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9 Substrate quality and the temperature sensitivity of soil organic matter decomposition

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19 Iain P. Hartley^{a,b,*}, Phil Ineson^a

20

21 *^aDepartment of Biology, Stockholm Environment Institute (SEI-York centre),*

22 *University of York, York, YO10 5YW, UK*

23

24 *^bCurrent address: School of Biological and Environmental Sciences, University of*

25 *Stirling, Stirling, FK9 4LA, UK*

26

27

28 **School of Biological and Environmental Sciences, University of Stirling, Stirling,*

29 *FK9 4LA, UK. Tel: +44 1786 467757; fax: +44 1786 467843.*

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

31

32 **Substrate quality and the temperature sensitivity of** 33 **soil organic matter decomposition**

34

35 **Abstract**

36

37 Determining the relative temperature sensitivities of the decomposition of the
38 different soil organic matter (SOM) pools is critical for predicting the long-term
39 impacts of climate change on soil carbon (C) storage. Although kinetic theory
40 suggests that the temperature sensitivity of SOM decomposition should increase with
41 substrate recalcitrance, there remains little empirical evidence to support this
42 hypothesis. In the study presented here, sub-samples from a single bulk soil sample
43 were frozen and sequentially defrosted to produce samples of the same soil that had
44 been incubated for different lengths of time, up to a maximum of 124 days. These
45 samples were then placed into an incubation system which allowed CO₂ production to
46 be monitored constantly and the response of soil respiration to short-term temperature
47 manipulations to be investigated. The temperature sensitivity of soil CO₂ production
48 increased significantly with incubation time suggesting that, as the most labile SOM
49 pool was depleted the temperature sensitivity of SOM decomposition increased. This
50 study is therefore one of the first to provide empirical support for kinetic theory.
51 Further, using a modelling approach, we demonstrate that it is the temperature
52 sensitivity of the decomposition of the more recalcitrant SOM pools that will
53 determine long-term soil-C losses. Therefore, the magnitude of the positive feedback
54 to global warming may have been underestimated in previous modelling studies.

55

56 *Keywords:* soil organic matter, temperature, labile, recalcitrant, CO₂, respiration,
57 climate change, feedback

58

59 **1. Introduction**

60

61 Modelling studies have suggested that C sequestration in terrestrial ecosystems
62 may be undermined by the positive response of SOM decomposition to temperature
63 (Cox et al., 2000; Jones et al., 2005). In fact simulations have shown that temperature-
64 induced soil-C losses could accelerate the rate of global warming by up to 40 % (Cox
65 et al., 2000). These predictions are, firstly, highly dependent on the exact
66 parameterization of the response of SOM decomposition to temperature (Jones et al.,
67 2003), and, secondly, based on the assumption that the decomposition of all the C
68 stored in soils is equally sensitive to temperature (Jones et al., 2005).

69 Contrary to the latter assumption, two highly cited studies concluded that the
70 decomposition of older, more recalcitrant SOM is insensitive to temperature (Liski
71 et al., 1999; Giardina and Ryan, 2000). Based on the amount and age of C stored in
72 the soils along a temperature gradient, Liski et al. (1999) argued that the
73 decomposition of old soil organic matter is insensitive to the influence of temperature.
74 Further, by reviewing the available literature, Giardina and Ryan (2000) demonstrated
75 that the decomposition of SOM in mineral soils was controlled more by substrate
76 quality than temperature. However, Ågren (2000) argued that the results of Liski et al.
77 (1999) may be due to particular properties of the model used (substrate quality
78 changed directly as a function of time and, due to fixed residence times and

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

92 More recently, empirical evidence from incubation studies has supported
93 model assumptions by suggesting that there is no difference in the temperature
94 sensitivity of the decomposition of labile and recalcitrant SOM (Fang et al., 2005;
95 Reichstein et al., 2005b; Conen et al., 2006). However, kinetic theory predicts that the
96 temperature sensitivity of decomposition should increase with substrate recalcitrance
97 (Bosatta and Ågren, 1999; Davidson and Janssens, 2006); the higher activation energy
98 associated with the breakdown of recalcitrant substrates should result in a greater
99 temperature sensitivity of decomposition. This logic appears to be supported by
100 measurements of the temperature sensitivity of leaf litter decomposition (Fierer et al.,
101 2005) but there remains little evidence from soil studies.

102 When soils are removed from the field, the links to photosynthesizing tissues
103 are severed and the input of labile substrates stopped. Due to inherent differences in
104 the turnover times of different carbon pools, labile substrates are progressively
105 depleted during the course of laboratory incubation (Townsend et al., 1997). Recent
106 studies have used this logic to demonstrate that the decomposition of recalcitrant
107 substrates is indeed temperature sensitive but they have failed to identify any
108 differences between recalcitrant and labile pools (Fang et al., 2005; Reichstein et al.,
109 2005b). However, the failure to detect significant differences could be due to large
110 inter-sample variability or errors associated with the measurement of soil CO₂
111 production between the different time points (Davidson and Janssens, 2006).

