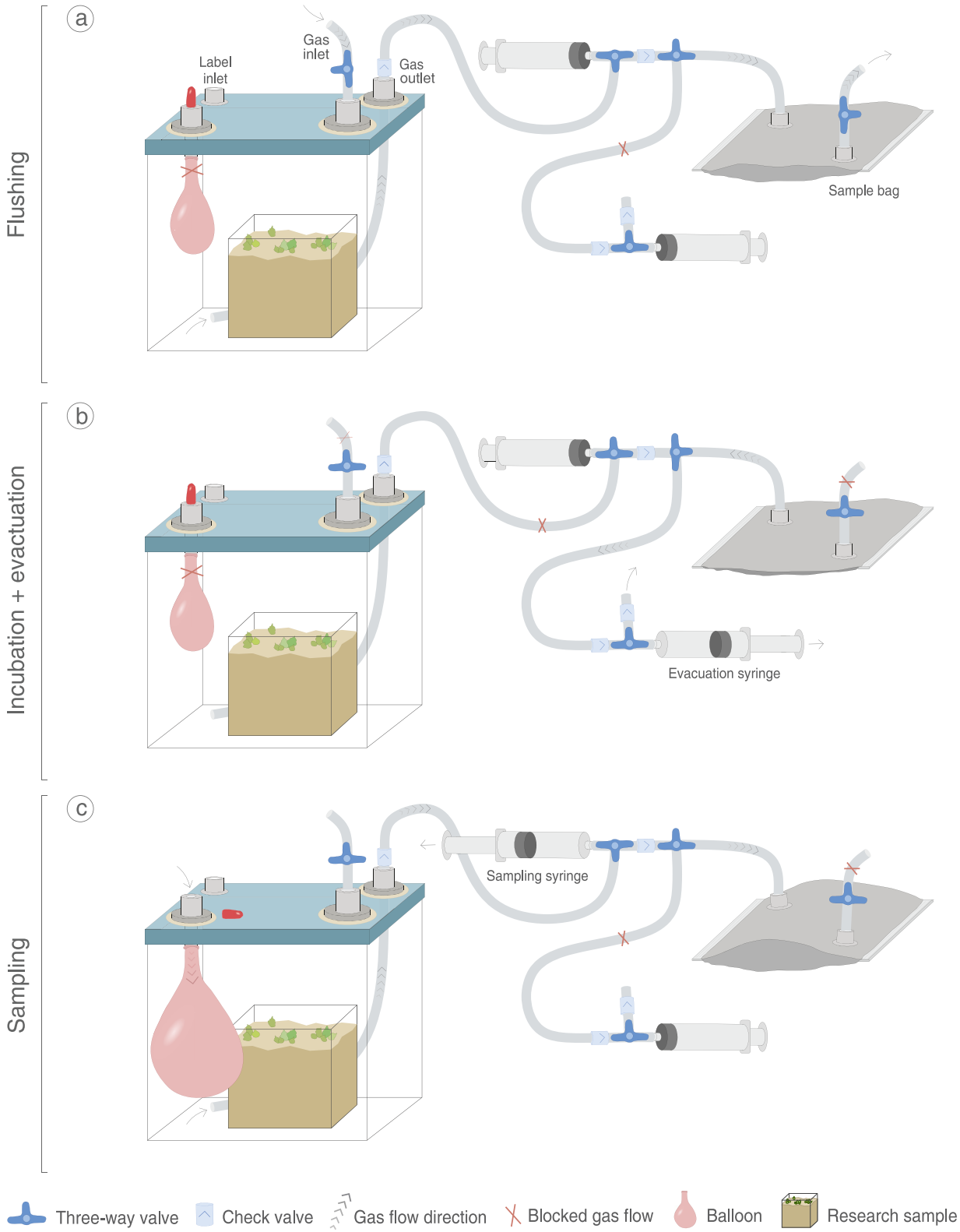


**Figure 1.** Schematic overview of the system setup and the three main working steps; (a) flushing of incubation box and sample bag with flush air of known $CO_2$ concentration and isotopic signature, (b) incubation during which incubation box is closed from all sides and the sample bag can be evacuated in parallel, and lastly, (c) sampling of the headspace air after a given incubation time, during which the balloon valve is opened to the outside to counterbalance underpressure. The check valves guaranteed a one-directional gas flow between the incubation box, syringes, and sample bag.

GmbH, Taufkirchen, Germany) creating an in situ $^{13}CO_2$ pulse. While not described in detail in this work, any vapor (e.g., $^{15}N_2$ pulses) could be added directly from the septum in dual-labeling approaches.

