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Recalcitrant carbon controls the magnitude of soil organic matter mineralization in temperate forests of northern China

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Abstract

Background: The large potential of the soil organic carbon (SOC) pool to sequester CO₂ from the atmosphere could greatly ameliorate the effect of future climate change. However, the quantity of carbon stored in terrestrial soils largely depends upon the magnitude of SOC mineralization. SOC mineralization constitutes an important part of the carbon cycle, and is driven by many biophysical variables, such as temperature and moisture.

Methods: Soil samples of a pine forest, an oak forest, and a pine and oak mixed forest were incubated for 387 days under conditions with six temperature settings (5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C) and three levels of soil moisture content (SMC, 30%, 60%, 90%). The instantaneous rate of mineralized SOC was periodically and automatically measured using a Li-Cor CO₂ analyzer. Based on the measured amount of mineralized SOC, carbon fractions were estimated separately via first-order kinetic one- and two-compartment models.

Results: During the 387 day incubation experiment, accumulative mineralized carbon ranged from 22.89 mg carbon (C) ·g⁻¹ SOC at 30 °C and 30% SMC for the mixed forest to 109.20 mg C·g⁻¹ SOC at 15 °C and 90% SMC for the oak forest. Mineralized recalcitrant carbon varied from 18.48 mg C·g⁻¹ SOC at 30 °C and 30% SMC for the mixed forest to 104.98 mg C·g⁻¹ SOC at 15 °C and 90% SMC for the oak forest, and contributed at least 80% to total mineralized carbon.

Conclusions: Based on the results of this experiment, the soil organic matter of the pure broadleaved forest is more vulnerable to soil microbial degradation in northern China; most of the amount of the mineralized SOC derived from the recalcitrant carbon pool. Labile carbon fraction constitutes on average 0.4% of SOC across the three forest types and was rapidly digested by soil microbes in the early incubation stage. SOC mineralization markedly increased with soil moisture content, and correlated parabolically to temperature with the highest value at 15 °C. No significant interaction was detected among these variables in the present study.

Keywords: Carbon mineralization, Soil carbon fraction, Long time incubation, Two-compartment model, Temperate forest

Background

The magnitude of soil organic matter (SOM) mineralization largely depends on its complex composition and its interaction with different biotic and abiotic variables. SOM is biophysically degraded to various biochemical products, e.g. low molecular weight compounds (mono-saccharide, cellulose, glucose, etc.), high molecular weight

matter (humus, lignin, etc.), or aliphatic or aromatic materials (fatty acid, ester, waxy, alkene, etc.) (von Lützw et al. 2007). These biodegradation products subsequently interact with mineral soil particles, or may be protected by the microenvironment of the mineral soil, and turn to become an organo-mineral matrix (Rasmussen et al. 2006). Based on its decomposability and availability to soil microorganisms, SOC is usually classified into labile and recalcitrant soil carbon. Labile carbon mainly consists of soil microbial biomass carbon, dissolved organic matter, and easily oxidative organic matter, whereas the recalcitrant carbon

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usually refers to the component of SOM that is resistant to microbial decomposition or protected by mineral soil particles (Fang et al. 2005; von Lützow et al. 2007). Apart from this classification, SOC is also partitioned into dissolved organic carbon (DOC), particulate organic carbon, microbial biomass carbon, oxidative carbon, and hydrolyzable carbon (von Lützow et al. 2007). Although many different methods have been applied to clearly extract different SOC fractions, a deeper understanding is urgently needed to elucidate the kinetic properties of SOC fractions and their roles in controlling the magnitude of SOM mineralization.

The responses of different SOC fractions to environmental factors were simulated extensively based on a conceptual model, as the analysis method of SOC fractionation could not explicitly predict the impact of future climate change on the SOC pool (Kirschbaum 2000, 2006). The quantity and the mean residence time of labile or recalcitrant carbon have been well established under different incubation conditions using a one- or two-compartment model, which is underpinned by the mechanism of the kinetic theory (Riffaldi et al. 1996; Rey and Jarvis 2006). The carbon mineralization could be estimated by these models as a function of time and temperature, both of which are the most important factors driving SOC mineralization. In contrast to field studies, these conceptual models could provide the least-biased estimates of the correlation between the decomposition of carbon fractions and incubation variables (Kirschbaum 2000), and increase the comparability between different study results. As expected, these conceptual models would help to identify the major contributor to the variations in SOM mineralization processes among the different forest types.

Incubating temperature and moisture are two well-known determinants influencing SOM decomposition. The co-occurrence of high moisture content and high temperature could significantly increase SOM decomposition rate, however only below the threshold value of moisture content does a positive relationship exist between soil CO₂ emission and temperature (Howard and Howard 1993). A moisture content between 50 and 60% has been considered optimal for microbial activity (Howard and Howard 1993). Apart from the roles of temperature and soil moisture, the time length of the incubation period is a critical factor in regulating the SOC mineralization process. The proportion of labile and recalcitrant SOC will obviously change with the mineralization process, because there is a large difference in the mean residence time of variant SOC fractions (Mills et al. 2014). The mineralization rate of soil carbon has also been found to decline with the incubation time extending (Reichstein et al. 2000), and the regime function of mineralization variables would substitute each other at different incubation stages (Giardina

and Ryan 2000; Rasmussen et al. 2006). Considering the complex properties of different SOC fractions and the uncertainty of their correlation to mineralization variables, the incubation experiment with a longer time period (more than one year) is of vital importance to clarify the mechanisms underlying SOC mineralization of different forest ecosystems.

