Original Articles

Measuring basal soil respiration across Europe: Do incubation temperature and incubation period matter?

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ABSTRACT

The European Commission recognises the essential role of soil biology in soil functioning and delivery of ecosystem services, but information is currently lacking evaluate of how these vary across soil and land-use types at a European scale. This study evaluated the measurement of the initial rate of soil basal respiration (BR) as a potential biological indicator of ecosystem service provision. The purpose of this study was to test ISO 16072:2002 (Soil Quality: Laboratory methods for the determining of microbial soil respiration). In the literature a range of pre-incubation temperatures (pre-inc) and experimental incubation temperatures (exp-inc) have been applied when using the ISO method for the establishment of basal respiration. This study evaluated whether the range of temperatures applied during pre- and exp- incubation had a significant effect on the rate of respiration determined when following the protocol established in ISO 16072:2002. The evaluation was carried out on a pedo-climatic gradient spanning ten countries across Europe and covering four biogeographical regions. Three sites were sampled in each country providing a range of soil and land-use parameters. Our results suggest that experimental incubation temperatures of 20 °C or above should be used in the application of the methodology ISO 16072:2002 (incubation at 15 °C resulted in erratic variation between replicates). However, pre-incubation temperature did not affect the soil basal respiration rate, when following the standard recommendations. The time interval with the best prediction of the initial rate of basal respiration was 6 h.

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1. Introduction

The development of biological indicators to assess changes in soil quality is an increasingly important research area. It is now accepted that soil biology is the driving force behind many soils processes (Francaviglia, 2008) and supports the delivery of soil and soil ecosystem functions such as nutrient transformations, water purification and carbon sequestration.

Currently, there is a plethora of data available on biological indicators. This information has been collected using a wide range of methods, each applied in specific environmental situations, complicating the meaningful comparison of data. The example addressed in this paper is the assessment of microbial basal respiration, frequently used to quantify changes in the activity of the soil microbial community (Winding et al., 2005; Bispo et al., 2009).

Soil basal respiration is defined as the steady rate of respiration in soil, which originates from the mineralisation of organic matter (Pell et al., 2006), and is estimated either on the basis of CO_2 evolution or O_2 uptake (Dilly and Zyakun, 2008). The measurement of soil basal respiration has been applied across a variety of research studies and both soil microbial respiration and the mineralisation of organic matter are commonly accepted as a key indicator for measuring changes to soil quality. Two stages of respiration response are quantified under the heading soil basal respiration

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Table 1
Evaluation of literature on the measurement of basal respiration rate for experimental- and pre-incubation periods and temperatures.

Authors	Year	Exp-inc sampling	Exp-inc temperature	Pre-inc period	Pre-inc temperature
Cěrnohlávková et al.	2009	24 h	20 °C	3 days	20°–22 °C
Uzun and Uyanoz	2011	24 h	25 °C	Not defined	
Hofman et al.	2004	24 h	Not defined	7 days	22 °C
Ritz and Wheatley	1989		25 °C	Not defined	
Grayston et al.	2001	24 h	15° and/or 30 °C	Not defined	
Maassen & Wirth	2004	8 h	20 °C	2 days	20 °C
Rousk et al.	2009	22–24 h	22 °C	1 week	22 °C
Bundy et al.	2004	6 h	Not defined	Not defined	
Mulder et al.	2005	5 weeks	20 °C	4 weeks	12 °C
Vanhala et al.	2005	6 h	14 °C	7 days	14 °C
Gömöryová	2011	24 h	22 °C	Not defined	
Bárcenas-Moreno	2011	4 days	25 °C	Not defined	
Fernandes	2005	10 days	Not defined	5 days	Not defined
Fließbach et al.	2007	72 h	Not defined	7 days	22 °C

within the literature: 1) an initial rate of respiration and 2) the growth response. This paper will only consider the initial response rate of basal respiration and its response to a range of incubation temperatures.

Anderson and Domsch (1977) emphasise the need for identical conditions of soil incubation, to be able to compare intrinsic differences across soils. However, a wide range of experimental incubation conditions were found within the literature; with ranges in experimental incubation temperature from 14° to 25 °C, and incubation periods from a minimum of 6 h up to a maximum of 6 weeks (Table 1).

The adoption of soil biological measurements as broad-scale monitoring indicators requires consistency and accuracy within a method across a range of environmental conditions such as soil type; vegetation type; and climate (Creamer et al., 2009). In order to adopt soil respiration as a universal tool for monitoring changes in soil quality it is essential to validate the precision of the international standard method (ISO 16072:2002 Soil Quality: Laboratory methods for the determining of microbial soil respiration). This study aimed to assess the implication of a range of experimental-incubation and pre-incubation temperatures on a range of soils representing a pedo-climatic gradient across Europe (Fig. 1). A pedo-climatic gradient represents a spectrum of the complex interactions between climate, geology and vegetation across Europe, ranging from dry, shallow soils in the Mediterranean to deeper, poorly drained soils under Boreal conditions.
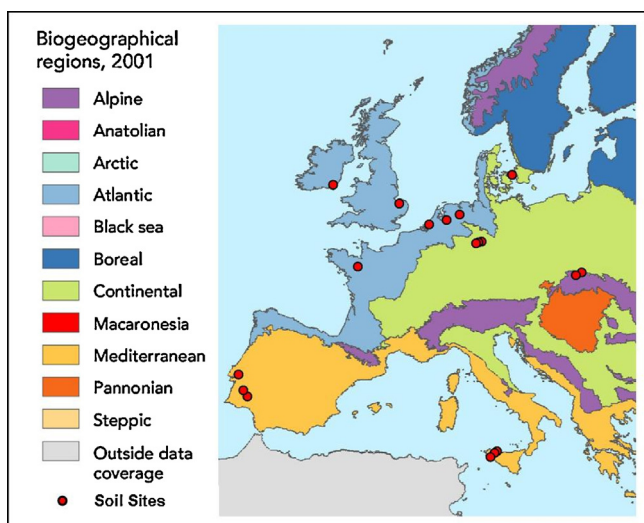

Fig. 1. Map depicting the distribution of sites across Europe, throughout the biogeographical regions (European Environment Agency, 2002).

