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## Physical protection of soil carbon in macroaggregates does not reduce the temperature dependence of soil CO<sub>2</sub> emissions

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### Abstract

In a warmer world, soil carbon dioxide (CO<sub>2</sub>) emissions are likely to increase. There is still much discussion about which soil organic carbon (SOC) pools are more sensitive to increasing temperatures. While the temperature sensitivity of carbon (C) stabilized by biochemical recalcitrance or by sorption to mineral surfaces has been characterized, few studies have been carried out on the temperature sensitivity – expressed as Q<sub>10</sub> – of C physically protected inside soil macroaggregates (0.2-2 mm). It has been suggested that increasing the availability of labile SOC by exposing C through macroaggregate crushing would decrease Q<sub>10</sub>, *i.e.* the temperature dependence of soil CO<sub>2</sub> emissions.

To test this hypothesis, the temperature dependence of CO<sub>2</sub> emissions from C physically protected in macroaggregates was measured through 21-day laboratory incubations of crushed and uncrushed soils, at 18°C and 28°C. 199 topsoil samples, acidic or calcareous, with SOC ranging from 2 to 121 g kg<sup>-1</sup> soil, were investigated.

The CO<sub>2</sub> emissions were slightly more sensible to temperature than to C deprotection: about 0.3 mg C g<sup>-1</sup> soil (= 13 mg C g<sup>-1</sup> SOC) and 0.2 mg C g<sup>-1</sup> (= 12 mg C g<sup>-1</sup> SOC) were additionally mineralized, in average, by increasing the temperature or by disrupting the soil structure, respectively. The mean Q<sub>10</sub> index ratio of CO<sub>2</sub> emitted at 28°C and 18°C was similar for crushed and uncrushed soil samples and equaled 1.6. This was partly explained because Q<sub>10</sub> of macroaggregate-protected C was 1. The results did not support the initial hypothesis of lower temperature dependence of soil CO<sub>2</sub> emissions after macroaggregate disruption, although a slight decrease of Q<sub>10</sub> was noticeable after crushing for soils with high

amounts of macroaggregate-protected C. Field research is now needed to confirm that soil tillage might have no effect on the temperature sensitivity of SOC stocks.

## 1. Introduction

Ecosystems play a major role in regulating the concentration of atmospheric greenhouse gases (carbon dioxide – CO<sub>2</sub> –, methane – CH<sub>4</sub> –, and nitrous oxide – N<sub>2</sub>O) concentrations (*Cerri et al., 2004; Smith, 2012*). The CO<sub>2</sub> fluxes between terrestrial ecosystems and the atmosphere resulted in a current global sink of about 2.4 Pg C yr<sup>-1</sup> over the period 2000-2009 (*Friedlingstein et al., 2010*). However, an increase in global ecosystem CO<sub>2</sub> emissions may change the global terrestrial ecosystem from a sink into a source, accelerating the increase in CO<sub>2</sub> concentration in the atmosphere. Temperature anomalies during recent years were positively and significantly correlated with soil CO<sub>2</sub> production, with an increase of 0.1 Pg C yr<sup>-1</sup> between 1989 and 2008 (*Bond-Lamberty and Thomson, 2010*). It is estimated that half of the CO<sub>2</sub> emissions from soils is produced by microbial activity involved in soil organic matter (SOM) decomposition, *i.e.* heterotrophic respiration (*Bond-Lamberty et al., 2004*). Hence determining the effect of increasing temperatures on heterotrophic soil respiration is a major priority.

On the other hand, SOC pools and turnover play key roles in building and sustaining soil fertility. Thus, the temperature dependence of SOC decomposition needs to be fully characterized in order to improve the modeling of SOC dynamics and to understand the dynamics of SOC stocks in agrosystems (*Gabrielle et al., 2002*). Decomposition and mineralization of SOC also depends on the frequency and intensity of soil disturbance by tillage practices. Conventional tillage causes additional CO<sub>2</sub> fluxes from the soil to the atmosphere in various pedoclimatic conditions (*Reicosky et al., 1997; Alvaro-Fuentes et al., 2007*). Mineralization of SOC is increased by the disruption of soil aggregates, which exposes SOC that was previously protected physically against microbial decomposition (*Balesdent et al., 2000; Six et al., 2004*).

Although several experimental studies and reviews have focused on the temperature sensitivity of SOC mineralization (*Kirschbaum 1995; Von Lützow and Kögel-Knabner 2009; Conant et al., 2011; Hamdi et al., 2013*), its accurate prediction for a given soil remains difficult. This is due to the diversity of factors that may influence a real or apparent temperature response, the difficulty of defining a measure of substrate quality, and the imprecise terminology of substrate recalcitrance (*Conant et al., 2011*). Substrate quality has usually been described in terms of biochemical processes, which generally follow the

Arrhenius and Van t'Hoff equations, showing that the decomposition of complex, recalcitrant SOC with a high activation energy is more temperature-dependent than the decomposition of simple, labile SOC with a lower activation energy (*Davidson and Janssens, 2006*). It is well established that SOC is not only stabilized by biochemical processes but also by chemical and physical processes such as adsorption on mineral surfaces and physical protection in soil aggregates (*Six et al., 2004; Leifeld and Kogel-Knabner, 2005; Razafimbelo et al., 2008;*). Although the physical protection of SOC inside macroaggregates represented only less than  $10 \text{ g } 100 \text{ g}^{-1}$  total SOC, it could represent up to 50% of SOC mineralizable in 21 days (*Chevallier et al., 2004*). While the temperature sensitivity of SOC stabilized by biochemical recalcitrance (*Conant et al., 2008*) or by sorption on mineral surfaces (*Leifeld and Fuhrer, 2005*) has been studied, only few studies have evaluated the temperature sensitivity of SOC physically protected inside soil aggregates (*Plante et al., 2009*). The SOC physically protected inside macroaggregates is usually estimated by comparing SOC mineralization from crushed ( $< 0.2 \text{ mm}$  or  $< 0.5 \text{ mm}$ ) and uncrushed ( $0.2\text{-}2 \text{ mm}$  or  $2\text{-}4 \text{ mm}$ ) soil samples (*Balesdent et al., 2000; Razafimbelo et al., 2008; Plante et al., 2009*). Plante et al. (2009) did not observe any difference in temperature sensibility of SOC, measured with  $Q_{10}$  values, between treatments comparing different levels of physical disruption. The  $Q_{10}$  index is the proportional change in respiration rates with a temperature increase of  $10^{\circ}\text{C}$ . A  $Q_{10}$  value higher than 1 means that respiration increases with temperature. On the one hand, deprotecting SOC and thus increasing the availability of labile SOC by soil crushing increases global soil respiration (*Balesdent et al., 2000*); on the other hand, adding labile substrates to decomposers could reduce  $Q_{10}$  and the global response of soil respiration to temperature (*Davidson and Janssens, 2006; Hamdi et al., 2011*). Plante et al. (2009) did not observe an effect of SOC protection on  $Q_{10}$  values, probably due to the higher quantity of unprotected SOC compared with the quantity of labile SOC exposed by soil disruption in the topsoils studied. Our hypothesis was that crushing increases the labile SOC pool and consequently the soil  $\text{CO}_2$  emissions. In the same time, adding labile SOC by soil crushing would decrease the temperature dependence of soil  $\text{CO}_2$  emissions ( $Q_{10}$ ), at least in soils with high levels of physically protected C. The present study aims to test the hypothesis by laboratory soil incubations. The temperature sensitivity of mineralization of physically protected C was measured by incubating uncrushed (2 mm) and crushed (0.2 mm) soil material of 199 topsoil samples originating from France (acid soils) and Tunisia (calcareous soils) at  $18^{\circ}\text{C}$  and  $28^{\circ}\text{C}$ .

