THIS MANUSCRIPT HAS BEEN PUBLISHED IN **SOIL BIOLOGY AND BIOCHEMISTRY**

The published version can be viewed at:

http://www.journals.elsevier.com/soil-biology-and-biochemistry/

Substrate quality and the temperature sensitivity of soil organic matter decomposition

Regular paper

29th August 2007

Pages: 29

Tables: 0

Figures: 6

Iain P. Hartley[a,b,*], Phil Ineson[a]

[a]*Department of Biology, Stockholm Environment Institute (SEI-York centre), University of York, York, YO10 5YW, UK*

[b]*Current address: School of Biological and Environmental Sciences, University of Stirling, Stirling, FK9 4LA, UK*

[*]*School of Biological and Environmental Sciences, University of Stirling, Stirling, FK9 4LA, UK*. Tel: +44 1786 467757; fax: +44 1786 467843.

E-mail address*:* i.p.hartley@stir.ac.uk

Substrate quality and the temperature sensitivity of soil organic matter decomposition

Abstract

Determining the relative temperature sensitivities of the decomposition of the different soil organic matter (SOM) pools is critical for predicting the long-term impacts of climate change on soil carbon (C) storage. Although kinetic theory suggests that the temperature sensitivity of SOM decomposition should increase with substrate recalcitrance, there remains little empirical evidence to support this hypothesis. In the study presented here, sub-samples from a single bulk soil sample were frozen and sequentially defrosted to produce samples of the same soil that had been incubated for different lengths of time, up to a maximum of 124 days. These samples were then placed into an incubation system which allowed CO_2 production to be monitored constantly and the response of soil respiration to short-term temperature manipulations to be investigated. The temperature sensitivity of soil CO_2 production increased significantly with incubation time suggesting that, as the most labile SOM pool was depleted the temperature sensitivity of SOM decomposition increased. This study is therefore one of the first to provide empirical support for kinetic theory. Further, using a modelling approach, we demonstrate that it is the temperature sensitivity of the decomposition of the more recalcitrant SOM pools that will determine long-term soil-C losses. Therefore, the magnitude of the positive feedback to global warming may have been underestimated in previous modelling studies.

Keywords: soil organic matter, temperature, labile, recalcitrant, CO_2, respiration, climate change, feedback

1. Introduction

Modelling studies have suggested that C sequestration in terrestrial ecosystems may be undermined by the positive response of SOM decomposition to temperature (Cox et al., 2000; Jones et al., 2005). In fact simulations have shown that temperature-induced soil-C losses could accelerate the rate of global warming by up to 40 % (Cox et al., 2000). These predictions are, firstly, highly dependent on the exact parameterization of the response of SOM decomposition to temperature (Jones et al., 2003), and, secondly, based on the assumption that the decomposition of all the C stored in soils is equally sensitive to temperature (Jones et al., 2005).

Contrary to the latter assumption, two highly cited studies concluded that the decomposition of older, more recalcitrant SOM is insensitive to temperature (Liski et al., 1999; Giardina and Ryan, 2000). Based on the amount and age of C stored in the soils along a temperature gradient, Liski et al. (1999) argued that the decomposition of old soil organic matter is insensitive to the influence of temperature. Further, by reviewing the available literature, Giardina and Ryan (2000) demonstrated that the decomposition of SOM in mineral soils was controlled more by substrate quality than temperature. However, Ågren (2000) argued that the results of Liski et al. (1999) may be due to particular properties of the model used (substrate quality changed directly as a function of time and, due to fixed residence times and

temperature sensitive respiration rates, the rate of transfer between model compartments varied with temperature), differences in litter decomposability across the temperature gradient and difficulties associated with ^{14}C dating SOM. In addition, Knorr et al. (2005) demonstrated that the conclusions of Giardina and Ryan (2000) may have been caused by a failure to take into account the heterogeneity (different pools) of SOM, while Ågren & Bosatta (2002) demonstrated that the relationship between SOM turnover times and temperature is not the same as the temperature response of a given soil and as such the results of Giardina and Ryan (2000) were not indicative of a short-tern temperature response. The confusion in the literature can be summarised by the fact that analyses of similar datasets have produced the contrasting conclusions that recalcitrant SOM decomposition is less temperature sensitive (Giardina and Ryan, 2000), equally temperature sensitive (Reichstein et al., 2005a), or more temperature sensitive (Knorr et al., 2005) than labile SOM decomposition.

More recently, empirical evidence from incubation studies has supported model assumptions by suggesting that there is no difference in the temperature sensitivity of the decomposition of labile and recalcitrant SOM (Fang et al., 2005; Reichstein et al., 2005b; Conen et al., 2006). However, kinetic theory predicts that the temperature sensitivity of decomposition should increase with substrate recalcitrance (Bosatta and Ågren, 1999; Davidson and Janssens, 2006); the higher activation energy associated with the breakdown of recalcitrant substrates should result in a greater temperature sensitivity of decomposition. This logic appears to be supported by measurements of the temperature sensitivity of leaf litter decomposition (Fierer et al., 2005) but there remains little evidence from soil studies.

When soils are removed from the field, the links to photosynthesizing tissues are severed and the input of labile substrates stopped. Due to inherent differences in the turnover times of different carbon pools, labile substrates are progressively depleted during the course of laboratory incubation (Townsend et al., 1997). Recent studies have used this logic to demonstrate that the decomposition of recalcitrant substrates is indeed temperature sensitive but they have failed to identify any differences between recalcitrant and labile pools (Fang et al., 2005; Reichstein et al., 2005b). However, the failure to detect significant differences could be due to large inter-sample variability or errors associated with the measurement of soil CO_2 production between the different time points (Davidson and Janssens, 2006).

We have used a novel experimental design in which sub-samples taken from the same initial soil sample were frozen and then sequentially defrosted to provide replicates that could be incubated at 15^{o}C for different periods of time. This allowed for direct and simultaneous comparison of temperature responses between the different samples without the problems associated with changes in potentially confounding factors, such as drift in incubator temperatures or flow rates, between measuring dates. The high-precision incubation system we used allowed statistically significant differences in CO_2 production rates to be identified even when the magnitude of such effects were small, allowing us to determine whether the temperature sensitivity of SOM decomposition changed with incubation time. Finally, to determine the implications of the results of our incubation study we carried out a modelling analysis investigating the effect of changing the temperature sensitivity of the decomposition of different soil-C pools on total soil-C losses.

2. Materials and Methods

2.1. Soil sampling and preparation

On September 1st 2004, 10 kg of soil were removed from the experimental garden at the University of York, UK. Soil comprising the upper horizons of the Escrick series (Matthews, 1971), was brought into this facility ~50 years ago, since when it has been repeated mixed whilst under cultivation with a variety of plant species. It is a sandy loam with a pH of 6.5 and a carbon content of 4 %. The soil was sieved through a 2 mm mesh and corrected to a gravimetric moisture content of 20 %. The main soil sample was then subdivided and samples were frozen at -20^{o}C. Sub-samples were then defrosted sequentially with an interval of approximately 5 weeks between four defrost dates, providing five 400 g samples on each date (a total of 20 samples). The defrosted samples were incubated in a constant temperature room at 15 ^{o}C and maintained at a moisture content of 20 % with frequent water addition. On February 2nd 2005, seven days after the final defrost date, the soil samples were added to the incubation system.

