Short-term temperature impact on soil heterotrophic respiration in limed agricultural soil samples

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Abstract This study sought to investigate the hourly and daily timescale responses of soil CO_2 fluxes to temperature in a limed agricultural soil. Observations from different incubation experiments were compared with the results of a model combining biotic (heterotrophic respiration) and abiotic (carbonate weathering) components. Several samples were pre-incubated for 8–9 days at three temperatures (5, 15 and 25 °C) and then submitted to short-term temperature (STT) cycles (where the temperature was increased from 5 to 35 °C in 10 °C stages, with each stage being 3 h long). During the temperature cycles (hourly timescale), the soil CO_2 fluxes increased significantly with temperature under all pre-incubation temperature (PIT) treatments. A hysteresis effect and negative fluxes during cooling phases were also systematically observed. At a given hourly timescale temperature, there was a negative relationship of the CO_2 fluxes with the PIT. Using the combined model allowed the experimental results to be clearly described, including the negative fluxes and the hysteresis effect, showing the potentially large contribution of abiotic fluxes to total fluxes in limed soils, after STT changes. The fairly good agreement between the measured and simulated flux results also suggested that the biotic flux temperature sensitivity was probably unaffected by timescale (hourly or daily) or PIT. The negative relationship of the CO_2 fluxes with the PIT probably derived from very labile soil carbon depletion, as shown in the simulations. This was not, however, confirmed by soil carbon measurements, which leaves open the possibility of adaptation within the microbial community.

Keywords Soil CO_2 flux · Temperature · Limed agricultural soil · Incubation experiment · Organic carbon model · Short-term

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Introduction

Soil heterotrophic respiration is the process by which carbon dioxide (CO_2) is released during the decomposition of organic carbon (OC) by soil organisms. In temperate ecosystems, temperature is the main

environmental driver of this process (Lloyd and Taylor 1994; Kätterer et al. 1998; Raich et al. 2002; Davidson and Janssens 2006). The interpretation of temperature impact on biotic soil CO_2 fluxes, however, depends on the timescale in question (Reichstein and Beer 2008). The activity of enzymes produced by bacteria and fungi (the most important soil heterotrophs) during decomposition directly increases with temperature, from minutes to hours (Willey et al. 2007). But temperature can also indirectly affect soil heterotrophic respiration through modifications of substrate quantity and availability, at daily to annual scales (Davidson et al. 2006). In addition, the microbial community itself is likely to be affected by temperature changes imposed over periods of several months to years (Luo et al. 2001; Bradford et al. 2008, 2010). Because of the interplay of these effects, the analysis of the temperature impact on heterotrophic respiration is rather complex. Given that the long-term temperature impact necessarily results from shorter-term influences, a better knowledge of heterotrophic respiration is needed.

Studying heterotrophic respiration in agricultural soils is of major interest because these ecosystems cover a significant proportion (about 30 %) of Western Europe's land surface (FAOSTAT 2009) and can behave as large net CO_2 sources (Smith et al. 2007; Ciais et al. 2010). These ecosystems have attracted research attention because of their large mitigation potential with regard to crop management practices (Smith et al. 2007).

Crops are regularly submitted to mechanical and chemical treatments that influence the soil ecosystem. More particularly, liming is a common management practice implemented to create a more favorable chemical environment for plant growth by increasing the soil pH. The main active added component is calcium carbonate ($CaCO_3$), which is involved in the $CaCO_3$–CO_2–H_2O system (Langmuir 1997):

$$CaCO_{3\,(s)} + H_2O + CO_2 \Leftrightarrow Ca(HCO_3)_{2\,(aq)} \qquad (1)$$

This equilibrium (Eq. 1) is highly dependent on soil pH. In alkaline soils, CO_2 can either be emitted from the soil (leading to carbonate precipitation) or taken up from the atmosphere (leading to carbonate dissolution). This latter process does not, however, occur in acidic soils, as the soil solution is saturated with carbonic acid (Stumm and Morgan 1996). Several

studies have shown that soil CO_2 emissions are greatly influenced by liming (Bertrand et al. 2007; Hamilton et al. 2007; Biasi et al. 2008) or natural calcareous soil properties (Emmerich 2003; Tamir et al. 2011). Thus, total CO_2 fluxes can come from two distinct sources: biotic (soil heterotrophic respiration) and abiotic (inorganic C fluxes linked to $CaCO_3$ dissolution and precipitation). For limed soil with high pH, the contribution of the abiotic component is non-negligible: it could constitute about 30 % of the total CO_2 fluxes under constant temperature conditions (Bertrand et al. 2007; Biasi et al. 2008; Tamir et al. 2011). These results show that ignoring inorganic C processes in limed soils could lead to incorrect estimates of the biotic mechanism of heterotrophic respiration (Tamir et al. 2011).

Although the equilibrium described by Eq. 1 is controlled by several reaction constants that are greatly affected by temperature changes (Stumm and Morgan 1996; Langmuir 1997), none of the above-mentioned studies investigated the impact of short-term (hourly) temperature changes on CO_2 fluxes produced by limed soils. Temperature influences the equilibrium between gaseous and water-dissolved CO_2, governed by Henry's Law, which implies that the $CaCO_3$–CO_2–H_2O system can either release or take up CO_2 (Eq. 1) when temperature increases or decreases, respectively. In limed soils, therefore, quantifying the response of heterotrophic respiration to temperature also requires obtaining good estimates of this response for the inorganic CO_2 fluxes.

The objectives of this study were (i) to separate biotic from abiotic exchanges in the total soil CO_2 fluxes from a limed crop in order to focus on the biotic component response to temperature and (ii) to investigate whether direct (enzymatic activity) and indirect (depletion of labile substrates) temperature impacts on soil respiration could be differentiated over a 8–9 days period. These objectives were achieved by conducting both experimentation and modeling. Short-term incubation experiments with different temperatures and soil water content (SWC) were performed on samples from limed and unlimed soils. Modeling was used to evaluate the abiotic exchange and to interpret the biotic flux dynamics. The incubation periods were kept short in order to limit the readily available C losses that often bias laboratory experiments (Kirschbaum 2006).

Materials and methods

General procedure

Soil samples were taken from two sites. They were pre-incubated at three temperatures (5, 15 and 25 °C) over 5 days (to eliminate perturbation due to sampling, see below) and were then submitted to a temperature cycle (Fig. 1), starting from the pre-incubation temperature (PIT), increasing by 10 °C stages up to 35 °C, then falling to 5 °C, and finally increasing again to the PIT. Each temperature stage lasted 3 h so as to allow temperature stabilization, and the cycle was therefore completed in about 22 h. The temperature during each cycle stage will be referred to here as the 'short-term temperature' (STT). At the end of each stage, sample respiration was measured. The temperature cycle was repeated 2 days later. The complete experiment was carried out three times, with two soil types (Exps. 1 and 2: limed soil; and Exp. 3: unlimed soil) and low or high SWC (Exps. 2a and 2b, respectively). The modality differences between these three experiments are given below ("Modalities of each experiment" section).