## 2. Materials and Methods

### 2.1. Sites and soils

The study analyzed 199 topsoil samples. Among them, 102 originated from France and 97 from Tunisia. The French soil samples were taken from the French Réseau de Mesures de la Qualité des Sols (RMQS), which is a national soil quality monitoring network (Jolivet et al., 2006). The RMQS surveys soils and their properties at *ca.* 2200 sites located on a regular 16-km grid across the French territory. At each site, 25 individual core samples of approximately 1 kg had been taken at 0–30 cm depth in a stratified unaligned systematic sampling design within a 20 m × 20 m area, and bulked to make a composite sample. For this study, soils were mainly from Brittany and the Massif Central. They were acidic and were mainly Luvisols, Cambisols, Regosols, sometimes Colluvic, and Leptosols (IUSS-ISRIC-FAO, 2006). These soil samples from France were mostly loamy soils (fine silt content > 300 g kg<sup>-1</sup> soil and sand content 300-500 g kg<sup>-1</sup> soil), but four were clayey (clay content > 300 g kg<sup>-1</sup> soil) and 45 sandy (sand content > 500 g kg<sup>-1</sup> soil). Clay content averaged 239 ± 5 g clay kg<sup>-1</sup> soil (mean ± standard error). The main land uses were forests, crops and grasslands. The SOC concentration averaged 28 ± 1 g C kg<sup>-1</sup> soil.

The Tunisian soil samples originated from the northern half of Tunisia and had been collected at 0-10 cm depth using a spade. These Tunisian soil samples were mostly loamy, but 23 were clayey and 14 sandy. Most Tunisian soil samples were calcareous. Soil inorganic C (SIC) ranged from 0 to 93 g C kg<sup>-1</sup> soil, averaged 43 ± 26 g C kg<sup>-1</sup> soil and the median was 48 g C kg<sup>-1</sup> soil. Even if the depth of the soil samplings in Tunisia was only 0-10 cm compared to 0-30 cm in France, the SOC concentrations in Tunisian samples were closed to those measured in the French samples. The SOC concentration averaged 21 ± 23 g C kg<sup>-1</sup> soil with a median at 14 g C kg<sup>-1</sup> soil. The Tunisian soils were mainly Calcaric Cambisols and Regosols, Kastanozems, Chromic and Vertic Cambisols (IUSS-ISRIC-FAO, 2006). The main land uses were forests, rangelands, orchards and crops.

### 2.2. Soil preparation and soil carbon determination

The samples were air-dried, gently broken up along natural faults, and sieved to < 2 mm. Coarse particles > 2 mm were discarded. One aliquot (50 g) of the air-dried, 2 mm sieved soil samples was then crushed with mortar and pestle and forced through a 0.2 mm sieve.

SOC concentration was determined on finely ground soil aliquots of 25-30 mg by dry combustion using an elemental analyser (Thermo Fisher Scientific CHN NA2000, Waltham, MA, USA). Calcareous soils were decarbonated prior to SOC determination: 10 mL of water

was added to 1 g of soil and 0.5 M HCl solution was then dripped onto the sample until there was no more effervescence; then samples were washed in water until the pH reached 7. This decarbonation procedure was applied only prior to SOC determination, it was not applied prior to mineralization assays.

SIC content was calculated as 0.12 times soil carbonate content, which was determined on finely ground air-dried soil samples using a Bernard calcimeter, according to the standard French procedure NF ISO 10693 (AFNOR, 1995). The carbonate content was calculated after calibration with a pure calcium carbonate standard.

### 2.3. Mineralization assays

Aliquots of 12 g of each of the uncrushed (2 mm sieved) and crushed (0.2 mm sieved) soil samples were incubated in triplicate at 18°C and 28°C for 21 days, with no pre-incubation, resulting in a total of more than 2400 incubations. As all incubations were not carried out simultaneously, time between soil crushing and incubation onset varied between 10 days and 2 months, during which the air dried soil samples were stored in closed plastic bags. A 3 bar pressure plate was used to determine water retention at -0.01 MPa for uncrushed and crushed soil samples. The water content at -0.01 MPa, which corresponds approximately to 80% of the water-filled pore space, was used as the optimum water content for microbial activity in laboratory conditions (Chevallier et al., 2011a). Deionized water was thus added to soils to a water potential of -0.01 MPa. Each sample was then placed in a 1-L airtight jar with a vial containing 19 mL of aqueous sodium hydroxide (NaOH) solution (0.5 M) to trap the CO<sub>2</sub> emitted. A vial containing 19 mL of deionized water was also added to the jar to keep the relative air humidity constant in the headspace. The amount of CO<sub>2</sub> emitted was determined by back titration (HCl 0.5 M; pH 8.6) of the NaOH solution with an excess of barium chloride (BaCl<sub>2</sub>; 1 M). The jars were opened and each CO<sub>2</sub> trap (NaOH) was replaced at day 7, and collected at day 21. At day 7, the water content of soil was checked by weighing, and deionized water was added when needed to reset soil water potential at -0.01 MPa. Water loss during the 21-day incubation ranged from 0 to 10 g H<sub>2</sub>O for 100 g initial H<sub>2</sub>O. Five jars without soil but with NaOH traps were also incubated at each temperature to determine the amount of initial atmospheric CO<sub>2</sub> in the jar. The cumulative CO<sub>2</sub> emissions over 7 and 21 days were expressed by mean ± standard error in mg C g<sup>-1</sup> soil or in mg C g<sup>-1</sup> SOC.

