Dear Anonymous Referee #3:

We highly appreciate the valuable comments you made on our paper. They were all enlightening and helped improve our manuscript. Below we would like to confirm the corrections made in response to your comments. Additional corrections have also been made on the previous manuscript following the remarks of other reviewers, so we would appreciate it if you could also refer to our responses made for other reviewers. We hope that the present correction will meet the criteria for your positive evaluation and fulfills your requirement for the paper's acceptance. We will be glad to receive any further suggestions for the paper's improvement that you may have.

Please take note that those written in **black** were your comments while those in **blue** were our responses

General Comments

This study conducted non factorial design experiments with only three treatments and obtained the contribution of heterotrophic respiration to total respiration and it response to climate warming. This kind of experiment has limited significance to global change ecology. Lack of necessary statistic analyses makes the conclusion inconveniencing.

We respect your opinion when you said that our study is of less significance to the global change ecology yet we are still counting on the impressions made by other two reviewers that our paper is interesting and is of high relevance in climate change research. Besides, we still believe that this kind of experiment is one of priority researches nowadays so that is why we presented data with very high data resolution.

Also, we made additional statistical analyses that will eventually convince you to believe on the conclusion of our research. Regarding the use of a non-factorial design experiment, please understand that our main concern is to study the effect of warming in heterotrophic respiration. Thus, trenching is common to two treatments (unwarmed-trenched and warmed-trenched) and is not considered a factor here while the third treatment is a control treatment. The only main factor in this case is warming. The contribution of heterotrophic respiration to total soil respiration is another story and is independent to soil warming for they were just simply the difference between the control chambers (where total soil respiration was obtained) and the unwarmed-trenched chambers (where we obtained the soil heterotrophic respiration) and we can say that trenching is the main factor in this case. Hence, we used a non factorial design as we simply studied the effect of one variable at a time (e.g. effect of warming on soil respiration, soil temperature, and soil moisture in comparison to non-warming plots).

Following your and other reviewer's comments, we determined daily Q_{10} and R_{10} in the equation, $F_c = R_{10} \times Q_{10}$^$((T_s-10)/10)$, for each chamber by least-squares method using hourly soil respiration and temperature data within 15 days moving windows (previous and following 7 days each) and conducted statistical analyses to show the significant difference among treatments. Figure I shows the seasonal variation in Q_{10} and R_{10}, and Table II shows the summary of statistics of each treatment.

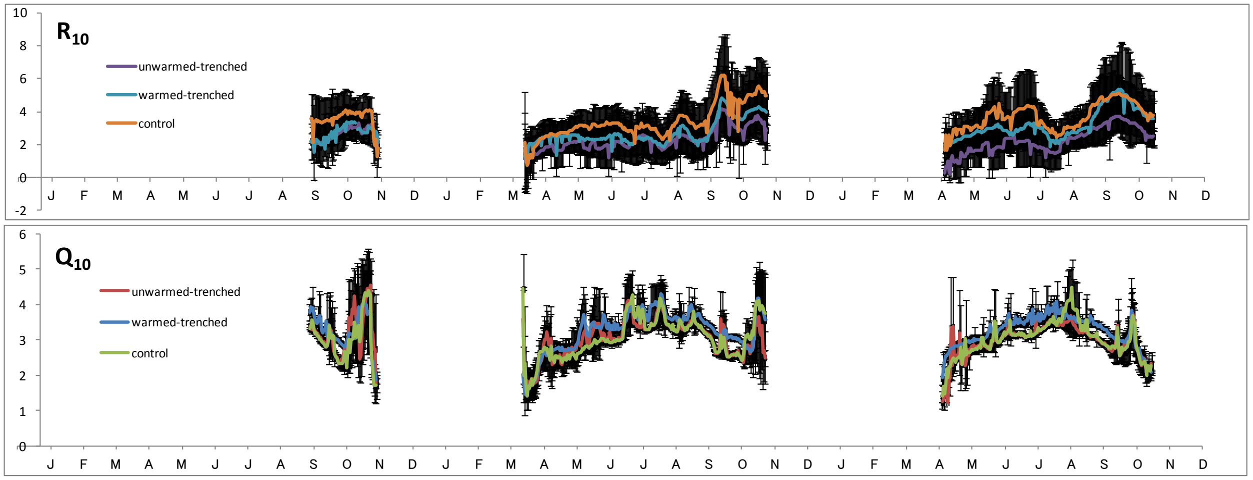

Figure I. Seasonal variation of Q_{10} and basal respiration rates at 10ºC (R_{10}). Colored lines indicate the daily average of 5 chambers for each treatment and vertical bars denote standard deviation for each day per treatment.