### 2.3. Evaluating the Performance of the Gas Sampling System

To verify setup performance and demonstrate the capacity of the sampling system, the most important system features were tested and quantified. In the tests described hereafter, soil sample prototypes (5 × 8 cm microcosms; polyvinyl chloride, KTK Kunststofftechnik GmbH, Germering, Germany; filled with sterilized quartz sand) were placed in the incubation boxes (1,000 mL total headspace volume). Gas measurements were conducted in five analytical replicates with a flow rate of 1.5 mL $s^{-1}$ and a total acquisition time of 150 s per sample. Before the measurement of each sample, a 60 s flush time was set in which the Delta Ray analyzer was flushed with sample air to stabilize the signal. All measurements were conducted using a Delta Ray Isotope Ratio Infrared Spectrometer™ URI (Thermo Fisher Scientific, Bremen, Germany) and the Qtegra™ ISDS 2.3.1487.49 software. Each measurement run was individually calibrated and corrected via bracketing using defined flush air and a reference gas.

#### 2.3.1. Feature 1: Airtightness of Sampling System

To test the airtightness of the gas sampling system, boxes ($n = 5$) were placed in airtight plastic bags. Each box held a container with 2 mg of 99% $Na_2{}^{13}CO_3$ to which 2 M $H_2SO_4$ was added in excess to create a highly enriched $^{13}C$ headspace atmosphere (~48,300‰ $\delta^{13}C$ V-PDB) within the box. The airtight plastic bags were then filled with the defined flush air (−38.3‰ $\delta^{13}C$ V-PDB) and sealed gas-tight after which the surrounding air around the boxes was measured at the start and after 3 hr (500 mL each). The relative source proportions of the box and the surrounding air to the resulting isotopic signature after incubation was determined using a two-end-member mixing model (i.e., IsoError, Phillips et al., 2005). For further impressions, see Figure S4 in Supporting Information S1.

#### 2.3.2. Feature 2: Storage Effects of Sample Bags

When an analyzer (e.g., IRIS/CRDS) is not accessible directly at the experimental site, gas samples are inevitably stored for hours, or days, before analysis. While the infrastructure around this setup did not require longtime sample storage, we tested for changes in $CO_2$ concentration and isotopic signatures of stored gas samples to quantify potential storage effects. This was realized by filling five sample bags with 500 mL air (1,850 µmol $mol^{-1}$, −76‰ $\delta^{13}C$ V-PDB). Gas samples were collected and measured at start, as well as after 2, 8, respectively, 24 hr ($n = 4$, stored at 21°C).

#### 2.3.3. Feature 3: Pressure Changes During Gas Sampling With and Without Balloon

The effectiveness of the balloons to counterbalance pressure changes in the incubation boxes during gas sampling was tested by sealing the balloon valve, thereby preventing air from entering the balloon, while retrieving gas samples ($n = 5$). Pressure changes were monitored using a manometer while repeatedly collecting 60 mL of gas until a total volume of 660 mL had been removed from the headspace. This was later repeated with the balloon valve open. The procedure of collecting gas was identical to that defined in "Operational steps of gas sampling" found in Supporting Information S1.

### 2.4. Example Application of Gas Sampling System

After the feature tests, the suitability of the gas sampling system for capturing low-$CO_2$ fluxes from dryland soils was finally tested in a phytotron incubation experiment of soil-biocrust systems.

#### 2.4.1. Experimental Background

Soil was collected from a semiarid site in Santa Gracia, Chile (−71.166, −29.757; 13.7°C MAT; 66 mm MAP; Bernhard et al., 2018), sieved (<2 mm) and filled into 5 × 8 cm microcosms. The incubation took place in an experimental walk-in climate chamber (Jákli et al., 2021) set to resemble the climatic setting of the sampling site. There were two watering treatments; half of the samples received 1.2 mL water per day ("normal water"), and the other half 0.6 mL to simulate dry conditions ("low water").

#### 2.4.2. Net Ecosystem Exchange (NEE) Measurements Under Light and Dark Conditions

The gas measurements reported in this study were carried out 3 months after incubation start. All samples received water and were left open for 2 hr before measurements. During NEE measurements under light conditions