1.1. Experimental incubation (exp-inc) and pre-incubation (pre-inc) temperature ranges

The International Standard ISO 16072:2002 was promoted within a recent large scale pan-European project to evaluate indicators for monitoring changes in soil quality (ENVASSO) (Bispo et al., 2009) as the accepted methodology for the measurement of soil basal respiration. The standard allows for the measurement of the initial rate of soil basal respiration at a range of temperatures (during the exp-inc and pre-inc periods of the assay).

Temperature is held to a controlled heat during the method (the exp-inc temperature), as temperature is considered to significantly affect the rates at which respiration takes place (Winding et al., 2005). The ISO 16072:2002 recommends a range of exp-inc temperatures between 20° to 30 °C, but states that other temperatures may be applied. The extent to which any exp-inc temperature effects are expressed or masked in sample sets containing soils of highly varied origin is not clear. We therefore set out to evaluate three exp-inc temperatures of 15°, 20° and 25 °C on a pedo-climatic gradient of soils, reflecting the temperature range found in the literature.

A pre-inc temperature is applied to settle and standardise the soil microbial community following disturbance of sampling and sieving, allowing the initial carbon flush to diminish (Bloem et al., 2006). Pre-inc temperatures and periods of duration have been defined by a group of experts across Europe (Table 2). These pre-inc temperatures provide a range of mesophilic temperature conditions which reflect different climatic zones. The Q_{10} established for each temperature by time period, results in the same incubation conditions having being applied to all soils when the endpoint is reached.

1.2. Length of experimental-incubation period

With the aim of achieving a high-throughput of samples, measurements of basal respiration rates are commonly derived from a single time point CO_2 measurement. This is beneficial in national or European monitoring systems where the labour intensity of a method is an important consideration. The timing of this one-off measurement of CO_2 concentration varies, with many

Table 2
Recommended soil pre-incubation periods prior to measurement of soil basal respiration (Jones et al., 2008).

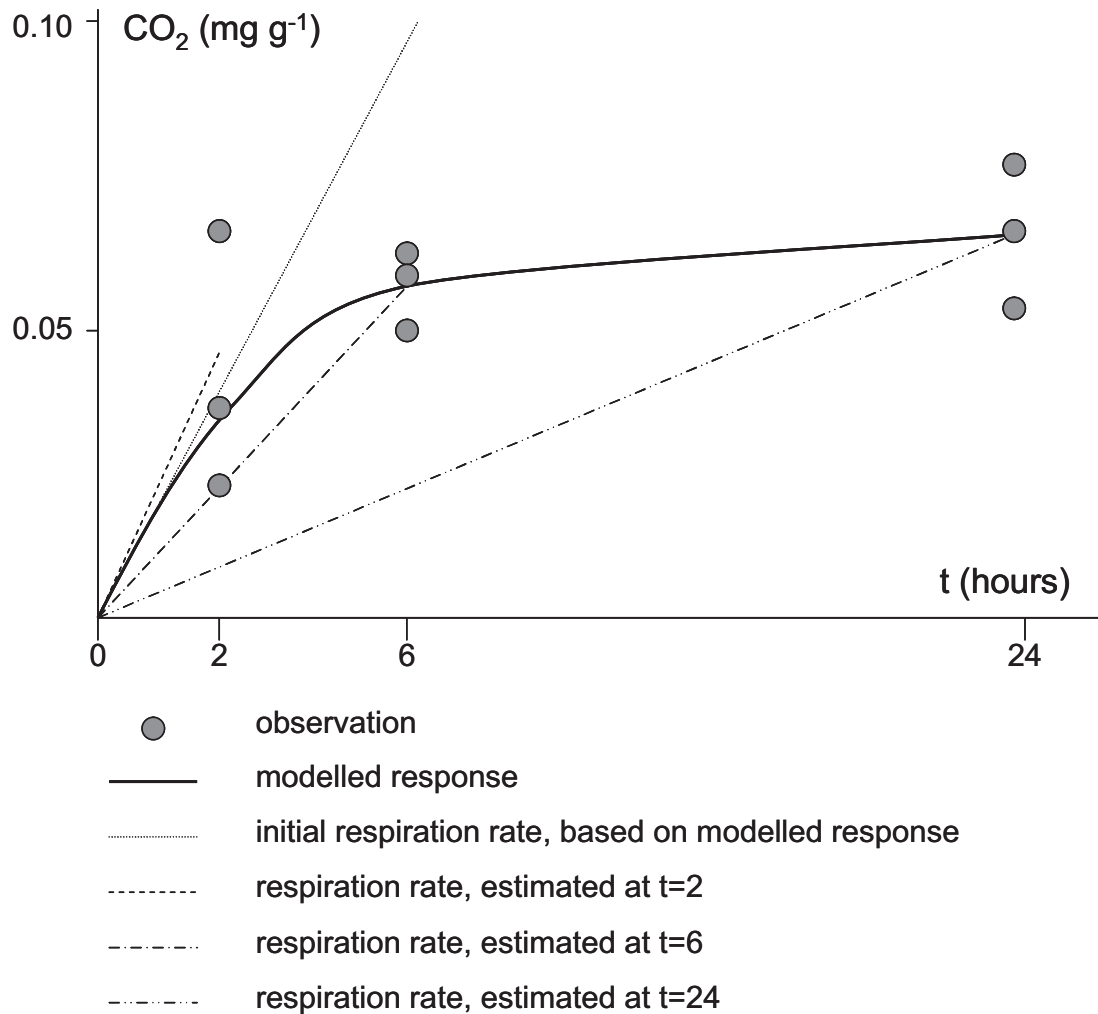| Period of pre-incubation	Temperature of pre-incubation
2 to 3 days	25° to 28 °C
1 week	20 °C
2 weeks	15 °C
4 weeks	10 °C

Fig. 2. This conceptual graph (hypothetical data) demonstrates how the estimate of the initial respiration rate (thin line), based on the modelled response (solid line), differs to estimates of the average respiration rates, based on individual measurements of respiration at times $t = 2$, 6 and 24 (the three dashed lines).