Site description

Soil sampling was carried out at the agricultural site of Lonzée in the Hesbaye region in Belgium and at the forest site of Vielsalm in the Belgian Ardennes (Table 1). The Lonzée field had been cultivated for more than 70 years and the most recent 4-year rotation cycle had been: winter wheat–seed potato–winter wheat–sugar beet. The climate is temperate maritime and the soil is a Luvisol (Hypereutric, Siltic) (IUSS Working Group WRB 2006).

In the context of this study, the most prominent site characteristic is the pH_{H2O}, which is 7.9. This is due to regular soil liming. The last input, 20 months before the sampling, was 10 t ha^{-1} of slime, residues from sugar beet treatment in sugar factories, corresponding to an input of 2,700 kg ha^{-1} $CaCO_3$, in addition to several classic NPK amendments. No information about the total inorganic C content of the Lonzée soil was available, however, at the time of sampling, as the loamy soils of the region naturally contain negligible amounts of carbonates when not amended (G. Colinet, pers. comm.). More details about this site are given in Moureaux et al. (2006).

Fig. 1 Experimental set-up and treatments. The pre-incubation period lasted for 5 days in all three experiments. Exp. 1 (Lonzée, spring 2009): as shown on the figure; Exp. 2 (Lonzée, summer 2009): two soil water content (SWC) treatments, 15 % by weight (Exp. 2a) and 26 % by weight (Exp. 2b); Exp. 3 (Vielsalm, autumn, 2009): only the 15 °C pre-incubation temperature (PIT) was used. Starting temperature in the short-term temperature (STT) variations is indicated in *bold-underlined*. The between-cycle period (Δt) lasted for 2 days in Exp. 1, and 1 day in Exps. 2 and 3
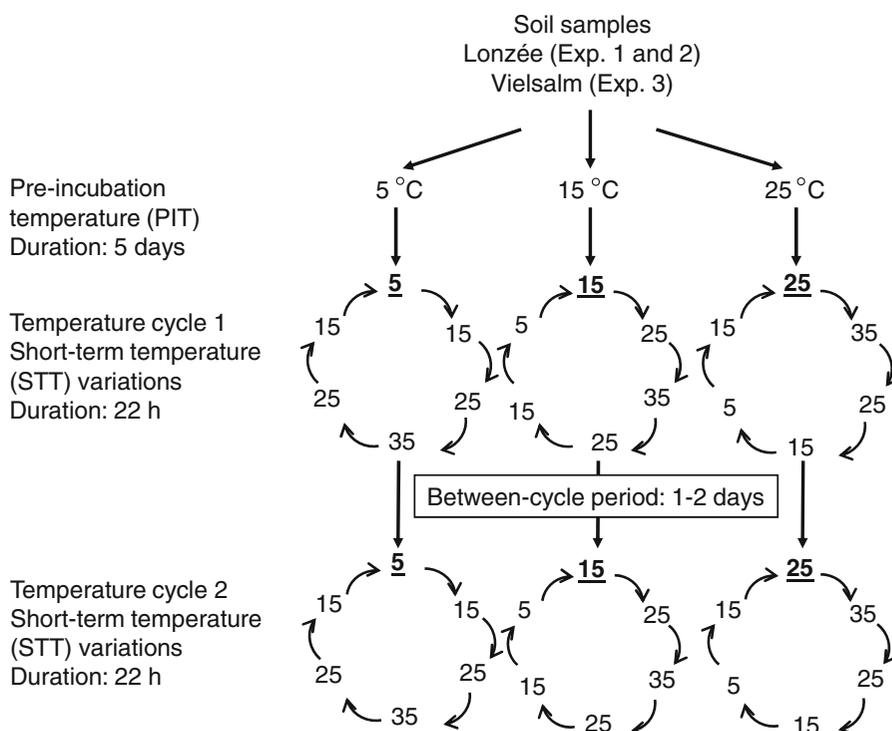
Table 1 Soil characteristics at the Lonzée and Vielsalm sites

Soil characteristics	Lonzée	Vielsalm
Sand (%)	5–10	27
Silt (%)	68–77	55
Clay (%)	18–22	18
% C (g/100 g)	1.08 ± 0.1	34.51 ± 13.33
% N (g/100 g)	0.12 ± 0.01	1.49 ± 0.53
Soil $C_{0-30 \text{ cm}}$ (kg m^{-2})	4.67 ± 0.19	5.60 ± 1.64
C/N ratio$_{topsoil}$	9.40 ± 0.09	19.98 ± 2.32
pH$_{(H2O)}$	7.9	4.3

The Vielsalm stand is of mixed composition, consisting mainly of 75-year-old Douglas fir trees (*Pseudotsuga menziesii* [Mirb.] FRANCO) and beeches (*Fagus sylvatica* L.) that are more than 100 years old. The soil is classified as Dystric Cambisol (IUSS Working Group WRB 2006); it is not limed and has a much lower mean pH$_{H2O}$ (4.3; Perrin 2005) than that at Lonzée. More details about this site are given in Table 1 and in Longdoz et al. (2000).

Sampling procedure

General procedure

For crop site core samplings, four plots (3 × 3 m) in the middle of the winter wheat field were weeded with glyphosate in order to create bare areas. Weeding was performed about 6 weeks before core sampling in order to prevent any bias in the measurements (Haney et al. 2002). The sampling was done by taking two soil cores (8 cm diameter, 14 cm depth) from each of the four bare areas. All cores were sieved (2 mm) and thoroughly homogenized so that the samples were representative of the average field characteristics (Franzluebbers 1999).

Nine samples (100.0 ± 0.2 g) of fresh homogenized soil were placed in 210 ml jars sealed with

metallic lids pierced with pipes for gas exchanges. The jars were placed in incubators set at 5, 15 and 25 °C-PIT (± 0.1 °C). Temperature sensors (thermocouples) were inserted into the soil samples at mid-depth (1.5 cm) in each jar. In order to eliminate perturbation caused by sampling (Franzluebbers 1999), the samples were kept at their respective PIT over a 5-day pre-incubation period. The length of this period was determined once preliminary tests had shown flux stabilization after 5 days. During this period, the SWC in each jar was checked gravimetrically every day and adjusted when its weight dropped by more than 1 %.

After the 5-day pre-incubation period, all the samples were submitted to the temperature cycle described above (cycle 1). A second similar temperature cycle (cycle 2) was carried out 2 days after the first one. Sample respiration was measured at the end of each temperature stage. The whole experiment lasted 8–9 days, including the pre-incubation period, the temperature cycles and the between-cycle period (Fig. 1).

Modalities of each experiment

The core samplings in Exps. 1 and 2 were performed at the Lonzée site (limed crop, the main site in this study) in spring (27 May) and summer (5 August) 2009, respectively. The Exp. 3 samplings were performed at the Vielsalm site (acidic forest soil) in autumn (5 October) 2009 in order to provide complementary information about a soil characterized by a low pH. In this case, the sampling was limited to three soil cores taken at a single location, and only one PIT treatment (15 °C) was applied (Table 2).

