



Lasting effect of soil warming on organic matter decomposition depends on tillage practices



Ruixing Hou ^{a, b}, Zhu Ouyang ^{a, b, *}, Dorodnikov Maxim ^c, Glenn Wilson ^d, Yakov Kuzyakov ^c

^a Key Lab. of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research Chinese Academy of Sciences, Beijing 100101, China

^b Yucheng Comprehensive Experiment Station, China Academy of Science, Beijing 100101, China

^c Department of Soil Science of Temperate Ecosystems, University of Göttingen, 37077 Göttingen, Germany

^d USDA-ARS, National Sedimentation Lab, Oxford, MS 38655, USA

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ABSTRACT

Global warming accelerates soil organic matter (SOM) decomposition with strong feedback to atmospheric CO₂. Such an effect should be especially important for no-till agricultural practices, where SOM accumulates in the topsoil as compared with conventional tillage. We incubated soil samples (0–5 cm) at three temperature levels (15, 21 and 27 °C) from long-term till and no-till systems that were *in situ* warmed and non-warmed to assess the temperature sensitivity of CO₂ efflux, labile organic carbon and extracellular enzyme activities. Thermal adaptation to prolonged warming was observed resulting in a lasting effect on SOM decomposition. On average, 26, 14 and 12% more CO₂ was emitted at each incubation temperature from the warmed soils compared to the non-warmed soils. The Q₁₀ value was lower for the warmed than the non-warmed soils. Soil microbial biomass C and dissolved organic C declined with warming. The activities of three extracellular enzymes, β-glucosidase, chitinase, and sulfatase, were higher under warming and no-till as compared to non-warmed and tilled soil. We concluded that the increased SOM decomposition due to the stimulation of microorganisms by warming was long-lasting. Predictions of C accumulation in the topsoil by no-till farming should be taken with caution, as this C pool is especially vulnerable to global warming.

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1. Introduction

Given the tremendous amount of organic carbon (C) in soil, understanding the feedback between soil organic matter (SOM) decomposition and global warming is critical for predicting future atmospheric CO₂ concentrations. The response of SOM decomposition to temperature changes has received considerable attention during the last decade (Melillo et al., 2002; Davidson and Janssens, 2006; Allison et al., 2010). How long the effects of warming could last on decomposition remains unclear: will this effect be ongoing for extended periods, or will the processes adapt to the warmer conditions and stabilize to the previous decomposition level after

exhausting available organics? Numerous studies have reported that increased temperature significantly alters microbial community structure and functioning by affecting substrate and nitrogen availability (Melillo et al., 2011; Bradford, 2013; Steinweg et al., 2013; DeAngelis et al., 2015). However, an understanding of microbial feedback to global warming remains limited, especially for the utilization of substrate and microbial activity associated with soil C cycling (Wallenstein et al., 2009).

Microbial controls on soil C cycling can be affected by global warming, primarily through two mechanisms. First, microbially-driven decomposition of SOM is regulated by the quantity and quality of substrates comprising the SOM, which can be affected by temperature increase. Warming often results in an increase in substrate input from litter and root exudates due to an increase in plant growth (Oberbauer et al., 2007). Warming can also stimulate the use of the easily available SOC pool, change the microbial community structure, and lead to an alteration in C use by microorganisms. There are debates on the relationship between the

* Corresponding author. Key Lab. of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research Chinese Academy of Sciences, Beijing 100101, China. Tel.: +86 01064856514; fax: +86 01064854230.

E-mail address: ouyz@igsnr.ac.cn (Z. Ouyang).

decline in available substrates and weaker response of warming-induced soil respiration (Bengtson and Bengtsson, 2007; Fissore et al., 2013). Second, warming may increase microbial activity, leading to acceleration of SOM decomposition. This acceleration may come from long-term (Billings and Ballantyne, 2013) or temporary thermal adaptation of the microbial community to the warmer conditions (Bradford, 2013). An increasing number of studies have shown that warming changes microbial community structure, and warming increases the rate of enzymatically-catalyzed reactions up to a temperature optimum (Wallenstein and Weintraub, 2008; Burns et al., 2013). However, only a few studies have measured extracellular enzyme activities (EEA) in field warming experiments and just some of those studies found warming to have a consistent positive effect on EEA (Bell and Henry, 2011; Jing et al., 2014; Schindlbacher et al., 2015). A reliable and sensitive proxy for the total microbial activity is the activity of key enzymes responsible for nutrient acquisition by microorganisms and SOM degradation.