#### 2.4. Calculations and statistical analyses

Extra C mineralized after crushing the soil has been attributed to the breakdown of soil macroaggregates (0.2-2 mm) and not to the grinding of plant residues (Bossuyt et al., 2002; Chevallier et al., 2011b). The amount of macroaggregate-protected C was calculated as the difference between the amounts of CO<sub>2</sub> emitted from crushed (0.2 mm sieved) and uncrushed (2 mm sieved) soil samples (Beare et al., 1994). It also represented the sensitivity of CO<sub>2</sub> emissions to soil crushing. Macroaggregate-protected C was calculated for each soil sample at 18°C and at 28°C.

The sensitivity of CO<sub>2</sub> emissions to temperature was calculated as the difference between the amounts of CO<sub>2</sub> emitted from uncrushed soil samples at 18°C and at 28°C. The temperature coefficient, Q<sub>10</sub>, is the relative change in respiration with a 10°C increase in temperature (Koepef, 1953; Lloyd and Taylor, 1994). In this experiment, Q<sub>10</sub> was calculated as the ratio between CO<sub>2</sub> emissions at 28°C and 18°C, for uncrushed and crushed soil samples. The Q<sub>10</sub> of the macroaggregate-protected C was also calculated. It was the ratio of CO<sub>2</sub> emitted from macroaggregate-protected C at 28°C and 18°C.

Crushing and temperature impacts on CO<sub>2</sub> emissions were tested by ANOVA using the R software (R-Core-team, 2014). Linear regressions between macroaggregate-protected C (*i.e.* difference between CO<sub>2</sub>-C emitted from crushed and uncrushed samples) or temperature-sensitive CO<sub>2</sub> emissions (*i.e.* difference between CO<sub>2</sub>-C emitted at 28°C and 18°C) and soil variables (*i.e.* SOC, SIC and clay content) or land use were realized by stepwise regression analyses with Akaike's Information Criterion, also using the R software (Efroyimson, 1960).

### 3. Results

The cumulative CO<sub>2</sub> emissions from 2 mm uncrushed and 0.2 mm crushed samples and from macroaggregate-protected C increased with incubation time (Table 1). The presentation will focus on the results after 21-day incubations, except when different trends had occurred at the beginning (7 days). The cumulative CO<sub>2</sub> emissions after 21 days increased significantly with incubation temperature ( $p < 0.001$ ), from 0.6 to 0.9 mg C g<sup>-1</sup> for uncrushed soils, and from 0.8 to 1.2 mg C g<sup>-1</sup> for crushed samples, in average; they also increased significantly with soil crushing, from 0.6 to 0.8 mg C g<sup>-1</sup> soil at 18°C and from 0.9 to 1.2 mg C g<sup>-1</sup> soil at 28°C, in average (Table 1). Relatively to SOC, mean cumulative CO<sub>2</sub> emissions increased significantly with soil crushing ( $p < 0.001$ ), from 29 to 41 mg C g<sup>-1</sup> SOC at 18°C, and from 42 to 54 mg C g<sup>-1</sup> SOC at 28°C. Thus macroaggregate-protected C accounted for 1.2% of total SOC, in average, and for about 26% and 21% of total CO<sub>2</sub> emissions from the crushed soil

after 21-day incubation at 18°C and 28°C, respectively. In other words, around 21-26% of labile SOC was protected inside aggregates 0.2-2 mm. However, 5% of the samples showed a significant decrease in CO<sub>2</sub> emissions after crushing. These soil samples were either acid or calcareous and had clay contents smaller than 250 g clay kg<sup>-1</sup> soil.

The amount of macroaggregate-protected C was positively correlated to total soil C ( $p < 0.001$ ; Figure 1a) and SOC ( $p < 0.001$ ; Figure 1b), and more closely after 7 than after 21 days. Incubation temperature modified slightly these linear relationships. After 7 days of incubation (after sample rewetting), the increase in temperature slightly but significantly increased the intercept of the linear regression ( $p < 0.02$ ) but not the slope; thus the increase in macroaggregate-protected C due to temperature was independent of initial total soil C or SOC contents at the beginning of the incubation. After 21 d of incubation, increasing the temperature also decreased the slope of the linear regressions ( $p < 0.05$ ); thus, the decrease of macroaggregate-protected C with temperature was especially noticeable in soil samples with high C contents.

The increase in mean macroaggregate-protected C from 18°C to 28°C was significant after 7 days of incubation (from 0.15 to 0.19 mg C g<sup>-1</sup> soil,  $p < 0.001$ ), but was no more significant after 21 days (from 0.22 to 0.25 mg C g<sup>-1</sup> soil;  $p > 0.1$ ). Thus the amount of C protected from mineralization in macroaggregates, as determined through soil crushing, was temperature dependent only at the beginning of incubation, *i.e.* the 7 first days after sample rewetting.

The temperature dependence of CO<sub>2</sub> emissions was the same for crushed and uncrushed soil samples. Indeed, the Q<sub>10</sub> ratio of CO<sub>2</sub> emissions between 28°C and 18°C was not affected significantly by crushing after either 7 or 21 days of incubation ( $p > 0.2$ ; Table 2) and equaled 1.6. It is noticeable that CO<sub>2</sub> emissions from 28 soil samples were smaller at 28°C than at 18°C and thus had Q<sub>10</sub> < 1 (Figure 2). The Q<sub>10</sub> index was lower in soils with high CO<sub>2</sub> emissions, *i.e.* with more labile C (Figure 2). After 21 days, the decrease of Q<sub>10</sub> with CO<sub>2</sub> emissions was slightly stronger for crushed (0.2 mm sieved) than for uncrushed (2 mm sieved) soil samples ( $p < 0.001$ ); thus exposing a larger amount of labile C by crushing reduced the temperature dependence of CO<sub>2</sub> emissions (Figure 2).

Although Q<sub>10</sub> calculated from incubation of uncrushed and crushed soil samples did not differ significantly, the difference in Q<sub>10</sub> between crushed and uncrushed samples was negatively correlated with macroaggregate-protected C ( $p < 0.001$ ; Figure 3). In other words, in soils with large amounts of macroaggregate-protected C, crushing decreased the Q<sub>10</sub> of CO<sub>2</sub> emissions. In addition, Q<sub>10</sub> of macroaggregate-protected C was quite low, with a median around 1, and was very variable (Table 2).



Though the sensitivities of CO<sub>2</sub> emissions to temperature and crushing were the same order of magnitude in average, around 0.22-0.32 mg C g<sup>-1</sup> soil or 12-13 mg C g<sup>-1</sup> SOC after 21 days (Table 1), increase in CO<sub>2</sub> emissions was 2.3 times higher after 10°C-temperature increase than after crushing (Figure 4).