In Exp. 1, some of the samples were used for microbial activity measurements (see "Soil carbon measurements" section). In Exp. 2, all the samples were used for respiration measurements, but two treatments were applied in order to analyze the SWC

Table 2 Experiment details and soil characteristics at the time of sampling

Experiments	Sampling date	Sampling site (pH$_{H2O}$)	Sample SWC	# Samples	PIT
Exp. 1	27 May	Lonzée (7.9)	Field (13 % by weight)	9	5, 15, and 25 °C
Exp. 2a	5 August	Lonzée (7.9)	Field (15 % by weight)	9	5, 15, and 25 °C
Exp. 2b	5 August	Lonzée (7.9)	Increased (26 % by weight)	9	5, 15, and 25 °C
Exp. 3	5 October	Vielsalm (4.3)	Field (15 % by weight)	3	15 °C

PIT pre-incubation temperature

impact: 18 samples were used, nine of them incubated at field SWC (Exp. 2a: 15 % by weight, about 75 % of field capacity) and nine at a higher SWC (Exp. 2 b: 26 % by weight, more than field capacity). In Exp. 2, the CO_2 fluxes were also measured once a day during the pre-incubation period in all three incubators in order to monitor the flux evolution during this period. The pH was also measured at both the beginning and the end of Exp. 2.

Incubation device and CO_2 flux measurements

Both the pre-incubation and temperature cycles were carried out in three home-made incubators able to keep the temperature constant at between 5 and 35 °C. They were completely insulated aluminum containers (22 cm diameter, 11.5 cm height) filled with water. The containers and the water were warmed up or cooled down using electronic heat pumps (RC12-6-01LS, Marlow Industries, Haywards Heath, UK) beneath each incubator. The jars containing the soil samples (see "Sampling procedure" section) were plunged into the water.

The inside jars were continuously ventilated at a flow rate of 1 l min^{-1} with outside air saturated in water vapor at the incubator PIT by passing through a bubbler. The ventilation prevented measurement biases due to CO_2 accumulation in soil pores between the measurements (Davidson et al. 2002) and the use of water-saturated air limited water loss from the samples.

During the flux measurements, the ventilation was cut off and the jar was connected manually to the soil CO_2 flux measurement system. This consisted of a dynamic closed loop system that included an infrared gas analyzer (Gascard II, Edinburgh Instruments Ltd, UK) and a pump (flow rate of 1.8 l min^{-1}), in addition to the jar. The total volume of the measurement system was 0.21 l. Fluxes were deduced from the slope of CO_2 concentration increase over time. The CO_2 concentrations in the jar were sampled every 10 s. The slopes were calculated using least squares regression and converted into fluxes expressed in μg CO_2 (g dry soil)$^{-1}$ h^{-1}. One flux corresponded to the mean of three consecutive repetitions. Between each repetition, the jar was flushed with outside air for 30 s to prevent an accumulation of CO_2. One measurement lasted for 180 s, the first 60 s being discarded in order to eliminate the flux variations caused by pressure fluctuations when closing the system (Curiel-Yuste

et al. 2007; Davidson et al. 2002). The slope of CO_2 concentration increase was then computed on the remaining 120 s.

Soil carbon measurements

In parallel to Exp. 1, fresh soil subsamples (45 g each) were taken from the jars in all the PIT treatments and used for OC measurements on three occasions: the beginning of cycle 1 (sampling 1), the end of cycle 1 (sampling 2) and during the cooling phase of cycle 2 (at 15 °C-STT, sampling 3). Each subsample was immediately placed in a new 210 ml-jar and refrigerated at 4 °C until processed. All the subsamples were treated 7 days after cycle 1 had started.

From the subsamples, a 40 g portion was used to assess microbial biomass C using the fumigation-extraction method (Jenkinson and Powlson 1976; Vance et al. 1987). Half of each soil portion was fumigated with ethanol-free chloroform for 3 days at 25 °C in the dark. Non-fumigated and fumigated soils were extracted with 50 ml 0.5 M K_2SO_4 and filtered (diameter 4.4 μm, filter 5951/2, Whatmann, Germany). Dissolved OC in fumigated and non-fumigated extracts was measured with a total OC analyzer (Labtoc, Pollution and Process Monitoring Limited, UK). Microbial C was calculated as the difference between the OC extracted from fumigated and non-fumigated samples, divided by a coefficient representing the efficiency of microbial C extraction (0.45; Joergensen 1996).

The rest of the subsamples were used for cold-water soluble C and hot-water extractable C measurements, using the extraction method described by Ghani et al. (2003). The soil portions were shaken (120 rpm) with deionized water (30 ml) for 30 min and the soil solutions were filtered (diameter 0.45 μm, Pall Corporation, Michigan, USA). The remaining wet soils were then mixed again with deionized water (30 ml) and placed in an oven for 16 h at 80 °C. These soil solutions were also filtered (diameter 0.45 μm, Pall Corporation, Michigan, USA). The filtrates collected after both filtrations were kept at 4 °C before analysis with the total OC analyzer.

Statistical analyses

Data were analyzed using ANOVA. One-way ANOVA was used to assess the STT impact on the soil CO_2 fluxes measured in each experiment.

Two-way ANOVA was used to study the impact of PIT or incubation time on soil microbial biomass, cold-water soluble C and hot-water extractable C (PIT and time as factors, $n = 3$).

Either one-way (first day of the pre-incubation period, with all samples at field SWC, and PIT as a factor, $n = 6$) and two-way (last day of the pre-incubation period, with PIT and SWC as factors, $n = 3$) ANOVA was used to study the influence of the pre-incubation conditions on CO_2 fluxes during the pre-incubation period in Exp. 2.

All statistical analyses were performed with MATLAB (R2009b version, *The Mathworks*, Natick, USA).

Biotic/abiotic model

In order to interpret the experimental results on the limed soil (Exps. 1, 2a and 2b), a model partitioning biotic and abiotic fluxes (biotic/abiotic model) was developed based on the assumptions that (i) the dynamics of biotic soil CO_2 fluxes (heterotrophic respiration, R) are driven by temperature, OC content in pools with different assimilability level and microbial biomass, and (ii) the abiotic flux component (F_A) results from shifts in the $CaCO_3$–CO_2–H_2O equilibrium following temperature changes.

The total CO_2 fluxes that we measured (F) can therefore be described as:

$$F = R + F_A \tag{2}$$

The heterotrophic respiration component, R, was computed by a first-order three-pool OC dynamics model based on the studies by Kirschbaum (2004) and Allison et al. (2010). The system consisted of one microbial biomass [C_M, (mg C (g dry soil)$^{-1}$)] and two OC substrate pools. Given the short duration of the experiments and the sample treatment (homogenization), we assumed that all the OC substrate taken into account in this model was labile C, the first pool representing very easily assimilated soil C [fast-cycling C pool, C_F, corresponding to cold-water extractable carbon (see "Soil carbon measurements" section) (mg C (g dry soil)$^{-1}$)] and the second representing easily assimilated soil C [slower-cycling C pool, C_S, corresponding to hot-water extractable C (see "Soil carbon measurements" section) (mg C (g dry soil)$^{-1}$)]. In addition, the dead microbial biomass at each time stage was assumed to flow half in C_F and

half in C_S. The temporal evolutions of C_F, C_S and C_M were then computed as:

$$\frac{dC_F}{dt} = -A_T \cdot k_F \cdot C_F + 0.5 \cdot k_M \cdot C_M \tag{3}$$

$$\frac{dC_S}{dt} = -A_T \cdot k_S \cdot C_S + 0.5 \cdot k_M \cdot C_M \tag{4}$$

$$\frac{dC_M}{dt} = CUE_T \cdot A_T \cdot (k_F \cdot C_F + k_S \cdot C_S) - k_M \cdot C_M \tag{5}$$

where k_M, k_F and k_S are the decomposition rate constants (h^{-1}) of the microbial biomass, fast-cycling and slower-cycling C pools, respectively (k_F and k_S being calibrated, see below); A_T is the temperature reduction factor (–); and CUE_T is the microbial carbon use efficiency (–) (i.e., the fraction of OC substrate used by microorganisms for their growth). CUE_T was assumed to decrease linearly with temperature, as reported by Allison et al. (2010):

$$CUE_T = 0.63 - 0.016 \cdot T_{soil} \tag{6}$$

where T_{soil} is the soil temperature (°C).

