Hardware Article

An open-source, automated, gas sampling peripheral for laboratory incubation experiments using cavity ring-down spectroscopy

Timothy D. Berry [a,*], Chance Creelman [b], Nick Nickerson [b], Akio Enders [c], Thea Whitman [a]

[a] Department of Soil Science, University of Wisconsin-Madison, United States
[b] Eosense Inc, Halifax, Nova Scotia, Canada
[c] Department of Biological and Environmental Engineering, Cornell University, United States

ARTICLE INFO

ABSTRACT

Spectroscopic instruments are becoming increasingly popular for measuring the isotopic composition and fluxes of a wide variety of gases in both field and laboratory experiments. The popularity of these instruments has created a need for automated multiplexers compatible with the equipment. While there are several such peripherals commercially available, they are currently limited to only a small number of samples (≤ 16), which is insufficient for some studies. To support researchers in constructing custom, larger-scale systems, we present our design for a scalable gas sampling peripheral that can be programmed to autonomously sample up to 56 vessels – the "multiplexer". While originally designed to be used with a Picarro cavity ring-down spectroscopy (CRDS) system, the multiplexer design and data processing approach implemented can be easily adapted to serve as a gas sampling/delivery platform for a wide variety of instruments including other cavity ring-down systems and infra-red gas analyzers. We demonstrate the basic capabilities of the multiplexer by using it to autonomously sample head-space CO_2 from 14 laboratory-incubated soils amended with ^{13}C-enriched pyrogenic organic matter for analysis in a Picarro G2201-i cavity ring-down spectroscopy system.

Specifications table

Hardware name	Multiplexer; scalable gas sampling peripheral
Subject area	• Biological Sciences • Environmental, Planetary, and Agricultural Sciences • Biogeochemistry
Hardware type	• Gas sample handling
Open Source License	CC-BY-ND-NC 4.0
Cost of Hardware	$4836
Source File Repository	https://doi.org/10.17632/km23j2bxwf.2

* Corresponding author.
  E-mail address: tdberry@wisc.edu (T.D. Berry).

1. Hardware in context

The availability of cavity ring-down spectroscopic (CRDS) and off-axis cavity spectroscopic instruments has resulted in major changes in the way trace gas fluxes and isotopic composition are measured [1,2]. In the past, isotopic and trace gas measurements required gas capture in the field and subsequent analysis by isotope ratio mass spectrometer (IRMS) and/ or gas chromatograph in the lab. While these techniques are still the gold standard for accuracy and precision, IRMS and gas chromatography are limited by their expense and limitations on sampling frequency and duration [3,4]. In contrast, modern cavity spectroscopy systems are relatively inexpensive, much more portable, and potentially more flexible than other analytical instruments. Because CRDS instruments continuously analyze the gas stream, researchers can collect hundreds of data points in the time it would take to complete one analysis on an IRMS and achieve similar precision, or they may choose to sacrifice precision to increase throughput for less sensitive measurements. One disadvantage of emerging instruments such as these is that they have only a small pool of third-party equipment manufacturers to produce peripherals, so as spectroscopy instruments continue to grow in popularity, there is an increasing need for compatible sampling peripherals. Still, there are currently several high-quality, commercially available gas sampling systems available that are compatible with the spectroscopy systems made by Picarro (Santa Clara, CA, USA) and Los Gatos Research (San Jose, CA, USA). Picarro, for example, produces a non-recirculating distribution manifold that samples from up to 16 sources and Eosense (Halifax, NS, CA) produces a recirculating multiplexer that can sample from up to 12 chambers. However, the relatively small capacity of these devices may be insufficient for some studies with multiple different experimental treatments or high levels of replication, leaving custom-built systems as the only viable option in such instances. Constructing such a system can be a daunting task for those with no experience in electronics or instrument design. Here, we share our design for a modular multiplexer system to assist researchers looking for a higher capacity and affordable gas sampling system.

The multiplexer presented here was designed to be compatible with Picarro CRDS instruments, but because it does not require compatibility with any specific software packages or interfaces, it can be modified to work with almost any instrument that regularly timestamps data. Alternatively, the multiplexer can also be operated without being connected to an analytical instrument as a programmable gas manifold system that is well suited for tasks requiring the automated delivery of gases (*e.g.*, purging headspace of reaction or incubation vessels).

2. Hardware description

The multiplexer is a modular, solenoid-driven gas sampling peripheral. A programmable relay control board is passed instructions to open and close specific relays according to a programmed schedule. Each relay controls a pair of solenoid valves that allow gas flow to and from a sample container via the connected inlet and outlet tubes and manifolds, and into the analytical instrument. A small auxiliary pump is used to speed gas flow to decrease the time necessary to flush and sample containers. Data are processed using an R script (provided in the design files section) to match the timestamped relay status (continually monitored and logged by interface software) to the corresponding timestamped data produced by the analytical instrument. With this approach, the multiplexer is compatible with any instrument capable of producing timestamped data files. In summary, this design:

- Allows programmable automation of gas sampling and delivery
- Is compatible with a wide range of instruments, including Picarro CRDSs
- Allows researchers to balance sampling requirements with construction costs

The version of the instrument presented here makes use of 128 solenoid valves (*i.e.*, 64 pairs of valves) controlled by 64 relays and allows for the sampling and flushing of 56 sample incubation chambers (additional relays are used to facilitate sample and gas line flushing; Fig. 1). Depending on the needs of the researcher, control boards supporting between 32 and 128 relays can be purchased and configured as described herein. Although it is possible to construct a multiplexer using this approach to sample <32 vessels, the availability of commercially produced gas sampling peripherals that support this number of vessels and the significant time invested in multiplexer construction (estimated at >100 person-hours) means that constructing these smaller systems is not likely cost-effective. It is also important to note that the design presented here is for laboratory (not field) use, as it is not weatherproof. Additional modifications to provide weatherproof housings for the electrical components would be possible but are beyond the scope of this work.
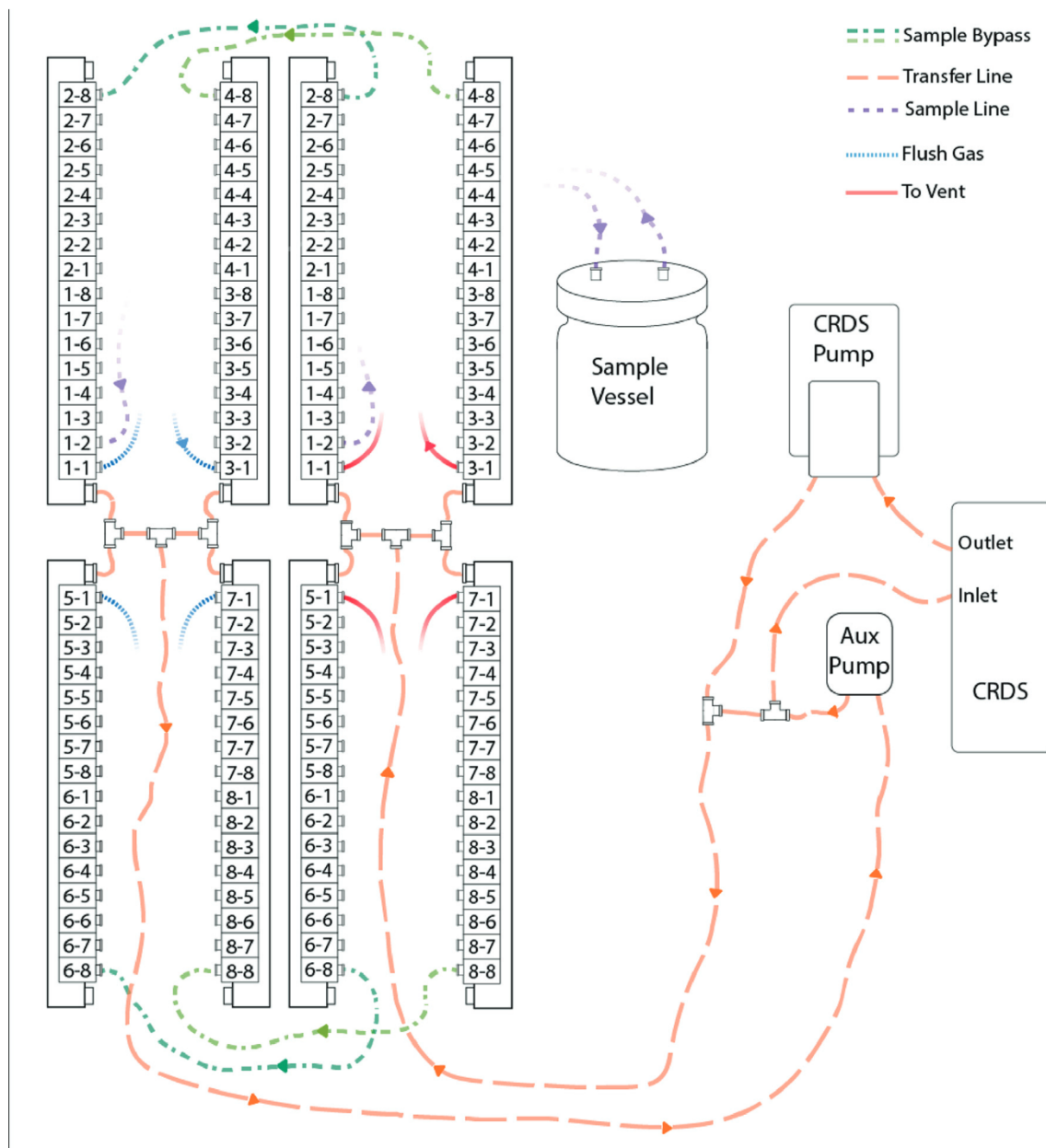
Fig. 1. Schematic of the sample flow paths for the 128-solenoid gas sampling multiplexer. Arrows indicate the flow of gas within the tubing during operation. Numbers indicate paired valves on manifolds.

3. Design files

Design file name	File type	Open source license	Location of the file
Relay controller logic	Text (.pdf)	CC-BY-ND-NC 4.0	https://doi.org/10.17632/km23j2bxwf.2
Demonstration processing script	R script (.R)	CC-BY-ND-NC 4.0	https://doi.org/10.17632/km23j2bxwf.2
Demonstration log file	Comma Separated (.csv)	CC-BY-ND-NC 4.0	https://doi.org/10.17632/km23j2bxwf.2
Demonstration data	Zip archive (.zip)	CC-BY-ND-NC 4.0	https://doi.org/10.17632/km23j2bxwf.2

Relay board logic: *Pseudocode documenting our approach to communicating with the relay board.* Many software packages and coding environments (*e.g.*, LabView) can be used to interface with the board, provided that it outputs a time stamped record of solenoid activation status. See the demonstration log file for an example output.