As SOC contents, SIC contents were positively correlated to CO<sub>2</sub> emissions from both crushed and uncrushed soil samples. The impacts of soil crushing and temperature on CO<sub>2</sub> emissions were affected significantly by SOC and SIC contents but not by clay content or land use (Table 3). The amount of SIC had a positive and significant impact on macroaggregate-protected C, and for uncrushed samples (2 mm sieved), on additional CO<sub>2</sub> emission due to 10°C increase.

#### 4. Discussion

If we consider that labile SOC can be defined as the SOC fraction mineralized in the first days of soil incubation (*Conant et al., 2008*), around 20-25% of this labile SOC fraction in the studied soils was protected inside 0.2-2 mm aggregates; this macroaggregate-protected labile SOC amounted to 7 to 12 mg C g<sup>-1</sup> SOC on a total of labile SOC representing 23 to 54 mg C g<sup>-1</sup> SOC (Table 1). The data confirmed previous results indicating that (i) the physical separation of substrates and decomposers, due to soil structure, reduces or delays the decomposition of otherwise available labile material (*Beare et al., 1994; Balesdent et al., 2000*), and (ii) the amount of protected labile C increases with total soil C (*Chevallier et al., 2004*). Carbon availability to microorganisms is dependent on soil structure, owing to the slow diffusion of oxygen or substrates to microorganisms or their enzymes located in small pores or in a pore geometry where diffusion pathways are non-continuous or tortuous (*Ruamps et al., 2011*). Soil crushing damages the soil structure and improves substrate diffusion rates and availability (*Balesdent et al., 2000*). The amount of protected SOC depends on the amount of total SOC itself. This process, called “self-protection” of SOC (*Balesdent et al., 2000*), meaning that SOC promotes soil aggregation as a binding agent (*Tisdall and Oades, 1982*), is also likely to promote the storage of SOC in a virtuous circle (*Angers, 1992*).

The amount of protected C estimated by crushing did not increase significantly with incubation temperature after 21 days. In addition soil crushing did not significantly affect the Q<sub>10</sub> values of soil. This indicates that increasing SOC oxidation by crushing did not lead to significant increase in the temperature dependence of CO<sub>2</sub> emissions. These observations on 199 topsoil samples from France and Tunisia confirmed the observations of *Plante et al.*

(2009): the physical protection of C within macroaggregates (0.2-2 mm) did not significantly reduce the temperature dependence of CO<sub>2</sub> emissions. The short incubation time (21 days) could not explain why the amount of protected C did not affect Q<sub>10</sub>. Indeed, C physically protected inside macroaggregates is especially labile, and 21 day incubation has usually been considered enough to estimate its amount (*Beare et al., 1994; Bossuyt et al., 2002; Plante et al., 2009*). The present results did not support the hypothesis that the limited effect of C protection on Q<sub>10</sub> observed in *Plante et al. (2009)* was due to the small proportion of protected C. Studying more soil samples and samples with larger amounts of protected C, as in the present study, did not lead to a significant and noticeable impact of that protection on Q<sub>10</sub>. However, this conclusion on the average results of CO<sub>2</sub> emissions from the 199 crushed and uncrushed soil samples at 18 and 28°C could be specified. Temperature seemed to slightly impact the amount of aggregate-protected C, positively at the beginning of the incubation, and in soils with high C content, negatively after 21 days of incubation (Figure 1). These contrasted results between the two durations of incubation likely stemmed from the absence of a pre-incubation before the first 7 days of incubation. Indeed, the amount of aggregate-protected C depends on the incubation duration (*Gupta and Germida, 1988*). If the soils had been pre-incubated, a large part of the very labile protected C would have been released before measurements, which was not appropriate considering the purpose of this study. Release of solutes and lysis of live microbial cells might explain the mineralization flush of SOC and microbial biomass just after wetting the soils (*Fierer and Schimel, 2002*). This initial flush of CO<sub>2</sub> increases with temperature (*Suseela et al., 2012; Poll et al., 2013*) and could explain a part of the positive effect of temperature on the amount of macroaggregate-protected C at the beginning of the incubation. After 21 days of incubation, and especially in soil samples with high C content, the temperature increase slightly reduced the amount of macroaggregate-protected C. Exposing a larger amount of labile C by crushing tended to reduce the temperature dependence of CO<sub>2</sub> emissions (Figure 2). Furthermore, in soils with high amounts of macroaggregate-protected C, results suggested that the hypothesis that crushing decreased slightly Q<sub>10</sub> could be true. The high lability of macroaggregate-protected C could explain these results. Adding labile substrate or increasing water-soluble C could reduce Q<sub>10</sub> (*Liu et al., 2006; Hamdi et al., 2011*). This effect was debated (*Gershenson et al., 2009*) and seemed to be dependent on the availability of the original substrate (*Davidson and Janssens, 2006*).

The response of CO<sub>2</sub> emissions to temperature was lower than usually reported in literature (1.6 vs. 2.0-2.5; *Hamdi et al., 2013*). Some of these responses, for 28 out of the 199 samples,

could even become negative. In the thermodynamic theory,  $Q_{10}$  can be below 1 when substrate limitation becomes evident (*Davidson and Janssens, 2006*). The explanation of the large variability of measured  $Q_{10}$  could be in the variability of the soil samples, which originated from different ecosystems, and thus in the variability in the quality and in the availability of substrates for decomposers (*Liu et al., 2006; Davidson et al., 2012*). However, in our experiment, the impact of soil management or soil type on  $Q_{10}$  was not significant, as in a metaanalysis study a large variability of measured  $Q_{10}$  remained largely unexplained (*Hamdi et al., 2013*). The C protected inside macroaggregates was not sensible to temperature, with a mean  $Q_{10}$  around 1. *Gillabel et al. (2010)* also observed that C protection reduced the temperature dependence of  $CO_2$  emissions. This could be explained by the different qualities and turnover times between unprotected and protected SOC. The high lability of C protected inside aggregates (*Golchin et al., 1995*) and lower  $Q_{10}$  indexes for labile than for recalcitrant SOC (*Conant et al., 2008*) could explain the low  $Q_{10}$  of macroaggregate-protected C.

Crushing the soil and increasing the temperature affect the metabolism and growth rates of microorganisms, and lead to higher respiration rates, approximately  $+12\text{mg C g}^{-1}\text{ SOC}$  in the present study. The increase in  $CO_2$  emissions was more marked with increasing temperature than with soil crushing, though similar in average (Figure 4 and Table 1). Both crushing and increasing temperature induce higher diffusion rates, higher C solubilization, higher cellular enzyme activity, and faster substrate uptakes (*Balesdent et al., 2000; Agren and Wetterstedt, 2007*). In addition, at higher temperatures substrate availability would be greater because the desorption of SOC from the mineral matrix increases with increasing temperature (*Kalbitz et al., 2000; Marschner and Bredow, 2002*), and also because high temperatures with optimum soil water content lead to increases in water-soluble C and labile C pools (*Liu et al., 2006*). Crushing at 0.2 mm does not modify the availability to microbial mineralization of the main protected SOC pool, within microaggregates ( $< 0.2\text{ mm}$ ), or through association with minerals (*Six et al., 2004; Zinn et al., 2007*). However the present experiment did not show conclusively that the same C pools were involved in the sensitivities to increasing temperature and to crushing.

