Automated sensor-based quantification of soil water retention and microbial respiration across drying conditions

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1	Automated Sensor-Based Quantification of Soil Water Retention and Microbial
2	Respiration Across Drying Conditions
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L8 L9	Abbreviations: SOM: Soil Organic Matter; SWC: Soil Water Content; WHC: Water Holding Capacity;

20	Abstract
21	Understanding soil responses to climate-induced precipitation variability is important for
22	improving global carbon models and the development of climate-resilient agriculture. However,
23	our knowledge remains limited regarding factors that influence soil water dynamics and
24	microbial respiration across drying conditions, partially hindered by the lack of easily accessible
25	methodologies. We developed a low-cost, automated, and integrated system to simultaneously
26	quantify microbial respiration and soil water retention in response to drought conditions. This
27	system integrates a CO ₂ sensor and a digital scale, enabling continuous measurement of CO ₂
28	concentration and soil water loss by evaporation when soils are incubated with desiccants inside
29	air-tight chambers. Here we show that the sensor platform provides accurate and repeatable CO_2
30	measurements when compared to a spectroscopy gas analyzer and the alkali trap method. Using
31	the sensor platform, we further demonstrated that averaged microbial respiration of rewetted soil
32	over 4-day incubation declined as management intensity increased following the order of Till \leq
33	$NoTill \leq Till_fallow \leq NoTill_fallow = Hay \ field \leq Forest. \ These \ soils \ were \ air-dried \ for \ two$
34	years before use in incubation experiments, and microbial respiration upon rewetting showed a
35	unimodal response, which rapidly increased and peaked within 12 hours except for in forest soils
36	that exhibited a delayed response with respiration peaking at 30 hours. Under drying conditions,
37	rewetted soil from the Hay field showed lower water loss than other soils. Volumetric water
38	content at the end of the drying period increased in the order of Till \leq NoTill \leq Till_fallow \leq
39	NoTill_fallow = Hay field< Forest. We posit that this sensor platform provides a powerful tool
40	for functional soil health assessments and fundamental understanding of soil water dynamics and
41	microbial activities in responses to climate-induced water stress.
42	
43	Keywords: CO ₂ sensor; Drought resistance; Microbial respiration; Soil health; Soil water
44	evaporation

1. Introduction

The frequency of severe floods and prolonged droughts is predicted to increase in many regions of the world due to intensified hydrological cycles associated with global warming (Dai, 2013). The ability of soil to buffer extreme weather events is critical in mitigating impacts of climate change on crop production. A soil that absorbs more water from rains without ponding or runoff and has slower evaporation afterward would particularly be desirable for climate-resilient agriculture (Baveye et al., 2020). Common measurements of soil hydraulic properties, such as water holding capacity, hydraulic conductivity, and water retention curves, provide important insights in predicting soil-water dynamics in response to variable precipitation (Seneviratne et al., 2010). However, obtaining data on these properties normally requires significant investments in time and labor and/or expensive analytical equipment. An inexpensive and rapid method for measuring soil water retention under drying conditions could facilitate the evaluation and prediction of soil responses to climate-induced precipitation variability.

Changes in soil water content (SWC) following precipitation have significant influences on soil microbial processes and soil organic matter (SOM) dynamics. The release of CO₂ and other greenhouse gases during wet-dry cycles is highly variable over time and difficult to predict (Borken and Matzner, 2009). Empirical studies often rely on discrete temporal measurements, which are likely to miss "hot moments" of gas flux from soils (van Groenigen et al., 2015). A flush in microbial activity is often observed upon rewetting dry soil, however, many unknowns remain regarding the underlying physiochemical and biological mechanisms of this flush (Schimel, 2018). Obtaining high-quality empirical data for an accurate representation of soil microbial responses to soil water variability is critical for improving biogeochemical models of greenhouse gas emissions and soil organic carbon (SOC) dynamics (Moyano et al., 2013).

The flush of CO₂ from short-term incubation of rewetted soils is also widely used as an indicator of soil quality or soil biological health (Franzluebbers, 2018; Wander et al., 2019). This indicator is referred to as short-term soil respiration, CO₂ burst, soil test biological activity, or C mineralization potential (Norris et al., 2020), and has been found to be responsive to soil management practices and to be associated with other soil properties such as soil microbial biomass, labile C pool, and N mineralization potential (Franzluebbers et al., 2000). Conventional methodologies for soil respiration measurements include the alkali trap method and the use of

76	gas analyzers to quantify CO ₂ accumulation from microbial respiration (Parkin et al., 1997).
77	These methods require substantial labor/time input and often only provide a few discrete
78	measurements during soil incubation. The use of hazardous chemicals or specialized instruments
79	has largely restricted such analyses to commercial soil testing labs or service-providing
80	universities, which makes routine soil respiration measurements cost prohibitive. There have
81	been attempts to make less expensive options using commercial CO2 sensors (Joshi Gyawali et
82	al., 2019), but the adoption of these approaches has been hindered by the complex engineering

The main objectives of our study were to: (1) develop a user-friendly sensor platform to provide continuous and simultaneous measurements of SWC and microbial respiration under drying conditions in a laboratory environment; (2) evaluate the reliability of this platform as compared with a spectroscopy gas analyzer and the alkali trap method; and (3) compare measurement results from soils with different land use and management histories to assess the usefulness of the sensor platform in soil health assessments. We expect that soils under low management intensity would have higher water retention and microbial respiration compared to intensively managed soils across drying conditions.

- 2. Materials and Methods
- 93 2.1. Construction of the sensor platform

experience required to assemble and use sensors.

94 2.1.1. Design overview

The platform consists of an air-tight chamber equipped with multiple sensors: a CO₂ sensor to measure CO₂ accumulation from microbial respiration, a digital scale to quantify changes in SWC during soil incubation, a temperature sensor, and a humidity sensor to track changes in temperature and humidity inside the chamber (Fig. 1). A desiccant is placed inside the chamber to prevent excessive humidity. The sensors and digital scale are controlled by a microcontroller equipped with a real-time clock and a data logger for automatic data storage as well as a display screen for real-time monitoring of results. All electronics are commercially available, cost less than USD \$150 (at the time of data collection), and can be assembled with little engineering experience.

The platform follows the principle of a modular design, allowing modification and independence of individual functional components. For example, the CO₂ sensor component can

106	be used in other containers (e.g., Mason jars) commonly used for soil incubation studies (Fig.
107	S3a) or adapted for use in the field independent of the digital scale. Similarly, the digital scale
108	can be used without desiccants in an open-air environment to monitor soil water evaporation
109	under natural air-drying conditions.
110	2.1.2. Hardware
111	The measurement system includes four environment sensors (a CO ₂ sensor, a load cell with a
112	signal converter, a temperature sensor, and a humidity sensor), a microcontroller, a data logger,
113	and a display screen. These components can be obtained from various suppliers with different
114	technical specifications and market prices. The CO2 sensor we used is a nondispersive infrared
115	(NDIR) sensor with a stated accuracy of \pm 30 μ mol $^{-1}$ mol $^{-1}$ (referred to ppm afterward) from
116	400-10,000 ppm (SCD-30, Adafruit Industries, New York, NY, USA). Such CO2 sensors are
117	highly sensitive and can respond to changes in CO2 concentration within seconds. This CO2
118	sensor includes temperature (accuracy: \pm 0.4 $^{\circ}$ C at 25 $^{\circ}$ C) and relative humidity sensors
119	(accuracy: \pm 3%). The load cell was obtained from a common household scale (500g Digital
120	Kitchen Scale US-KA8B, AMIR) and then connected to an analog-to-digital converter
121	(NAU7802 Qwiic Scale, SparkFun Electronics, Boulder, CO, USA). Many microcontrollers can
122	be used to configure and control these sensors; we used an ESP32-based board (HUZZAH32
123	Feather Board, Adafruit Industries, New York, NY, USA) because of its ease in incorporating
124	data-loggers and display screens. These electronics can be powered by portable batteries (9 V-12
125	V) or common USB ports. The environmental sensors were stationed inside an air-tight chamber
126	$(25 \text{ cm} \times 18 \text{ cm} \times 15 \text{ cm}, \text{LocknLock Inc., South Korea})$, with wire connections to the
127	microcontroller and other electronics secured outside of the chamber.
128	2.1.3. Software and sensor calibration
129	The programing languages to control the electronics are compiled through the open-source
130	Arduino Software (https://www.arduino.cc/en/software), a widely used integrated development
131	environment (IDE) for writing and uploading code to circuit boards. Commercially available
132	sensors usually contain detailed instructions on how to configure sensors and example codes on
133	Arduino IDE for data acquisition. Example codes for sensor communication and data acquisition
134	used in this study is available on Adafruit learning guides (https://learn.adafruit.com/adafruit-
135	scd30/arduino).