Table I. Temperature sensitivity (Q_{10} and R_{10}) of soil CO_2 efflux rate for each treatment. Whole data obtained from 5 chambers at a certain period (3 years or each year) were used to analyze the significant difference among treatments. Values were shown as average±standard deviation (number of data). Tukey-Kramer HSD test was used to check the significant difference among treatments and values in a row followed by different superscript letters denote significant difference ($p < 0.001$) among treatments.

	Unwarmed–trenched	Warmed–trenched	Control
		Q_{10}	
3 years	3.03±0.65^{a} (2275)	3.24±0.60^{b} (2304)	3.03±0.64^{a} (2307)
2007	3.20±0.90^{ab} (311)	3.29±0.75^{a} (303)	3.04±0.78^{b} (312)
2008	3.03±0.66^{a} (1095)	3.25±0.65^{b} (1123)	3.05±0.65^{a} (1114)
2009	2.98±0.49^{a} (869)	3.21±0.47^{b} (878)	3.00±0.58^{a} (881)
		R_{10}	
3 years	2.50±0.89^{a} (2275)	2.94±1.52^{b} (2289)	3.59±1.45^{c} (2307)
2007	2.65±0.57^{a} (311)	2.68±1.09^{a} (288)	3.64±1.15^{b} (312)
2008	2.41±0.97^{a} (1095)	2.77±1.42^{b} (1123)	3.47±1.55^{c} (1114)
2009	2.55±0.86^{a} (869)	3.24±1.69^{b} (878)	3.72±1.42^{c} (881)

These analyses showed that warming treatment increased not only the heterotrophic and basal respiration rate but also Q_{10}, thus we revised the manuscript (including the title, abstract, materials and method) according to these new results. However these results still support one of our conclusions that "if we predict the soil heterotrophic respiration rate in future warmer environment using the relationship between soil temperature and soil heterotrophic respiration obtained at present climatic condition, the rate can be underestimated.". The new title is "Soil warming in a cool-temperate mixed forest with peat soil enhanced heterotrophic respiration rate and the temperature sensitivity".

In agreement with the recommendation from Reviewers #1 and #2, fitting regressions for shorter periods and for each chamber has the advantage of showing the seasonal variation and the daily standard deviation for each treatment. In addition, we can check the significant difference among the treatments, so we decided to use the newly computed results. All new results were reflected in the revised paper. Especially we fully revised sub-section "3.2 Soil CO_2 efflux and the warming effect" as follows, by adding Figures I, II, III, and IV and Table I in this response, and deleting Table 1, Figures 1, 2, 3, 4, 6 and 8 in the previous manuscript following the recommendation by other reviewers. However, we remained Figure 5 just to show the overall temperature sensitivity for each treatment (but the regression equation was changed to the new one). Figures II, III, and IV are listed at the last part of this response. We also revised related sentences in the manuscript with describing new methods to determine seasonal variations in Q_{10} and R_{10}.

"3.2 Soil CO_2 efflux and the warming effect

Soil CO_2 effluxes in all the treatments roughly paralleled to the seasonal variation of soil temperature. Increasing the rate at the start of growing season in spring until summer and decreases towards leaf fall in autumn (Figure IV). Soil warming increased the heterotrophic respiration rate consistently across the entire measurement period ($p < 0.001$). The efflux rate of control chamber was almost the same with that of warmed-trenched chamber in 2007, but was intermediate between the effluxes of warmed and unwarmed trenched chambers. Mean heterotrophic respiration rate was 4.67, 5.87, and 6.91 (μmol C m^{-2} s^{-1}) during snow-free period in 2007, 2008, and 2009, respectively, at warmed-trenched treatment, showing increasing trend from 2007 towards 2009. This increase is likely caused by the increasing temperature during this period. Across all seasons within the 3-yr warming period, soil CO_2 efflux was greatest in the warmed-trenched chambers (Figure IV). Warming increased the efflux by 74% (or around 25% per ºC) (mean 6.11±3.07SD μmol C m^{-2} s^{-1}) compared with that of the unwarmed-trenched treatments (mean 3.52±1.74μmol C m^{-2} s^{-1}) (p<0.001), while the control chambers obtained 4.98±2.44 μmol C m^{-2} s^{-1}.

