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Coping with artifacts induced by $CaCO_3$–CO_2–H_2O equilibria in substrate utilization profiling of calcareous soils

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ABSTRACT

This study was undertaken to cope with artifacts derived from $CaCO_3$–CO_2–H_2O equilibria that occur in substrate-induced respiration (SIR) measurements in calcareous soils. Considering that partitioning of respired CO_2 between headspace and soil solution becomes increasingly important above a pH value of 6 and when headspace-to-solution ratios are low, an underestimation in respiration measurements are to be expected under such conditions.

In order to evaluate the relative magnitude of this bias in respiration measurements under a wide range of soil conditions, we investigated soils from four sites along a climatic gradient in Israel. Even though all soils were formed over calcareous bedrock, their $CaCO_3$ contents increased with increasing climate aridity.

We calculated the theoretical distribution of CO_2 between the gaseous and solution phases for each soil, based on the equilibrium reactions between CO_2, water, and the various carbonate species. Theoretically calculated factors required for the correction of biased CO_2 evolution data were compared to those obtained by actually measuring CO_2 retention in the soils, and were found to be smaller, although reasonably close to them. CO_2 retention levels determined by experiment were substantial and followed the order: Mediterranean (74% of initial headspace CO_2) $>$ semi-arid (70%) $>$ humid-Mediterranean (64%) $>$ arid soil (58%).

An additional potential artifact in substrate utilization profiling was addressed when soil respiration was found to be overestimated when induced by considerably acidic substrates such as carboxylic acids. This overestimation results from soil pH lowering which reduces the CO_2 retention potential. Hence, if CO_2 retention correction factors are applied to carboxylic acid-induced respiration measurements, they must be reduced according to any pH decline. Moreover, $CaCO_3$ dissolution may follow pH decline, and be accompanied by abiotic CO_2 evolution that may be mistakenly regarded as respired CO_2.

We suggest taking the following sequence of measures when generating substrate utilization profiles for calcareous soils: (1) experimental determination of CO_2 retention levels and retention correction factors; (2) comparison with theoretical calculations; (3) assessment of pH changes induced by substrates and modification of retention factors necessitated by these changes; (4) measurement of abiotic substrate-induced CO_2 evolution rates and their subtraction from measured SIR data; and (5) multiplication of biotic SIR data by CO_2 retention correction factors.

The comparative analysis of utilization profiles obtained from corrected SIR data with those obtained from the originally measured CO_2 evolution rates emphasized the immense underestimation of non-acidic SIRs, concurrent with an overestimation of SIRs obtained for appreciably acidic substrates such as carboxylic acids, in ordinary SIR assays.

1. Introduction

Sole-carbon-source utilization tests have been widely used since the 1990s for assessing soil microbial functional profiles at the community level (Garland and Mills, 1991). The technique behind this method is substrate-induced respiration (SIR) (Anderson and Domsch, 1975). CO_2 evolved from the soil is measured and considered to be microbially respired. CO_2 evolution rate is further used for the estimation of soil microbial biomass (Anderson and Domsch, 1978).

In practice, however, a fraction of the respired CO_2 avoids detection by the measuring device since CO_2 is partitioned

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between the headspace of the container used for soil incubation, and soil solution, according to the following aqueous carbonate equilibrium reactions:

(1) $CO_2\ (gas) + H_2O \leftrightarrow H_2CO_3^{\circ}$
(2) $H_2CO_3^{\circ} \leftrightarrow H^+ + HCO_3^-$
(3) $HCO_3^- \leftrightarrow H^+ + CO_3^{2-}$ (Lindsay, 1979).

Since the amount of CO_3^{2-} formed is negligible at the pH prevailing in most soils (<8), the main controlling equilibrium is the amount of HCO_3^- that is formed, which increases with pH, as emerges from the Henderson–Hasselbach equation:

(4) $pH = pK + \log[(HCO_3^-)/(CO_2)]$

Sparling and West (1990) calculated the theoretical distribution of CO_2 between the gaseous and solution phases in SIR assays, under varying headspace-to-solution volume ratios, and under an artificially produced range of soil pH values. Their results emphasized the potential for substantial retention of CO_2 in the solution phase, especially with low headspace-to-solution ratios and pH values greater than 6. In such situations, correction factors need to be applied to the CO_2 measurements in order to avoid underestimation of respiration. This would be of particular importance for calcareous soils, which encompass a pH range of 7.5–8.5 (Chen and Barak, 1982). Although such soils are generally more common in arid and semi-arid regions (Stevenson and Verburg, 2006), they still cover more than 30% of the Earth's land surface (Chen and Barak, 1982).

Another possible source of deviation from reliable assessment of microbial respiration in carbon-source utilization tests is the regular inclusion of organic acid substrates. A sufficiently acidic substrate might lower soil pH and, in fact, reduce the factor required to compensate for CO_2 retention. If the factor is not reduced accordingly, an overestimation would ensue for the microbial respiration of this substrate in comparison with pH-neutral substrates.