The forest type will manipulate the quality and quantity of SOM through the above- and belowground litter inputs (Fissore et al. 2008). The first objective of this study was to quantify the pool sizes of labile and recalcitrant SOC and their contribution to the total amount of mineralized SOC for different forest types. Secondly, we aimed to explore the predominant factor controlling the mineralization magnitude of SOC and its fraction. It was hypothesized that the magnitude of SOC mineralization is largely determined by substrate availability in the late incubation stage, and that the incubation factors will affect the supply of SOM to soil microbial decomposers.

Methods

Study site and soil sampling

This study has been carried out in the Taiyueshan Long-Term Forest Ecology Research Station, which is located at the National Forest Park of Lingkongshan (36°33′–36°42′ N, 111°59′–112°07′ E; elevation ranging from 1100 to 1950 m a.s.l.). This forest park is located about 200 km southwest of Taiyuan in the Shanxi Province of northern China. The geographic and meteorological conditions and species composition of the forest communities for this study area had already been introduced in detail by (Zhou et al. 2013). The tree species extensively distributed across this forest park are *Pinus tabulaeformis* Carrière, *Quercus wutaishanica* Mayr, *Betula dahurica* Pall, *Betula platyphylla* Suk, *Juglans mandshurica* Maxim, *Tilia mongolica* Maxim, *Populus davidiana* Dode, *Malus baccata* Borkh. The major shrub species are *Corylus mandshurica* Maxim, *Corylus heterophylla* Fisch, *Acer ginnala* Maxim, *Lespedeza bicolor* Turcz, *Philadelphus incanus* Koehne, *Rosa bella* Rehd. The herbaceous community mainly consists of *Carex lanceolata* Boott, *Spodiopogon sibiricus* Trin, *Rubia chinensis* Regel et Maack, *Thalictrum petaloideum* Linn, *Melica pappiana* Hempel.

Five forest plots of 20 m × 20 m were established in 2013, for each of three forest types: a *Pinus tabulaeformis* forest, a *Quercus wutaishanica* forest, and a mixed forest of *P. tabulaeformis* and *Q. wutaishanica*. The plots were laid out along the topography of the Xiaoshegou catchment area, at a distance of 3 km west of the research station. Five soil samples at 20 cm depth were taken using a 4-cm diameter soil auger to assess the soil condition of each forest plot in late August; these samples were then mixed and combined into a single sample after passing

through a 2 cm sieve screen to separate rocks and debris. Each composite soil sample was separated into two sub-samples. One sub-sampled was transported in an ice-cooled container to the Key Laboratory for Forest Resources & Ecosystem Processes of the Beijing Forestry University. Soil physicochemical properties were analyzed with air-dried soil of 20 g being ground and passed through a 0.18-mm sieve screen. SOC content was analyzed via the standard Mebius method (Nelson and Sommers 1982). TN content was analyzed following the Kjeldahl digestion procedure and ammonium nitrogen ($\text{NH}_4 - \text{N}$) was colorimetrically measured by the alkali method with a Tector Kjeltac 1025 Distilling system (Gallaher et al. 1976). The soil pH was measured in deionized H_2O with a water to soil ratio of 2.5:1 using the Sartorius AG method (PB-10, Sartorius, Germany). The water holding capacity (WHC) was determined by saturating 20 g fresh soil above a filter paper in a 10-cm glass funnel, and then permitting the water to drain for 4–6 h before being weighed ($n = 5$). The saturated soils were then oven-dried at 105 °C to a constant mass, until the WHC was equal to the percentage of water retained after several hours' drainage (for 100% WHC). Soil microbial biomass carbon and nitrogen contents were measured using the chloroform-fumigation method with a mean calibration factor k_C of 0.38 (Vance et al. 1987a, 1987b). Concurrently with soil sampling, soil bulk density was measured by dividing the mass of oven-dried soil (at 105 °C) by the cylinder volume (100 cm^3) after subtracting the detritus volume for each forest plot.

Incubation and measurement of SOC mineralization rate

The second sub-sampled soil was immediately (< 2 d) delivered in a cooler with blue ice to the Key Laboratory of Ecosystem Network Observation and Modeling, Chinese Academy of Sciences. The sub-sampled soil of each forest plot was divided into eighteen 40 g equivalent aliquots; the soil moisture content (SMC) of every six aliquots was individually adjusted to 30, 60 and 90% WHC. The moisture-adjusted soil samples were placed in 200 cm^3 plastic flasks with lids perforated to allow gas diffusion, and connected directly to the CO_2 measurement system. After getting static and equilibrated for 5 days, six soil samples separately at 30, 60 and 90% of WHC for each forest plot were respectively incubated for 387 days in a microcosm with temperatures of 5 °C, 10 °C, 15 °C, 20 °C, 25 °C and 30 °C and a stable air moisture content ($n = 5$ per incubator). To prevent anaerobiosis, the perforated hole of the flask lid was not sealed until 24 h before the measurement of soil organic carbon mineralization rate. Throughout the whole incubation period, the flask was periodically weighed and the required proportion of deionized water was added to maintain soil moisture content at 30%, or 60%, or 90% of WHC.