Temperature was considered to be the only factor influencing soil C pool depletion. It was assumed to affect the two pools similarly, given the short time-scale of the experiment and the assumption that they were made of relatively easily assimilated substrate. A_T was computed as a Q_{10} function formulated to increase with temperature from 0 to 1, when 35 °C (maximum temperature imposed during incubation experiments) was reached:

$$A_T = Q_{10}^{\frac{T_{soil}-35}{10}} \tag{7}$$

where Q_{10} is the temperature sensitivity parameter (–) (calibrated; see below).

The heterotrophic respiration component [R, µg CO_2 (g dry soil)$^{-1}$ h^{-1}] was finally computed as the fraction of OC consumed by microorganisms and not used for their growth:

$$R = 3667 \cdot A_T \cdot (1 - CUE_T) \cdot (k_F \cdot C_F + k_S \cdot C_S) \tag{8}$$

where the numerical factor 3667 allows conversion from mg C into µg CO_2.

The abiotic component, F_A, was evaluated using an empirical model that estimates the CO_2 fluxes resulting from equilibrium shifts of Eq. 1. This was computed as:

$$F_A = -\frac{\Delta C_{CO2}}{\Delta t} \cdot \frac{V_w}{m} \tag{9}$$

where V_w is the volume of water in the soil samples (m^3); m is the dry soil mass (g); Δt is the time interval (3 h) between two measurements; and ΔC_{CO2} is the aqueous CO_2 concentration change following a 10 °C-temperature change ($\mu g\ CO_2\ m^{-3}$).

The aqueous CO_2 concentration was estimated by a function of soil CO_2 partial pressure (pCO_2, kPa) and soil temperature, thus:

$$C_{CO2} = 10^{-6} \cdot \left(53.6 + 27.8p\ CO_2 + 525.8\sqrt{pCO_2}\right) \\ \cdot \left(98120 - 578.6\ T_{soil} + 0.87\ T_{soil}^2\right) \tag{10}$$

This equation was established empirically by Maier et al. (2010) for alkaline soils by relating to T_{soil} and $pCO_2\ C_{CO2}$ values computed with a more fundamental model describing the thermodynamic equilibrium of the $CaCO_3$–CO_2–H_2O system (see more details in Maier et al. (2010)]. The pH was assumed to be constant. They validated this empirical model in laboratory and field measurements taken from a calcareous soil. It was found to be applicable to soils with a pH > 7, for 272.15 K < T_{soil} < 302.15 K and 0.045 kPa < pCO_2 < 1.2 kPa.

The biotic/abiotic model was run with MATLAB (R2009b version, *The Mathworks*, Natick, USA).

Strategies of model calibration, validation and use

Initial conditions (C_M, C_F and C_S) were obtained from the soil OC measurements ("Soil carbon measurements" section). The model was calibrated with the data from experiments with 15 °C-PIT and validated with those corresponding to 5 and 25 °C-PIT. Independent calibration and validation were performed for Exps. 1, 2a and 2b. Calibration of k_F, k_S, Q_{10} and pCO_2, k_M was assumed to be fixed at $2 \times 10^{-4}\ h^{-1}$ (Allison et al. 2010). It was performed by minimizing the sum of squared differences between the measured (F, Eq. 2) and modeled ($R + F_A$, Eq. 2) CO_2 fluxes, after checking for model robustness by avoiding local minima.

Uncertainties about model outputs were supposed to result from the propagation through the model of the uncertainties on the parameters and initial conditions. This was estimated using Monte Carlo simulation.

Uncertainty about each parameter was estimated as the standard deviation of the distribution frequency of the parameter values that led to a sum of squared differences falling between the minimum sum of squared differences and this one increased by 10 % (the 10 % increase corresponded roughly to the mean standard error of the flux measurements). Uncertainties about the initial conditions were estimated as the standard deviation of the soil OC measurements ("Soil carbon measurements" section).

Results

Observations from the laboratory incubations

In all the situations, soil CO_2 flux increased significantly ($p < 0.05$) and monotonically with STT regardless of the experiment, the cycle, the SWC treatment or the PIT (Fig. 2).

A clear dependence of fluxes on PIT also appeared when comparing them at the same STT, the highest CO_2 fluxes being systematically observed in the samples with the lowest PIT (Fig. 2; Supplementary Table).

An important clockwise hysteresis effect, with lower CO_2 fluxes during the cooling phase, was observed in all cases related to the Lonzée samples (Fig. 2a–f), suggesting that the direct temperature impact also depended on recent thermal history in these limed soil samples. This hysteresis effect was more pronounced in samples with the lowest PIT (Fig. 2a–f) and the highest SWC (Fig. 2e–f; Supplementary Table). No hysteresis was observed in the acid forest soils from Vielsalm (Fig. 2g–h).

Negative fluxes were measured during all the cooling phases for the Lonzée samples (Fig. 2a–f). They were systematically observed at 5 °C-STT (Fig. 2; Supplementary Table) and, in most cases, at 15 °C-STT (Fig. 2a–f). At 5 °C-STT, they reached the same order of magnitude as the positive fluxes during the warming phase. They were more important in the samples kept at a higher SWC (Fig. 2c–d vs. e–f; Supplementary Table). At Vielsalm, the fluxes measured at 5 °C-STT after cooling phases were always positive, with a mean of $0.74 \pm 0.14\ \mu g\ CO_2.(g\ dry\ soil)^{-1}\ h^{-1}$ (Fig. 2g–h).

