ESTIMATING THE BIOMASS OF MICROBIAL FUNCTIONAL GROUPS USING RATES OF GROWTH-RELATED SOIL RESPIRATION

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Summary—We developed a simple method that can be used to estimate the biomass and growth rates of microbial functional groups in soil. The method is derived from basic principles and can be used to estimate the biomass of organisms that can mineralize specific substrates added to soil. We adapted the substrate-induced growth-response (SIGR) model that was originally used to analyze curves of substrate disappearance or cumulative CO₂ production. The present model utilizes data describing the rate of CO₂ production from substrates added to soil. We used two unique systems to demonstrate the applicability of this method. In one test of the model we added glucose to alpine tundra soil to estimate the biomass that could respond to a labile carbon source. We also derived biomass estimates from a widely used substrate-induced respiration (SIR) model for the same soil. Overall the SIGR method yielded conservative biomass estimates (mean = 194 pg C g⁻¹ soil) when compared to the SIR estimates (mean = 436 pg C g⁻¹ soil). In the second test we used a soil to which a known biomass of a specific functional group (i.e. pentachlorophenol-mineralizers) was added. In this case the SIGR method also gave a conservative estimate of 0.05 pg C g⁻¹ compared to a death-rate adjusted value of 0.11 pg C g⁻¹ for the actual inoculum added to the soil. The SIGR model also estimated maximum specific growth rates (0.11–0.12 h⁻¹) similar to those measured in independent experiments (0.09 h⁻¹) for the Sphingomonas sp. that was added to the soil. Using our model we were able to obtain biomass estimates and growth rates for microbial functional groups without using calibrations needed for other physiological methods. Overall, the SIGR approach gives conservative estimates of the active biomass that can mineralize specific carbon substrates added to soil. © 1997 Elsevier Science Ltd

INTRODUCTION

Despite the broad recognition of the role of microorganisms in biogeochemical cycles, very little is known about the population dynamics of those soil microorganisms which drive these cycles. This is true not only at the taxonomic level, but also at the metabolic and functional group levels. Recent attempts at identifying microbial functional groups include the use of molecular techniques (Stahl and Kane, 1992; Ka et al., 1994) and methods based on the metabolic activity of specific microbial groups (Van de Werf and Verstraete, 1987; Schmidt and Gier, 1990; Smolander et al., 1993). We have used the growth response of microbial functional groups to substrates added to soil to estimate populations of those microbial functional groups (Schmidt, 1992a; Schmidt et al., 1995). This substrate-induced growth-response (SIGR) method is similar to the substrate-induced respiration (SIR) method that is widely used in studies of soil ecology (e.g. Anderson and Domsch, 1978; Ocio and Brookes, 1990; Wardle and Parkinson, 1990; Kaiser et al., 1992; Hintze et al., 1994; Hopkins et al., 1994; Smolander et al., 1994). One advantage of the SIGR method is that its theoretical underpinnings come from basic principles of microbial population dynamics (Schmidt, 1992a,b). In contrast, the SIR method utilizes conversion factors that are empirically derived and often vary for different soil types (Kaiser et al., 1992; Hintze et al., 1994) and microbial functional groups. Thus, the SIGR method provides not only a method for estimating microbial biomass and activity but also gives us a theoretical and testable framework for understanding microbial communities in soil.

We describe modifications to the original SIGR method and demonstrate its use in two very different soil systems. We found it necessary to modify the original SIGR method since it was initially developed for studies in which discrete values of CO₂ production were measured and accumulated over time (Schmidt, 1992a; Schmidt et al., 1995). It has been pointed out, however, that accumulating data results in a smoothing effect that can dampen errors and yield unreliable parameter estimates.
To address this problem, we re-derived the basic SIGR equation so it can be used to analyze non-accumulated rate-based data. We used this new SIGR method to estimate the biomass of microbial functional groups from two different soils. We then compared the SIGR-derived parameter estimates to estimates obtained using several other methods.