Demonstration processing script (for Picarro-generated data): *An R script that takes the relay* log *from the controller (.log file) and instrument data files (.dat, contained within .zip archive), matches them based on shared time stamps, plots gas concentration and isotopic composition, and applies a 2-member mixing model to partition the source of gases.* This script was used to conduct the analysis described in section 7.4 and produce Fig. 20. This script uses the Broom [5], Zoo [6], and Tidyverse [7] packages in addition to base R functions.

Demonstration log file: *The .csv file produced by the software communicating with the relay controller; this file contains timestamped records of which solenoid valves are active during the analysis.* These time stamps are matched with the timestamps in the demonstration data by the processing script.

Demonstration data: *A compressed zip-archive (0.98 GB when uncompressed) containing the 173 .dat files produced by the Picarro instrument during the demonstration run.* This dataset was processed with the processing script to produce Fig. 20.

Bill of materials

Designator	Component	Supplier	Unit Number	Unit Cost (USD)	Total Cost (USD)
Manifold and Valve Assembly					
Manifold Fittings (A1)	Male connector fitting #10–32 thread port, 1/8″ Tubing (KQ2H01-32A)	SMC	128	0.82	104.96
Mainline Fittings (A2)	Male connector fitting 1/8″ NPT port, 1/8″ Tubing (KQ2H01-32AS)	SMC	8	1.11	8.88
Manifolds (A3)	16 Station manifold 2x Supply ports 1/8″ NPT, #10–32 Outlet ports, 2x Exhaust ports 1/8″ NPT (E15M-16)	Clippard	8	41.93	335.44
Plastic Plug (A4)	1/8″ NPT Natural Polypropylene Threaded Plug (64724)	US Plastics	10	0.42	4.20
Solenoid Valves (A5)	15 mm Miniature Pneumatic Valve (E215F-2C012)	Clippard	128	17.38	2224.64
Electronics					
Break-out Board (B1)	ATX Power Supply Breakout board (RB-Cyt-101)	Cyton	1	7.71	7.71
Connection Board (B2)	Solderable Breadboard - Large (PRT-12699)	Sparkfun	2	8.95	17.90
Diodes (B3)	Schottky 60 V 1A Diode	Digikey	64	0.359	22.98
Relay Board (B4)	64 Channel SPDT Relay Controller w/ TLEE module and AC adapter (FLL_FXR32x_FXR32xL2)	National Control Devices	1	1159.00	1159.00
Solenoid connector (B5)	Valve connector w/1 m Leads (C2A-RB1000)	Clippard	128	2.96	378.88
Power Supply (B6)	550 W ATX12V & EPS12V Modular Power Supply	Corsair	1	64.99	64.99
Wire	25 ft. spool solid core, pre-tinned, 22 AWG	Sparkfun	8	2.5	20
Tubing and Fittings					
T-fittings (C1)	SMC KQ2T Union Tee (KQ2T01-00A)	SMC	12	1.18	14.16
Pump (C2)	Pump DC-V 12 V (UNMP015.1.2KN B)	KNF	1	341.00	341.00
Nut and Ferrule Set (C3)	Nut and Ferrule Set for 1/4″ tube (SS-400-NFSET)	Swagelok	5	4.07	20.35
MNPT-Tube fitting (C4)	MNPT to 1/4″ tube adapter (SS-4-TA-1-4)	Swagelok	1	6.20	6.20
FNPT-Tube fitting (C5)	1/4″ FNPT to 1/8″ tube adapter (KQB2F01-N02)	SMC	2	5.6	11.20
MNPT-VCR Fitting (C6)	1/4″ VCR to 1/4″ MNPT adapter (SS-4-WVCR-1-4)	Swagelok	1	36.52	36.52
Tubing	500 ft roll Polyurethane tubing 1/8″ OD, 0.08″ ID (TIUB01R-33)	SMC	2	28.26	56.52

3.1. Build instructions

Additional tools and resources needed:

- Socket and/or open-end wrenches (9/32, 3/8, 7/16, and 11/16 in.)
- Precision screwdriver set
- Soldering iron and solder
- Needle-nosed pliers
- Wire stripper
- Teflon thread-tape

Optional tools and resources:

- Electrical multimeter (for troubleshooting)
- Plastic zip ties and/or heat-shrink tubing (for organization of cables)

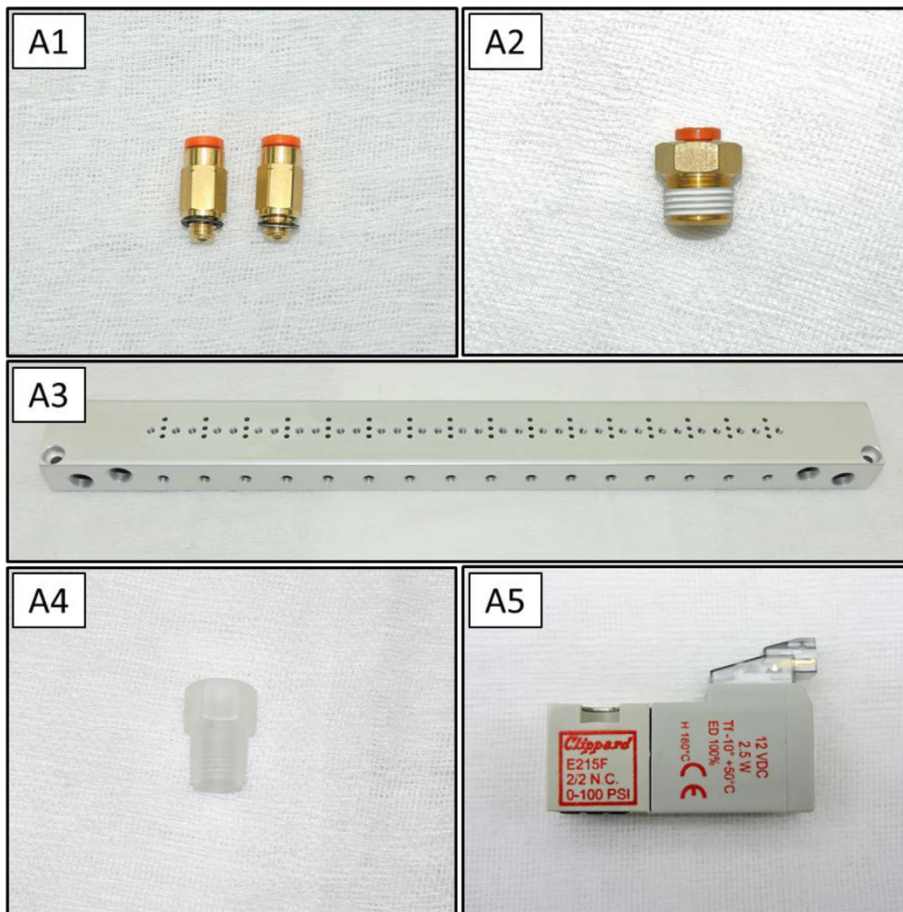### 3.2. Manifold assembly (components and construction summarized in Fig. 2 and Fig. 4)

Fig. 2. Parts list for manifold system. Components are labelled as in the bill of materials. (A1) Manifold fittings; (A2) Mainline fittings; (A3) Manifolds; (A4) Plastic plug; (A5) Solenoid valve.

1. Install the #10–32 threaded manifold fittings (**component A1**) into each manifold (**A3**) until finger-tight, making sure that the gasket seals included with the fittings are in place. Tighten an additional ¼ turn with a 9/32-inch wrench or socket driver.

2. Install a 1/8-inch NPT mainline fitting (**A2**) into one of the ports marked S (supply) on each manifold (**A3**) until finger-tight. If using the recommended manifold configuration, these fittings should be placed so that the fittings can face each other when placed in a mirrored orientation (Fig. 3). Tighten an additional ¼ turn with a 7/16-inch wrench or socket driver.

3. Install a 1/8-inch NPT plastic plug **(A4)** into the free S-port on each manifold and tighten using a 7/16-inch wrench or socket drive. Be careful not to cross-thread or overtighten these fittings as the aluminum manifold will easily deform the threads on the plugs and compromise the seal.
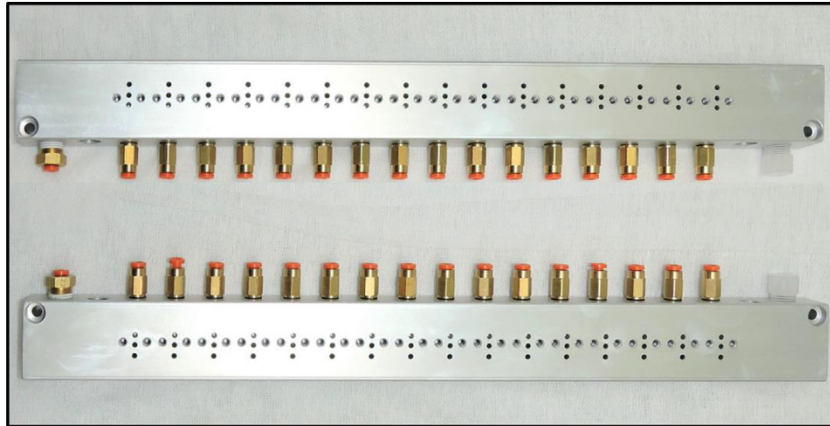

Fig. 3. Correct manifold orientation. Note the mirrored orientation.

