

Soil Basal Respiration as a Method to Determine The Influence of Soil Management and Microbial Activity

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Abstract

Soil respiration represents the transfer of CO₂ from the soil to the atmosphere and is one of the largest terrestrial carbon fluxes after gross primary production. Because it is driven largely by the metabolic activity of soil organisms, it is widely recognized as a sensitive indicator of soil biological activity, carbon turnover, and the impacts of environmental or management-related disturbances. Soil respiration integrates multiple CO₂ sources, including autotrophic respiration from plant roots and rhizosphere microorganisms, and heterotrophic respiration associated with microbial decomposition of organic matter. When measured in the absence of external substrates or recent nutrient inputs, microbial respiration is referred to as soil basal respiration (SBR).

This article presents an infrared gas analyzer (IRGA) based protocol to quantify SBR under controlled laboratory conditions. To isolate the heterotrophic component attributable to microbial metabolism alone, plant-derived CO₂ fluxes are excluded by using preconditioned, sieved, and homogenized soil samples incubated under standardized moisture and temperature conditions, allowing quantification of cumulative C-CO₂ evolution over time, providing a robust proxy for microbial biomass, activity, and soil health. The protocol includes soil preparation, moisture adjustment, sealed-vial incubation, IRGA-based CO₂ measurement, and calculation of cumulative respiration.

In this study, we assessed SBR using an infrared gas analyser (IRGA), which allows monitoring of soil CO₂ dynamics under controlled conditions. Representative results showed that compost addition enhanced microbial respiration in arid soils from the Tabernas Desert, indicating a strong stimulation of microbial processes following organic amendment. These findings highlight the usefulness of SBR as an indicator of soil management effects on microbial activity, particularly in degraded arid environments. Methodologically, the IRGA-based protocol offers a practical tool for research and teaching applications related to soil carbon dynamics.

Introduction

Soil respiration is a central biogeochemical process that encompasses the flux of carbon dioxide (CO₂) from the soil surface to the atmosphere. It is estimated to be the second largest terrestrial carbon flux after gross primary production¹ and contributes significantly to the global carbon cycle². This process is key to carbon turnover in terrestrial ecosystems and is increasingly utilized as a sensitive indicator of soil biological activity and soil carbon sequestration dynamics^{3,4}.

Soil respiration is derived from multiple sources and is generally categorized into two main biological components: autotrophic respiration, originating from plant root metabolism and rhizosphere-associated microorganisms, and heterotrophic respiration, associated with the microbial decomposition of soil organic matter^{5,6}. Non-biological sources such as carbonate weathering⁷ and photodegradation⁸ may also contribute under specific conditions. To isolate the heterotrophic component attributable to microbial metabolism alone, plant-derived CO₂ fluxes must be excluded.

Within this context, microbial respiration is defined as the production of CO₂ or uptake of O₂ by microorganisms—including bacteria, fungi, protozoa, and algae—as a result of aerobic or anaerobic metabolic processes⁹. When measured in the absence of external substrates or recent nutrient inputs, microbial respiration is referred to as basal respiration¹⁰. Basal respiration reflects the endogenous metabolic activity of the native microbial community and is often employed as a proxy for microbial biomass, activity, and sensitivity to environmental or anthropogenic disturbances^{11,12}.

To accurately assess basal respiration, laboratory-based measurements are performed on preconditioned, sieved, and homogenized soil samples. This pre-treatment effectively removes roots and macrofauna, thus eliminating autotrophic respiration and enabling a focus on heterotrophic microbial activity. Although sieving alters the original soil structure, it improves sample homogeneity and reproducibility, and facilitates the study of microbially driven processes in isolation⁹. Incubation is typically conducted under controlled environmental conditions, such as 25 °C and 60% of the soil field capacity, to optimize microbial activity without favoring specific functional groups^{13,14}. In this context, soil moisture and field capacity are critical parameters because microbial respiration is highly sensitive to water availability, which regulates substrate diffusion, oxygen supply, and microbial metabolism. For this reason, basal respiration measurements are commonly performed under standardized moisture conditions expressed as a proportion of field capacity. The C-CO₂ evolved over an incubation period is quantified as a measure of basal respiration¹⁵.