The measurement of SOC mineralization started at different time intervals after the equilibrated soil samples had been incubated in microcosms. The instantaneous rate of SOC mineralization was measured as CO_2 efflux from soil within flask on day 1, 3, 5, 8, 16, 23, 29, 36, 43, 57, 75, 118, 151, 180, 225, 252, 293, 361, 387 in sequence. Sixteen flasks of soil samples were placed in an electronic water bath at the same temperature as that at which the soil samples were incubated. These flasks were connected to a PRI-8800 Automatic Temperature Control Soil Flux System (PRI-8800; Pre-Eco, Beijing, China). This automated system was mainly composed of a Li-Cor CO_2 analyzer (Li-7000), an air-flow controller, soda-lime equipment to manipulate the initial CO_2 concentration, and a data collector. Comprehensive information and the schematic configuration of this automated system were introduced in detail by He et al. (2013). The SOC mineralization rate was estimated using the following equation:

$$R_s = (L \times V \times \alpha \times \beta) / m \quad (1)$$

where R_s represents the instantaneous rate of SOC mineralization ($\mu\text{g C}\cdot\text{g}^{-1}\text{ soil}\cdot\text{h}^{-1}$); L refers to the slope of the CO_2 concentration; V is the volume of the incubation flask and gas tube (cm^3); m is the dry weight of the soil sample (g); α is the transformation parameter of the CO_2 mass; β is the transformation coefficient of time.

Based on the SOC mineralization rate, the cumulative mineralized SOC for a specific period of time was also calculated following the empirical function below.

$$C_{cum} = 1/SOC_f \times \sum \{ (R_{si} + R_{s(i+1)}) \times (t_{i+1} - t_i) / 2 \} \quad (2)$$

where C_{cum} is the cumulative mineralized SOC ($\text{mg C}\cdot\text{g}^{-1}\text{ SOC}$); SOC_f refers to soil organic carbon content ($\text{g}\cdot\text{kg}^{-1}$) for the corresponding forest plot; R_{si} and $R_{s(i+1)}$ are the instantaneous rates of SOC mineralization measured consecutively at time i and $i + 1$, as calculated by Eq. (1).

Calculation of soil carbon fractions

Different soil carbon pools were estimated by the first-order kinetic one-compartment and two-compartment models based on the relationship of cumulative mineralized SOC and the time length of the incubation period. Firstly, the potentially mineralizable carbon pool in soil was calculated via fitting the mean cumulative mineralized carbon data at a different incubation temperature and moisture for each forest type to the following model (Sanford and Smith 1972; Rey and Jarvis 2006):

$$C_{cum}(t) = C_0 \times (1 - e^{-k_0 t}) \quad (3)$$

where $C_{cum}(t)$ is the mean cumulative mineralized SOC

until time t (mg C·g⁻¹ SOC), C_0 is the ‘potential’ mineralizable C (mg C·g⁻¹ SOC), k_0 is the decomposition rate constant for mineralization carbon (day⁻¹), and t is the incubation time (day).

Secondly, the first-order two-compartment model was applied to calculate labile carbon fraction and recalcitrant carbon fraction (Andr en and Paustian 1987; Rey and Jarvis 2006). This equation was displayed as below:

$$C_{cum}(t) = C_1 \times (1 - e^{-k_1 t}) + C_2 \times (1 - e^{-k_2 t}) \quad (4)$$

where $C_{cum}(t)$ is the mean cumulative carbon mineralized during the incubation period time t (mg C·g⁻¹ SOC), C_1 is the labile carbon fraction (mg C·g⁻¹ SOC), C_2 is the recalcitrant carbon fraction (mg C·g⁻¹ SOC), k_1 and k_2 are the first-order kinetic decomposition rate constants for the labile and recalcitrant carbon fractions (day⁻¹) respectively. The cumulative mineralized SOC (the emitted CO₂) is expressed on a basis of SOC content (mg C·g⁻¹ SOC) when fitting models (3) and (4) to the incubation data, and the modeled result essentially represents the SOC fraction that was mineralized. More detailed information about the law to employ these models may be found in Rasmussen et al. (2006) and Rey and Jarvis (2006).

In order to get the reasonable parameters from functions (3) and (4), several criteria were set up: that (i) k_0 and k_1 were larger than 0, and that (ii) k_1 was larger than k_2 , and that (iii) C_0 , C_1 and C_2 were larger than zero. Additionally, one assumption was followed that ($C_1 + C_2$) was equal to 1000 mg C·g⁻¹ SOC (i.e. the two carbon fractions add up to the total amount of initial organic carbon in the sample). Based on the functions (2), (3) and (4) and the estimations of different carbon pools, the quantity of mineralized recalcitrant carbon was assumed to be the difference between the total amount of mineralized carbon and that of C_0 (potential mineralizable C) or C_1 (labile carbon fraction). In the present study, C_0 was used to estimate the amount of mineralized recalcitrant carbon, as most C_1 could not meet the criterion (iii) at a moisture content of 30% for all forest types (Additional file 1).