136	Load cells for the digital scales were calibrated using a two-point calibration (zero weight,
137	and a standard 50 g weight) assuming a liner response. The CO2 sensors were calibrated to 400
138	ppm when exposed to fresh outdoor air. For this study, we configured the sensors to record CO ₂
139	concentration every minute.
140	2.2. Testing of sensor accuracy, noise, and signal drift
141	CO ₂ sensors were validated by comparing their readings with readings from a spectroscopy
142	gas analyzer with high precision (± 5 ppb CO ₂ , Picarro G2301, Picarro Inc., Santa Clara, CA,
143	USA). CO2 sensors with portable batteries and other associated electronics were placed in a
144	Mason jar (8-cm in diameter × 18 cm in height, Ball Corporation, Broomfield, Colorado, USA).
145	The lid of the jar had two customized tubing fittings, which were connected to the Picarro gas
146	analyzer and an air pump to allow air inside the jar to circulate through the gas analyzer in a
147	closed loop (Fig. 2b). Five measurement runs were conducted with a gradient of CO ₂
148	concentrations ranging from ambient to over 8000 ppm. Elevated CO2 concentrations were
149	created by breathing into the Mason jar right before closing the lids. For each run, readings from
150	CO ₂ sensors and the Picarro gas analyzer were recorded every 5 minutes for 15 minutes.
151	To evaluate the noise level and potential signal drift of the CO ₂ sensors over time, a gradient
152	of CO ₂ concentrations (~500 ppm to ~6000 ppm) was created inside incubation chambers.
153	Similarly, a series of standard weights (0 g, 10 g, 30 g, 50 g, 70 g, and 100 g) were placed on the
154	load cells to test accuracy and precision of the digital scales. High CO2 concentrations were
155	created by breathing into chambers before closing lids, which were further sealed with duct tape
156	to minimize air leakage. The sensor chambers were then placed inside an incubator at 28.5 °C
157	(Model 1535 Incubator, VWR Scientific Inc., Pennsylvania, USA). Sensors and other electronics
158	were powered through USB ports inside the incubator and sensor readings were recorded every
159	minute for 48 hours.
160	2.3. Experiment I: Comparing sensor-based microbial respiration with the conventional alkali
161	trap method
162	2.3.1. Soil collection
163	Soils were collected from June to July 2020 from six field sites under diverse land use and
164	management practices: silage corn (Zea mays L.) fields with conventional tillage (Till) for more
165	than 50 years or no-till practice (NoTill) for more than 10 years, research plots with a history of

166	more than 40 years of conventional tillage (Till_fallow) or no-till practices (NoTill_fallow)
167	before being fallow for 5 years, a grassy field managed for hay production for more than 100
168	years (Hay), and a mixed deciduous forest that has been developing on abandoned cropland for
169	more than 150 years (Forest). Within each field, three soil pits sized 1-m deep and 1-m wide
170	were excavated for pedon description. Surface soils (0-5 cm) were collected from each pedon.
171	There were two pedons within the hay fields, resulting in a total of 17 soils for use in this study.
172	Field-moist soils were sieved to < 8 mm and then air-dried and stored in plastic bags for two
173	years before use in the rewetting experiments. All soils have a sandy loam texture, but soil total
174	C content increases as management decreases following the order of Till = NoTill=Till_fallow <
175	NoTill_fallow=Hay <forest (table="" 1).<="" td=""></forest>
176	2.3.2. Microbial respiration of rewetted soils in 4-day incubations
177	To evaluate the reliability of the CO ₂ sensor in measuring microbial respiration compared to
178	the conventional alkali trap method, we incubated soils inside sensor chambers following a
179	procedure commonly described as short-term C mineralization or CO2 flush of re-wetted soils
180	(Franzluebbers, 2018). Briefly, 20 g of air-dry soils were re-wetted with 7.5 mL of water inside a
181	150-mL plastic container (5.8-cm diameter \times 7.2-cm height). The re-wetted soil was then placed
182	inside the sensor chamber (regular, 4500 mL in volume, Fig 1), which was incubated at 25 $^{\circ}$ C in
183	a controlled environment incubator (Intellus Environmental Controller, Percival Inc., Iowa,
184	USA). Readings for CO ₂ concentrations, temperature, and relative humidity inside the chamber
185	were recorded every minute for 4 days. To evaluate whether the chamber volume would affect
186	sensor detection of CO ₂ emission, we tested a subset of soils (two NoTill soils and two Forest
187	soils) placed inside Mason jars of different sizes (473 mL, and 1890 mL) that are commonly
188	used for soil incubation (Fig. S3).
189	For the alkali trap method, re-wetted soils with the same soil water ratio (20 g air-dry soil:
190	7.5 mL water) were placed inside Mason jars (8-cm diameter × 12-cm height) following the
191	procedure described in the Cornell Comprehensive Assessments of Soil Health Manual
192	(Schindelbeck et al., 2016). A 10-mL glass beaker consisting of 9 mL of 0.5 M KOH was placed
193	on a stand over the soil surface to serve as an alkali trap. The jars were sealed with the two-part
194	lids and then placed in the same incubator used for the sensor study at 25 °C. Electrical
195	conductivity (EC) of the KOH trap solution was measured before and after a 4-day incubation