2.2. Respiration measurements

A temperature-controlled incubation system was constructed at the University of York which allowed frequent measurements of respiration of up to 20 soil samples to be made. An infra-red gas analyzer (ADC-225 MK3, ADC Bioscientific Ltd., Herts, UK) connected to a 24-channel gas-handling unit (Model: WA-161, ADC

Bioscientific Ltd., Herts, UK) was used to measure the CO_2 concentration in each of 24 lines, connected to individual incubation chambers, with a sampling frequency of once every 2 hours. The flow rate of air through each line was maintained at 50 cm^3 min^{-1} throughout and was monitored with a digital flow meter (Model: GFM 171, 0-500 cm^3 min^{-1}, Aalborg Instruments and Controls Inc., New York, USA). Soil samples were added to 20 of the chambers with the final four left empty to allow the CO_2 concentration of the incoming air to be measured. Respiration rates were calculated based on the mass of soil incubated (dry weight measured at end of incubation), the flow rate through the lines (50 cm^3 min^{-1}) and the difference between the CO_2 concentration in the incoming air and the CO_2 concentration in each of the soil lines. The temperature of the soils in the incubation chambers was controlled by a heating, large volume water bath maintained within a dedicated cold room, with a precision of temperature control of 0.1 ^oC (Electronics Workshop, Biology Department, University of York, UK).

The response of soil CO_2 production to temperature was determined by first increasing soil temperatures from 10 ^oC to 15 ^oC to 20 ^oC before reducing the temperature back to 15 and 10 ^oC. The mean rate at each temperature was then calculated allowing any fluctuations in the baseline rate of respiration to be included in the calculation of the temperature response (Fang et al., 2005). Each temperature was maintained for a total of 48 hours to allow respiration rates to stabilise. These temperatures are regularly experienced during the growing season in the experimental garden at the University of York (Hartley et al., 2007).

2.3. Model

A two-pool SOM decomposition model was constructed. No passive or inert pool was included, with the recalcitrant pool in this model mainly representing the slow pool *sensu* CENTURY (Parton et al., 1987) or the humus pool *sensu* RothC (Jenkinson, 1990). Equation 1 describes the dynamics of the labile pool. C enters the labile pool at a constant rate while decomposition losses are dependent on temperature and the size of the labile SOM pool.

$$dC_l/dt = -k_l Q_{10l}^{(T-Tref)/10} C_l + I \qquad \text{(Equation 1)}$$

Where k_l is the rate constant applied to the decomposition of labile SOM, Q_{10l} is the Q_{10} value assigned to labile SOM decomposition, C_l is the size of the labile pool and I is the rate of input into the labile pool.

Equation 2 describes the dynamics of the recalcitrant pool. C enters the recalcitrant pool as a function of the rate of decomposition occurring in the labile pool, while decomposition losses are again dependent on temperature and the size of the recalcitrant SOM pool.

$$dC_r/dt = -k_r Q_{10r}^{(T-Tref)/10} C_r + hk_l Q_{10l}^{(T-Tref)/10} C_l \qquad \text{(Equation 2)}$$

Where k_r is the rate constant applied to the decomposition of recalcitrant SOM, Q_{10r} is the Q_{10} value assigned to recalcitrant SOM decomposition, C_r is the size of the

197 recalcitrant pool and *h* is the fraction of labile substrate converted to recalcitrant
198 material.

199 Based on the soil incubated in the study presented above, the total C content of
200 the soil was 4 % and the initial rate of heterotrophic (microbial) soil respiration was
201 set to 7.2 μg C gdw^{-1} day^{-1}. It was not possible to determine the size of the labile SOM
202 pool from our incubation study as labile substrates appeared to be depleted throughout
203 (see below). Therefore, to reflect pool sizes used in similar modelling studies, the
204 large recalcitrant SOM pool was assumed to represent 95% of soil C (Kirschbaum,
205 2004; Eliasson et al., 2005; Knorr et al., 2005; Rey & Jarvis, 2006), although the
206 effect of varying the size of the labile SOM pool between 5 and 15 % of total SOM
207 was also investigated.

208 Two temperature scenarios were investigated: 1) ambient temperature and 2)
209 ambient + 3^{o}C. To reflect the incubation, the temperature in the ambient scenario was
210 considered to be 15^{o}C, which is higher than the mean annual temperature in York but
211 may take into account the exponential relationship between temperature and
212 respiration (the mean annual rate of respiration is often higher than the respiration rate
213 at the mean annual temperature in non-water stressed environments). However, as the
214 main aim of the modelling investigation was to determine which parameters are most
215 important in determining soil C-losses in response to warming, rather than to precisely
216 quantify losses, the assigning of the ambient temperature was not critical.

217 Respiration rates and the sizes of the different SOM pools were recalculated
218 on a daily time step and no seasonal changes in temperature or substrate input were
219 included in the model. In the ambient temperature scenario, C inputs exactly equalled
220 C losses from the labile pool through respiration and C transfer to the recalcitrant pool

($I = k_l C_l$), which in turn exactly balanced respiratory losses from the recalcitrant pool ($hk_l C_l = k_r C_r$). Under the scenario in which temperature was increased by 3°C, the rates of decomposition were altered by the Q_{10} values assigned to each pool.

Given the uncertainty in the exact parameterization of the temperature sensitivities of recalcitrant and labile SOM decomposition from our empirical data (see below), the model was used to determine how sensitive total soil-C losses were to varying the temperature sensitivity of decomposition between Q_{10} values of 2 and 4, firstly for the large recalcitrant SOM pool and secondly for the smaller labile SOM pool. In conjunction with these simulations the relative contributions of the recalcitrant and labile SOM pools to total heterotrophic soil respiration (microbial respiration *i.e.* not including roots) were also varied. This was achieved by altering the rate constants k_l and k_r, with h also having to vary to maintain steady state conditions in the ambient scenario.

The sensitivity analysis investigated the effect of varying the different parameters after two different timescales, 1 and 20 years. The 1 year timescale is potentially relevant to short-term field experiments, while the 20 year timescale is relevant in terms of decadal responses of soil respiration to climate change. However, it was also possible to determine steady state pool sizes and the time period required for the pools to approach these new steady state conditions.

The steady state pool sizes for the labile and recalcitrant pools can be determined from Equations 3 and 4, respectively, where C_{lss} and C_{rss} are the steady state pool sizes.

$$C_{lss} = I / (k_l Q_{10l}^{(T\text{-}Tref)/10}) \qquad \text{(Equation 3)}$$

$$C_{rss} = hI/(k_r Q_{10r}^{(T\text{-}Tref)/10}) \quad \text{(Equation 4)}$$

The rates at which the labile and recalcitrant pools arrive at new steady state conditions were determined by Equations 5 and 6, respectively, where T_{lss} and T_{rss} represent the times taken to approach steady state. The sensitivity analyses could then be placed within the context of longer-term dynamics. However, it should be emphasised that these equations only indicate the time taken to approach steady state conditions, and, in fact, only ~63 % of C losses from each pool have occurred over the time scales determined by these equations.

$$T_{lss} = 1/(k_l Q_{10l}^{(T\text{-}Tref)/10}) \quad \text{(Equation 5)}$$

$$T_{rss} = 1/(k_r Q_{10r}^{(T\text{-}Tref)/10}) \quad \text{(Equation 6)}$$

Finally, a second, slightly modified, version of the model was constructed. Many SOM decomposition models (e.g. RothC (Jenkinson, 1990) and CENTURY (Parton et al., 1987)) apply temperature functions to intrinsic turnover rates associated with the different SOM pools. The model presented above, investigated the effect of altering the relative contributions of recalcitrant SOM and labile SOM to total heterotrophic soil respiration. As the initial C pool sizes were not altered during these simulations, it could be argued that it was effectively the turnover times of the different SOM pools that were being manipulated. In the modified model, the sizes of the two pools (C_l and C_r) were modified so that the mean residence times remained constant when the relative contributions to total heterotrophic respiration were varied in the ambient scenario (mean residence times changed temperature).

2.4. Data analysis

Statistical analyses were carried out using SPSS (Version 11, SPSS Science, Birmingham, UK). One-way ANOVAs were used to determine whether the rate of CO_2 production and the temperature responses differed between the samples that had been incubated for different lengths of time.