($NEE_{light}$), the photosynthetic photon flux density (PPFD) in the chamber was increased to 600 µmol $m^{-2}$ $s^{-1}$ to account for the PAR transmittance of the plastic lids (73.8 ± 0.3%) and samples were collected after ∼45 min closure time. The closure time was decided based on preliminary measurements to determine approximate $CO_2$ fluxes of the different treatments. This allowed us to select a time point for reliable quantification at which a sufficient change in headspace $CO_2$ had been achieved. After $NEE_{light}$ measurements, lights were turned off for 20 min before the flushing procedure started again—this time in complete darkness—to determine heterotrophic respiration ($NEE_{dark}$). Although this includes dark respiration of autotrophic organisms, $NEE_{dark}$ will hereafter be referred to as heterotrophic respiration. Any work conducted during the dark incubation was carried out under green light (PPFD < 15 µmol $m^{-2}$ $s^{-1}$). The output from the spectrometer (µmol $CO_2$ $mol^{-1}$) was converted to µmol $CO_2$ using the ideal gas law, after which the $NEE_{light}$ and $NEE_{dark}$ fluxes were calculated as:

$$NEE_{light/dark} = \frac{CO_{2_{headspace}} - CO_{2_{flush}}}{A * t}$$

with

$NEE_{light/dark}$: net ecosystem exchange of $CO_2$ under light/dark incubation (in µmol $m^{-2}$ $s^{-1}$)

$CO_{2\ headspace}$: $CO_2$ content in the headspace after incubation (in µmol)

$CO_{2\ flush}$: $CO_2$ content before incubation (here: ∼16.8 µmol)

$A$: surface area of sample (in $m^2$)

$t$: time of incubation (in s)

To isolate the $\delta^{13}C$ of respired $CO_2$ from mixing air residing in the headspace, a two-end-member mixing model was applied (Hafner et al., 2021)

$$\delta^{13}C_{respiration} = \frac{\delta^{13}C_{headspace} * CO_{2_{headspace}} - \delta^{13}C_{flush} * CO_{2_{flush}}}{CO_{2_{headspace}} - CO_{2_{flush}}}$$

with

$\delta^{13}C_{respiration}$: $\delta^{13}C$ signal of respired $CO_2$ (in ‰ $\delta^{13}C$ V-PDB)

$\delta^{13}C_{headspace}$: $\delta^{13}C$ signal in the headspace (in ‰ $\delta^{13}C$ V-PDB)

$CO_{2\ headspace}$: $CO_2$ concentration in the headspace after incubation (in µmol $mol^{-1}$)

$\delta^{13}C_{flush}$: $\delta^{13}C$ signal of flush air before incubation (here: −38.3‰ $\delta^{13}C$ V-PDB)

$CO_{2\ flush}$: $CO_2$ concentration of flush air before incubation (here: 405.8 µmol $mol^{-1}$)

The $NEE_{light}$ and $NEE_{dark}$ measurements presented in this manuscript were conducted 5 days after $^{13}CO_2$ pulse labeling (according to the method described in Section 2.2). For further impressions, see Figure S5 in Supporting Information S1.

### 2.5. Statistics

All statistical testing was carried out in the R statistical environment (version 4.2.2, R Development Core Team, 2008) in RStudio (version 2022.12.0 Build 353, RStudio Team, 2015). For all tests, data were tested for homogeneity of variances (Levene test) beforehand, and the residuals of every model used were tested for normality (Shapiro test/Q-Q-Plot, Kozak & Piepho, 2018). The effect of storage time on the gas signature inside the bags was tested with a one-sample $t$ test (two-sided and the mean tested for was 0). A linear mixed effect model ("lme" function) was calculated to test for differences in I: pressure changes inside the box with and without balloon as well as the differences in II: $NEE_{light/dark}$ and $\delta^{13}C$ signal. For model I: the volume which was extracted and balloon (yes versus no) were used as a fixed effect and the ID of the box as a random effect and II: treatment (normal water versus low water) and species (biocrust versus bare soil) were used as fixed effects and the chamber was used as a random effect (package: nlme, version: 3.1–137). The R code for all tests is included in supplements. If the mixed effect model showed significant effects, we did a post hoc test with the "emmeans"