The negative values sometimes observed for protected C (Figures 1, 3 and 4) mostly occurred in soils with clay content  $< 250\text{ g kg}^{-1}$  soil. There were several explanations: (i) the “protective capacity” of macroaggregates has been described to be linked to high clay and SOC contents (*Balesdent et al., 2000*), thus soil samples with lower clay content might have small or no significant amount of C protected; (ii) negative values could be also related to highly porous macroaggregates with a low physical protection potential of C within larger

pores (*Pulleman and Marinissen, 2004*); and (iii) some fractions of macroaggregate-protected C could be not labile in the 21 days of incubation. However, that last explanation was unlikely since macroaggregate-protected C was usually considered especially labile in short term (e.g., *Gupta and Germida, 1988*). Carbonate dissolution could not explain the negative protected C values because they were measured from both acidic and calcareous soil samples. In addition, SIC was positively correlated to CO<sub>2</sub> emissions from both crushed and uncrushed soil samples. Carbonate dissolution may be increased through reaction with organic acids produced by SOM decay or biological activities resulting from warming or soil crushing (*Bertrand et al., 2007*). Carbonate dissolution could explain the positive relationships found between SIC and macroaggregate-protected C or temperature sensibility of CO<sub>2</sub> emissions (Table 3), even if re-crystallization of calcium carbonate might compensate for the contribution of SIC to total CO<sub>2</sub> emissions (*Tamir et al., 2012*). The positive interactions between SOC and SIC contents on CO<sub>2</sub> emissions from macroaggregate-protected C at 18°C (Table 3) confirmed that SIC dynamics would not be independent from SOC dynamics and CO<sub>2</sub> emissions.

## **5. Conclusion**

The protection of C within 0.2-2 mm aggregates did not affect the temperature dependence of CO<sub>2</sub> emissions, unlike SOC stabilization by biochemical recalcitrance (*Conant et al., 2008*) or sorption on mineral surfaces (*Leifeld and Fuhrer, 2005*). Even if macroaggregate crushing in the laboratory represents a more intense disruption of the soil structure than soil tillage in the field, this study attempted to provide preliminary evidence on whether intensive tillage might influence the sensitivity of SOC stocks to increasing temperature. Field research is now needed to confirm that soil tillage might have no effect on the sensitivity of SOC stocks to increasing temperature.

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## References

- AFNOR-Association-Française-de-Normalisation* (1995): Qualité du Sol - Détermination de la Teneur en Carbonate - Méthode Volumétrique, Paris, France.
- Agren, G.I., Wetterstedt, J.A.M.* (2007): What determines the temperature response of soil organic matter decomposition? *Soil Biol Biochem* 39, 1794-1798.
- Alvaro-Fuentes, J., Cantero-Martinez, C., Lopez, M.V., Arrue, J.L.* (2007): Soil carbon dioxide fluxes following tillage in semiarid Mediterranean agroecosystems. *Soil Tillage Res.* 96, 331-341.
- Angers, D.A.* (1992): Changes in soil aggregation and organic carbon under corn and alfalfa. *Soil Sci Soc Am J* 56, 1244-1249.
- Balesdent, J., Chenu, C., Balabane, M.* (2000): Relationship of soil organic matter dynamics to physical protection and tillage. *Soil Tillage Res.* 53, 215-230.
- Beare, M.H., Cabrera, M.L., Hendrix, P.F., Coleman, D.C.* (1994): Aggregate-protected and unprotected organic matter pools in conventional-tillage and no-tillage soils. *Soil Sci Soc Am J* 58, 787-795.
- Bertrand, I., Delfosse, O., Mary, B.* (2007): Carbon and nitrogen mineralization in acidic, limed and calcareous agricultural soils: Apparent and actual effects. *Soil Biol Biochem* 39, 276-288.
- Bond-Lamberty, B., Thomson, A.* (2010): Temperature-associated increases in the global soil respiration record. *Nature* 464, 579-582.
- Bond-Lamberty, B., Wang, C.K., Gower, S.T.* (2004): A global relationship between the heterotrophic and autotrophic components of soil respiration? *Glob Change Biol* 10, 1756-1766.
- Bossuyt, H., Six, J., Hendrix, P.F.* (2002): Aggregate-protected carbon in no-tillage and conventional tillage agroecosystems using carbon-14 labeled plant residue. *Soil Sci Soc Am J* 66, 1965-1973.
- Cerri, C.C., Bernoux, M., Cerri, C.E.P., Feller, C.* (2004): Carbon cycling and sequestration opportunities in South America: the case of Brazil. *Soil Use. Manage.* 20, 248-254.
- Chevallier, T., Blanchart, E., Toucet, J., Bernoux, M.* (2011a): Methods to estimate the protection of soil organic carbon within macroaggregates 1- Does soil water status affect the estimated amount of soil organic carbon protected inside macroaggregate? *Communication in Soil Science and Plant Analysis* 42, 1522-1536.
- Chevallier, T., Blanchart, E., Toucet, J., Bernoux, M.* (2011b): Methods to estimate aggregate protected soil organic carbon - 2 Does the grinding of the plant residues affect the estimations of the aggregate protected soil organic carbon? *Communications in Soil Science and Plant Analysis* 42, 1537-1543.