The larger soil organic C content at the Vielsalm site (Table 1) probably did not have a great impact on

Fig. 2 Short-term responses of the soil CO_2 fluxes ($n = 3$) to temperature changes, with the samples pre-incubated at 5 °C (*full line*), 15 °C (*dashed line*) and 25 °C (*dotted line*) during all experiments (Exp.): Exp. 1 cycle 1 (**a**) and cycle 2 (**b**); Exp. 2a (soil samples at an SWC of 15 % by weight), cycle 1 (**c**) and cycle 2 (**d**); Exp. 2b (soil samples at an SWC of 26 % by weight), cycle 1 (**e**) and cycle 2 (**f**) and Exp. 3 cycle 1 (**g**) and cycle 2 (**h**). The *arrows* in the subplot (**a**) indicate how the fluxes were affected either by warming or cooling in all (**a–f**) situations. The error bars represent the standard error of the mean ($n = 3$)
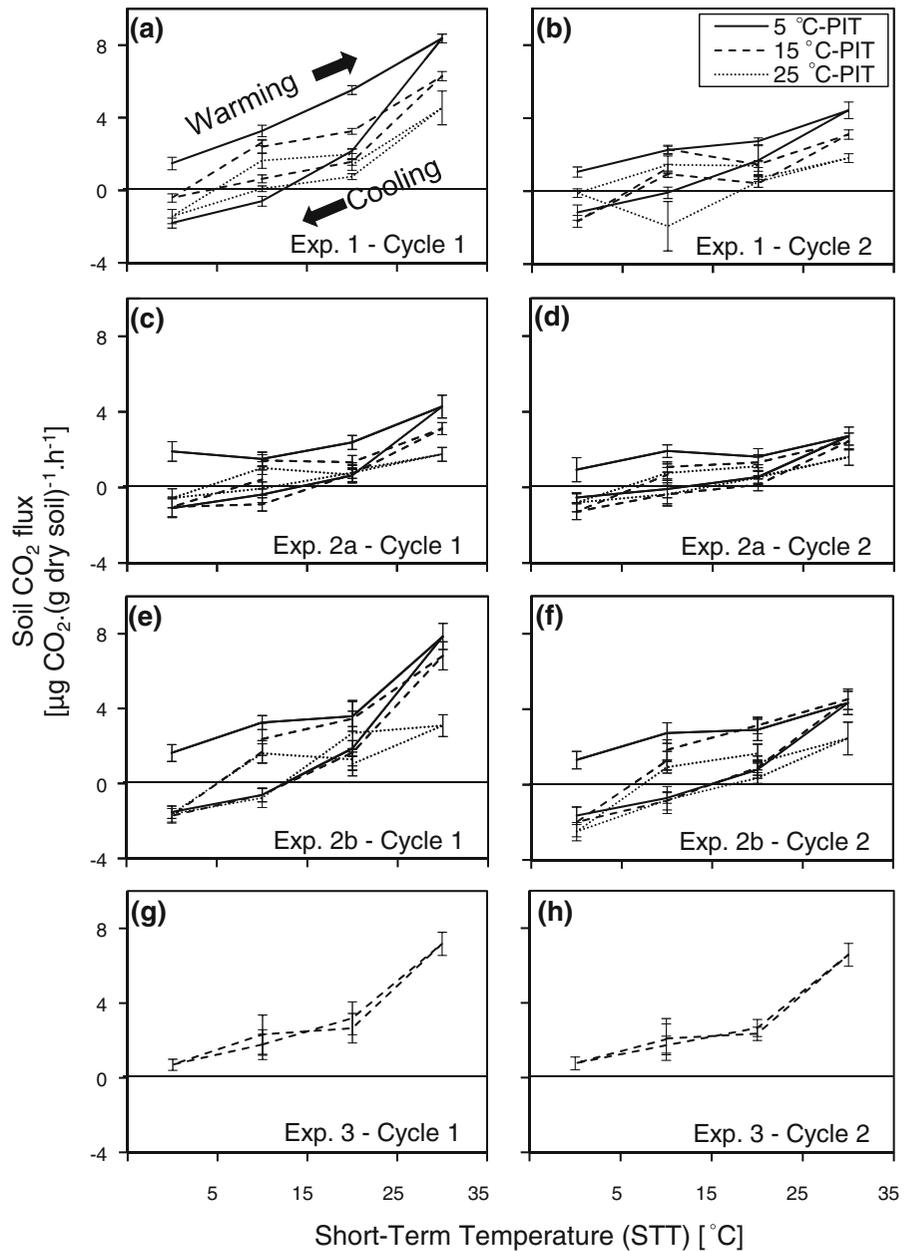
the CO_2 flux response to temperature because it was of the same order of magnitude at both sites (Fig. 2).

The pH_{H2O} measurements performed during Exp. 2 showed no significant evolution, regardless of the PIT or SWC treatments. Its value was 7.94 ± 0.03 just after soil sampling (all eight samplings together) and 7.97 ± 0.04 after the experiment (all samples from all PITs confounded).

At the beginning of the pre-incubation period, differences were observed between fluxes at different PITs (Fig. 3), samples at a higher PIT producing significantly higher fluxes ($p < 0.001$). These differences decreased during the pre-incubation period, and after 5 days no significant difference was observed between treatments ($p > 0.05$).

Neither dependency on PIT nor time ($p > 0.05$), nor the interaction between these two factors ($p > 0.05$; Fig. 4), was found for microbial biomass, cold-water soluble and hot-water extractable C. Averaged over time and the PIT treatments, the
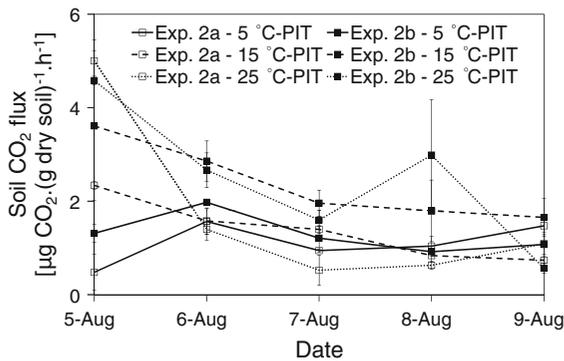
Fig. 3 Daily timescale evolution of the soil CO_2 fluxes ($n = 3$) during the pre-incubation period of Exp. 2, with the samples pre-incubated at 5 °C (*full lines*), 15 °C (*dashed lines*) and 25 °C (*dotted lines*). For each pre-incubation temperature treatment, the *two lines* represent the SWC treatments (Exp. 2a (SWC = 15 % by weight): *open symbols*; Exp. 2b (SWC = 26 % by weight): *closed symbols*). The error bars represent the standard error of the mean ($n = 3$)

microbial biomass was 0.14 ± 0.04 mg C (g dry soil)$^{-1}$ and cold-water soluble and hot-water extractable C contents were 0.027 ± 0.009 and 0.27 ± 0.02 mg C (g dry soil)$^{-1}$, respectively.

Biotic/abiotic model application

The calibration provided model parameters that were both realistic and fairly repeatable from one experiment to another (Table 3). The k_F and k_S values were, however, in the upper range of the usual values and the Q_{10} values were generally smaller than 2 (see in "Discussion"). The uncertainties were greater in Exp. 1 than in the other two experiments.

The model simulations agreed fairly well with the observed flux dynamics (Fig. 5), especially in the 5 and 15 °C-PIT treatments during the temperature cycles, and in Exps. 2a (25 °C-PIT) and 2b (5 and 15 °C-PIT) during the pre-incubation periods. Flux measurements overall fell within model output uncertainties (in Fig. 5, compare the black dots with the area inside the grey lines). In addition to the flux response to STT variations, it correctly reproduced (i) the negative relationship between maximum fluxes at 35 °C and PIT (for each experiment, compare peak heights between Fig. 5a, d and g, between Fig. 5b, e and h and between Fig. 5c, f and i); (ii) the flux reduction during the pre-incubation periods (Fig. 5b, c, e, f, h, i); and (iii) the lower fluxes observed in cycle 2 compared with cycle 1 in all experiments (Fig. 5a–i).