The difference in soil CO2 efflux between unwarmed-trenched and control chambers showed that heterotrophic respiration contributed 71% of the total soil respiration and the remaining 29% was assumed to be the autotrophic respiration (Figure 7 in the previous manuscript). Autotrophic respiration peaked in advance (June to July) from that of heterotrophic respiration (August) in both 2008 and 2009. For over 20-month period, total soil respiration rate reached 2.74 $kgCm^{-2}$ wherein 1.94 $kgCm^{-2}$ of it had been contributed by heterotrophic respiration. Calculating for an equal period of measurement from 22 April to 19 November for both 2008 and 2009 showed that total soil respiration rate dropped from 1.20 $kgCm^{-2}$ in 2008 to 1.13 $kgCm^{-2}$ in 2009 while soil heterotrophic respiration decreased from 0.86 kgCm−2 in 2008 down to 0.81 kgCm−2 in 2009. A higher average soil temperature in 2008 (15.5 and 15.6 ºC for control and unwarmedtrenched treatment, respectively) than that in 2009 (14.8 and 15.0 ºC, respectively) was observed from June to September, and this could cause the decrease in the soil respiration rates in 2009. The rate of decrease in the total soil respiration from 2008 to 2009 (0.07 $kgCm^{-2}$) was primarily driven by the decrease in the soil heterotrophic respiration (0.05 $kgCm^{-2}$).

An exponential function described the relationship between the soil CO_2 efflux and soil temperature for each treatment (Figure 5 in the previous manuscript, but changing the regression equation). Q_{10} values in unwarmed-trenched, warmed-trenched and control were 2.44, 2.44, and 2.54, respectively, with little difference between unwarmed- and warmed-trenched treatments. Meanwhile, basal respiration rate at 10ºC in soil temperature (R_{10}) differs among treatments with a higher R_{10} in warmed-trenched chambers (3.59 μmol C m^{-2} s^{-1}) compared with unwarmed-trenched chambers (2.75 μmol C m^{-2} s^{-1}). Control chambers showed the highest (3.91 μmol C m^{-2} s^{-1}) owing to the contribution of root respiration.

Seasonal variation in Q_{10} and R_{10} (Figure I) showed that Q_{10} tended to be high in summer except very high values obtained jut after snow melt and before snow accumulation, while R_{10} tended to be high in Autumn. Comparing whole 3-yr data set, R_{10} in warmed-trenched chambers (2.94 ± 1.52 μmol C m^{-2} s^{-1}) was significantly higher than that in unwarmed-trenched chambers (2.50 ± 0.89 μmol C m^{-2} s^{-1}) (Table I). Control chambers showed the highest R_{10} (3.59 ± 1.45 μmol C m^{-2} s^{-1}) owing to the contribution of root respiration. The significant difference caused by warming was observed in 2008 and 2009, but not in 2007. The increase in R_{10} was observed in 2009 compared with that in 2008 for all treatments.

Similar to the case for R_{10}, warming increased the Q_{10} significantly, if we compare data in whole 3 years, 2008, and 2009, while there was no significant difference between unwarmed-trenched and control treatments for the 3 years. Contradictory to the case for R_{10}, Q_{10} in 2009 was lower than that in 2008 for all treatments. The short term determination and averaging of Q_{10} and R_{10} in Table I increased the values compared with the case obtained as in Figure 5."

Specific Comments

Introduction

A critical reference concerning climate warming and temperature sensitivity (Luo et al. 2001. Nature) was missing.

Page 6418, Line 15-20: Luo et al. (2001) has long been shown that climate warming could decrease temperature sensitivity of soil respiration. Authors intentionally avoid to cite this reference when making the argument.