196	using a benchtop EC meter (Orion Versa Star Pro, Thermo Scientific, Waltham, MA, USA).
197	Total amount of CO ₂ respired was calculated based on changes in EC in the alkali trap relative to
198	EC in pure KOH and saturated K ₂ CO ₃ solution (Schindelbeck et al., 2016). Averaged microbial
199	respiration rate (µg CO ₂ -C g ⁻¹ soil hr ⁻¹) over the 4-day incubation was calculated for
200	comparisons with the sensor method.
201	2.4. Experiment II: Soil water dynamics and microbial respiration under drying conditions in 7-
202	day incubations
203	To simulate a wet-dry cycle, soils were rewetted until field capacity and then incubated
204	together with desiccant inside the sensor chambers. Soil columns were created by packing air-
205	dried soil into 50-mL conical plastic tubes (3-cm diameter × 11-cm height, Corning Inc., NY,
206	USA). The top half of the plastic tube was cut off to allow faster dispersion of water vapor, and a
207	small hole (~2 mm in diameter) was drilled in the bottom of the tube to allow water drainage. A
208	piece of filter paper (Whatman no. 42, Cytiva Life Science, MA. USA) was placed inside the
209	tube, and wetted with deionized water. The weight (W_{tube}) of the tube with wet filter paper was
210	recorded.
211	A total of 22.5 mL of soil (5 cm in height) was loosely packed in the tubes by gently tapping
212	the side of the tubes while adding soil. Differences in bulk density among the repacked soils
213	(Table 1) resulted in net dry soil weight (W_{soil_dry}) ranging from 14.9 g (Forest soil) to 29.7 g
214	(Till soil). For each soil column, 25 mL of water was added to the top, simulating a 3.5 cm
215	precipitation event. The re-wetted soil columns were allowed to drain for about one hour until
216	water stopped dripping from the bottom, which was considered to be field capacity SWC. The
217	soil columns were then placed on the digital scale inside the chambers with the total initial
218	weight (W_{T0}) recorded, and SWC at field capacity was then calculated as the difference between
219	W_{T0} and $(W_{tube} + W_{soil_dry})$. Inside each sensor chamber, 35 g of anhydrous CaCl ₂ (Fisher
220	Chemical, Fisher Scientific, NH, USA) was spread out evenly in a petri dish (10-cm in diameter)
221	to maintain a low relative humidity (15-30%) during the incubation. All sensor chambers were
222	then placed inside an incubator at 28.5 °C to accelerate drying for seven days. All 17 soils from
223	Experiment I were used for this experiment. To examine within-soil variability, a few soils from
224	the NoTill_fallow, Till_fallow, and the Hay fields were replicated, which resulted in 21
225	independent incubations.

2.5. Experiment III: Comparing microbial respiration under consistent vs. drying moistureconditions in 7-day incubation

Six soil samples from NoTill_fallow and Till_fallow sites were incubated for approximately seven days at 28.5 °C under both consistent moisture conditions as described in Experiment I and drying conditions as described in Experiment II. To allow direct comparisons based on mass, all 12 incubations started with 20 g of dry soil. Gravimetric SWC was maintained at $3.75~\rm cm^3~g^{-1}$ for soils under consistent moisture conditions. In comparison, SWC declined from $0.50~\rm cm^3~g^{-1}$ to $0.10~\rm cm^3~g^{-1}$ when incubated under drying conditions.

2.6. Data processing and statistics

Senor readings are stored in a micro-SD card in a text file, which can be easily exported into data analysis software. To reduce data noise, raw CO₂ and weight readings of every minute were first averaged by the hour before further analyses.

Cumulative CO₂ respired (ppm) at time t was calculated by subtracting initial CO₂ concentrations (t = 0) from CO₂ concentrations at time t. To further reduce sensor noise, hourly moving averages of CO₂ concentrations were calculated using a moving window of four hours; the hourly respiration rates (ppm per hour) were then calculated by taking the difference of CO₂ moving averages between consecutive hours. The moving window of four hours was chosen to minimize the effects of short-term fluctuation in sensor response (Fig. S2). To examine whether reducing the data to the hourly averages would affect the temporal respiration pattern, we also calculated respiration rates for a subset of soils using the raw data with the frequency of every minute. The respiration rate was further converted to the amount of CO₂-C per unit of soil per unit time (μ g CO₂-C g⁻¹ soil hr⁻¹) based on the ideal gas law, the volume and the temperature of the incubation chamber, and the amount of soil used for each sample. Averaged respiration rates within each day or over the entire incubation period were calculated by averaging the hourly respiration rates within specific time windows.

Cumulative water evaporated at time t was calculated by subtracting weight at time t from the initial weight (W_{T0}), assuming 1 g of weight loss from the soil column equals to 1 mL of water evaporated. Relative water evaporation (%) was calculated by dividing cumulative water evaporation by initial SWC at field capacity. Volumetric SWC (cm³ H₂O cm⁻³ soil) at time t was calculated by taking the difference between initial SWC and cumulative water evaporation

at time t . Gravimetric SWC (cm ³ H ₂ O g ⁻¹ soil) was also calculated based on volumetric SWC
and the net dry soil weight of each soil column. To calculate evaporation rates, a moving average
of weight data were first calculated using a moving window of four hours, and hourly
evaporation rates (cm ³ H ₂ O cm ⁻³ soil hr ⁻¹) were calculated by taking the difference of moving
averages between consecutive hours. Averaged evaporation rates within each day or over the
entire incubation period were calculated by averaging the hourly evaporation rates within
specific time windows.

For Experiment I, a linear model was conducted to compare microbial respiration between the sensor method and the alkali trap method, and Wald test was used to evaluate if the slope of the linear model deviated from 1 (Fox et al., 2007). A pairwise t-test was used to evaluate if the two methods provided similar measurements of microbial respiration. For Experiment I and II, a two-way (land use × day) ANOVA was used to evaluate how land management and time since rewetting affected respiration and evaporation rates. Pearson correlation coefficients were calculated to evaluate the relationships between soil C content, SWC, and microbial respiration. For Experiment III, a three-way ANOVA (land use × day × incubation method) was used to evaluate if microbial respiration differed between the two incubation conditions (rewetted and maintained at consistent moisture levels vs. rewetted to field capacity and continued drying). Due to the high number of soil groups to compare (treatment levels = 6) and the small sample size per group (n = 3), we used alpha = 0.10 to increase the power to detect differences among groups. When source effects were significant, treatment means were separated using *post hoc* pairwise comparisons with Tukey's HSD tests at α = 0.10.

All data analyses and visualization were performed in R software version 2.15 (R Core Team, 2016) with the following packages: *zoo* for calculating the moving averages (Zeileis and Grothendieck, 2005), *ggplot2* for data visualization (Wickham, 2016), *mgcv* for fitting smooth lines for hourly respiration and evaporation data (Wood, 2017), *car* for the Wald test of the linear models for method comparisons (Fox et al., 2007), and *emmeans* for ANOVA and pairwise comparisons (Lenth and Lenth, 2018).

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3.1. Sensor accuracy, noise, and signal drift

The digital scales provided accurate readings of known standard weight ranging from 0 g to 100 g (Fig. S1). There was no apparent signal drift over 48 hours under consistent temperature, with a noise of approximately \pm 0.3 g around expected values (Fig. S1). Consistent scale readings are critical to accurately track changes in soil weight induced by water evaporation as soil dries.

CO₂ readings from sensors were highly correlated with gas analyzer readings within the range of 500–8000 ppm (Fig. 2a, $r^2 = 1$, p < 0.001). The slope of the regression did not deviate from 1, although CO₂ sensors had consistently higher readings (+ 46 ppm) than the gas analyzer (paired *t*-test, p = 0.03). There was no apparent drift of CO₂ readings inside empty incubation chambers for concentrations ≤ 4900 ppm (Fig. S2). CO₂ readings of ambient indoor air averaged 522 ppm over 48 hours with a minimum of 491 ppm and a maximum of 542 ppm, consistent with the stated noise level of \pm 30 ppm. However, incubation chambers with very high CO₂ concentrations (>4900 ppm) declined between 300–400 ppm after 48 hours, possibly because chambers were not completely air-tight (Fig. S2). A downward drift of CO₂ concentrations would underestimate microbial respiration; however, such effects may be negligible if losses are small relative to microbial respiration rates.

3.2. Soil microbial respiration of rewetted soils: sensor-based method vs. alkali trap method

The sensor platform detected increases in CO₂ concentrations within hours after re-wetting
(Fig. 3a). Rates of CO₂ accumulation did not follow a linear pattern during the 4-day incubation. Instead, respiration rates increased rapidly and peaked within 12 hours for most soils, except the forest soils that peaked in respiration approximately 30 hours after re-wetting (Fig 3b). Similar respiration peaks (10–12 hours for cropland soils vs. ~30 hours for forest soils) were observed when soils were incubated using smaller Mason jars (Fig. S3b). Respiration rates calculated using minute rather than four-hour frequency showed similar respiration patterns, although there was much higher variability with the higher temporal resolution (Fig. S4). The temperature inside all soil chambers was maintained at 26 °C during the 4-day incubation, and relative air humidity rapidly increased to near 100% saturation soon after the incubation started (Fig. S5).