No-till (No-Till) farming sequesters more C close to the soil surface than does conventionally tilled (Till) farming, whereas Till stores more C deeper in the profile (Baker et al., 2007; Hou et al., 2012). However, the fate of this surface-sequestered C in No-Till soil is unclear under warming and post-warming conditions. To better project the responses of SOM decomposition under two tillage systems to future warmer world, we incubated the topsoil (0–5 cm) from four years *in situ* warming field experiment, analyzed the released CO₂ at three temperatures (15, 21 and 27 °C) and estimated the temperature sensitivity of SOM decomposition (Q_{10}). The objective of this study was to determine the lasting effect of warming on microbial activity under long-term No-Till and Till management systems to answer the following research questions: (i) is there a difference in CO₂ production and extracellular enzyme activities between *in situ* warmed and non-warmed soils? (ii) are the differences in CO₂ and enzyme activities temperature sensitive and (iii) are the sensitivities dependent upon the tillage system?

2. Materials and methods

2.1. Site description

This study was conducted on long-term (since 2003) experimental field plots in the North China Plain (NCP) (36°50'N, 116°34'E, 20 m above sea level). The site is located in a temperate semi-arid climate, with an annual mean temperature of 13.6 °C and mean precipitation of 553 mm during the past 29 years (from 1985 to 2013). Approximately 70% of the annual precipitation occurs between June and September. The soil is classified as a Calcaric fluvisol according to the FAO-UNESCO system, and the surface soil texture is silt loam (12% sand, 66% silt; 22% clay), according to the USDA classification system, with a pH of 6.9. Winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.) were double cropped, which is common in the NCP. Depending on precipitation, winter wheat was irrigated two to three times each season (70–80 mm each time), while summer maize was irrigated only in dry summers. Each year, crop residues (including straw and stover) were retained on the soil surface for No-Till but removed for Till. The study involved four treatments: Till with and without warming (TW and TN, respectively) and No-Till with and without warming (NW and NN, respectively). The warmed block in each pair was continuously heated using an MSR-2420 infrared heater (Kalglo Electronics Inc., Bethlehem, PA, USA) beginning on the 4th of February, 2010. The infrared heater was placed 3 m above ground. Soil moisture and temperature were measured by probes at 5 cm depth. The details of the set-up are provided in the previous study of the same field (Hou et al., 2014).

2.2. Sampling and incubation

Soil samples (0–5 cm) were taken on 13th May, 2013 and stored at 4 °C before incubation. Soil sampled from the four long-term field treatments (TN, TW, NN, NW) were weighed in equivalent 30-g air-dried soil samples and placed into air-tight vessels (120 ml). These samples were incubated at 15, 21 and 27 °C for 59 days, with four replications for each treatment and temperature. The soil moisture was kept at 70% of its water holding capacity (i.e. 30% gravimetric moisture content) with deionized water. Thus, the microbial activities observed were in response to the soil properties established by the long-term management systems and exposure or non-exposure to warming at the time of sampling.

2.3. Measurements

Soil CO₂ efflux was trapped by 3 ml of 1 M NaOH in small vials placed in vessels. The traps were changed eight times during the 59-day incubation, and the CO₂ efflux was determined. The temperature sensitivity (Q_{10}), which is a measure of the rate of a parameter change as a consequence of increasing the temperature by 10 °C, was estimated based on the CO₂ fluxes at three temperatures at the beginning and end of the incubation periods (0–6th day and 35th to the 59th day, respectively). Mean respiration rates at each incubation temperature were fitted with an exponential model to calculate Q_{10} value:

$$Rs = ae^{bT} \quad (1)$$

$$Q_{10} = e^{10b} \quad (2)$$

where Rs is soil respiration, T is soil temperature, and a and b are two regression coefficients (Luo et al., 2001).

Soil pH was measured using a 1:2.5 (w/v) soil to 0.01 M CaCl₂ ratio with a glass electrode. The concentrations of soil organic C and total N were determined with a LECO CN2000 analyzer. Soil microbial biomass carbon (MBC) and K₂SO₄-extracted carbon – dissolved organic carbon (DOC) were determined by the fumigation-extraction method before and after incubation (Vance et al., 1987). A K_C value of 0.45 was used to calculate the C content of the SMBC, and this factor was empirically defined in earlier studies (Vance et al., 1987) based on cell survival after fumigation with chloroform to correct for the carbon that could not be extracted.