The simulated abiotic fluxes (F_A) were positive when the temperature increased and negative when it decreased (Table 4). Their magnitude was larger in the samples with a higher SWC (Exp. 2b vs. Exp. 2a) and for colder STT variations (Table 4). The inclusion of F_A in the model reduced the sum of squared differences between simulated and measured fluxes by 52, 51 and 67 % for Exps. 1, 2a and 2b respectively. This corresponded to a 1–35 % reduction of the standard error of the measured CO_2 flux mean. Subtracting the abiotic component from the measured fluxes also led to an important reduction of the hysteresis effect (Fig. 6).

Contrary to our observations (Fig. 4), the simulated OC pool contents changed during the experiments (data not shown): C_F decreased continuously, the pool being almost totally empty by the end of each experiment. This decrease was sharper in the samples with the 25 °C-PIT than with the 5 and 15 °C-PIT. C_S also decreased in all three experiments, although more smoothly, while microbial biomass (C_M) increased in all experiments, particularly in Exps. 1 and 2b (up to 25 % of its initial value).

Discussion

Hysteresis effect and negative fluxes: a consequence of the occurrence of short-term abiotic CO_2 fluxes?

The fact that the clockwise hysteresis and the negative fluxes were observed in only two of the three experiments reasonably eliminates the risk of a measurement factor attributable to the device. As they appeared only in experiments with the Lonzée samples, which were characterized by a high pH (pH$_{H2O}$ = 7.9) and an effervescent reaction to HCl, and not with the Vielsalm samples (pH$_{H2O}$ = 4.3, no effervescent reaction), this rather suggests the presence of abiotic fluxes resulting from carbonate weathering in the limed soil.

These fluxes would be triggered by STT variations that would provoke CO_2 dissolution constant changes (Stumm and Morgan 1996). As a result, positive abiotic fluxes would be observed during STT increases and negative abiotic fluxes during STT decreases. Their combination with heterotrophic respiration, itself controlled by STT, would generate the
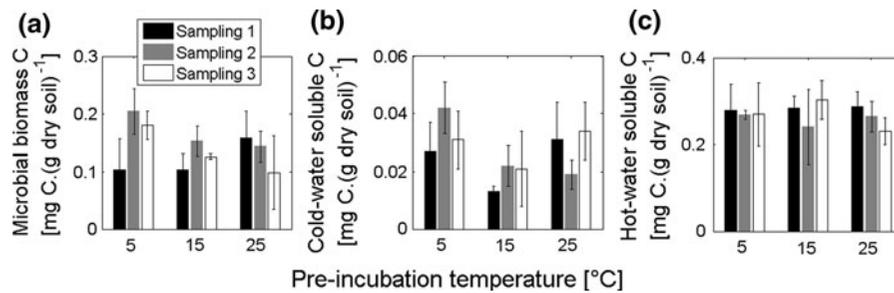
Fig. 4 Microbial biomass (**a**), cold-water soluble carbon (**b**) and hot water-extractable carbon (**c**) evolution over the three samplings carried out during Exp. 1 in each incubator. Sampling 1 (*black bars*) was done at the beginning of cycle 1, sampling 2 (*grey bars*) at the end of cycle 1 and sampling 3 (*white bars*) during the cooling phase of cycle 2. The error bars represent the standard error of the mean ($n = 3$)

Table 3 Parameter values [decomposition constants of the fast-cycling (k_F) and slower-cycling (k_S) carbon pools, soil heterotrophic respiration temperature sensitivity (Q_{10}), soil CO_2 partial pressure (pCO_2)] determined during calibration of the biotic/abiotic (BA) model in the 15 °C-pre-incubation temperature (PIT) treatment for each experiment related to the limed soil samples

Experiments	Calibrated parameter values				Minimum sum of squared differences
	k_F (h^{-1})	k_S (h^{-1})	Q_{10} (–)	pCO_2 (kPa)	
Exp. 1	0.141 [0; 0.70]	0.027 [0.023; 0.031]	1.44 [1.34; 1.53]	0.71 [0.035; 1.346]	6.74
Exp. 2a	0.124 [0.09; 0.158]	0.0022 [0.0018; 0.0025]	1.88 [1.60; 2.15]	0.33 [0.17; 0.50]	4.75
Exp. 2b	0.103 [0.047; 0.159]	0.0066 [0.0052; 0.0079]	1.60 [1.38; 1.82]	0.42 [0.20; 0.63]	9.27

The minimum sum of squared differences [(µg CO_2 (g dry soil)$^{-1}$ h^{-1})^2] resulting from model fitting is also given for each experiment. The values between brackets represent the parameter range ($m \pm \sigma$)

hysteresis. In addition, total negative fluxes could appear when the abiotic CO_2 sink exceeded the biotic CO_2 source (i.e., during temperature decreases) at a low STT.

The larger hysteresis at low temperature (Fig. 2) and high SWC (comparison of Exps. 2a and 2b) are consequences of this process: in both cases, it results from larger positive or negative abiotic fluxes. In the first case, it results from a larger CO_2 dissolution at low temperature. In the second, it is because humid soils contain more water and constitute a larger reservoir for CO_2 dissolution or emission.

The biotic/abiotic model confirmed this hypothesis and made the hysteresis quantification possible. The introduction in the simulation of the abiotic term considerably reduced the sum of squared differences (by more than 50 %) between simulated and measured fluxes. The agreement was not perfect, however, because the simulated hysteresis was always smaller than the observed one and because the model did not reproduce the negative fluxes in all cases. In addition,

the approach could probably be improved: first, the model depends on soil CO_2 partial pressure (pCO_2), which was not measured during the experiment, so no direct validation could be done. The values predicted by the model were, however, compatible with the pCO_2 generally measured in the field, varying between 0.04 kPa (400 ppm) and 4.05 kPa (40,000 ppm) (Gocke et al. 2011). Second, the model did not take pH variations into account. The absence of a significant pH evolution during Exp. 2 suggests, however, that this should not greatly affect the results, at least at the timescale considered in this study.