This protocol is particularly suitable for controlled laboratory incubation studies aimed at assessing microbial activity through basal respiration in preconditioned soils. It is especially useful for comparing soils subjected to different management practices, such as organic amendment, or other disturbances, using homogenized samples under standardized temperature and moisture conditions. Because plant-derived CO₂ inputs are excluded, the method is most appropriate for evaluating the heterotrophic component of soil respiration rather than total soil respiration under field conditions.

Several analytical techniques have been developed for quantifying soil respiration, each one varying in sensitivity, throughput, and operational complexity^{9,16}. Classical approaches include alkali absorption followed by titration, conductimetric systems, gas chromatography, and infrared gas analysis. Alkali-based methods are robust and relatively inexpensive, but they provide limited temporal resolution and may be affected by incomplete CO₂ absorption or leakage. Conductimetric methods allow continuous measurements, although they may be influenced by temperature and ionic strength. Gas chromatography provides precise headspace CO₂ quantification and allows the simultaneous determination of other gases, but it requires specialized instrumentation and trained personnel, and throughput may be limited. By contrast, infrared gas analyzers (IRGA) allow rapid and repeated monitoring of CO₂ concentration under controlled incubation conditions, making them particularly useful for time-resolved measurements of microbial respiration. However, IRGA measurements also require appropriate calibration and careful control of incubation conditions to ensure reproducibility, and may be affected by signal drift, water vapour interference, and reduced reliability at high CO₂ concentrations. Recent developments have expanded the availability of infrared- and NDIR-based soil respiration systems, including lower-cost chamber-based and sensor-based platforms for laboratory and field applications^{24,25}. In closed-vial incubations, the headspace-to-soil ratio and the interval between measurements should be adjusted according to the expected respiration rate in order to avoid excessive CO₂ accumulation during incubation. Recent IRGA-based protocols have also been published to support standardized laboratory measurements of soil respiration under controlled incubation conditions^{26,27}, and standardized methodological guidance for soil respiration measurements has also been proposed in recent FAO protocol²⁸. In this broader context, basal respiration can also be considered one component of soil health assessment in long-term agricultural experiments. It reflects microbial metabolic activity and C mineralization, while indicators such as CO₂ flush, permanganate-oxidizable carbon, and soil organic carbon capture complementary aspects of soil functioning²⁹. In this study, we used an IRGA to quantify basal respiration rates from incubated soil samples. This method enables repeated monitoring of CO₂ efflux over the incubation period, facilitating the assessment of microbial

metabolic activity under defined moisture and thermal conditions. The following protocol describes the laboratory procedure used to measure basal soil respiration in soils subjected to different management conditions.

Protocol

1. Preparations

1. Sample collection:

1. Collect samples per soil horizon or between 0-10 cm in case of surficial studies.
NOTE: Use disinfected tools and gloves to avoid microbial contaminations.
2. Place samples in isothermal bags and transport to the laboratory in a refrigerated box.
3. If soil pre-processing can not be done immediately, keep samples at 4°C (preferably no longer than 48 hours).
4. Allow samples to equilibrate to room temperature for at least 2 h before processing and incubation.

2. Material preparation and soil pre-processing:

1. Disinfect laboratory materials by immersing them in 10% bleach or 70% ethanol for 30 min, then rinse thoroughly with distilled water and allow them to air-dry before use.

CAUTION: Use gloves, lab coat and glasses when operating with these chemicals.

2. Leave samples at room temperature for 2 hours for stabilization.
3. Sieve samples to 2 mm. Discard roots and coarse debris using tweezers.
4. Clean the sieve after each sample with the disinfection solutions and dry with a paper towel.