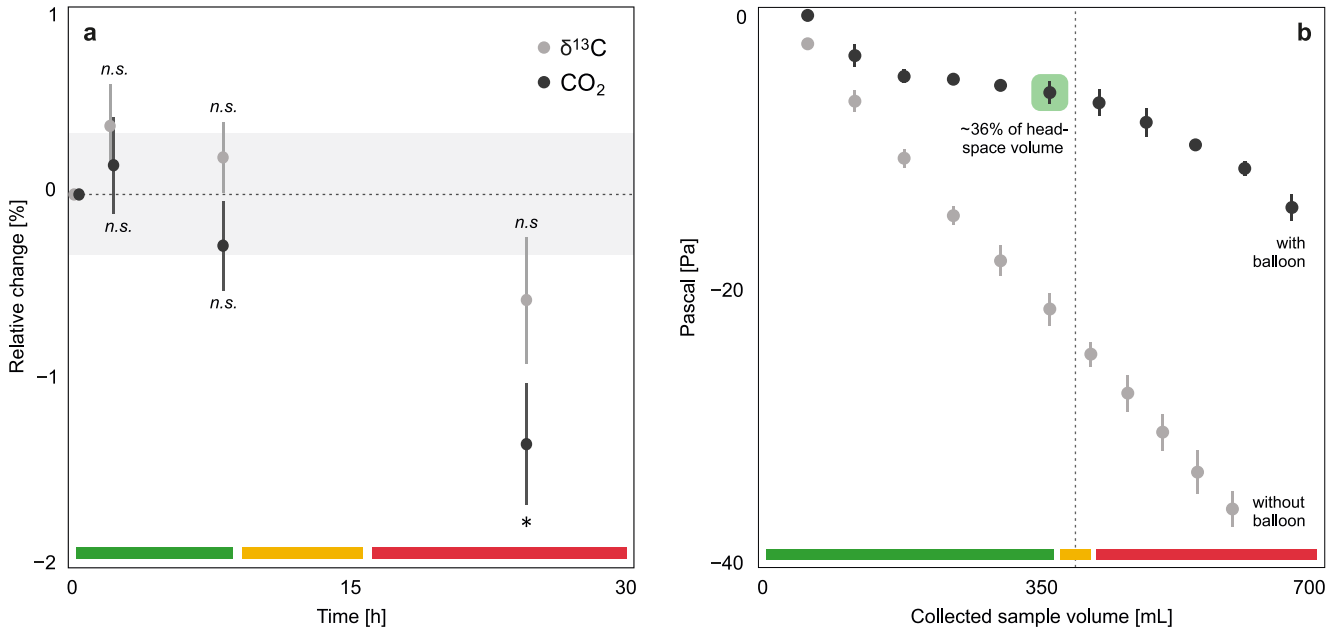


**Figure 2.** The gas sampling system was evaluated via feature tests, here showing (a) the relative change (%) in $CO_2$ concentration and $\delta^{13}C$ of gas samples stored in sample bags over time (h). Points represent means of independent replicates ($n = 4$). The shaded area shows the range of measurement uncertainty set by the Isotope Ratio Infrared Spectrometer (IRIS) instrument. The significant difference between the original $CO_2$ concentration and that measured after 20 hr is indicated by an asterisk ($p < 0.05$). (b) Pressure changes in the chamber headspace (Pa) as a function of collected gas sample volume (mL) in sampling systems with and without balloons ($n = 5$). Due to high underpressure in systems without balloons during the last five measurement points, it was no longer possible to retrieve the full 60 mL. The pressure difference between systems with and without balloon was significant ($p < 0.001$) in all measurement points. The green box marks the selected sample volume used in this experiment (360 mL, corresponding to 36% of headspace volume). Data are shown as mean ± SD.

function with Tukey correction (package: emmeans, version: 1.3.1). Data are given in text and tables as the means ± 1 standard deviation.

## 3. Results

### 3.1. Incubation Test Confirms Gas Tightness of Sampling System

When having placed the boxes (containing ~48,300‰ $\delta^{13}C$ V-PDB $CO_2$) in airtight plastic bags, the surrounding air in the bags was determined to be −23.6 ± 1.1‰ $\delta^{13}C$. After 3 hr incubation, the measurements revealed an average of −21.3 ± 1.9‰ $\delta^{13}C$, accounting for an increase of 2.3 ± 0.9‰ $\delta^{13}C$ during the incubation. The two-end-member mixing model confirmed that >99.99 ± 0.00% of the sampled air was in fact original surrounding air, meaning that <0.01 ± 0.00% was from leakage.

### 3.2. Slight Effect on Isotopic Signal and Concentration of Gas Samples Only After Long-Term Storage

The storage and consequent measurements of gas samples showed no notable changes during the first hours of storage. The relative change compared to the baseline in $\delta^{13}C$, +0.4 ± 0.2% and +0.2 ± 0.2% after 3 and 8 hr, and in $CO_2$ concentration, +0.2 ± 0.3% respectively −0.3 ± 0.2%, remained within the region of measurement uncertainty (Figure 2a). After 24 hr, however, a storage effect could be detected, with an average of 0.6 ± 0.3% change in the isotopic signal and 1.3 ± 0.3% change in $CO_2$ concentration ($p < 0.05$). Overall, this accounted for an average decrease of 0.4 ± 0.3‰ $\delta^{13}C$ and an increase of +24.9 ± 6.0 μmol $mol^{-1}$ $CO_2$ after 24 hr.