3. Results

3.1. Temperature sensitivity of soil respiration

As expected, the rate of soil respiration declined significantly with increasing incubation time ($P < 0.001$), reflecting the fact that the most labile substrates were progressively depleted (Fig. 1). However, the temperature sensitivity of respiration, expressed as a Q_{10}, increased significantly with incubation time (Fig. 2). Based on the reduction in the rate of respiration between days 7 and 124, the contribution of the most labile SOM pool to respiration in the samples that had been incubated for the shortest time was estimated to be approximately 45%. Q_{10} values for "labile" and "recalcitrant" SOM decomposition could then be calculated, by mass balance, assuming the Q_{10} of the 124 day-incubated samples represented recalcitrant SOM decomposition. The calculated Q_{10} values were 2.85 and 3.25, respectively. However, a significant difference was observed in the temperature sensitivity of the respiration of samples incubated for 50 and 124 days despite the rate of respiration declining by

293 only ~12 % between these two periods (Figs. 1 and 2). This suggests that the type of
294 substrate being utilised was changing even after respiration rates had become
295 relatively constant and that labile substrates were being depleted throughout.
296 Therefore, the labile pool probably contributed more than 45 % of initial respiration
297 and magnitude of the difference in Q_{10} values between the two pools is probably
298 underestimated.
299
300 *3.2. Model results*
301
302 The modelling exercise was designed to determine the implications of the
303 apparent relationship between substrate quality and the temperature sensitivity of
304 SOM decomposition observed in the incubation study. When simulations were run for
305 1 year, the temperature sensitivity of both labile SOM decomposition (Fig. 3a) and
306 recalcitrant SOM decomposition had a major effect on the magnitude of soil-C losses
307 (Fig. 3b). However, after 20 years only recalcitrant SOM dynamics were important in
308 determining temperature-induced C losses. The temperature sensitivity of recalcitrant
309 SOM decomposition had a major effect on total soil C losses after twenty years of
310 enhanced soil temperatures (Fig. 4a), especially in simulations in which recalcitrant
311 SOM decomposition contributed substantially to total heterotrophic respiration.
312 In contrast, the temperature sensitivity of labile SOM decomposition played
313 only a minor role in determining C losses after 20 years (Fig. 4b). In addition, almost
314 identical results were produced when the sizes of the two pools were altered to reflect
315 the changes in their contributions to respiration, so maintaining the mean residence
316 time of C in each pool (modified model, data not shown). The temperature sensitivity

317 of recalcitrant SOM decomposition and its relative contribution to total heterotrophic
318 soil respiration, this time altered through changes in pool size, remained the key
319 determinants of soil C losses after 20 years, with the temperature sensitivity of the
320 decomposition of the labile pool having little effect.

321 The importance of recalcitrant SOM dynamics can be further illustrated by
322 showing how the contributions of the two pools to soil-C losses changed over time.
323 Within two years of imposing the 3^{o}C warming treatment, losses of C from the
324 recalcitrant pool exceeded labile pool C-losses, and, after approximately 5 years,
325 losses of C from the labile pool had ceased (Fig. 5). In addition, altering the size of
326 the labile SOM pool had relatively little effect on soil C losses after 20 years; tripling
327 the size of the labile C pool (from 5-15 % of SOM) increased C losses from 9.4 to
328 11.6 % of total soil C. However, the size of the labile SOM pool was found to control
329 the speed with which soil respiration rates declined following the onset of the
330 warming treatment. Increasing the size of the labile SOM pool reduced the rate of the
331 decline in the initial positive response of soil respiration to elevated temperature
332 (Fig. 6).

333 In terms of steady state conditions, when Q_{10} values were increased from 2
334 to 4, total losses increased from 18.8 % to 34.0 % of the C stored in each pool, while
335 the rate at which steady state conditions were approached also increased by 23 %. In
336 the labile pool steady state conditions were approached within 263 to 324 days,
337 although, as shown in Figs. 5 and 6, it took considerably longer for the final
338 equilibrium to be reached. The contribution of the recalcitrant pool to total
339 heterotrophic respiration also affected the rate at which steady state conditions were
340 reached, but had no effect on total C losses. Increasing the contribution from 5 to

50 % increased the rate at which the steady state was approached by one order of magnitude (average of 262 and 26.2 years, respectively), highlighting the importance of determining the mean residence time of SOM in the recalcitrant pool for predicting the rate at which C will be lost from soils. Steady conditions in the recalcitrant pool were not approached within the 20 year period investigated in the sensitivity analysis in any of the simulations carried out.

4. Discussion

4.1. Substrate chemistry and the temperature dependence of decomposition

In our study it appeared that as the most labile substrates were used up, the temperature sensitivity of soil respiration increased (Figs. 1 and 2). The magnitude of this change in the temperature sensitivity of soil respiration was relatively low yet significant differences were observed (Fig. 2). Even after respiration rates had become relatively constant, the temperature sensitivity of soil respiration continued to increase (Figs. 1 and 2) suggesting that the quality of the substrates being utilised was changing throughout. As SOM represents a continuum of substrates of differing recalcitrance it is debateable as to whether the temperature sensitivity of truly recalcitrant SOM decomposition can investigated by relatively short-term incubations; directly determining the temperature sensitivity of the decomposition of SOM, with turnover times of hundreds or thousands of years, would require an extremely long-term incubation. Therefore, the we propose that the calculated Q_{10} values probably still underestimate the temperature sensitivity of truly recalcitrant SOM

365 decomposition, although this suggestion requires extrapolation of our results beyond
366 the range of substrate recalcitrance that we were able to directly measure.
367 Over the course of our incubation, as labile substrates were progressively
368 depleted, there was the potential for shifts in microbial community structure. The
369 flush of available substrates immediately after defrosting may have selected for a
370 community of *r*-selected microbes (Fierer et al., 2007) whose decomposition activities
371 may have differed in their response to temperature as compared with the more *K*-
372 selected community which may have developed subsequently. Had significant
373 differences in the temperature sensitivity of respiration only existed between time 7
374 and the other dates, then it could have been argued that microbial community
375 adaptation was primarily responsible for the observed patterns. In our study, the lack
376 of a significant difference between time 7 and time 50 as compared with the
377 significant difference between time 50 and time 124 (Fig. 2) suggest that differences
378 were generated slowly and continuously over time which is consistent with changes in
379 substrate chemistry being the main driver. However, it remains extremely difficult to
380 determine whether substrate chemistry *per se* or differences in the temperatures
381 sensitivities of the microbial communities adapted to decompose the different
382 substrates, determined the pattern observed in this study.
383 Studies using both stable and radioactive C isotopes, have attempted to
384 determine whether the contribution of older, more recalcitrant SOM to soil CO_2
385 production changes with incubation temperature. These studies have generally
386 identified increases (Biasi et al., 2005; Bol et al., 2003; Waldrop and Firestone, 2004;
387 Vanhala et al., 2007) or no change (Conen et al., 2006; Czimczik and Trumbore,
388 2007; Dioumaeva et al., 2002) in the contributions of older SOM at higher

389 temperatures. However, where increased contributions have been observed it has not
390 been clear whether these results were caused by shifts in substrate utilisation patterns
391 or by differences in the temperature responses of young versus old SOM
392 decomposition, and differences in the intrinsic stability of material derived from the
393 different plants types may limit the utility of C_3-C_4 plant shifts (Wynn and Bird,
394 2007).

