Effects of Soil Moisture on the Temperature Sensitivity of Soil Heterotrophic Respiration: A Laboratory Incubation Study

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Abstract

The temperature sensitivity (Q₁₀) of soil heterotrophic respiration (Rₘ) is an important ecological model parameter and may vary with temperature and moisture. While Q₁₀ generally decreases with increasing temperature, the moisture effects on Q₁₀ have been controversial. To address this, we conducted a 90-day laboratory incubation experiment using a subtropical forest soil with a full factorial combination of five moisture levels (20%, 40%, 60%, 80%, and 100% water holding capacity - WHC) and five temperature levels (10, 17, 24, 31, and 38 °C). Under each moisture treatment, Rₘ was measured several times for each temperature treatment to derive Q₁₀ based on the exponential relationships between Rₘ and temperature. Microbial biomass carbon (MBC), microbial community structure and soil nutrients were also measured several times to detect their potential contributions to the moisture-induced Q₁₀ variation. We found that Q₁₀ was significantly lower at lower moisture levels (60%, 40% and 20% WHC) than at higher moisture level (80% WHC) during the early stage of the incubation, but became significantly higher at 20%WHC than at 60% WHC and not significantly different from the other three moisture levels during the late stage of incubation. In contrast, soil Rh had the highest value at 60% WHC and the lowest at 20% WHC throughout the whole incubation period. Variations of Q₁₀ were significantly associated with MBC during the early stages of incubation, but with the fungi-to-bacteria ratio during the later stages, suggesting that changes in microbial biomass and community structure are related to the moisture-induced Q₁₀ changes. This study implies that global warming’s impacts on soil CO₂ emission may depend upon soil moisture conditions. With the same temperature rise, wetter soils may emit more CO₂ into the atmosphere via heterotrophic respiration.

Introduction

Temperature sensitivity of soil respiration, usually termed as Q₁₀, is defined as the increase of soil respiration rate by a 10 °C rise in temperature [1]. Q₁₀ has been considered an important model parameter in predicting terrestrial ecosystem carbon cycle and feedback to climate warming [2]. In the past several decades, Q₁₀ has been investigated extensively, particularly through field-observed soil respiration and environmental factor data [3,4]. It has been found that Q₁₀ is not a constant of 2, but varies with vegetation and edaphic conditions such as temperature, moisture, and substrate availability [2]. As global temperature continues to rise [5], it is of paramount importance to understand how Q₁₀ is influenced by these factors individually and interactively. Since under field conditions, effects of soil temperature and moisture on Q₁₀ are often confounded with each other and with other factors, laboratory incubation has the advantage of deriving the primary and interactive effects of the environmental factors on Q₁₀.

Many studies have demonstrated that Q₁₀ can be influenced by a variety of biological and environmental factors [1,6,7]. Soil temperature itself has been found to have a negative correlation with Q₁₀. For example, at lower temperature regions (e.g., tundra), Q₁₀ tends to be higher than the estimates at warmer temperature regions (e.g., warm desert) [8]. A manipulated warming experiment also demonstrates that Q₁₀ is significantly lower at high temperature treatments than at the low temperature control [1].

Thus, the temperature effects on Q₁₀ have been generally consistent; i.e., Q₁₀ decreases with increasing temperature. However, the effects of other factors such as soil moisture on Q₁₀ have been less certain and deserve more research.

