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# Warming mineralises young and old soil carbon equally

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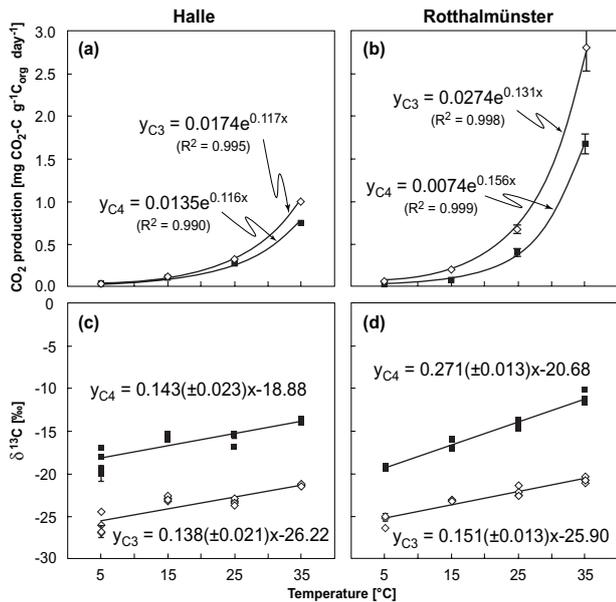
**Abstract.** The temperature sensitivity of soil organic carbon decomposition is critical for predicting future climate change because soils store 2–3 times the amount of atmospheric carbon. Of particular controversy is the question, whether temperature sensitivity differs between young or labile and old or more stable carbon pools. Ambiguities in experimental methodology have so far limited corroboration of any particular hypothesis. Here, we show in a clear-cut approach that differences in temperature sensitivity between young and old carbon are negligible. Using the change in stable isotope composition in transitional systems from C3 to C4 vegetation, we were able to directly distinguish the temperature sensitivity of carbon differing several decades in age. This method had several advantages over previously followed approaches. It allowed to identify release of much older carbon, avoided un-natural conditions of long-term incubations and did not require arguable curve-fitting. Our results demonstrate that feedbacks of the carbon cycle on climate change are driven equally by young and old soil organic carbon.

## 1 Introduction

Climate change is a process happening over decades. It is mainly driven by increasing concentrations of atmospheric CO<sub>2</sub> (Houghton, 2005). Predictions of future CO<sub>2</sub> concentrations significantly depend on the effect of warming on the release of CO<sub>2</sub> from decomposing soil organic matter (Jones et al., 2003, 2005) and on the possible duration of this effect. If only young and more labile soil organic matter are sensitive to warming (Melillo et al., 2002), any feedback between climate change and soil carbon would be minor and short-lived because the greatest fraction of soil organic matter is of greater age and more stable. A larger effect would

be expected, if all soil carbon would have the same temperature sensitivity (Townsend et al., 1997; Diomaeva et al., 2002; Fang et al., 2005, 2006), or if old carbon would react more strongly to warming than young carbon (Bol et al., 2003; Knorr et al., 2005; Fierer et al., 2005; Leifeld and Fuhrer, 2005). The latter, currently most discussed proposition, would imply that global warming leads to an even stronger increase in the mobilisation of carbon pools than suggested by any previous hypothesis. Investigating the temperature sensitivity of different fractions of soil organic matter has proven difficult (Reichstein et al., 2005; Davidson and Janssens, 2006). Three approaches have been followed so far. One has been to expose soils to different temperatures over several months or years while measuring CO<sub>2</sub> release (Townsend et al., 1997; Giardina and Ryan, 2000; Melillo et al., 2002; Knorr et al., 2005, Fang et al., 2005). Usually, CO<sub>2</sub> effluxes were observed to decrease with time as the fraction of young compounds gets smaller (Townsend et al., 1997; Waldrop et al., 2004). Response to temperature changes towards the end of the observation period were assumed representative for older fractions of soil organic matter. Yet, only a small proportion of the young carbon is lost in the process and most of the CO<sub>2</sub> at the end of the observation periods might still originate from the young fraction. Thus, information about older carbon is very limited. Interpretation of results from this type of experiment strongly depend on the applied model. The same set of data can be used to support contrasting hypotheses (Knorr et al., 2005; Reichstein et al., 2005; Fang et al., 2006). The second approach to study temperature sensitivity is to compare soil carbon of different qualities by means of physical-chemical fractionation and to investigate them separately (Leifeld and Fuhrer, 2005) or to incubate soil from different soil horizons (Fierer et al., 2003; Fang et al., 2005) under the premise that they represent different stabilities. Yet, the destructive nature of the first approach hampers the transferability of its results to natural conditions, and an unequivocal assignment of the evolved