395 The results presented here provide empirical evidence that the temperature
396 sensitivity of SOM decomposition increases with substrate recalcitrance, and so
397 represent one of the first studies to directly support kinetic theory (Bosatta and Ågren,
398 1999; Davidson and Janssens, 2006). The results are also in agreement with the study
399 of Leifeld and Fuhrer (2005) which measured the temperature sensitivity of the
400 decomposition of separate SOM fractions. Their data suggested that Q_{10} values
401 increased dramatically as substrate quality declined, although the fractionation
402 procedure undertaken was extremely destructive and substantially altered the physical
403 properties of the soil (Conen et al., 2006). In contrast to our results, a study, which
404 modelled the temperature-sensitivity of decomposition based on long-term incubation
405 of soils at different temperatures, suggested that Q_{10} values decreased as substrates
406 became more recalcitrant (Rey and Jarvis 2006). As incubation temperature may
407 affect the way in which material decomposes (Ågren & Bosatta, 2002; Dalias et al.,
408 2001), parallel incubations at different temperatures may fail to determine the actual
409 relationship between substrate chemistry and the temperature sensitivity of SOM
410 decomposition. Determining the reasons for the discrepancies observed between
411 studies utilising different methodologies remains of key importance (Kirschbaum,
412 2006).

Previous studies which have utilised similar methodologies as in the study presented here have demonstrated that the decomposition of more recalcitrant SOM is highly temperature sensitive but have failed to identify a change in the temperature sensitivity of CO_2 production with incubation time (Fang et al., 2005; Reichstein et al., 2005b). There are a number of possible explanations for this discrepancy. Firstly, in contrast to the study of Fang et al., (2005), we measured the response of CO_2 production to changes in temperature across a relatively narrow range regularly experienced by the soil. Secondly, in contrast to the study of Reichstein et al. (2005b), our slower temperature fluctuations resulted in the entire soil sample experiencing a common temperature while respiration measurements were made. Thirdly, given the relatively small magnitude of the changes in the temperature response, the high-precision incubation system utilised here (see tight error bars Fig. 1) may have permitted significant differences to be detected that have not been previously possible. Finally, it should be emphasises that our results were derived from a single mineral soil type. The extent to which this relationship holds true across soils differing in physical and chemical properties requires further research (Rasmussen et al., 2006).

4.2. Model implications

Many soil decomposition models take into account the wide range of substrates present in SOM by modelling SOM dynamics using a series of C pools with different intrinsic turnover times (e.g. CENTURY (Parton et al., 1987) and RothC (Jenkinson, 1990)). However, these models generally apply the same temperature functions to the decomposition of each pool, regardless of whether the substrates present are assumed

437 to be predominantly labile or recalcitrant. The aim of our modelling study was to
438 determine how sensitive C-loss estimates are to uncertainty in the temperature
439 sensitivity of the decomposition rates of the different pools.

440 The modelling analysis highlighted the temperature sensitivity of the large
441 recalcitrant SOM pool as a critical parameter in determining long-term soil-C losses
442 (Fig. 4). As the incubation study suggested that the temperature sensitivity of SOM
443 decomposition increases with substrate recalcitrance, and given that most SOM
444 decomposition models have mainly been parameterised by short-term incubations and
445 field studies (which are likely dominated by mainly labile SOM dynamics), it seems
446 that global warming-induced soil-C losses may have been underestimated in previous
447 studies (e.g. Jones et al., 2005).

448 As the labile pool rapidly approached new steady state conditions (Figs. 5
449 and 6), the temperature sensitivity of labile SOM decomposition had little effect on
450 soil-C losses after a modelled twenty year period. However, the size of the labile
451 SOM pool did determine the rate at which the initial positive response of soil
452 respiration to the increase in temperature declined. In agreement with other modelling
453 studies (Kirschbaum, 2004; Eliasson et al., 2005; Knorr et al., 2005), this indicates
454 that it is the labile pool dynamics that control the apparent thermal acclimation of soil
455 respiration. Therefore, even investigations into how soil respiration responds to
456 relatively long-term soil warming (1-5 years) will mainly provide information on the
457 size of the labile pool but may tell us little about the potential for long-term C losses
458 (Fig. 6).

459 Our sensitivity analyses investigated which factors determine soil-C losses
460 after a fixed amount of time (1 or 20 years). In terms of steady state conditions,

461 although the recalcitrant pool takes a longer time to arrive at a new equilibrium, the
462 effect of temperature on the proportion of C lost is identical between the labile and
463 recalcitrant pool; when the Q_{10} value was increased from 2 to 4, total C losses from
464 each pool increased from 18.8 to 34.0 %. Therefore, for steady state conditions, SOM
465 dynamics could be modelled as a single pool. However, in terms of the transient,
466 decadal response of soil-C stocks to a change in temperature it is clear that the relative
467 sizes of the different pools, the mean residence time of C in the different pools, as
468 well as the temperature sensitivity of decomposition will combine to determine soil-C
469 losses.

470 In contrast to our findings, a recent modelling study, based on current litter
471 input rates and soil-C stocks, estimated that the global temperature sensitivity of SOM
472 decomposition equated to a Q_{10} value of just 1.37 (Ise and Moorcroft, 2006). SOM
473 accumulation is the result of small differences between inputs and outputs, and links
474 between plant productivity and soil respiration, which probably cannot be represented
475 simply by differences in current litter input rates (Ise and Moorcroft, 2006), may
476 affect the relationship between temperature and SOM accumulation. In our model, the
477 input rate remained constant throughout but one consequence of higher decomposition
478 rates could be increased nutrient availability, which could feedback on plant
479 productivity and therefore the rate of C input to the soil. Our study focused solely on
480 the temperature sensitivity of SOM decomposition and the consequences for soil-C
481 stocks, however, holistic approaches which measure the response of all components of
482 the C-cycle to environmental drivers are clearly urgently required.

483

484 *4.3. Future directions*

The results of the incubation study suggest that the temperature sensitivity of SOM decomposition increases with substrate recalcitrance and the modelling results show that it is the response of more recalcitrant SOM decomposition to changes in temperature, and its contribution to total soil respiration, that will determine the magnitude of any positive feedback to climate change. In light of this, there may need to be a shift in the way belowground C-cycling is investigated; distinguishing between recently-fixed C mineralization and older recalcitrant SOM decomposition is more critical than distinguishing between microbial and plant root respiration *per se*.

The great difficulties associated with directly measuring changes in the sizes of soil C stocks, have led to research focusing mainly on measuring C fluxes (Valentini et al., 2000). However, when measurements are made *in situ*, changes in the rate of recalcitrant SOM decomposition tend to be obscured by the activity of roots (Hanson et al., 2000) and the response of the dynamic labile SOM pool (Gu et al., 2004). New methods for increasing our ability to measure the dynamics of more recalcitrant SOM must be developed. Radiocarbon dating of soil CO_2 can be used to partition soil respiration into recently fixed and older C sources (Schuur and Trumbore, 2006; Trumbore, 2006), and has demonstrated that the contribution of old SOM to total soil respiration tends to be greater at high latitudes (Trumbore, 2000). The model results presented above suggest that ecosystems in which respired CO_2 is mainly modern, arising from relatively small labile pools, are unlikely to respond positively to temperature in the long term, and therefore may have the potential to act as C sinks, whilst in ecosystems in which there is a substantial contribution from the larger, older SOM pools, sustained C losses are probable. Extending radiocarbon

dating of soil respired CO_2 to a broad range of ecosystems may provide important information as to the vulnerability of soil C stores.

4.4. Conclusions

It has been recognized for some time that the response of SOM decomposition to temperature has the capacity to alter C sequestration in terrestrial ecosystems (Jenkinson et al., 1991; Kirschbaum, 1995). Whilst the pool structures utilised by models have improved our ability to investigate the effects of climate change on soil C storage, our study also highlights both how difficult, and important, it is to empirically parameterize these models, both in terms of the size of the pools and the exact temperature dependence of decomposition in each pool. Worryingly, the results of our incubation suggest that the temperature sensitivity of SOM decomposition increases with substrate recalcitrance and therefore predictions of future soil-C losses may be underestimated.

Acknowledgements

We are extremely grateful to Göran Ågren and an anonymous reviewer whose comments were of great help in revising this manuscript. This work was carried out within the Natural Environment Research Council (NERC) funded UK Centre for Terrestrial Carbon Dynamics (CTCD). Zoe Billings assisted with the construction of the incubation system and the precise temperature control was made possible by controllers custom-built by the University of York, Biology Department, Electronics

533 Workshop. IPH's PhD was joint-funded by NERC and Forest Research, the executive
534 research Agency of the Forestry Commission.