- Chevallier, T., Blanchart, E., Albrecht, A., Feller, C. (2004): The physical protection of soil organic carbon in aggregates: a mechanism of carbon storage in a Vertisol under pasture and market gardening (Martinique, West Indies). *Agr Ecosyst Environ* 103, 375-387.
- Conant, R.T., Drijber, R.A., Haddix, M.L., Parton, W.J., Paul, E.A., Plante, A.F., Six, J., Steinweg, J.M. (2008): Sensitivity of organic matter decomposition to warming varies with its quality. *Glob Change Biol* 14, 868-877.
- Conant, R.T., Ryan, M.G., Agren, G.I., Birge, H. E., Davidson, E.A., Eliasson, P.E., Evans, S.E., Frey, S.D., Giardina, C.P., Hopkins, F.M., Hyvönens, R., Kirschbaum, M.U.F., Lavalley, J.M., Leifeld, J., Parton, W.J., Steinweg, J.M., Wallenstein, M.D., Wetterstedt, J.A.M., Bradford, M.A. (2011): Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. *Glob Change Biol* 11, 3392–3404.
- Davidson, E.A., Janssens, I.A. (2006): Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440, 165-173.
- Davidson, E.A., Samanta, S., Caramori, S.S., Savage, K. (2012): The Dual Arrhenius and Michaelis-Menten kinetics model for decomposition of soil organic matter at hourly to seasonal time scales. *Glob Change Biol* 18, 371-384.
- Efroymson, M.A. (1960): Multiple regression analysis. In A. Ralston and H. S. Wilf: *Mathematical Methods for Digital Computers*. Wiley: New York, 191-203.
- Fierer, N., Schimel, J.P. (2002): Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology & Biochemistry* 34, 777-787.
- Friedlingstein, P., Houghton, R.A., Marland, G., Hackler, J., Boden, T.A., Conway, T.J., Canadell, J.G., Raupach, M.R., Ciais, P., Le Quéré, C. (2010): Update on CO<sub>2</sub> emissions. *Nature Geoscience* 3, 811-812.
- Gabrielle, B., Mary, B., Roche, R., Smith, P., Gosse, G. (2002): Simulation of carbon and nitrogen dynamics in arable soils: a comparison of approaches. *Eur J Agron* 18, 107-120.
- Gershenson, A., Bader, N.E., Cheng, W.X. (2009): Effects of substrate availability on the temperature sensitivity of soil organic matter decomposition. *Glob Change Biol* 15, 176-183.
- Gillabel, J., Cebrian-Lopez, B., Six, J., Merckx, R. (2010): Experimental evidence for the attenuating effect of SOM protection on temperature sensitivity of SOM decomposition. *Glob Change Biol* 16, 2789-2798.
- Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P. (1995): Structural and dynamic properties of soil organic matter as reflected by C-13 natural abundance, pyrolysis mass spectrometry and solid-state C-13 NMR spectroscopy in density fractions of an oxisol under forest and pasture. *Aust. J. Soil. Res.* 33, 59-76.
- Gupta, V.V.S.R., Germida, J.J. (1988): Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. *Soil Biol Biochem* 20, 777-786.

- Hamdi, S., Chevallier, T., Ben Aïssa, N., Ben Hammouda, M., Gallali, T., Chotte, J.L., Bernoux, M. (2011): Short-term temperature dependence of heterotrophic soil respiration after one-month of pre-incubation at different temperatures. *Soil Biol Biochem* 43, 1752-1758.
- Hamdi, S., Moyano, F., Sall, S.N., Bernoux, M., Chevallier, T. (2013): Synthesis analysis of the temperature sensitivity of soil respiration from laboratory studies in relation to incubation methods and soil conditions. *Soil Biol Biochem* 58, 115-126.
- IUSS-ISRIC-FAO (2006): World Reference Base for Soil resources 2006: World Soil Resources Reports No103. FAO: Rome, Italy.
- Jolivet, C., Arrouays, D., Boulonne, L., Ratié, C., Saby, N. (2006): Le Réseau de mesures de la qualité des sols de France (RMQS). Etat d'avancement et premiers résultats. *Etude et Gestion des Sols* 13, 149-164.
- Kalbitz, K., Solinger, S., Park, J.H., Michalzik, B., Matzner, E. (2000): Controls on the dynamics of dissolved organic matter in soils: A Review. *Soil Science* 165, 277-304.
- 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 Biol Biochem* 38, 2510-2518.
- Koepf, H. (1953): Die Temperatur/Zeit-Abhängigkeit der Bodenatmung. *Zeitschrift für Pflanzenernährung, Düngung und Bodenkunde* 61, 29-48.
- Leifeld, J., Fuhrer, J. (2005): The temperature response of CO<sub>2</sub> production from bulk soils and soil fractions is related to soil organic matter quality. *Biogeochemistry* 75, 433-453.
- Leifeld, J., Kogel-Knabner, I. (2005): Soil organic matter fractions as early indicators for carbon stock changes under different land-use? *Geoderma* 124, 143-155.
- Liu, H.S., Li, L.H., Han, X.G., Huang, J.H., Sun, J.X., Wang, H.Y. (2006): Respiratory substrate availability plays a crucial role in the response of soil respiration to environmental factors. *Appl Soil Ecol* 32, 284-292.
- Lloyd, J., Taylor, J.A. (1994): On the temperature dependence of soil respiration. *Funct Ecol* 8, 315-323.
- Marschner, B., Bredow, A. (2002): Temperature effects on release and ecologically relevant properties of dissolved organic carbon in sterilised and biologically active soil samples. *Soil Biology & Biochemistry* 34, 459-466.
- Plante, A.F., Six, J., Paul, E.A., Conant, R.T. (2009): Does physical protection of soil organic matter attenuate temperature sensitivity? *Soil Sci Soc Am J* 73, 1168-1172.
- Poll, C., Marhan, S., Back, F., Niklaus, P., Kandeler, E. (2013): Field-scale manipulation of soil temperature and precipitation change soil CO<sub>2</sub> flux in a temperate agricultural ecosystem. *Agr Ecosyst Environ* 165, 88-97.
- Pulleman, M.M., Marinissen, J.C.Y. (2004): Physical protection of mineralizable C in aggregates from long-term pasture and arable soil. *Geoderma* 120, 273-282.

- R-Core-team* (2014): R: A language and environment for statistical computing. Available at <http://www.R-project.org/> Vienna, Austria.
- Razafimbelo, T., Albrecht, A., Oliver, R., Chevallier, T., Chapuis-Lardy, L., Feller, C.* (2008): Aggregate associated-C and physical protection in a tropical clayey soil under Malagasy conventional and no-tillage systems. *Soil Tillage Res.* 98, 140-149.
- Reicosky, D.C., Dugas, W.A., Torbert, H.A.* (1997): Tillage-induced soil carbon dioxide loss from different cropping systems. *Soil Tillage Res.* 41, 105-118.
- Ruamps, L., Nunan, N., Chenu, C.* (2011): Microbial biogeography at the soil pore scale. *Soil Biol Biochem* 43, 280-286.
- Six, J., Bossuyt, H., Degryze, S., Denef, K.* (2004): A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil Tillage Res.* 79, 7-31.
- Smith, P.* (2012): Agricultural greenhouse gas mitigation potential globally, in Europe and in the UK: what have we learnt in the last 20 years? *Glob Change Biol* 18, 35-43.
- Suseela, V., Conant, R.T., Wallenstein, M.D., Dukes, J.S.* (2012): Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment. *Glob Change Biol* 18, 336-348.
- Tamir, G., Shenker, M., Heller, H., Bloom, P.R., Fine, P., Bar-Tal, A.* (2012): Dissolution and re-crystallization processes of active carbonate in soil developed on Tufa. *Soil Sci Soc Am J* 76, 1606-1613.
- Tisdall, J.M., Oades, J.M.* (1982): Organic matter and water-stable aggregates in soils. *J. Soil Sci.* 33, 141-163.
- Von Lützow, M., Kögel-Knabner, I.* (2009): *Temperature sensitivity of soil organic matter decomposition - what do we know?* *Biol Fertil Soils* 46, 1-15.
- Zinn, Y.L., Lal, R., Bigham, J.M., Resck, D.V. S.* (2007): Edaphic controls on soil organic carbon retention in the Brazilian Cerrado: Texture and Mineralogy. *Soil Sci Soc Am J* 71, 1204-1214.



