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Weaker priming and mineralisation of low molecular weight organic substances in paddy than in upland soil

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ABSTRACT

Although soil organic matter (SOM) and microbial biomass pools in flooded paddy soils are generally larger than they are in upland soils, the processes (i.e., slower mineralisation, other types of C stabilization, and a negative priming effect) underlying higher SOM stocks in paddy soil are unclear. To elucidate these processes, three ^{13}C labelled low molecular weight organic substances (^{13}C-LMWOS) (i.e., glucose, acetic acid, and oxalic acid) were incubated in upland and paddy soils under simulated field conditions. Within 30 days of incubation, acetic acid exhibited the highest mineralisation in both soils. The amount of mineralisation of glucose in upland soil was higher than that of oxalic acid ($p < 0.05$), whereas the opposite was observed for paddy soil. Mineralisation of all three LMWOS was lower in paddy soil than that in upland soil ($p < 0.05$), illustrating that the molecular structure of the LMWOS as well as soil management determined the mineralisation rate. The priming effect evoked by oxalic acid and glucose was lower in paddy than in upland soil ($p < 0.05$). Therefore, the generally weaker mineralisation and priming effect of LMWOS observed in paddy soil contributed to higher carbon accumulation than they did in upland soil. Priming effect was positively correlated with fungal abundance, which was lower in paddy soil than in upland soil. Thus, slow organic C turnover in paddy soil is partly attributed to the suppression of fungal activity by flooding.

1. Introduction

Terrestrial ecosystems play an important role in the global carbon (C) cycle. Low molecular weight organic substances (LMWOS), e.g., sugars, carboxylic acids, and amino acids, are derived from root exudates [1,2], leached litter products [3,4], microbial residues, and metabolic products [5]. The rapid mineralisation and turnover of LMWOS appears to dominate the total CO_2 emission of soil, despite their low concentration of these substances [4,6]. The mineralisation rates of LMWOS are generally very fast, ranging from minutes to days [7–9]. For example, in one study [10], 50% of glucose-C was observed to have been released as CO_2 within 20 days (d) in grassland soil, and more than 50% of applied ^{13}C amino acids (alanine and glutamate) were observed to have been mineralised after 10 d in an arable soil in another study [11]. Mineralisation is LMWOS-specific, e.g., a higher proportion of amino acids (19.4% of the total ^{14}C added) than of glucose (14%) are mineralised to CO_2 within 2 d in arctic tundra soil [12]. Moreover, C in a −COOH group oxidizes to CO_2 faster than C in a $-CH_3$ group [7]. Thus, the $-CH_3$ group contributes more to the formation of soil organic matter (SOM) than does the −COOH group. In short, the chemical nature of LMWOS largely

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determines their mineralisation processes in soil [9].

LMWOS may evoke the priming effect, which could be positive or negative [13,14]. Microbial activation by LMWOS is one of the causes of the priming effect, where positive priming could result from microbial growth and the concomitant increased production of enzymes that degrade SOM [15,16]. Nobili et al. [17] suggested that some microorganisms invest low amounts of energy in maintaining a cellular state of "metabolic alertness", and they react more rapidly to substrates than to dormant cells. The input of LMWOS in soil accelerates the turnover of bacteria, especially that of *r*-strategists, thus triggering a positive priming effect [18]. Some microbial groups that preferentially utilize poorly available substrates from bacterial necromass remain alive after the exhaustion of easily available organics [19]. These organisms are considered to be *K*-strategists [20], which are stimulated by moribund bacteria and their lysates, thereby continuing to promote the decomposition of SOM and the positive priming effect. However, sometimes the exhaustion of microbially available substrates and the subsequent decrease in enzymatic activities can lead to negative priming [21,22].

In China, paddy fields account for approximately 26% of the farmlands and are primarily distributed in subtropical regions [23]. Under the same geomorphic units and climatic conditions, organic C content in surface-flooded paddy soil is greater than it is in upland soil [24]. In comparison to upland agro-ecosystems, flooded paddy ecosystems have specific physical and chemical soil properties and associated microbial communities [25]. Compared with upland soil, the processes—e.g., higher organic carbon input, slower mineralisation, other types of C stabilization, slower turnover, and negative priming effect—that lead to higher SOM stocks in paddy soil are unclear. The objective of this study was to distinguish the mineralisation and priming effects of three ^{13}C-LMWOS (glucose, acetic acid, and oxalic acid) in upland and paddy soils based on a simulated field experiment. The working hypotheses for this study were (1) the mineralisation differs among the three ^{13}C-LMWOS owing to their discrepancies in the types and numbers of their functional groups and microbial utilization [7,11]. (2) The slower turnover rate of SOM in anaerobic paddy soil will lead to a lower proportion of mineralised ^{13}C-LMWOS in paddy soil than that in upland soil, thus resulting in the accumulation of SOM in paddy soil [25]. (3) Lower microbial metabolic quotient (qCO_2, the ratio of CO_2 production per unit microbial biomass C) in paddy soil leads to a weaker priming effect than that in upland soil [26–29].

2. Materials and methods

2.1. Soil sampling and preparation

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

2.2. Experimental design and soil incubations

Four treatments were applied to upland and paddy soils: the addition of each of the three ^{13}C-labelled LMWOS—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. (2) Acetic and oxalic acids contain different numbers of chemical functional groups (i.e., one or two −COOH, respectively), which could affect microbial activity and SOM mineralisation. All three ^{13}C-labelled substances, i.e., ^{13}C-glucose (U-^{13}C, 99 atom%), ^{13}C-acetic acid (1, 2−^{13}C2, 99 atom%), and ^{13}C-oxalic acid (1, 2−^{13}C2, 99 atom%), were purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA), and were mixed with their corresponding unlabelled substances, resulting in 5.1 atom% ^{13}C of glucose, 4.46 atom% ^{13}C of acetic acid, and 6.01 atom% ^{13}C of oxalic acid.

Experiments were conducted under open-air field conditions. A certain amount of sampled soil (equivalent to 200 g dry soil) was weighed and added to each polyvinylchloride (PVC) tube (hereafter referred to as 'soil columns': 20 cm height × 5 cm diameters; the thickness of soil in PVC tube was approximately 8 cm). During the pre-incubation (2 weeks) and incubation (30 d) periods, the water-holding capacity was gravimetrically controlled at approximately 40% in upland soil, while the paddy soil was maintained under flooding conditions (with 3 cm water) by supplementing with distilled water. At the beginning of incubation, 1 mL of ^{13}C-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 tracer was equal to 20 mg C (about 20% of the C stored within the microbial biomass in paddy soil) [30]. Subsequently, 5 mg N was added as an $(NH_4)_2SO_4$ solution to all the treatments for microbial growth. A plastic cover was used to prevent rainfall for the 30 d of experiments. At 0.25, 0.5, 1, 2, 5, 10, 20, and 30 d of incubation, redox potential (Eh) in 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 two subparts for the measurement of different properties: (1) one subpart was used to analyse microbial biomass C (MBC) within 24 h, and (2) the other subpart was stored at −80 °C to analyse microbial abundance and the microbial community.