535

536 **References**

537

538 Ågren, G.I., Bosatta, E., 2002. Reconciling differences in predictions of temperature
539 response of soil organic matter. Soil Biology & Biochemistry 34, 129-132.
540
541 Ågren, G.I., 2000. The temperature dependence of old soil organic matter. Ambio 29,
542 55.
543
544 Biasi, C., Rusalimova, O., Meyer, H., Kaiser, C., Wanek, W., Barsukov, P., Junger,
545 H., Richter, A., 2005. Temperature-dependent shift from labile to recalcitrant carbon
546 sources of arctic heterotrophs. Rapid Communications in Mass Spectrometry 19,
547 1401-1408.
548
549 Bol, R., Bolger, T., Cully, R., Little, D., 2003. Recalcitrant soil organic materials
550 mineralize more efficiently at higher temperatures. Journal of Plant Nutrition and Soil
551 Science-Zeitschrift fur Pflanzenernahrung und Bodenkunde 166, 300-307.
552
553 Bosatta, E., Ågren, G.I., 1999. Soil organic matter quality interpreted
554 thermodynamically. Soil Biology & Biochemistry 31, 1889-1891.
555
556 Conen, F., Leifeld, J., Seth, B., Alewell, C., 2006. Warming mineralises young and
557 old soil carbon equally. Biogeosciences 3, 515-519.
558
559 Cox, P.M., Betts, R.A., Jones, C.D., Spall, S.A., Totterdell, I.J., 2000. Acceleration of
560 global warming due to carbon-cycle feedbacks in a coupled climate model. Nature
561 408, 184-187.
562

563 Czimczik, C.I., Trumbore, S.E., 2007. Short-term controls on the age of microbial
564 carbon sources in boreal forest soils. Journal of Geophysical Research-Biogeosciences
565 112, doi: 10.1029/2006JG000389.
566
567 Dalias, P., Anderson, J.M., Bottner, P., Couteaux, M.M., 2001. Long-term effects of
568 temperature on carbon mineralisation processes. Soil Biology & Biochemistry 33,
569 1049-1057.
570
571 Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon
572 decomposition and feedbacks to climate change. Nature 440, 165-173.
573
574 Dioumaeva, I., Trumbore, S., Schuur, E.A.G., Goulden, M.L., Litvak, M., Hirsch,
575 A.I., 2002. Decomposition of peat from upland boreal forest: Temperature
576 dependence and sources of respired carbon. Journal of Geophysical Research-
577 Atmospheres 108, doi:10.1029/2001JD000848.
578
579 Eliasson, P.E., McMurtrie, R.E., Pepper, D.A., Stromgren, M., Linder, S., Ågren, G.I.,
580 2005. The response of heterotrophic CO_2 flux to soil warming. Global Change
581 Biology 11, 167-181.
582
583 Fang, C.M., Smith, P., Moncrieff, J.B., Smith, J.U., 2005. Similar response of labile
584 and resistant soil organic matter pools to changes in temperature. Nature 433, 57-59.
585
586 Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification
587 of soil bacteria. Ecology 88, 1354-1364.
588
589 Fierer, N., Craine, J.M., McLauchlan, K., Schimel, J.P., 2005. Litter quality and the
590 temperature sensitivity of decomposition. Ecology 86, 320-326.
591
592 Giardina, C.P., Ryan, M.G., 2000. Evidence that decomposition rates of organic
593 carbon in mineral soil do not vary with temperature. Nature 404, 858-861.

Gu, L.H., Post, W.M., King, A.W., 2004. Fast labile carbon turnover obscures sensitivity of heterotrophic respiration from soil to temperature: A model analysis. Global Biogeochemical Cycles 18, Art. No. GB1022.

Hartley, I.P., Heinemeyer, A., Evans, S.P., Ineson, P., 2007. The effect of soil warming on bulk soil vs. rhizosphere respiration. Global Change Biology 13, 2654-2667.

Hanson, P.J., Edwards, N.T., Garten, C.T., Andrews, J.A., 2000. Separating root and soil microbial contributions to soil respiration: A review of methods and observations. Biogeochemistry 48, 115-146.

Ise, T., Moorcroft, P.R., 2006. The global-scale temperature and moisture dependencies of soil organic carbon decomposition: an analysis using a mechanistic decomposition model. Biogeochemistry 80, 217-231.

Jenkinson, D.S., 1990. The Turnover of Organic-Carbon and Nitrogen in Soil. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 329, 361-368.

Jenkinson, D.S., Adams, D.E., Wild, A., 1991. Model Estimates of CO_2 Emissions from Soil in Response to Global Warming. Nature 351, 304-306.

Jones, C., McConnell, C., Coleman, K., Cox, P., Falloon, P., Jenkinson, D., Powlson, D., 2005. Global climate change and soil carbon stocks; predictions from two contrasting models for the turnover of organic carbon in soil. Global Change Biology 11, 154-166.

Jones, C.D., Cox, P., Huntingford, C., 2003. Uncertainty in climate-carbon-cycle projections associated with the sensitivity of soil respiration to temperature. Tellus Series B-Chemical and Physical Meteorology 55, 642-648.

Kirschbaum, M.U.F., 1995. The Temperature-Dependence of Soil Organic-Matter Decomposition, and the Effect of Global Warming on Soil Organic-C Storage. Soil Biology & Biochemistry 27, 753-760.

Kirschbaum, M.U.F., 2004. Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? Global Change Biology 10, 1870-1877.

Kirschbaum, M.U.F., 2006. The temperature dependence of organic-matter decomposition - still a topic of debate. Soil Biology & Biochemistry 38, 2510-2518.

Knorr, W., Prentice, I.C., House, J.I., Holland, E.A., 2005. Long-term sensitivity of soil carbon turnover to warming. Nature 433, 298-301.

Leifeld, J., Fehrur, J., 2005. The temperature response of CO_2 production from bulk soils and soil fractions is related to soil organic matter quality. Biogeochemistry 75, 433-543.

Liski, J., Ilvesniemi, H., Makela, A., Westman, C.J., 1999. CO_2 emissions from soil in response to climatic warming are overestimated - The decomposition of old soil organic matter is tolerant of temperature. Ambio 28, 171-174.

Matthews, B., 1971. Soil Survey Record No. 6, Soils in Yorkshire I. Sheet SE 65 (York East). Soil Survey of England and Wales. Rothamsted Experimental Station, Harpenden, Herts.

Parton, W.J., Schimel, D.S., Cole, C.V., Ojima, D.S., 1987. Analysis of Factors Controlling Soil Organic-Matter Levels in Great-Plains Grasslands. Soil Science Society of America Journal 51, 1173-1179.

Rasmussen, C., Southard, R.J., Horwath, W.R., 2006. Mineral control of organic carbon mineralization in a range of temperate conifer forest soils. Global Change Biology 12, 834-847.

Reichstein, M., Katterer, T., Andren, O., Ciais, P., Schulze, E.D., Cramer, W., Papale, D., Valentini, R., 2005a. Temperature sensitivity of decomposition in relation to soil organic matter pools: critique and outlook. Biogeosciences 2, 317-321.

Reichstein, M., Subke, J.A., Angeli, A.C., Tenhunen, J.D., 2005b. Does the temperature sensitivity of decomposition of soil organic matter depend upon water content, soil horizon, or incubation time? Global Change Biology 11, 1754-1767.

Rey, A., Jarvis, P., 2006. Modelling the effect of temperature on carbon mineralization rates across a network of European forest sites (FORCAST). Global Change Biology 12, 1894-1908.

Schuur, E.A.G., Trumbore, S.E., 2006. Partitioning sources of soil respiration in boreal black spruce forest using radiocarbon. Global Change Biology 12, 165-176.

