



## Effects of biotic and abiotic factors on soil organic matter mineralization: Experiments and structural modeling analysis



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### ABSTRACT

Soil organic matter (SOM) mineralization is affected by various abiotic and biotic factors, as well as the input of exogenous organic substances. Our previous studies have shown that SOM mineralization in flooded rice paddies is lower than that in adjacent upland soils in subtropical agro-ecosystems. However, the main factors contributing to the differences in SOM mineralization remain unclear. To compare the effects of biotic and abiotic factors on SOM mineralization between upland and paddy soils, we incubated upland and paddy (flooded) soils with three low molecular weight organic substances (LMWOS, i.e., glucose, acetic acid, or oxalic acid) for 30 days under field conditions. Generally, the average CO<sub>2</sub> efflux from upland soil was higher than that in paddy soil with the same LMWOS addition. The total content of phospholipid fatty acids (PLFAs) in paddy soil was 2–5 times higher than that of upland soil, irrespective of the LMWOS added. Redundancy analyses indicated that microbial community composition was influenced mainly by the low redox potential (Eh) and dissolved organic carbon in paddy soil. Structural equation modeling revealed that, among abiotic factors, temperature exerted indirect effects on SOM mineralization by influencing biotic factors in both soils; Eh has a positive and direct effect on SOM mineralization in paddy soil. In terms of biotic factors, SOM mineralization in upland soil was mainly regulated by the quantity of bacteria. In paddy soil, SOM mineralization was largely influenced by the ratio of fungal to bacterial PLFAs and peroxidase activity.

### 1. Introduction

Soil organic matter (SOM) is particularly important for sustaining the productivity of agro-ecosystems [1], in which mineralization of SOM is regarded as an important process in regulating global C cycling [2]. Abiotic factors such as climate (e.g., temperature and precipitation) and soil physicochemical properties (e.g., soil moisture and aeration, etc.) are typically identified as key predictors of SOM mineralization. An exponential relationship has been observed between SOM mineralization and temperature [3–6]. Furthermore, changes in soil moisture can alter the temperature sensitivity of SOM mineralization [7]. High soil moisture levels limit oxygen availability, and thus contribute to a decrease in redox conditions [8], which leads to a decrease in SOM mineralization via effects on microbial activity and metabolism [9]. Biotic factors such as exoenzyme activity, microbial composition, and microbial activity also play important roles in regulating SOM

mineralization [10,11]. Exoenzyme activity influences SOM mineralization by regulating soil biochemical processes, including the formation and decomposition of labile organic substances. Bacteria and fungi generally comprise 90% of the total soil microbial biomass, and are responsible for the majority of SOM mineralization [12]. The biomass and ratio of bacteria and fungi are correlated with the microbial metabolic quotient, which reflects the carbon-use efficiency [13,14]. Overall, the exoenzymes are responsible for the hydrolysis and humification of SOM [15], whereas microbial composition and microbial activity determine the rate of carbon loss from the soil. To date, however, few studies have simultaneously considered both abiotic and biotic factors as predictors of SOM mineralization. Therefore, there is an urgent need to determine the direct and indirect contributions of individual factors and the key drivers of SOM mineralization.

Soils contain many types of LMWOS with distinct properties that can be utilized by microorganisms, e.g., glucose, acetic acid, and oxalic

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acid derived from root exudates, decomposition of SOM, organic fertilization, and microbial metabolites [16]. Previous studies have shown that microbial uptake and utilization of LMWOS-C in soil depends on the oxidation state of carbon [17]. Different types of LMWOS have been shown to determine the metabolic intensity and direction of LMWOS-C. The input of LMWOS stimulates microbial competition for use of exogenous carbon, which accelerates the consumption of soil oxygen. Changes in soil aeration can lead to variations in microbial biomass, community structure, and activity [18–20]. Some microbial taxa preferentially metabolize LMWOS-C compounds and respond on a community level to inputs of LMWOS-C substrates (e.g., increase in relative abundance) in a predictable manner [21,22]. Input of LMWOS stimulates changes of microbial composition and activity [23,24], and enables investigation of biotic effects.

In general, SOM mineralization has mainly been investigated in forests [13,25], grasslands [26,27], and dry land systems [28,29]. In China, paddy fields account for approximately 26% of farmlands, and are primarily distributed in subtropical regions [30]. Within the same geomorphic unit, the organic carbon content of flooded paddy soils is higher than that of upland soils, whereas paddy soils exhibit lower mineralization rates [31]. The differences between these two soil habitats could be a key factor in the variations in SOM mineralization rates. Compared with upland soil, paddy soil generally maintains a higher level of soil moisture, lower Eh, higher microbial biomass, and distinct microbial communities [32,33]. Although several studies have explored the effect of these factors on SOM mineralization in upland soils [10,29], it remains unclear how and which abiotic and biotic factors lead to low SOM mineralization in flooded paddy soil [33–35]. Structural equation modeling (SEM) effectively distinguishes specific cause-and-effect relationships between observed variables, and is the preferred method for screening the key factors that determine SOM mineralization in soils. Thus, the objective of the present study was to compare the effects of biotic and abiotic factors on SOM mineralization between paddy and upland soils. In a field experiment, LMWOS (glucose, acetic acid, and oxalic acid—the common components of root exudates) were added to soil, which was subsequently incubated for 30 days. Structural equation modeling was used to separate the biotic and abiotic effects on CO<sub>2</sub> efflux depending on the dynamics of microbial biomass properties and environmental conditions.