2.3. CO_2 flux

The flux and the ^{13}C atom% of the released CO_2 were analysed at 0, 0.25, 0.5, 1, 2, 5, 10, 15, 20, 25, and 30 d after the addition of the LMWOS (i.e., glucose, acetic acid, and oxalic acid). CO_2 flux was determined using the static chamber chromatography method [31]. Briefly, CO_2 gas was sampled between 9:00 and 11:00 a.m. on each sampling day. Prior to sampling, the concentration and ^{13}C atom% of CO_2 in each column were maintained at ambient levels. Then, CO_2 in the ambient air was taken as a background value (0 min). After sealing the column for 30 min within a chamber (the volume of the chamber was approximately 300 mL), two 30 mL samples were collected from each chamber using a syringe and were subsequently injected into separate pre-evacuated 12-mL vacuum bottles fitted with butyl-rubber lids to measure CO_2 concentrations and δ^{13}C values. CO_2 concentration was analysed using a gas chromatograph (Agilent 7890A; Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector for CO_2 analyses at 250 °C. The δ^{13}C value of CO_2 was determined using an ultra high-resolution isotope ratio mass spectrometer (MAT 253; Thermo Scientific, Waltham, MA, USA). The CO_2 flux (F) (mg d^{-1} kg^{-1} soil) was calculated as follows:

Table 1
Basic properties of upland and paddy soils.

	SOC, g kg^{-1}	TN, g kg^{-1}	SOC/TN	pH	DOC, mg kg^{-1}	MBC, mg kg^{-1}	Sand, %	Silt, %	Clay, %
Upland soil	10.05	0.95	10.58	4.81	22.60	140.7	15.69	58.53	25.78
Paddy soil	16.29	1.56	10.44	5.14	14.25	455.8	6.31	63.07	30.62

Note: SOC, soil organic carbon; TN, total nitrogen; DOC, dissolved organic carbon; MBC, microbial biomass C.

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

where C is the concentration of CO_2 determined by gas chromatography (mg L^{-1}); M is the molecular weight of CO_2 (44 g mol^{-1}); V is the volume of the static chamber (L); V_0 is the molar volume of gas in standard state (22.4 L mol^{-1}); T_0 is the temperature of 0 °C 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 sampling time (d).

2.4. Analysis of carbon in soil and microbial biomass

MBC was determined by applying the fumigation-extraction technique [32] and was calculated as the difference between organic C in fumigated and unfumigated samples with 0.45 as a correction factor. Briefly, 20 g of fresh soil was placed into a plastic vial and exposed to chloroform for 24 h. After the complete removal of chloroform, soil MBC was extracted with 80 mL of 0.05 M K_2SO_4. Organic C was measured using a high-temperature combustion total organic C analyser (Phoenix 8000; Teldyne Tekmar, Mason, OH, USA). In addition, the liquid samples extracted from fumigated and unfumigated soil were freeze-dried, and $\delta^{13}C$ values of the freeze-dried samples were determined using an ultra high-resolution isotope ratio mass spectrometer (MAT 253; Thermo Scientific, Waltham, MA, USA).

2.5. Quantitative PCR

qPCR was performed using the primers 1369f (5′-CGGTGAA-TACGTTCYCGG-3′) and 1492r (5′-GGWTACCTT-GTTACGACT-3′) [33], and NS1 (5′-ATTCCCCGTTACCCGTTG-3′), and Fung (5′-GTAGTCATATGCTTGTCTC-3′) to quantify bacterial 16S and fungal 18S rDNA, respectively [34]. qPCR conditions were as follows: one cycle at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s and 60 °C for 30 s. A melting cycle was added to the end of amplification at a temperature based on the melting curve from 60 °C to 95 °C for both genes. Threshold cycle (Ct) values were determined using 7900HT system SDS software 2.3 (Applied Biosystems, Foster City, CA, USA). Each sample was repeated in triplicate, and the mean values were used for the subsequent analyses. Standard curves were prepared using serial dilutions of positive plasmids containing 16S or 18S rDNA genes. Amplification efficiency was >90%, and R^2 was >0.99 for all bacterial and fungal calibration curves. Standard curves, negative controls, and soil DNA samples were amplified in a single 384-well plate. The composition of the microbial community was assessed as the relative abundance of fungi to bacteria [35].

2.6. Carbon dynamic calculations

To calculate the contribution of the added LMWOS to CO_2 evolution and MBC, a two end-member mixing model was used based on equation (2), where the C content of the soil in equation (3) was substituted according to equation (2):

$$C_{total} = C_{LMWOS} + C_{soil} \quad (2)$$

where C_{total}, C_{LMWOS} and C_{soil} represent the total C that was captured from each jar, from the LMWOS and from the soil, respectively.

Regarding isotopes, equation (3) can be rearranged in terms of ^{13}C as follows:

$$^{13}F_{total} \times C_{total} = {}^{13}F_{LMWOS} \times C_{LMWOS} + {}^{13}F_{soil} \times C_{soil} \quad (3)$$

where $^{13}F_{total}$, $^{13}F_{LMWOS}$ and $^{13}F_{soil}$ represent the ^{13}C atom % of C of each jar, of the LMWOS and of the soil, respectively.

Priming effect was calculated based on the experimental data as follows:

$$\text{Priming effect} = \text{T-}CO_2 - \text{C-}CO_2 - \text{L-}CO_2 \quad (4)$$

where T-CO_2 is the total CO_2-C from both soils amended with a labelled LMWOS (mg C kg^{-1} soil); C-CO_2 is the CO_2-C derived from the control (mg C kg^{-1} soil); and L-CO_2 is the CO_2-C derived from the added organic substance (mg C kg^{-1} soil).

The microbial metabolic quotient (qCO_2) is the ratio of CO_2 production per unit MBC:

$$qCO_2 = CO_2 /MBC \quad (5)$$

where CO_2 is the CO_2 emission rate, MBC is the microbial biomass C determined by fumigation extraction.

2.7. Statistical analysis

Means and standard errors were calculated in Microsoft Excel 2007. Paired-sample *t*-test was performed for each LMWOS between upland and paddy soils. A one-way ANOVA (Tukey's HSD) was used to assess the effects of treatments on cumulative CO_2 evolution, MBC, and the priming effect at each sampling time for each soil. A two-way ANOVA was performed to assess the effects of two factors (i.e., soil management and LMWOS type) on the cumulative CO_2 evolution, LMWOS mineralisation, and priming effect. Spearman's correlation coefficients were calculated to quantify the relationships between microbial metabolic quotient (qCO_2), microbial abundance, CO_2 flux, and the priming effect in both soils. Statistical analyses were carried out using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA), and a $p < 0.05$ was considered the upper limit for statistical confidence.