312	Average 4-day microbial respiration increased as soil management intensity decreased,
313	following the order of Till \leq NoTill \leq Till fallow \leq NoTill fallow $=$ Hay $<$ Forest (Fig. 3c), and
314	was positively correlated with soil total C content ($r = 0.92, p < 0.001$). Average microbial
315	respiration rates calculated from the sensor method were highly correlated with those of the
316	alkali trap method (Fig. 4, $r^2 = 0.89$), and the slope of the linear regression did not deviate from
317	1. Respiration measurements did not differ between the two methods based on the paired <i>t</i> -test
318	comparison ($p = 0.15$).
319	3.3. Soil water dynamics under drying conditions
320	The combined use of the digital scale and the CO2 sensor enabled simultaneous
321	quantification of soil water dynamics and microbial respiration under drying conditions. As
322	expected, the total weight on the digital scale decreased as water evaporated over time (Fig. 5a).
323	Initial net weight of the 5-cm saturated soil columns (22.5 mL) decreased in the order of Till >
324	$NoTill > Till_fallow \ge NoTill_fallow \ge Hay = Forest (Table 1)$, driven mainly by differences in
325	soil bulk density (Table 1). Cumulative water evaporation was similar among soils in the first
326	three days but was significantly lower in hay soil than other soils after four days (Fig. 5b, Fig. 6).
327	In comparison, relative water evaporation, after accounting for the initial difference in SWC,
328	decreased as management intensity decreased in the order of Till \geq NoTill = Till_fallow =
329	NoTill_fallow = Hay ≥ Forest (Fig. 5c, Fig. 7). An average of 92% of the initial water in Till soil
330	had evaporated by day 7 while 73% had evaporated in Forest soil (Fig. 7). Differences in
331	evaporation led to differences in SWC during incubation (Fig. 5d, Fig. 8). For instance,
332	volumetric SWC in Hay soil was similar to that of Till soil at day one but was 156% higher than
333	that of Till soil by day 7 (Fig. 8). The observed differences in water evaporation among soils
334	were not caused by variability in incubation conditions because temperature was maintained
335	between 28 °C to 28.5 °C and relative humidity between 15% to 35% throughout the incubation
336	(Fig. S6).
337	Volumetric SWC at field capacity was similar among cropland soils, which were lower
338	than that in the forest soils before the drying experiment (Table 1). In comparison, volumetric
339	SWC at the end of the drying period increased in the order of Till ≤ NoTill < Till_fallow ≤
340	NoTill_fallow = Hay < Forest (Fig. 8). Gravimetric SWC followed a similar pattern (Fig. S7).
341	Both volumetric and gravimetric SWC at the end of the drying period were positively correlated

with soil total C content (r = 0.77 and r = 0.92 for volumetric SWC and gravimetric SWC 342 respectively). Water evaporation rates for most soils showed a typical two-stage pattern: 343 evaporation was relatively constant within the first three or four days (stage I) and then rapidly 344 declined afterward (stage II) (Fig. 9). However, evaporation rates for NoTill fallow and Hay 345 soils continued to decline without an apparent plateau (Fig. 9). 346 3.5. Comparison of soil microbial respiration under drying conditions vs. consistent soil moisture 347 Despite SWC declining more than 70% after 7 days, dynamics of microbial respiration 348 under drying conditions were broadly similar to when soils were kept at consistent SWC (Fig. 10 349 vs. Fig 3b). Microbial respiration at the end of the drying period increased in the order of Till \leq 350 NoTill \leq Till fallow = Hay \leq NoTill fallow \leq Forest (Fig. 10), and showed positive correlations 351 with volumetric SWC (r = 0.53, p = 0.016), gravimetric SWC (r = 0.57, p = 0.010), and soil total 352 C (r = 0.58, p = 0.008). 353 Direct comparisons of a subset of the soils (i.e., Till fallow and NoTill fallow) at the 354 same incubation temperature revealed that cumulative microbial respiration tended to be higher 355 in soils with consistent moisture level as compared to soils incubated under drying conditions 356 (paired t-test, p = 0.07). The lower respiration in drying soils was apparent on day 1 and day 7 357 (Fig. 11). Respiration was consistently higher in the NoTill fallow soil than the Till fallow soil 358 over the 7-day incubation (Fig. 11, Soil management, p < 0.001). 359 360 4. Discussion 361 4.1. Using CO₂ sensors to quantify the temporal dynamics of soil microbial respiration The ability of the sensor platform to detect differences in CO₂ flux from various land uses 362 and management practices and its congruence with the conventional alkali trap method suggests 363 that inexpensive CO₂ sensors can serve as a promising alternative for quantifying soil microbial 364 respiration. Consistent with our findings, previous studies have showed that similar NDIR CO₂ 365

sensors using a first-order calibration (i.e., calibrated to 400 ppm when exposed to outdoor air)

readings with other gas analyzers (Joshi Gyawali et al., 2019). CO2 sensors have been used to

to quantify microbial responses to different herbicide levels (Djerdj et al., 2021). In addition,

detect respiration differences in soils with or without diesel contamination (Kaur et al., 2015) and

provided accurate measurements of CO₂ gas standards (Djerdj et al., 2021) or comparable

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quantifying short-term microbial respiration as a biological indicator of soil health is becoming popular among researchers and other stakeholders working on soil conservation (Wander et al., 2019; Liptzin et al., 2022). The CO₂ sensor platform is portable and does not require hazardous chemicals, making it appealing for use outside of conventional soil analysis labs.

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Continuous measurements from CO₂ sensors provided a high temporal resolution in microbial responses to changes in soil water content. Patterns of microbial respiration upon rewetting have been intensively studied and two types of response patterns have been observed (Meisner et al., 2015; Brangarí et al., 2021). The type I response is characterized by respiration being highest immediately (i.e., within minutes) upon rewetting then decreasing gradually (Fraser et al., 2016; Brangarí et al., 2021), and seems to occur when soils were exposed to a short period of drying (i.e., within weeks) (Iovieno and Bååth 2008; Sawada et al., 2016; Slessarev et al., 2020) or when soils only partially dry before rewetting (Meisner et al., 2017). In contrast, the type II response occurs when soils were completely air-dried for a long period of time (i.e., one year or more) before rewetting (Meisner et al., 2013), wherein respiration remains low for an extended period before rapidly increasing with a lag in respiration peaks at ~ 20 hours following rewetting (Meisner et al., 2013, 2015). Consistent with the type II response, soils in our study were air-dried for two years and respiration upon rewetting showed a universal unimodal pattern with respiration peaked at 10-30 hours after rewetting. The mechanism driving the long delay in respiration peaks in our soil, as suggested by Meisner et al. (2013), is likely due to the reduced size of the microbial community that survived the prolonged drying. The resulting high ratio of dissolvable organic C: living microbial biomass after rewetting soils following a prolonged drying period could drive exponential growth for hours coupled with increases in respiration rates. For soils that are air-dried for a short period of time, the population size of surviving microbes remains high, leading to immediately high respiration following rewetting (Type I response). We used the same sensor setup to test soils that were air-dried for two weeks, which showed the same type I respiration pattern as expected (Fig. S8). Our results also revealed a narrow period of time for microbial respiration peaks, suggesting that shape patterns between Type-I and Type-II can be misidentified if discrete measurements fail to capture such "hot moments" in CO₂ flux (Slessarev et al., 2020).