3. Determine soil moisture (amount of water present) and field capacity (water that can retain soil)¹⁷.

1. Soil moisture calculation: Determine soil moisture by oven-drying (105°C) 20 g of sample in a ceramic capsule during 24 hours¹⁸ [Eq 1].

$$\text{Soil moisture (\%)} = \frac{\text{weight fresh sample} - \text{weight 105°C dried sample}}{\text{weight 105°C dried sample}} \times 100$$

[Eq 1]

2. Determine field capacity using a "Richards" pressure-membrane apparatus¹⁹.

1. Place soil samples over the ceramic plates of the device delimited by plastic rings.

2. Add distilled water up to saturation and leave a minimum of 24 h. Later, apply a pressure of -0.33 bar (-33 kPa) for other 24 h. Then determine soil moisture again according to Equation 1.

4. Determine soil density (g·mL⁻¹). Fill a tared and known volume jar with the oven-dried soil sample and record the weight of the soil. The soil density is the ratio weight/volume of the jar.

5. Sample preparation for incubation:

1. Set the incubator temperature to 25 °C. The UNE-EN ISO 16072:2002 standard (Soil quality: Laboratory methods for determination of microbial respiration, <https://www.iso.org/standard/32096.html>) suggests that temperature for comparability between studies).
2. Estimate moisture conditions: Calculate the volume of distilled required for each sample to reach 60% of field capacity from its initial soil moisture content^{20,21}. This will be the moisture level at the start of the experiment and no water adjustment during the incubation period is needed.
3. Selection of soil amount: The amount of soil used may vary depending on its organic matter content (the higher the organic matter content, the less soil is needed for the experiment)²¹. However, 20 g is suggested as a reference quantity.
4. Place samples in 100 mL glass vials and add distilled water up to the 60% field capacity moisture percentage. Seal the vials with a septum and place them in the incubator.

2. CO₂ concentration measurements

1. Interval of measurement:

1. Perform CO₂ measurements at selected intervals according to the expected respiration rate of the soil in order to avoid the excessive CO₂ accumulation in the vial headspace.
2. Avoid concentrations higher than 10% (100 000 ppm) to prevent inhibition of microbial activity. Shorten the measurement intervals if concentrations exceed this threshold. Perform measurements daily during the first week and every 3 or 4 days thereafter in the representative example shown later.

2. Measurements of CO₂ concentrations (%) with an Infra-Red Headspace Gas Analyzer (IRGA)

1. Calibrate the IRGA before each measurement session according to the manufacturer instructions or using a zero standard (CO₂-free air or nitrogen) and at least

one span standard of known CO₂ concentration within the measurement range. Verify instrument stability and correct for baseline drift according to the manufacturer's instructions. Record calibration parameters prior to sample measurements.

2. In the IRGA device described in the material list, connect a needle with one capillar to the instrument..

3. Insert the needle through the septum into the vial to extract an aliquot of gas and record the CO₂ concentration (%).

4. Perform repeated readings per sample until the signal stabilizes.

3. Sample reconditioning

1. Leave the vials open in a clean area protected from dust and air flows (preferably in a laminar flow chamber) for half an hour after each measurement to renew the air and prevent anaerobic conditions. Do not mix or agitate the soil during this aeration step.

2. Reseal the vials using the septum and return them to the incubation chamber.

4. CO₂ correction

1. Add a vial without soil but with the same operation conditions to account for a blank.

2. Subtract the values of the blank to the samples to remove the enviromental CO₂ conditions.

NOTE: Be careful of exhaling close to the opened vials. This can bias the results.