3. Results

3.1. CO_2 evolution

The proportion of mineralisation of the three LMWOS in flooded paddy soil was lower than that of upland soil ($p < 0.05$; Fig. 1a and b). In comparison with the control soil, the addition of glucose, acetic acid, and oxalic acid ($p < 0.05$) increased cumulative CO_2 evolution from the soil by 54%, 79%, and 81% in upland soil,

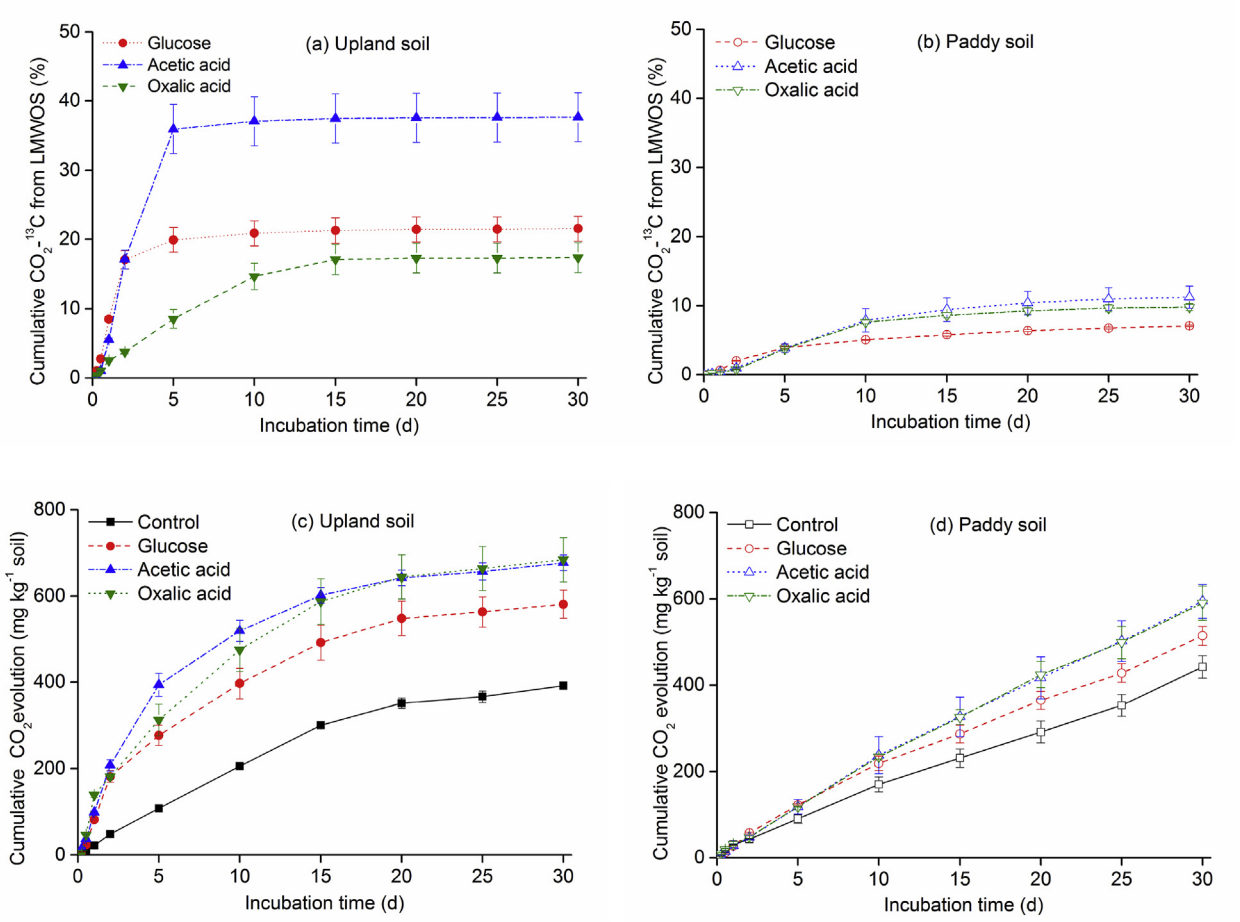

Fig. 1. Cumulative CO_2-^{13}C from exogenous organic carbon (%) (a, b) and cumulative CO_2 evolution (c, d) after low molecular weight organic substances addition in upland and paddy soils. Error bars indicate one standard error, values are means ± standard deviations (n = 3).

respectively (Fig. 1c), and by 21%, 42%, and 41% in paddy soil, respectively (Fig. 1d). No significant differences in cumulative CO_2 evolution were observed between acetic acid and oxalic acid addition treatments. However, cumulative CO_2 evolution from both soils was higher with acetic acid and oxalic acid addition than that with the glucose addition ($p < 0.05$). Cumulative CO_2 evolution from upland soil was higher after adding glucose, acetic acid, and oxalic acid ($p < 0.05$) than it was in paddy soil by 32%, 31%, and 33%, respectively.

3.2. Priming effect

In upland soil, the positive priming effect increased sharply and peaked at 3–5 d before quickly decreasing, whereupon it became negative at 20–30 d (Fig. 2a). In paddy soil with acetic and oxalic acid, the priming effect was negative during the first 6 h of incubation, after which it became positive until the end of the incubation period. Regarding the addition of glucose, the priming effect became negative after 25 d of incubation (Fig. 2b). The priming effects with glucose and oxalic acid additions in upland soil were approximately 3 and 1.5–2 folds higher than in paddy soil, respectively ($p < 0.05$; Fig. 2c).

3.3. Dynamics of microbial biomass and carbon use efficiency

In upland soil, the percentages of ^{13}C incorporated microbial biomass (^{13}C-MB) after the addition of glucose (16.0% of LMWOS-^{13}C) or acetic acid (14.5% of LMWOS-^{13}C) was higher ($p < 0.05$; Fig. 3c) than that after oxalic acid (7.8% of LMWOS-^{13}C) at the end of the 30 d incubation. In paddy soil, the percentages of ^{13}C-MB differed ($p < 0.05$; Fig. 3d) depending on the types of LMWOS (22.3%, 13.8%, and 2.7% of ^{13}C-MB in response to glucose-^{13}C, acetic acid-^{13}C, and oxalic acid-^{13}C, respectively). In general, the abundance of bacteria from paddy soil was higher than that in upland soil, while the abundance of fungi exhibited the opposite trend ($p < 0.05$; Fig. 4). As shown in Table 2, MBC was negatively correlated with the ratio of the abundance of 18S rDNA genes to the abundance of 16S rDNA genes ($p < 0.05$), while the priming effect was positively correlated with the gene abundance of fungi ($p < 0.05$).