**MATERIALS AND METHODS**

**Indigenous glucose mineralizers**

The soil used was from a tundra meadow located at an elevation of 3500 m on Niwot Ridge in the Colorado Front Range. The dominant plant species in this meadow is *Kobresia myosuroides* (Villars) Fiori and Paoli. The pH of the soil was approximately 5.0 (Fisk and Schmidt, 1995) with an organic matter content of 27% (May and Webber, 1982). Additional and more detailed characteristics of this site and the soil used have been described by May and Webber (1982), Neff et al. (1994), Fisk and Schmidt (1995) and Fisk and Schmidt (1996). Soil was collected from fertilized plots (last fertilized in 1991) in October 1992, sieved (2 mm) and refrigerated for 15 days prior to use. Soil samples equivalent to 12 g dry wt were put in biometer flasks (Scow et al., 1986, 1989) and allowed to equilibrate for 12 h before the experiment began. Then unlabeled glucose, 14C-labeled glucose (1.75 × 10^4 dis. min^-1 per flask) and (NH₄)₂SO₄ were added in deionized water to obtain a gravimetric water content of 50%, a glucose concentration of 4 mg g^-1 and a C-to-N ratio of 3. This concentration of glucose was needed to induce growth of the standing biomass of glucose mineralizers as determined in preliminary tests (data not shown). The incubation temperature was 22.5 ± 1°C.

**Soil inoculation experiment**

The bacterium used in the soil inoculation experiments is a PCP-mineralizing *Sphingomonas* sp. that has been characterized by Radehaus and Schmidt (1992) and Schmidt et al. (1995). The soil used was a sandy loam with 5% organic matter and a pH of 7.0 and was described as "soil #2" by Schmidt and Gier (1990). Sieved (2 mm) samples, equivalent to 44.5 g dry wt of soil, were placed in 250 ml biometer flasks with enough deionized water to bring the soil to 50% of field capacity. Field capacity was determined on subsamples of the soil using the method of Hanks and Ashcroft (1980). After incubating the flasks for 12 h at 22.5 ± 1°C, 14C-labeled (4000 dis. min^-1 g^-1 soil) and unlabeled PCP were added in deionized water to achieve a PCP concentration of 300 µg g^-1 soil and a moisture content equivalent to 70% of field capacity. Inoculum consisted of 1 ml of a stationary phase culture of *Sphingomonas* sp. RA2 that contained approximately 2.7 × 10^8 cells ml^-1. Thus, the final cell density after inoculation was 6.0 × 10^6 cells g^-1 soil. The inoculum and substrate were thoroughly mixed into the soil using a Teflon-coated spatula. The incubation temperature was 22.5 ± 1°C. Evolved 14CO₂ was quantified as described above. Uninoculated flasks were run as controls to show that PCP mineralization was due to the added inoculum (data not shown).

**Chemicals**

Uniformly 14C-labeled PCP (11.9 m Ci mmol^-1) and glucose (296 m Ci mmol^-1) were purchased from Sigma Chemical Company (St Louis, Missouri). Unlabeled reagent grade PCP was purchased from Fluka Chemical Company (Ronkonkoma, New York) and reagent grade glucose was obtained from Mallinckrodt, Inc. (Paris, Kentucky).

**Data analysis and theory**

The basis of the SIGR approach is that the biomass of organisms, that can mineralize a given substrate, can be estimated based on the amount of substrate needed to induce growth of that biomass. The starting point for our derivation is the substrate depletion form of the logarithmic growth equation (Simkins and Alexander, 1984):

\[-dS/dt = \mu_{max}(S_0 + X_0 - S)\]

in which \( S \) is the substrate concentration, \( S_0 \) is the initial substrate concentration, \( \mu_{max} \) is the specific growth rate and \( X_0 \) is the initial biomass of organisms respiring the substrate expressed in the same units as \( S \). Equation 1 can be integrated to obtain:

\[S = S_0 + X_0(1 - \exp(\mu_{max}t))\]

Equation 1 expresses the rate of substrate depletion as a function of \( S \). In order to analyze data from time course experiments, however, we need a form of the equation in which the rate of substrate depletion is expressed as a function of time. To obtain such an equation the first step is to re-
arrange equation 2 to get:

\[ S_0 + X_0 - S = X_0 \exp(\mu_{\text{max}} t) \]  

(3)

By substituting the right side of equation 3 into equation 1 we obtain:

\[-\frac{dS}{dt} = \mu_{\text{max}}(X_0 \exp(\mu_{\text{max}} t)) \]  

(4)

Equation 4 expresses the rate of substrate depletion as a function of time and can be used to analyze curves of rate vs time.