Townsend, A.R., Vitousek, P.M., Desmarais, D.J., Tharpe,A., 1997. Soil carbon pool structure and temperature sensitivity inferred using CO_2 and $^{13}CO_2$ incubation fluxes from five Hawaiian soils. Biogeochemistry 38, 1-17.

Trumbore, S., 2000. Age of soil organic matter and soil respiration: Radiocarbon constraints on belowground C dynamics. Ecological Applications 10, 399-411.

Trumbore, S., 2006. Carbon respired by terrestrial ecosystems - recent progress and challenges. Global Change Biology 12, 141-153.

Valentini, R., Matteucci, G., Dolman, A.J., Schulze, E.D., Rebmann, C., Moors, E.J., Granier, A., Gross, P., Jensen, N.O., Pilegaard, K., Lindroth, A., Grelle, A., Bernhofer, C., Grunwald, T., Aubinet, M., Ceulemans, R., Kowalski, A.S., Vesala, T.,

Rannik, U., Berbigier, P., Loustau, D., Guomundsson, J., Thorgeirsson, H., Ibrom, A., Morgenstern, K., Clement, R., Moncrieff, J., Montagnani, L., Minerbi, S., Jarvis, P.G., 2000. Respiration as the main determinant of carbon balance in European forests. Nature 404, 861-865.

Vanhala, P., Karhu, K., Tuomi, M., Sonninen, E., Jungner, H., Fritze, H., Liski, J., 2007. Old soil carbon is more temperature sensitive than the young in an agricultural field. Soil Biology & Biochemistry 39, 2967-2970.

Waldrop, M.P., Firestone, M.K., 2004. Altered utilization patterns of young and old soil C by microorganisms caused by temperature shifts and N additions. Biogeochemistry 67, 235-248.

Wynn, J.G., Bird, M.I., 2007. C4-derived soil organic carbon decomposes faster than its C3 counterpart in mixed C3/C4 soils. Global Change Biology 13, 2206-2217.

Figure Legends

Fig. 1. The rate of respiration in the samples defrosted for 7 days (black bars), 50 days (horizontally hashed bars), 87 days (open bars) and 124 days (diagonally hashed bars) at the three incubation temperatures. Within a temperature, bars labelled with different letters differ significantly (One-way ANOVAs, $P < 0.001$). Error bars represent +1SE (n = 5). Note that the y-axis is log transformed.

Fig. 2. The relationship between the temperature sensitivity of respiration (Q_{10}) and the length of time the samples had been incubated for prior to respiration measurements commencing. Bars labelled with different letters differ significantly (One-way ANOVA: d.f. = 3,16, F = 6.018, $P = 0.007$). Error bars represent +1SE (n = 5). Note log-transformed y-axis.

Fig. 3. The effect of varying the temperature sensitivity of (a) recalcitrant SOM decomposition (Recalcitrant Q_{10}) and (b) labile SOM decomposition (Labile Q_{10}), and the contribution of recalcitrant SOM to total heterotrophic soil respiration (Recalcitrant contribution (%)) on the percentage loss of C from a soil after <u>one</u> year in which the soil temperature was 3^{o}C above ambient. To reflect the results of our incubation, for the model presented in panel (a) Q_{10l} is set to 2.85, and in panel (b) Q_{10r} is set to 3.25.

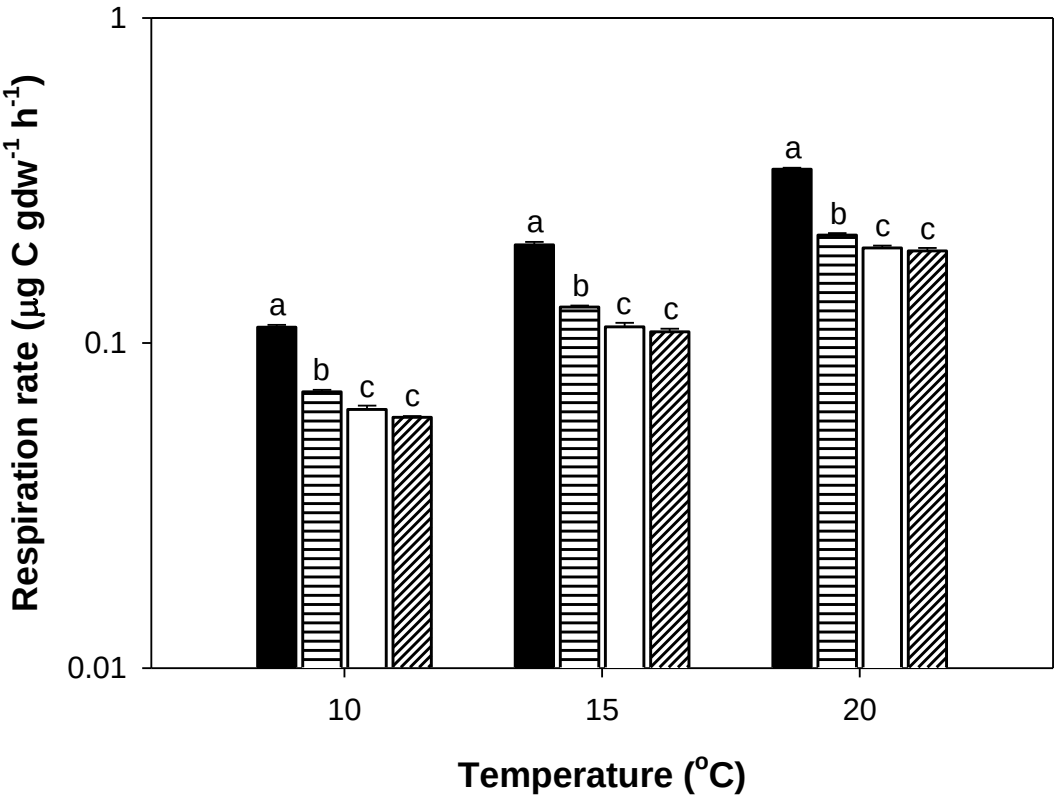
Fig. 4. The effect of varying the temperature sensitivity of (a) recalcitrant SOM decomposition (Recalcitrant Q_{10}) and (b) labile SOM decomposition (Labile Q_{10}), and the contribution of recalcitrant SOM to total heterotrophic soil respiration

(Recalcitrant contribution (%)), on the percentage loss of C from the soil after twenty years in which the soil temperature was 3^{o}C above ambient. To reflect the results of our incubation, for the model presented in panel (a) Q_{10l} is set to 2.85, and in panel (b) Q_{10r} is set to 3.25.

Fig. 5. Daily soil-C losses from the labile (solid line) and recalcitrant pools (dotted line), as a percentage of total soil-C, over a twenty-year period in which soil temperatures were 3^{o}C above ambient. In the modelled scenario, the labile pool represented 5 % of soil C and initially contributed 80 % to total heterotrophic respiration. The Q_{10} values for labile and recalcitrant SOM decomposition were 2.85 and 3.25, respectively.

Fig. 6. The change in the rate of heterotrophic soil respiration over time in the 3^{o}C warming scenario, expressed as a proportion of the respiration rate in the ambient scenario, when the labile SOM pool constituted 5 % (solid line), 10 % (dotted line) and 15 % (hashed line) of total soil C. This graph was produced from a simulation in which the decomposition of labile SOM initially contributed 80% of total heterotrophic soil respiration and the Q_{10} values associated with labile and recalcitrant SOM decomposition were 2.85 and 3.25 respectively.