4. Discussion

4.1. Temporal dynamics and cumulative mineralisation of LMWOS

The mineralisation of acetic acid was higher than that of glucose in both soils (Fig. 1a and b). Previous studies have shown that differences in the metabolic pathways and chemical structure of LMWOS led to different mineralisation rates [11,36]. Carboxylic acids are more completely converted to CO_2 owing to the oxidation of a higher proportion of carboxylic acid C into the citric acid cycle than of glucose entering glycolysis and pentose phosphate pathways [37], especially under anaerobic conditions. In our study, after 30 d incubation, the difference in ^{13}C-MB between the soils, to which LMWOS had been added, may be due to the discrepancy of microbial metabolic pathways. The microbial carbon use efficiency

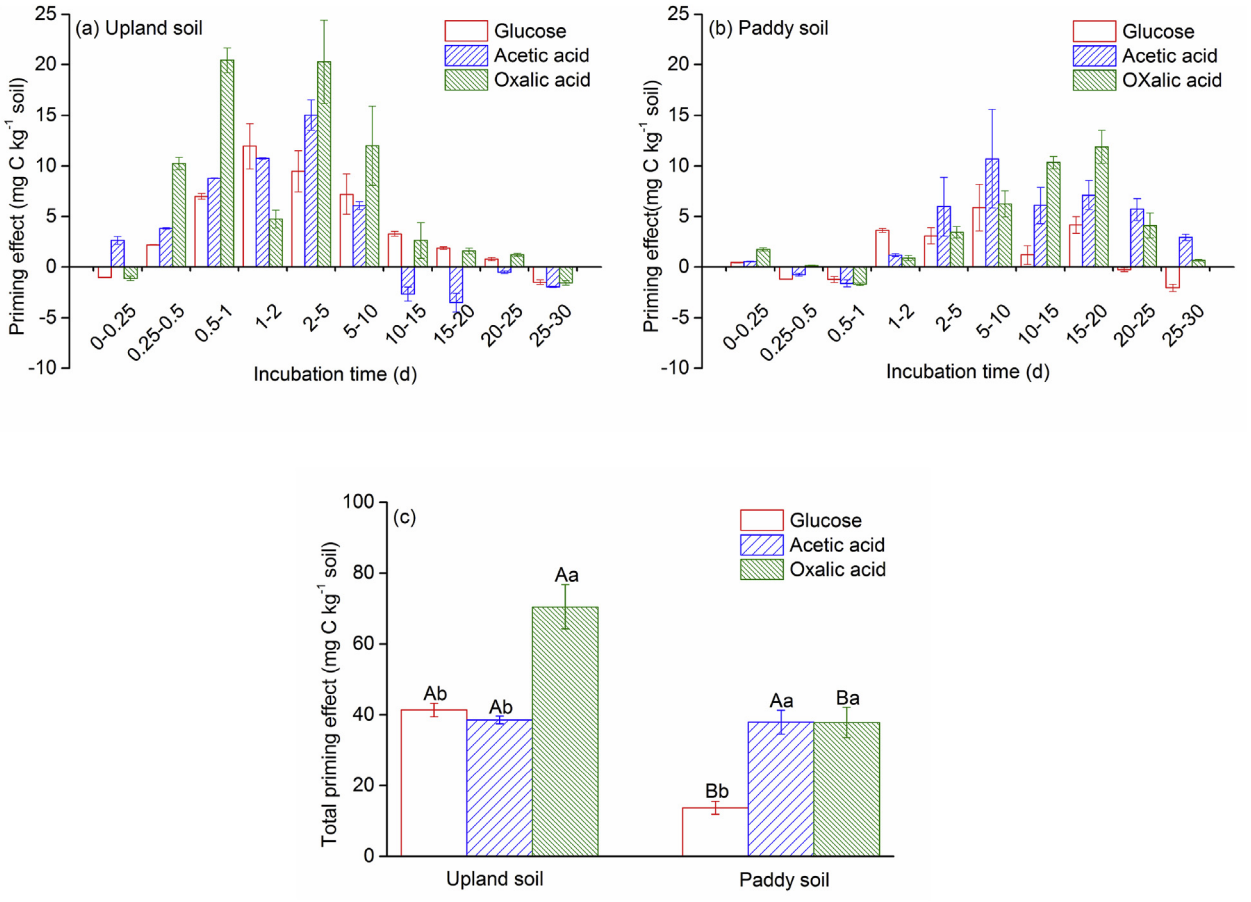

Fig. 2. Priming effect dynamics (a, b) and total priming effect (c) during incubation period after low molecular weight organic substances (LMWOS) addition in upland and paddy soils. Different uppercase and lowercase letters represent significant differences between both soils with the same LMWOS addition and different LMWOS addition within the same soil, respectively, at $p < 0.05$. Error bars indicate one standard error, values are means ± standard deviations (n = 3).

can be predetermined based on the molecular structure of an LMWOS. For example, microbes prefer to use C from a $-CH_3$ group for protoplasm synthesis, whereas C from a $-COOH$ group can be converted to CO_2 faster [7]. Therefore, the proportion of ^{13}C from acetic acid incorporated into microbial biomass was higher than that from oxalic acid. Microbial alanine transformation pathways convert the C1 atom ($-COOH$) to CO_2, whereas C2 and C3 remain bonded for either fatty acid synthesis or entry into the citric acid cycle [38]. Although oxalic acid is a di-carboxylic acid, the differences in C atom positions may affect the rate at which $-COOH$ decomposes to CO_2, and it could explain the faster mineralisation of carboxylic acids in upland soils.

The mineralisation of LMWOS [39] and SOM are also affected by soil characteristics (e.g., pH, soil texture and soil nutrients) [36,40]. In particular, soil pH may play a role in CO_2 emission. The lower pH in upland soil (Table 1) accelerates decarboxylation thus promoting the production of CO_2 with the addition of carboxylic acid [7].

4.2. CO_2 evolution

Cumulative CO_2 evolution with the addition of carboxylic acids (acetic acid and oxalic acid) was higher than that of the glucose addition in both soils (Fig. 1c and d), reflecting the differences in the microbial utilization and metabolism of the three substances [15]. Microbial activity slows under anaerobic condition [41], which was observed in this study with the lower qCO_2 in paddy soil in comparison to upland soil (Fig. A3). As shown in Fig. A1, at the beginning of incubation, the Eh for all treatments was below −50 mv, and values remained at −250 mv after 10 d of incubation. This is a reflection of the reductive or anaerobic condition in paddy soil. Under anaerobic condition, depolymerization relies primarily on hydrolytic enzymes, and fermenting microorganisms compete for lower-molecular weight products. Because hydrolysis is restricted to a limited set of chemical bonds, depolymerization is often regarded as the rate-limiting step in SOM decomposition under anaerobic conditions [42]. As a result, the concentration of mineralised LMWOS in flooded paddy soil was lower than it was in upland soil. Anaerobic microbes outcompete aerobic organisms under submerged condition with low O_2 availability, and the final mineralisation products are not completely oxidized to CO_2, thus leading to a decrease in the total CO_2 emission. Moreover, compared with upland soil, the flooded conditions in paddy soil leads to a high partial pressure of CO_2 (pCO_2) and a low CO_2 diffusion rate [43].

