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Relation between substrate-induced respiration and heat loss from soil samples treated with various contaminants ☆

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Abstract

Heat loss and respiration following amendment with glucose were recorded for soils contaminated with heavy metals and organic pesticides over a period up to 28 days. For this purpose, measuring ampoules were adapted to enable measurement of heat loss and CO_2 evolution on the same soil sample and at the same time. Heat loss and CO_2 evolution were highly correlated ($r^2 = 0.83$), which is explained by the fact that in our studies heat loss from soils could be almost entirely attributed to metabolic reactions transforming glucose into CO_2. These results demonstrate that the described adapted ampoules can be useful instruments to monitor microbial activity both accurately and economically by heat loss and CO_2 production on the same soil samples during decomposition of various C substrates.

Keywords: Heavy metal; Pesticide; Respiration; Soil

1. Introduction

Terrestrial ecotoxicity tests are still in their infancy compared with their aquatic analogues. Among others, one reason certainly is the lack of understanding on how toxicants affect soil biological activity and how such effects can be measured.

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Soil metabolic processes of relevance to toxicity measurements in ecological studies include respiration, carbon and nitrogen cycling (including mineralization and immobilization), adenosine triphosphate (ATP) measurement, soil microbial biomass estimations, and enzymatic activity measurements. The rationale for this is that soil microorganisms form a cornerstone in many nutrient cycles. The metabolic processes selected for toxicity testing depend on the specific characteristics of each system and the type of information needed. A simple method cannot be expected to meet all requirements. Most research programmes on toxicity testing therefore combine a number of tests to reveal information on potential toxicity.

Microcalorimetric techniques, however, are providing both an alternative and a supplementary means of studying the effect of toxic substances on soil microorganisms [1]. Microcalorimetry can detect the varying specific rates of heat output or heat changes (dQ/dt per unit biomass) which accompany all biological activities and reflect the total metabolic activity of a microbial community. A preliminary study of the effects of carbofuran and monocrotophos on heat output and on carbon and nitrogen mineralization in a Northern Thailand soil showed that microcalorimetry is the most sensitive tool to monitor the changes in metabolic processes, energy transformations and the kinetics of biological processes in soil microorganisms [2]. The sensitivity of heat output measurements is typically in the microwatt (μW) range. However, in order to obtain a more complete understanding of the measured signals, heat output still needs to be compared with other tests at this stage.

Current methods for measuring CO_2 evolution from soils usually require relatively large soil samples. This contrasts with the small sample sizes necessary for microcalorimetry, and may lead to large amounts of toxic waste. It thus seems mandatory to develop a micro-technique that could combine measurement of both heat output and CO_2 evolution on one and the same small soil sample, thereby using low cost supplies to allow for adequate replication.

Heilmann and Beese [3], Sparling [4] and Gustafsson and Gustafsson [5] reported highly significant correlations between heat production and respiration rates. Using such relationships, the development of accurate and economical tools for combined measurement of heat output and CO_2 evolution on the same, small soil sample seems a logical next step.

Since 1980, many methods have been proposed for developing a miniaturized method for CO_2 evolution measurement by direct incubation in disposable plastic syringes, but none was completely satisfactory [3,6]. In order to make available a technique allowing soil microbial activities to be monitored by heat loss and CO_2 evolution on a large number of soil samples, an economical micro-technique was developed and tested in this paper.

2. Materials and methods

2.1. Soils

An uncontaminated soil from Chiangmai (Northern Thailand) was used. After sampling, the soil was passed through a 2-mm sieve and air dried at ambient temperature. The soil analysis data are shown in Table 1.