3. Calculation of soil basal respiration (SBR)

1. Determine the headspace volume in the vial (V_{hs}) by subtracting the volume occupied by soil (bulk density) and added water from the total vial volume [Eq 2].
2. Estimate soil volume using bulk density measurements and calculate water volume from added moisture. Use this corrected headspace volume in all subsequent calculations.
3. Determination of the headspace volume in the vial (V_{hs}): It refers to the volume of the vial minus the volume of the soil and water content [Eq 2].

$$V_{hs} (mL) = 100 mL (vial) - \frac{20 g \text{ soil}}{\text{Soil density } (g \cdot mL^{-1})} - H_2O (mL) \text{ at field capacity}$$

[Eq 2]

4. Estimation of CO₂ volume in the vial

1. Obtain CO₂ volume in the vial from the IRGA reads of CO₂ concentration (%), considering the effective headsapce of the volume of the vial [Eq 3].

$$V_{CO_2} (Litres) = V_{hs} (mL) \cdot \left(\frac{L}{1000mL} \right) \times \frac{[\%CO_2]}{100} = V_{hs} \cdot [\%CO_2] \cdot 10^{-5} \text{ [Eq 3]}$$

5. Estimation of CO₂ moles exhaled from the soil samples:

1. Obtain CO₂ moles exhaled from the soil samples by solving for n in the ideal gas law equation ($P \cdot V = n \cdot R \cdot T$) [Eq 4], after replacing the previously calculated V in [Eq 3].

$$n (CO_2 \text{ mols}) = \frac{P \cdot V}{R \cdot T} = \frac{P \cdot V_{hs} \cdot [\%CO_2]}{100000 \cdot R \cdot T} \text{ [Eq 4]}$$

6. Estimation of CO₂ flux (mg C-CO₂ kg⁻¹ dry soil day⁻¹)

1. Individual measurements of %CO₂ must be added and the final CO₂ flux is estimated using [Eq 5] with the following considerations: i) correct sample weight considering soil moisture; ii) consider that one mole of CO₂ accounts for 12 g of C (12000 mg C).

$$mg C - CO_2 \cdot kg^{-1} \text{ dry soil} \cdot day^{-1} = \frac{12000 (mg C - mol CO_2) \cdot P \cdot V_{hs} \cdot [\%CO_2]}{100000 \cdot R \cdot T \cdot \text{dry soil}(kg) \times t(days)} = \frac{P \cdot V_{hs} \cdot [\%CO_2]}{12 \cdot R \cdot T \cdot \text{dry soil}(kg) \times t(days)} \text{ [Eq 5]}$$

where:

P = gas pressure inside the vial. This should be equivalent to atmospheric (atm). Correct to you location values.

V_{hs} = gas headspace volume in the vial (L)

[% CO₂] = CO₂ concentration measured with the IRGA

R = gas constant = 0.082 L·atm·K⁻¹·mol⁻¹

T = Incubation temperature in Kelvin

dry soil = dry soil mass in kg

t = incubation time in days

A summary workflow of the protocol is shown in **Figure 1**.