4.3. Temporal dynamics of the priming effect

During the incubation period, the addition of LMWOS accelerated the total CO_2 evolution in comparison to the control soil. The addition of LMWOS supplied C and energy to microbes and promoted the acceleration of LMWOS mineralisation and the breakdown of native SOM [44]. The priming effect in upland soil changed sharply and became stronger than that of flooded paddy soil (Fig. 2). At the start of incubation, nearly all LMWOS induced a

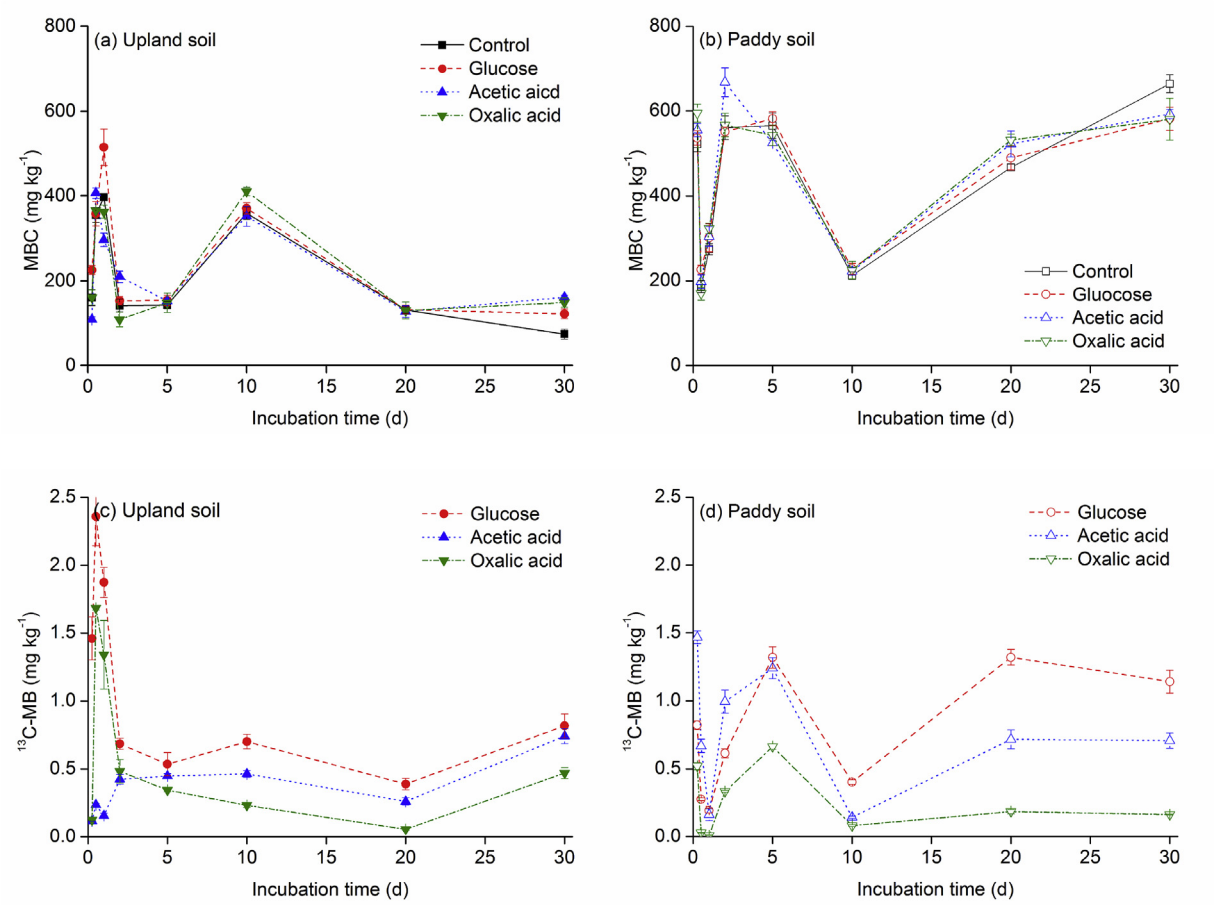

Fig. 3. Changes of microbial biomass C (MBC) (a, b) and ^{13}C-MB (c, d) in upland and paddy soils after different low molecular weight organic substances addition. Error bars indicate one standard error, values are means ± standard deviations (n = 3).

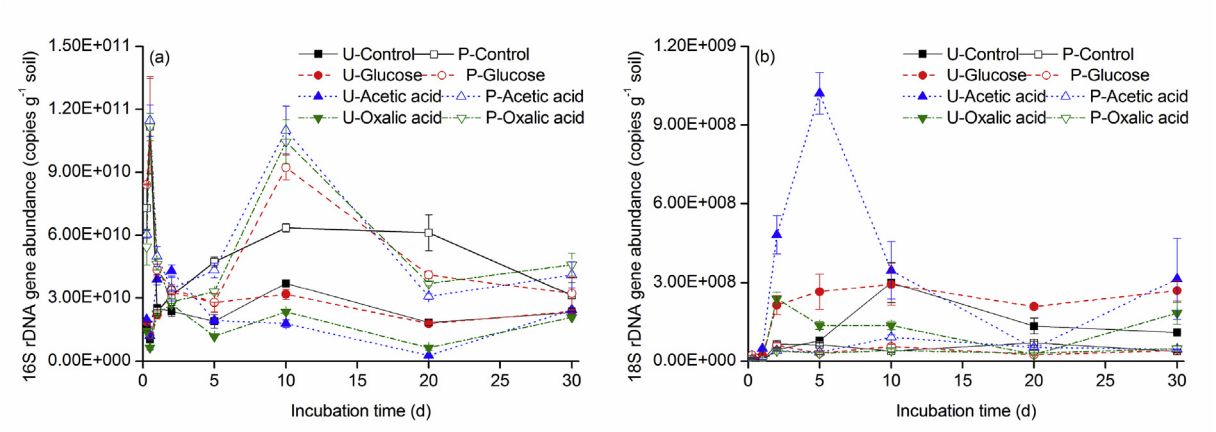

Fig. 4. Changes of 16S rDNA and 18S rDNA gene abundances in upland (U) and paddy (P) soils after different low molecular weight organic substances addition. Error bars indicate one standard error, values are means ± standard deviations (n = 3).

negative priming effect on native SOM, which was likely the result of microbes switching from SOM decomposition to the freshly added organic substances [13]. After the addition of LMWOS, *r-K* competition was assumed to be negligible, and SOM decomposing *K*-strategists switched to utilizing the added LMWOS, thus leading to a negative priming effect [20]. However, the priming effect became positive at 2–20 d (Fig. 2a and b) after readily available substrates were provided, and after some microbial species had begun to grow [19]. When the amount of added available organic C was not sufficient to minimize the competition between *r*- and *K*-strategists, microbes needed to increase SOM utilization, in order to meet their energy and nutrient requirements, leading to positive priming [40,45]. According to Fontaine [19], *r*-strategists likely used the added C quickly. At the end of the 30 d, there was insufficient C

Table 2
Correlations coefficients among microbial metabolic quotient (qCO_2), microbial abundance, CO_2 flux and priming effect.