We mentioned earlier that there was a slight reduction in Q_{10}, so we referred Lou et al. (2001) to have observed the same trend and we stated it in P6430 L25-28 as follows;

“Reductions in the Q_{10} under induced temperature were observed in a tallgrass prairie (Luo et al., 2001), suggesting acclimation of respiration to climate warming and/or alteration of substrate supply”.

We did not refer their study here in Introduction because their study site vegetation (tall grass prairie) is different from ours (forest), however, we referred their study following your suggestion.

Material and Methods

P6419, L15: what were the diameter and depth for the “100 cm3” soil cores?

The diameter is 5 cm, and we added this information in the revised paper.

P6419, L23: what did “SD” mean? Define it before use the abbreviation. What was the size of the chambers?

SD is the standard deviation and we explained this abbreviation at the first part of revised manuscript. P6421 L8-9 states that “Each of the 15 chambers had a dimension of 0.9 × 0.9 × 0.5 m high”.

P 6420L9-10: Trenching itself could stimulate root decomposition and soil respiration and the phenomenon can last for more than 6-9 months (See Zhou et al. 2007. GCB). Thus, inclusion the 2007 data could have overestimated the contribution of heterotrophic respiration to total respiration and the response of heterotrophic respiration to warming.

We remained the data obtained in 2007, however we mentioned about the uncertainties in the data obtained in 2007in the sub-section 4.3 in Discussion as follows;

“4.3 Contribution of heterotrophic respiration to the total soil respiration

Our result showed that heterotrophic respiration rate (not associated with warming) governs the total soil respiration rate given its 71% contribution. Several studies report the similar contribution of heterotrophic respiration such as, 67% for a mixed hardwood forest in Massachusetts (Bowden et al., 1993); 77% for a lowland old-growth beech (*Nothofacus*) in New Zealand (Tate et al., 1993); >70% for *Picea abies* stands in Northeast Bavaria, Germany (Buchmann, 2000); and 56 to 69% for a subalpine forest dominated by lodgepole pine (*Pinus contorta*) trees in Niwot Ridge, Colorado (Scott-Denton et al., 2006).

However, because we applied trenching method to separate heterotrophic and autotrophic respirations, it could have altered the microorganism activities and thus, decomposition of soil organic matter due to the absence of living roots (Kuzyakov, 2006), which may had caused the underestimation of the observed heterotrophic respiration. On the other hand, because trenching itself could stimulate root decomposition and soil respiration and the phemenon can last for more than 6 to 9 months (Zhou et al., 2007), the data obtained in 2007 could have overestimated the contribution of heterotrophic respiration to total respiration. Uncertainties still remain as to the contribution of root respiration below the trenching depth (30 cm), although our root biomass survey shows minor contribution of the root below 30 cm to the whole root biomass within the surface 45 cm soil layer. Contribution of roots in deep layer could cause an overestimation of the heterotrophic respiration in the unwarmed-trenched treatment, and underestimation of the autotrophic respiration estimated as the difference between the two treatments (unwarmed-trenched and control). Given these disadvantages in the method, our estimated values may include uncertainties to some extent."

Here, we did not mention the possibility of overestimating the response of heterotrophic respiration to warming in 2007, because there was no significant difference in Q_{10} and R_{10} between warmed- and unwarmed-trenched treatments only in 2007 (Table I).

P 6420L21-25: What was the distribution of plant roots in soil profile? I do not think 30cm trenching can exclude plant roots in forest.

In order to prove whether trenching depth is sufficient or not, we established three 15 × 15 cm^2 plots beside the chambers in control plots. We collected 15 × 15 × 15 cm^3 soil blocks each for the three layers (i.e. 0-15, 15-30, 30-45 cm deep) and coarse and fine roots were collected on each soil block, then washed and ovendried to determine their biomass contents. The root biomass for each layer was 664±64SD, 156±22, and 41±8 gDW m^{-2}, for 0-15, 15-30, and 30-45 cm deep, respectively. Based on the new results, we considered that the contribution of roots below 30 cm deep to soil respiration could be minor at trenched treatments.

Supporting our result, a previous soil coring and minirhizotron study made within Teshio Experimental Forest have shown that the fine roots of both bamboos (*Sasa senanensis* and *Sasa kurilensis*) and prevailing trees (the same species within our site) were concentrated in the surface soil (0-15 cm) and decreased with increasing soil depth (Fukuzawa et al., 2007, Ecological research, 22, 485-495). This similar pattern was observed in our site.