To analyze the responses of microorganisms to warming, the activity of three extracellular enzymes was determined: β -Glucosidase, N-acetyl- β -D-Glucosaminidase (chitinase) and sulfatase, which reflect C (β -Glucosidase, chitinase), N (chitinase) and S (sulfatase) cycling, respectively. Extracellular enzyme activities were measured using fluorogenically labeled substrates according to a modified technique (Marx et al., 2001; Stemmer, 2004). Three fluorogenic enzyme substrates based on 4-methylumbelliferone (MUF) were used: MUF-b-d-glucopyranoside (MUF-G; EC 3.2.1.21, for the detection of β -glucosidase), MUF-N-acetyl-b-d-glucosaminide dihydrate (MUF-NAG; EC 3.2.1.14) for chitinase, and MUF-sulfate potassium salt (MUF-S; EC 3.1.6) for sulfatase activity. To dissolve the MUF-substrates, 2 ml of 2-methoxyethanol was used. Pre-dissolved MUF-substrates were further diluted with sterile distilled water to give the desired concentrations. The soil samples (1 g) were suspended in water (20 ml) and shaken on an overhead shaker for 15 min at room temperature at maximum speed to ensure thorough mixing. A subsample of the soil suspension (50 μ L) was added to 100 μ L MUF-substrate solution and 50 μ L MES-buffer, which were pre-pipetted in deep-well microplates (96-well, 0.5 ml, HJ-Bioanalytik GmbH, Monchengladbach, Germany). Fluorescence was measured at an excitation wavelength of 360 nm and an

emission wavelength of 460 nm at 0, 30 and 60 min after reaction, with a Victor3 1420–050 Multilabel Counter (PerkinElmer, Waltham, MA, USA). Calibration curves were included in every series of enzyme measurements. Enzyme activities were expressed as MUF release in nanomoles per gram bulk soil dry weight per hour ($\text{nmol g}^{-1} \text{h}^{-1}$).

2.4. Statistical analysis

Data were analyzed by ANOVA procedure using the SPSS for Windows, version 11.5 (SPSS Inc., Champaign, IL). Three-way ANOVA was used to analyze the individual and interactive effects of warming, incubation temperature and tillage systems on the CO_2 effluxes, soil labile carbon and extracellular enzyme activities. Differences were considered significant at $P < 0.05$. Means of main effects were compared using the least significant difference (LSD) test after a significant ANOVA test.

3. Results

3.1. CO_2 efflux and Q_{10}

CO_2 efflux from soil incubated at three temperatures showed lasting effects of field warming on SOM decomposition (Fig. 1). CO_2 efflux rates were significantly increased in tilled soil under warming (TW) compared to non-warmed (TN) at all three incubation temperatures by 26, 13 and 12% for 15, 21 and 27 °C incubation temperatures, respectively. Similar results were found between NW and NN, with 26, 15 and 12% higher CO_2 fluxes under the warmed, no-till soil. Thus, 17% more CO_2 was released on average due to exposure to warming regardless of the tillage system. Between tillage systems, CO_2 effluxes tended to higher under NN than TN by 2.8, 7.9 and 19.2% for 15, 21 and 27 °C, respectively; while greater increases were found under NW than TW with 3.2, 7.9 and 18.8%. CO_2 efflux was significantly affected by warming (<0.001), incubation temperature (<0.001) and tillage system (<0.001) (Table 1), but the significantly effect of the interaction of three factors was only observed between incubation \times tillage system (<0.001).

The patterns of the ratio of the cumulative CO_2 flux for warmed to non-warmed soil were similar for No-Till and Till systems (Fig. 2), indicating that the CO_2 flux was not sensitive enough to

reflect the differences between land use systems. However, considering the incubation temperatures, the greatest relative effect of warming on cumulative CO_2 efflux was observed at 15 °C for both No-Till and Till systems. For 27 °C incubation, a sharp increase was observed during the first 6 days, followed by slow deceleration back to the initial level. These changes in relative CO_2 flux between warmed and non-warmed soil indicated that with increasing incubation temperature, the pool of easily available C for decomposition decreased much faster under warmed vs. non-warmed treatments. Thus, the rate of SOM decomposition was not constant during incubation.