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Bursts of respiration upon rapid rewetting of dry soils are well documented in the literature and are often referred to as the "Birch Effect" (Birch and Friend, 1956; Borken and Matzner, 2009). Mechanisms driving this C-flush are not fully understood (see reviews in Schimel, 2018; Barnard et al., 2020), and include microbial metabolism of cytoplasmic solutes that were accumulated to maintain hydration during drying (Fierer and Schimel, 2003; Warren, 2014) as well as microbial utilization of other substrates released from cell lysis upon rewetting (Blazewicz et al., 2020) or previous physically protected SOM (Navarro-García et al., 2012). Respiration peaks observed in our study are consistent with the rapid microbial responses to high availability of labile compounds (either cellular or extracellular) from rewetting. As easily accessible substrates were consumed, microbial respiration rapidly declined. Similar respiration peaks occurred when soils were rewetted to field capacity, suggesting that high CO₂ flux can be dominated by anaerobic respiration, which may promote denitrification processes and trigger pulses of N₂O emission (Leitner et al., 2017). Delayed responses in the Forest soil could be due to its microbial communities being distinct from cropland soils (Gan et al., unpublished data), and it has been shown that different microbial groups resuscitate at different times following rewetting (Placella et al., 2012). Combined with other microbial analyses such as transcriptomics and stable isotope probing analyses (Aanderud et al., 2015), this sensor platform could be a powerful tool in experiments to elucidate temporal dynamics of soil microbial communities and their functional response to dry-wet cycles and other disturbances.

One concern with our incubation system is that increases in CO₂ concentration in the airtight chambers may suppress microbial respiration (Daniels et al., 1985). MacFadyen (1973) reported that soil respiration measured at a CO₂ concentration of 1700 ppm was 0 to 63% lower than in a CO₂-free atmosphere. Koizumi et al. (1991) found that soil respiration was stimulated when CO₂ concentration was below ambient level, although respiration rates were consistent when CO₂ levels were between 500–1000 ppm. Because the alkali trap method would lower CO₂ concentration below the ambient level, it may over-estimate soil microbial respiration when compared with other closed-chamber or open-flow methods (Bekku et al., 1995). The similarity in microbial respiration obtained between the alkali trap method and the sensor method suggests that the CO₂ concentration effects may be negligible when compared with differences in microbial respiration induced by soil heterogeneity. In addition, respiration rates remain similar when smaller incubation chambers were used (Fig. 3b vs Fig. S3b), suggesting that high CO₂

concentration (up to 10,000 ppm) had little effect on microbial respiration during short-term 431 incubation (i.e., 4–7 days). For longer-term incubation, extremely high cumulated CO₂ 432 433 concentrations can be avoided by reducing the soil: chamber volume ratio or by including periodic ventilation during incubation. 434 4.2. Tracking soil water content and water evaporation using digital scales 435 The evaporation method is a well-established technique to determine soil water retention 436 437 curves and other soil hydraulic properties in the laboratory (Peters et al., 2015). Similar to data collected by commercial devices such as the HYPROP system (Bezerra-Coelho et al., 2018), 438 water evaporation quantified by the inexpensive sensor platforms exhibited the typical two-stage 439 characteristics: evaporation rate is relatively constant at the beginning (stage I) and then rapidly 440 441 drops (stage II) when the soil surface becomes dry (Campbell and Norman, 1998). The evaporation rate of the first stage is determined by evaporative demand of the atmosphere 442 (Campbell and Norman, 1998), which was kept consistent across all soil incubations in our 443 study. As a result, the magnitude of stage I evaporation was similar among soils for the first three 444 445 days (Fig. 6). At the onset of the second drying stage, evaporation rate is determined by the ability of soils to conduct water to the evaporating surface (Lehmann et al., 2008; Han and Zhou, 446 2013). We believe such empirical evaporation curves generated from the sensor platform can be 447 valuable in modeling soil water evaporation and advancing the theoretical understanding of soil 448 water dynamics in land-surface processes (Bittelli et al., 2008). 449 Temporal dynamics of SWC is determined by water infiltration and retention following 450 precipitation and water loss to evaporation under drying conditions. SWC at field capacity 451 generally increases as SOM increases (Hudson, 1994), as seen in our study that Forest soil had 452 453 the highest total C content and the highest SWC at field capacity. In comparison, soil water evaporation is a complex process that is determined by environmental conditions and soil 454 455 properties such as soil pore size distribution, soil matric potential, soil surface roughness, etc. (Yiotis et al., 2003; Lehmann et al., 2008). Soil structure can be important in affecting water 456 457 evaporation, and undisturbed soil cores generally have lower evaporation than disturbed soil

(Burt et al., 2005). Soils in our study were disturbed (i.e., sieved < 8mm then air dried) before

they were loosely packed in soil columns. Hay soils had a lower evaporation and did not have an

apparent steady constant rate stage (Fig. 9), which may be partially due to its lower percentage of

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water saturation at the beginning (Table 1). However, there is cumulative evidence that mucilage
excreted from plant roots and extracellular polysaccharides (EPS) produced by soil bacteria can
form biofilms and aggregations that reduce water evaporation and thus conserve more water in
soils (Roberson and Firestone, 1992; Kroener et al., 2018; Zheng et al., 2018). Moreover, the
addition of EPS in soil has been shown to smoothen the transition between stage-I and stage-II
evaporation, reducing the period of the stage I evaporation and total soil water evaporation
(Zheng et al. 2018). While more research is needed to confirm the lower evaporation in Hay soils
and to investigate links between water evaporation and soil EPS and aggregation levels, we
suggest that improving soil resistance to evaporation can be an important strategy to conserve
soil water across drying conditions.

4.3. Land use and management effects on soil response to drought

Land use and management practices can profoundly alter soil physicochemical and biological properties, which would further affect a soil's ability to withstand extreme weather events. Consistent with our expectation, volumetric SWC at the end of the drying period increased in the order of Till ≤ NoTill < Till_fallow ≤ NoTill_fallow = Hay < Forest. The ability to retain soil water under drying conditions is essential to maintain soil biological functions, as evidenced from the positive correlation between SWC and microbial respiration at the end of the drying period. Meanwhile, difference in soil C content along the management gradient explained a substantial amount of variation in SWC and microbial respiration, highlighting the central role of SOM in regulating soil water and soil functioning (Rawls et al., 2003; Murphy, 2015). Increase in SOM improves soil water-holding capacity (Lal, 2020) and may also reduce water evaporation by altering aggregation and pore size distribution (Ding et al., 2014). As such, restoring SOM with conservation management practices will be key in improving soil resilience and the development of climate-resilient agriculture (Baveye et al., 2020).

4.4. Caveats and future improvements

The sensor platform consists of inexpensive commercial sensors and electronics and provides continuous and automated measurements of microbial respiration and soil water evaporation during soil incubation. As commercial sensors may come with a wide range of technical specifications, it is important to verify the accuracy, noise levels, and signal drift when using environmental sensors for continuous soil measurements. However, the absolute accuracy

of the sensor readings may not be critical when changes in the measurement values are the point
of interest. In addition, the digital scales and CO2 sensors can be used separately or in
combination depending on research interests, and can be configured to integrate other sensors
such as soil nitrogen sensors (Fan et al., 2022) to assess soil biological activity for mechanistic
evaluations and soil health studies. The simplicity and low cost of the sensor platform has the
potential to advance soil resilience research and increase the involvement of stakeholder groups
in the development and evaluation of climate-resilient agriculture.