Table 1
Selected characteristics of soil from Chiangmai, Thailand

pH_{H_2O} (1 : 1)	5.71	$K_{exch.}$/[mg (kg soil)$^{-1}$]	258
Organic matter/%	2.82	$Na_{exch.}$/[mg (kg soil)$^{-1}$]	2
N/%	0.092	$Ca_{exch.}$/[mg (kg soil)$^{-1}$]	229
P_{avail}/[mg (kg soil)$^{-1}$], Bray II	3.8	$Mg_{exch.}$/[mg (kg soil)$^{-1}$]	82
P_{tot}/[mg (kg soil)$^{-1}$]	284	Soil texture	clay

2.2. Treatment with pesticide and heavy metal

The soil was preincubated at 40% water holding capacity (WHC) for 7 days at 25°C. The pesticide pentachlorophenol (PCP, 86%, Janssen Chimica) and the heavy metal salt mercury chloride ($HgCl_2$, 99.5%, Janssen Chimica) were used as examples and mixed into the soils at rates of 20 or 200 mg (kg dry soil)$^{-1}$. After adding the pesticide or heavy metal, the soils were adjusted to a moisture content up to 70% WHC. The batches of soil were put into jars and incubated at 25°C. At 1, 3, 7, 14, 21 and 28 days after treatment (DAT) with toxicants, the soils were treated with 0.1% of glucose. Of this batch, 1 g samples were weighed into the combined syringe–ampoules and were lowered into the microcalorimetry apparatus 30 min after the glucose treatment.

2.3. The tools

2.3.1. Adapted ampoule–syringe

The measuring ampoules were adapted to allow measurement of both heat output and CO_2 evolution (Fig. 1). A small glass ampoule (1.5 cm^3 clear vial, Alltech) was used to monitor heat output in a microcalorimeter from 1 g soil samples. This glass ampoule was covered by the rubber piston of a 5 cm^3 single-use syringe (Omnifix) and so inserted into such a 5 cm^3 single-use syringe (Plastipak) as to obtain a head space of 2 cm^3. The syringe was connected with a single-use needle (Terumo No. 17) and the tip of the needle was blocked with a small piece of a gas chromatograph septum.

The combined syringe–ampoule was lowered into the microcalorimeter and heat output was recorded for 48 h. After 24 and 48 h the adapted ampoules were taken out and the headspace atmosphere was analysed for CO_2 by gas chromatography (see below).

2.3.2. Heat output measurement

A four independent channel microcalorimeter (LKB 2277 Bio Activity Monitor, LKB, Bromma, Sweden) was used to monitor heat loss from the contaminated and the uncontaminated soil samples for periods up to 48 h. In each channel two positions are available, and the difference in heat loss between the two was registered. One position contains the adapted syringe–ampoule and the other

