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Short Communication

Substrate-induced respiration as a measure of microbial biomass in vermicomposting studies

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ABSTRACT

Here it is evaluated the relationship between substrate-induced respiration (SIR) and microbial biomass C (C_{mic}, estimated by chloroform fumigation–extraction) in order to establish SIR as a quick technique to determine microbial biomass in vermicomposting processes. For this, there were designed continuous feeding reactors in which new layers of manure were added sequentially to form an age gradient inside the reactors. Six reactors were set up with and without earthworms (three reactors per treatment). In reactors, with and without earthworms, values of C_{mic} and SIR ranged from 1690 to 42,900 μg g^{-1} dw and from 43 to 2300 μg CO_2 h^{-1} g^{-1} dw, respectively. SIR was significantly related to C_{mic} ($r = 0.63$, $P < 0.0001$). It is proposed an equation to convert SIR values into C_{mic}, SIR (μg CO_2 h^{-1} g^{-1} dw) = 25.97 + 0.04 C_{mic} (μg g^{-1} dw) which will be useful for comparison between studies which used different techniques for determining microbial biomass.

1. Introduction

The methods currently available to measure microbial biomass and microbial activity in soils include fumigation–extraction, fumigation–incubation, substrate-induced respiration, arginine ammonification, ATP, total amount of phospholipid fatty acids (PLFAs) and DNA analysis (reviewed in Paul, 2007). Many of these methods carry large and tedious procedures making them unsuitable as rapid estimates of microbial biomass. In addition, most of these techniques have not been applied to study microbial biomass dynamics in composting and vermicomposting processes, being only widely used fumigation–extraction (Domínguez et al., 2003; Mondini et al., 2004; Aira et al., 2007), with some studies using ATP measurements and PFLAs profiles (García et al., 1992; Klamer and Bååth, 1998); although the use of molecular techniques is increasing (reviewed in Insam et al., 2002).

The substrate-induced respiration (SIR) consists in the measurement of microbial respiration of samples after amending them with an excess of a readily nutrient source, usually glucose, to trigger microbial activity. Thus, the initial maximum respiratory response, which has to be optimized for every new kind of sample, is related with the current size of living microbial biomass (Anderson and Domsch, 1978). In this way, the calibration of SIR with microbial biomass C from fumigation–incubation in a wide array of soils with pH ranging between 3.8 and 7.1, and C/N ratio from 7.9 to 28.0 lead Anderson and Domsch (1978) to the following equation: biomass C (μg g^{-1} soil) = (μl CO_2 g^{-1} soil h^{-1}) × 40.04 + 0.37, this relationship being valid for incubations at 22 °C. A lot of work performed following this procedure in a wide range of soils tended to support this finding.

Vermicomposting is a process microbiologically characterized by strong changes of microbial biomass with periods of rapid growth as well as low activity, unlike the steady-state situation of most soils. It has been shown that microbial biomass and activity decreases during vermicomposting processes (reviewed in Domínguez, 2004) despite in initial stages both microbial parameters can be enhanced (Aira et al., 2007). In addition, vermicomposting processes also produces considerable changes in physicochemical properties of substrates (like pH, N and C pools), making difficult the fine-tuning of analytical procedures to study microbial communities characteristics like microbial biomass and substrate-induced respiration.

The aim of this work was to test the accuracy of substrate-induced respiration as a method to estimate microbial biomass in vermicomposting processes. To do this, SIR values were compared with the microbial biomass C obtained by the fumigation–extraction, a standard technique for determining microbial biomass.

2. Methods

2.1. The substrates

Fresh pig manure (80% moisture and pH 8.1) was obtained from a pig-breeding farm near the University of Vigo, NW, Spain. The

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continuous feeding reactors to obtain the substrates used in this experiment were comprised of modules that were added sequentially to the system. The modules, which resembled sieves, were made of PVC. The external diameter of each was 30 cm, with a height of 2 cm, giving a volume of 1413 cm^3. The bottom of the modules was a mesh size 5 mm, which allowed earthworms to move between modules. Each reactor was initially composed of one module containing vermicompost, in which earthworms were placed, and another module containing a layer of 1.5 kg of fresh pig manure (300 g of dry mass, moisture content 80 T 10%). Then, reactors were fed with layers containing 1.5 kg of fresh pig manure; we set up three reactors without and three with earthworms containing an initial population of 500 mature earthworms (*Eisenia fetida*). Due to moisture content of pig manure we did not need to add water to reactors, which showed a mean moisture content of 81 ± 0.02% through time in all the modules. In the same way, there was little variation in the pH values through the vermicomposting processes with a mean value of 7.1 ± 0.07. At the end of the experiment (i.e. after 36 weeks), each reactor comprised 12 layers with an increasing gradient of age from upper to lower layers of: 2, 4, 7, 8, 11, 18, 21,25, 27, 29, 33 and 36 weeks. Composite samples (three samples per module) were taken from all the modules for the quantification of bacterial biomass C and substrate-induced respiration. The total number of replicates was 72, that is 12 modules per reactor × six reactors.

2.2. Analytical methods

Microbial biomass C was determined by the chloroform fumigation–extraction method (Vance et al., 1987) with field-moist samples (5 g fresh weight). The filtered extracts (0.5 M K_2SO_4) of both fumigated and unfumigated samples were analyzed for soluble organic C using a Microplate Reader (Bio-Rad Microplate Reader 550, 590 nm). Microbial biomass C was estimated as the difference between the organic C extracted from the fumigated and that from the non fumigated sample, multiplied by the K_2SO_4 extract efficiency factor for microbial C (k_c = 2.64) as previously done (Domínguez et al., 2003; Mondini et al., 2004; Aira et al., 2007).