4. Attach the solenoid valves **(A5)** to each position on the manifolds using the included screws such that the body of solenoid protrudes above the fittings. Tighten screws evenly to ensure that the rubber solenoid gasket is compressed evenly above the holes on the top of each manifold. Overtightening of one side will result in a poor seal.
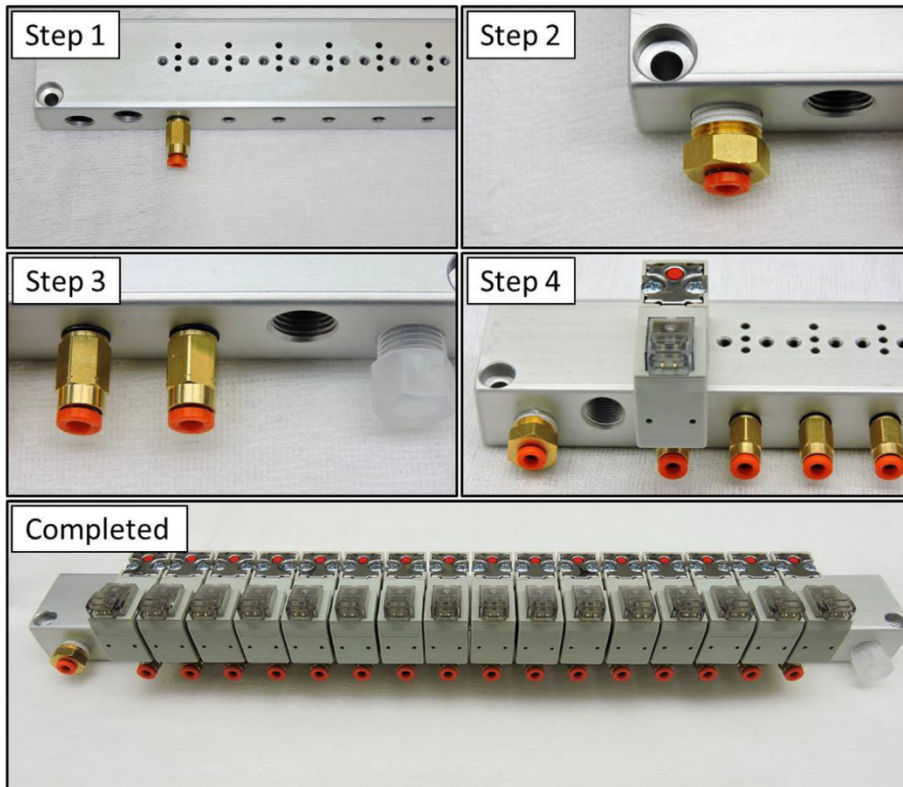

Fig. 4. Steps in the assembly of the solenoid manifolds. Panel labels correspond to instructions in the section 5.1 in the body of the text. Step 1 – install manifold fittings into each manifold. Step 2 – install mainline fittings into one supply port. Step 3 – install plastic plug into other supply port. Step 4 – attach solenoid valves to manifolds. A completed single manifold is shown.

5. Repeat steps 1–4 for each manifold. There are 4 pairs of mirrored manifolds housing 16 solenoids each in the 128-solenoid design.

Optional: Mount the manifolds to a plywood or high-density plastic sheet using 2 #10 screws for each manifold. The ideal placement of the manifolds is demonstrated in Fig. 5. A completed manifold has a mass of approximately 1.1 kg – care should be taken to use a sturdy mount if the manifolds are to be mounted vertically.
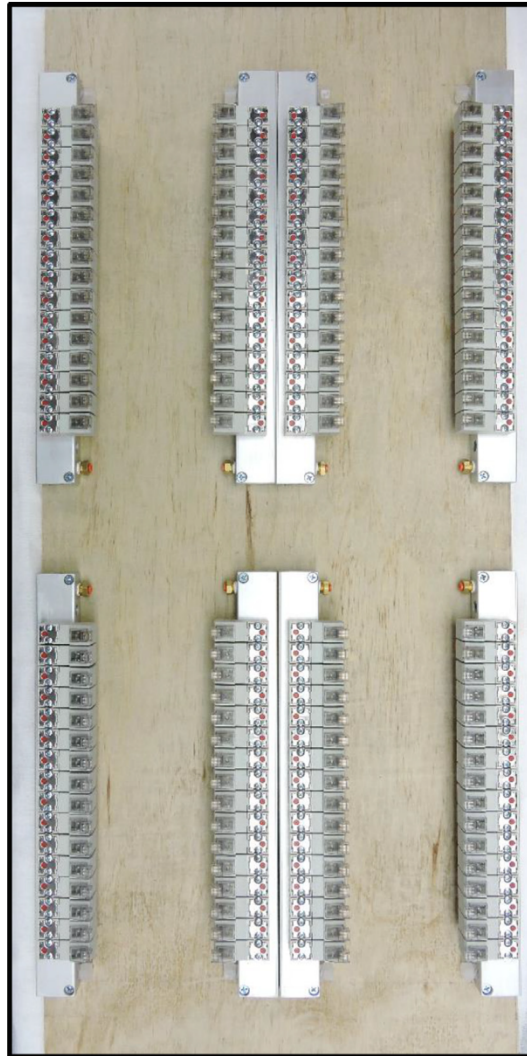

Fig. 5. Assembled and mounted manifolds.

3.3. Electronics assembly (components and construction summarized in Fig. 6 and Fig. 8)

Note: The instructions provided are for assembling the multiplexer exactly as pictured, using solid-core 22-AWG wires. The color, length, and composition of the wires and the orientation of circuits on the relay board can be customized when assembling your own multiplexer. Similarly, any solderable prototyping board may be used for construction. The board used here was chosen because its clear labelling makes it easy for beginners to work with. Note that, as with a standard

prototyping board, each numbered row on the board is electrically isolated while columns within a row are connected. Always take proper safety precautions when working with electricity. Make sure that all workpieces have been disconnected from their power supply before use. Remove all conductive jewelry (rings, watches, bracelets, *etc.*) and work with clean, dry hands.
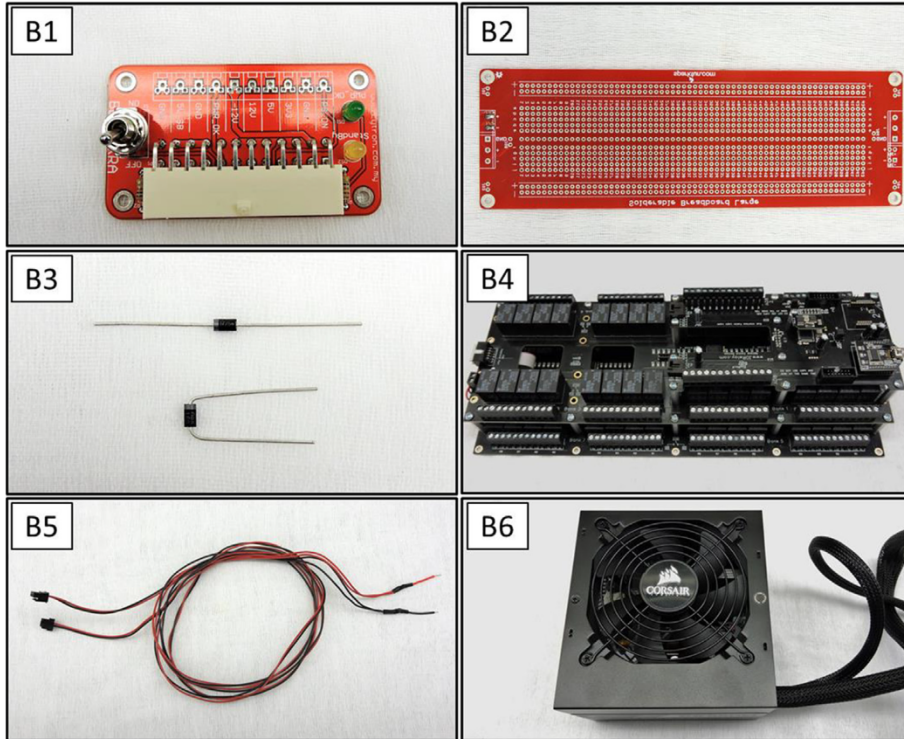

Fig. 6. Parts list for electrical system. Components are labelled as in the bill of materials. (B1) Breakout board; (B2) Connection board; (B3) Diodes; (B4) Relay board; (B5) Solenoid connector; (B6) Power supply.
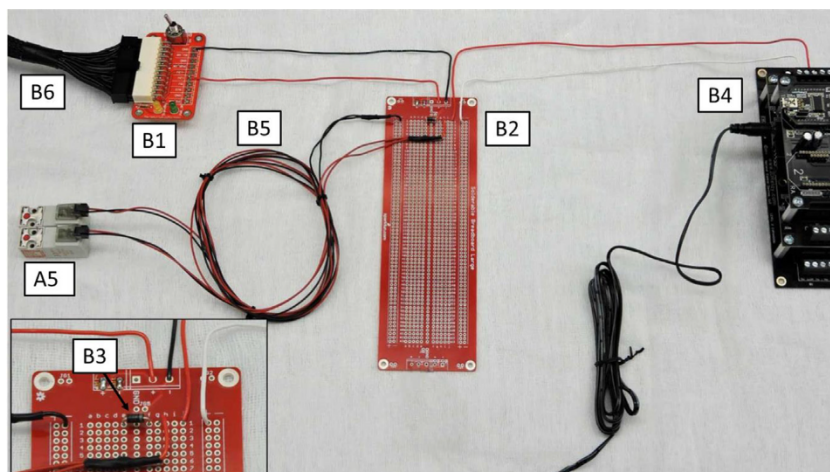

Fig. 7. Overview of multiplexer electrical system. Components are labelled as in the bill of materials. **Inset.** Magnified view of component B3 in circuit.

Optional: Using a small piece of solder, connect the two metal contacts labelled TIE (+) on the top of the connection board to each other; doing so connects the (+) rails on each side of the board to one another. Connect the (−) rails by using a second small blob of solder to do the same with the contacts labelled TIE (−). This step is optional because only one of each of the rails is used in assembly. However, having the rails on each side connected makes certain troubleshooting tasks easier once wires are connected to the board.