IRGA-based soil basal respiration workflow

Schematic overview of the methodological pipeline used for SBR assessment

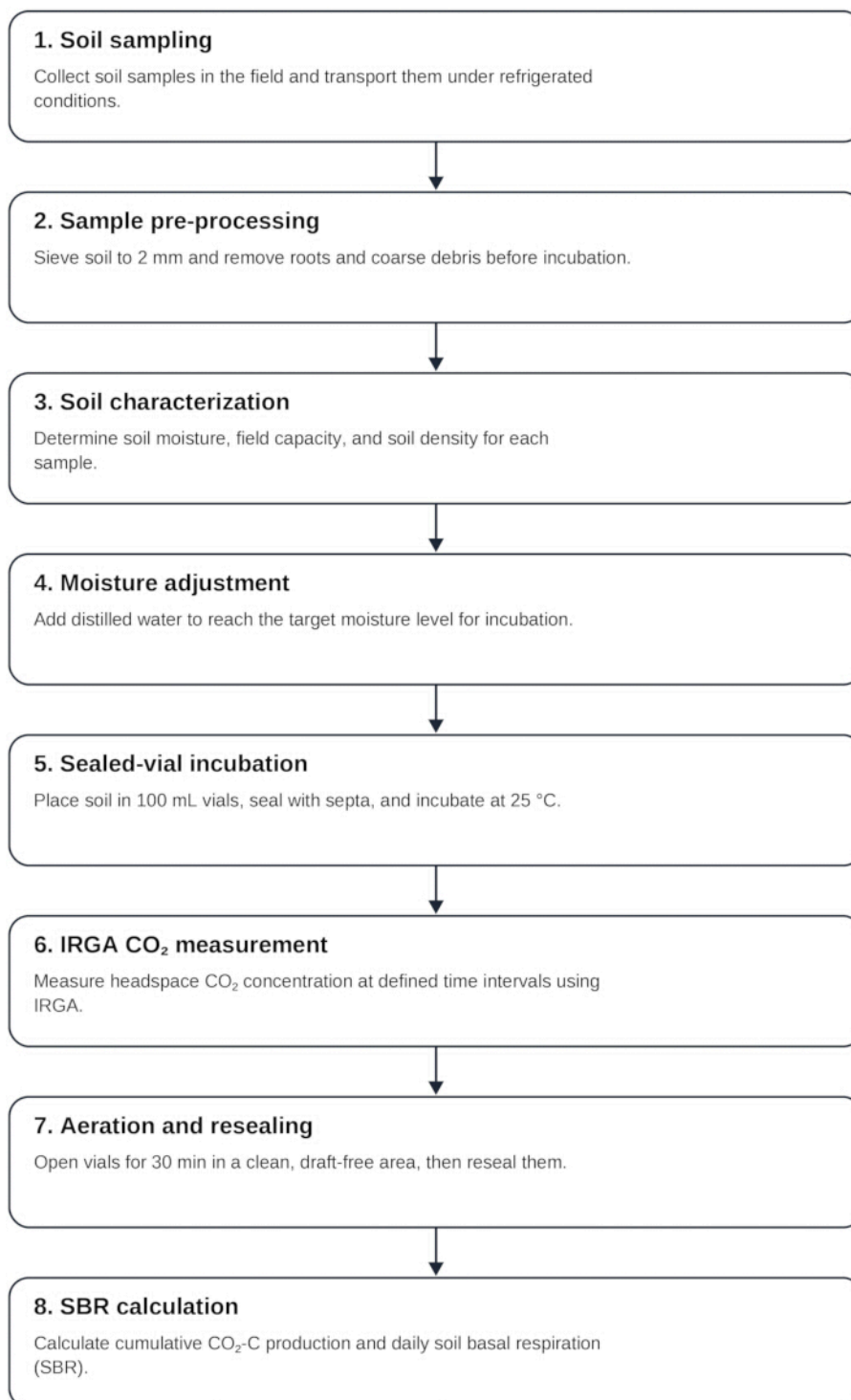


Figure 1 Workflow of the IRGA-based soil basal respiration protocol, from soil sampling and preconditioning to incubation, IRGA measurement, and calculations. [Please click here to view a larger version of this figure.](#)

Results

The results showed in this article correspond to one experiment where low fertile soils were amended with compost to increase the fertility parameters and microbiological activity. The original soils were surveyed at the “Tabernas desert” (Southeastern Spain), a region with a semiarid climate and poor developed soils²². Approximately, 40 kg of the upper 30 cm of one agricultural field

were sampled, taken to the laboratory and 2 mm sieved to obtain the fine earth fraction. This was distributed in 6 pots (10 L volume) and three of them were amended on a 10% dose (w:w) with compost derived from sewage sludge and crop residues.

Samples were periodically mixed and moistured (60% field capacity) to facilitate the integration of the compost in the soil. After one and three months of the start of the experiment, each pot was sampled. The soils main characteristics are shown in **Table 1**.