studies reporting 24 h of incubation (e.g. Grayston et al., 2001; Hofman et al., 2004; Uzun and Uyanoz, 2011), while incubation times as short as 6 h have been reported (Bundy et al., 2004; Vanhala et al., 2005). ISO 16072:2002 indicates that one reading after 20–24 h is sufficient (in addition to the initial T0 reading). However, a single measurement of CO_2 concentration assumes a linear increase in CO_2 concentration over time, rendering timing of gas sampling inconsequential within the first 24 h; this is shown by the variety of sampling periods indicated in Table 1. But non-linear increases in CO_2 concentration over time have been reported, with CO_2 concentrations either accelerating or approaching a maximum value within 24 h, (e.g. Ritz et al., 2006). Therefore in an ideal scenario CO_2 concentrations should be measured multiple times during the exp-inc period to facilitate assessment of the linearity of the response over time. This is exemplified in Fig. 2: in this (hypothetical) scenario, CO_2 concentrations of each of the three hypothetical replicates were measured three times during the incubation period, i.e. at $t = 2$, 6 and 24 h (dots on the graph). These longitudinal measurements allow the (non-linear) increase in CO_2 concentration to be fitted by an appropriate model (solid line), from which the initial slope of the model, i.e. the initial respiration rate, can be established (dotted line). If only a single observation is applied after one given length of time, this can lead to both underestimation and overestimation

of the initial respiration rate, for two different reasons: in this example (Fig. 2), a single reading of the average respiration rate at $t = 24$ (dash-dot-dotted line) will underestimate the initial respiration rate at $t = 0$, due to the non-linearity of the increase in CO_2 concentration. In addition, under or overestimation of the initial respiration rate may arise from random errors in the measurements at one single point in time. In the example of Fig. 2, the average respiration rate at $t = 2$ (dashed line) overestimates the initial respiration rate, as a result of the large random variation in CO_2 concentration between replicates for this specific time interval.

As with variations in exp-inc temperature, the potential range of exp-inc periods creates problems when comparing published studies. Therefore, in order to present a detailed evaluation of ISO 16072:2002, we investigated the accuracy of using an average respiration rate, based on one single reading at the end of the exp-inc period, to calculate the initial rate of basal respiration, in comparison to the initial respiration rate derived from multiple readings throughout the exp-inc period.

1.3. Objectives

The overall objectives of this study were to evaluate the methodological measurement of the initial rate of basal soil respiration,

Table 3
Climatic and land-use properties of sites.

Country	Land-use	Average soil temperature ($^\circ$C)	Average temperature of the growing season ($^\circ$C)
Ireland	Grassland	11.12	10.67
Ireland	Tillage	11.12	10.67
Ireland	Forest	11.12	10.67
France	Forest	11.49	12.04
France	Tillage	11.49	12.04
France	Grassland	11.49	12.04
Germany	Tillage	11.6	13.16
Germany	Grassland	11.6	13.16
Germany	Fallow	11.6	13.18
Denmark	Arable	8.66	12.20
Denmark	Arable	8.66	12.20
Denmark	Arable	8.66	12.20
Hungary	Grassland	No data	15.25
Hungary	Tillage	No data	15.25
Hungary	Forest	No data	12.50
UK	Grassland	9.90	12.10
UK	Tillage	9.90	12.10
UK	Fallow – long-term	9.90	12.10
Portugal	Grassland	No data	23 ± 3
Portugal	Fallow	No data	23 ± 3
Portugal	Fallow	No data	23 ± 3
Italy	Tillage	No data	17.19
Italy	Grassland	No data	14.79
Italy	Forest	No data	14.79
Greenland	Dwarf shrub heath	2.08	7.18
Greenland	Dwarf shrub heath	2.08	7.18
Greenland	Dwarf shrub heath	2.08	7.18
Netherlands	Tillage	10.70	12.60
Netherlands	Forest	10.70	12.60
Netherlands	Tillage – Organic	10.40	12.46

using a range of soils sampled from a pedo-climatic gradient across Europe. The key questions to be answered were;

- Does experimental-incubation (exp-inc) temperature affect the response of the initial respiration rate for basal respiration and if so, what exp-inc temperature gives the most consistent results?
- Does pre-incubation (pre-inc) temperature affect the response of the initial respiration rate for basal respiration and if so, what pre-inc temperature gives the most consistent results?
- What is the optimum length of exp-inc period, if only one gas measurement can be facilitated?

2. Material and methods

2.1. Site selection

Samples were collected from ten countries to achieve a range of climatic zones (Mediterranean, Continental, Atlantic and Boreal) (Fig. 1). Within each country three sites of contrasting land-use (i.e. arable, grass and woodland) were sampled to provide a spectrum of soils for analyses. The sampling sites were prescribed to provide a range of soil physical and chemical characteristics and to ensure a range and contrast in the soil microbiological properties being considered in this study. Each country sampled a clay loam, loam and sandy loam soil. Only one sample per site was taken, as this was a method comparison exercise and we merely required a spectrum of soil properties under a range of land-uses. Samples were taken over a two-week period in June 2009. Data relating to the site location and land-use was collected and climatic data obtained from the nearest weather station (Table 3).

2.2. Sample collection and preparation

A protocol was developed for sampling, following ISO 10381-6:1993a. Sites were sampled to a depth of 10 cm, with the removal of surface vegetation. Soil samples were sieved fresh to <2 mm and kept in a cold room (<4 $^\circ$C). All samples were and posted within ten days of sampling, postage packages were kept cool (approx. <4 $^\circ$C) during transit by the insertion of coolpacks. On arrival samples were stored at <4 $^\circ$C in the dark during the process of preparation for the measurement of basal respiration. Sample storage did not exceed four weeks from sampling date.

Sub-samples were taken during the storage period to measure water holding capacity and moisture content. Water-holding capacity of sieved soil was measured using a Haines-funnel system, where 100 ml of water was added to 50 g of fresh soil in a funnel for 30 min. Excess water was collected from the funnel system and its volume measured (Jenkinson and Powlson, 1976). Moisture content was determined using the Gravimetric method as laid out in the Soil Quality ISO Section 3.1 (ISO 11465:1993b), with the modification of using only 5 g soil per sample. In accordance with ISO 16072:2002, moisture contents of soil samples were adjusted to a water holding capacity of 40–60%.

Following coning and quartering a 200 g sub-sample of soil was incubated in a plastic bag with a wet wick to maintain constant moisture content. Soils were pre-incubated in the dark at a range of temperatures and corresponding time periods following the recommendation of Jones et al. (2008), (Table 2).