1. Solder one end of a ~15 cm long black wire to either GRND connection on the power supply break-out board **(B1)** and the other end to the (−) connection at the top of the connection board **(B2)**

2. Solder one end of a ~15 cm long red wire to the 12 V connection on the power supply break-out board **(B1)** and the other end to the (+) connection at the top of the connection board **(B2).**

3. Connect positions E1 to F1 on the connection board **(B2)** using a diode **(B3)**. Make sure that the cathode side (marked with a band on the diode body) is closest to column F (as in Figs. 7 and 8). This diode protects the relay board from any voltage spikes induced when deactivating solenoids.
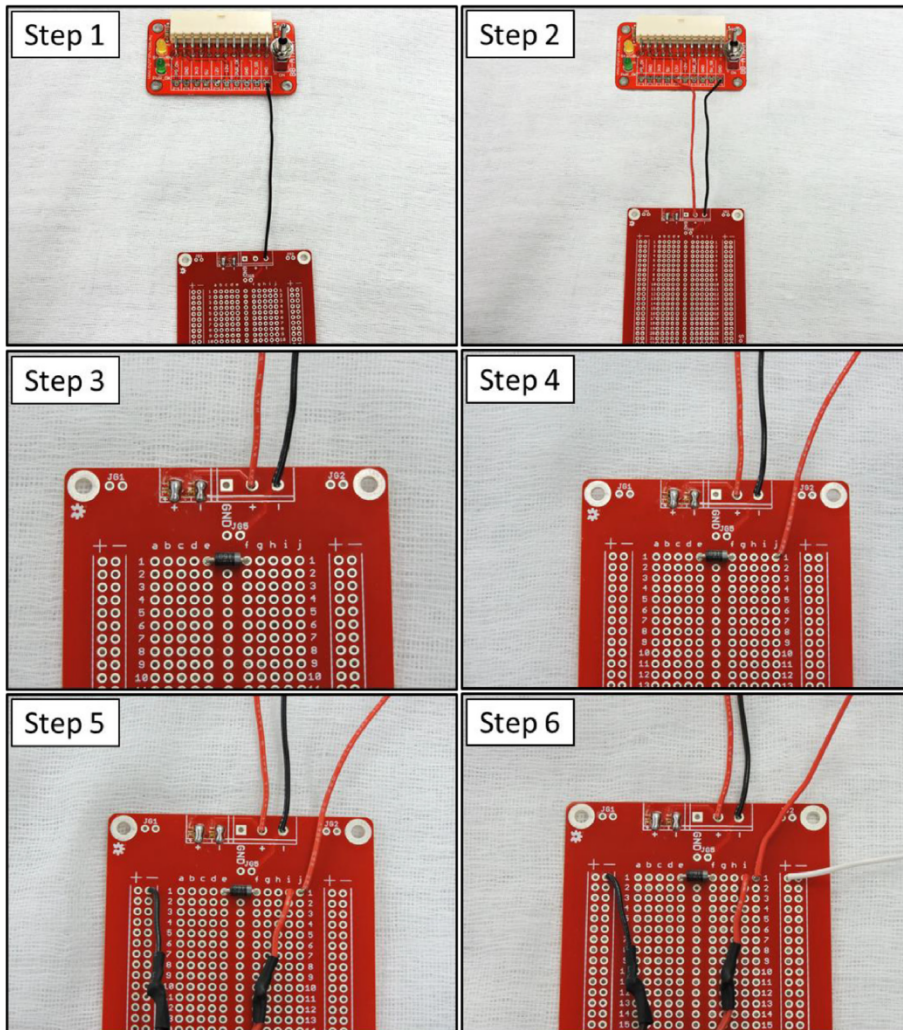

Fig. 8. Steps in the assembly of the first solenoid/relay circuit. Panel labels correspond to instructions in section 5.2 in the body of the text. This process is repeated for each solenoid pair. Step 1 – connect breakout board GRND to connection board (−) with black wire; Step 2 – connect breakout board 12 V to connection board (+) with red wire; Step 3 – connect diode; Step 4 – solder red wire to connection board J1; Step 5 – solder solenoid connector wires to I1 and the (−) rail; Step 6 – solder white wire to (+) rail on the connection board. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Solder one end of a red wire to connection J1 on the connection board **(B2)**. The length of this wire should be at least 30 cm to ensure that it is able to reach the terminal for the corresponding relay on the relay board **(B4)**. Wire length must be increased when building circuits using the distal relays. The recommended wire lengths when using the layout presented here are:

35 cm for rows 1–8 and 33–40, which will connect to relays in banks 1 and 2, respectively
40 cm for rows 9–16 and 41–48, which will connect to relays in banks 5 and 6, respectively
50 cm for rows 17–24 and 49–56, which will connect to relays in banks 3 and 4, respectively
60 cm for rows 25–32 and 57–64, which will connect to relays in banks 7 and 8, respectively.

Note: Even-numbered and odd-numbered banks are on opposite sides of the relay board. Layout was chosen to eliminate the need to cross wires across the connection board so that users can more easily trace the connections.

5. Solder the two red wires and two black wires from a pair of solenoid connectors **(B5)** to I1 and the (-) rail, respectively, on the connection board **(B2)**. Alternatively, matching wires can be soldered to a short jumper wire to make connecting multiple leads to a single connection point easier, as shown in Fig. 9.

Repeat steps 3–5 for each solenoid pair, constructing each circuit on a new row of the connection board. If constructing a multiplexer with ≥64-solenoid pairs, a second connection board can be used.

Note: Managing the large number of wires needed can be a significant challenge. It is recommended that leads be gathered into groups of 8 or 16 (to correspond either the number of relays in a relay bank, or solenoids on a manifold) using either zip ties or heat-shrink tubing (*e.g.*, Fig. 10). Additionally, it is helpful to label the top and bottom of each pair of leads with matching identifiers (*e.g.*, the relay number that they will connect to) – this makes connecting each relay to the corresponding solenoid pair easier later.
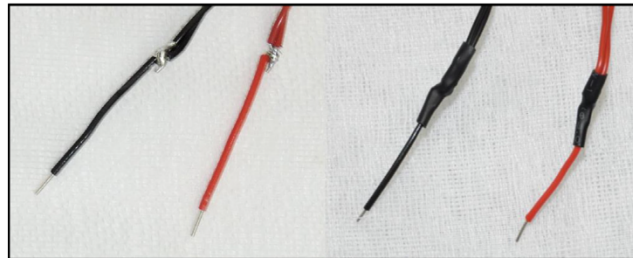

Fig. 9. Matching leads from two different solenoid connectors **(B5)** can be consolidated by connecting them to a single jumper wire as show here before (left) and after (right) the application of heat-shrink insulation.
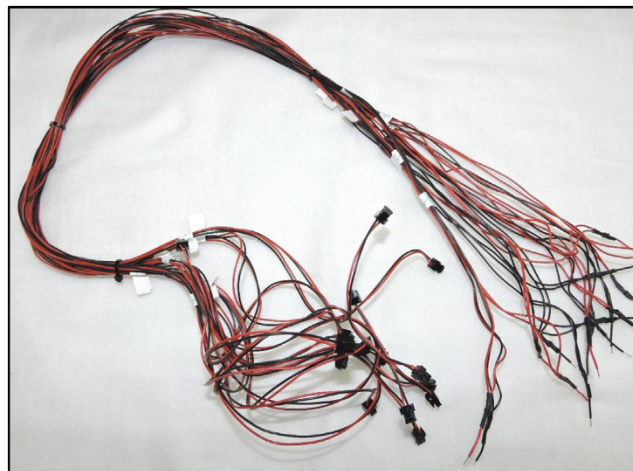

Fig. 10. It is recommended that solenoid connectors be bundled and labelled in sets of 8 or 16.

6. For each bank of relays to be used, solder a ~25 cm length of white wire to the (+) rail on the connection board **(B2)** (as in Figs. 8 and 9). Since all positions on the (+) rail are connected, any row can be used. A relay bank supports 8 relays/solenoid pairs; 8 total connections are made in a system with 64 solenoid pairs like the one assembled here.

7. Connect the free end of each of the white wires soldered to the connection board **(B2)** in step 6 to the COM (common) terminal on the first relay in each bank on the relay board **(B4)**. The COM terminal will carry the current necessary to power the solenoids when the relay on the board is opened.

8. To power the other relays in the bank, connect the COM terminals of each relay to the adjacent relays in the bank on the relay board **(B4)** with short lengths of white wire, as done in Fig. 11. **Note:** It is possible to connect all COM terminals in series

but because voltage drops off as resistance increases with longer circuit lengths it is recommended that no more than 8 relays be connected in series as shown here.

9. Connect the free end of each of the red wires soldered to the connection board **(B2)** in step 4 to the NO (normally open) position on one of the relays (shown for relay 1 in Fig. 11) on the relay board **(B4)**. This completes the relay-solenoid circuit.

10. Finally, plug the AC adapter (comes with **B4**) into the relay board **(B4)** to power the controller.
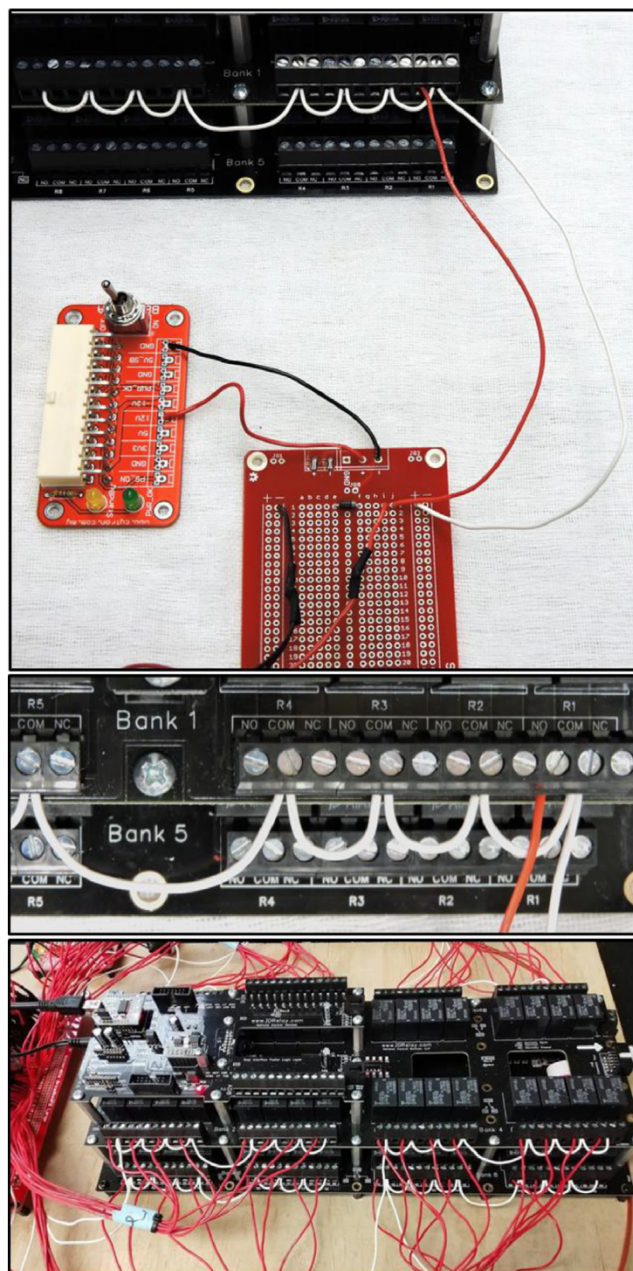

Fig. 11. Completed connections between connection board and relay board (top), magnified view of relay bank terminals (center), and the relay board with all connections made (bottom).