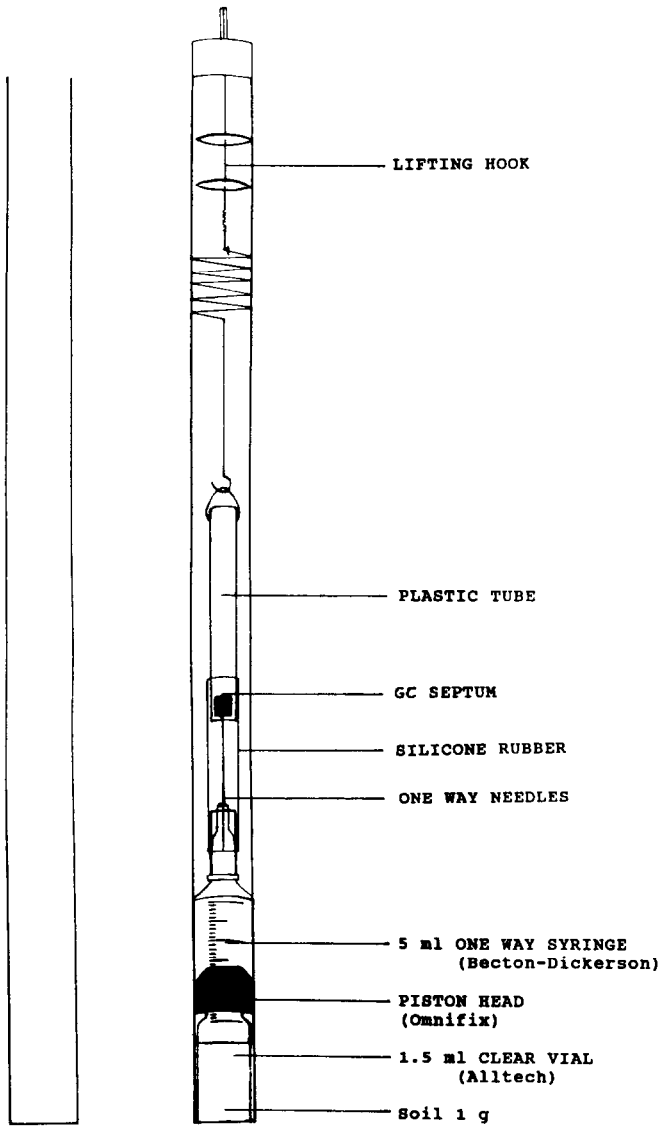

Fig. 1. Combined ampoule–syringe for simultaneously monitoring microbial activity by heat output and CO_2 production on one soil sample.

contains as a reference an ampoule filled with sterile sand at the same moisture content as the soil sample. The internal calibration was carried out in the range 0–100 μW. The detection limit was 1 μW and the baseline stability over 24 h was ± 1 μW. The microcalorimeter working temperature was 25°C. Before lowering into the measuring position, ampoules were equilibrated at this temperature for 30 min. The heat output was recorded from the beginning, but the results were used in

calculations only after a 1.5 h stabilization period. The data were recorded continuously for 48 h.

2.3.3. Soil respiration measurement

Soil gas in the 2 cm^3 headspace of the adapted ampoule–syringe was injected into a gas chromatograph (Hewlett-Packard 5800hp) equipped with a thermal capture detector (TCD). The gas chromatograph (GC) was run isothermally at 100°C and at an oven temperature of 150°C. The results were integrated and collected by using a Shimadzu CR3A (Kyoto, Japan). The samples were subjected to CO_2 measurement at 24 and 48 h from the start of the heat output measurement.

3. Results and discussion

The experiment confirmed that the combination of various parts of disposable syringes can give good results, and allows combined measurement of heat output data and CO_2 evolution data on the same soil sample of 1 g.

Comparing the ratio between heat output and CO_2 evolution in uncontaminated soil following glucose amendment, the results show that the maxima in heat output rate are correlated with the amounts of CO_2 evolved. The heat output rates show the highest maximum for the 1 DAT sample, and a decrease for the 3 DAT sample to a minimum for the 7 DAT sample. From then on, maxima of heat output again increase for the 14, 21 and 28 DAT samples in that order (Fig. 4). Comparing this trend with the amount of CO_2 evolved from the control soils over 48 h, results are similar at all stages (Fig. 2). A constant ratio between heat output and soil

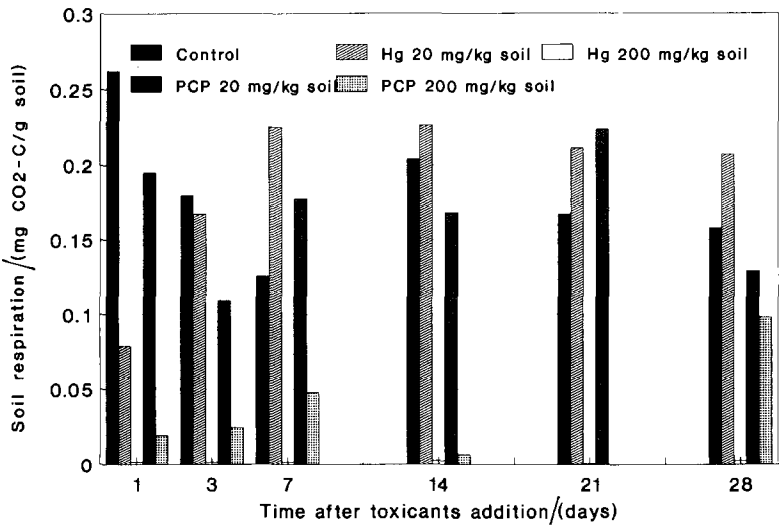

Fig. 2. CO_2 production from control and contaminated soil samples as measured with the combined ampoule–syringe.