2.3. Determination of basal respiration

Basal respiration was determined in serum bottles following ISO 16072:2002 – Section 5.5. Samples were randomly allocated numbered bottles and from that point on were blind sampled (i.e. no knowledge of sample origin). Three replicates of each soil sample were processed. To pressurise the system we added 21 cm^3 of laboratory air and the sampling syringe was pumped twice in the laboratory atmosphere before injection. After every three bottles pressurised, an ambient air sample from the laboratory was taken and analysed to measure background conditions. Gas samples were taken from the bottle headspace at regular intervals. Samples were inserted into pre-evacuated vials, the syringe was pumped twice in the headspace before the 7 cm^3 samples were taken. Gas vials were analysed using a Varian CP-3800 GC fitted with a packed column and the carrier gas was argon. CO_2 concentration was calculated for each sample replicate using Star Chromatography Workstation (2004). Calculation of the rate of CO_2 formation was determined using the method provided by ISO 16072:2002.

To answer the objectives laid out in the introduction, two experiments were conducted, using three replicate sub-samples of the soils from each site for each incubation temperature;

1) An Exp-inc experiment which applied a standardised pre-inc temperature of 20 $^\circ$C for a period of 7 days. Soils were subsequently incubated at 15°, 20° and 25 $^\circ$C for a period of 24 h. Gas samples were taken at time intervals 0, 6 and 24 h. This experiment did not include samples collected from Greenland.
2) A Pre-inc experiment, which assessed a range of pre-inc temperatures (at 10°, 15°, 20° or 25 $^\circ$C, at the corresponding time periods – see Table 2). Soils were experimentally incubated at a standardised temperature of 20 $^\circ$C, following gas sampling T0, for a period of 24 h. Gas samples were taken at time intervals 0, 2, 6 and 24 h.

2.4. Data analysis

Cumulative CO_2 production at each time period was calculated by subtracting the ambient CO_2 concentrations at time = 0 from the corresponding CO_2 concentrations measured at time intervals 2, 6 and 24 h.

We evaluated three descriptive models to describe the observed increases in CO_2 concentration over time and to derive the initial respiration rate; these were the non-rectangular hyperbola (Eq. (1)), the Michaelis–Menten equation (Eq. (2)) and the common negative exponential curve (Eq. (3)). These models were selected as they each quantify biological scenarios where a rate variable (in our case the increase in CO_2-concentrations) changes over time before "settling" at a constant rate.

2.4.1. Non-rectangular hyperbola

Non-rectangular hyperbola, which is a complex but versatile three-parameter saturation curve that can be denoted as:

$$CO_2 = \frac{r_0 \cdot t + CO_{2,max} - \sqrt{(r_0 \cdot t + CO_{2,max})^2 - 4 \cdot q \cdot r_0 \cdot t \cdot CO_{2,max}}}{2q}$$

$$(1)$$

where CO_2 (mg CO_2 g^{-1} soil) is the quantity of CO_2 produced, t (h) is the time at which CO_2 concentration was measured, $CO_{2,max}$ (mg CO_2 g^{-1} soil) is the asymptotic maximum quantity of CO_2 produced, r_0 (mg CO_2 g^{-1} soil h^{-1}) is the initial respiration rate, and q (no dimensions) is a parameter describing the "acuteness" of the curve.

2.4.2. Michaelis-Menten equation

Michaelis–Menten equation, which is a simple two-parameter saturation curve commonly used to describe chemical and biological processes; this equation can be written as:

$$CO_2 = CO_{2,max} \cdot \frac{t}{t+K}$$

$$(2)$$

In which CO_2, $CO_{2,max}$ and t are as described above, and K (h) is the half-response time, i.e. the time at which the quantity of CO_2 concentration equates to half of the (asymptotic) $CO_{2,max}$. The Michaelis–Menten curve approaches linearity for $K \to \infty$; as such it is capable of fitting linear data.

2.4.3. Negative exponential equation

For curves originating from the origin, the common negative exponential curve can be denoted as the following two-parameter curve:

$$CO_2 = CO_{2,max} \cdot (1 - e^{c.t})$$

$$(3)$$

In which CO_2, $CO_{2,max}$ and t are as described above, and c (h) is a parameter, usually taking a negative value, describing the shape of the curve.

We fitted each of these models to each of the soils × treatments, by minimising the residual sum of squares between observations and model predictions.

3. Results

3.1. Model evaluation

A wide variety of responses of CO_2 concentration to exp-inc temperature and length of exp-inc were observed, both in terms of the magnitude of the responses and their temporal patterns. Fig. 3 shows an example of a typical response (Hungary, grassland site, loam soil, pH 8.3) of observed CO_2 concentration (diamonds), measured at $t = 6$ and $t = 24$, for the three exp-inc temperatures. Other sites followed similar patterns: in general, higher exp-inc
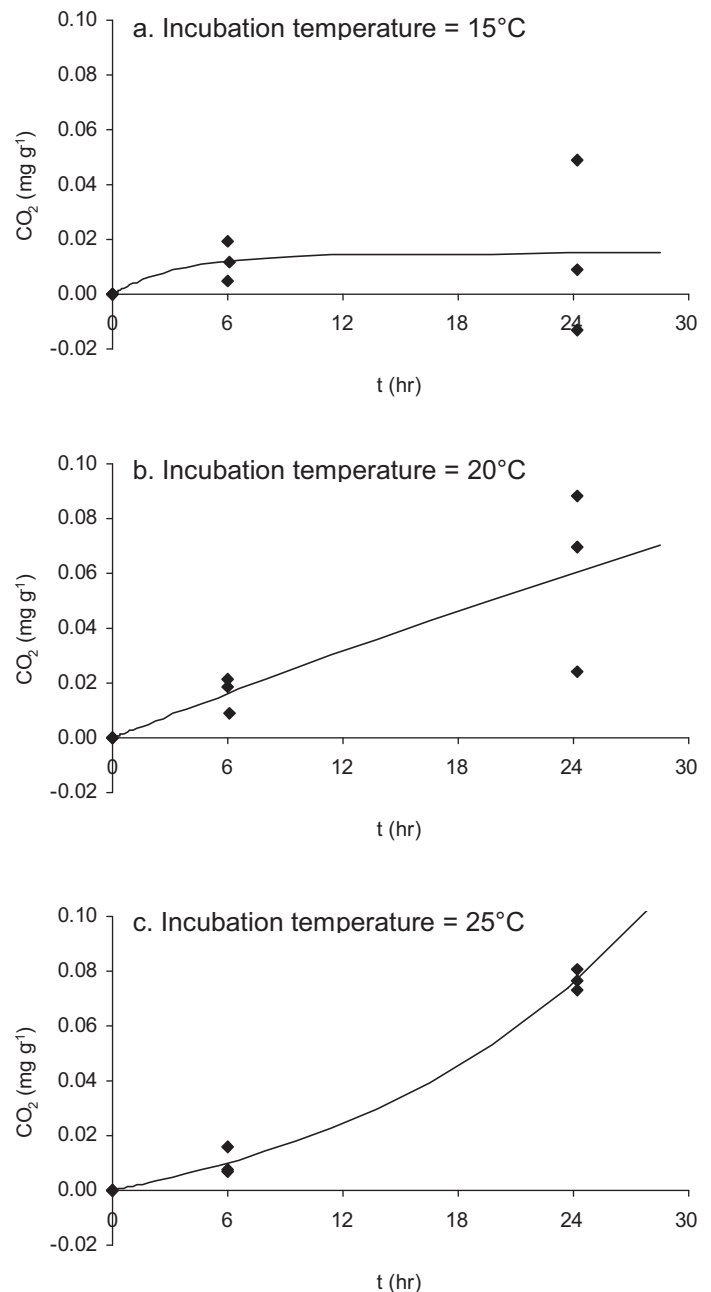