Finally, one surprising result was the importance of the abiotic flux contribution to the total hourly CO_2 fluxes (20–100 %, depending on the temperature stage). It was indeed higher than those obtained from field observations in a limed peatland by Biasi et al. (2008) and in sandy loams developed on glacial till by Hamilton et al. (2007) (10–15 % of total CO_2 emissions). It was also higher than observations reported by Bertrand et al. (2007) and Tamir et al. (2011) in
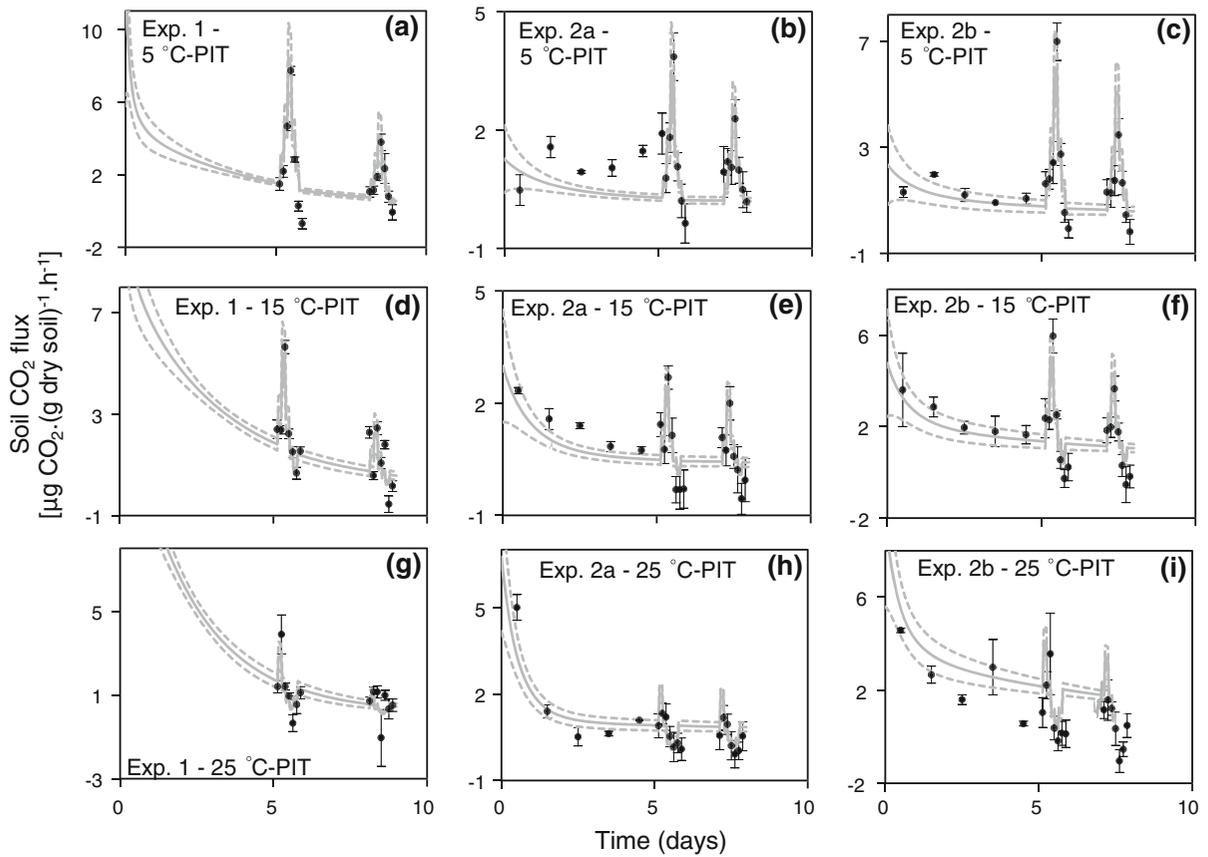
Fig. 5 Comparisons between the temporal evolutions of the 'corrected' (= $F - F_A$, Eq. 2) CO_2 fluxes (mean values, $n = 3$; *black dots*) and the fluxes calculated using the soil respiration component of the biotic/abiotic model (mean R: *full grey lines*) for the three PIT treatments in Exps. 1, 2a (soil samples at an SWC of 15 % by weight) and 2b (soil samples at an SWC of 26 % by weight). The two peaks coincide with cycles 1 and 2. The uncertainty ranges ($m \pm \sigma$) of simulated R are given by the *two dotted grey lines* around each *grey line*. Notice the different scales on the y-axes

Table 4 Abiotic fluxes [F_A, Eq. 2, (µg CO_2 (g dry soil)$^{-1}$ h^{-1})] calculated with the calibrated soil CO_2 partial pressure ($p CO_2$, kPa) for each experiment and each short-term temperature (STT) change (either increasing or decreasing)

STT change	Abiotic flux (= F_A) [µg CO_2 (g dry soil)$^{-1}$ h^{-1}]		
	Exp. 1	Exp. 2a	Exp. 2b
$p CO_2$ (kPa)	0.71	0.33	0.42
5 → 15 °C/15 → 5 °C	1/−1	0.8/−0.8	1.6/−1.6
15 → 25 °C/25 → 15 °C	0.8/−0.8	0.6/−0.6	1.3/−1.3
25 → 35 °C/35 → 25 °C	0.6/−0.6	0.5/−0.5	0.9/−0.9

Soil CO_2 emission is counted positively

laboratory incubations performed with samples of different types of limed soils. In addition, at the Lonzée site, where the samples were taken we observed neither negative soil CO_2 fluxes nor any hysteresis (Suleau et al. 2011). A possible explanation

could be the sieving procedure, which probably caused soil aggregate disruption and released the carbonates protected within them. The amount of carbonates in the samples was not, however, determined in our study. Another explanation could be related to the

Fig. 6 Comparisons between the measured (*full lines*) and corrected (= $F - F_A$, Eq. 2, *dashed lines*) fluxes for the 5 °C-PIT treatment in Exps. 1, 2a (soil samples at an SWC of 15 % by weight) and 2b (soil samples at an SWC of 26 % by weight). At each temperature stage the same symbols are used for the measured and the corrected fluxes. The *arrows* in the subplot (**a**) indicate how the fluxes were affected either by warming or cooling in all (**a**–**f**) situations. Notice the different scales on the *y*-axes. The error bars represent the standard error of the mean (*n* = 3)
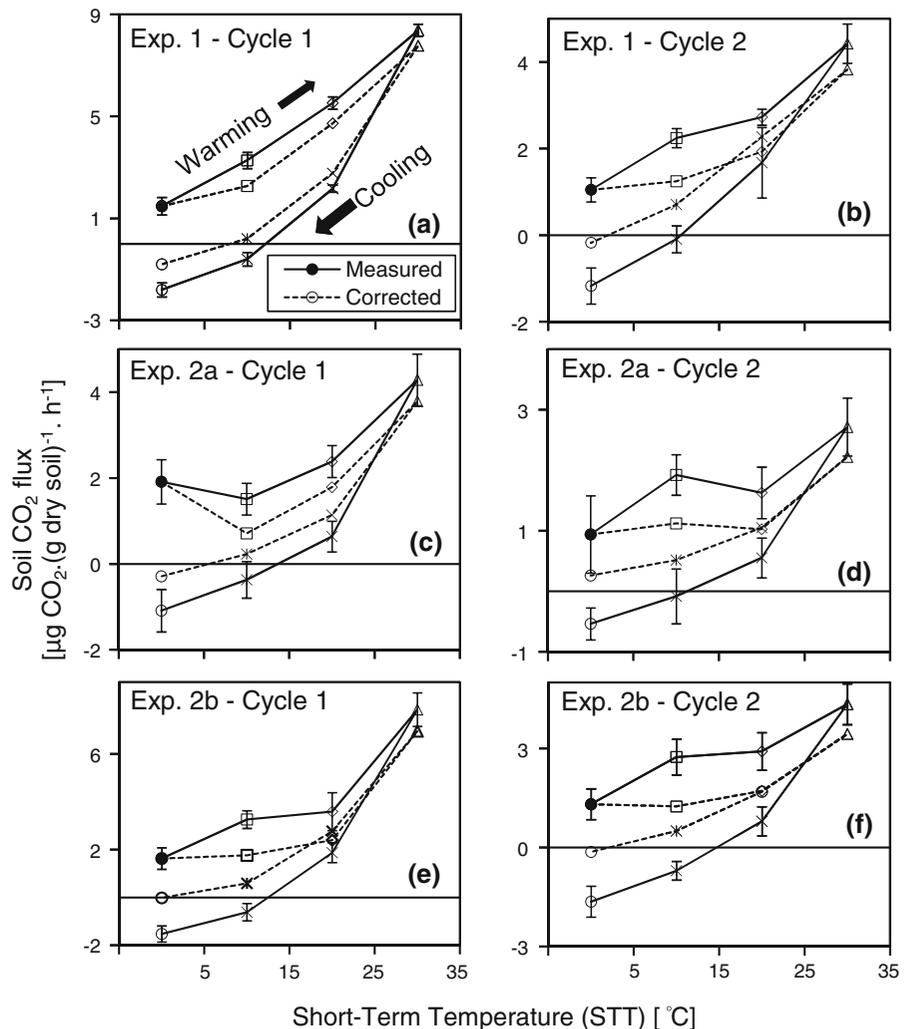
temperature change rate that we used in this experiment (about 3.3 °C h^{-1}), which was higher than that commonly observed in the field in temperate regions.