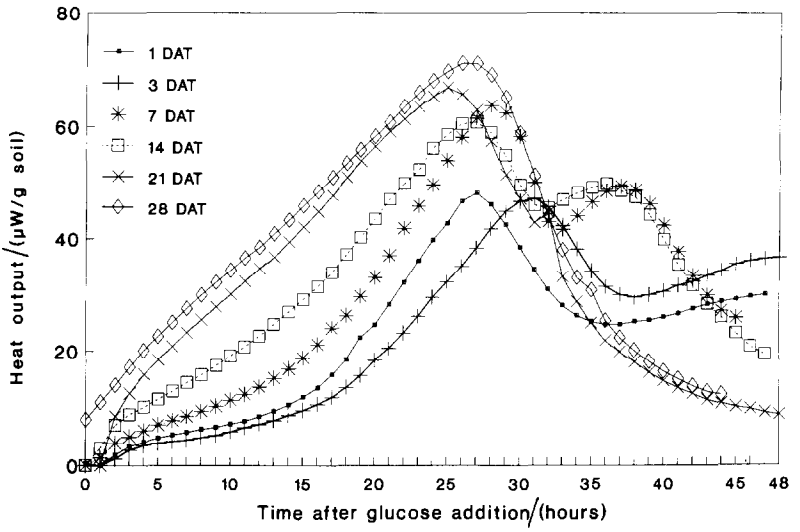

Fig. 3. Heat output from soil samples contaminated with 20 mg Hg (kg soil)$^{-1}$ at 1, 3, 7, 14, 21 and 28 days after treatment (DAT) and measured with the combined ampoule–syringe.

respiration can thus be expected in uncontaminated situations for aerobic metabolism. In our case, heat loss from uncontaminated soils can be almost entirely attributed to metabolic reactions transforming glucose to carbon dioxide. Sparling [4] reported an average amount of heat evolved per cm^3 of CO_2 gas respired equal to 21.1 J cm^{-3} for a 1–6 h period in aerobic conditions. In our case the average ratio was 50.1 J for 1 cm^3 of respired CO_2 over a 1–48 h period from the 1 DAT sample.

Following glucose amendment, soil samples treated with $HgCl_2$ and pentachlorophenol (PCP) at a rate of 200 mg (kg soil)$^{-1}$ showed both a reduced heat loss and a lower CO_2 evolution rate during the entire 28 days of the incubation period (Fig. 2; data of heat output not shown). The effect was especially marked for Hg-treated soils, for which a reduction in heat loss and CO_2 evolution by almost 100% was observed. Soil samples treated with Hg or PCP at a rate of 20 mg (kg soil)$^{-1}$ showed a reduction in CO_2 evolution, as compared with control samples, at 1 and 3 days after addition of the toxicant (Fig. 2). Heat loss from glucose-amended soils was adversely affected by Hg addition at a rate of 20 mg (kg soil)$^{-1}$ up to 14 days after treatment with Hg (Fig. 3). Twenty-one days after the addition of Hg, heat loss was completely restored and similar to that of untreated control soils. Addition of PCP at a rate of 20 mg (kg soil)$^{-1}$ caused a decrease in the heat production at 1 day and 3 days after pesticide application, but had a positive effect on the heat loss from 7 days onward.

In this respect, one might anticipate that toxicants would change the ratio between heat output and CO_2 evolution, which should be a constant for aerobic metabolism in uncontaminated situations. By following heat loss during the first 24 h after glucose treatment, curves differed depending on the time elapsed