In contrast to the relative increase in CO_2 flux by warming, the Q_{10} parameter revealed differences between warmed and non-warmed soils. The field warmed soils had a significantly greater Q_{10} of CO_2 flux during days 0–6 of incubation than non-warmed soils, while the reverse was observed for days 35–59 (Fig. 3). The Q_{10} changes implied that the temperature sensitivity of SOM decomposition was strongly affected by the 4-years of exposure to warming due to either altered structure and activity of the microbial communities or the quality of the SOM. Clearly, both features are closely connected and most likely changed together.

3.2. Labile organic carbon

The labile extracted organic compounds significantly declined during incubation. After 59 days, soil microbial biomass carbon (MBC) declined during incubation on average for the three temperatures by 33 and 42% for TN and TW, respectively, compared to the start of incubation. The decreases for NN and NW were smaller and amounted to 23 and 30%, respectively (Fig. 4). In contrast, the average decline in dissolved organic carbon (DOC) between warmed and non-warmed soils was similar at 28% for Till and No-Till systems.

3.3. Extracellular enzyme activity

All extracellular enzyme activities (EEA) were significantly higher in warmed soils under No-Till, whereas no effect of warming was observed between TN and TW (Fig. 5). Warmed soil under No-Till demonstrated significantly higher EEA than non-warmed soils for chitinase and sulfatase at 15 °C by 35 and 182%, and by 96 and 63% at 21 °C, respectively. A significant increase in β -Glucosidase (responsible for the splitting of complex organics, e.g., cellulose to glucose) indicated an increase in the organic substrates available for microorganisms. The highest activity of β -Glucosidase, occurring at 21 °C, may reflect the optimum temperature of this enzyme, whereas available N and S were not limiting for the microorganisms (the activities of chitinase and sulfatase were lower or unchanged from the initial soil, respectively) (Fig. 5). All three EEA were significantly affected by warming, incubation temperature and tillage system (all $P < 0.05$). The interactive effects of warming with tillage, and incubation temperature with tillage also significantly affected the three enzyme activities (all $P < 0.05$) (Table 1).

4. Discussion

4.1. Lasting effect on soil CO_2 efflux

Higher CO_2 efflux from field warmed soils relative to non-warmed was observed under same incubation temperatures (Fig. 1). Warming accelerated SOM decomposition and strongly increased soil respiration (Lu et al., 2013; Wang et al., 2014). The lasting positive effects of warming on soil respiration has been observed, i.e. there was increased CO_2 efflux from pre-warmed soil relative to a control after warming was switched off under field

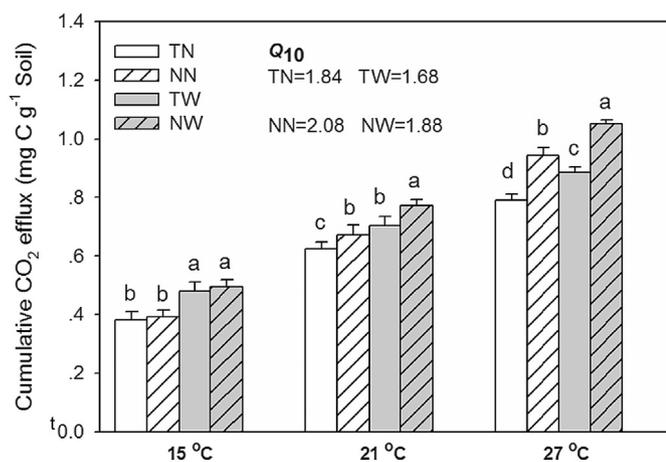


Fig. 1. Cumulative CO_2 effluxes (\pm SE) from TN, TW, NN and NW under three temperatures (15, 21 and 27 °C). Different lowercase letters show the significant differences at same temperatures among the four treatments. TN, Till + no warming; TW, Till + warming; NN, No-Till + no warming; NW, No-Till + warming.

Table 1
Results (P-values) of three-way ANOVA on the effects of warming (W), incubation temperature (I), tillage system (T) and their interactions on cumulative CO₂ efflux, soil microbial biomass carbon (MBC), dissolved organic carbon (DOC), β-Glucosidase, Chitinase and Sulfatase.