### 3.3. Reduced Underpressure During Gas Sampling

A distinct effect of the balloon was detected in the build-up of underpressure as a function of gas volume retrieved from the headspace. After 60 mL, the vacuum level in the systems without balloons was four times larger

**Table 1**
*Headspace Parameters Monitored During Two Incubation Phases; Incubation in Light ($NEE_{light}$) and in Darkness ($NEE_{dark}$)*

| | Normal water | | Low water | |
|---|---|---|---|---|
| $NEE_{light}$ | Biocrust | Bare soil | Biocrust | Bare soil |
| Chamber temperature (°C) | | 21.0 ± 0.1 | | |
| Soil temperature before incubation (°C) | 21.3 ± 0.4 | 21.3 ± 0.9 | 21.1 ± 0.7 | 20.9 ± 0.7 |
| Soil temperature after incubation (°C) | 25.3 ± 0.2 | 25.2 ± 0.9 | 24.9 ± 0.8 | 25.0 ± 0.7 |
| $\Delta T$ before and after incubation | 4.0 ± 0.3 | 3.9 ± 0.1 | 3.8 ± 0.3 | 4.2 ± 0.0 |
| $CO_2$ concentration in headspace after incubation (µmol $mol^{-1}$) | 318.2 ± 44.1 | 485.8 ± 11.9 | 425.6 ± 19.7 | 486.8 ± 19.5 |
| Total $CO_2$ change during incubation (µmol $mol^{-1}$) | −88.0 ± 44.3 | 79.5 ± 11.7 | 19.4 ± 19.7 | 81.9 ± 17.1 |
| $CO_2$ flux rate (µmol $mol^{-1}$ $min^{-1}$) | −1.7 ± 0.8 | 1.5 ± 0.2 | 0.4 ± 0.4 | 1.6 ± 0.3 |
| | Normal water | | Low water | |
| $NEE_{dark}$ | Biocrust | Bare soil | Biocrust | Bare soil |
| Chamber temperature (°C) | | 20.9 ± 0.0 | | |
| Soil temperature before incubation (°C) | 23.1 ± 0.6 | 23.4 ± 1.3 | 22.9 ± 0.8 | 23.0 ± 1.1 |
| Soil temperature after incubation (°C) | 21.9 ± 0.2 | 21.9 ± 0.5 | 21.7 ± 0.3 | 21.8 ± 0.4 |
| $\Delta T$ before and after incubation | −1.2 ± 0.4 | −1.5 ± 0.8 | −1.1 ± 0.5 | −1.2 ± 0.7 |
| $CO_2$ concentration in headspace after incubation (µmol $mol^{-1}$) | 561.8 ± 61.0 | 465.3 ± 7.4 | 460.0 ± 19.4 | 459.4 ± 21.3 |
| Total $CO_2$ change during incubation (µmol $mol^{-1}$) | 161.7 ± 63.2 | 59.5 ± 7.4 | 54.2 ± 19.2 | 53.5 ± 21.3 |
| $CO_2$ flux rate (µmol $mol^{-1}$ $min^{-1}$) | 3.9 ± 1.5 | 1.5 ± 0.2 | 1.3 ± 0.5 | 1.3 ± 0.5 |

*Note.* All values are based on means of $n = 4$ replicates.

compared to those with balloons (−2.5 ± 0.5 compared to −0.4 ± 0.4 Pa, $p < 0.001$). This distinction prevailed as the collected sample volume increased; at 360 mL the pressure reached −21.6 ± 1.2 in the systems without balloons compared to −6.1 ± 0.8 Pa in the systems with balloons (Figure 2b, $p < 0.001$), demonstrating that the balloon counterbalanced 72% of the underpressure. For the respiration and photosynthesis measurements described later, 360 mL gas samples were collected, representing 36% of the total headspace volume.

### 3.4. Practical Application of the System

#### 3.4.1. Soil Temperature and $CO_2$ Changes During Incubation

Headspace parameters were closely monitored during closure time. During dark incubation, the $CO_2$ change was largest in respiration measurements of biocrust samples (161.7 ± 63.2 µmol $mol^{-1}$) and smallest in the dry controls (53.5 ± 21.3 µmol $mol^{-1}$; Table 1). Photosynthetic fluxes were only determined in normal watered biocrusts during light measurements (−1.7 ± 0.8 µmol $mol^{-1}$), while no photosynthetic activity was detected in bare soil controls.

The continuous soil temperature measurements revealed an averaging +4.0 ± 0.2°C temperature increase during the photosynthesis measurements, reaching a maximum soil temperature of around 25°C. During respiration measurements in darkness, only a slight decrease in temperature was determined (−1.3 ± 0.6°C on average).