Samples	pH (1:2.5)	EC (1:5)	SOC	Available P	Field capacity (%)
		(μS/cm)	(%)	(mg P Kg soil ⁻¹)	
T-control 1 month	7.89 ± 0.14	180.83 ± 69.6	0.56 ± 0.03	4.43 ± 0.51	5.00 ± 0.25
T-control 3 months	7.80 ± 0.04	246 ± 78.82	0.53 ± 0.02	6.35 ± 0.38	5.02 ± 0.24
T+ compost 1 month	6.96 ± 0.04	2506.67 ± 17.56	2.17 ± 0.08	128.8 ± 3.19	8.83 ± 0.33
T+compost 3 months	7.16 ± 0.03	2976.67 ± 70.24	2.25 ± 0.03	150.29 ± 6.52	8.79 ± 0.30

Table 1: Physicochemical characteristics of soils used in the soil basal respiration experiment. Physicochemical properties of non-amended Tabernas Desert soil (T-control) and 10% compost-amended soil (T+compost) measured at 1 month and 3 months after treatment application. Parameters include pH, electrical conductivity (EC), soil organic carbon (SOC), available phosphorus (P), and field capacity. Values are presented as mean ± SEM (n = 3).

For SBR estimation, 20 g of soil were collected and placed in 100 mL glass vials where each sample was incubated under constant temperature (25 °C) and moisture conditions (60% field capacity) during 31 days. % CO₂ in the vials was periodically monitored and calculations were done to estimate cumulative emissions (mg C–CO₂ kg⁻¹ soil) and daily respiration rates (mg C–CO₂ kg⁻¹ soil day⁻¹). Cumulative soil respiration (**Figure 2**) increased during the 31-day incubation period in both soil types, treated with compost (T

+compost) and without organic amendment (T- control), but with particular emphasis in the first days of T+compost (1 month). Composted soils (T+compost) showed the highest accumulated CO₂ values, but with differences between dates. T compost (1 month) reached maximum values over 450 mg C–CO₂ kg⁻¹ (**Figure 2.A**), and T compost (3 months) around 272 mg C–CO₂ kg⁻¹ (**Figure 2.B**). On the contrary, control soils did not exceeded the value of 100 mg C–CO₂ kg⁻¹.

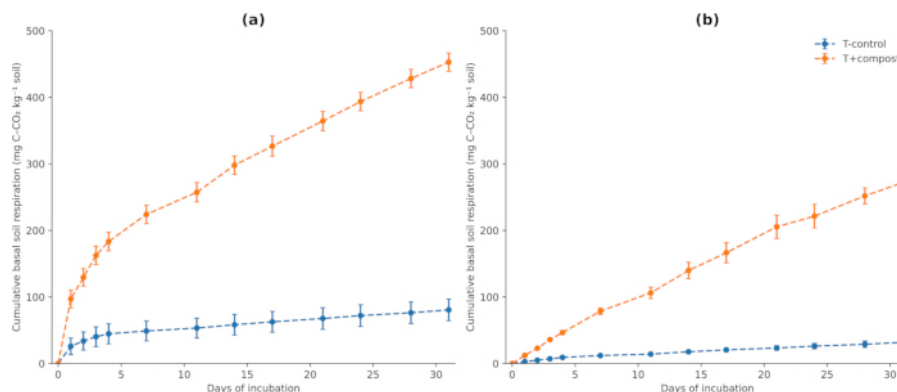


Figure 2. Cumulative soil basal respiration (SBR; mg C-CO₂ kg⁻¹) in non-amended Tabernas desert soil (T-control) and 10% compost amended (T +compost). (A) shows soils sampled 1 month after the start of the pot experiment, and panel (B) shows soils sampled 3 months after the start of the experiment. Points and whiskers shows means $\pm$ SEM (Standard Error of the Mean) values (replicates n = 3). [Please click here to view a larger version of this figure.](#)

The curves shown in **Figure 2** depicts how the methodology proposed clearly reports the changes in soil management with differences in and between 1-m and 3-m soil surveys. In the 1-m T + compost samples can be seen the “priming effect” due to the recent organic amendment addition. This result in a steep curve during the first days, which later turns into a less steep line. In T-control samples this effect did not happen. Besides, in 3-m T +compost samples the priming effect was reduced, but the effect of the amendment was obviously stated with a steeper curve than

in non-amended soils. It is important to check the curves reflect the experimental design to validate the experiment results.