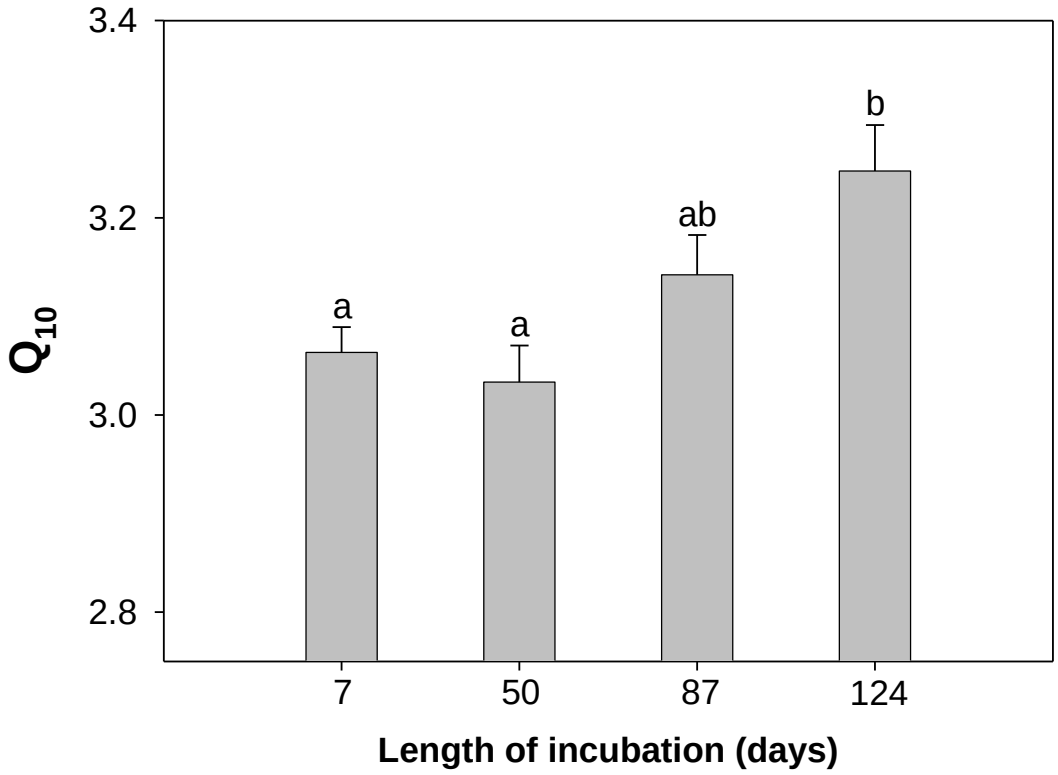
763 Fig. 1.



764
765

766

Fig. 2.