112 We have used a novel experimental design in which sub-samples taken from
113 the same initial soil sample were frozen and then sequentially defrosted to provide
114 replicates that could be incubated at 15°C for different periods of time. This allowed
115 for direct and simultaneous comparison of temperature responses between the
116 different samples without the problems associated with changes in potentially
117 confounding factors, such as drift in incubator temperatures or flow rates, between
118 measuring dates. The high-precision incubation system we used allowed statistically
119 significant differences in CO₂ production rates to be identified even when the
120 magnitude of such effects were small, allowing us to determine whether the
121 temperature sensitivity of SOM decomposition changed with incubation time. Finally,
122 to determine the implications of the results of our incubation study we carried out a
123 modelling analysis investigating the effect of changing the temperature sensitivity of
124 the decomposition of different soil-C pools on total soil-C losses.

125

126 **2. Materials and Methods**

127

128 *2.1. Soil sampling and preparation*

129

130 On September 1st 2004, 10 kg of soil were removed from the experimental
131 garden at the University of York, UK. Soil comprising the upper horizons of the
132 Escrick series (Matthews, 1971), was brought into this facility ~50 years ago, since
133 when it has been repeatedly mixed whilst under cultivation with a variety of plant
134 species. It is a sandy loam with a pH of 6.5 and a carbon content of 4 %. The soil was
135 sieved through a 2 mm mesh and corrected to a gravimetric moisture content of 20 %.
136 The main soil sample was then subdivided and samples were frozen at -20°C. Sub-
137 samples were then defrosted sequentially with an interval of approximately 5 weeks
138 between four defrost dates, providing five 400 g samples on each date (a total of 20
139 samples). The defrosted samples were incubated in a constant temperature room at
140 15 °C and maintained at a moisture content of 20 % with frequent water addition. On
141 February 2nd 2005, seven days after the final defrost date, the soil samples were added
142 to the incubation system.

143

144 *2.2. Respiration measurements*

145

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

150 Bioscientific Ltd., Herts, UK) was used to measure the CO₂ concentration in each of
151 24 lines, connected to individual incubation chambers, with a sampling frequency of
152 once every 2 hours. The flow rate of air through each line was maintained at 50 cm³
153 min⁻¹ throughout and was monitored with a digital flow meter (Model: GFM 171,
154 0-500 cm³ min⁻¹, Aalborg Instruments and Controls Inc., New York, USA). Soil
155 samples were added to 20 of the chambers with the final four left empty to allow the
156 CO₂ concentration of the incoming air to be measured. Respiration rates were
157 calculated based on the mass of soil incubated (dry weight measured at end of
158 incubation), the flow rate through the lines (50 cm³ min⁻¹) and the difference between
159 the CO₂ concentration in the incoming air and the CO₂ concentration in each of the
160 soil lines. The temperature of the soils in the incubation chambers was controlled by a
161 heating, large volume water bath maintained within a dedicated cold room, with a
162 precision of temperature control of 0.1 °C (Electronics Workshop, Biology
163 Department, University of York, UK).

164 The response of soil CO₂ production to temperature was determined by first
165 increasing soil temperatures from 10 °C to 15 °C to 20 °C before reducing the
166 temperature back to 15 and 10 °C. The mean rate at each temperature was then
167 calculated allowing any fluctuations in the baseline rate of respiration to be included
168 in the calculation of the temperature response (Fang et al., 2005). Each temperature
169 was maintained for a total of 48 hours to allow respiration rates to stabilise. These
170 temperatures are regularly experienced during the growing season in the experimental
171 garden at the University of York (Hartley et al., 2007).

172

173

174 2.3. Model

175

176 A two-pool SOM decomposition model was constructed. No passive or inert pool was
177 included, with the recalcitrant pool in this model mainly representing the slow pool
178 *sensu* CENTURY (Parton et al., 1987) or the humus pool *sensu* RothC (Jenkinson,
179 1990). Equation 1 describes the dynamics of the labile pool. C enters the labile pool at
180 a constant rate while decomposition losses are dependent on temperature and the size
181 of the labile SOM pool.