3.4. Tubing and pump preparation (components and construction summarized in Fig. 12, Fig. 13, and Fig. 14)

Note: The lengths of tubing used in this section will vary depending on the layout and positioning of each component of the system. Any tubing lengths provided here are suggestions and can be adjusted to fit the researcher's needs. In general,

tubing length should be chosen to minimize length while still preventing tension on the tubing, which might contribute to leaking. The gas volume within the tubing specific to each system will be corrected for during validation and use of the instrument.
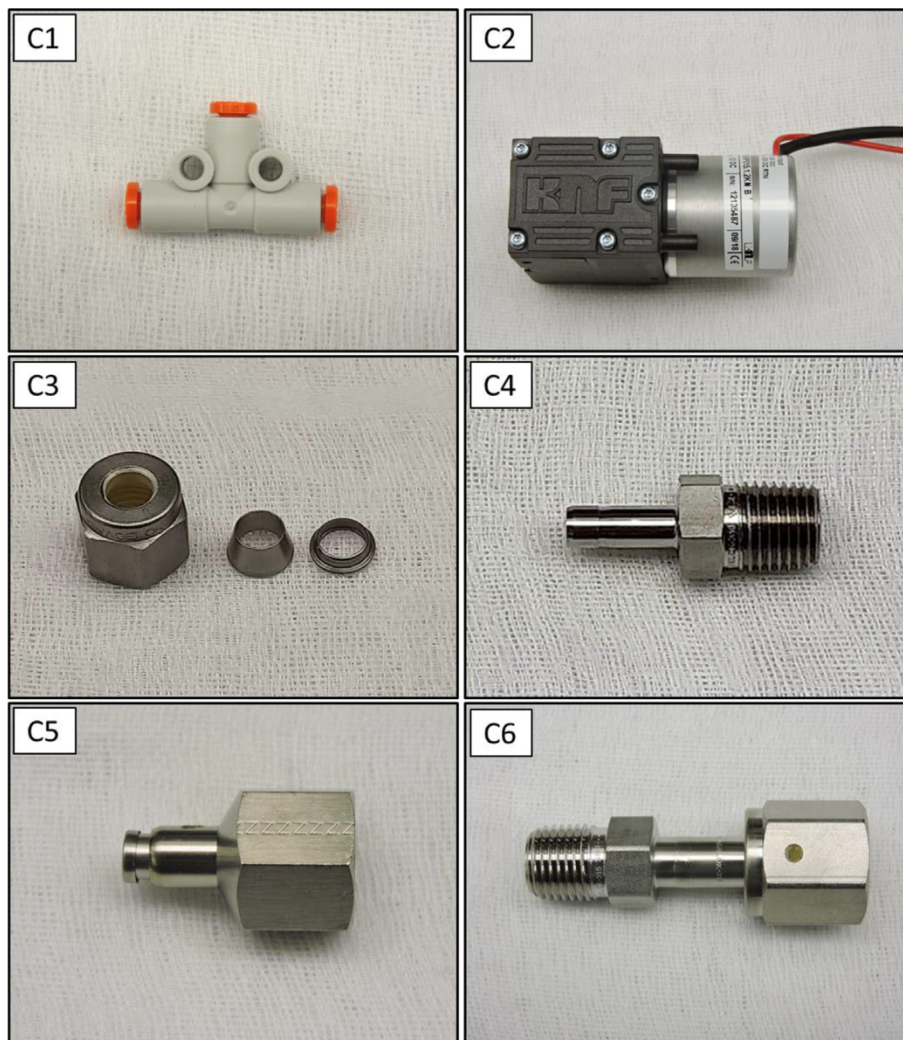

Fig. 12. Parts list for tubing system. Components are labelled as in the bill of materials. (C1) T-fitting; (C2) Pump; (C3) Nut and Ferrule; (C4) MNPT-Tube fitting; (C5) FNPT-Tube fitting; (C6) MNPT-VCR Fitting.
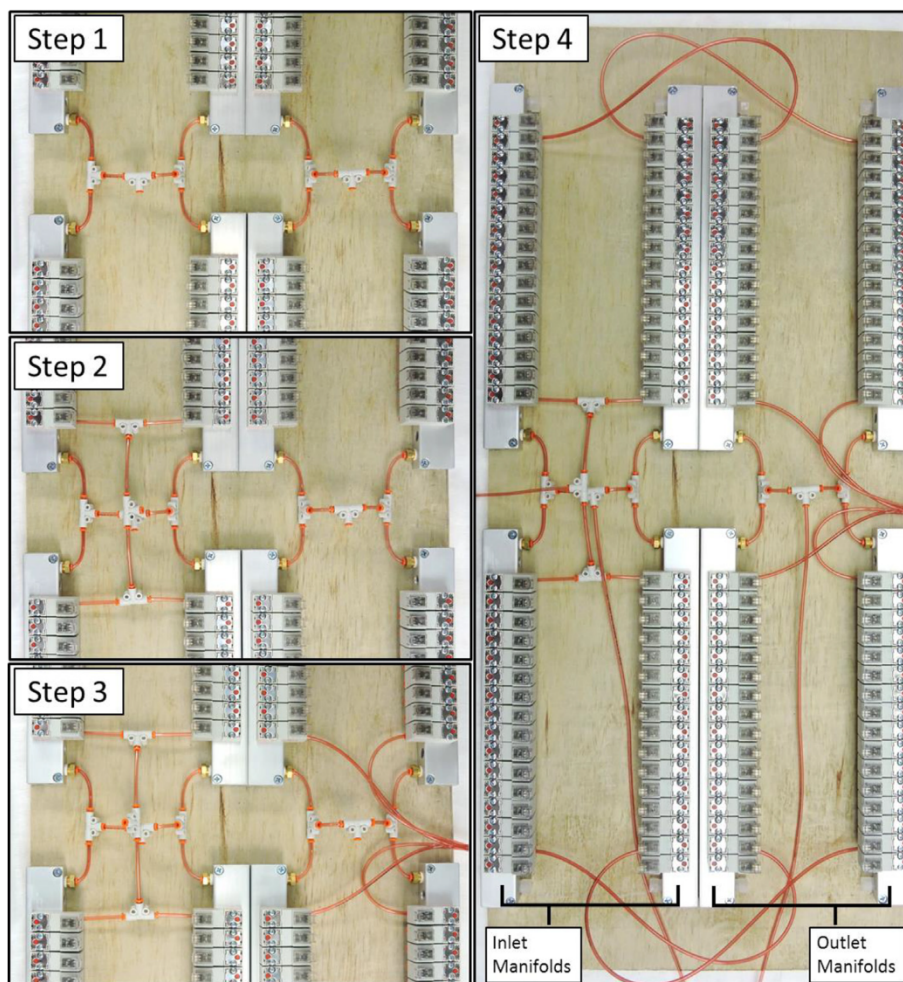
Fig. 13. Steps in the assembly of the multiplexer manifold tubing. Panel labels correspond to instructions in section 5.3 in the body of the text. Step 1 – connect each group of 4 manifolds together with T-fittings; Step 2 – connect the first fitting of each manifold on the inlet side together with T-fittings; Step 3 – connect the first fitting of each manifold on the outlet side to separate lengths of tubing; Step 4 – connect the last fitting on each manifold on each inlet manifold to the corresponding fitting on an outlet manifold.

1. Connect three T-fittings **(C1)** together using short lengths of tubing (1/8-inch outer diameter, 0.08-inch inner diameter) before connecting the four endmost connection points to the mainline fittings **(A2)** on each of four neighboring manifolds as in Fig. 13. Repeat to connect the second set of manifolds. These combine the separate manifolds into inlet (left) and outlet (right) manifolds that will connect to the pumping system.

2. Using a similar set of three T-fittings, connect the first manifold fitting **(A3)** of the leftmost group of manifolds together. These connections will be used to supply flushing gas to the manifolds on the inlet side of the instrument.

3. Connect tubing to the first manifold fittings of the rightmost group of manifolds and position the free ends away from the mounted manifolds. These connections will be used to vent gas from the system during flushing from an external gas cylinder and maintain a safe operating pressure.

4. Connect the last fitting on each inlet manifold to the last fitting on the corresponding outlet manifold. These connections form a sample bypass, which allows the multiplexer to pass air between manifolds without affecting sample vessels.
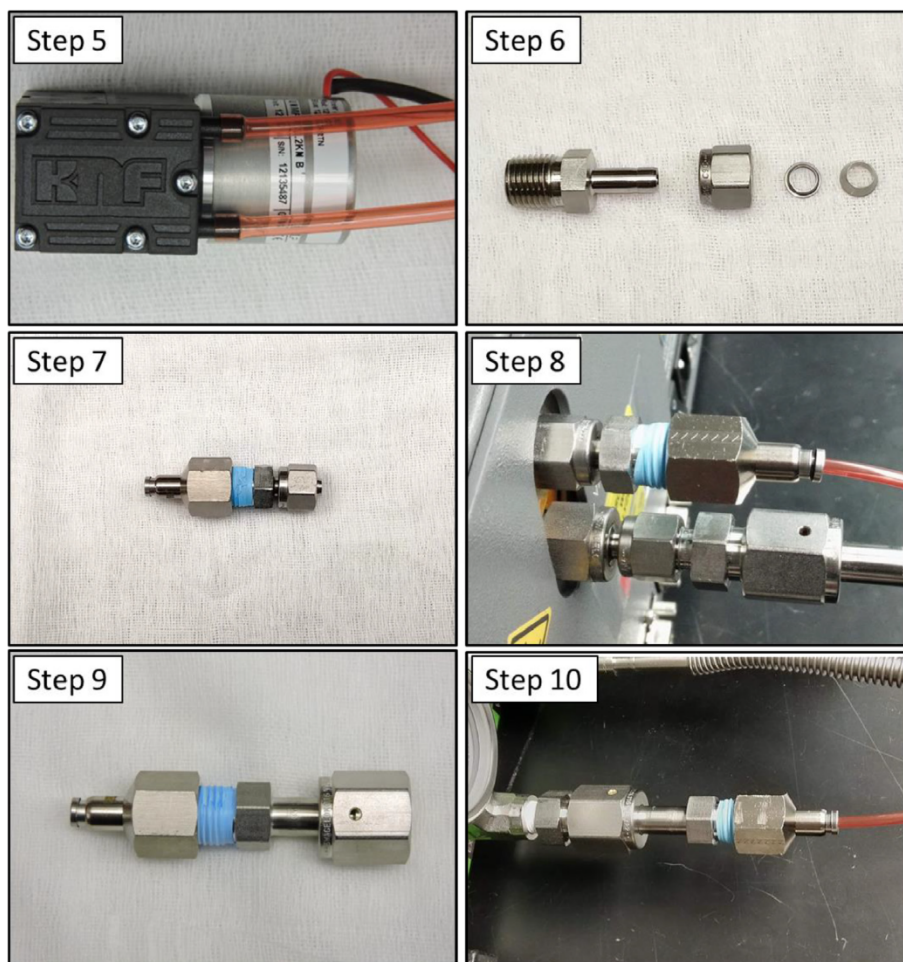
Fig. 14. Steps in the assembly of pumping system. Panel labels correspond to instructions in section 5.3 in the body of the text. Step 5 – connect tubing to auxiliary pump; Step 6 – connect a Swagelok nut to the MNPT-tubing adapter; Step 7 – connect the MNPT-tubing fitting to one of the FNPT-tube fittings separate lengths of tubing; Step 8 – connect the combined fitting to the inlet of the analytical instrument; Step 9 – connect the remaining FNPT-tube fitting to the MNPT-VCR fitting; Step 10 – connect this combined fitting to the outlet of the analytical instrument.