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**Fig. 1.** Mean temperature-dependent respiration rates at (a), Halle; (b), Rotthalmünster under continuous C3 vegetation (open diamonds) and where vegetation had changed to C4 (closed squares) (error bars indicate  $\pm 1$  s.d.;  $n=4$ ). Effect of temperature on  $\delta^{13}\text{C}$  values of CO<sub>2</sub> released at (c), Halle; (d), Rotthalmünster for soils under continuous C3 vegetation (open diamonds) and soils, where vegetation had changed to C4 (closed squares) (error bars indicate the internal analytical error, where larger than the symbol). Values in brackets in the regression equations in (c) and (d) indicate the error of the slope.

CO<sub>2</sub> to young vs. old organic matter is hampered for both. The third approach makes use of different isotopic signatures in CO<sub>2</sub> derived from young and old soil carbon, either after a transition from C3 to C4 vegetation or after spiking with <sup>14</sup>C that occurred after 1950, and has been applied in long-term incubation studies (Dioumaeva et al., 2002; Bol et al., 2003; Waldrop and Firestone, 2004). Here, we follow the third approach in a short-term incubation study to avoid alteration of the soil through exhaustion of labile carbon or changes in microbial community structure as common in long-term incubation studies.

## 2 Material and methods

Change of vegetation from C3 to C4 type allows to distinguish between soil carbon pools originating from the time before and after the changes (Balesdent et al., 1987) as well as their respective contribution to CO<sub>2</sub> production (Townsend et al., 1997; Waldrop et al., 2004). The fraction of new carbon equals the difference in  $\delta^{13}\text{C}$  between C3 soil (or CO<sub>2</sub>) and C4 soil (or CO<sub>2</sub>) divided by the difference in  $\delta^{13}\text{C}$  between previous C3 and new C4 carbon input. To make use of such natural isotope enrichment, we collected soil

samples during the winter of 2005/2006 from the plough-layer (0–30 cm) of two sets of split cereal fields in Germany where the vegetation on one part had changed from continuous C3 to a monoculture of C4 vegetation, while the other part remained as C3 (Tab. 1). Litter inputs on the C4 plots were smaller in Halle (HL), where whole plants were harvested, than in Rotthalmünster (RM), where only grain was removed from the field. Fresh samples were sieved (2 mm), homogenised and incubated at 15°C for several days before being split into 32 units of 5.0 g per site and vegetation type. The 5.0 g samples were placed into 12 ml Exetainer vials (Labco, High Wycombe, UK), three times evacuated and flushed with CO<sub>2</sub>-free air, equilibrated to atmospheric pressure and immediately placed in one of four temperature controlled water baths (5, 15, 25 and 35°C, 8 replicates per temperature treatment). The evolution of CO<sub>2</sub> concentrations within the vials was monitored on four of the eight replicates through repeated extraction of 1 ml headspace gas and its immediate analysis on a gas chromatograph with methaniser and flame ionisation detector (model 8610C, SRI Instruments, Torrance, CA, USA). When concentrations of around 1000 ppm CO<sub>2</sub> were reached, headspace CO<sub>2</sub> in the remaining four replicates of each treatment were measured for carbon isotope ratios using a continuous flow isotope ratio mass spectrometer (CF-IRMS, DELTA<sup>plus</sup> XP, Thermo Finnigan, Bremen, Germany) equipped with a GasBench II following standard processing techniques and a backflushing system (Seth et al., 2006). This allowed for the comparison of essentially the same substrate quality independent of temperature. The duration of the incubations ranged from a few hours at 35°C to up to 6 days at 5°C. Isotope ratios are reported as  $\delta^{13}\text{C}$  values relative to VPDB [‰]. Isotope ratios of soil carbon were obtained using an elemental analyzer (Flash-EA 1112, Thermo Electron, Milano, Italy) connected to our CF-IRMS.