182

183
$$dC_l/dt = -k_l Q_{10l}^{(T-T_{ref})/10} C_l + I$$
 (Equation 1)

184

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

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

192

193
$$dC_r/dt = -k_r Q_{10r}^{(T-T_{ref})/10} C_r + h k_l Q_{10l}^{(T-T_{ref})/10} C_l$$
 (Equation 2)

194

195 Where k_r is the rate constant applied to the decomposition of recalcitrant SOM, Q_{10r} is
196 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 $7.2 \mu\text{g C gdw}^{-1} \text{ day}^{-1}$. 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°C . To reflect the incubation, the temperature in the ambient scenario was
210 considered to be 15°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

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

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

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

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

243
244
$$C_{lss} = I / (k_l Q_{10}^{(T-T_{ref})/10}) \quad \text{(Equation 3)}$$

245 $C_{r_{ss}} = hI / (k_r Q_{10r}^{(T-T_{ref})/10})$ (Equation 4)

246

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

254

255 $T_{l_{ss}} = 1 / (k_l Q_{10l}^{(T-T_{ref})/10})$ (Equation 5)

256 $T_{r_{ss}} = 1 / (k_r Q_{10r}^{(T-T_{ref})/10})$ (Equation 6)

257

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

269

270 2.4. Data analysis

271

272 Statistical analyses were carried out using SPSS (Version 11, SPSS Science,
273 Birmingham, UK). One-way ANOVAs were used to determine whether the rate of
274 CO₂ production and the temperature responses differed between the samples that had
275 been incubated for different lengths of time.

276

277 3. Results

278

279 3.1. Temperature sensitivity of soil respiration

280

281 As expected, the rate of soil respiration declined significantly with increasing
282 incubation time ($P < 0.001$), reflecting the fact that the most labile substrates were
283 progressively depleted (Fig. 1). However, the temperature sensitivity of respiration,
284 expressed as a Q_{10} , increased significantly with incubation time (Fig. 2). Based on the
285 reduction in the rate of respiration between days 7 and 124, the contribution of the
286 most labile SOM pool to respiration in the samples that had been incubated for the
287 shortest time was estimated to be approximately 45%. Q_{10} values for “labile” and
288 “recalcitrant” SOM decomposition could then be calculated, by mass balance,
289 assuming the Q_{10} of the 124 day-incubated samples represented recalcitrant SOM
290 decomposition. The calculated Q_{10} values were 2.85 and 3.25, respectively. However,
291 a significant difference was observed in the temperature sensitivity of the respiration
292 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°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

341 50 % increased the rate at which the steady state was approached by one order of
342 magnitude (average of 262 and 26.2 years, respectively), highlighting the importance
343 of determining the mean residence time of SOM in the recalcitrant pool for predicting
344 the rate at which C will be lost from soils. Steady conditions in the recalcitrant pool
345 were not approached within the 20 year period investigated in the sensitivity analysis
346 in any of the simulations carried out.

347

348 **4. Discussion**

349

350 *4.1. Substrate chemistry and the temperature dependence of decomposition*

351

352 In our study it appeared that as the most labile substrates were used up, the
353 temperature sensitivity of soil respiration increased (Figs. 1 and 2). The magnitude of
354 this change in the temperature sensitivity of soil respiration was relatively low yet
355 significant differences were observed (Fig. 2). Even after respiration rates had become
356 relatively constant, the temperature sensitivity of soil respiration continued to increase
357 (Figs. 1 and 2) suggesting that the quality of the substrates being utilised was
358 changing throughout. As SOM represents a continuum of substrates of differing
359 recalcitrance it is debateable as to whether the temperature sensitivity of truly
360 recalcitrant SOM decomposition can be investigated by relatively short-term incubations;
361 directly determining the temperature sensitivity of the decomposition of SOM, with
362 turnover times of hundreds or thousands of years, would require an extremely long-
363 term incubation. Therefore, we propose that the calculated Q_{10} values probably
364 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 CO₂
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 C₃-C₄ 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 Q₁₀ 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 Q₁₀ 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).

413 Previous studies which have utilised similar methodologies as in the study
414 presented here have demonstrated that the decomposition of more recalcitrant SOM is
415 highly temperature sensitive but have failed to identify a change in the temperature
416 sensitivity of CO₂ production with incubation time (Fang et al., 2005; Reichstein
417 et al., 2005b). There are a number of possible explanations for this discrepancy.
418 Firstly, in contrast to the study of Fang et al., (2005), we measured the response of
419 CO₂ production to changes in temperature across a relatively narrow range regularly
420 experienced by the soil. Secondly, in contrast to the study of Reichstein et al. (2005b),
421 our slower temperature fluctuations resulted in the entire soil sample experiencing a
422 common temperature while respiration measurements were made. Thirdly, given the
423 relatively small magnitude of the changes in the temperature response, the high-
424 precision incubation system utilised here (see tight error bars Fig. 1) may have
425 permitted significant differences to be detected that have not been previously possible.
426 Finally, it should be emphasised that our results were derived from a single mineral
427 soil type. The extent to which this relationship holds true across soils differing in
428 physical and chemical properties requires further research (Rasmussen et al., 2006).