Soil moisture plays a critical role in soil respiration and may have a significant impact on Q₁₀. Many researchers have discussed the effects of soil moisture on soil respiration under different intermediate levels, above or below which soil respiration decreases [15]. At the optimum soil moisture, the macropore spaces are filled with...
adequate amounts of air and water which can facilitate the diffusion of both oxygen and soluble substrates [16]. In very wet soils, oxygen limitation occurs, and in very dry soils, the movement of soluble substrates via water films is restricted. Although the mechanistic understanding on the effects of soil moisture on R_h has been largely advanced, its influence on the Q_{10} of R_h is still inconclusive. For example, Wang et al. [17] reported that Q_{10} increased with soil moisture until reaching a threshold, and then declined in six temperate forests of China. Carlyle and Than [18] showed that soil moisture limited the Q_{10} of soil respiration beneath a *pinus radiata* stand in south-eastern Australia. But Reichstein et al. [19] found that Q_{10} was insensitive to the drying of a spruce forest soil. The inconsistency of soil moisture effects on Q_{10} is probably due to the confounding influences of different environmental factors under field conditions. One recent incubation study showed that soil moisture indeed influenced Q_{10} and the moisture-Q_{10} relationship differed between soils obtained at different topographic positions [20], but the underlying mechanisms remained unclear.

Effects of soil moisture on Q_{10} may be ascribed to changes in microbial biomass and community structure, and the physical and chemical properties of the soil [7,21]. Changes in soil moisture can affect the composition and function of soil microbial community due to differences in drought tolerance among taxonomic and functional groups of microorganisms [22]. For example, fungi can survive drought stress better than bacteria due to their ability to grow at lower matric potentials [23,24]. Soil moisture can also affect the quantity of soil microbial biomass carbon (MBC) and dissolved organic carbon (DOC) [25,26]. Despite a general understanding of the above processes, whether soil moisture effects on Q_{10} can be related to its influences on soil properties such as MBC, DOC, nutrient availability, and microbial community structure is still in active debate.

In this study, we investigated soil moisture effects on Q_{10} by incubating a subtropical forest soil under five temperature levels and five moisture levels over 90 days. Soil R_h and other properties such as MBC and DOC, nitrogen and phosphorous contents, and microbial community phospholipid fatty acids (PLFAs) were measured several times during the incubation period. Our objectives were first to analyze how changes in soil moisture influenced Q_{10}, and second to explore whether the moisture effects on Q_{10} could be related to its impacts on the soil microbial and chemical properties measured.

### Materials and Methods

#### Ethics Statement

Soils were sampled from a study site that is maintained by the South China Botanical Garden, Chinese Academy of Sciences. All necessary permits were obtained for the described study. This study did not involve endangered or protected species.

#### Site Description

Incubation soils used in this study were collected from an evergreen broadleaf forest stand at the Heshan Hilly Land Interdisciplinary Experimental Station (22°34'N, 112°50'E) in Guangdong Province of China. The region has a subtropical humid monsoon climate with apparent dry and wet seasons. The wet season starts in April and ends in October, and the dry season begins in November and lasts through March of the following year. The mean annual precipitation and temperature are 1700 mm and 21.7°C, respectively. The forest stand is 29 years old and mainly dominated by native tree species (*Schima superba* and *Michelia macclurei*) with an average height of 15 m and an average diameter at breast height (DBH) of 30 cm. The soil is categorized as Oxisols based on the US Soil Classification System [27,28], with a bulk density of 1.4 g cm⁻³, total organic carbon (TOC) of 2.80%, total nitrogen (TN) of 0.15%, and total phosphorus (TP) of 0.02% at the depth of 0–20 cm.

#### Incubation Experimental Design

In the field, four sampling areas, with a distance of at least 10 m between each, were selected to collect the incubation soils. In each area, five sampling sites (20×10 cm²) were randomly selected and sampled to the depth of 20 cm. These five random samples were homogenized to form a composite sample. Before sampling, the uppermost layer of litter with visible un-decomposed materials was excluded. We had four composite samples as four experimental replicates, each one weighing about 50 kg in fresh weight. All soil samples were transported to the laboratory and passed through a 2 mm sieve with apparent plant roots and stones being removed.