**Table 1.** Amount of CO<sub>2</sub> emissions (in mg C g<sup>-1</sup> soil and in mg C g<sup>-1</sup> SOC) after 7- and 21-day incubation according to sample preparation (2 mm uncrushed and 0.2 mm crushed soil) and incubation temperature (18°C and 28°C). Data are presented as mean ± standard error (*n* = 199, *i.e.* 199 soil samples were incubated uncrushed and crushed at 18 and 28°C in triplicate; data presented here are averages over the 199 means for each treatment).

Incubation conditions	Uncrushed soil (2 mm)		Crushed soil (0.2 mm)		Macroaggregate-protected C	
	mg C g <sup>-1</sup> soil	mg C g <sup>-1</sup> SOC	mg C g <sup>-1</sup> soil	mg C g <sup>-1</sup> SOC	mg C g <sup>-1</sup> soil	mg C g <sup>-1</sup> SOC
7 days						
18°C	0.33 ± 0.01	15 ± 1	0.48 ± 0.02	23 ± 1	0.15 ± 0.01	7 ± 1
28°C	0.49 ± 0.02	23 ± 1	0.68 ± 0.03	32 ± 1	0.19 ± 0.01	9 ± 1
21 days						
18°C	0.61 ± 0.03	29 ± 1	0.83 ± 0.04	41 ± 2	0.22 ± 0.02	11 ± 1
28°C	0.93 ± 0.04	42 ± 1	1.17 ± 0.05	54 ± 2	0.25 ± 0.02	12 ± 1

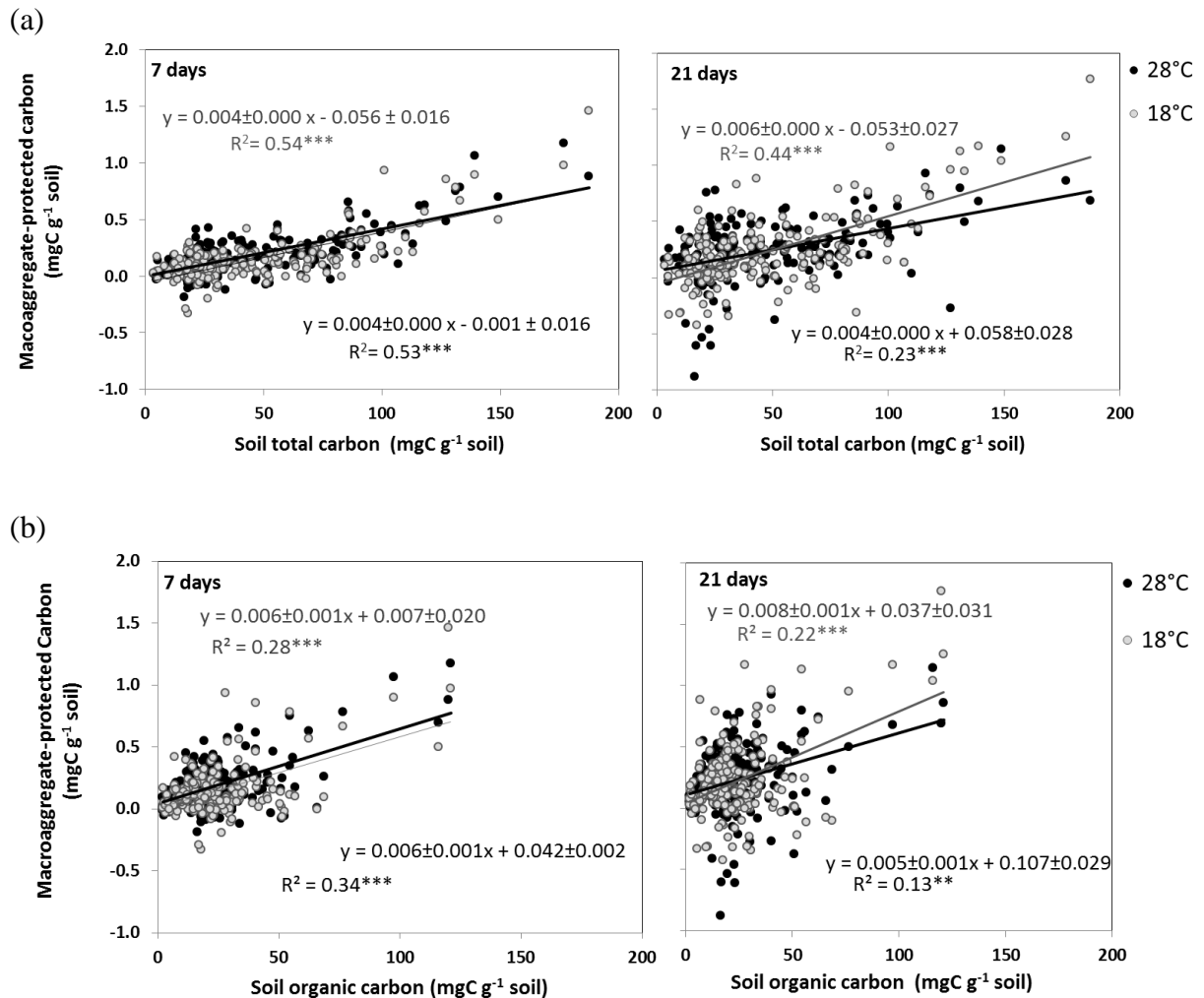
**Table 2.** Q<sub>10</sub> index ratio of CO<sub>2</sub> emitted at 28°C and 18°C after 7- and 21-day incubation according to sample preparation (2-mm uncrushed and 0.2-mm crushed soil), *n* = 199.