#### 3.4.2. Net Ecosystem Exchange (NEE) and $\delta^{13}C$ Signal

The respiration rates showed a clear distinction between biocrusts and bare soil systems, with $NEE_{dark}$ reaching 0.5 ± 0.1 µmol $CO_2$ $m^{-2}$ $s^{-1}$ in soils with biocrusts under normal watering compared to 0.2 ± 0.0 µmol $CO_2$ $m^{-2}$ $s^{-1}$ in the corresponding controls ($p = 0.00$; Figure 3a). The rates in low water biocrusts, however, were identical to those in bare soil controls (0.2 ± 0.0 and 0.2 ± 0.1 µmol $CO_2$ $m^{-2}$ $s^{-1}$ in biocrust, respectively, bare soils, $p = 0.99$). The $NEE_{light}$ reached 0.3 ± 0.1 µmol $CO_2$ $m^{-2}$ $s^{-1}$ in biocrusts under normal watering, representing the only net C gain of the systems studied.

Similar to these patterns, the $\delta^{13}C$ signal of respired $CO_2$ during dark incubation, determined with the two-end-member mixing model, again showed the highest signal in normal watered biocrusts 5 days after labeling; 655.7 ± 288.9‰ compared to 104.2 ± 23.4‰ $\delta^{13}C$ V-PDB in the corresponding controls (Figure 3b, $p = 0.00$). This distinction was also reflected in low-watered biocrusts and controls (542.1 ± 220.2‰ compared to 115.9 ± 42.1‰, $p = 0.00$).

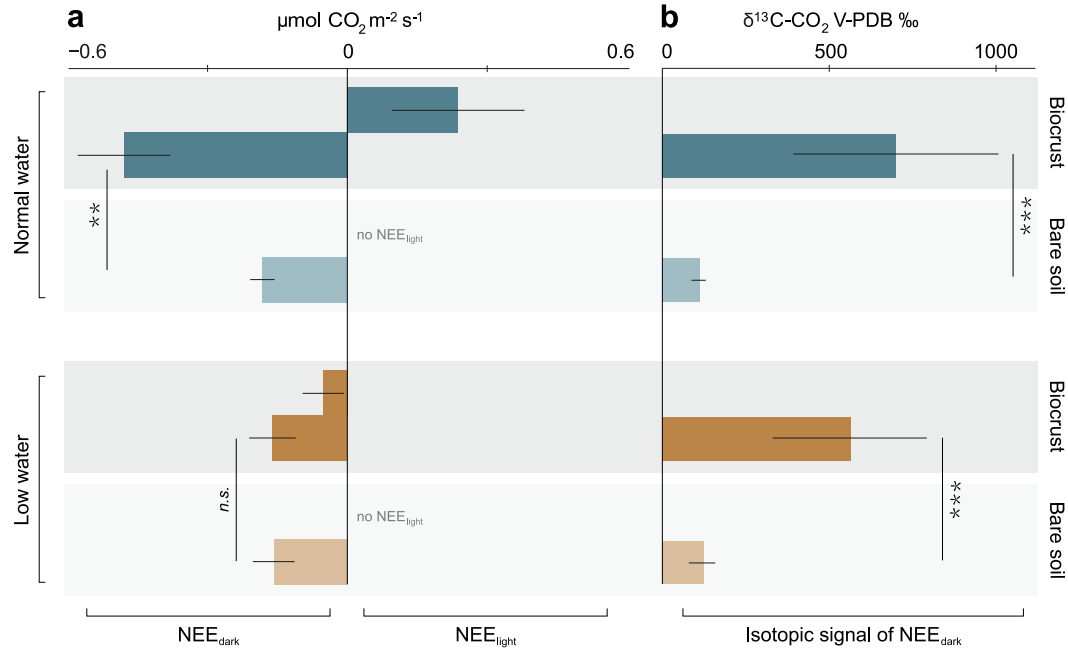


**Figure 3.** (a) Net ecosystem exchange during light ($NEE_{light}$) and dark ($NEE_{dark}$) incubation in μmol $CO_2$ $m^{-2}$ $s^{-1}$ and (b) the isotopic signal of $NEE_{dark}$ $CO_2$ in $\delta^{13}C$ V-PDB‰ from soils with biocrust (in bold colors) and bare soil controls (in faded colors), either with normal (blue) or low (brown) water addition. Data are shown as mean ± SD ($n = 4$) and asterisks represent significant differences between biocrusts and corresponding controls.

## 4. Discussion

In this work, we present a closed static chamber system (nonflow-through) developed for measurements of soil respiration and photosynthesis of low-$CO_2$ soil systems, with the possibility of conducting $^{13}CO_2$ and $^{15}N_2$ pulse labeling using the same setup. The suitability of the system for capturing low-$CO_2$ fluxes was evaluated by respiration and photosynthesis measurements, as well as in a set of feature tests.