To investigate soil moisture effects on the temperature sensitivity (Q_{10}) of soil R_h, we used five soil moisture levels: 20%, 40%, 60%, 80%, and 100% water holding capacity (WHC). For each moisture level, soils were incubated under five temperature levels: 10, 17, 24, 31, and 38°C. A full factorial combination of the two factors and five levels for each factor produced 25 experimental treatments. Each treatment had four replicates from the four composite samples. Each replicate further had 6 duplicates, with one duplicate for measuring R_h and the other 5 for destructive sampling. As a result, we had 600 incubation soil samples in total with 120 (= 5 moisture levels × 4 replicates × 6 duplicates) in each of the five static temperature incubators (RXZ-600B, Southeast Instrument Co., Ltd., Ningbo, China). The temperature and relative humidity deviations of the incubators are ±1.5°C and ±7%, respectively. Each air-dried incubation soil sample (equivalent to 50 g of oven-dried soil) was added to each triangle flask and its soil water content was adjusted to the corresponding soil moisture level by adding deionized water. The flasks were covered by rubber stoppers with small holes to reduce water loss via evaporation and maintain gas exchange. In order to maintain constant soil moisture levels, water loss was checked and corrected weekly by weighing each flask and adding water as necessary. At most, 1 ml of water (equivalent to 3.3% of changes in 100% WHC) was added every week to the flask at the temperature level of 38°C.

#### Measurements of Soil R_h, Microbial and Chemical Properties

The incubation experiment lasted for 90 days. Soil R_h rates were measured using the Li-6262 Infrared Gas Analyzer (Li-Cor Inc., Lincoln, NE) on days 1, 2, 3, 4, 6, 7, 13, 18, 27, 34, 41, 53, 62, 74, and 90. Electrical fans blowing air into each incubator for 30 min every four days were used to maintain an aerobic incubation environment. Before R_h measuring, each triangle flask was ventilated for 3 minutes to minimize gas accumulation in the headspace. After ventilation, another type of rubber stoppers with two plastic tubes for gas inlet and outlet was used to seal the flask and the tubes were connected to Li-6262 for measuring headspace CO₂ concentration. The CO₂ concentration in the headspace was recorded every second for 2 minutes and R_h rate was calculated using the linear portion of the response curve of CO₂ concentration versus time [29]. At each moisture level and each measurement day, Q_{10} was calculated by fitting an exponential function to the measured R_h against the 5 temperature levels:

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R_h = ae^{bT}
\]
Figure 1. Responses of soil heterotrophic respiration ($R_h$) to changes in soil temperature after 7 (A), 30 (B), and 90 (C) days of incubation. Each data point is the mean of four replicates under each soil moisture treatment. Error bars represent standard errors ($n = 4$).

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where $R_h$ is the measured soil heterotrophic respiration rate ($\mu$g g$^{-1}$ oven dried soil h$^{-1}$), $T$ is the incubation temperature ($^\circ$C), $a$ and $b$ are the fitted model parameters. $Q_{10}$ is calculated individually for each of the four replicates using the following equation:

$$Q_{10} = e^{10b}$$

As intrinsic $Q_{10}$ is based on the kinetic reactions of molecular structure changes with temperature [2], the $Q_{10}$ of this study is the apparent temperature sensitivity.

For measuring MBC, DOC, total organic carbon (TOC), total nitrogen (TN), total phosphorous (TP), inorganic N (NH$_4^+$ and NO$_3^-$), and PLFAs, three flasks (or replicates) of each treatment were harvested on days 7, 30, and 90. Part of the soil in each flask was collected and stored at $-20^\circ$C for later analysis of PLFAs. The remaining soil was used to analyze microbial biomass and chemical properties. It is noted here that TOC, TN, and TP were only analyzed for the samples harvested on days 7 and 90.