5. Connect a 1 m long length of tubing to the inlet barb and a 25 cm long length of tubing to the outlet barb on the pump **(C2)**. Insert the free end of the tube connected to the pump inlet into the combined inlet manifold fitting created in step 1 of this section.

Note: The nominal outer diameter of the tubing used, and the pump tubing barbs are both 1/8-inch – thus, the tubing must be expanded slightly to fit the barb. It is recommended that a gentle heat source (hair-drier or heat gun) be used to warm the end of the tubing prior to inserting the tip of a #2 Phillips-head screwdriver into the tubing to dilate it. The tubing can then be placed on the pump barbs to form a very tight seal which can be further reinforced with a small zip tie. The manufacturer of the pump recommends that adhesives NOT be used.

6. Place the 1/4-inch nut and ferrule assembly **(C3)** onto the tubing adapter of the 1/4-inch MNPT-tubing fitting **(C4)**. After ensuring that the ferrules are oriented correctly, tighten the fitting onto another 1/4-inch Swagelok fitting to compress the ferrule and permanently bind the nut and tube adapter fitting. Loosen the nut to remove the combined fitting (**C3 + C4**) fitting.

Note: While it is possible to compress the ferrule using the 1/4-inch Swagelok inlet fitting of the analytical instrument itself, it is highly recommended that a separate fitting that can be held with a wrench be used instead; this avoids putting unnecessary torque to the instrument inlet.

7. After applying PTFE-thread tape to the male threads on the fitting assembled above (winding the tape in the same direction that the fitting will be tightened), tighten into the 1/4-inch to 1/8-inch FNPT-tubing fitting **(C5)**.

8. Install the combined fitting **(C3 + C4 + C5)** onto the inlet port of the analytical instrument. Tighten using an 11/16-inch wrench and making sure to avoid cross-threading. Insert a 50 cm length of urethane tubing into the fitting.

9. Apply PTFE-thread tape to the MNPT end of the MNPT-VCR fitting **(C6)** (winding the tape in same direction that the fitting will be tightened) and connect it to a second Tubing-NPT fitting **(C5)**.

Note: This step may not be necessary if the outlet fitting on the analytical instrument is already a NPT fitting. In this case, steps 6, 7, and 8 may be repeated for the outlet fitting. The Picarro recirculating pump used in this system has a VCR fitting which necessitates the use of the MNPT-VCR fitting **(C6)** as an adapter.

10. Install the combined fitting produced in step 7 **(C5 + C6)** on the outlet fitting of the analytical instrument to be used. Tighten using a 11/16-inch wrench, making sure to avoid cross-threading. Insert a 50 cm length of urethane tubing into the fitting.

Note: The number of fittings used to make a connection between instruments increases the likelihood of leaks in a system. Thus, the fewest number of fittings needed to make a connection should always be used. This complex series of fittings was chosen to allow for quick disconnection of tubing from the instrument's vent – if users desire a less complex fitting, components C5 and C6 can be substituted for a VCR to 1/8-inch compression fitting.

11. Using two additional T-fittings **(C1)** connected by a short length of tubing, connect the two lengths of tubing from steps 8 and 10 to the outlet side of the auxiliary pump. Insert the tubing from the pump's outlet (Step 5) into one of the T-fittings. Using a final 1 m length of tubing, connect the combined manifold outlet (from step 1 of this section) and the remaining position on the T-fitting. The completed junction should resemble that in Fig. 15.
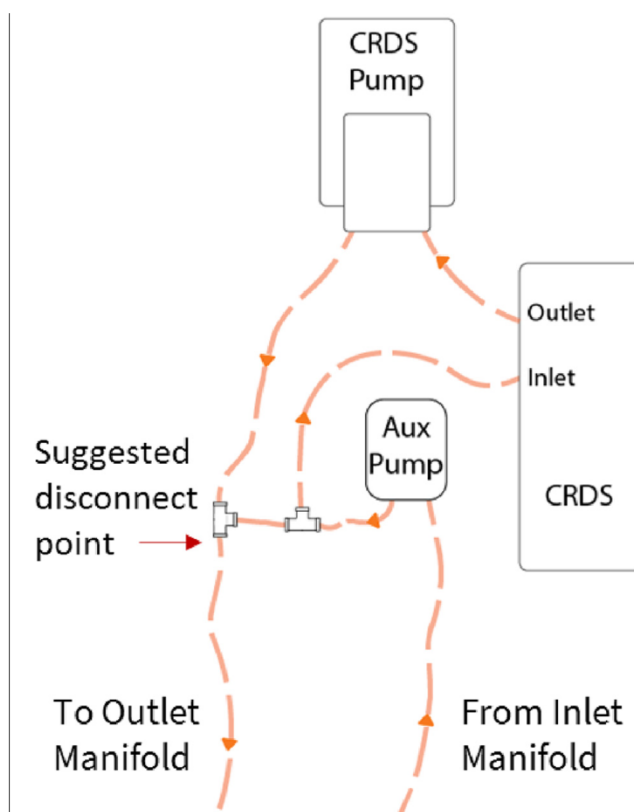

Fig. 15. Schematic of the junction connecting the auxiliary pump, manifolds, and analytical instruments. When first starting the instrument, it is recommended tubing be disconnected from one fitting to prevent over-pressurization before a solenoid can be activated.

4. Operating instructions

4.1. Powering up

1. Power on the instrument and computer that will interface with the multiplexer.
2. Switch the switch on the power supply unit **(B6)** into the on position. The yellow LED labelled "Standby" on the breakout board **(B1)** should now be lit.

3. Switch the power switch on the break-out board **(B1)** into the on position. The green LED on the break-out board labelled "PWR_OK" should now be lit.

4. Connect the relay board (**B4**) to the computer that will be logging the data. While this process varies depending on the connectivity options chosen when purchasing the board and the operating system of the computer, the relay board ships with instructions from the manufacturer on installing the correct device drivers on the computer.

4.2. Controlling the multiplexer

A wide variety of software applications can be used to communicate with the relay controller used. NCD provides software that allows for automated control of their relay boards. Researchers with familiarity with LabView may choose to integrate control of the multiplexer into a LabView terminal and can do so using drivers provided by NCD. As another approach, because the command set utilized by the NCD relay controller is open source, researchers may choose to design their own applets to use with the instrument. For researchers interested in this approach, we have included pseudocode in the relay controller logic document that offers an outline of the general program requirements. Briefly, in our design, relay control included: (1) starting with all relays off, (2) loading a .csv file with two inputs per line – the relays to be opened for each step and the duration of each step, (3) creating a log file that records which relays were open at which timestamps. After running the experiment, the multiplexer log file is matched with the instrument output log file by timestamp, as illustrated in detail in the provided R script.

5. Validation and characterization

5.1. Leak testing

After assembly, the multiplexer and all attached tubing should be thoroughly tested for leaks. The preferred method of leak testing in this setting is to flush the system with a gas of known composition. After a baseline is established, individual fittings are then exposed to a directed flow of a gas with a distinct composition to check for ingress of outside gas into the system. With this approach, the instrument itself is used to detect leaks into the system. When using this technique, it is important to ONLY use gases that are compatible with your instrument. In the specific demonstration of the multiplexer described here, the system was tested as follows:

1) System is flushed with clean air with a CO_2 concentration of 393 ppm for 5 min.
2) Flow of the flush gas is stopped.
3) The baseline is observed – no rapid drops or spikes are expected.
4) A trickle flow of pure CO_2 is directed through a long flexible 1/8-inch tube for use as a probe.
5) The flow of pure CO_2 is briefly passed directly over a fitting or connection and removed after 1 s.
6) The CO_2 concentration in the system is watched for 2 min to verify that no pulse in CO_2 is observed. If a sudden spike in CO_2 is observed, it indicates outside gas is entering the system at or near the testing fitting.
7) After tightening and/or verifying the integrity of the fitting, retesting is performed to confirm the leak has been fixed.

Additionally, the opposite approach can be taken to assess leakage of gas OUT of the system. Here the system is flushed with a gas of a known composition and an external leak detector is passed over each fitting to check for leak-tightness. The downside of this approach is that it requires the use of an external leak detector and that many such devices are tuned to be more sensitive to helium leaks than leaks of nitrogen, carbon dioxide, or air (helium in a common carrier gas in gas chromatography and mass spectrometry but is not recommended for use with a Picarro).

NOTE: As with the previous leak testing technique, it is vital that the instrument never be flushed with a gas that is not compatible with the system, as this could damage the instrument.

The demonstration system described here was extensively leak tested during development and assembly and was found to perform well under relevant experimental conditions (*e.g.* measuring soil CO_2 fluxes when surrounded by an atmosphere of approximately 400 ppm CO_2). It is important to note, however, that a system with this design will likely never be completely leak-free, as the plastic push-connector fittings used are a convenient low-cost alternative to more leak-proof fittings such as metal compression fittings. In the unlikely event that an instrument will be used to analyze gases in a pressurized environment or one with an extremely high concentration of the gas of interest, it is recommended that all push connector fittings be substituted for 1/8-inch compression fittings (with the understanding that this will significantly increase the total cost of the system).