In addition, we collected soil core samples beside the chambers in control plots at 5, 10, 20, and 40 cm deep in the soil with 5 replicates to determine C contents and stock within the surface 30 cm soil layer, following the recommendation from reviewer #2. Accordingly, we deleted the sentences on the soil carbon content at P6419L15-25 and P6429L11-13 in the previous manuscript, then we added new sub-sections "Soil and root biomass measurements" in the Material and method section, and "Soil carbon content and root biomass" in the Results section to show this newly observed data as follows.

"2.5 Soil and root biomass measurements

In August 2011, soil sample cores of 100 cm^3 (5 cm in diameter) each were collected beside the five chambers in control plots at 4 depths (5, 10, 20 and 40 cm) to evaluate the soil carbon content and density in the study area. Dry bulk density was obtained by weighing the samples after 2 days of oven-drying at 80 ˚C. Soil carbon content was analyzed using an automatic NC analyzer (Sumigraph NC-900, Sumika Chemical Analysis Service, Japan), attached to a gas chromatograph (GC-8A, Shimadzu Corp., Japan). Three homogenized soil samples with 49 to 52 mg weight were analyzed to get the average for each core.

In addition, root biomass (> 0.5 mm in diameter) was measured every 15 cm soil layer down to 45 cm deep at three of the five points where the soil cores were sampled. Soil blocks with 15×15×15 cm were collected at each layer, and roots in the blocks were collected. The root samples were washed and oven-dried at 80 ˚C for two days and weighed."

"3.1 Soil carbon content and root biomass

The soil carbon content was 99±32 SD, 111±32, 188±22 and 233±45 g kg^{-1}, at 5, 10, 20, 40 cm deep, respectively and evaluated soil carbon density at surface 30 cm soil layer was 17.6±1.6 kgC m^{-2}. The root biomass was 664±64, 156±22, and 41±8 gDW m^{-2}, for 0-15, 15-30, and 30-45 cm soil layers, respectively. The root biomass sharply decreased with the increase in depth and >95% of the roots in the collected soil was in the surface 0-30 cm soil layer."

However, the large proportion of heterotrophic respiration to total soil respiration obtained in this study might be partly caused by incomplete trenching, thus in the discussion section, we acknowledged the possibility in the sub-section 4.3 as mentioned above.

P6421: When did the soil respiration measurement begin in each year? How long did it last in each year?

We added this following sentence in sub-section 2.3.

"These measurements were conducted from 4 September to 20 November in 2007, from 22 March to 20 November in 2008, and from 22 April to 20 November."

P6423L27: What were the criteria for identifying the outliers of the data?

P6422L15-25 until P6423L1-4 will give you the details about outliers checking and handling.

Results

P6424L14: Were "4 and 3%" absolute or relative differences? It seems absolute differences. Specify it.

It is absolute and we added this information.

P6424L24-26: The results supported the above comments on P 6420L9-10: trenching itself stimulate root decomposition and soil respiration.

We would like to clarify that in 2007, the efflux at control (neither trenching nor warming) was almost equal to that of warmed-trenched treatment but was still higher than that of unwarmed-trenched treatment. It simply shows trenching decreased soil respiration rate.

P6424L26- P6425L3: This kind comparison makes nonsense unless the snow-free periods over the 3 years had the same time length. Why did the soil CO2 efflux increase with year?

An increasing temperature from 2007 towards 2009 had likely increased soil CO_2 efflux rates.

Because we used the mean value to show the inter-annual variation, we considered that this comparison has a value to be informed.

We revised this sentence as, "Mean heterotrophic respiration rate was 4.67, 5.87, and 6.91 (μmol C m^{-2} s^{-1}) during snow-free period in 2007, 2008, and 2009, respectively at warmed-trenched treatment, showing increasing trend from 2007 towards 2009. This increase is likely caused by the increasing temperature during this period."

P6425L13-15: Why?