429

430 *4.2. Model implications*

431

432 Many soil decomposition models take into account the wide range of substrates
433 present in SOM by modelling SOM dynamics using a series of C pools with different
434 intrinsic turnover times (e.g. CENTURY (Parton et al., 1987) and RothC (Jenkinson,
435 1990)). However, these models generally apply the same temperature functions to the
436 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*

485

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

494 The great difficulties associated with directly measuring changes in the sizes
495 of soil C stocks, have led to research focusing mainly on measuring C fluxes
496 (Valentini et al., 2000). However, when measurements are made *in situ*, changes in
497 the rate of recalcitrant SOM decomposition tend to be obscured by the activity of
498 roots (Hanson et al., 2000) and the response of the dynamic labile SOM pool (Gu et
499 al., 2004). New methods for increasing our ability to measure the dynamics of more
500 recalcitrant SOM must be developed. Radiocarbon dating of soil CO₂ can be used to
501 partition soil respiration into recently fixed and older C sources (Schuur and
502 Trumbore, 2006; Trumbore, 2006), and has demonstrated that the contribution of old
503 SOM to total soil respiration tends to be greater at high latitudes (Trumbore, 2000).
504 The model results presented above suggest that ecosystems in which respired CO₂ is
505 mainly modern, arising from relatively small labile pools, are unlikely to respond
506 positively to temperature in the long term, and therefore may have the potential to act
507 as C sinks, whilst in ecosystems in which there is a substantial contribution from the
508 larger, older SOM pools, sustained C losses are probable. Extending radiocarbon

509 dating of soil respired CO₂ to a broad range of ecosystems may provide important
510 information as to the vulnerability of soil C stores.

511

512 *4.4. Conclusions*

513

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

524

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526

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717 **Figure Legends**

718

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

724

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

730

731 Fig. 3. The effect of varying the temperature sensitivity of (a) recalcitrant SOM
732 decomposition (Recalcitrant Q_{10}) and (b) labile SOM decomposition (Labile Q_{10}), and
733 the contribution of recalcitrant SOM to total heterotrophic soil respiration
734 (Recalcitrant contribution (%)) on the percentage loss of C from a soil after one year
735 in which the soil temperature was 3°C above ambient. To reflect the results of our
736 incubation, for the model presented in panel (a) Q_{10i} is set to 2.85, and in panel (b)
737 Q_{10r} is set to 3.25.

738

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

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

746

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

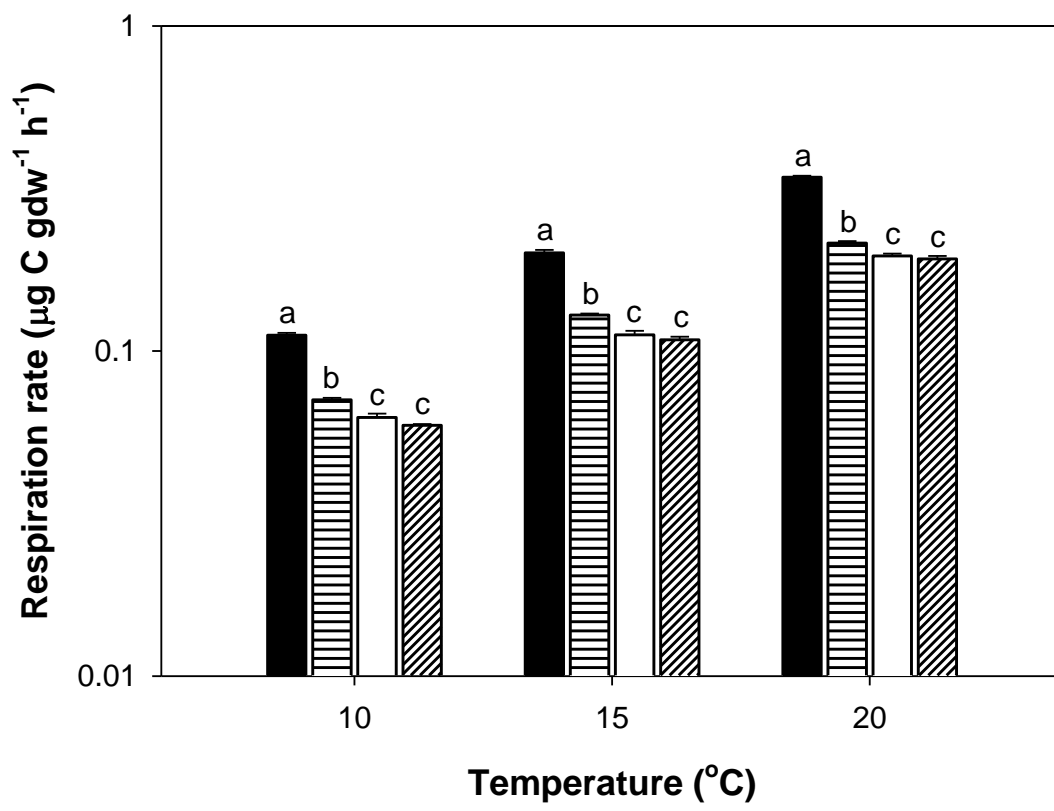
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754 Fig. 6. The change in the rate of heterotrophic soil respiration over time in the 3°C
755 warming scenario, expressed as a proportion of the respiration rate in the ambient
756 scenario, when the labile SOM pool constituted 5 % (solid line), 10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