Statistical analyses

The data presented in the tables and figures represent the mean value under different incubation conditions for each forest type. The differences of soil carbon pools for various incubation conditions and forest types were tested using one way ANOVA or multi-factor analysis of variance. Equations (3) and (4) were fitted using non-linear regression analysis (*nls* of R language). The starting value for each parameter was obtained from the published literature. Canonical correspondence analysis (CCA) was applied using the function ‘*cca*’ in the *vegan* package of R.

All of the statistical analyses were done using the software R3.4.0. The figures in this paper were compiled using SigmaPlot 10.0 and R software.

Results

Cumulative mineralized carbon

The cumulative carbon mineralized (C_{cum} , C respired per g initial SOC) over the incubation period (387 days) significantly varied with forest type, incubation temperature and soil moisture content, but no significant interaction effect was found on C_{cum} among these three variables (Table 1). Under the same incubation temperature, C_{cum} showed an increasing trend with SMC from 30 to 60 to 90% for each forest type; the forest of *Q. wutaishanica* had a higher C_{cum} value than those of the *P. tabulaeformis* forest and the mixed forest, under similar incubation conditions except at 5 °C; a parabolic correlation existed between C_{cum} and incubation temperature at the same SMC gradient for each forest type. The highest value of C_{cum} was 96.74, 109.20, and 100.08 mg C·g⁻¹ SOC respectively for the *P. tabulaeformis* forest, the *Q. wutaishanica* forest, and the mixed forest under an incubation condition of 15 °C and 90% SMC (Fig. 1; Additional file 2).

Potential mineralizable carbon

Potential mineralizable carbon (C_0) was calculated by fitting the first-order kinetic one-compartment model to the data of C_{cum} . The estimation of C_0 was lowest at 3.70 mg C·g⁻¹ SOC at 10 °C and 30% SMC, and highest at 4.51 mg C·g⁻¹ SOC under 15 °C and 90% SMC for the *P. tabulaeformis* forest, it varied from 3.60 mg C·g⁻¹ SOC (20 °C + 30% SMC) to 4.24 (25 °C + 90% SMC) mg C·g⁻¹ SOC for the *Q. wutaishanica* forest, and ranged from 3.59 mg C·g⁻¹ SOC (30 °C + 60% SMC) to 4.75 (5 °C + 30% SMC) mg C·g⁻¹ SOC for the mixed forest (Table 2). Although the results of multi-factor analysis of variance indicated that C_0 and its decomposition rate constant (k_0) were markedly influenced by forest type, incubation temperature and SMC, there was not a clear trend of C_0 changing with increasing temperature. SMC had a weak positive effect on C_0 (Table 3).

Table 1 Effects of temperature, moisture and forest type on cumulative mineralized carbon (C_{min})

Variable	Df	Sum Sq	Mean Sq	F	P
F	2	5989	2994	7.066	0.0011
M	2	87,270	43,635	102.98	<2e-16
T	5	15,736	3147	7.427	1.89e-06
F × M	4	3387	847	1.998	0.959
F × T	10	3683	368	0.869	0.563
M × T	10	5403	540	1.275	0.246
F × M × T	20	3041	152	0.359	0.995

F, M and T represent forest type, moisture content, and temperature, respectively