Fig. 3. An example (Hungary, grassland site, loam soil) of a typical response of CO_2 during the incubation period, for the three contrasting experimental incubation temperatures of a) 15 °C; b) 20 °C and c) 25 °C. Solid line shows best fit using the negative exponential model.

temperatures resulted in a) lower coefficients of variance on individual sampling occasions and b) a change in response type from asymptotic to linear and even exponential.

Of the three models, the non-rectangular hyperbola tended to overfit the data, as a result of its high number of free parameters. This means that a "perfect" model fit was obtained for a wide range of parameter combinations, rendering the optimised parameter values meaningless. The Michaelis–Menten equation satisfactorily described the asymptotic and linear curves, and, having only two parameters, resulted in exclusive best estimates of the parameters. However, this model failed to adequately describe the scenarios in which CO_2 concentration increases exponentially; in these scenarios the model converged to linearity, resulting in overestimation of r_0. By contrast, the negative exponential model

Table 4

Incubation temperature	Average coefficient of determination		% of samples in which MM > NE
	Michaelis–Menten (MM)	Negative exponential (NE)	
15 °C	0.239	0.224	25%
20 °C	0.679	0.686	0%
25 °C	0.789	0.817	0%

satisfactorily described all scenarios, i.e. asymptotic, linear and exponential curves; this is exemplified by the model fits in Fig. 3 and by the comparison of the coefficients of determination between the Michaelis–Menten equation and the negative exponential curve in Table 4.

Differentiation of Equation 3 and subsequent substitution of 0 for t in the derivative shows that, using this equation, the initial respiration rate (r_0) is defined by $-c \cdot CO_{2,max}$. Over time, this equation approaches the asymptotic maximum value of $CO_{2,max}$; the pace of this approach is determined by parameter c, with more negative values for c resulting in faster approaches. The negative exponential curve approaches linearity for $c \to 0$; as such it is capable of fitting linear data. Moreover, the same equation can be used to describe alternative scenarios in which CO_2 concentration accelerates over time, i.e. to describe an exponential increase. In these scenarios, parameter c takes a positive value; this results in artificially negative asymptotic values for $CO_{2,max}$; however, it can be shown that in such cases, r_0 is still accurately defined by the product of $-c \cdot CO_{2,max}$. Therefore, this model was adopted and employed for further data analysis.

3.2. Effect of exp-inc temperature and pre-inc temperature on respiration response over time

Incubation temperature had two effects on the response of CO_2 concentration over time:

1. The first effect of exp-inc was that at lower exp-inc temperatures, variation within replicates was negatively affected. At 15 °C exp-inc temperature, there was more random variation between replicates (e.g. Fig. 3). In addition, at this temperature occasional erratic responses were observed, where CO_2 concentration at $t = 24$ was significantly lower than at $t = 6$, or on occasions lower than at $t = 0$. At exp-inc temperatures of 20 °C and 25 °C, both the random variation between replicates and the incidence of erratic responses were progressively reduced. As a result the coefficient of determination for the negative exponential model increased with higher exp-inc temperatures. Fig. 4 visualises the cumulative frequency distribution of this coefficient of determination for each of the exp-inc temperatures; it shows that at 15 °C (dotted line) the coefficient was lower than 0.70 for all samples and lower than 0.50 in most cases. Exp-inc temperatures of 20 °C (dashed line) and 25 °C (solid line) resulted in higher coefficients of determination, mostly in excess of 0.50 and 0.80, respectively. Contrastingly, the pre-inc temperature did not have any effect on random variation between replicates, nor on the incidence of erratic responses. Fig. 5 shows that the coefficient of determination exceeded 0.80 for more than 75% of the samples in the pre-inc experiment, irrespective of pre-inc temperature.

2. The second effect of exp-inc temperature was that, in general, it affected the shape of the response. An exp-inc temperature of 15 °C resulted in mostly asymptotic responses, while linear and exponential responses were progressively more frequently observed at exp-inc temperatures of 20 °C and 25 °C (see Fig. 3 for a typical example). We have visualised this in Fig. 6, which shows the cumulative frequency distribution of the estimates for
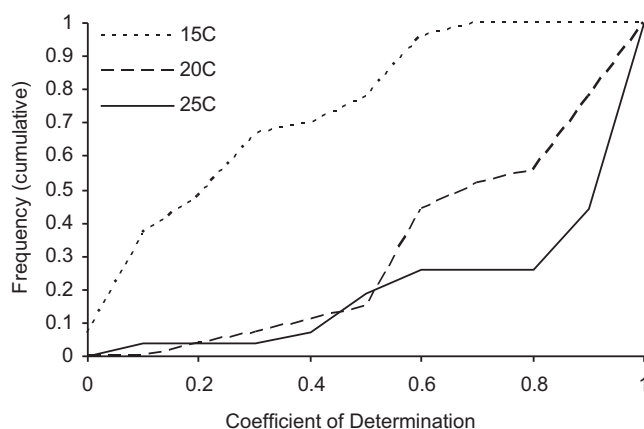