Differential temperature responses: a consequence of fast-cycling carbon consumption?

Our experimental results indicated that temperature affects the biotic soil CO_2 fluxes through both direct (hourly timescale) and indirect (daily timescale) effects. At the hourly scale, biotic fluxes increased clearly with temperature under all experimental conditions and this could be quantified by a Q_{10} curve, as in the biotic/abiotic model. The Q_{10} values obtained through the model application were slightly lower than those usually determined for this parameter

(Kätterer et al. 1998) and those reported by Suleau et al. (2011) when characterizing the heterotrophic respiration seasonal response at Lonzée. In addition, the fact that a good agreement between the biotic/abiotic model simulations and measurements was obtained by using a unique Q_{10} value in each experiment suggests that temperature sensitivity was the same at the hourly and daily timescales and was not affected by the PIT. Bradford et al. (2010) and Hamdi et al. (2011) did not find a PIT impact on temperature sensitivity either when pre-incubating the samples in the same temperature range as ours (10–30 °C).

At the daily scale, the temperature response was more complex. First, at the same STT, the lower the PIT, the larger the respiration rate. Second, after 5 days, no significant difference between the CO_2 fluxes was observed between the three PITs.

The ability of the biotic/abiotic model to reproduce the flux dynamics suggests that temperature-induced substrate depletion could be the main cause of the lower respiration rates in samples pre-incubated at higher temperatures. Higher temperatures could indeed induce a larger very labile C depletion due to greater consumption by microorganisms during pre-incubation periods. The lower respiration rates in the second cycle could also result from a reduction of very labile C content in each sample. Finally, the flux convergence between all PIT treatments during the pre-incubation period of Exp. 2, as shown in Fig. 3, could be explained by compensation between the simultaneous decrease in soil labile C and increase in enzyme activity with temperature. These results tend to confirm the important role played by labile substrate availability, as also shown in other recent studies (Larionova et al. 2007; Gershenson et al. 2009; Curiel-Yuste et al. 2010; Karhu et al. 2010; Wetterstedt et al. 2010).

The decomposition constant values selected during model calibration were higher than most values reported in the literature (e.g., Kätterer et al. 1998). This could indicate that the total OC of these homogenized samples was mainly made of very easily and rapidly assimilated substrate, so that their amount decreased at a very high rate. This is probably due to sample homogenization, which made carbon more available, as also suggested by Lömander et al. (1998) who observed higher decomposition rates in laboratory incubations than in the field. This procedure remains necessary, however, in order to eliminate flux spatial variability, and it did not affect the comparison of temperature effects between PIT conditions, as all the samples were similarly pre-treated.

The soil OC measurements performed during Exp. 1, however, did not support the hypothesis of labile C depletion and microbial biomass variation. Neither a significant decrease in OC nor an increase in microbial biomass was measured. The variability surrounding the OC measurements, however, indicates that the method should have been precise enough to detect C losses up to 0.03 mg C (g dry soil)$^{-1}$ (cold-water soluble C). Ghani et al. (2003) showed that their method was sensitive enough to assess differences between management types at the field scale, with C amounts exhibiting large spatial and temporal variability. Given that based on the results of many previous incubation experiments (e.g., Kirschbaum

2006; Curiel-Yuste et al. 2010; Gershenson et al. 2009; Karhu et al. 2010) the labile C depletion hypothesis is still the most likely explanation of our observations, this does not exclude the possibility that sample mishandling during laboratory analysis prevented us from obtaining more reliable results.

Other explanations for our observations can be given, but they are more speculative. Acclimation processes that could encompass genotypic, phenotypic and ecological changes within the microbial community (Bradford et al. 2008) could be considered, even though their importance in soils at the ecosystem scale is controversial (Davidson and Janssens 2006; Hartley et al. 2007, 2008; Malcolm et al. 2009). In this study, however, the lack of experimental data on enzymatic activity and heterotrophic community composition or activity prevented us from checking these hypotheses.

As suggested by Curiel-Yuste et al. (2010), the OC substrate depletion and acclimation hypotheses could be complementary in explaining STT response mediation by indirect longer-term temperature effects.

Model uncertainties

Parameter estimation was more accurate when the biotic/abiotic model was applied to Exps. 2a and 2b, probably because more calibration points were available (there were no measurements during the pre-incubation period in Exp. 1). This was observed particularly for k_F and pCO_2, for which the uncertainties represented 400 and 90 % of the mean value in Exp. 1, respectively, while they decreased to 27 and 51 %, and 54 and 50 % in Exps. 2a, and 2b, respectively. The output model uncertainties were small enough for the model to reproduce the observed impacts of PIT and STT on the CO_2 fluxes, the hysteresis effect and the negative fluxes. These uncertainties could be further reduced by reducing the initial condition uncertainties and increasing the number of flux measurements necessary to calibrate the parameters.

Conclusion

The fairly good agreement between simulation with the biotic/abiotic model and experimental results suggested that, as well the hysteresis that appeared in the short-term flux response, the negative fluxes that were observed probably resulted from abiotic processes

related to temperature-driven CO_2 dissolution. This was made firmer by (i) the basic chemical nature of Lonzée soil, (ii) the overall good agreement between the experimental results and those from the biotic/abiotic model application and (iii) their fitting improvement by adding this abiotic component. These findings suggest that caution should be taken when interpreting the CO_2 flux results from a limed soil, as they can represent between 20 and 100 % of the total measured CO_2 fluxes, in response to hourly temperature changes.

With regard to biotic fluxes, the analysis suggested that their temperature sensitivity was probably not affected by timescale (hourly and daily) or PIT. On the contrary, the respiration rate at a given STT was negatively related to the PIT, which resulted from differential OC substrate depletion in the soil samples (daily timescale). In terms of OC pool dynamics, however, the model disagreed with the soil OC measurements. This would therefore indicate that OC depletion might not constitute the sole hypothesis accounting for daily timescale variations of soil CO_2 fluxes in response to temperature, and leaves open the possibility of a microbial community adaptation to temperature changes, with a less active community at higher PIT. Further investigations, including characterization of microbial community enzymatic activity, composition and activity and soil organic matter degree of protection, could be performed to provide a better understanding of how the soil biological environment reacts to STT changes.

Finally, this study highlighted the need to keep studying the impact of soil sample preparation and pre-treatment on CO_2 fluxes and soil OC content measurements performed in laboratory incubations.

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