Daily respiration rates provided more information on the overall dynamics (**Figure 3**). Composted soils showed significantly higher values (p -value < 0.01) compared with control soils, with differences also found between the sample dates. Microbial soil respiration was significantly higher after 1 month of sampling than after 3 months in both type of samples (T control and T + compost).

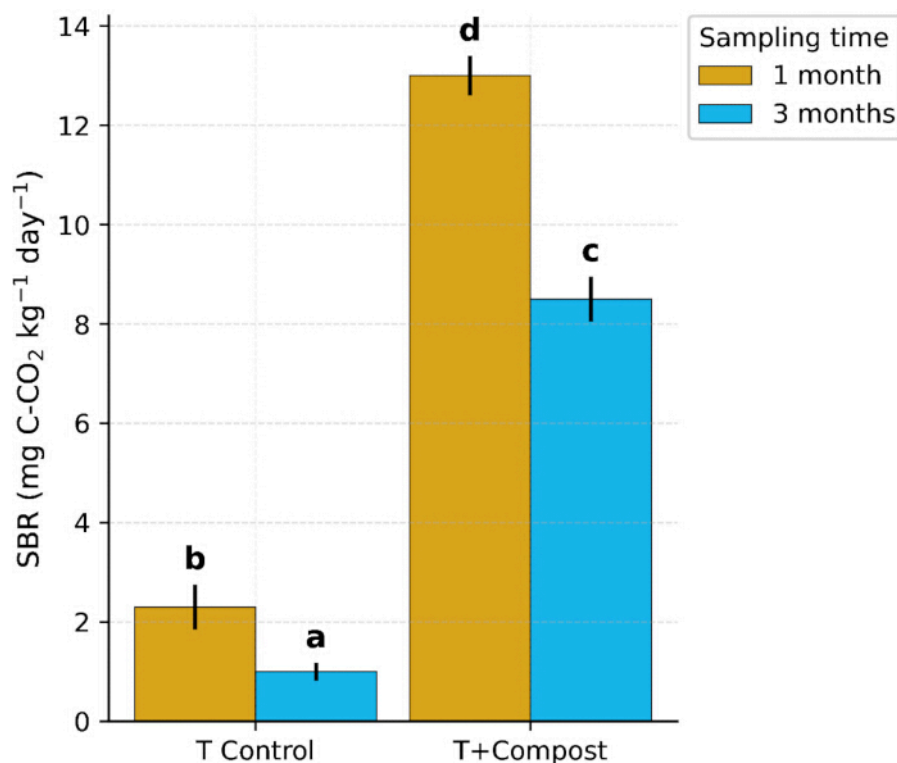


Figure 3. Daily soil basal respiration rates in control and compost-amended soils. Daily soil basal respiration (SBR) rates measured in non-amended Tabernas Desert soil (T Control) and soil amended with 10% compost (T+Compost) after 1 month and 3 months of incubation. Values are expressed

as mg C-CO₂ kg⁻¹ dry soil day⁻¹. Bars represent mean ± SEM (n = 3). Different letters indicate statistically significant differences among treatments and sampling times (one-way ANOVA, p < 0.01). [Please click here to view a larger version of this figure.](#)

The results show that adding compost effectively improved microbial respiration in the arid soils of the Tabernas Desert. These findings highlight the strong stimulation of soil microbial processes following organic amendment and provide an accessible model for understanding carbon dynamics, microbial functioning, and the role of compost in improving soil quality and fertility in degraded arid environments.