Source of variance	CO ₂ efflux	MBC	DOC	β-Glucosidase	Chitinase	Sulfatase
W	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
I	<0.001	<0.001	<0.001	<0.001	0.004	0.001
T	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
W × I	0.703	0.919	0.789	0.307	0.002	0.024
W × T	0.337	0.187	0.303	<0.001	<0.001	<0.001
I × T	<0.001	0.128	0.791	<0.001	0.031	<0.001
W × I × T	0.987	0.956	0.760	0.380	0.018	0.001

conditions (Hartley et al., 2007). In our study, the lasting effect indirectly indicated there was *in situ* thermal adaptation of microbial processes (Bradford, 2013). The lower Q_{10} values during the whole incubation period under warmed than non-warmed soils for both tillage practices meant that four years warming decreased temperature sensitivity (Fig. 1). Thus, thermal adaptation of soil respiration involved weaker response of mass-specific respiration to temperature change (Atkin and Tjoelker, 2003). Our results were supported by previous findings demonstrating that long-term warming can change the mechanisms of SOM turnover (Billings and Ballantyne, 2013).

4.2. Response of labile organic carbon and Q_{10}

Our results showed a rapid decline in the relative increase of soil respiration rate with time especially at 15 and 27 °C (Fig. 2) and

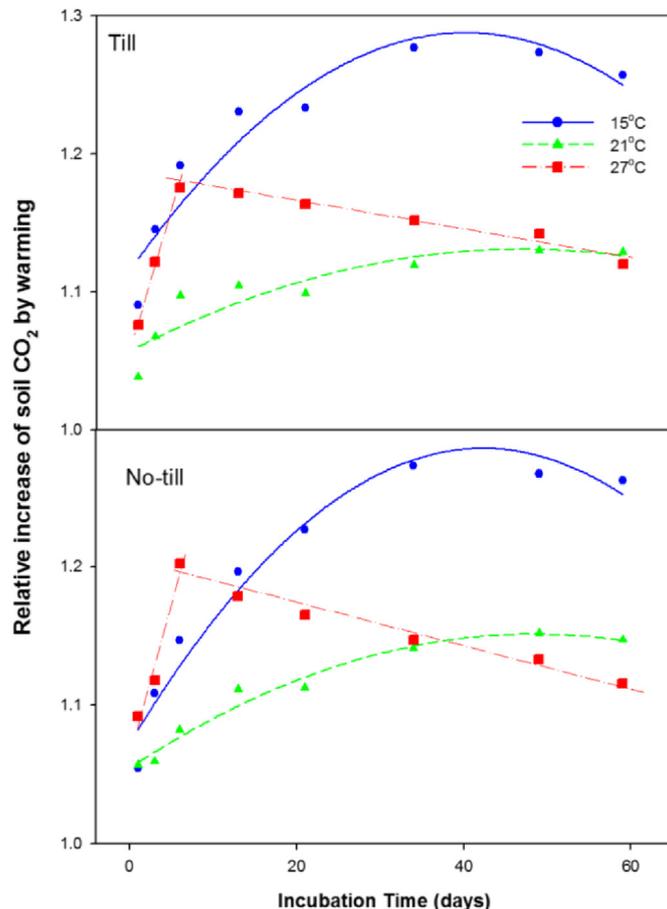


Fig. 2. Relative increase in CO₂ efflux from soil by warming, i.e., ratio of Warmed/Non-warmed for tilled soil (Top) or soil without tillage (Bottom).

more intensive substrate depletion in warmed soils with higher incubation temperature (Fig. 4). The supply of available substrate could govern the sensitivity of microbial decomposition of SOM to temperature (Bell et al., 2010; Bell and Henry, 2011). Similarly, substrate depletion under sustained experimental warming was indicated by higher CO₂ efflux under warmed relative to non-warmed soils (Melillo et al., 2002; Bradford et al., 2008). The decline in MBC with increasing temperature (Fig. 4) was likely caused by higher microbial activity which resulted in rapid depletion of cell reserves and easily available substrates in soil (Knorr et al., 2005; Bradford et al., 2008).