In most soil respiration experiments, however, data collected are in the form of product formation (e.g. CO₂) rather than substrate depletion. We, therefore, derived an equation to analyze curves of the rate of product formation vs time. To do this we used a proportionality constant, \( c \), that is equal to the amount of product produced per unit of substrate consumed. The constant \( c \) is equal to \( 1 - Y_c \) if it is assumed that all substrate initially present is converted to either biomass or product and \( Y_c \) is equal to the yield coefficient with units of \( \mu \) C-biomass \( \mu \) g⁻¹ C-substrate. The rate of product formation should be proportionate to the inverse of the rate of substrate depletion and therefore we can write:

\[ \frac{dP}{dt} = \frac{-dS}{dt}(c) \]  

(5)

where \( P \) equals the amount of product (CO₂) produced. Multiplying both sides of equation 4 by \( c \) and substituting the left side of equation 5 for \( -\frac{dS}{dt}(c) \) we obtain:

\[ \frac{dP}{dt} = \mu_{\text{max}}(c X_0 \exp(\mu_{\text{max}} t)) \]  

(6)

Because \( c \) and \( X_0 \) cannot be independently estimated using nonlinear regression analysis we combine them into one parameter, \( X_1 \), and thus equation 6 can be rewritten as:

\[ \frac{dP}{dt} = \mu_{\text{max}}(X_1 \exp(\mu_{\text{max}} t)) \]  

(7)

where \( X_1 \) represents biomass in terms of product produced and with the same units as product (i.e. \( \mu \) g C-CO₂ g⁻¹).

To convert \( X_1 \) to units of \( \mu \) g biomass-C g⁻¹ it is multiplied first by \( 1/c \) to convert to units of \( \mu \) g C substrate g⁻¹ and then by \( Y_c \) to convert units of substrate to biomass. Combining these two conversion steps into one factor we can write:

\[ X_a = X_1(Y_c/1 - Y_c) \]  

(8)

in which \( X_a \) is the actual biomass with units of \( \mu \) g C-biomass g⁻¹. We used a value of \( Y_c = 0.5 \) g biomass-C g⁻¹ substrate-C (Seto and Alexander, 1985) for glucose amended soils. For FCP experiments we used a value for \( Y_c \) of 0.2 g biomass-C g⁻¹ substrate-C as determined in pure culture experiments with Sphingomonas sp. RA2 (Radehaus and Schmidt, 1992).

For comparative purposes we also analyzed some of our data using the substrate induced respiration (SIR) method (Anderson and Domsch, 1978) using the empirically derived equation:

\[ X_a = 400.4p + 3.7 \]  

(9)

where \( X_a \) is the initial biomass with units of \( \mu \) g biomass-C g⁻¹ and \( p \) is the initial rate of CO₂ production with units of ml CO₂ 100 g⁻¹ soil h⁻¹ (Ocio and Brookes, 1990). We also analyzed our glucose data with a new relationship observed by Hintze et al. (1994):

\[ X_a = 28.8(p^{1/11}) \]  

(10)

Estimates of active biomass (\( X_a \)) and growth rates (\( \mu_{\text{max}} \)) were obtained by fitting models such as equation 7 to mineralization data using a modification of the non-linear regression approach of Schmidt (1992b) and Hess and Schmidt (1995).

Equation 7 describes the exponentially increasing portion of ¹⁴CO₂ data. Since this equation only works with growing populations, it was necessary to remove data points from the lag period and points after the rate was not increasing exponentially. To do this, the curves of ¹⁴CO₂ accumulation were re-drawn in the form of rate vs time, as suggested by Hess and Schmidt (1995). The amount of ¹⁴CO₂ counted at a given sampling time was divided by the hours between two consecutive samples and plotted between the last two sampling times at the midpoint. When the mineralization curve is viewed in such a manner it is clear at what stage the rate begins continually increasing and when the lag is over. Before analyzing the data using equation 7, the lag was removed and \( t_0 \) was set to 0 h.

To determine when exponential growth ended, an \( F \)-test was used in a similar manner to that used by other authors for model discrimination purposes (Robinson, 1985; Motulsky and Ransnas, 1987; Hess and Schmidt, 1995). Firstly, the data points up to the apex of the rate vs time curve were all fit to equation 7. The last point was then removed and the model was run again. The sum of squares and degrees of freedom from both fits were inserted into the following equation which we adapted from Motulsky and Ransnas (1987):

\[ F = (SS_1 - SS_2)/(df_1 - df_2)/(SS_2/df_2) \]  

(11)

where \( SS_1 \) is the sum of squares of the fit of equation 7 to the data and \( SS_2 \) is the sum of squares of the fit to the same data set minus the last point. Likewise, \( df_1 \) and \( df_2 \) are the degrees of freedom associated with \( SS_1 \) and \( SS_2 \), respectively. Thus, a large \( F \)-value indicates an improved fit even at the expense of losing a degree of freedom. Alternatively, the generation of a small \( F \)-value
Fig. 1. Replicate curves of $^{14}$CO$_2$ accumulation from alpine tundra soils incubated with 4 mg glucose g$^{-1}$ soil. The open and closed circles represent the two replicate samples.