Index	16S	18S	18S: 16S	MBC	qCO_2	Priming effect	CO_2 flux
16S	1	−0.174	−0.500**	0.218	0.115	0.000	0.051
18S		1	0.910**	−0.326*	−0.131	0.308*	−0.257
18S: 16S			1	−0.334*	−0.219	0.195	−0.245
MBC				1	−0.313*	0.073	0.101
qCO_2					1	0.389**	0.821**
Priming effect						1	0.378**
CO_2 flux							1

Note: *p < 0.05; **p < 0.01; 16S, 16S rDNA gene abundance; 18S, 18S rDNA gene abundance; 18S: 16S, Ratio of 18S rDNA gene abundance to 16S rDNA gene abundance.

for the slow growing *K*-strategists to make the switch from SOM to added C for their energy, which likely lead to a limitation of the ability of *K*-strategist microorganisms to decompose and utilize recalcitrant soil C. In upland soil, a negative priming effect occurred as a result of the exhaustion of energy-rich compounds available to microbes [21].

The easily available organic substance can stimulate fast-growing r-strategist microbes that would not be able to decompose SOM [19]. The real priming effect is partially controlled by slow-growing K-strategist microbes whose size and activity are stimulated by recalcitrant SOM [46]. Fungi are the dominant microbes in the degradation of the polymerized fraction of SOM [47]. Previous studies have indicated that fungal abundance is positively correlated with the decomposition of recalcitrant soil C, where fungi might play an important role in the turnover rate of SOM [48,49]. As can be seen in Table 2, the priming effect was positively related to qCO_2 and fungal abundance, and was observed to be lower in paddy than in upland soil. High soil moisture and anaerobic conditions can limit the abundance and activity of fungi [50]. Thus, as observed in other studies [46], slow C turnover and the low priming effect in paddy soil in this study was partially attributed to the flooding condition associated with soil management, thereby, suppressing fungal abundance and activity.

5. Conclusions

Both total LMWOS-derived CO_2 and SOM-derived CO_2 were higher in upland than in paddy soil, and affected by the type of LMWOS. The generally lower LMWOS mineralisation and associated priming effect in paddy soil likely led to the higher accumulation of SOM in comparison to that in upland soil. Weak microbial activity reflected here as metabolic quotient (qCO_2) leads to low priming effect values. In paddy soil, the abundance and activity of fungi was limited by the anaerobic conditions and high moisture level in comparison to upland soil, thereby, resulting in a weak priming effect. In summary, the priming effect was attributed to the abundance and activity of fungi, indicating that fungi may be the major contributors to accelerated SOM decomposition in upland soil.

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Appendices

Table A.1
Results of ANOVA for effects of soil management (SM) and low molecular weight organic substance (LMWOS) on cumulative CO_2 evolution, LMWOS mineralisation, and priming effect after 30 d incubation.

Parameter	df	Cumulative CO_2 evolution		LMWOS mineralisation		Priming effect	
		F value	*P value*	*F value*	*P value*	*F value*	*P value*
SM	1	71.24	<0.001	112.5	<0.001	283.1	<0.001
LMWOS	2	56.42	<0.001	684.5	<0.001	56.96	<0.001
SM × LMWOS	3	6.45	0.005	112.5	<0.001	1.10	0.36
R^2		0.92		0.99		0.96	

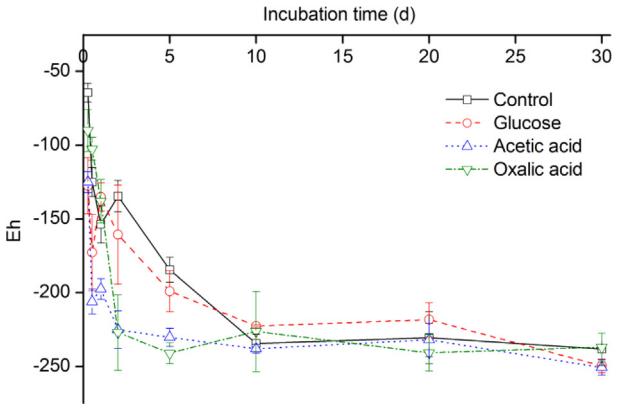

Fig. A.1. Dynamics of Eh in paddy soil after different low molecular weight organic substances addition. Error bars indicate one standard error, values are means ± standard deviations (n = 3).

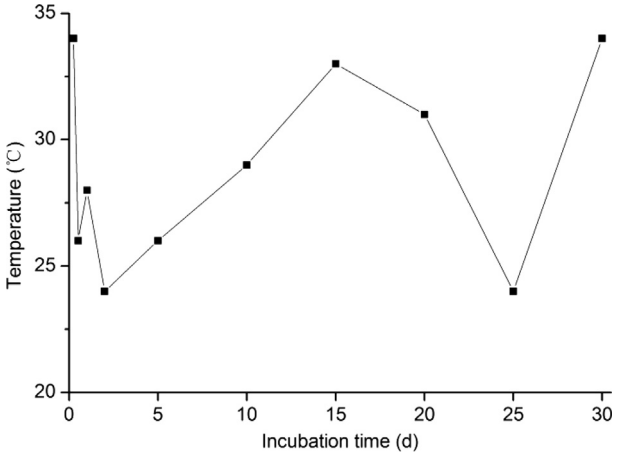

Fig. A.2. Change of ambient temperature during gas sampling time.