The decrease of easily available substrate could limit the temperature sensitivity of SOM decomposition (Eliasson et al., 2005; Fissore et al., 2013). In the laboratory, ample substrate leads to higher Q_{10} in warmed soil than in non-warmed soil during the first 1–2 days of incubation (Piva et al., 2012; Nie et al., 2013). This is consistent with our results (Fig. 3) in which higher microbial activity in warmed soils decomposed labile substrates faster, leading to higher ($P < 0.05$) Q_{10} values for warmed compared to non-warmed soils during the first 6 days. The substrates depleted faster during the incubation under higher temperature, which may have consequently resulted in the decreased temperature sensitivity. The depletion of labile substrates declined the initially high CO₂ efflux to lower levels at the end of incubation. This decline led to lower ($P < 0.05$) Q_{10} in warmed soils than the non-warmed (Fig. 3) and decreased Q_{10} in warmed vs. non-warmed treatments during the complete 59-day incubation (Fig. 1). Thus, our results indicated that the lasting effect of warming on SOM decomposition could be restricted by substrate depletion.

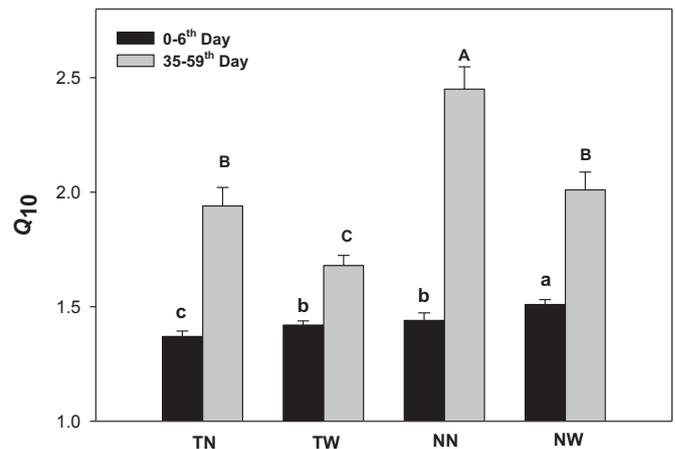


Fig. 3. Q_{10} values during the beginning and end periods: first 6 days and from day 35 to 59 of incubation. The lowercase/uppercase letters represent significant differences of 0–6th day/35–59th day among four treatments respectively. TN, Till + no warming; TW, Till + warming; NN, No-Till + no warming; NW, No-Till + warming.

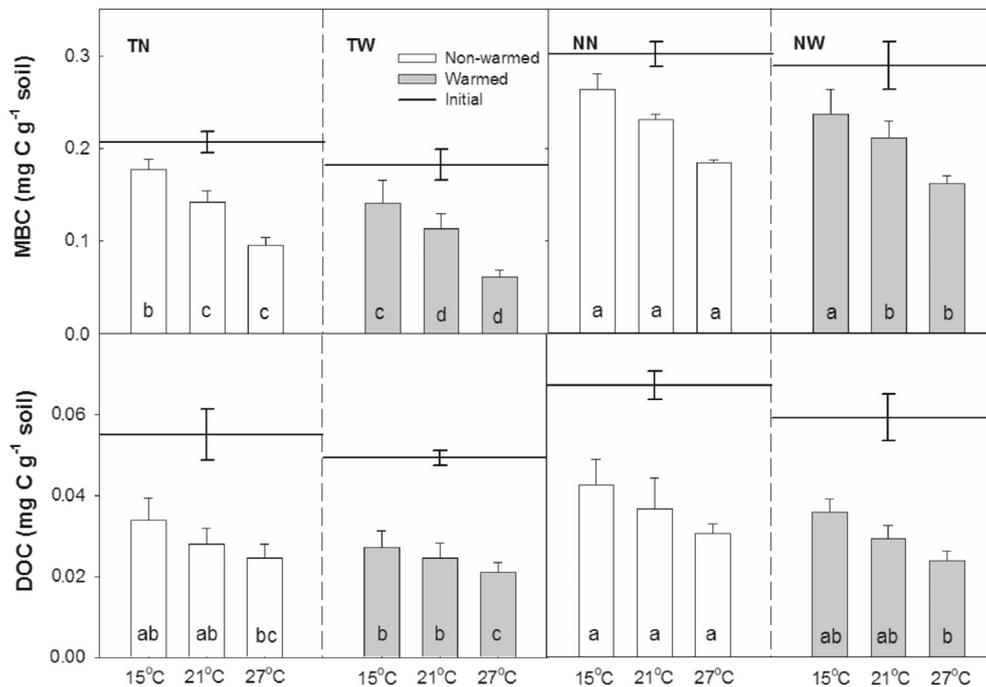


Fig. 4. Soil microbial biomass carbon (SMBC) and K_2SO_4 -extracted (dissolved) organic carbon (DOC) in control and warmed soils before (Initial, horizontal lines) and after incubation (bars). All values of SMBC and DOC at the end of incubation decreased significantly ($P < 0.05$). Different lowercase letters show the significant differences of MBC or DOC at same temperatures among the four treatments. TN, Till + no warming; TW, Till + warming; NN, No-Till + no warming; NW, No-Till + warming.