Discussion

Soil respiration is a sensitive indicator of microbial activity and organic matter decomposition, providing noteworthy information on the effects of soil management^{23,24}. The experimental approach described in this article provides a simple and powerful framework to quantify soil microbial activity through CO₂ emission measurements under controlled laboratory conditions²⁵, and it was based and updated from García et al. (2003)²¹. The incubation setup with repeated measurements make it possible to analyse cumulative and daily CO₂ fluxes, which provide valuable insights into the temporal dynamics of soil respiration and the functionality of the ecosystem^{29,3}.

The experiment described in the previous section evaluated the microbiological response of arid soils to an increase in organic matter through the addition of compost. The results clearly showed that organic matter enhanced the respiration dynamics of the soil, increasing both cumulative and daily CO₂ emissions. Amended arid soils (T+compost) exhibited markedly higher cumulative CO₂ emissions compared with the non-amended control, consistent with previous observations on the use of organic amendments in soils^{24,31,32}. The composition of organic matter has important implications for the microbial response. Labile carbon compounds provide easily degradable substrates that are immediately available for microbial metabolism²⁴, which can explain the initial notable increase in respiration shown in **Figure 2**. At the end of the experiment, the curves became less steep, which could suggest a shift in the availability of easily decomposable organic substrates to more recalcitrant ones^{33,34}. This temporal evolution of respiration, with an initial pulse linked to labile carbon decomposition ("priming effect") followed by stabilization as microbial activity adapted to more recalcitrant substrates, is typical after the addition of compost based organic amendments³³. The previous is also supported by the daily respiration rates, where significant differences were found due to both factors, organic matter addition and time since the addition (**Figure 3**). Therefore, the results suggest that organic amendments can quickly stimulate microbial activity in semiarid Mediterranean soils, leading to improved soil biological functioning and soil fertility. However, increased soil respiration

should not be interpreted exclusively as a positive response to soil management. Although higher CO₂ fluxes may reflect enhanced microbial activity and substrate availability, they may also indicate accelerated carbon loss from the soil, which may be undesirable when long-term carbon sequestration is a management goal. In addition, compost inputs may induce priming effects, stimulating not only the mineralization of the added organic material but also that of native soil organic matter³⁵.

Beyond the ecological interpretation, this experiment has a strong pedagogical value. It enables students and scientifics to connect microbial metabolic processes with broader ecological concepts such as ecosystem resilience, carbon cycling, and soil recovery. By visually comparing respiration curves across treatments, users could directly learn how soil management practices influence biological activity and environmental sustainability. This visualization reinforces theoretical knowledge of soil ecology through empirical data and strengthens the link between experimental work and environmental management.

In terms of methodology, the infrared gas analysis (IRGA) technique used in this study, provides a practical approach for monitoring CO₂ dynamics under controlled conditions offers a high precision, temporal resolution, stability, and reproducibility^{3,29}. Compared with classical methods, it allows repeated measurements over time and facilitates the analysis of cumulative and daily respiration patterns. Several protocol steps are critical for obtaining reproducible basal soil respiration measurements, including sample homogenization, accurate moisture adjustment relative to field capacity, standardized incubation temperature, and consistent timing of CO₂ measurements. Reliable IRGA readings also depend on appropriate calibration, stable signal acquisition, and avoidance of excessive CO₂ accumulation in the vial headspace.

A further methodological limitation is that the vials were opened for 30 min after each measurement to renew the headspace air and prevent anaerobic conditions. This step may introduce minor airborne contamination and repeated shifts in CO₂ concentrations, so the procedure should be interpreted as a series of closed incubation intervals with periodic reconditioning rather than as a continuous sealed incubation. A less disruptive alternative would be to extract only a small headspace aliquot without fully opening the vial; however, in the present protocol, opening the vials was considered necessary to restore aerobic conditions between measurements. Despite these limitations, the method remains useful for comparative assessment of treatment effects under standardized laboratory conditions and for future applications in soil management, organic amendment studies, and teaching contexts.

Disclosures

The authors have no conflicts of interest to disclose.

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