For the 20-month study period, there were 38,340 soil CO_2 efflux data for each treatment. In every ºC change in temperature, enormous number of data fell within the range. Usually 30 or less data fell at extreme upper temperature range which led to uncertain average value, so we decided to discard them and considered them unusual cases.

P6425L15-16: This result was contradictory with that in P6424L24-26.

P6424L24-26 conveyed the same meaning as that of P6425L15-16. Both implied that the efflux rate of both warmed-trenched and control chambers were higher than that of unwarmed-trenched chambers. The result in P6424L24-26 explained about the inter-annual variation in the efflux rate while that in P6425L15-16 described the efflux rates within the same temperature range.

P6425L19-29: Were the temperature sensitivity and base respiration statistically significant from each other? If yes, how did you do the statistics? Similar comment on P6426. These kinds of comparisons qualitative rather than quantitative and make nonsense unless statistics were conducted.

As mentioned above, we fully revised this part by determining seasonal variation in Q_{10} and R_{10}, and applying statistical test to show the significant difference among treatments.

P6426L24-26: Again, inclusion the 2007 data overestimated the contribution of heterotrophic respiration to total respiration

Please refer to above replies.

P6427L11-16: Many previous studies and this study have already shown temperature sensitivity of soil respiration changes with soil temperature itself. Why did the authors use the same temperature sensitivity to estimate the soil respiration in snow season?

We deleted this part in the revised paper according with your and other reviewer's suggestions.

Discussions

P6431L15-25: It's over-extrapolation from one site results to regional or national scale.

It's somehow over-extrapolation, however we would like to remain this part after following minor revision on sentences in order to show possible effects on the global carbon cycle. If this explanation still cannot be accepted, we will remove this part.

"Gorham (1991) estimated that total release of carbon by drainage of boreal and subarctic peatlands could be 8.5 to 42 TgC yr^{-1}. If we assume 74% increase in soil heterotrophic respiration rate, it would correspond to an increased release of 6 to 31 Tg C yr^{-1}. This is 10% of Japan's current industrial CO_2 emission of 330 Tg C yr^{-1} in 2008 (GIO and CGER- NIES, 2010), and could provide a strong positive feedback to global atmospheric CO_2 concentrations and, consequently, warming."

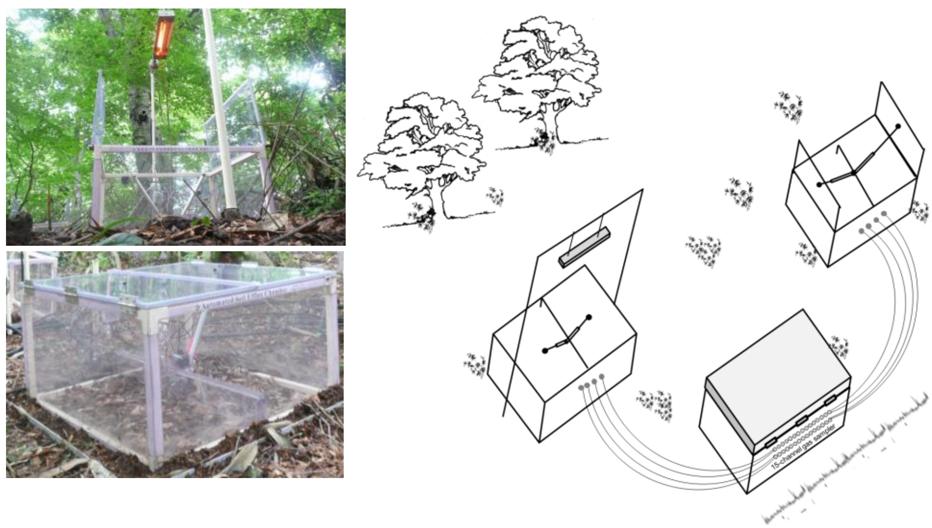

Figure II Images of warmed-trenched plot (upper left); a closed chamber with trenching (lower left); and a schematic illustration of the multi-channel automated system.