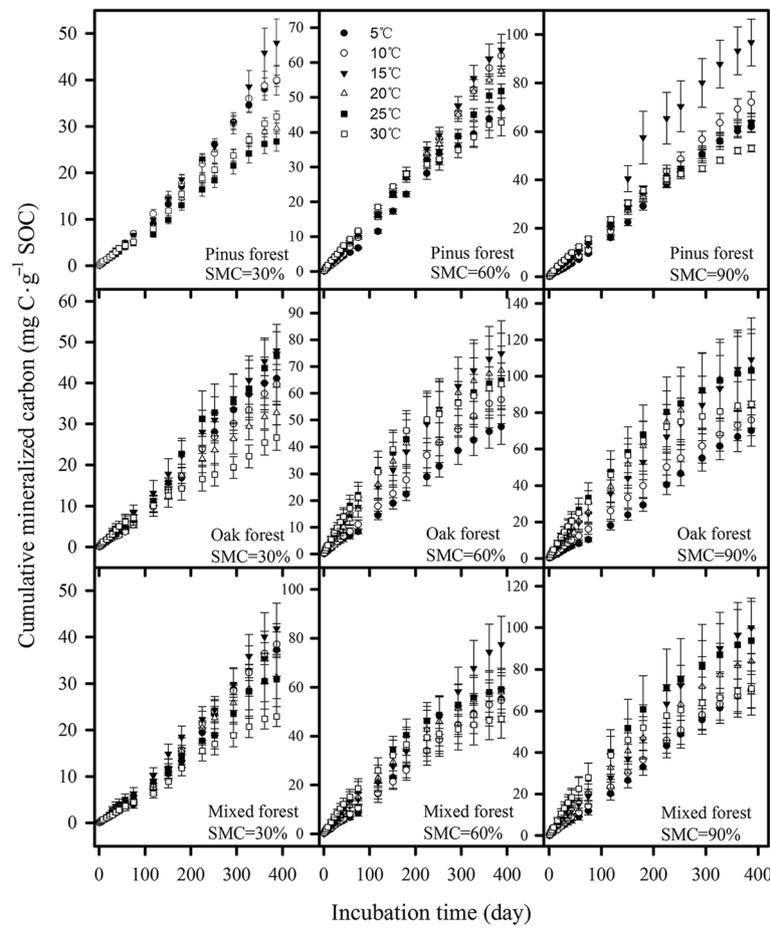


Fig. 1 Cumulative mineralized carbon under different temperature and soil moisture gradients for temperate forests

The decomposition rate constant (k_0) was prominently affected and significantly increased by SMC under the same temperature gradient, and kept increasing slightly with temperature under the same SMC for each forest type. Obviously, k_0 under 30 °C and 90% SMC condition was highest at 25.06×10^{-3} ,

33.93×10^{-3} and $32.04 \times 10^{-3} \text{ day}^{-1}$ for the *P. tabulaeformis* forest, the *Q. wutaishanica* forest, and the mixed forest, respectively (Table 4).

We only presented the data of C_1 , k_1 and k_2 at 60%, 90% SMC as supportive materials, since the first-order kinetic two-compartment model did not fit well the data

Table 2 Potential mineralizable soil carbon (C_0) obtained through fitting the model ($C_{cum}(t) = C_0 \times (1 - e^{-k_0 t})$) to cumulative mineralized carbon (C_{cum}) at temperature and moisture incubation conditions

Forest type	Moisture (%)	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C
Pinus	30	4.12 a	3.70 a	3.97 a	3.87 a	3.72 a	3.75 a
	60	3.95 a	4.15 a	4.08 ab	4.00 a	3.89 a	3.77 a
	90	4.22 a	4.26 a	4.51 b	4.08 a	4.09 a	3.95 a
Oak	30	3.86 a	4.11 a	4.04 a	3.60 a	4.06 a	3.34 a
	60	3.73 a	3.76 a	4.01 a	3.91 a	3.84 a	3.89 b
	90	4.10 a	4.06 a	4.23 a	4.22 a	4.24 a	4.14 b
Mixed	30	4.75 b	4.46 b	4.04 a	3.83 a	4.05 a	4.41 a
	60	3.92 a	3.86 a	4.06 a	3.88 a	3.90 a	3.59 a
	90	4.09 ab	4.07 ab	4.31 a	4.06 a	4.16 a	3.95 a

Variant lowercase letter means significant difference at $P < 0.05$ among moisture gradients ($n = 5$)

Table 3 The effects of temperature, moisture and forest type on potential mineralizable carbon (C_0)

Variable	Df	Sum Sq	Mean Sq	F	P
F	2	1.57	0.787	3.622	0.028
M	2	2.73	1.366	6.288	0.002
T	5	2.69	0.537	2.472	0.033
F × M	4	2.63	0.658	3.029	0.019
F × T	10	1.37	0.138	0.633	0.785
M × T	10	1.54	0.154	0.707	0.717
F × M × T	20	4.52	0.226	1.041	0.416

F, M and T represent forest type, moisture content, and temperature respectively

of C_{cum} at 30% SMC for all forest types. C_1 was on average lower than C_0 by one unit.

Contribution of recalcitrant carbon to the cumulative mineralized carbon

The amount of recalcitrant carbon mineralized during the incubation period was indirectly calculated from the cumulative mineralized carbon (C_{cum}) minus the potential mineralizable carbon (C_0). The value of mineralized recalcitrant carbon varied from 18.48 mg C·g⁻¹ SOC at 30 °C and 30% SMC for the mixed forest to 104.98 mg C·g⁻¹ SOC at 15 °C and 90% SMC for the oak forest (Table 5). Similar to C_{cum} and C_0 , the mineralized recalcitrant carbon was also greatly influenced by forest type, incubation temperature and SMC (Table 6).

The mineralized recalcitrant carbon took over more than 80% of cumulative mineralized carbon for all forest types, under any incubation condition. This percentage was significantly manipulated and markedly increased by SMC for each forest type and temperature gradient (Table 7).

Soil biophysical properties and their effects on carbon mineralization

Soil resource content and physicochemical properties were influenced by forest type: the *Q. wutaishanica* forest had higher values than the *P. tabulaeformis* forest in SOC, TN, but was lower in SBD (Table 8).

The results of the canonical correspondence analysis indicated that the recalcitrant carbon mineralization was affected jointly by forest type, SOC, SBD, SMBN and soil pH, and its 68.33% variance was mainly ascribed to these five factors ($P = 0.003$). SOC and SBD had a converse effect and clustered along the first axis explaining 50.42% of the total variance, and SMBN and pH along the second axis comprising 20.02% of the total variance. SBD played an important role in determining the recalcitrant carbon mineralization in the *P. tabulaeformis* forest, SOC and pH were important factors in the *Q. wutaishanica* forest, and SMBN and pH had a great function in the mixed forest (Fig. 2).