Incubation time		Uncrushed soil (2 mm)	Crushed soil (0.2 mm)	Macroaggregate-protected C
7 days				
	mean ± standard error	1.63 ± 0.04	1.55 ± 0.05	0.61 ± 0.28
	1 <sup>st</sup> quart - median - 3 <sup>rd</sup> quart	1.3 - 1.6 - 1.9	1.3 - 1.5 - 1.7	0.2 - 1.2 - 1.7
21 days				
	mean ± standard error	1.67 ± 0.05	1.63 ± 0.09	0.83 ± 0.37
	1 <sup>st</sup> quart - median - 3 <sup>rd</sup> quart	1.3 - 1.6 - 1.9	1.3 - 1.5 - 1.7	0.1 - 1.0 - 1.5

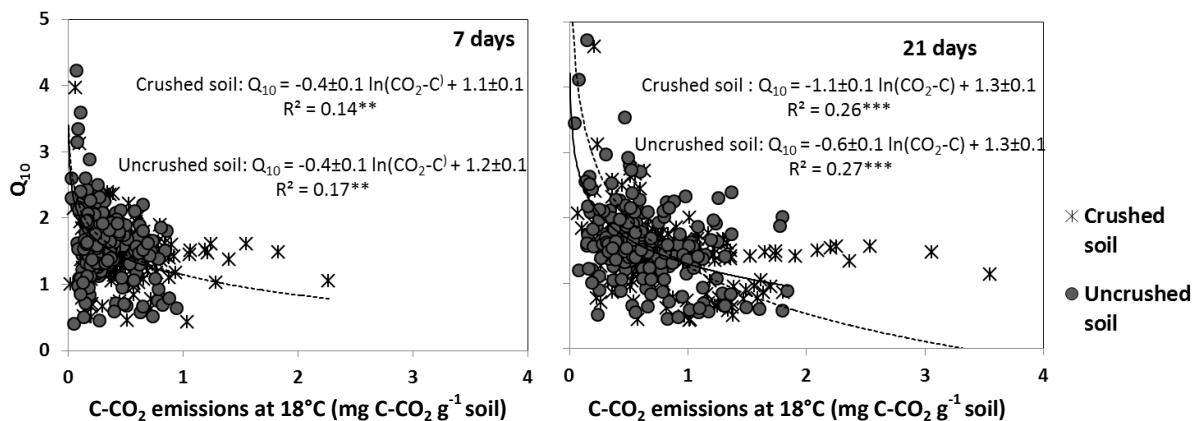
**Table 3.** Effects of soil organic (SOC) and inorganic carbon contents (SIC) and of land use and clay content on macroaggregate-protected carbon (*i.e.* difference between C-CO<sub>2</sub> emitted from crushed and uncrushed samples) and temperature-sensitive CO<sub>2</sub>-C emissions (*i.e.* difference between C-CO<sub>2</sub> emitted at 28°C and 18°C) after 21 days of incubation. P values (> F) were calculated after stepwise analysis.

Variable	Macroaggregate-protected carbon after 21 days		Temperature-sensitive CO <sub>2</sub> -C emitted after 21 days	
	at 28°C	at 18°C	Uncrushed	Crushed
SOC	< 0.001	< 0.001	< 0.001	< 0.001
SIC	0.013	< 0.001	< 0.001	0.634
Land use	0.421	0.689	0.298	0.428
Clay content	0.185	0.210	0.954	0.840
SOC × SIC	0.668	< 0.001	0.006	0.574
SOC × land use	0.216	0.850	0.321	0.990
SOC × clay content	0.948	0.322	0.570	0.342
SIC × land use	0.530	0.635	0.019	0.023
Clay content × SIC	0.084	0.136	0.645	0.160
Residuals (mg C g <sup>-1</sup> soil)	0.29	0.21	0.31	0.36

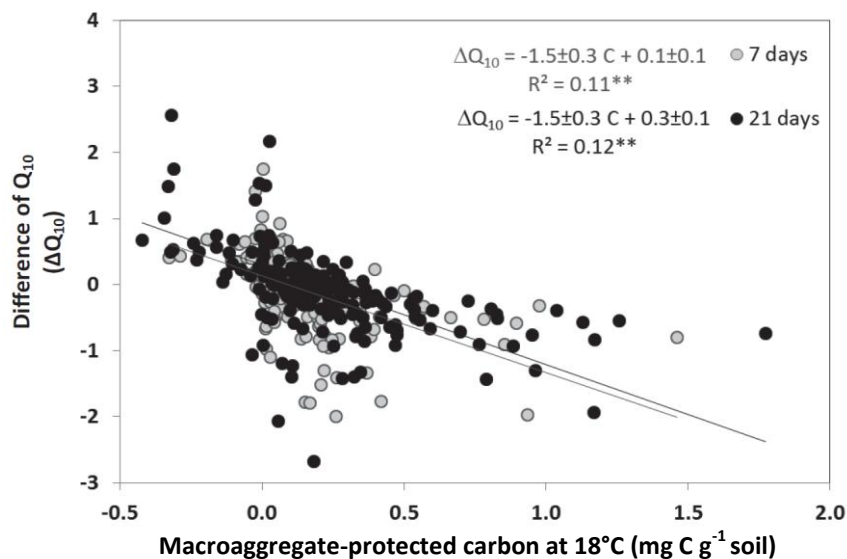
**Figure 1.** Correlations between macroaggregate-protected C (*i.e.* difference between C-CO<sub>2</sub> from crushed and uncrushed soil samples) at 18°C (grey circles) or 28°C (black circles) and total soil carbon (a) or soil organic carbon (b), after 7 and 21 days of incubation, respectively. Regression parameter uncertainties ( $\pm$ ) are standard errors, \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  ( $n = 199$ ).



**Figure 2.** Relationships between Q<sub>10</sub> calculated on uncrushed (2 mm sieved) and crushed (0.2 mm sieved) soil and the amount of C-CO<sub>2</sub> emitted at 18°C after 7 and 21 days of incubation. Dotted and plain lines are fitted to logarithm functions for crushed and uncrushed samples, respectively. Regression parameter uncertainties are standard errors, \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  ( $n = 199$ ).



**Figure 3.** Relationships between the difference in  $Q_{10}$  between crushed and uncrushed samples (*i.e.* crushing impact on the temperature dependence of soil  $CO_2$  emissions), and macroaggregate-protected C at  $18^\circ C$  after 7 and 21 days, respectively. \*\*  $p < 0.01$  ( $n = 199$ ).



**Figure 4.** Relationship between the sensitivities of  $CO_2$  emissions to crushing (difference between C- $CO_2$  emitted from crushed and uncrushed samples at  $18^\circ C$ ) and to temperature (difference between C- $CO_2$  emitted from uncrushed samples at  $18^\circ C$  and  $28^\circ C$ ) after 21 days. Regression parameter uncertainties are standard errors; \*\*  $p < 0.01$  ( $n = 199$ ).