Fig. 4. Cumulative frequency distribution of coefficient of determination, as a function of incubation temperature, using the negative exponential model.
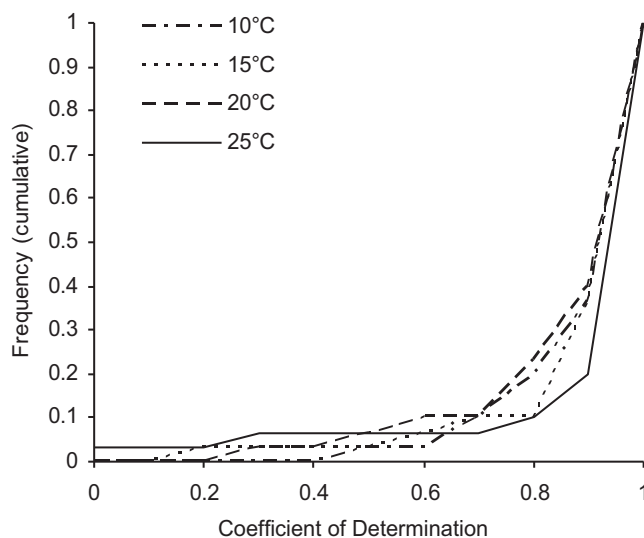

Fig. 5. Cumulative frequency distribution of coefficient of determination, as a function of pre-incubation temperature, using the negative exponential model.

parameter c of the negative exponential model. At 15 °C (dotted line) the estimate for c was negative for the majority of samples, indicating an asymptotic response. Contrastingly, at 25 °C (solid line) estimates for c were either close to zero or positive for the majority of the same samples, signifying linear and exponential responses, respectively. At 20 °C (dashed line), estimates for c were close to zero for over 50% of the samples (linear response), and either negative (asymptotic response) or positive (exponential response) for the remainder of the samples. The pre-inc temperature did not affect the shape of the CO_2 concentration response over time. Fig. 7 demonstrates that estimates for c were either negative or close to zero for most of the samples, irrespective of pre-inc temperature.
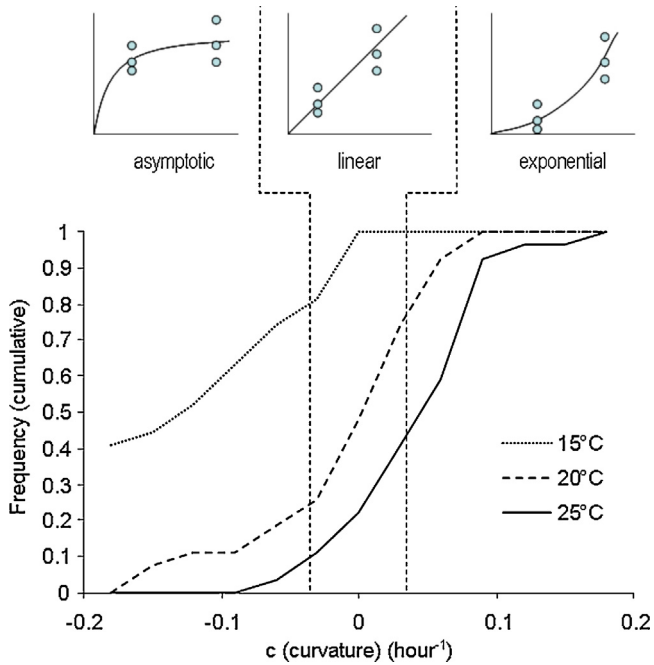
Fig. 6. Cumulative frequency distribution of parameter c, as a function of incubation temperature, using the negative exponential model. Parameter c describes the shape of the response of respiration over time.

3.3. Effect of exp-inc temperature and pre-inc temperature on initial respiration rate

The flexibility of the negative exponential model in adequately describing a variety of response types, allowed us the use this model to estimate the initial respiration rate (r_0), which, by definition, equates to $-c \cdot CO_{2,max}$. We estimated r_0 only for model fits that yielded a coefficient of determination of at least 0.50, in order to eliminate aforementioned erratic responses from further analysis. Figs. 8 and 9 visualise the distribution of r_0 as a function of temperature. Neither exp-inc temperature (Fig. 8), or pre-inc temperature (Fig. 9), had a consistent affect on the initial respiration rate.
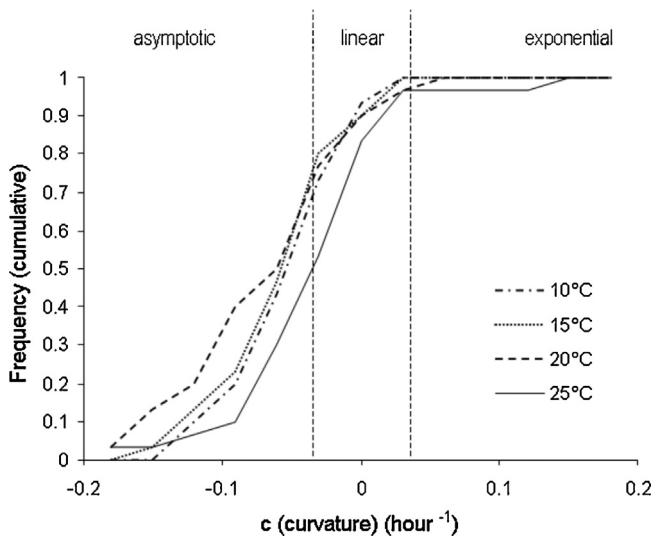

Fig. 7. Cumulative frequency distribution of parameter c, as a function of pre-incubation temperature, using the negative exponential model. Parameter c describes the shape of the response of respiration over time.
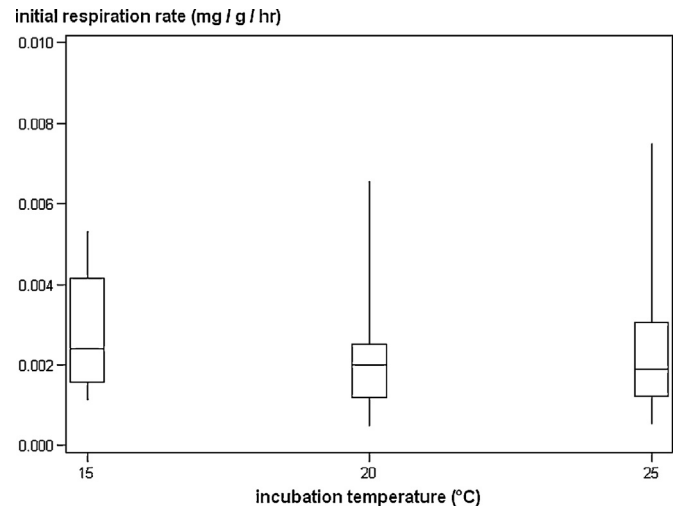

Fig. 8. Initial respiration rate, derived from the negative exponential model, as a function of incubation temperature. Line = median, box = inter-quartile range, whiskers = 95 percentile range.