Substrate-induced respiration (SIR) has two main requirements. First, the samples must be saturated with substrate with respect to the response, and second the response to the addition of substrate must be measured before any increase in microorganism biomass (Anderson and Domsch, 1978; Bailey et al., 2008). In this way, we first calculated the saturation curve for glucose solutions when using organic manures by incubating samples with solutions of 0, 20, 40, 60, 80 and 100 mg glucose g^{-1} dw during 2 h, using five replicates per glucose concentration. Then, once the optimal glucose solution is chosen we calculated the microbial growth curve, incubating samples with the optimal glucose solution during 2, 4, 8 and 12 h with 10 replicates per incubation time. In all tests respiration from samples was measured by capturing the evolved CO_2 from samples in NaOH traps after adding 0.75 ml of the corresponding glucose solution, and then measured by titration with HCl to a phenolphthalein endpoint, after adding excess $BaCl_2$.

2.3. Statistical analysis

Data from glucose saturation curve and microbial growth curve were analyzed with a one-way ANOVA, followed a post hoc comparison (Tukey HSD test). Relationships between SIR and microbial biomass C were analyzed with regression analysis. Assumptions of regression analysis (linearity, independence of errors, homoscedasticity and normality) were checked by inspecting plots of residuals. All statistical analyses were performed using R 2.6.2 (2008).

3. Results

The glucose saturation curve (Fig. 1a) showed that from the five glucose concentrations assayed the highest concentration of glucose (100 mg g^{-1} dw) produced the maximum value of respiration (ANOVA, $F_{5,24}$ = 13.693, P < 0.0001). Once the optimal glucose solution was found, we build up a microbial growth curve with different incubation times. This curve (Fig. 1b) indicates that 8 and 12 h can be used to measured SIR, since there was not a significant effect of time of incubation, that is, there were not any increase in microbial biomass, that would invalidate the test (ANOVA, $F_{3,36}$ = 1.56, P = 0.22; Fig. 1b).

Concentrations of microbial biomass C and values of respiration from SIR were highly related (r = 0.63, P < 0.0001) showing a strong and linear relationship (Fig. 2), being the values of respiration from SIR proportional to the concentration of microbial biomass C. This relationship is defined by the equation SIR = 25.97 + 0.04 (C_{mic}). Values of C_{mic} significantly decreased with the age of layers (r = −0.31, P = 0.007); further, this relationship increased in reactors with earthworms (r = −0.63, P < 0.0001; Fig. 3a) and disappeared in reactors without earthworms (r = 0.27, P = 0.10). However, values of SIR significantly decreased with time (r = −0.67, P < 0.0001; Fig. 3b), relationship that decreased in reactors without earthworms (r = −0.54, P < 0.001) and increased in reactors with earthworms (r = −0.81, P < 0.0001).

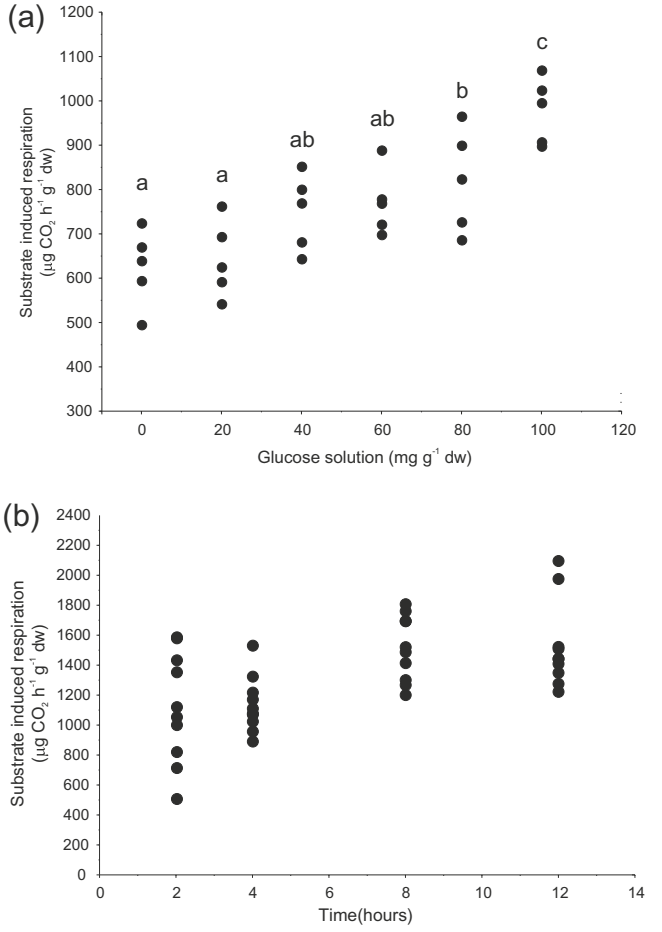

Fig. 1. Determination of substrate-induced respiration parameters, (a) saturation curve for substrates after adding glucose solutions (range from 0 to 100 mg glucose g^{-1} dw pig manure), and (b), microbial growth curve for substrate-induced respiration after the addition of 0.75 ml of glucose solution (equivalent to 100 mg glucose g^{-1} dw pig manure). Different letters indicate significant differences (Tukey HSD test).