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TABLE

Table 1. Soil physicochemical properties. Values are averages (\pm SD) from three pedons within each field except for two pedons from the hay field. Different lowercase letters within the same rows indicate statistical differences among soils collected from different land use and management practices according to Tukey's HSD test (p = 0.10).

Soil properties †	Till	NoTill	Till_fallow	NoTill_fallow	Hay	Forest
Soil Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
% Clay	8.6 (0.53)	8.2 (0.36)	7.7 (1.19)	10.1 (0.72)	11.3 (1.41)	9.3 (5.81)
% Sand	64.4 (2.25)	60.5 (1.89)	58.2 (4.55)	55.6 (2.12)	57.7 (0.21)	61.4 (12.56)
Total C, mg g ⁻¹	23.5 (2.14) a	26.9 (1.1) a	31.3 (2.82) a	52.2 (16.18) b	50.7 (1.95) b	100.6 (7.66) c
Bulk density, g cm ⁻³	1.29 (0.04) d	1.09 (0.03) c	0.99 (0.07) c	0.88 (0.03) b	0.87 (0.04) b	0.7 (0.03) a
Porosity, cm ³ cm ⁻³	0.51 (0.01) a	0.59 (0.01) b	0.63 (0.02) b	0.67 (0.01) c	0.67 (0.02) c	0.74 (0.01) d
SWC at field capacity, cm ³ cm ⁻³	0.41 (0.01) a	0.45 (0.01) a	0.46 (0.03) a	0.46 (0.02) a	0.41 (0.01) a	0.54 (0.05) b
SWC at field capacity, cm ³ g ⁻¹	0.32 (0.0004) a	0.41 (0.003) b	0.47 (0.01) c	0.52 (0.03) d	0.47 (0.01) c	0.77 (0.04) e
Saturation level at field capacity, %	0.81 (0.04) b	0.76 (0.03) b	0.74 (0.07) b	0.69 (0.03) ab	0.61 (0.04) a	0.73 (0.08) ab

†Soil texture (% sand and % clay) was determined using the hydrometer method (Gee and Bauder, 1979); Total C is measured using a total combustion elemental analyzer (ECS 4010, Costech Analytical Technologies Inc.). Bulk density is calculated based on the net soil weight of the 22.5ml of soil in the loosely packed columns in Experiment II; Porosity is calculated based on bulk density and a mineral density of 2.65 g cm⁻³. Volumetric and gravimetric SWC (soil water content) at field capacity is described in the method section for Experiment II; Saturation level is calculated based on the volumetric SWC and porosity.

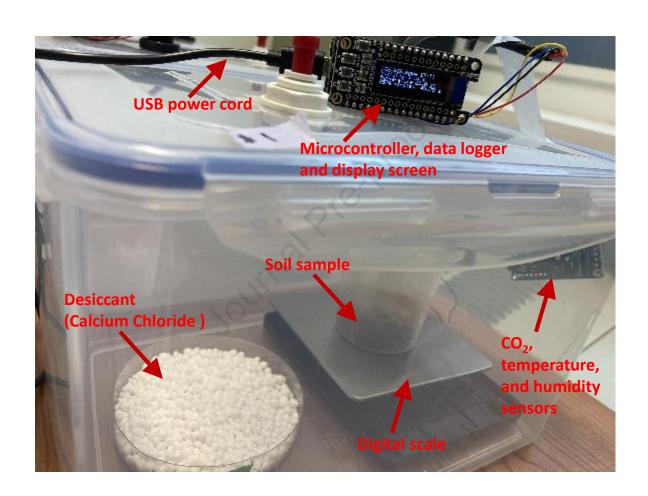
FIGURES LEGENDS

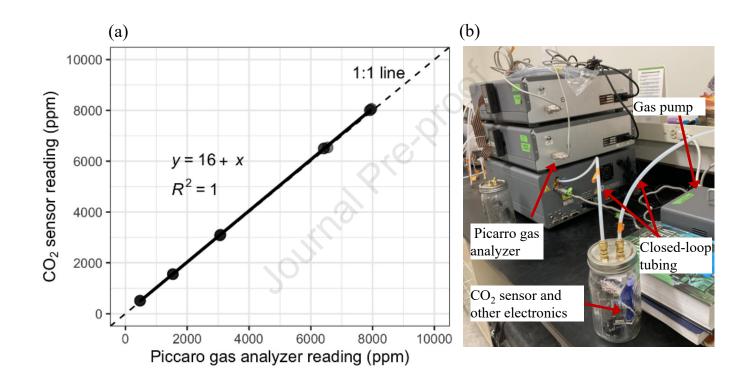
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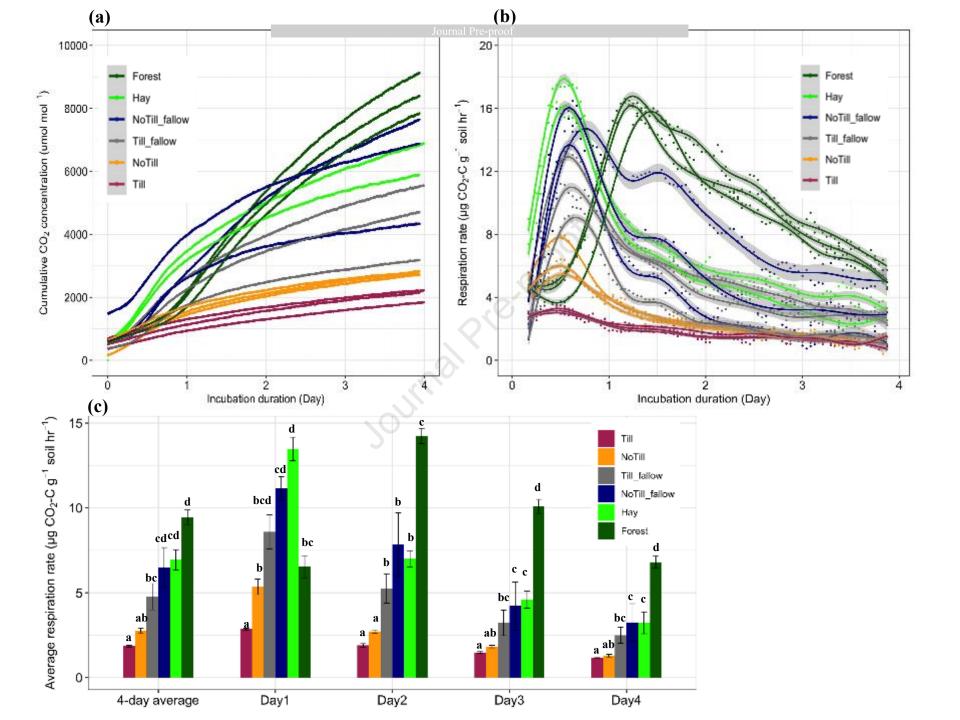
- Fig. 1. The novel sensor platform with soil sample, desiccant, environmental sensors, and digital
- scale inside the air-tight chamber. The micro-controller, display screen and data logger are
- stationed outside the chamber.
- 699 Fig. 2. (a) Relationships between CO₂ concentrations measured by the Piccaro gas analyzer and
- 700 the CO₂ sensor. (b) Laboratory setup for measuring gas concentration inside the glass jar using
- 701 the CO₂ sensor and the gas analyzer.
- Fig. 3. (a) Cumulative CO₂ concentrations from soil incubations with consistent gravimetric
- water content (3.75 cm⁻³ g⁻¹). (b) Hourly microbial respiration rates. (c) Averages of microbial
- respiration rates over 4 days or within each day. Different lowercase letters within the same days
- 705 indicate statistical differences among soils collected from different land use and management
- practices according to Tukey's HSD test (p = 0.10). Maroon: corn field with conventional till
- practices; Orange: corn field with no-till practices; Grey: fallow corn field with a history of
- conventional till practices; Blue: fallow corn field with a history of no-till practices; Green: hay
- 709 field; Dark green: unmanaged forest.
- Fig. 4. Correlation between microbial respiration quantified by the sensor method and the alkali
- trap method. For both methods, soils were incubated under consistent moisture conditions (3.75)
- 712 cm⁻³ g⁻¹). Color legend is the same as that in Fig. 3.
- Fig. 5. Soil water dynamics when rewetted soils were incubated under drying conditions. (a)
- 714 Total weight recorded by the digital scales. (b) Cumulative amount of water evaporated. (3).
- Proportion of cumulative water evaporation. (4) Volumetric soil water content. Color legend is
- 716 the same as that in Fig. 3.
- 717 Fig. 6. Daily averages of cumulative water evaporation when rewetted soils were incubated
- 718 under drying conditions. Different lowercase letters within the same days indicate statistical
- 719 differences among soils collected from different land use and management practices according to
- Tukey's HSD test (p = 0.10). Color legend is the same as that in Fig. 3.
- Fig. 7. Daily averages of relative water evaporation when rewetted soils were incubated under
- drying conditions. Different lowercase letters within the same days indicate statistical differences
- among soils collected from different land use and management practices according to Tukey's
- HSD test (p = 0.10). Color legend is the same as that in Fig. 3.
- Fig. 8. Volumetric soil water content when rewetted soils were incubated under drying
- conditions. Different lowercase letters within the same days indicate statistical differences
- among soils collected from different land use and management practices according to Tukey's
- HSD test (p = 0.10). Gravimetric soil water content showed a similar pattern in the end of the
- 729 incubation and was provided in Fig. S5. Color legend is the same as that in Fig. 3.