(usually less than 1) indicates the fit was not improved by removing the last datum from the curve. When this occurred the datum was added back to the curve and the previous model estimates (associated with $SS_1$ in equation 7) were used to estimate values for the parameters of equation 7. This iterative process gave a consistent and unbiased method for determining when exponential growth had ceased.

Once an acceptable fit was obtained according to the $F$-test, the residuals were plotted and examined for randomness and outliers. Residuals not falling within the 99% confidence limits for the normal distribution were removed as outliers, and the model was run again under the same goodness of fit criteria ($F$-test and analysis of residuals). Additional criteria such as standard error values and Pearson's $r$ were also used to assess the statistical validity of the model fits (Anscombe and Tukey, 1963; Motulsky and Ransnas, 1987; Hess and Schmidt, 1995). All curve fitting and data analyses were carried out using Kaleidagraph$^\text{TM}$ (Synergy Software, Reading, PA).

RESULTS AND DISCUSSION

The major goal of our work was to derive a broadly applicable model that could be used to estimate the biomass of specific microbial functional groups in soil. This goal is similar to that of our

Fig. 2. Non-accumulated $^{14}$CO$_2$ rate data from glucose mineralization (4 mg g$^{-1}$ soil) in alpine tundra soil. The open and closed circles represent the two replicate samples.
previous SIGR studies (Schmidt, 1992a; Schmidt et al., 1995), except that the original SIGR equation (Schmidt, 1992a) could only be used with CO₂ data that were accumulated over time. As has been pointed out by a number of workers (e.g. Taylor and Parkinson, 1988; Hess and Schmidt, 1995), cumulative curves of CO₂ evolution should not be used to estimate parameters of microbial biomass dynamics. In contrast, the approach derived in this paper can be used to estimate statistically valid parameters of microbial biomass dynamics from soil respiration data. In the following sections we use equation 7 and equation 8 to estimate the biomass \( X_a \) and maximum growth rates \( \mu_{max} \) of specific microbial functional groups using CO₂ production curves from two different soils and substrate types.

Indigenous glucose mineralizers

As part of our ongoing studies of below ground processes in alpine tundra (Mullen and Schmidt, 1993; Neff et al., 1994; Fisk and Schmidt, 1995; Fisk and Schmidt, 1996) we wished to quantify the community of microorganisms that could respond to an influx of a labile substrate such as glucose. To use the modified SIGR model (equation 7) for this purpose, the data were treated in two ways that differ from previous SIGR analyses (Schmidt, 1992a,b; Schmidt et al., 1995). Firstly, we used the data in a rate-based form, rather than a product accumulation form. The smoothing effect of accumulating data (cf. Hess and Schmidt, 1995), is illustrated by comparing the curves of CO₂ production over time (Fig. 1) to those of the rate of CO₂ production at each time point (Fig. 2). The curve in Fig. 1 is deceptively flawless, and only by examining the rate data in Fig. 2 can the true magnitude of the scatter of the data be seen. As shown by Hess and Schmidt (1995), accumulated curves such as those in Fig. 1 do not provide true estimates of error for parameters of microbial community dynamics. Thus, in this case we have modified the original SIGR approach (Schmidt, 1992a,b), in a manner that allows us to analyze the rate-based data of Fig. 2 using a differential form of the SIGR model; i.e. equation 7.

In a second modification of the data before application of the SIGR model, we removed the portions of the data set that did not correspond to the exponential growth phase. Using the criteria described in Section 2, we removed the non-accelerating portions at the beginning and the end of the curves (Fig. 3) to isolate the exponential growth phase for use in the SIGR model. Point-by-point removal by this method proved to be an objective way to avoid potential errors associated with initial lag times or deceleration of CO₂ efflux. Nonlinear regression estimates of the growth rate and initial biomass concentration from the fit of equation 7 to these data are presented in Table 1.