## 3 Results and discussion

Isotopic signatures of C4 vegetation are enriched in <sup>13</sup>C relative to C3 vegetation by 16.8‰ in Halle (John et al., 2003) (HL) and by 14.1‰ in Rotthalmünster (Ludwig et al., 2005) (RM). Isotopic values of C3 and C4 litter compared to the  $\delta^{13}\text{C}$  of soils allows the calculation of old carbon (e.g. originating from before the change to C4) and young carbon. At HL, 13% of soil carbon were younger than 45 years and contributed 44% of CO<sub>2</sub> between 5 and 35°C on average. In RM, 43% of soil carbon were less than 26 years old and contributed 55% of the total CO<sub>2</sub> emission. The smaller proportion of C4 soil carbon at HL despite the earlier change to this vegetation type results from smaller litter inputs. At both sites, respiration rates were somewhat smaller at C4 sites. Values of Q<sub>10</sub> were identical (3.2) on both plots at HL (Fig. 1a). At RM, the Q<sub>10</sub> value of the soil with C4 vegetation was greater (4.8) than under contin-

**Table 1.** Summary information on sites from where the studied soil samples were taken at a depth of 0–30 cm in winter 2005–2006.

	Halle		Rotthalmünster	
	C3 continuous	C3 changed to C4	C3 continuous	C3 changed to C4
Vegetation before change	Rye	Rye	Wheat	Wheat
Year of change to C4		1961		1979
Vegetation since change	Rye	Maize	Wheat	Maize
Annual precipitation	465 mm		886 mm	
Mean annual temperature	9.2°C		8.7°C	
Soil texture (sand, silt, clay)	69%, 23%, 8%		10%, 73%, 17%	
Soil organic carbon	1.09%	1.12%	1.27%	1.37%
$\delta^{13}\text{C}$ in soil organic carbon	-25.7‰	-23.5‰	-26.7‰	-20.6‰

uous C3 vegetation (3.7) (Fig. 1b). Interestingly, the  $\delta^{13}\text{C}$  value in  $\text{CO}_2$  was temperature-dependent under continuous C3 vegetation. At both sites, increasing temperatures led to increasingly enriched  $\delta^{13}\text{C}$  values of about 1.38 ( $\pm 0.21$ )‰ and 1.51 ( $\pm 0.13$ )‰ per 10°C at HL and RM, respectively. These values were calculated from the slope of regression lines through plots of  $\delta^{13}\text{C}$  in  $\text{CO}_2$  against incubation temperature (Figs. 1c, d). Trends were reproducible when samples first incubated at 5 and 35°C were incubated a second time at the opposite end of the temperature range. These trends stress the importance of control treatments in studies based on C3 to C4 vegetation change. Since we have not further investigated the cause of the temperature dependence of  $\delta^{13}\text{C}$  in our control treatments, we can only speculate about possible mechanisms involved. They could indicate increasing decomposition at higher temperatures of compounds generally enriched in  $^{13}\text{C}$  against background, such as sugars, amino-acids, hemicellulose or pectin (Glaser, 2005, and references therein). They could also be caused by a growing importance of fungal respiration relative to microbial respiration at higher temperatures (Kelliher et al., 2005, and references therein). However, it is more likely that they are caused by the Raleigh fractionation in combination with metabolic branching (Schmidt, 2003). At moderate temperatures soil microbial organisms exhibit low metabolic quotients suggesting that only between 70 to 50% of carbon is respired. In this case, isotope fractionation during the pyruvate-dehydrogenase reaction (Melzer and Schmidt, 1987) liberates depleted carbon for respiration. However, at higher temperatures the metabolic quotient is decreasing and most carbon is used for respiration, so that the pyruvate-dehydrogenase reaction is almost complete and no fractionation occurs. Consequently, respired carbon will be isotopically enriched with increasing temperature. A further possibility to explain the temperature dependent fractionation in the C3 soil could be a decrease in the activity of Gram-negative bacteria relative the activity of Gram-positive bacteria with increasing temperature. Gram-negative bacteria preferentially degrade isotopically more depleted plant car-