### 4.1. Limited Pressure Changes Enabled Larger and More Representative Gas Sample Volumes

The restricted $CO_2$ fluxes of the low-activity dryland samples posed two main requirements for the system layout; the headspace volume needed to be kept small enough to capture $CO_2$ release from a dryland soil without extending closure times, while still accommodating the collection of gas sample volumes large enough for thorough flushing and analytical replication of the IRIS measurements. Flushing for >47 s is required to reach 99% of the maximum accuracy of the Delta Ray analyzer (Boudoire et al., 2020). As we estimated a further risk due to alternating measurements of highly labeled and natural abundance samples, we set 60 s flushing prior to measurement (accounting for 90 mL at 1.5 mL $s^{-1}$ flow rate). To rule out measurement error, each gas sample was then replicated ($n = 5$, with 30 s measurement time per analytical replicate), which in total required a sample volume of 315 mL.

Collecting gas samples from a static chamber with a small headspace volume causes distinct air pressure differences in the headspace, which lead to risks of high-$CO_2$ air leaking from soil pores into the headspace during sampling (Fang & Moncrieff, 1996). We tackled this problem by installing a balloon open to the outside to counterbalance the build-up of underpressure, while still retrieving enough sample volume to meet the measurement requirements described above. To the best of our knowledge, this is a novel system feature that has not previously been implemented in other comparable static chamber-based systems. When collecting the set sample volume (360 mL, representing 36% of the total headspace volume; Figure 2b), the balloon reduced underpressure by 72%. Thus, we were able to considerably lower the risk of high $CO_2$ air being drawn from internal soil pore spaces. Especially for setups in which only smaller sample volumes are required, the balloon limit underpressure very effectively; after withdrawing 60 mL, the underpressure remained at −0.4 Pa (compared to −2.5 Pa without balloon). However, since gas does not homogenize inside a static chamber (Hutchinson & Livingston, 2001),

we argue that caution must be taken when only sampling such small fractions of the headspace, as the question remains whether this accurately represents the true headspace mean. To further counteract the issue with soil pore air mixing into the sample, we encourage thorough flushing before and in between measurements. Before measurements, each sample system (including incubation boxes and sample bags) was flushed with defined flush air at 5 mL $s^{-1}$ for >15 min, equivalent to flushing each box with >70 times of its volume.

### 4.2. One-Step Flushing and Subsequent Evacuation of Compartments Counteracts Cross-Contamination

Handling isotopically highly enriched samples together with unlabeled samples at natural abundance increases risks of cross-contamination, for example, via surface exchange effects (adsorption and desorption) within the analyzing instrument (Meijer et al., 2000) and increases the need of flushing exetainer vials and other sampling compartments to reduce contamination effects (Reinsch & Ambus, 2013). Furthermore, one of the most prevalent risks in incubation experiments with repeated measurements within short timeframes is when residual gas remains in sampling equipment and is transferred between sampling runs. We approached these issues by connecting system compartments (including sample bags) to enable one-step flushing without having to attach or detach any compartments. Further, the following evacuation step where all residual flush air in sample bags and the system was removed added an extra level of security. Consequently, during photosynthesis and respiration measurements, we could not determine any traces of contamination between samples or measurements caused by gas-wall interactions or residual $^{13}CO_2$ remaining in the system. In addition, the setup with three-way valves and check valves meant that a one-directional air flow was guaranteed, reducing the risk of spoiling samples due to human error.

### 4.3. Storage of Gas Samples for Hours Did Not Cause Changes in Sample Composition

We conclude that after 8 hr of gas sample storage in the aluminum sample bags, changes in $CO_2$ concentration and $\delta^{13}C$ remained within the range of measurement uncertainty, thereby excluding any notable storage effects during the first few hours. However, after 20 hr, a notable reduction of both parameters occurred. Consequently, in cases where sampling logistics require samples to be stored for longer periods of time, we suggest other gas storage options, such as septum-capped vials (Hardie et al., 2010) or multi-layered foil sample bags (Boudoir et al., 2020) proving reliable conditions for long-term sample storage. Alternatively, including separate reference samples as "external standards" with defined air of similar $CO_2$ concentration and isotopic composition in the aluminum sample bags presented in this study would allow for the quantification of possible fractionation or leakage effects that could arise during long-term storage.