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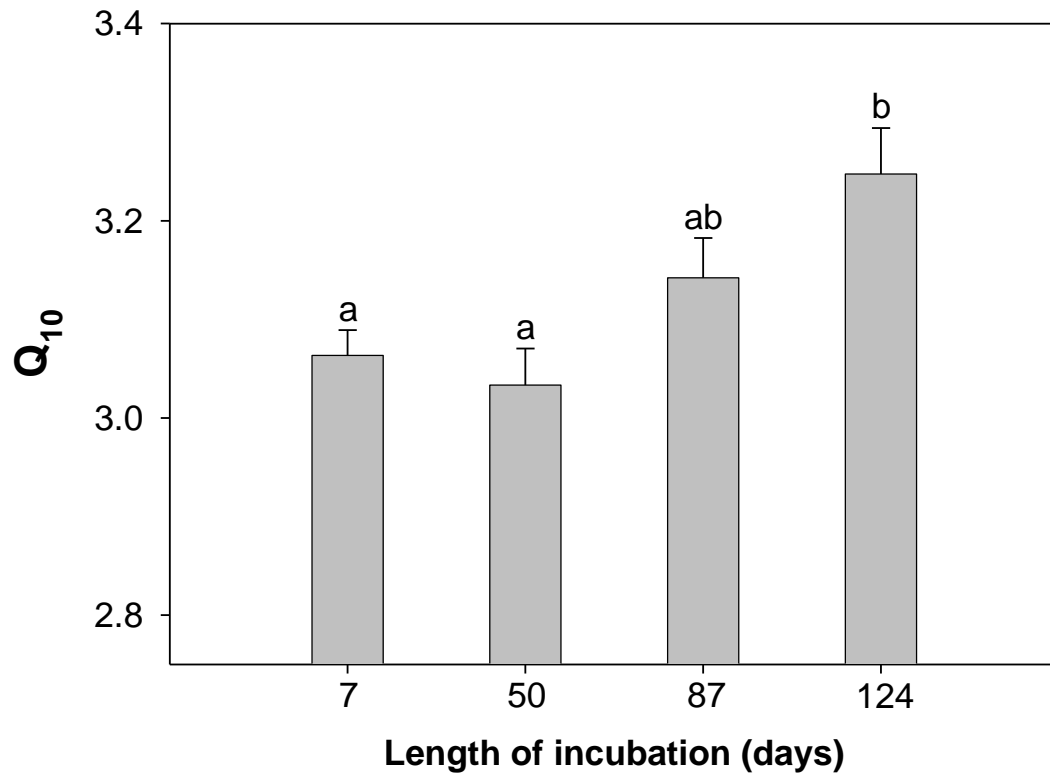
763 Fig. 1.



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Fig. 2.