Discussion

The magnitude of SOC mineralization and its fraction

Although invariant correlations were not detected between the amount of mineralized SOC and some incubation variables, there existed a constant and gradually increasing trend of cumulative mineralized SOC with incubation time in the present study. The longer the incubation time period, the larger the amount of mineralized SOC. At the end of this incubation experiment, the broadleaved forest at 15 °C and 90% SMC was found to have the highest value of mineralized SOC at 109.20 mg C·g⁻¹ SOC, which was about four times higher than the lowest value of the mixed forest soil at 30 °C and 30% SMC. The total amount of the mineralized SOC in the present study averaged 58.37 mg C·g⁻¹ SOC across all incubation conditions, which was in agreement with other results of Rey et al. (2005, 2008)

Table 4 Decomposition constant ($k_0, \times 10^{-3}, \text{day}^{-1}$) obtained through fitting the model ($C_{cum}(t) = C_0 \times (1 - e^{-k_0 t})$) to cumulative mineralized carbon (C_{cum}) at temperature and moisture incubation conditions

Forest type	Moisture (%)	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C
Pinus	30	6.79 a	7.42 a	9.36 a	9.67 a	8.92 a	9.21 a
	60	10.59 ab	12.05 a	14.62 a	14.96 ab	16.26 ab	21.27 b
	90	11.90 b	14.04 a	15.40 a	19.36 b	20.43 b	25.06 b
Oak	30	6.89 a	6.72 a	10.14 a	10.08 a	8.80 a	12.85 a
	60	12.30 b	13.71 a	20.05 ab	19.85 ab	25.81 b	27.53 b
	90	11.68 b	16.18 a	24.71 b	25.43 b	29.31 b	33.93 b
Mixed	30	4.63 a	4.94 a	7.99 a	6.85 a	6.95 a	6.32 a
	60	10.87 b	12.83 b	15.56 ab	17.23 b	20.02 b	26.43 b
	90	13.56 b	15.64 b	18.99 b	22.08 b	25.91 b	32.04 b

Variant lowercase letter means significant difference at $P < 0.05$ among moisture gradients ($n = 5$)

Table 5 Mineralized recalcitrant carbon ($C_r = C_{cum} - C_0$) during the incubation period for three forest types under different incubation conditions

Forest type	Moisture (%)	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C
Pinus	30	35.68 a	36.37 a	44.05 a	25.72 a	23.12 a	28.33 a
	60	43.11 b	57.81 b	59.59 a	53.60 b	48.03 b	39.29 b
	90	57.87 c	67.87 b	92.29 b	59.23 b	59.79 b	49.29 c
Oak	30	37.25 a	35.55 a	43.88 a	29.10 a	42.60 a	23.31 a
	60	43.69 ab	53.85 ab	70.87 ab	64.54 ab	60.90 ab	59.44 b
	90	66.02 b	71.81 b	104.98 b	99.87 b	89.94 b	80.57 b
Mixed	30	32.50 a	33.94 a	37.77 a	27.56 a	26.94 a	18.48 a
	60	51.35 ab	50.65 ab	73.44 b	54.16 ab	55.29 ab	43.40 ab
	90	66.18 b	65.50 b	95.77 b	79.83 b	89.54 b	66.87 b

Variant lowercase letter means significant difference at $P < 0.05$ among moisture gradients ($n = 5$)

and of the permafrost peatland in northeast China (Wang et al. 2010). This mean value of total mineralized SOC also fell well within the range of mineralized carbon compiled by Hamdi et al. (2013). The results of the present study agree with other research results where SOC had been mineralized under similar incubation conditions.

The cumulative mineralized carbon had a curvilinear relationship with the incubation time, and was fitted to the first order kinetic one-compartment or two-compartment model to estimate the soil carbon fractions for each forest type. Only the estimates from the one-compartment model were presented in this study, as the two-compartment model did not fit well the data of cumulative carbon mineralized at 30% SMC. The potential mineralizable carbon (C_0) averaged $4.00 \text{ mg C}\cdot\text{g}^{-1} \text{ SOC}$, contributing on average 8.36% to the total mineralized carbon across all incubation conditions, and taking over 0.4% of soil carbon. Its decomposition constants (k_0) varied around $1.23 \times 10^{-2} \text{ day}^{-1}$ for all incubation conditions. As recalcitrant carbon was assumed to be SOC or cumulative mineralized carbon minus potential mineralizable carbon, it contributed more than 90% to the SOC pool and almost 99% to the total mineralized carbon. The lowest proportion of labile SOC was also found by the studies

of Yang et al. (2006), Rey and Jarvis (2006), but k_0 was slightly lower in the report of Rey and Jarvis (2006). This variation in k_0 value may be ascribed to the different moisture content during the incubation process.