### 4.4. Detecting Low-$CO_2$ fluxes During Short Closure Times

The $NEE_{dark/light}$ data presented in this study confirm the suitability of the sampling setup for $CO_2$ measurements of low flux soil systems, for example, with respiration rates ($NEE_{dark}$) as low as 0.1 µmol $CO_2$ $m^{-2}$ $s^{-1}$ in the low-water bare soil controls. Respiration rates in the biocrust systems overall ranged from 0.1 to 0.2 µmol $CO_2$ $m^{-2}$ $s^{-1}$ and 0.3–0.7 µmol $CO_2$ $m^{-2}$ $s^{-1}$ in "low water" and "normal water" biocrusts, respectively. These rates are consistent with, or at the lower end of, respiration measurements reported from field studies in similar ecosystems, for example, around 0.4 µmol $CO_2$ $m^{-2}$ $s^{-1}$ in lichen-dominated and moss-dominated biocrusts in the Tengger Desert (Guan et al., 2019), 0.1–7.6 in moss-dominated biocrusts on the semiarid Chinese Loess Plateau (Yao et al., 2020), <2 µmol $CO_2$ $m^{-2}$ $s^{-1}$ in cyanobacteria-dominated biocrusts on the semiarid Colorado Plateau (Bowling et al., 2011) or 0.7 µmol $CO_2$ $m^{-2}$ $s^{-1}$ in lichen-dominated biocrusts in the Tabernas Desert (Chamizo et al., 2021).

We were able to quantify $CO_2$ fixation via photosynthesis ($NEE_{light}$) in biocrusts, demonstrating that the setup is suitable for capturing $CO_2$ uptake in systems with lower organisms such as bryophytes and cyanobacteria. Water availability clearly affected fluxes, with biocrusts under normal watering being the only systems with net C uptake ($0.3 \pm 0.1$ µmol $CO_2$ $m^{-2}$ $s^{-1}$). These rates are slightly lower than those reported from field measurements, for example, around 0.5 µmol $CO_2$ $m^{-2}$ $s^{-1}$ (Bowling et al., 2011) or 0.7 µmol $CO_2$ $m^{-2}$ $s^{-1}$ (Chamizo et al., 2021). However, some studies have reported rates as high as 3.6–4.0 µmol $CO_2$ $m^{-2}$ $s^{-1}$ in algae-dominated biocrusts in the Mojave Desert (Brostoff et al., 2005) and 5.9 µmol $CO_2$ $m^{-2}$ $s^{-1}$ in lichen-dominated biocrusts under optimal conditions in the Namib Desert (Lange et al., 1994). Lange (2001) points out how crust density and composition are responsible for the large variation in measured net photosynthesis between studies. As the biocrusts in this study were established in the laboratory only 3 months before the flux measurements, this is likely a reason why the $NEE_{light}$ fluxes are lower compared to some of the field observations.

The design of the sampling system to facilitate the detection of small-range differences in $CO_2$ build-up meant that incubation times could be kept short—presumably even shorter than the 45 min tested in this work. Limiting closure times is of particular importance so that artifacts caused by changing headspace conditions, a commonly acknowledged challenge in soil $CO_2$ measurements (Rochette & Hutchinson, 2005), can be avoided. For instance, during the 45 min closure time, we could detect a +4°C soil temperature change, accounting for a ∼20% relative increase between the start and end of incubation. Soil temperature can drive respiration rates, for example, via accelerated rates of organic matter mineralization (Eliasson et al., 2005), which can result in overestimated fluxes and should, thus, not be disregarded or underestimated. In addition, as headspace $CO_2$ increases during incubation, the natural diffusion gradient is altered which could, in turn, lead to an underestimation of the flux (Conen & Smith, 2000). Consequently, $CO_2$ build-up in the headspace and the duration of which samples are exposed to these conditions needs to be restricted. This further underlines the need not only for incubation systems enabling short closure times, but for continuous monitoring of parameters, such as temperature and $CO_2$ concentrations, for later correction.

## 5. Conclusions

We have developed a sampling setup for the quantification of soil and plant-soil systems with very low $CO_2$ fluxes, which allows in situ $^{13}CO_2$ pulse labeling within the same setup. The system can be applied to a wide range of experimental systems, from desert biocrust-soil and small-scale vascular plant-soil systems to low C subpolar and alpine soils. The unique feature of the presented setup, the application of a balloon for air pressure compensation, helped mitigate >70% of the negative pressure build-up in the chamber headspace during sampling. This allowed a larger fraction of the headspace volume to be sampled, resulting in representative and sufficient sample volumes for isotopic measurements.

The one-step flushing and evacuation procedure minimized the risk of cross-contamination between samples while maintaining convenient handling. This was strengthened by the implementation of check valves to ensure unidirectional flow, which significantly reduced human error during sampling. In summary, this system provides a low-cost and accessible solution for both $^{13}CO_2$ labeling and $CO_2$ measurements that is applicable to a wide range of settings—including low-$CO_2$ flux ecosystems.

## Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

## Data Availability Statement

Data from all measurements supporting the findings of this study are presented in the main article or are publicly available via https://doi.org/10.5281/zenodo.8179391 (Witzgall et al., 2023). The soil material used in this study is registered under the following IGSN numbers: GFTHS0001 and GFTHS0002.

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