Soil MBC was measured using the modified fumigation-extraction method [30]. MBC was calculated as the difference in extractable C concentrations between the fumigated and un-fumigated samples divided by a KEC factor of 0.38 [30]. The extractable C concentrations of un-fumigated samples were the soil DOC [31]. Soil NH$_4^+$ and NO$_3^-$ were measured by the method proposed in Dorich and Nelson [32]. Soil TOC was measured using the potassium bichromate-concentrated sulphuric acid heating method. Soil TN and TP were measured using the Kjeldahl resolution Auto Flow Injection method. Microbial community PLFAs were analyzed according to Bossio and Scow [33]. Concentrations of each PLFA were calculated relative to the 19:0 internal standard concentrations. 15:0, i15:0, a15:0, i16:0, 16:1w7c, 17:0, i17:0, a17:0, cy17:0, 18:1w7c, 19:0 cyclow8c were selected as bacterial biomarkers and 18:2w6, 9c were selected as fungal biomarkers [34,35].

### Statistical Analysis

Repeated-measures ANOVA was employed to determine the effects of sampling time and soil moisture on $Q_{10}$ and $R_h$ on the 15 measuring days. One-way ANOVA was used to analyze soil moisture effects on $Q_{10}$ for days 7, 30, and 90; Tukey’s HSD multiple comparison method was used to test $Q_{10}$ differences among soil moisture levels. Regression analysis was used to derive the relationships between $R_h$ and temperature, and between $Q_{10}$ (or $R_h$) and incubation time. Pearson correlation analysis was applied to detect the potential contributions of soil microbial and chemical properties on the $Q_{10}$ variations with moisture. All these statistical analyses were performed using SPSS software 16.0 (SPSS Inc., Chicago, IL).

### Table 1. Repeated-measures ANOVA for the temperature and time effects on $R_h$ under different soil moisture treatments.

| Moisture (%WHC) | Temperature (°C) | Time (day) | Temperature × Time |
|----------------|-----------------|------------|-------------------|
|                | $F_{(4, 15)}$   | $P$        | $F_{(14, 210)}$   | $P$            | $F_{(56, 210)}$ | $P$        |
| 20%            | 36.96           | <0.01      | 26.59             | <0.01          | 5.46            | <0.01      |
| 40%            | 35.77           | <0.01      | 50.03             | <0.01          | 3.88            | <0.01      |
| 60%            | 10.90           | <0.01      | 45.26             | <0.01          | 6.63            | <0.01      |
| 80%            | 12.24           | <0.01      | 52.44             | <0.01          | 6.47            | <0.01      |
| 100%           | 139.15          | <0.01      | 159.63            | <0.01          | 17.60           | <0.01      |

%WHC: percent of water holding capacity.

### Table 2. Regression equations of heterotrophic respiration ($R_h$) with temperature under different moisture treatments.

| Soil moisture (%WHC) | Day 7                          | Day 30                       | Day 90                       |
|---------------------|--------------------------------|------------------------------|------------------------------|
|                     | $R_h = 0.1684e^{0.0318T}$      | $R_h = 0.1333e^{0.0335T}$    | $R_h = 0.062e^{0.0546T}$     |
|                     | $R^2 = 0.72^*$                 | $R^2 = 0.72^*$               | $R^2 = 0.98^*$               |
| 40%                 | $R_h = 0.3014e^{0.0324T}$      | $R_h = 0.3526e^{0.0383T}$    | $R_h = 0.1896e^{0.2273T}$    |
|                     | $R^2 = 0.95^*$                 | $R^2 = 0.72^*$               | $R^2 = 0.97^*$               |
| 60%                 | $R_h = 0.6639e^{0.0311T}$      | $R_h = 0.6382e^{0.0218T}$    | $R_h = 0.6148e^{0.0217T}$    |
|                     | $R^2 = 0.92^*$                 | $R^2 = 0.78^*$               | $R^2 = 0.25$                 |
| 80%                 | $R_h = 0.2136e^{0.0537T}$      | $R_h = 0.1774e^{0.0532T}$    | $R_h = 0.2484e^{0.0342T}$    |
|                     | $R^2 = 0.92^*$                 | $R^2 = 0.99^*$               | $R^2 = 0.77^*$               |
| 100%                | $R_h = 0.1926e^{0.0485T}$      | $R_h = 0.1469e^{0.0201T}$    | $R_h = 0.19e^{0.2333T}$      |
|                     | $R^2 = 0.98^*$                 | $R^2 = 0.99^*$               | $R^2 = 0.81^*$               |

%WHC: percent of water holding capacity.