For comparative purposes, we also used the first 2 h of rate data to estimate the biomass of glucose mineralizers using the well-established SIR method (Anderson and Domsch, 1978; Ocio and Brookes, 1990). The SIR equation of Anderson and Domsch (1978) yielded biomass-C estimates that averaged 428 µg C g⁻¹ soil, considerably higher than the average of 196 µg C g⁻¹ soil using the SIGR method (Table 1). This higher estimate occurred despite the fact that only labeled CO₂ was used for biomass measurements; SIR typically uses total CO₂ respired. The difference between the two methods was even greater when we used an estimate of the

![Fig. 3. Non-accumulated rate data from Fig. 2 after non-accelerating portions were removed. The lines represent the curve fits from equation 7 and parameter estimates are listed in Table 1. The open and closed circles represent the two replicate samples.](image)
Table 1. Estimates of microbial biomass carbon (+standard errors of the parameter estimates or mean) using the SIGR approach [equation 7] and the SIR method [equation 9] of Anderson and Domsch (1978) and [equation 10] (Hintze et al., 1994)

<table>
<thead>
<tr>
<th>Test</th>
<th>$X_a$ (µg C g$^{-1}$)</th>
<th>$\mu_{max}$ (h$^{-1}$)</th>
<th>$X_a$ (µg C g$^{-1}$)</th>
<th>$X_a$ (µg C g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test 1</td>
<td>191 ± 13</td>
<td>0.031 ± 0.001</td>
<td>468</td>
<td>59 ± 1</td>
</tr>
<tr>
<td>Test 2</td>
<td>201 ± 16</td>
<td>0.031 ± 0.001</td>
<td>464</td>
<td>33.6</td>
</tr>
<tr>
<td>Means</td>
<td>194 ± 15</td>
<td>0.031 ± 0.002</td>
<td>436 ± 28</td>
<td>31 ± 2</td>
</tr>
</tbody>
</table>

rate of total glucose-induced CO$_2$. We did this by adding the rate of basal respiration of 0.72 µg C-CO$_2$ g$^{-1}$ soil h$^{-1}$ (Fisk and Schmidt, 1996) to our rate of $^{14}$C-CO$_2$ production and re-calculated $X_a$ to be 496 µg biomass-C g$^{-1}$. Other workers have also commented on the apparent overestimation of microbial biomass-C by the SIR method, especially in soils with low biomass contents (Sparling and West, 1988; Ocio and Brookes, 1990; Hintze et al., 1994). Another explanation for the different estimates from the two methods is that the SIGR estimates are based on the biomass of organisms that can grow on glucose during the incubation period, whereas the SIR estimates are based on total glucose induced respiration. Total glucose induced respiration includes both basal rates of respiration and respiration due to the addition of glucose.

Hintze et al. (1994) have proposed a modified SIR approach that utilizes equation 10 to estimate biomass-C in soils. When we applied this equation to our data, it under-estimated biomass-C as compared to the SIGR approach (Table 1). This under-estimation might be due to the fact that, as with the standard SIR approach (Anderson and Domsch, 1978), the equation of Hintze et al. (1994) was formulated to relate the total amount of glucose-induced respiration to microbial biomass-C. We therefore added a measured rate of basal respiration of 0.72 µg C-CO$_2$ g$^{-1}$ soil h$^{-1}$ (Fisk and Schmidt, 1996) to our rate of $^{14}$C-CO$_2$ production and recalculated $X_a$ using equation 10. This only increased the estimate of $X_a$ from 31 to 36 µg biomass-C g$^{-1}$ soil, still well below the SIGR value of 194 µg biomass-C g$^{-1}$ soil (Table 1).

Based on the comparisons presented above, the rate-based SIGR method gives values of glucose-induced biomass that fall between the values predicted by the approach of Anderson and Domsch (1978) and that of Hintze et al. (1994). Because none of the methods currently available for estimating microbial biomass-C in soils can necessarily be considered a benchmark measurement (Wardle and Ghani, 1995), comparing the SIGR and SIR approaches does not necessarily validate or invalidate either approach. Given that the SIGR approach is based on basic theories of microbial growth dynamics and the SIR is based on an empirical relationship, it is encouraging that the two approaches give estimates of glucose induced microbial biomass-C that are even within an order of magnitude of each other.