4.3. Response of extracellular enzyme activity

In our study, warming significantly increased EEA under No-Till with small but significant effect on Till soils. This finding answered our research questions in a way that warming promoted change of EEA through a set of direct and indirect effects such as increased plant depositions, possible shifts in microbial community structure and other effects (discussed below). Our study showed that such positive effects of warming on EEA were dependent upon the tillage system and only held true for No-Till (Fig. 5). In addition to the direct temperature effects on EEA, warming indirectly controls EEA through soil moisture and plant physiology (Bell et al., 2010; Henry, 2012) which may offset the apparent warming-induced EEA increase. Excessive drying of soils under warming is expected to decrease the EEA because of desiccation and limited substrate supply of plant-derived deposits. However, no clear effects of soil moisture loss on EEA under warming have been reported (Allison and Treseder, 2008; Jing et al., 2014). Irrigation maintained the soil at a relatively high moisture level (Hou et al., 2014) thereby preventing any negative effects of warming on EEA. Plant physiology may also affect substrate availability by deposition of labile C (Tilman et al., 2009; Allison et al., 2010). On the same experimental site, greater root biomass was measured under No-Till relative to Till treatment in the 0–10 cm soil layer (Hou et al., 2015). Direct positive relationship between root biomass and EEA increase was reported (Geisseler et al., 2011). Altogether, maintenance of soil moisture and greater root biomass near the surface both should contribute to the increased EEA with warming in our experimental site.

Between the two tillage treatments, the response of EEA to warming was much less pronounced under Till as compared to No-Till. This was explained by differences in amount and quality of SOM between the two treatments. Thus, significantly lower SOC, total N (See Supplementary Tab. S1), MBC and DOC (Fig. 4) contents were measured near the surface under Till than No-Till resulting in

overall lower availability of substrate for microorganisms in the Till system.

4.4. Effects of tillage systems

Higher CO_2 efflux was measured under No-Till relative to Till for warmed and non-warmed soils, especially at 21 and 27 °C (Fig. 1). No-Till has been observed to strongly increase soil microbial biomass and activity, and to release more CO_2 during incubation relative to tilled soil (Wagai et al., 2013). However, the findings of higher CO_2 efflux due to warming under no-till compared to tillage treatment measured in the laboratory should be cautiously extended to field conditions. The natural distribution of crop residue under No-Till as compared to tilled soil provides physical protection by occlusion in aggregates which limits SOM mineralization in the field. Sampling of soil inevitably affects soil structure and decreases the physical protection of SOM. As all of the soil samples were collected and sieved in a similar manner and there was no significant difference in total soil organic carbon content between soils of each tillage treatment (See Supplementary Tab. S1), the observed differences in temperature sensitivity of CO_2 flux of the two tillage systems cannot be explained solely by changes in physical protection of SOM. Furthermore, our field observations revealed a decrease in soil CO_2 emissions over time under Till, while it consistently increased under No-Till treatment over the three wheat seasons and two maize seasons under warming (Hou et al., 2014), hereby supporting the observed patterns in the current study.

To the best of our knowledge, this is the first study that compared the response of SOM decomposition to warming under long-term Till and No-Till farming. The lasting positive effects of warming on the SOM decomposition and decline of Q_{10} were observed for the two tillage systems indicating thermal adaptation of microbial community to higher *in situ* temperature. Comparing the two tillage practices, higher Q_{10} under No-Till than Till (Fig. 1)