bon whereas Gram-positive bacteria degrade relatively more enriched soil organic matter. This was recently confirmed for both study sites using compound specific  $^{13}\text{C}$  and  $^{14}\text{C}$  contents of microbial phospholipid fatty acids (Kramer and Gleixner, 2006). Trends in  $\delta^{13}\text{C}$  of respired  $\text{CO}_2$  with temperature were also found in other studies. Biasi et al. (2005) and Andrews et al. (2000) found increasing temperature to result in increasingly depleted  $\delta^{13}\text{C}$  of respired  $\text{CO}_2$ , which is in contrast to our findings and probably the result of changes in microbial community structure as a reaction to a long-term temperature change.

To test differences in temperature sensitivity of young and old carbon, we compared changes in the isotopic signature of  $\text{CO}_2$  across the studied temperature range between the C3 soils and the soils where vegetation changed from C3 to C4 type. At HL, the mean increase in  $\delta^{13}\text{C}$  values of released  $\text{CO}_2$  was the same for both soils (Fig. 1c). The difference (0.05‰) per 10°C increase in temperature was much smaller than the combined errors of the slopes ( $\pm 0.31$ ‰). This indicates that at this site, carbon older or younger than 45 years respectively has the same temperature sensitivity. At RM,  $\delta^{13}\text{C}$  values of soil 26 years under C4 vegetation were slightly more enriched with increasing temperatures (Fig. 1d). A temperature increase of 10°C resulted on average in an enrichment 1.20 ( $\pm 0.18$ )‰ larger than in soil under continuous C3 vegetation. In other words, a conceivable temperature increase of 5°C above the current mean annual temperature of 8.7°C by the end of this century, would cause a 9.6% increase in stimulation of the decomposition of soil carbon younger than 26 years, compared with older soil carbon. The statistical significance of this result underlines the precision of our approach. However, unlike at HL, the  $Q_{10}$  values differed considerably at RM between the plots. This might result from differences in substrate availability (Davidson and Janssens, 2006). Almost twice the amount of litter is incorporated annually by C4 compared to C3 vegetation at RM (Ludwig et al., 2005) because maize produces larger litter quantities than wheat. Thus, sugars, amino-acids, hemicellulose or pectin enriched in  $\delta^{13}\text{C}$  against the rest of

the younger carbon background as well as the ratio of Gram-negative to Gram-positive bacteria might not be exactly the same under C4 and C3 vegetation at the RM site. Thus, the greater enrichment of CO<sub>2</sub> in <sup>13</sup>C with increased temperature at the C4 site might not necessarily indicate a greater temperature sensitivity of younger soil organic carbon. It could equally result from a greater influence of the same factors that have possibly caused the <sup>13</sup>C enrichment of CO<sub>2</sub> at the C3 control sites. At HL, grain and straw were removed from both parts of the field (John et al., 2003) and therefore annual carbon inputs to soil and Q<sub>10</sub> values were more similar. Taking this into consideration, we conclude that albeit detectable, the small effect observed at RM is likely to be the result of different amounts of annual carbon input rather than being a real difference in temperature sensitivities between young and old carbon.

This result and the robustness of our findings may help to reduce the uncertainty in predicting changes in atmospheric CO<sub>2</sub> concentrations associated with the long-term response of soil carbon decomposition to increasing temperatures. Our study was limited to agricultural soils but natural soils may behave similarly. Unlike previous studies based on a shift from C3 to C4 vegetation, our results were neither influenced by changes in microbial community composition (Waldrop and Firestone, 2004) nor by different substrate qualities in our temperature treatments (Bol et al., 2003). Our results suggest that the carbon cycle feedback on climate change will neither be small and short lived until young carbon pools have reached a new dynamic equilibrium, nor accelerating with a growing share of CO<sub>2</sub> from old carbon, but steady and persistent in agreement with Townsend et al. (1997) and Fang et al. (2005, 2006). Similarly, for northern peatlands, Dioumaeva et al. (2002) found increasing rates of respiration with temperature but an unchanged relative proportion of <sup>14</sup>C in respired CO<sub>2</sub>, which may suggest an equal sensitivity of old and young carbon to warming also for this important source of CO<sub>2</sub>.

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