In calcareous soils, however, such an acid-induced pH decline would be significantly lessened due to calcium carbonate dissolution and the formation of CO_3^{2-}:

(5) $CaCO_3 \leftrightarrow Ca^{2+} + CO_3^{2-}$

CO_3^{2-} can react with H^+ ions to form HCO_3^- and $H_2CO_3^{\circ}$, with consequential abiotic CO_2 evolution. This evolution can also be depicted in the overall reaction portraying the dissolution of $CaCO_3$ in soil, obtained by adding up the equilibrium equations of the CO_2–H_2O system (1–3) and the calcium carbonate system (5):

(6) $CaCO_3 + 2H^+ \leftrightarrow Ca^{2+} + H_2O + CO_2\ (gas)$

In order to obtain reliable substrate utilization profiles for microbial communities in calcareous soils, it is, therefore, essential to address the above-mentioned possible artifacts, and find ways to compensate for them. This requires the determination of CO_2 retention in unamended, as well as substrate-amended soil, in order to make up for the underestimated respiration. On the other hand, it requires the determination of abiotic substrate-induced CO_2 evolution rates, which otherwise would be erroneously regarded as biotic, bringing about an overestimation of microbial respiration.

In this study, a series of calcareous soils sampled in different climatic regions, and which were highly variable in their physical and chemical properties, were assessed for their potential to retain CO_2 in soil solution in the course of SIR assays. In addition, they were assessed for the effects of application of carbon substrates varying in acidity level on CO_2 retention and abiotic CO_2 evolution. After that, it became possible to establish the correction factors needed for determination of the microbial respiration rate by the use of theoretical, in conjunction with experimentally-based, calculations.

2. Materials and methods

2.1. Sampling sites

Soil sampling was conducted at the end of November 2007 at four sites, located along a 245-km-long climatic gradient stretching the length of Israel from north to south. Environmental conditions at these sites represent humid-Mediterranean, Mediterranean, semi-arid, and arid climate types. The basic climate in the region is Mediterranean, characterized by rainy winters (October–April) and prolonged rainless summers. The plant growing season commences soon after the first rains, between October and December.

Soil (0–10 cm) was randomly sampled in quadruples from the open spaces between perennial plants. Samples were taken from south-facing slopes, overlying calcareous bedrock. Upon arrival at the laboratory, soil samples were sieved (<2 mm) and kept at room temperature (~15 °C) until use. All analyses were completed within 2 weeks of sampling.

The humid-Mediterranean site is located near Ein Ya'aqov (N 33°0′ E 35°14′) in the northern Galilee Mountains, at an elevation of 500 m above sea level (a.s.l.), on montmorillonitic Terra Rossa. Average annual rainfall at this site amounts to 780 mm, and the mean annual temperature is 18.1 °C. The vegetation varies from closed oak maquis to more open garrigue dominated by shrubs. Herbaceous vegetation, mainly composed of annuals, coexists with shrubs.

The Mediterranean site is located 18 km south-west of Jerusalem (N 31°42′ E 35°3′) at 620 m a.s.l., on Terra Rossa. Average annual rainfall is 537 mm, and the mean annual temperature is 17 °C. The vegetation is dominated by shrubs and large numbers of herbaceous (mostly annual) plant species.

The semi-arid site is located near Lahav, between the southern Judean Mountains and the northern Negev (N 31°23′ E 34°54′) at 590 m a.s.l., on light brown Rendzina. Average annual rainfall is 300 mm, and the mean annual temperature is 18.4 °C. The vegetation is dominated by dwarf shrubs associated with herbaceous (chiefly annual) plants.

The arid site is situated on the Negev plateau (N 30°52′ E 34°46′) near Sde Boker, at an elevation of 470 m a.s.l., on desert Lithosol. Average annual rainfall is 90 mm, and the mean annual temperature is 19.1 °C. The open vegetation of this site is dominated by small shrubs and sparsely growing desert annuals and geophytes (Fleischer and Sternberg, 2006).

2.2. Laboratory analysis

Soil moisture (SM) content was determined gravimetrically by drying soil samples for 24 h at 105 °C (Black, 1965). For the determination of soil water-holding capacity (WHC), 100-g soil samples were flooded with tap water in a bottom-perforated vessel for 5 min. The WHC was inferred from the amount of residual water remaining following draining of gravitational water.

Total organic carbon (TOC) content was determined by oxidation with 1 N potassium dichromate in acidic medium, according to Rowell (1994).

pH was determined with a combined pH electrode in the filtered supernatant of a mixture of 20 g soil and 40 ml distilled H_2O following shaking for 10 min (160 rpm).

Particle size analysis and associated texture of the investigated soils were obtained from Ravikovitch, 1981, and their bulk densities