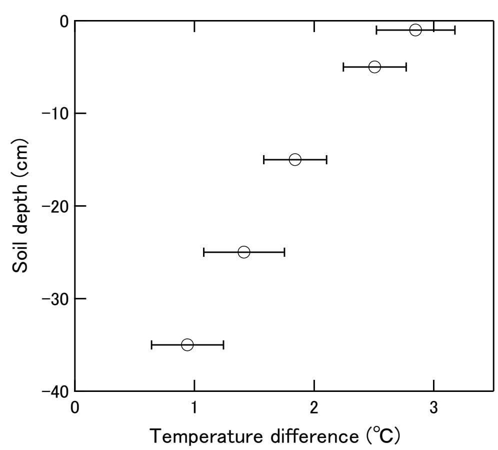

Figure III Difference in the soil temperature profile between unwarmed- and warmed trenched treatments from 22 August to 7 September 2011.

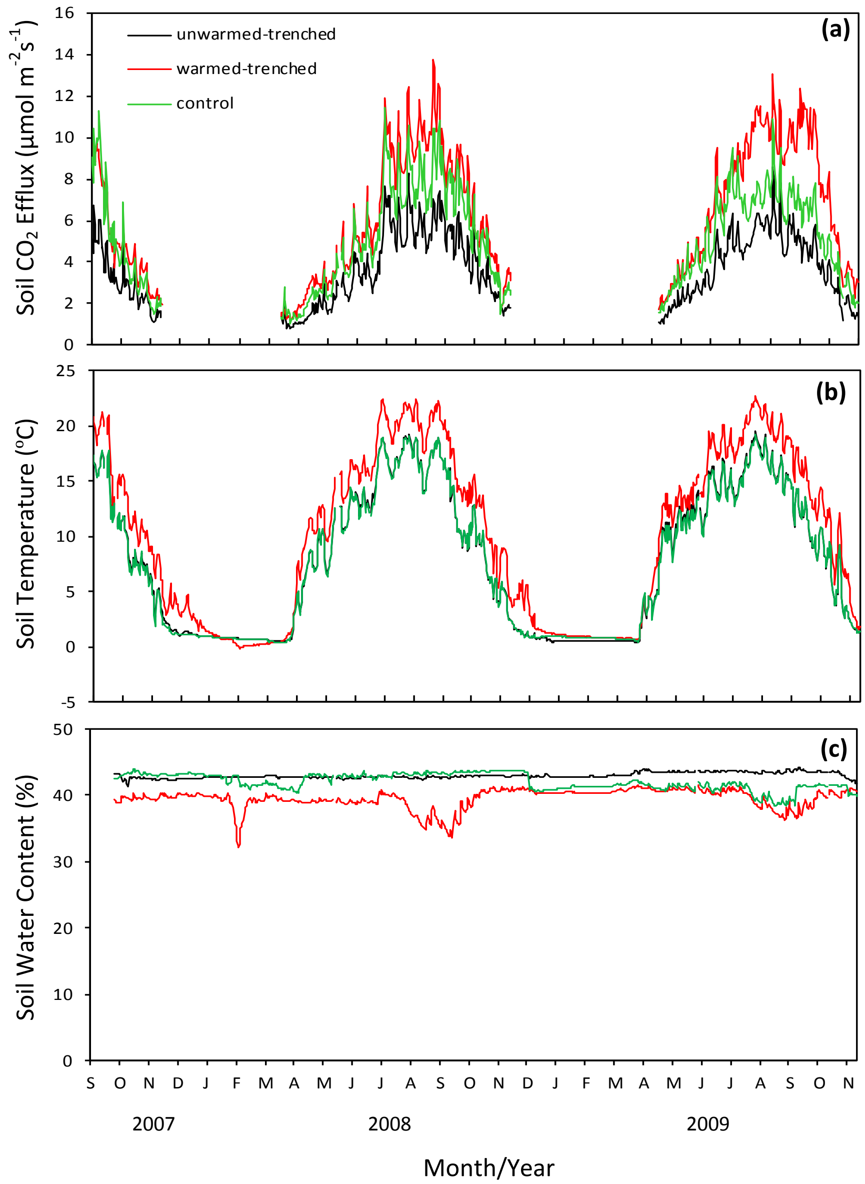

Fig IV Interannual variation of (a) soil CO_2 efflux; (b) soil temperature; and (c) soil water content in unwarmed-trenched, warmed-trenched, and control treatments during the study period in 2007–2009. All data are daily averages.

Thank you very much.

Respectfully yours,

The Authors