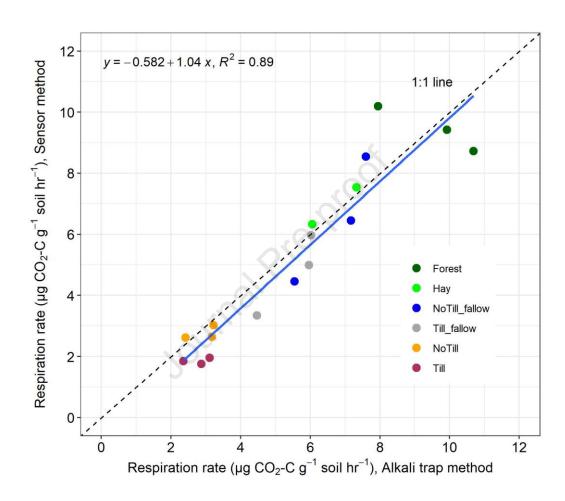
Fig. 9. Hourly water evaporation rates when rewetted soils were incubated under drying 730 conditions. Different lowcase letters indicate statistical differences between days according to 731 Tukey's HSD test (p = 0.10). The shaded areas indicate Stage I evaporation with a relatively 732 steady constant rate. Color legend is the same as that in Fig. 3. 733 Fig. 10. Hourly microbial respiration rates when rewetted soils were incubated under drying 734 conditions. Color legend is the same as that in Fig. 3. 735 Fig. 11. Comparison of soil microbial respiration when incubated under consistent moisture 736 737 conditions (blue) or drying conditions (orange). Asterisks on day 1 and day 7 indicate significant difference between the two incubation conditions. T/F: Till Fallow; NT/F: NoTill Fallow

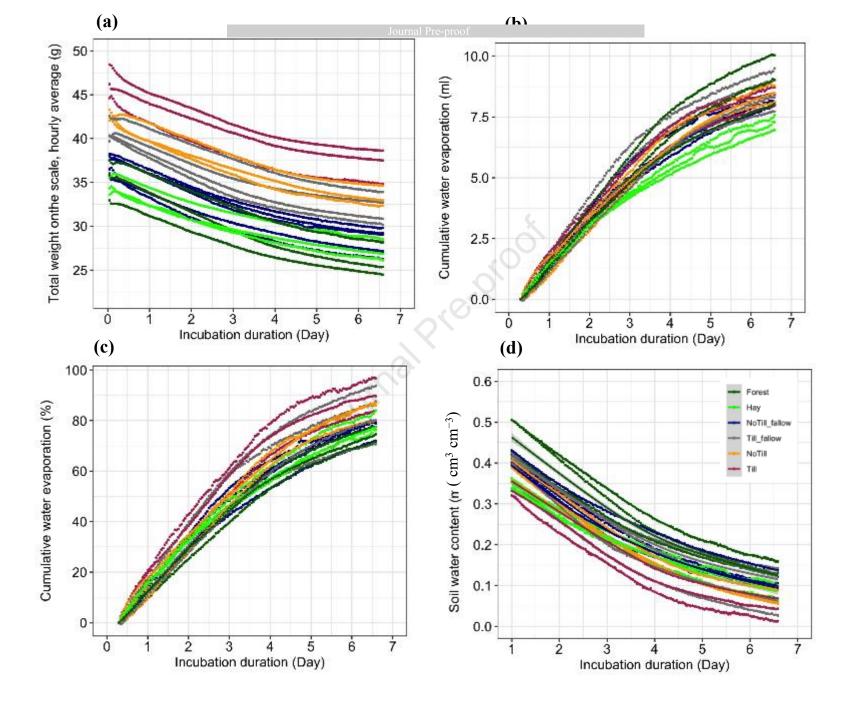
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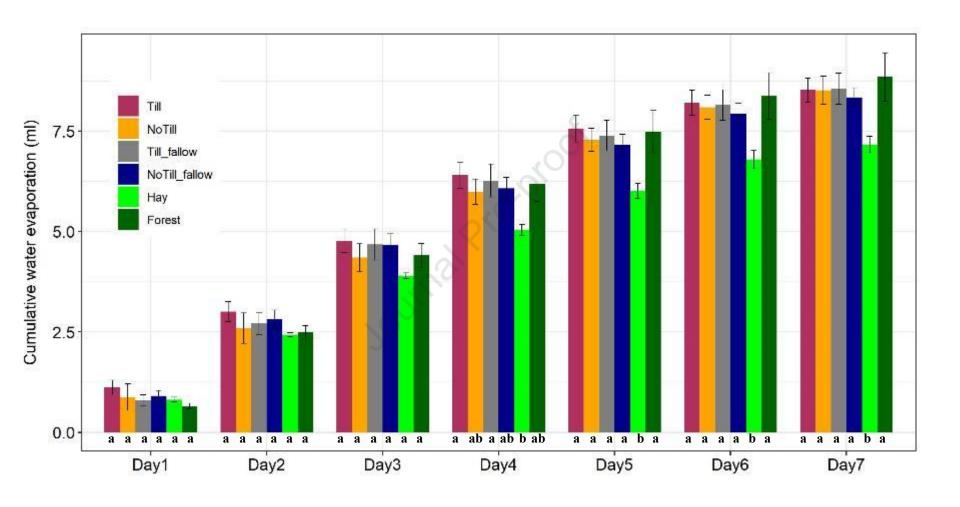