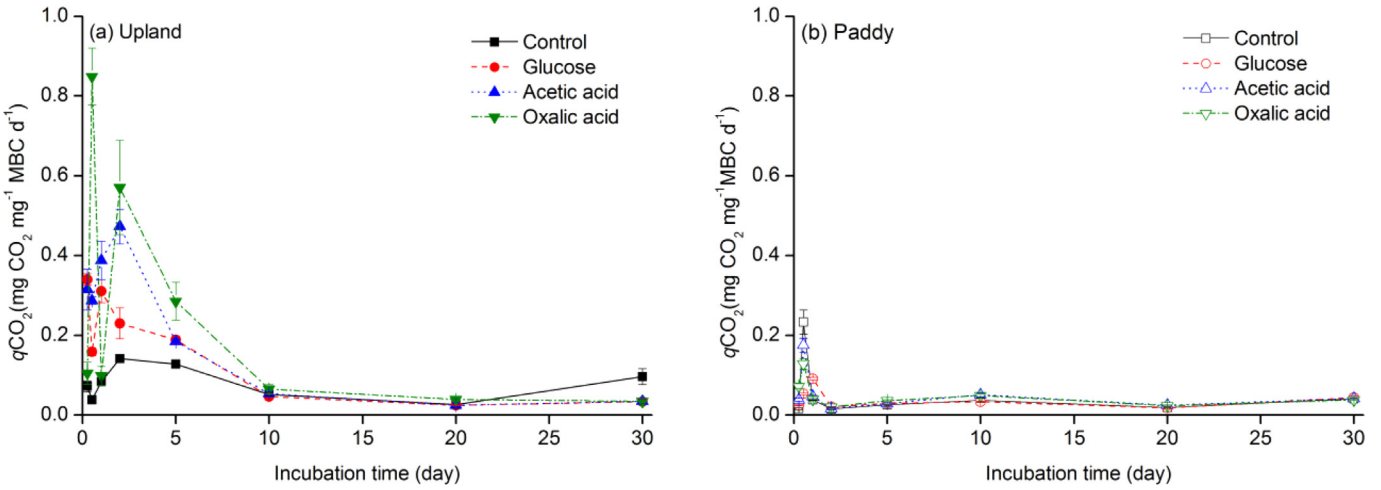

Fig. A.3. Changes in the microbial metabolic quotient (qCO_2) in upland and paddy soils after different low molecular weight organic substances addition. Error bars indicate one standard error, values are means ± standard deviations (n = 3).

References

[1] A. Hartmann, M. Schmid, D.V. Tuinen, G. Berg, Plant-driven selection of microbes, Plant Soil 321 (2009) 235–257.

[2] H. Fischer, K.U. Eckhardt, A. Meyer, G. Neumann, P. Leinweber, K. Fischer, Y. Kuzyakov, Rhizodeposition of maize: short-term carbon budget and composition, J. Plant Nutr. Soil Sci. 173 (2010) 67–79.

[3] K.G. Eilers, C.L. Lauber, R. Knight, N. Fierer, Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil, Soil Biol. Biochem. 42 (2010) 896–903.

[4] H. Fischer, A. Meyer, K. Fischer, Y. Kuzyakov, Carbohydrate and amino acid composition of dissolved organic matter leached from soil, Soil Biol. Biochem. 39 (2007) 2926–2935.

[5] W. Zech, N. Senesi, G. Guggenberger, K. Kaiser, J. Lehmann, T.M. Miano, A. Miltner, G. Schroth, Factors controlling humification and mineralization of soil organic matter in the tropics, Geoderma 79 (1997) 117–161.

[6] J. Rousk, P.C. Brookes, H.C. Glanville, D.L. Jones, Lack of correlation between turnover of low-molecular-weight dissolved organic carbon and differences in microbial community composition or growth across a soil pH gradient, Appl. Environ. Microb. 77 (2011) 2791–2795.

[7] H. Fischer, Y. Kuzyakov, Sorption, microbial uptake and decomposition of acetate in soil: transformations revealed by position-specific ^{14}C labeling, Soil Biol. Biochem. 42 (2010) 186–192.

[8] K. Fujii, C. Hayakawa, P.A.W.V. Hees, S. Funakawa, T. Kosaki, Biodegradation of low molecular weight organic compounds and their contribution to heterotrophic soil respiration in three Japanese forest soils, Plant Soil 334 (2010) 475–489.

[9] A. Gunina, A.R. Smith, Y. Kuzyakov, D.L. Jones, Microbial uptake and utilization of low molecular weight organic substrates in soil depend on carbon oxidation state, Biogeochemistry 133 (2017) 89–100.

[10] H. Glanville, J. Rousk, P. Golyshin, D.L. Jones, Mineralization of low molecular weight carbon substrates in soil solution under laboratory and field conditions, Soil Biol. Biochem. 48 (2012) 88–95.

[11] A. Gunina, M.A. Dippold, B. Glaser, Y. Kuzyakov, Fate of low molecular weight organic substances in an arable soil: from microbial uptake to utilisation and stabilization, Soil Biol. Biochem. 77 (2014) 304–313.

[12] E. Boddy, P. Roberts, P.W. Hill, J. Farrar, D.L. Jones, Turnover of low molecular weight dissolved organic C (DOC) and microbial C exhibit different temperature sensitivities in Arctic tundra soils, Soil Biol. Biochem. 40 (2008) 1557–1566.

[13] Y. Kuzyakov, R. Bol, Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar, Soil Biol. Biochem. 38 (2006) 747–758.

[14] Y. Kuzyakov, J.K. Friedel, K. Stahr, Review of mechanisms and quantification of priming effects, Soil Biol. Biochem. 32 (2000) 1485–1498.

[15] B. Guenet, J. Leloup, X. Raynaud, G. Bardoux, L. Abbadie, Negative priming effect on mineralization in a soil free of vegetation for 80 years, Eur. J. Soil Sci. 61 (2010) 384–391.

[16] R.L. Sinsabaugh, M.E. Gallo, C. Lauber, M.P. Waldrop, D.R. Zak, Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition, Biogeochemistry 75 (2005) 201–215.

[17] M.D. Nobili, M. Contin, C. Mondini, P.C. Brookes, Soil microbial biomass is triggered into activity by trace amounts of substrate, Soil Biol. Biochem. 33 (2001) 1163–1170.

[18] A.T. Nottingham, H. Griffiths, P.M. Chamberlain, A.W. Stott, E.V.J. Tanner, Soil priming by sugar and leaf-litter substrates: a link to microbial groups, Appl. Soil Ecol. 42 (2009) 183–190.

[19] S. Fontaine, A. Mariotti, L. Abbadie, The priming effect of organic matter: a question of microbial competition, Soil Biol. Biochem. 35 (2003) 837–843.

[20] E.V. Blagodatskaya, S.A. Blagodatsky, T.H. Anderson, Y. Kuzyakov, Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies, Appl. Soil Ecol. 37 (2007) 95–105.

[21] M.W.I. Schmidt, M.S. Torn, S. Abiven, T. Dittmar, G. Guggenberger, I.A. Janssens, M. Kleber, I. Kögel-Knabner, J. Lehmann, D.A.C. Manning, P. Nannipieri, D.P. Rasse, S. Weiner, S.E. Trumbore, Persistence of soil organic matter as an ecosystem property, Nature 478 (2011) 49–56.