5.2. Flow characterization

CRDS systems typically receive gas flows of less than 300 mL/minute. While this flow rate is enough for the instrument to run, it means that flushing and sampling of each sample vessel would take a prohibitively long time if using only the recirculating pump supplied with the CRDS instrument. To accelerate the rate at which vessels can be flushed and/or sampled, we have included in this design an auxiliary gas sampling pump to boost the maximum flow rate and increase throughput. The pump chosen for this is a KNF diaphragm pump that has been specified to have a maximum flow rate of 2.1 L/minute. When incorporated into the system as per the instructions above (and thus not receiving maximum power) the pump produces a flow of 1.61 L/minute. Because this flow rate is much higher than that required by the CRDS instrument, several design decisions were made to prevent over-pressurization of the system (as indicated by a non-zero PSIG reading on the Picarro pump pressure gauge).

When operating as a closed-loop system (*i.e.*, a vessel is being sampled (Fig. 18B) or the instrument is being run in an "idle" position (Fig. 18D) and is not being flushed with cylinder gas), the auxiliary pump creates a pressure difference that drives the flow through the whole system. In this mode, the pressures within the system do not cause over-pressurization of the system or the Picarro, because the instrument regulates the pressure in its cavity by adjusting the rate of flow at its inlet and outlet, allowing the gas to circulate through the unrestricted loop driven by the auxiliary pump.

Over-pressurization can occur if the auxiliary pump is running without a loop and there is no outlet for the gas flow coming from the pump. Because of this, the system should never be left to run with all connections in place and no solenoids activated. During an experiment and between sampling windows, the instrument should be kept in an idle position (Fig. 18D), with the outlet manifold flowing directly into the inlet manifolds through the sample bypass solenoids. When the instrument is not in use (*e.g.*, when first booting system before an experiment) disconnecting the tubing from the outlet side of the auxiliary pump will prevent over-pressurization (Fig. 15).

Finally, when being flushed with gas from a cylinder, the pressure in the system will increase, causing over-pressurization in the system if not given an outlet. Because of this, the solenoids controlling the introduction of flush gas (labelled 1-1, 3-1, 5-1, and 7-1 in Fig. 18) into the system and those controlling the vent outlets (labelled 2-1, 4-1, 6-1, and 8-1) share a relay on the control board – thus, it is not possible to unintentionally pressurize the system, because activating a solenoid to flush the system (*e.g.*, 1-1) will activate a corresponding solenoid to vent the system (*e.g.*, 2-1).

5.3. Standardization and calibration

As with any gas handling system, the multiplexer itself has a non-zero volume which will contain non-sample gases that mix with the samples and contribute to the measured concentrations or isotopic compositions. This effect can be controlled by using a gas with a known composition to flush the system before analyzing samples and by determining the contribution of the multiplexer volume to the combined multiplexer/sample vessel system. It is recommended that, for each volume of sample vessel used on the multiplexer (*e.g.*, if you use different-sized glass jars for different experiments), a "dilution factor" be determined empirically via the measurement of known volumes and concentrations of a standard gas. It is important to note that this should be done IN ADDITION to regular standardization and calibration of the measurement instrument itself. Determining the dilution factor does not need to be repeated frequently, as the system volume should remain the same, if the sample vessel volume and multiplexer setup remain the same.

The dilution factor for the multiplexer system used in the use case below was determined by injecting 200, 400, and 600 μL of pure CO_2 into 473 mL sample vessels that had been flushed with CO_2-free air. By plotting the measured concentration of these samples against the calculated concentrations for the volume of the gas injected, we can determine the relationship between a measured value and the actual value by taking the slope of the plotted line (Fig. 16). Using this approach, the dilution factor of the system was calculated to be 0.754 – thus, 75.4% of a measured concentration will be derived from the actual concentration of the sample, while the balance of the measurement is made up of background gas in the system.

This information can be entered into the data analysis script to allow for it to correct for the dilution factor during analysis. The specific volumes of CO_2 used were chosen because the corresponding CO_2 concentration would bracket the expected CO_2 concentrations in the upcoming experiment. Because the Picarro G2201-I has a working range of 100–4000 ppm and a guaranteed spec range of 380–2000 ppm, the user may wish to expand the range of their linearity measurements to better bracket their expected measurements.
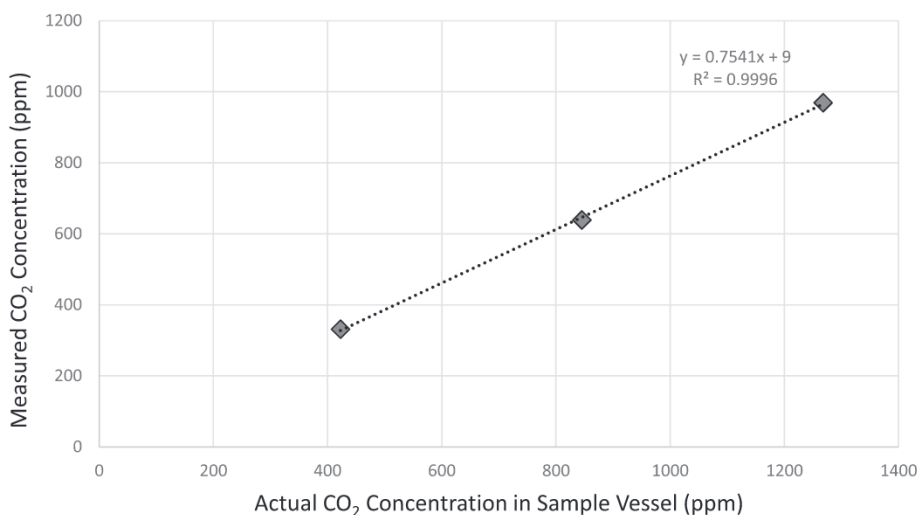

Fig. 16. The dilution effect caused by non-sample gases in the multiplexer system can be determined by plotting the measured concentration of a gas against the expect concentration of the gas in a vessel of known volume at a known pressure in a background of CO_2-free synthetic air. The slope of the regression line from this figure was used in the data processing script to correct the measured CO_2 concentrations in the demonstration experiment.

5.4. Use case

As an example of how the multiplexer can be used, we present a small-scale laboratory soil incubation experiment. Soil scientists often are interested in partitioning CO_2 fluxes between two (or more) different sources. Here, we illustrate an example of partitioning CO_2 fluxes from soil organic matter and pyrogenic organic matter (PyOM). In this experiment, 50 g of Sparta series sandy loam soil was added to each of 14 100 mL glass vials. ^{13}C-enriched pyrogenic organic matter (PyOM) produced from the pyrolysis of sugar maple (*Acer saccharum*) wood at 350 °C was mixed with the dry soil in 7 of these vials at a rate of 10 mg PyOM per gram dry soil. The PyOM used was produced as in Whitman *et al.* from twigs collected in 2011 from saplings ^{13}C-labelled by Horowitz *et al.* [8,9]. The PyOM lot used in this study has an isotopic composition of +73.32‰ δ^{13}C relative to the vPDB standard.

After thorough mixing, the soil in each jar was wetted with 6.2 mL of distilled water to bring the soil to 60% of its water-holding capacity. Immediately after wetting, each vial was set inside of a 1-pint (473 mL) Mason jar also containing 100 mL of water that had been acidified to a pH of 4.5 with H_3PO_4 to help maintain soil moisture levels during the experiment. The acidification was chosen to reduce potential dissolution of CO_2 in the water. Jars were immediately sealed and attached to randomly selected positions on the multiplexer manifolds using 1.5 m lengths of polyurethane tubing (Fig. 17). In addition to these 14 samples, one empty vessel was also included to serve as a blank. The multiplexer was then attached to a Picarro G2131i cavity ring-down spectroscopy system on which the concentration and isotopic composition of CO_2 respired from soil was measured.
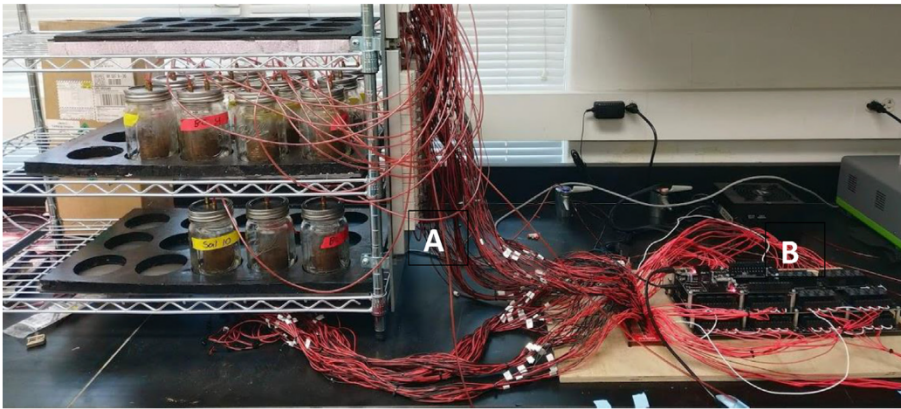
Fig. 17. The soils attached to the multiplexer during the incubation experiment. The manifolds (A) can be seen mounted vertically on the side of the shelving unit holding the samples. The control board (B) sits nearby (uncovered for this picture).

The relay command file used to control sampling of the vessels for this experiment is included, but briefly, consists of a cycle repeated for each vessel as follows for 21 cycles (see Fig. 18 for details):

1. Flushing of gas lines and manifolds with flush gas (synthetic air with a CO_2 concentration of 395 ppm) from a compressed gas cylinder for 60 s. Flush gas is vented to the atmosphere during this period.