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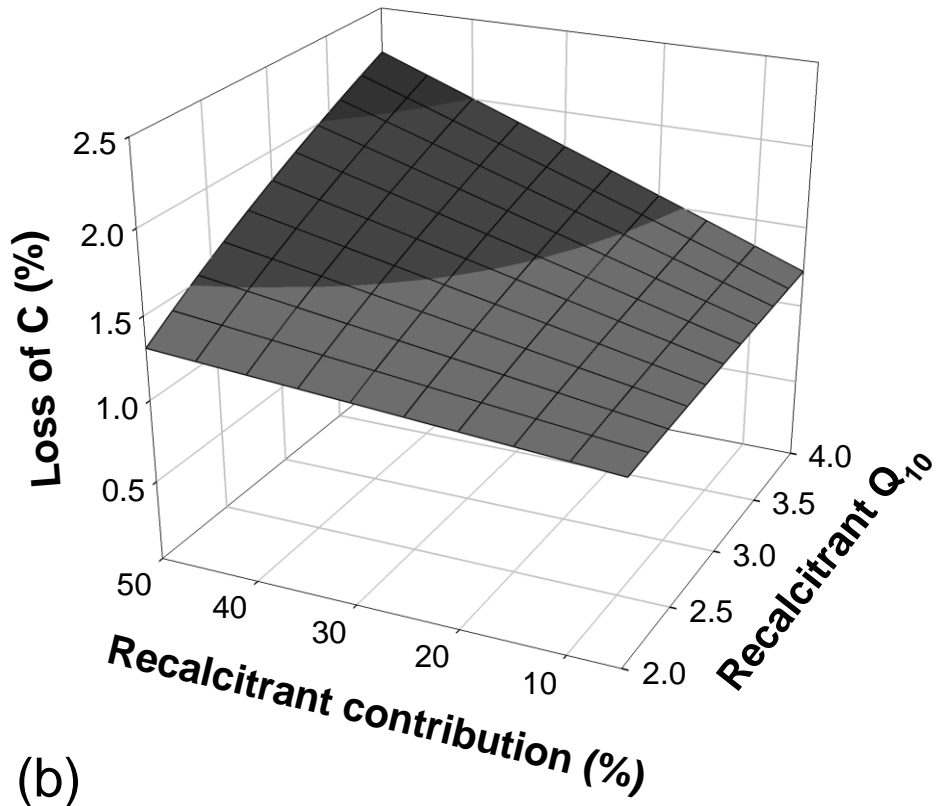
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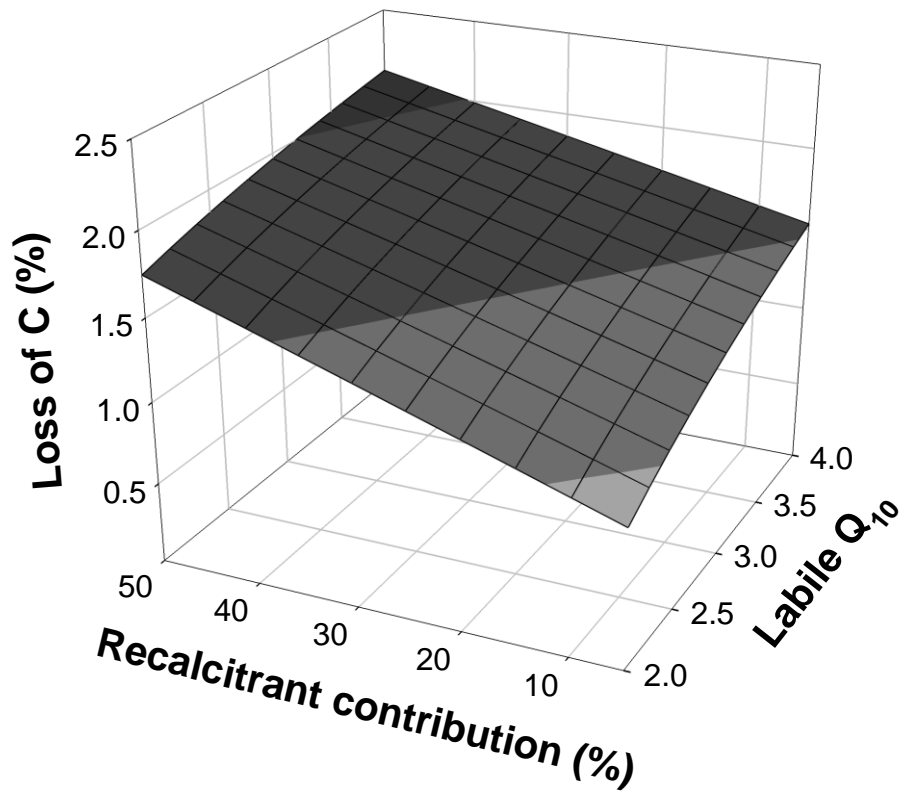
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771 Fig. 3.

(a)