The effects of temperature and moisture on SOC mineralization rate

Temperature and moisture content are two important variables manipulating the magnitude of SOC mineralization. When other factors, e.g. soil moisture and pH, are in optimal status, carbon mineralization often exponentially increases with temperature, and their correlation is frequently described by the Arrhenius function or the Michaelis-Menten model (Davidson et al. 2012). In this study, the cumulative mineralized carbon was significantly influenced by the incubation temperature, however, it reached the highest value at 15 °C under each moisture gradient for all forest types. In most cases, no significant difference was found in the total amount of mineralized carbon between 30 °C and 5 °C, except at 30 and 90% SMC for the *P. tabulaeformis* forest and at 30% SMC for the mixed forest.

The above discrepancy between the changing trend of cumulative mineralized carbon and incubation temperature was mainly attributed to the complexity of soil carbon fraction. In the present study, the labile carbon (C_0 or C_1) constituted a small amount of the SOC pool, and the larger proportion of mineralized carbon mainly derived from the recalcitrant carbon pool. The carbon pool mineralized during the first 60 days of the incubation period equaled the estimated labile carbon pool, and the instantaneous mineralization rate positively increased with temperature during this first incubation stage (≥ 70 days), and then the mineralization rate began decreasing and was lowest at 30 °C as the incubation time was longer than 70 days. The lower amount of cumulative mineralized carbon at 30 °C may be accounted for by the faster depletion of labile carbon

Table 6 Effects of temperature, moisture and forest type on mineralized recalcitrant carbon (C_r)

Variable	Df	Sum Sq	Mean Sq	F	P
F	2	5943	2971	7.154	0.001
M	2	86,705	43,352	104.38	<2e-16
T	5	15,476	3095	7.452	1.8e-06
F × M	4	3413	853	2.054	0.088
F × T	10	3608	361	0.869	0.563
M × T	10	5250	525	1.264	0.252
F × M × T	20	2906	145	0.35	0.996

F, M and T represent forest type, moisture content, and temperature respectively

Table 7 Contribution (%) of recalcitrant carbon (C_r) to cumulative mineralized carbon (C_{cum})

Forest type	Moisture (%)	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C
Pinus	30	89.70 a	90.67 a	91.49 a	86.72 a	85.83 a	88.18 a
	60	91.66 b	93.33 ab	93.61 b	93.21 b	92.72 b	91.39 b
	90	93.28 b	94.14 b	95.27 c	93.80 b	93.81 b	92.92 b
Oak	30	89.88 a	89.17 a	91.16 a	88.56 a	90.81 a	86.40 a
	60	91.56 ab	92.89 b	94.07 ab	93.21 b	93.19 ab	93.56 b
	90	93.68 b	94.41 b	95.49 b	95.17 b	95.23 b	94.68 b
Mixed	30	85.19 a	88.27 a	89.27 a	86.56 a	83.62 a	79.70 a
	60	92.08 ab	92.68 b	94.10 ab	92.45 ab	92.90 ab	91.42 ab
	90	93.80 b	93.82 b	95.40 b	94.41 b	94.83 b	93.81 b

Variant lowercase letter means significant difference at $P < 0.05$ among moisture gradients ($n = 5$)

during the prior incubation stage and the difficult availability of recalcitrant carbon at the late incubation stage. The reason for carbon mineralization keeping a higher value at 15 °C was mainly because the mean annual temperature fluctuates around 15 °C in this study area, and because microbial organisms will maintain a higher activity for a longer period at this temperature gradient.

The soil moisture content significantly increased carbon mineralization in the present incubation experiment. Unlike the effect of temperature, the highest moisture content, i.e. 90% SMC, led to the largest amount of cumulative mineralized carbon for all forest types. Soil moisture not only ameliorated the dormant status of microbes, but also increased the dissolution of organic or mineral resources, which in turn supplied a large quantity of substrate to microbial decomposition (Rey et al. 2005; Demoling et al. 2007). Although 60% SMC was suggested to be optimal for microbial activity (Howard and Howard 1993), the higher proportion of sandy particulate modified the aeration of incubating soil samples (Rasmussen et al. 2006), but still promoted the SOC mineralization at 90% SMC.

The effects of forest type and soil properties on SOC mineralization

Forest type is a great biotic manipulator in regulating the turnover of soil organic matter via the quantity and quality of litter input or through the modification of soil biophysical properties. Cellulose and lignin constitute the largest composition of forest ecosystem production, and are the primary determinants of SOM quality and

microbial decomposition (Rasmussen et al. 2006; Rey et al. 2008). Generally, the broadleaf forest has a litter production of high quality and quantity, which enhances soil physicochemical properties. In our study, the *Q. wutaishanica* forest had a higher SOC and TN content and lower soil bulk density than the *P. tabulaeformis* forest. Using the data of the measured soil characteristics and the cumulative mineralized carbon, the canonical correspondence analysis indicated that carbon mineralization was significantly influenced by SOC, soil pH, soil microbial biomass nitrogen, and soil bulk density. However, soil bulk density had a negative effect on carbon mineralization along the first CCA axis.