$i R_h$ represents soil heterotrophic respiration rate and $T$ represents temperature.

$R^2$ is the coefficient of determination; * and ** indicate significance at $P\leq0.05$ and $P\leq0.01$, respectively.

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Results

Relationships between Soil $R_h$ and Temperature under Different Soil Moisture Treatments

Soil $R_h$ varied significantly with temperature and incubation time under all the 5 moisture treatments (Table 1). $R_h$ responses to temperature changes on days 7, 30, and 90 were displayed to represent the early, middle, and late incubation stages (Fig. 1). Throughout the whole incubation period, soil $R_h$ was highest at 60% WHC, lowest at 20% WHC, and intermediate at the other moisture levels (Fig. 1). Among the three measurement days, soil $R_h$ had the highest values on day 7, especially at 60% and 80% WHC, compared to those on days 30 and 90. Soil $R_h$ declined with incubation time, declining in smaller magnitudes at 20% WHC and 100% WHC.

The temperature response of soil $R_h$ could be well fitted using the exponential model for each soil moisture treatment and measurement day (Fig. 1). Model parameters for the three representative days are presented in Table 2. All models were significant with the coefficient of determination ($R^2$) ranging from 0.53 to 0.84. The $Q_{10}$ regression functions are quadratic for 20%, 40% and 60% WHC and cubic for 80% and 100% WHC. Error bars (n = 4) represent standard deviations. $R^2$ is the coefficient of determination. P is the significance level.

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Changes of $Q_{10}$ with Incubation Time under Different Soil Moisture Treatments

Under all the 5 moisture treatments, $Q_{10}$ values ranged from 0.95 to 1.97 and varied markedly with incubation time. $Q_{10}$ was higher in the beginning of the incubation, and declined with time around day 60, then increased slightly to the end of the experiment (Fig. 2A). Among different soil moisture treatments, $Q_{10}$ at 80% and 100% WHC was higher than at the other soil moisture treatments. $Q_{10}$ at 60% WHC had the lowest value, especially after day 30. $Q_{10}$ at 20% WHC was among the lowest in the beginning of the incubation, but increased with incubation time and had the highest values at the end of the experiment (Fig. 2A).

To better show the $Q_{10}$ variation pattern with incubation time, polynomial regression models were used to fit $Q_{10}$ with incubation time. Findings indicate that a quadratic regression model could fit $Q_{10}$ well at 20%, 40% and 60% WHC with $R^2 \approx 0.50$ (Fig. 2A), while a cubic regression model should be applied at 80% and 100% WHC ($R^2 \approx 0.80$; Fig. 2A). Further inspection revealed the days on which the minimum and maximum $Q_{10}$ appeared. At 20%, 40%, and 60% WHC, the lowest $Q_{10}$ appeared on days 53, 62, and 62 with their values being 1.11, 1.15, and 0.95, respectively. At 80% and 100% WHC, $Q_{10}$ showed the highest values of 1.94 and 1.74 on day 18, and the lowest values of 1.28 and 1.22 on day 62, respectively.

Effects of Soil Moisture on $Q_{10}$

Repeated-measures ANOVA showed that $Q_{10}$ was significantly influenced by soil moisture ($F(4, 15) = 18.41, P<0.01$), incubation time ($F(14, 210) = 15.41, P<0.01$) and the interaction of the two ($F(56, 210) = 4.44, P<0.01$). Averaged over the 15 measurement times, $Q_{10}$ was significantly lower at 60%, 40% and 20% WHC compared to those at 80% and 100% WHC (Fig. 3B). $Q_{10}$ at 80% WHC had the highest value but was not significantly different from that at 100% WHC (Fig. 3A).