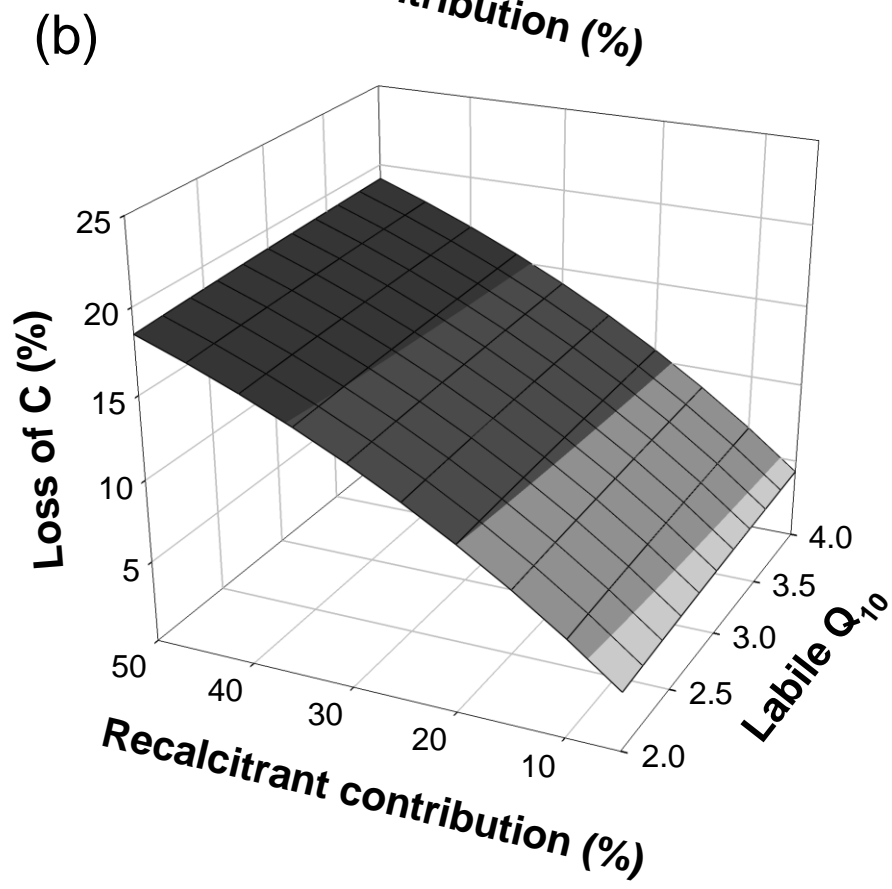
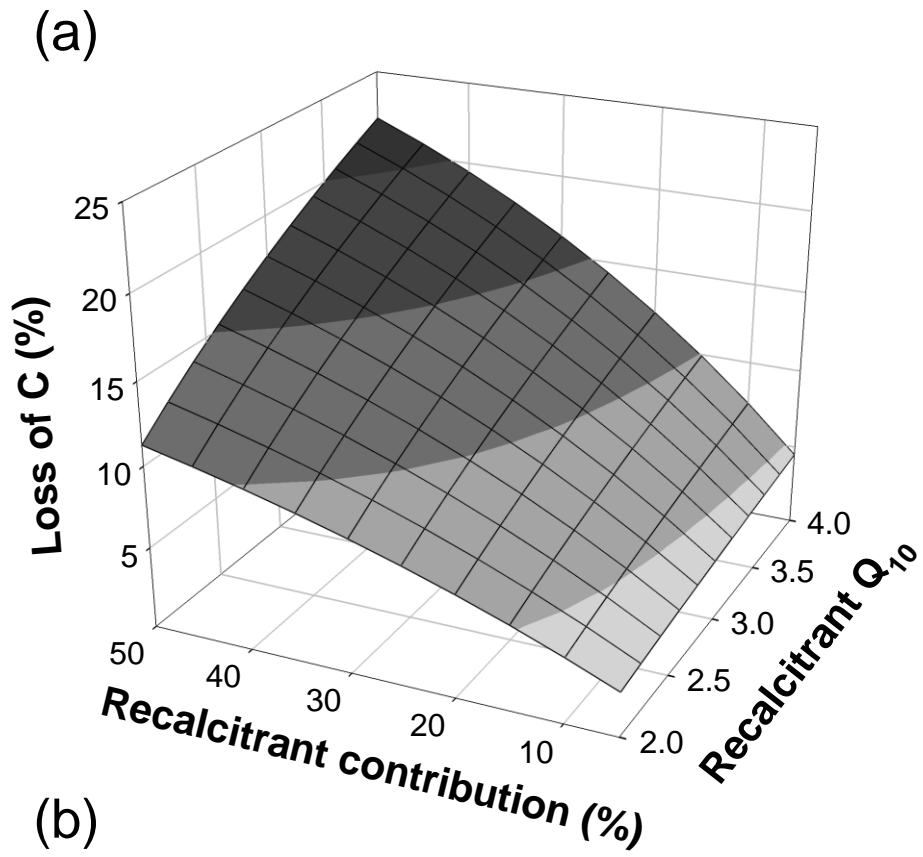


(b)