3.4. Relationship between initial respiration rate and average respiration rates

We used only the data from the pre-inc experiment to test the extent to which the average respiration rate, derived from one temporal measurement, corresponds to the initial respiration rate, as estimated by the model using all temporal measurements over time. Fig. 10 visualises this relationship for estimates of the average respiration rate, measured at time intervals $t = 2$ (solid diamonds), $t = 6$ (crosses) and $t = 24$ (open squares), for each of the incubation temperatures (colours). Most of the open squares ($t = 24$) lie below the line (underestimation of initial response rate), whereas the solid diamonds and crosses both fall closer to the line ($t = 2$ and $t = 6$ respectively). While the correlation between the logarithmic transformations of the average respiration rate, established at $t = 2$ and the initial respiration rate was 0.79, the average respiration rate at $t = 2$, tended to overestimate the initial respiration rate in most cases (note logarithmic scales), resulting in a relatively large root mean square error (RMSE) of 0.00338 mg g^{-1} h^{-1}. Large deviations between the average and initial rates of respiration were observed specifically at low rates of respiration. The correlation between the
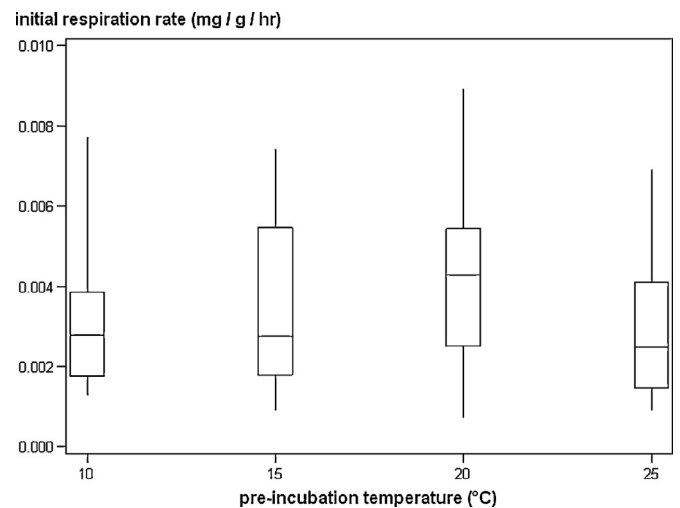

Fig. 9. Initial respiration rate, derived from the negative exponential model, as a function of pre-incubation temperature. Line = median, box = inter-quartile range, whiskers = 95 percentile range.
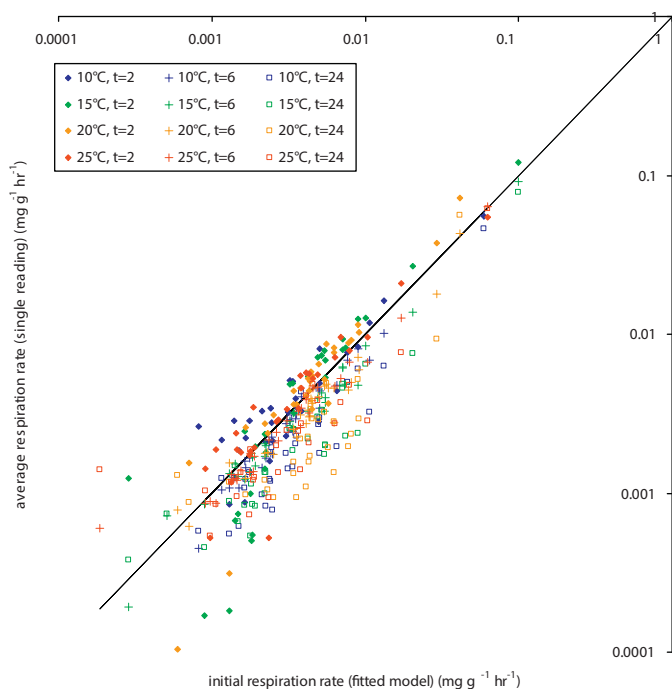
Fig. 10. Relationship between initial respiration rate, derived by fitting the negative exponential model, using all readings, and average respiration rate derived from single readings at $t = 2$ (solid diamonds), $t = 6$ (crosses) and $t = 24$ (open squares) for contrasting pre-incubation temperatures (colours). Note logarithmic scales on both axes.

logarithmic transformations of the average respiration rate, established at $t = 6$ and the initial respiration rate was higher and equated to 0.95, with a lower RMSE of $0.001994 \, \mathrm{mg \, g^{-1} \, h^{-1}}$, although the average respiration rate at this time interval consistently underestimated the initial respiration rate. The average respiration rate measured at $t = 24$, gave rise to even larger underestimations of the initial respiration rate, with a weaker logarithmic correlation of 0.75 and higher RMSE of $0.00423 \, \mathrm{mg \, g^{-1} \, h^{-1}}$.

4. Discussion

4.1. Model application

Descriptive models were used to calculate initial rates of basal respiration due to the non-linear response of CO_2 concentrations over the exp-inc period. Of the three models evaluated in this paper, we selected the negative exponential model as the most versatile and robust model to accurately describe the wide variety of observed rates of basal respiration. However, care should be taken in employing the negative exponential model, as its parameters require manual initialisation for the optimisation process, i.e. it requires the data analyst to set arbitrary values for the parameters at the start of the computerised optimisation process. This initialisation should pay particular cognisance to the discontinuity in the value of $CO_{2,max}$ as a function of the value of c: if c is negative, then $CO_{2,max}$ must, by definition, be positive. As c approaches 0, $CO_{2,max}$ asymptotically approaches infinity for linear responses of the respiration rate, switching to negative values as soon as c exceeds 0. In practice this means that the $CO_{2,max}$ must be correctly initialised by choosing either a positive or negative initialisation value, depending on the shape of the observed respiration response. Where linear responses are observed, this choice can be ambiguous. In such cases we conducted the optimisation process twice, i.e. once with a positive initial value for $CO_{2,max}$ and a negative initial value for c, and

vice versa, and selected the outcome which produced the lowest residual sum of squares.