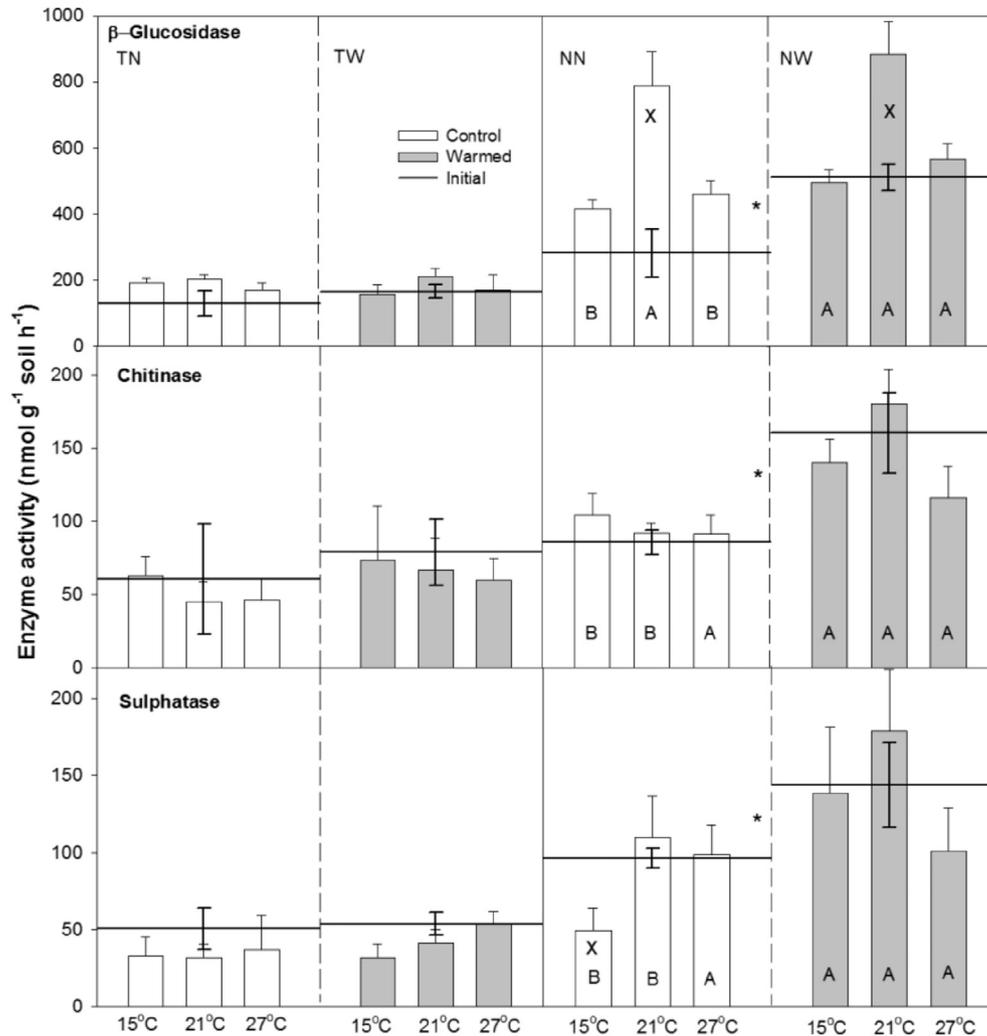


Fig. 5. Activities of three enzymes: β -Glucosidase, Chitinase, and Sulphatase before and after incubation of non-warmed and warmed soils. Horizontal lines represent the original values of each soil. Stars (*) represent a significant difference in the activity value of initial soils, crosses (X) represent a significant difference between final and initial soils in same treatment; letters represent significant differences in activity at the same temperature after incubation ($P < 0.05$). TN, Till + no warming; TW, Till + warming; NN, No-Till + no warming; NW, No-Till + warming.

meant higher temperature sensitivity of SOM decomposition thereby answering our second research question. Thus, the retention of residues on the surface by No-Till makes the easily available substrate more vulnerable to warming effects on C decomposition and CO₂ efflux. Our results indicate that there is a need to consider contrasting responses to tillage management for global warming when predicting soil C pools in croplands.

5. Conclusions

After four years of *in situ* soil warming, the SOM decomposition was more intensive in warmed soil as compared with non-warmed soil. This effect of warming on soil microorganisms lasted for at least two months. Based on the increased activities of measured enzymes (β -Glucosidase, Chitinase and Sulphatase), we conclude that the microorganisms were more active under warming, probably because of thermal adaptation of microorganisms. Such warming-induced EEA were most pronounced under No-Till system. At the same time, substrate depletion was found to limit the soil CO₂ efflux and the temperature sensitivity of SOM decomposition. Thus, C sequestration in the topsoil under No-Till system would be more vulnerable to environmental changes as compared with the Till system. The C balance in croplands should be evaluated to consider

differences in SOM decomposition between Till and No-Till systems under increasing temperature.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2015.12.008>.

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