Since the interactive effect of soil moisture and incubation time was significant, we further compared $Q_{10}$ values among soil moisture treatments on three typical measurement days (Fig. 3B-D). On days 7 and 30, $Q_{10}$ at 80% WHC was not significantly different from that at 100% WHC but significantly higher than those at the three lower moisture levels, which is similar to the all-day average results shown in Fig. 3A. On day 90, $Q_{10}$ at 20%
WHC was significantly higher than at 60% WHC, but there was no significant difference among the other three soil moisture treatments.

Correlations between $Q_{10}$ and Soil Properties

Pearson correlation analysis showed that on days 7 and 30, $Q_{10}$ was positively correlated with MBC and the ratio of MBC to DOC (Fig. 4A–D). On day 90, no significant correlation was found between $Q_{10}$ and MBC or MBC/DOC; instead, $Q_{10}$ positively correlated with the F:B ratio (Fig. 4E) and TP (Fig. 4F). We did not find significant correlations between $Q_{10}$ and other soil chemical properties (inorganic nitrogen, DOC, TOC, and TN), which was therefore not presented.

Discussion

Moisture Effects on $R_h$ Responses to Temperature

Similar to many previous results [2,36,37], soil $R_h$ increased with temperature exponentially in our study; however response curves varied among different soil moisture treatments (Fig. 1, Table 2). Some previous studies showed that soil respiration may be decoupled from temperature under certain soil moisture levels resulting in soil respiration that is unaffected by temperature under water stress [38]. For example, Yu et al. [39] found that temperature was the determinant factor and $R_h$ increased with it exponentially only when soil moisture was not limited. However, our results showed that soil $R_h$ could still increase with temperature, even at 20% WHC, though at a relatively slow rate (Table 2). The discrepancy between our results and theirs may be because the studied soils differ in chemical, physical and microbial properties.
Regardless of temperature variations, soil RH at 60% WHC tended to be higher than at both lower and higher soil moisture treatments. This result is also consistent with some other studies that reported higher RH at intermediate moisture content [40–42]. The decrease of soil RH at lower soil moisture has been attributed to soluble substrate limitation, whereas at higher soil moisture level, especially at saturated soil moisture, RH was mainly limited by oxygen [12,36]. The decrease of soil RH over incubation time was probably caused by the depletion of labile substrate [43,44]. However, the persistently high RH at 60% WHC during the 90-day incubation could be due to the persistently high microbial activity, because this was the optimal moisture level and high microbial activity might override the influence of substrate limitation.

Variations of Q10 with Incubation Time

The variation of Q10 with time has been found not to be uniform [45]. A laboratory incubation study found that Q10 increased with incubation time, which was ascribed to substrate quality change from labile to recalcitrant [46]. In some long-term warming experiments, Q10 was found to decline over time [1,45]. In our relatively short-term incubation experiment, we found that Q10 declined with incubation time initially but increased during later incubation stages, and quadratic or cubic regression models were fitted to quantify the changes of Q10 at different soil moisture treatments (Fig. 2A). Over the 90-day incubation period, mean Q10 values was mostly <2.0, which is lower than the conventional estimates (2.0–2.6) probably due to less confounding factors involved in our incubation experiment [47]. The changes of Q10 might be related to the changes of soil RH, as many laboratory studies have shown that soil RH decreases with incubation time [48]. The underlying mechanisms were ascribed to substrate depletion [43,44]: the longer the incubation time, the more time microbes had to consume the labile carbon, leaving less to remain in the soil. In the absence of labile carbon, microbial mediated soil RH tends to have lower Q10 [49,50]. Similar variation patterns of Q10 with incubation time have been observed by Tuomi et al. [51] and Hamdi et al. [52], in which quadratic and cubic functions were also used to describe the relationships between Q10 and incubation time.