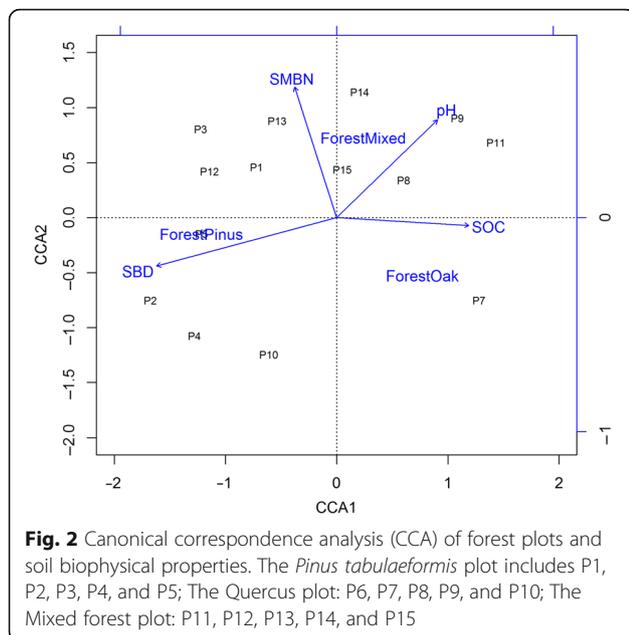
The proportion of labile and recalcitrant carbon specifies the quality of SOC, which determines the magnitude of SOC decomposition. The low quality SOC with more recalcitrant carbon needs a longer time to turnover (Leifeld and Führer 2005). Soil bulk density has a negative correlation with soil organic carbon content, and reflects the mineral soil percentage in the soil regime, the higher its value the slower the carbon mineralization. The change of soil microbial biomass nitrogen may represent the transition of microbial community composition and the substrate use strategy. After the depletion of labile carbon during the first weeks, microbial activity turned to be C-constrained (Demoling et al. 2007). The K-strategist bacteria decomposing recalcitrant carbon (Fontaine et al. 2003) or the fungi using N-rich SOM would become predominant in the microbial community during the late stage of the incubation period (Boberg et al. 2010). Soil pH not only

Table 8 Soil biophysical characteristics for three forest types

Forest type	SOC ($\text{g}\cdot\text{kg}^{-1}$)	TN ($\text{g}\cdot\text{kg}^{-1}$)	C:N	SMBC ($\text{mg}\cdot\text{g}^{-1}$ SOC)	SMBN ($\text{mg}\cdot\text{g}^{-1}$ TN)	pH	SBD ($\text{g}\cdot\text{cm}^{-3}$)
<i>P. tabulaeformis</i> forest	17.80 a	1.46 a	12.13 a	50.77 a	60.91 a	5.98 a	1.11 b
<i>Q. wutaishanica</i> forest	33.90 b	2.79 b	12.05 a	26.97 a	38.48 a	6.16 a	0.99 a
Mixed forest	28.63 ab	2.22 ab	12.66 a	36.63 a	59.75 a	5.96 a	0.94 a

Variant lowercase letter means significant difference at $P < 0.05$ among moisture gradients ($n = 5$)

SOC soil organic carbon, TN total nitrogen content, SMBC soil microbial biomass carbon, SMBN soil microbial biomass nitrogen, SBD soil bulk density



modulates the soil micro-environment and microbial community composition (Strickland and Rousk 2010), but also governs the breakdown processes of SOC decomposition (Riffaldi et al. 1996). The soil mineralization process including the adsorption of SOC by colloids and the substitution between different multivalent cations within organo-mineral complexes (Yuan et al. 2000; Rasmussen et al. 2006) is greatly influenced by soil pH, which ultimately controlled the availability of recalcitrant carbon in the late incubation stage of our experiment.

Conclusions

SOC mineralization was significantly influenced by forest type, incubation temperature and soil moisture content, and reached the highest value for the *Q. wutaishanica* forest under the incubation condition at 15 °C and 90% soil moisture content. The amount of mineralized SOC slightly increased with soil moisture content and had a parabolic relationship with the incubation temperature. The simulation results of the first-order kinetic two-compartment model indicated that more than 80% of mineralized SOC derived from the recalcitrant pool of soil organic matter for the temperate forests of northern China.

Additional files

Additional file 1: Labile carbon pool and decomposition constants estimated by a two-compartment model under different incubation conditions for three forest types. (DOC 54 kb)

Additional file 2: Raw data for mineralized SOC during a given incubation period under different conditions for three forest types. (DOCX 27 kb)

Abbreviations

C: Carbon; C_g : Potential mineralizable carbon; CCA: Canonical correspondence analysis; C_{cum} : Cumulative mineralized SOC; C_r : Mineralized recalcitrant carbon; DOC: Dissolved organic carbon; SBD: Soil bulk density; SMBC: Soil microbial biomass carbon content; SMBN: Soil microbial biomass nitrogen content; SMC: Soil moisture content; SOC: Soil organic carbon; TN: Total nitrogen content; WHC: Water holding capacity

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Availability of data and materials

All raw data are provided in the Additional files.

Authors' contributions

HZ carried out the field investigations, analyzed the soil samples in the laboratory, and performed the statistical analysis. ZZ designed the experiment and drafted this manuscript. Both authors agreed to this submission.

Competing interests

The authors declare that they have no competing interests.

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