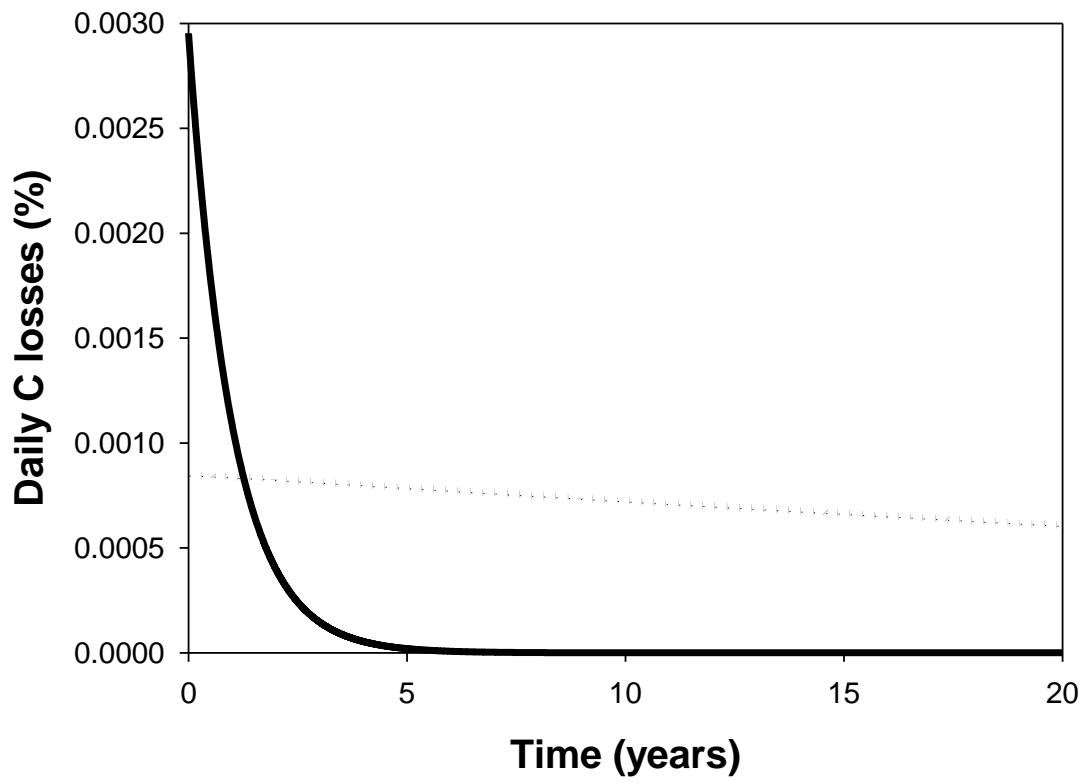
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774 Fig. 4.



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777 Fig. 5.



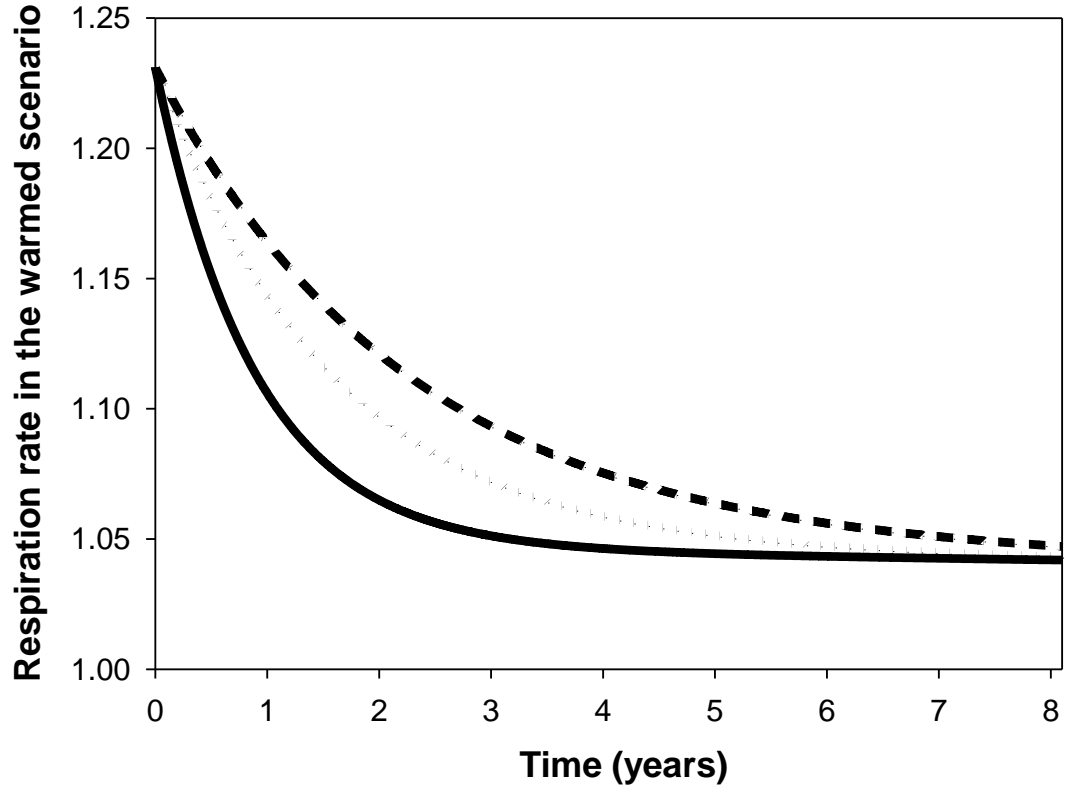
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782 Fig. 6.
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