4.2. Effect of exp-inc temperature and pre-inc temperature on initial respiration rates

We did not observe any obvious or consistent effects of either exp-inc temperature or pre-inc temperature on the initial respiration rates (Figs. 8 and 9). This implies that initial respiration rates are not directly impacted by exp-inc or pre-inc temperature. However, exp-inc at 15 °C gave rise to anomalous respiration responses, and large variation between replicates, for a large number of samples originating throughout the variety of pedo-climatic zones. This research agrees with the recommended protocol of the International Organization for Standardization ISO 16072:2002, which proposes that an experimental incubation temperature should be used within the range of 20–30 °C. Similarly Kirschbaum (1995) reported a temperature sensitivity as expressed by Q10 calculated from a comprehensive literature survey, was far greater at low <10 °C compared to moderate to high temperatures of 20–30 °C. Therefore, we do suggest caution at applying temperatures below 20 °C, as this may result in large variation between replicates, when assessing the initial rate of basal respiration. Following the pre-inc recommendations from Jones et al. (2008), this research found no effect of pre-inc temperature on initial rates of basal respiration.

The samples used in this study were derived from a wide variety of pedo-climatic regions across Europe, and displayed an equally wide array of soil properties and land-use types. Nevertheless, we did find a consistent effect of the exp-inc temperature on the shape of the initial respiration response; in other words, on the change in initial respiration rate during the exp-inc period. At lower exp-inc temperatures (15 °C), respiration tended to slow down within the duration of the exp-inc period, giving rise to asymptotic response curves. At an exp-inc temperature of 20 °C the initial respiration rate tended to remain constant within the 24 h period, resulting in linear responses, whereas an exp-inc temperature of 25 °C tended to accelerate the initial rate of respiration within the 24 h period, leading to exponential responses. It is somewhat surprising that this phenomenon was consistent for the majority of samples, irrespective of their pedo-climatic zone of origin. The mechanism behind the phenomenon is yet unclear: in biology an asymptotic response curve commonly signifies exhaustion of a limited resource, whereas an exponential response commonly indicates either population growth, or additional provision of growth-limiting resources over time (e.g. Schulte et al., 2003). From first principles, we expected higher exp-inc temperatures to lead to more rapid depletion of easily available C resources; hence we expected asymptotic responses to be more prevalent at higher temperatures. In this study however, we found the opposite to be the case. One potential explanation is niche-differentiation within microbial communities in relation to temperatures, with heat-tolerant communities having access to larger quantities of substrate resources, this may be reflected by the time of year that samples were taken, in this case June. Alternatively, it is conceivable that the shapes of the curves reflect the differences between the pre-inc temperature and the exp-inc temperatures. During the experimental incubation experiment all treatments were preincubated at 20 °C, therefore the asymptotic response at exp-inc temperatures of 15 °C could merely reflect the "slowing down" of the respiration rate while the sample cools down from 20 °C to 15 °C during incubation, while, symmetrically, the exponential response at exp-inc temperatures of 25 °C would merely reflect the acceleration of the respiration rate while the sample heats up from 20 °C to 25 °C. Hamdi et al. (2011) reported a similar heating effect causing an increase in the soil respiration rate over time in a similar study looking at the impact of pre-incubation (basal)

temperatures of 20 to 50 °C on soil sampled at an experimental research station in North-west Tunisia. In experiments for the assessment of initial basal respiration, such artefacts arising from samples "cooling down" and "heating up" during the incubation could be negated by applying identical pre-inc and exp-inc temperatures.

4.3. Optimum duration of incubation

In this study, we measured CO_2 concentration at multiple intervals during the exp-inc period. This enabled us to reliably estimate the initial respiration rate and detect temporal changes in initial respiration rate throughout the exp-inc period. However, multiple measurements of CO_2 concentration are often associated with challenges in terms of logistics and resource requirements, in particular in monitoring applications and large scale experiments. In this paper, we demonstrated that, even in such cases, there is significant merit in temporal measurements to model the shape of the curve of the initial response rate. Nevertheless, in this paper we also showed that, where resources or logistical arrangements are limiting, the initial respiration rate can be satisfactorily estimated from the average respiration rate, measured at a single point in time. However, in this case, length of the exp-inc period, (the time interval of CO_2 concentration measurement), is pivotal for the accuracy of this estimation. Our data, based on samples from 27 sites from contrasting pedo-climatic regions across Europe, shows that the initial respiration rates, calculated from average respiration rates from measurements at T24, will significantly underestimate the response. The initial respiration rates calculated from average respiration rates measured after 2 and 6 h slightly overestimated and underestimated the response respectively, with the 6 h measurement giving the lowest root mean square error.

This suggests that, for the purpose of a single point estimation of the initial respiration rate, the optimum length of the incubation period is likely to be between 4 and 6 h. This agrees with the findings of Campbell et al. (2003), who suggested an experimental incubation period of 6 h when assessing multiple substrate induced respiration (which includes a basal measurement); this has since been applied by numerous studies for basal respiration (e.g. Bundy et al., 2004 and Vanhala et al., 2005).

5. Conclusions

We have demonstrated in this study that the measurement of initial rate of basal soil respiration is most consistent at an experimental incubation temperature of 20 °C and 25 °C, with large variation between sample replicates found at 15 °C. This is consistent with the recommendations of ISO 16072:2002. However, we do see a change in the shape of the initial respiration curve with differing exp-inc temperatures, suggesting a possible shift in the microbial community. This study found that the time interval/temperature combinations recommended for pre-incubation of soils prior to soil basal respiration have no effect on initial respiration rates, though it may be prudent to equalise the pre-inc temperature to the exp-inc temperature to avoid the cooling down or heating up of the sample during the exp-inc period. Finally, if only a single point estimation of the initial respiration rate can be measured, the optimum length of the exp-inc period is between 4 and 6 h.

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