The increases of Q10 at the later stage might be related to soil substrate quality changes (Fig. 2A). As the labile carbon decreased, recalcitrant carbon could be decomposed. It has been previously reported that Q10 tends to be higher in this situation [53,54]. In this study, it was not clear what caused the higher Q10 at 20% WHC at the later stage, but NH4+-N and TP were also higher at 20% WHC, which may be related to the higher Q10.

Moisture Effects on Q10

We found that soil moisture had a significant effect on Q10, which aligns with the findings from several previous studies [17,20,55]. Our results showed that at the intermediate soil moisture level (i.e. 60% WHC), Q10 was lower than at the other soil moisture levels. While there was no significant difference of Q10 among 60%, 40% and 20% WHC, Q10 at 60% WHC was significantly lower than at 80% and 100% WHC (Fig. 3). Previous studies have shown that drying can decrease Q10 of soil respiration and total ecosystem respiration [2,56], and this may be largely due to substrate limitation caused by the limited diffusion of solutes in thin soil water films [57,58].

We further tested which soil properties would influence Q10 at different incubation days and found that, at the early and middle incubation stages, Q10 had a significant positive correlation with MBC and the ratio of MBC to DOC (Fig. 4). The higher MBC and MBC to DOC ratio were particularly associated with higher soil moisture levels, under which labile substrate might be more available to microbes due to less water limitation. However, the Arrhenius equation shows that reactants with lower activation energies (i.e. more reactive and less recalcitrant) should have lower temperature sensitivity [2]. Our incubation results indicated that Q10 might not only be determined by substrate availability, but also by microbial properties such microbial biomass.

At the late stage of incubation, Q10 was significantly related to F:B and TP. The tight correlation of Q10 with F:B ratio was quite interesting. Both fungi and bacteria are important decomposers, but their structures and chemical compositions are very different. Fungi have hyphae that allow them to move, colonize and degrade surface litters, and fungal cell walls are the polymers of melanin and of chitin, much more resistant to degradation [59,60]. At the late stage, labile substrate diminishing may favor fungal communities which can degrade more recalcitrant substrate. As suggested by the carbon quality hypothesis [2,61], soils with more fungi or higher F:B ratio would have larger Q10, as demonstrated here. Bradford et al. [62] also reported a shift in microbial community structure could alter the Q10 of RH. The positive correlation between Q10 and TP suggested that P availability might also influence Q10. For example, the Q10 value for the 20% WHC was higher than those for the other moisture levels (Fig. 2A) and TP was correspondingly higher, probably due to the lower rate of consumption by microbes at lower moisture levels. A field study also showed that summer drought caused a 22-64% reduction of microbial phosphorus [63], indicating lower microbial consumption of P under water stress. Furthermore, forest soils in subtropical China are often phosphorous limited [64]. The phosphorous saved by the lower rate of consumption might therefore contribute to the higher Q10 at 20% WHC during the late stage of the incubation.

Conclusions

By incubating a subtropical forest soil under five temperature levels and five moisture levels and measuring soil RH and microbial and chemical properties throughout the incubation, we found that: 1) soil moisture significantly influenced Q10, with Q10 being higher at higher soil moisture levels than at the lower moisture levels during the early stage of the incubation; 2) soil heterotrophic respiration was highest at intermediate moisture and lowest when the soil was very dry; 3) Q10 mostly declined with incubation time and could be best described by quadratic or cubic functions; and 4) moisture-induced Q10 changes were associated with soil microbial biomass at the early stage of incubation, but to the ratio of fungi-to-bacteria at the late stage. These results imply that the response of soil RH to future global warming may be shaped by changes in precipitation patterns. In dry conditions, global warming may stimulate less soil CO2 emission, but in wet conditions, relatively more soil CO2 may be emitted. Considering that more soil organic carbon has often been accumulated in the wet areas, with the same temperature rise high Q10 would mean more soil CO2 emission to the atmosphere from these areas in the future.

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Author Contributions
Conceived and designed the experiments: WS DH. Performed the experiments: WZ. Analyzed the data: WZ DH WS. Wrote the paper: WZ DH WS.

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