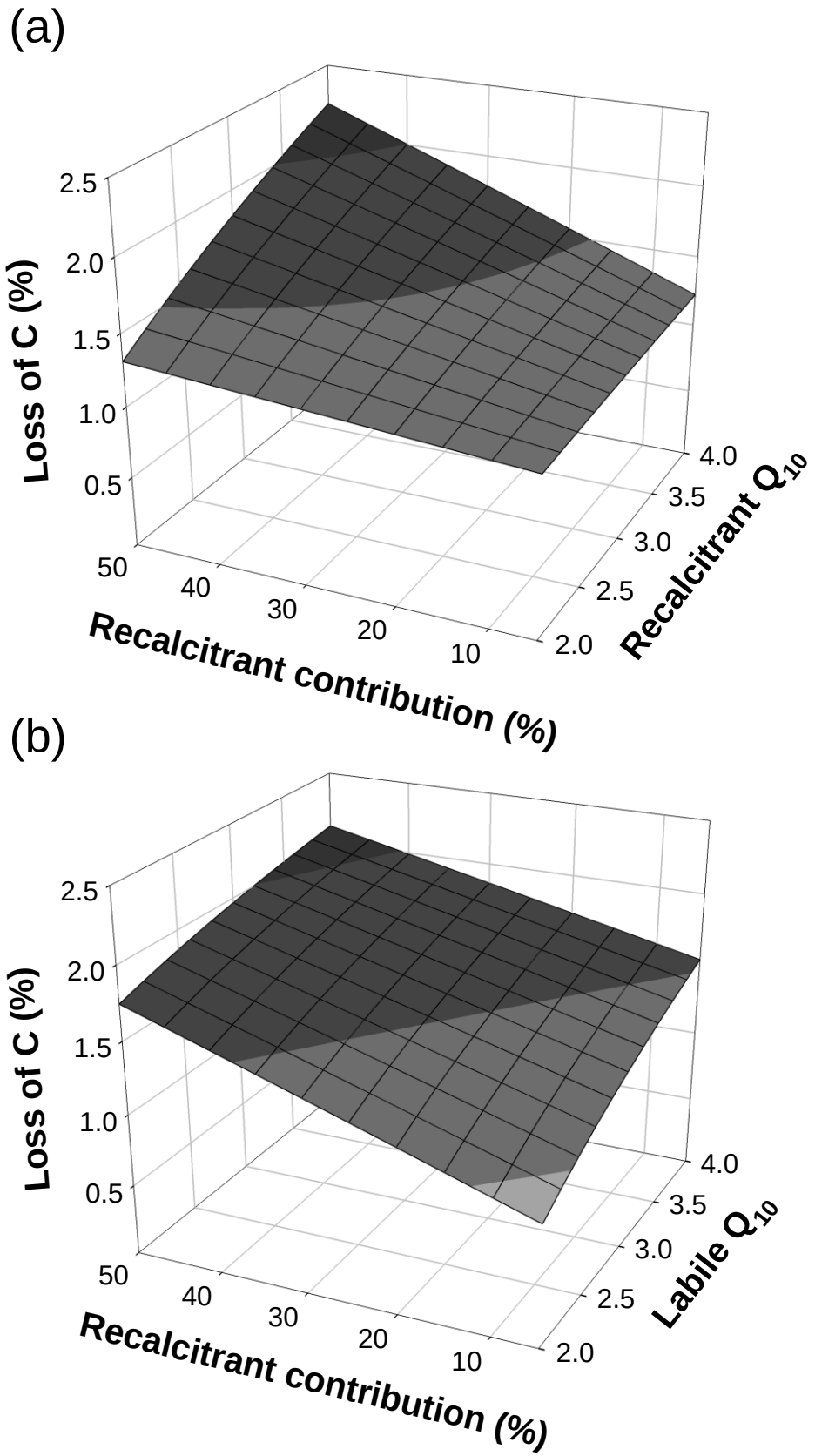
767

768

769

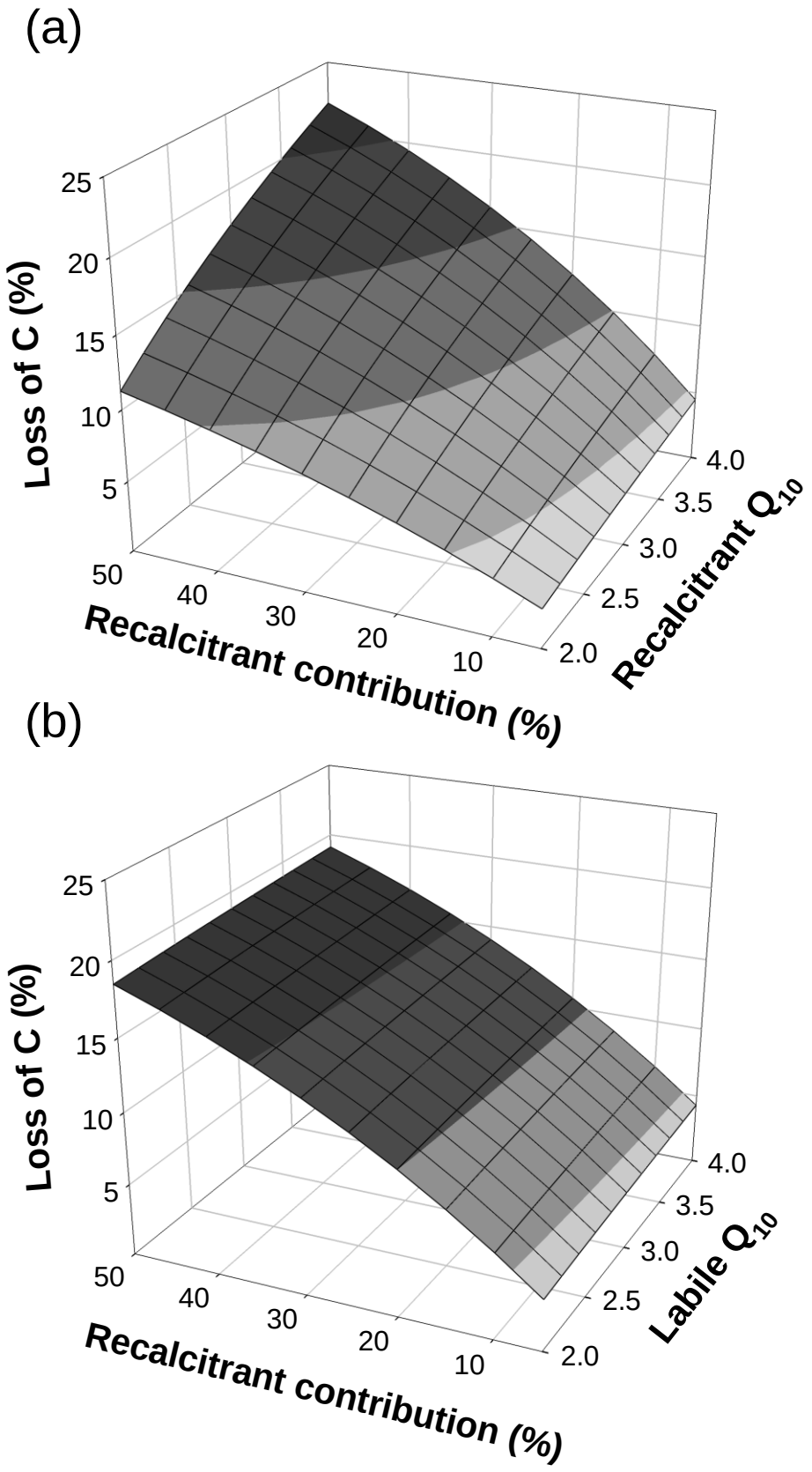
770

771 Fig. 3.



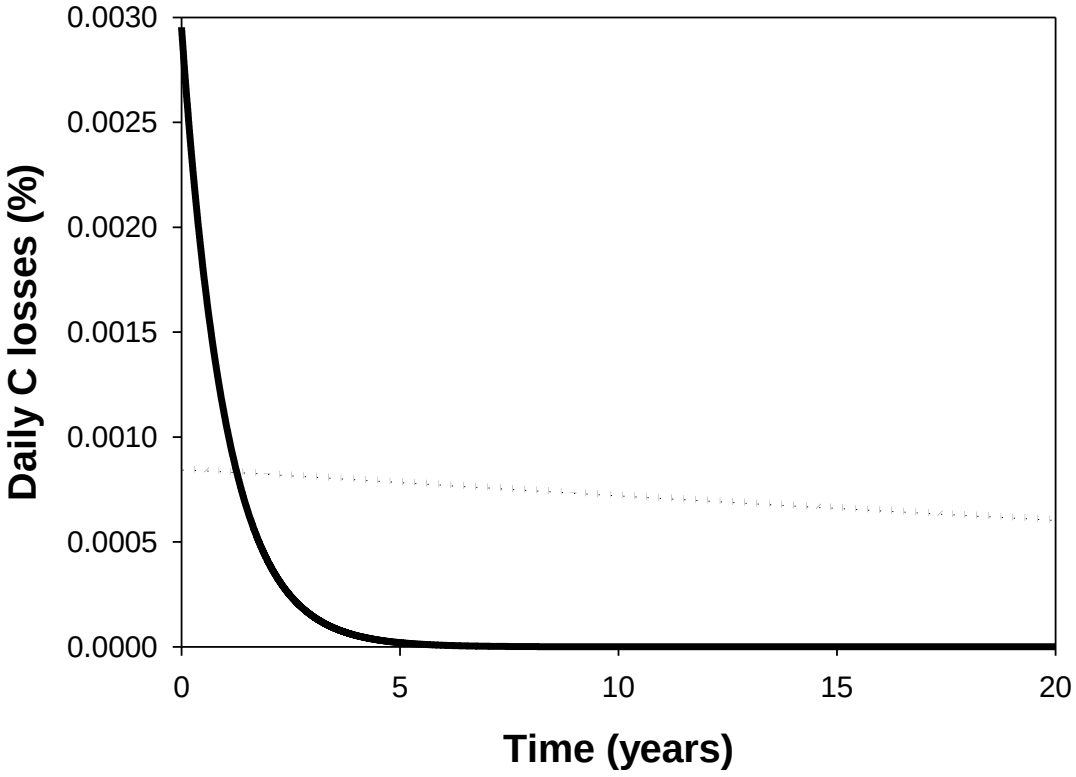
772
773

774 Fig. 4.



775
776

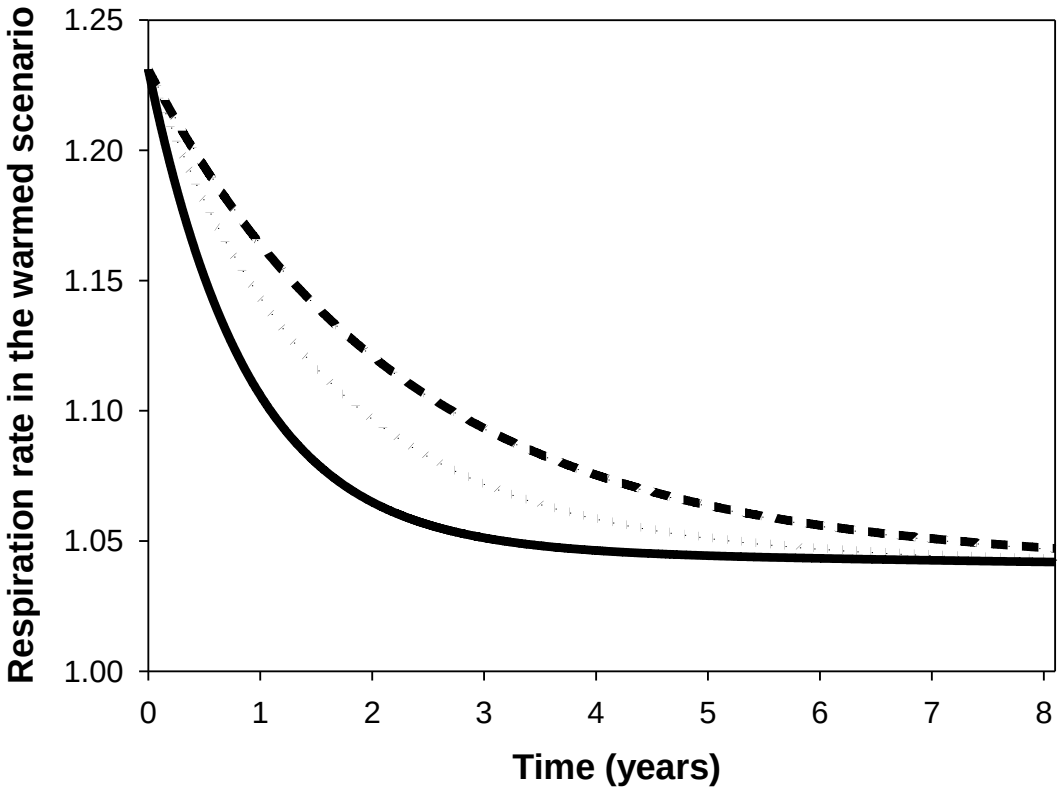
777 Fig. 5.



778
779
780
781

782 Fig. 6.

783



784

785

786