**Soil inoculation studies**

In addition to applying the SIGR model to the generalized functional group of glucose mineralizers, we illustrate the versatility of the SIGR...
method by using it with PCP mineralizers, a more specific metabolic group. The inoculation of a PCP-mineralizing \textit{Sphingomonas} sp. into soil containing 300 \(\mu\)g PCP g\(^{-1}\) resulted in growth shaped curves of \(^{14}\)CO\(_2\) evolution (Fig. 4), from which we removed non-accelerating portions and then fit the data to equation 7 (Fig. 5). The soil used showed no previous evidence of PCP-mineralizing activity at the concentrations tested (Colores et al., 1995).

Parameter estimates generated from fitting equation 7 to these curves are presented in Table 2. Estimates of \(\mu_{\text{max}}\) of 0.12 and 0.11 h\(^{-1}\) are close to the growth rate for the same organism measured in pure culture (0.09 h\(^{-1}\)) at a PCP concentration of 40 \(\mu\)g ml\(^{-1}\) (Radehaus and Schmidt, 1992). Model estimates of \(X_a\) can be compared with an independent estimate of \(X_a\) calculated from the amount of inoculum that was added to the soil. The independent estimate of 340 ng biomass-C \(g^{-1}\) is higher than the SIGR estimates of 50 ng biomass-C \(g^{-1}\). The difference between these two estimates is probably due to the fact that the SIGR approach estimates only active biomass. Stationary-phase cells were used as the inoculum, and therefore the actual population of living cells added to the soil may have been less than the theoretical maximum of 340 ng biomass-C \(g^{-1}\). It is also likely that there was a die-off of \textit{Sphingomonas} sp. RA2 cells when the inoculum was added to the soil. \textit{Sphingomonas} sp. RA2 is not indigenous to the soil used in this experiment and had been cultured on laboratory media for several years prior to this attempt to re-introduce it into contaminated soil. We have also noticed a significant die-off of cells when liquid cultures containing PCP are inoculated with \textit{Sphingomonas} sp. RA2. In one experiment, there was a three-fold die-back of viable cells from \(1.4 \times 10^6\) to \(5 \times 10^5\) cells ml\(^{-1}\) in the first 12 h after inoculation (Radehaus and Schmidt, unpublished data). If this also happened in the present study, our independent estimate of \(X_a\) would be lowered from 340 to 110 ng biomass-C \(g^{-1}\), a value that is closer to the SIGR estimates of \(X_a\) of 50 ng biomass-C \(g^{-1}\).

Because the SIR method of Anderson and Domsch (1978) was developed for glucose-amended soil we did not use it to analyze our data from PCP-amended soils. Hopkins \textit{et al.} (1994) used the SIR method with amino acid-amended soil and pointed out the need to re-derive a relationship between SIR and biomass-C for different substrates. In contrast, the SIGR method can be used to estimate the biomass of a broad range of metabolic groups as long as the CO\(_2\) produced from a particular substrate can be quantified.

In conclusion, we developed a rate-based SIGR model that can be used to obtain estimates of microbial biomass and growth rates from soil respiration experiments. By using only accelerating portions of the growth curve we avoid complications due to enzyme induction and other lags, and get reproducible estimates of the active microbial biomass. This model is simple and is based on

Table 2. Parameter estimates (+standard errors) from the fit of equation 7 to the mineralization curve depicted in Fig. 4. Estimates for the inoculum were calculated based on the number of cells added to the soil.

<table>
<thead>
<tr>
<th></th>
<th>(X_a) (ng C (g^{-1}))</th>
<th>(\mu_{\text{max}}) (h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum</td>
<td>340</td>
<td>0.09 (\pm) 0.008</td>
</tr>
<tr>
<td>1</td>
<td>50 (\pm) 6</td>
<td>0.11 (\pm) 0.004</td>
</tr>
<tr>
<td>2</td>
<td>50 (\pm) 6</td>
<td>0.12 (\pm) 0.005</td>
</tr>
</tbody>
</table>

Fig. 5. Non-accumulated rate data from Fig. 4 after non-accelerating portions have been removed. The lines represent the curve fits from equation 7 and parameter estimates are listed in Table 2. The open and closed circles represent the two replicate samples.
fundamental principles of microbial growth dynamics, thus avoiding some of the assumptions and calibrations of other physiological methods of estimating biomass. Therefore, its application should not be limited to a specific substrate or soil type. We examined two examples of its use; one that demonstrated its usefulness in estimating the biomass of a large physiological group (the glucose mineralizers) and another example that showed it could be used to study a much more restricted functional group (Pseudomonas sp. that mineralizes high concentrations of pentachlorophenol). Applied and Environmental Microbiology 58, 2879–2885.

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