## 2. Material and methods

### 2.1. Soil sampling and preparation

During the fallow season, surface soils (0–15 cm) were collected from an upland field (29°15′49.7″N, 111°31′57.5″E) and a paddy field (29°15′22.0″N, 111°31′38.1″E) in Pantang, Hunan Province, China. These fields have been under tillage for at least 30 years. The upland field was under crop rotation with cotton and canola, whereas the paddy field was under monocropping with rice (drained in the fallow season). Fresh soils were sieved (< 2 mm) and mixed. After removal of the visible roots, plant residues, and rocks, the soil was subsequently stored at 4 °C prior to incubation experiments. The basic soil properties are listed in Table 1.

**Table 1**  
Basic properties of upland and paddy soils.

	SOM (g C kg <sup>-1</sup> )	TN (g N kg <sup>-1</sup> )	C/N	pH	DOM (mg C kg <sup>-1</sup> )	MBC (mg C kg <sup>-1</sup> )
Upland soil	10.1	0.9	10.6	4.81	22.6	140.7
Paddy soil	16.3	1.6	10.4	5.14	14.3	455.8

Note: SOM, soil organic matter; TN, total nitrogen; DOM, dissolved organic matter; MBC, microbial biomass carbon.

### 2.2. Experimental design and soil incubation

Four treatments were applied to upland and paddy soils: the addition of each of the three types of LMWOS, i.e., glucose, acetic acid, oxalic acid, and a control treatment that did not contain any exogenous LMWOS. The rationale for choosing these substances was based on the following considerations: (1) Carbohydrates and organic acids are relatively abundant LMWOS of root exudates [16], and (2) acetic acid and oxalic acid contain different numbers of chemical functional groups (one or two –COOH groups), which could affect microbial activity and SOM mineralization [17]. Each LMWOS was added to the soil to simulate root exudates.

The experiment was conducted under field conditions. Sampled soil (equivalent of 200 g dry soil) was weighted and added to each polyvinylchloride (PVC) tube (hereafter referred to as ‘soil columns’: 20 cm height, 5 cm diameter; the thickness of soil in PVC tube was approximately 8 cm). During the pre-incubation (2 weeks) and incubation (30 days [d]) periods, the water-holding capacity was gravimetrically controlled at approximately 40% in upland soil, whereas the paddy soil was maintained under flooding conditions (with 3 cm of water) by supplementation with distilled water. At the beginning of incubation, 1 ml of LMWOS solution was injected into each column with a syringe. The injection of each LMWOS solution was performed at 5 vertical points in each soil column (approximately every 1.5 cm), with each injection point receiving 0.2 ml of solution. The amount of applied LMWOS was equal to 20 mg of C. Subsequently, 5 mg of N was added (in the same manner described above) as an (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution to all the treatments for microbial growth. A plastic cover was used to shield the soil from rainfall during the 30 d of the experiment. At 0.25, 2, 10, and 30 d of incubation, the redox potential (Eh) of paddy soil was measured by inserting the probe of an InLab Redox sensor (Mettler Toledo Columbus, OH, USA) to a depth of 4–5 cm below the soil surface, and the Eh (mV) was recorded until the value was stable. Next, the water layer on paddy soil samples was removed using syringes, and separate columns for upland and paddy soils were destructively sampled. Each soil sample was divided into three subparts for the measurement of different soil properties: (1) The first subpart was used to analyze microbial biomass C (MBC) within 24 h (2) The second subpart was frozen (–20 °C) to analyze enzyme activity and microbial PLFAs. (3) The third subpart was air-dried for pH measurement.

### 2.3. CO<sub>2</sub> efflux

The CO<sub>2</sub> efflux from each treatment was analyzed at 0.25, 0.5, 1, 2, 5, 10, 15, 20, 25, and 30 d after addition of LMWOS (i.e., glucose, acetic acid, or oxalic acid). CO<sub>2</sub> efflux was determined using static chamber chromatography. Briefly, CO<sub>2</sub> gas was sampled between 9:00 and 11:00 AM on each sampling day. Two gas samples were collected from the static chamber using a 30-ml syringe at 0 and 30 min after closing of the chamber, and were subsequently injected into 12-ml pre-evacuated vacuum bottles fitted with butyl-rubber lids. The ambient temperature in the columns was measured using a thermocouple (JM624; Tianjin Jinming Instrument Co., Ltd., Tianjin, China). The CO<sub>2</sub> concentrations was analyzed using a gas chromatograph (Agilent 7890A; Agilent Technologies, Santa Clara, CA, USA), equipped with a flame ionization detector for CO<sub>2</sub> analyses at 250 °C, the CO<sub>2</sub> efflux ( $F$ ) (mg kg<sup>-1</sup> d<sup>-1</sup>) was calculated as follows:

$$F = C \times M \times V / (V_0 \times (T_0 + T) / T_0) / m / t, \quad (1)$$

where  $C$  is the concentration of CO<sub>2</sub> determined by gas chromatography (mg L<sup>-1</sup>);  $M$  is the molecular weight of CO<sub>2</sub> (44 g mol<sup>-1</sup>);  $V$  is the volume of the static chamber (L);  $V_0$  is the molar volume of gas in standard state (22.4 L mol<sup>-1</sup>);  $T_0$  is the temperature in standard state (273 K);  $T$  is the ambient temperature (°C);  $m$  is the weight of dry soil incubated in the column (kg); and  $t$  is the sampling time (d).

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