2. Sampling of vessel for 600 s.

3. Flushing of vessel with flush gas (as in step 1) for 300 s.

4. Repeat steps 1–3 for each of 15 vessels.

5. Idle for 14,400 s (4 h), allowing soil samples time to respire. During this time the manifolds (but NOT sample vessels) vent to the atmosphere.
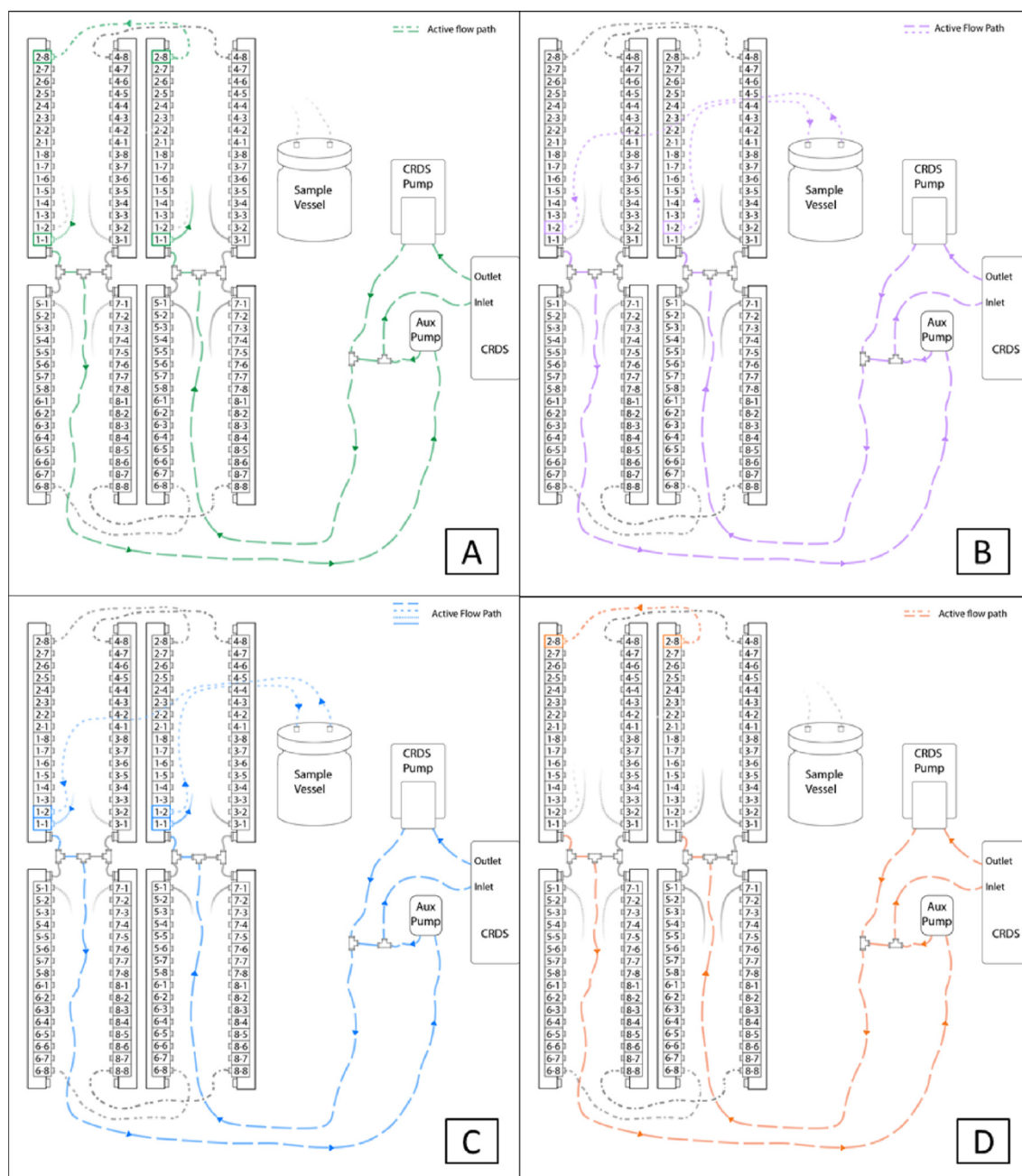
6. Repeat steps 1–5 for each cycle.

Fig. 18. Flow paths of gas during flushing of lines and manifolds (A), sampling (B), flushing of sample vessels (C), and idle (D) phases of the multiplexer sample cycle. Colored lines with arrows depict tubing with active gas flow in the direction indicated while gray lines indicate tubing that is unused at that step.

This measurement cycle resulted in high quality data with low background gas concentrations for the first 15 cycles (see example trace for 3 samples in Fig. 19). After this measurement, the soils respired more CO_2 than could be completely flushed in the 300 s flushing window. This elevated background is corrected for in the script used to process the data, but future experiments using this soil have had vessel flush times increased to 600 s.

The script included partitions the CO_2 fluxes between two sources and generates a collection of figures and data tables that are relevant for those interested in soil gas fluxes and biochemical questions. (Fig. 20 for example, demonstrates that the addition of PyOM suppressed microbial respiration of soil organic matter in this study.) A previous short-term study where a similar PyOM material was added to a different soil also resulted in short term decreases in microbial respiration of soil organic matter, which may be due to short-term substrate switching, where microbes previously consuming soil-derived C switch to the small fraction of PyOM-C that is easily respired [8]. While a full discussion of these observations

is beyond the scope of this paper, we highlight that the frequency of measurement offered by this system allows for the detection of fine-temporal-scale dynamics.
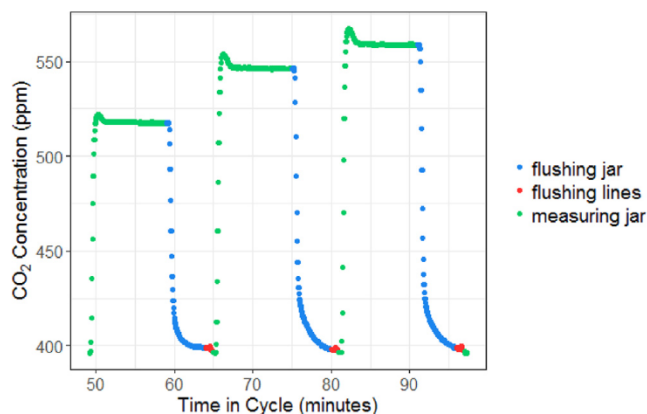

Fig. 19. An example trace demonstrating the measurement of CO_2 respired by soil samples – in this trace three soil microcosms were analyzed (green) and flushed with synthetic zero air (blue) before being allowed to accumulate CO_2 for future measurements. Manifolds and gas lines were flushed and vented to atmosphere in between analyses (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
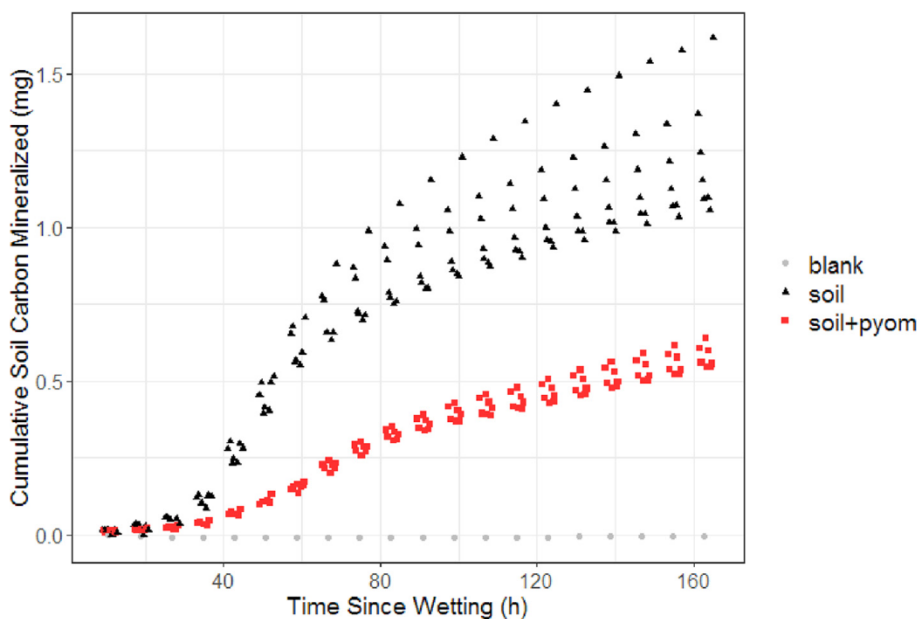

Fig. 20. An example of a figure generated by the data processing script using the data from the experiment described. This figure shows the cumulative mineralization of soil organic matter over time in PyOM amended and un-amended soils. Points are individual measurements.

6. Summary

The multiplexer described herein is a modular gas sampling and delivery system originally designed to operate with cavity spectroscopy instruments such as those made by Picarro and Los Gatos Research, but is compatible with any instrument capable of producing timestamped data files. Configured as described above, the multiplexer can support sampling from 56 vessels, but can be scaled as required to meet scientific and budgetary requirements. We have demonstrated the capabilities of the multiplexer system in a typical use case by using it to autonomously sample head-space CO_2 from 14 laboratory-incubated soils amended with ^{13}C-enriched pyrogenic organic matter for analysis in a Picarro G2201-i cavity ring-down spectroscopy system.

CRediT authorship contribution statement

Timothy D. Berry: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft. **Chance Creelman:** Conceptualization, Software. **Nick Nickerson:** Conceptualization, Software, Writing - review & editing. **Akio Enders:** Conceptualization, Writing - review & editing. **Thea Whitman:** Conceptualization, Resources, Supervision, Project administration, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

Authors Nickerson and Creelman are employed by Eosense Inc., a for-profit company that produces gas multiplexer units for similar applications. No additional interests are present.

Acknowledgements

References

[1] P. Zalicki, R.N. Zare, Cavity ring-down spectroscopy for quantitative absorption-measurements, J. Chem. Phys. 102 (7) (1995) 2708–2717.
[2] E.R. Crosson et al, Stable isotope ratios using cavity ring-down spectroscopy: determination of $^{13}C/^{12}C$ for carbon dioxide in human breath, Anal. Chem. 74 (9) (2002) 2003–2007.
[3] A.J. Midwood, P. Millard, Challenges in measuring the delta C-13 of the soil surface CO_2 efflux, Rapid Commun. Mass Spectrom. 25 (1) (2011) 232–242.
[4] E. Kerstel, L. Gianfrani, Advances in laser-based isotope ratio measurements: selected applications, Appl. Phys. B-Lasers and Optics 92 (3) (2008) 439–449.
[5] Robinson, David and Alex Hayes, Broom: Convert statistical analysis objects into tidy tibbles. 2018. R package: version 0.5.0.
[6] A. Zeileis, G. Grothendieck, Zoo: S3 infrastructure for regular and irregular time series, J. Stat. Softw. 14 (6) (2005) 27.
[7] H. Wickham et al, Welcome to the tidyverse, J. Open Source Software 4 (43) (2019) 1686.
[8] T. Whitman, A. Enders, J. Lehmann, Pyrogenic carbon additions to soil counteract positive priming of soil carbon mineralization by plants, Soil Biol. Biochem. 73 (2014) 33–41.
[9] M.E. Horowitz et al, Patterns of late-season photosynthate movement in sugar maple saplings, Can. J. For. Res. 39 (12) (2009) 2294–2298.

Dr. Timothy Berry received his B.S. in Microbiology from Michigan State University in 2010 and his Ph.D. in Interdisciplinary Life Sciences from Purdue University in 2015. Since 2017 he has worked in the Whitman Soil Microbial Ecology and Biogeochemistry lab in the Department of Soil Science at the University of Wisconsin-Madison. His current work focuses on using stable isotopes to trace the degradation of highly aromatic carbon substrates in soils.