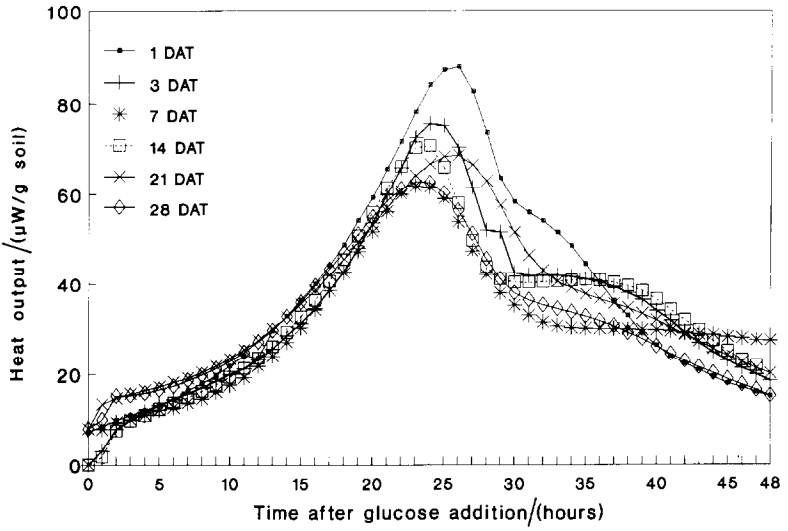

Fig. 4. Heat output from uncontaminated soil samples at different times, measured with the combined ampoule–syringe.

between toxicant addition and measurement (Figs. 3 and 4). With regard to the heat loss 24 h after glucose addition, the heat loss from soil amended with Hg at a rate of 20 mg kg^{-1} first decreases from the 1 DAT sample to the 3 DAT sample but then increases stepwise the longer the time between toxicant addition and heat loss

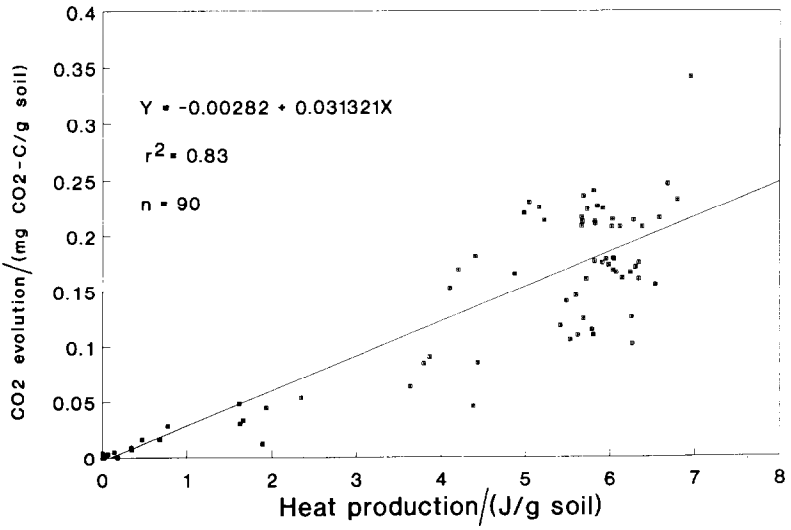

Fig. 5. Correlation between CO_2 evolution and heat production as measured with the combined ampoule–syringe.

measurement (Fig. 3). In contrast to this, the heat output curves, at least for the same 24 h period from uncontaminated soils, remain quite similar, irrespective of the length of incubation period (Fig. 4). The same calculation as for the uncontaminated 1 DAT soil yields ratios of heat evolved per cm^3 of CO_2 respired equal to 93.6 and 65.4 J cm^{-3} for soils treated with 20 mg Hg (kg soil)$^{-1}$ or PCP (kg soil)$^{-1}$ respectively and 1 day after this treatment (1 DAT). This shows that toxicants do change the ratio of heat output versus CO_2 evolution by comparison with the uncontaminated control.

A combined ampoule–syringe was constructed to provide an economical way of simultaneously measuring CO_2 evolution and heat loss from the same soil sample. Heat loss and CO_2 evolution from all samples were highly correlated ($r^2 = 0.83$, $P = 0.001$; Fig. 5), proving that this objective was met. In the various treatments both respiration and heat loss responded to toxicant stress. Here in this experiment only two toxicants were tested, leaving opportunities for a more detailed investigation as to how toxicants may distort the ratio between heat loss and respiration and thereby providing a sensitive soil toxicity index.

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