[22] B.W. Sullivan, S.C. Hart, Evaluation of mechanisms controlling the priming of soil carbon along a substrate age gradient, Soil Biol. Biochem. 58 (2013) 293–301.

[23] Z.H. Liu, Z.G. Li, P.Q. Tang, Z.P. Li, W.B. Wu, P. Yang, L.Z. You, Change analysis of rice area and production in China during the past three decades, J. Geogr. Sci. 23 (2013) 1005–1018.

[24] J.S. Wu, Carbon accumulation in paddy ecosystems in subtropical China: evidence from landscape studies, Eur. J. Soil Sci. 62 (2011) 29–34.

[25] J.S. Wu, P. Zhou, L. Li, Y.R. Su, H.Z. Yuan, J.K. Syers, Restricted mineralization of fresh organic materials incorporated into a subtropical paddy soil, J. Sci. Food Agric. 92 (2012) 1031–1037.

[26] R. Zornoza, C. Guerrero, J. Mataix-Solera, V. Arcenegui, F. García-Orenes, J. Mataix-Beneyto, Assessing the effects of air-drying and rewetting pretreatment on soil microbial biomass, basal respiration, metabolic quotient and soluble carbon under Mediterranean conditions, Eur. J. Soil Biol. 43 (2007) 120–129.

[27] S.R. Holden, A.A. Berhe, K.K. Treseder, Decreases in soil moisture and organic matter quality suppress microbial decomposition following a boreal forest fire, Soil Biol. Biochem. 87 (2015) 1–9.

[28] T. Bölscher, L. Wadsö, G. Börjesson, A.M. Herrmann, Differences in substrate use efficiency: impacts of microbial community composition, land use management, and substrate complexity, Biol. Fert. Soils 52 (2016) 547–559.

[29] F. Bastida, I.F. Torres, T. Hernández, P. Bombach, H.H. Richnow, C. García, Can the labile carbon contribute to carbon immobilization in semiarid soils, Priming effects and microbial community dynamics, Soil Biol. Biochem. 57 (2013) 892–902.

[30] E. Blagodatskaya, Y. Kuzyakov, Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review, Biol. Fertil. Soils 45 (2008) 115–131.

[31] S.M. Collier, M.D. Ruark, L.G. Oates, W.E. Jokela, C.J. Dell, Measurement of greenhouse gas flux from agricultural soils using static chambers, J. Vis. Exp. 90 (2014) e52110.

[32] J. Wu, R.G. Joergensen, B. Pommerening, R. Chaussod, P.C. Brookes, Measurement of soil microbial biomass C by fumigation-extraction—an automated procedure, Soil Biol. Biochem. 22 (1990) 1167–1169.

[33] C.E. Dandie, M.N. Miller, D.L. Burton, B.J. Zebarth, J.T. Trevors, C. Goyer, Nitric oxide reductase-targeted real-time PCR quantification of denitrifier populations in soil, Appl. Environ. Microb. 73 (2007) 4250–4258.

[34] L.A. May, B. Smiley, M.G. Schmidt, Comparative denaturing gradient gel electrophoresis analysis of fungal communities associated with whole plant corn silage, Can. J. Microbiol. 47 (2001) 829–841.

[35] C.L. Lauber, M.S. Strickland, M.A. Bradford, N. Fierer, The influence of soil properties on the structure of bacterial and fungal communities across land-

use types, Soil Biol. Biochem. 40 (2008) 2407–2415.
[36] K.Z. Mganga, Y. Kuzyakov, Glucose decomposition and its incorporation into soil microbial biomass depending on land use in Mt. Kilimanjaro ecosystems, Eur. J. Soil Biol. 62 (2014) 74–82.
[37] C. Apostel, M. Dippold, Y. Kuzyakov, Biochemistry of hexose and pentose transformations in soil analyzed by position-specific labeling and ^{13}C-PLFA, Soil Biol. Biochem. 80 (2015) 199–208.
[38] P.W. Hill, J.F. Farrar, D.L. Jones, Decoupling of microbial glucose uptake and mineralization in soil, Soil Biol. Biochem. 40 (2008) 616–624.
[39] P.K. Mutuo, K.D. Shepherd, A. Albrecht, G. Cadisch, Prediction of carbon mineralization rates from different soil physical fractions using diffuse reflectance spectroscopy, Soil Biol. Biochem. 38 (2006) 1658–1664.
[40] S. Chowdhury, M. Farrell, N. Bolan, Priming of soil organic carbon by malic acid addition is differentially affected by nutrient availability, Soil Biol. Biochem. 77 (2014) 158–169.
[41] R.E. Drenovsky, D. Vo, K.J. Graham, K.M. Scow, Soil water content and organic carbon availability are major determinants of soil microbial community composition, Microb. Ecol. 48 (2004) 424–430.
[42] M. Keiluweit, P.S. Nico, M. Kleber, S. Fendorf, Are oxygen limitations under recognized regulators of organic carbon turnover in upland soils, Biogeochemistry 127 (2016) 157–171.
[43] E.A. Hasenmueller, L.X. Jin, G.E. Stinchcomb, H. Lin, S.L. Brantley, J.P. Kaye, Topographic controls on the depth distribution of soil CO_2 in a small temperate watershed, Appl. Geochem 63 (2015) 58–69.
[44] B. Degens, G. Sparling, Changes in aggregation do not correspond with changes in labile organic C fractions in soil amended with ^{14}C-glucose, Soil Biol. Biochem. 28 (1996) 453–462.
[45] J.A.J. Dungait, D.W. Hopkins, A.S. Gregory, A.P. Whitmore, Soil organic matter turnover is governed by accessibility not recalcitrance, Glob. Change Biol. 18 (2012) 1781–1796.
[46] S. Fontaine, C. Henault, A. Aamor, N. Bdioui, J.M.G. Bloor, V. Maire, B. Mary, S. Revaillot, P.A. Maron, Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect, Soil Biol. Biochem. 43 (2011) 86–96.
[47] W.D. Boer, L.B. Folman, R.C. Summerbell, L. Boddy, Living in a fungal world: impact of fungi on soil bacterial niche development, Fems Microbiolo. Rev. 29 (2005) 795–811.
[48] J. Garcia-Pausas, E. Paterson, Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon, Soil Biol. Biochem. 43 (2011) 1705–1713.
[49] E. Paterson, A. Sim, Soil-specific response functions of organic matter mineralization to the availability of labile carbon, Glob. Change Biol. 19 (2013) 1562–1571.
[50] M.S. Strickland, J. Rousk, Considering fungal:bacterial dominance in soils – methods, controls, and ecosystem implications, Soil Biol. Biochem. 42 (2010) 1385–1395.