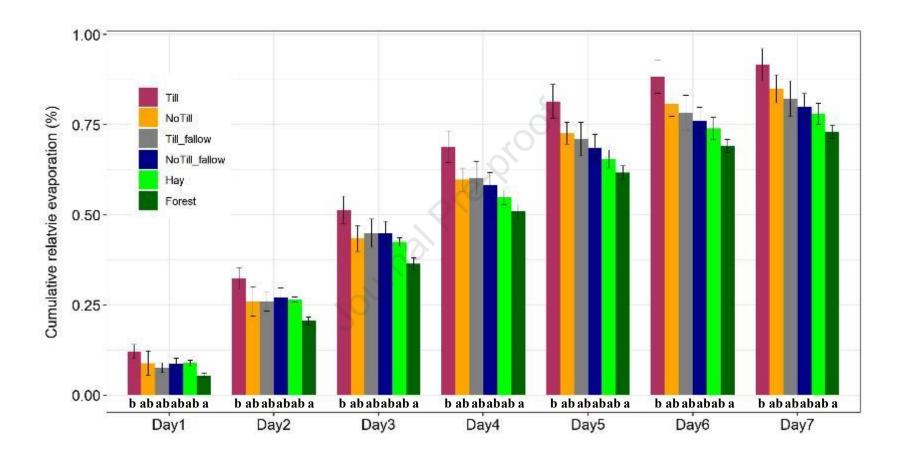


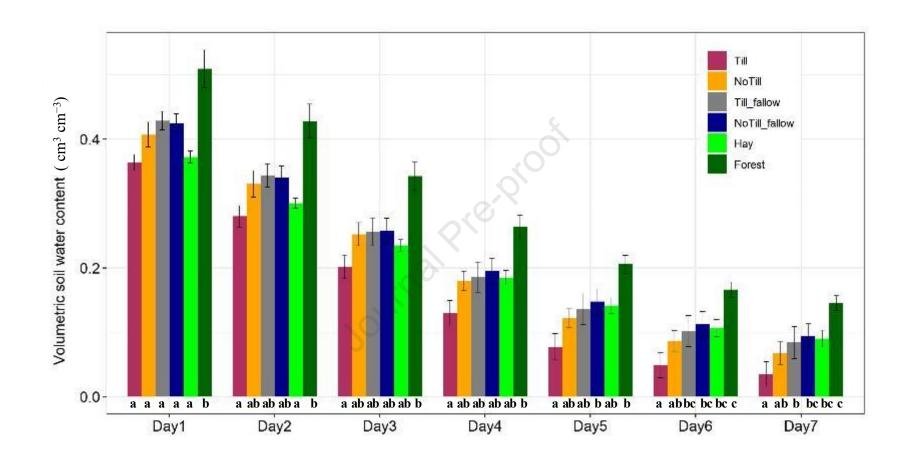


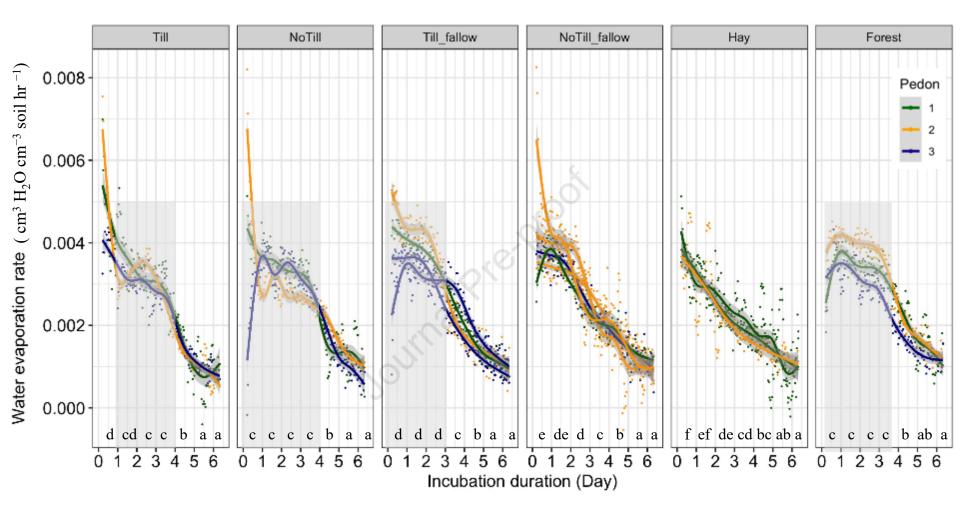


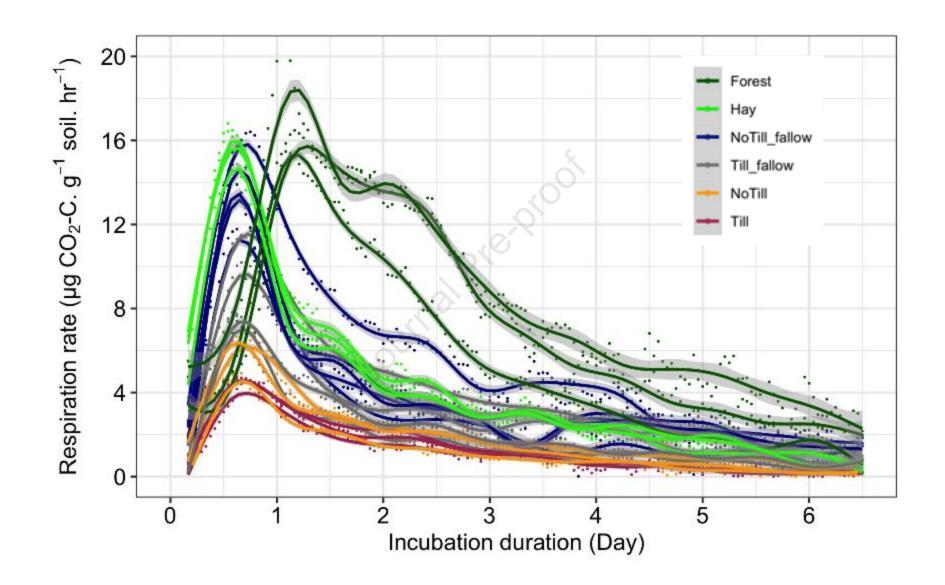


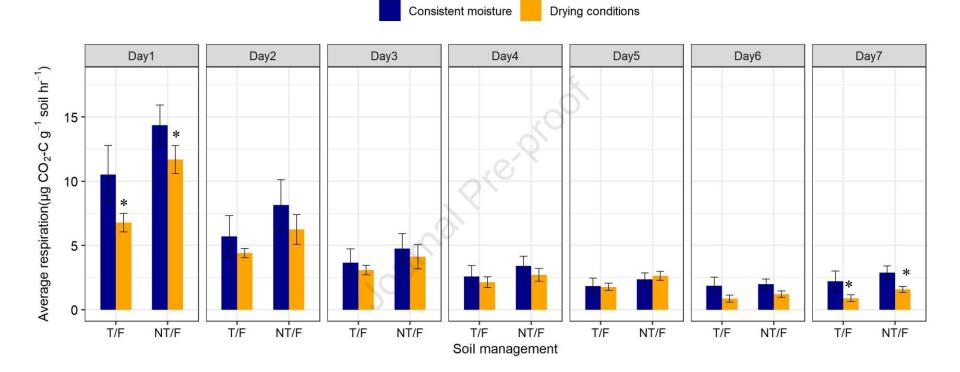












Highlights

- > Soil water retention and microbial respiration was quantified using a novel system
- > CO₂ sensors provided reliable measurements compared with the alkali trap method
- > Respiration was highest in forest soils and declined as management intensified
- ➤ Soils with prolonged drying had respiration peaked at 10–30 hr upon rewetting
- Rewetted soil from hay fields had lower evaporation than other soils when drying

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☑ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
\Box The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

John Reight Cook