Article title: Controls on mineral-associated organic matter formation in a degraded Oxisol

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Abstract

Oxisols are the dominant soil type in humid tropical and subtropical regions and are subjected to both drying–rewetting (DRW) cycles and fluctuating oxygen (O_2) availability driven by warm temperatures and abundant rainfall in surface layers. Drying-rewetting cycles and O_2 fluctuations may critically affect the microbial transformation of plant litter and subsequent stabilization as mineral-associated organic carbon (MAOC), but experimental data are still limited. We examined the impacts of DRW cycles, and variable O_2 regimes with constant moisture, on carbon (C) and iron (Fe) dynamics in a degraded Oxisol (under long-term fallow) with added plant residues. In laboratory incubations (> 3 months), both DRW cycling and fluctuating O_2 availability induced a flush of respiration and a temporary increase in microbial biomass C (MBC) following soil rewetting or O_2 exposure, although MBC was consistently suppressed in these treatments relative to the control (60 % water holding capacity under constantly aerobic condition). Consequently, DRW cycles significantly increased but O_2 fluctuations significantly decreased cumulative C mineralization relative to the control. Concentrations of short-range-ordered Fe oxides peaked immediately after litter addition and decreased five-fold during the remainder of the experiment. Mineral-associated C (defined as the chemically dispersed < 53 μm soil fraction) increased 42-64% relative to initial values but was significantly lower under DRW cycling and fluctuating O_2 relative to the control. Correspondingly, these treatments had greater fine particulate organic C (53 – 250 μm), despite increased CO_2 production under DRW cycling. Our data indicate the potential for rapid and

significant accrual of MAOC in a degraded Oxisol, but environmental factors such as DRW cycling and fluctuating O_2 can inhibit the conversion of plant litter to MAOC—possibly by suppressing microbial biomass formation and/or microbial transformations of organic matter.

Keywords: Birch effect; Drying–rewetting; Oxygen fluctuations; Litter decomposition; Iron redox; Mineral-organic associations

1. Introduction

The occurrence of heavy rainfall, episodically high C inputs, and rapid soil water evaporation in humid tropical and subtropical terrestrial ecosystems often causes periods of drying and rewetting (DRW) cycles, and thus creates oscillations between anaerobic and aerobic conditions (Silver et al., 1999; Fierer and Schimel, 2003; Wen et al., 2018). As climate change is intensifying the hydrological cycle, leading to both increased evapotranspiration and precipitation (IPCC, 2007), many surface soils are likely to experience more frequent DRW in response to rainfall events that occur following dry conditions (O'Connell et al., 2018).

It has been well documented that DRW cycles can strongly affect soil organic carbon (SOC) dynamics. Drying of soil often reduces microbial respiration, which is rapidly enhanced following subsequent wetting (Birch, 1958; Lund and Goksøyr, 1980). In dry soils, low microbial activity is mainly due to physiological stress and limited diffusion of substrates (Shi et al., 2015). The pronounced pulses of microbial respiration after soil rewetting (the "Birch Effect"; Birch, 1958) are generally

attributed to increased substrate availability from intracellular solutes due to microbial cell lysis and release of osmolytes (Halverson et al., 2000), microbial death following drying and rewetting (Wu and Brookes, 2005) and exposure of labile organic C following aggregate disruption (Utomo and Dexter, 1982; Denef et al., 2001). Most empirical studies observed that DRW cycles resulted in more cumulative CO_2 efflux compared to constant moisture conditions (Miller et al., 2005; Xiang et al., 2008), although there are exceptions (Mikha et al., 2005). In the long run, DRW cycles may accelerate C loss from soil, but an uncertainty still exists concerning their effects on net SOC balance. Long-term net soil C balance depends on both the amount of extant SOC that is mineralized to CO_2 and the amount of new C inputs that become stabilized (at least transiently) via biophysical or geochemical mechanisms. Therefore, it is necessary to understand the effects of DRW on both microbial respiration and the fate of residual C following residue inputs to soils.

Interaction with the soil mineral phase, particularly short-range-ordered iron (SRO Fe) oxides, is a major mechanism that stabilizes C in mineral soils (Kleber et al., 2015). Microbial necromass produced during decomposition of plant residues often constitutes a major component of mineral-associated organic C (Cotrufo et al., 2013, 2015). Thus, higher microbial activities and growth may increase microbial biomass, and facilitate formation of mineral-associated C despite promoting the loss of litter to CO_2. However, some studies observed that DRW cycles significantly increased microbial respiration but reduced microbial biomass in soils with plant litter incorporation (Miller et al., 2005; Cosentino et al., 2006). These results indicate DRW

may force microbes to shift resource allocation from anabolism to catabolism, potentially making litter C vulnerable to loss and limiting mineral-associated C formation (Schimel et al., 2007). However, experiments evaluating the effects of DRW cycles on litter transformation and formation of organo-mineral associations are still lacking.

Drying–rewetting cycles not only cause moisture fluctuation but also lead to soil oxygen (O_2) fluctuations, resulting in alternating aerobic, hypoxic and anaerobic conditions (O'Connell et al., 2018). Generally, O_2 deficiency can retard residue decomposition and limit the transformation of residue into mineral-associated C due to limited microbial growth and suppressed oxidative enzyme activities (Keiluweit et al., 2016). However, periodic changes in redox conditions resulting from fluctuating aerobic and anaerobic conditions can drive substantial ferric Fe [Fe(III)] reduction and ferrous Fe [Fe(II)] oxidation which could stimulate residue decomposition via mechanisms such as desorption and/or production of reactive oxygen species (Lovley et al., 1986; Hall et al., 2015; Yang and Liptzin, 2015). In addition, recent studies found that repeated reductive dissolution and oxidative re-precipitation of Fe oxides under continuous alternation of redox conditions could lead to an increase in SRO Fe oxides (Vogelsang et al., 2016; Winkler et al., 2018), suggesting that Fe redox cycling may increase the opportunity for formation of organo-mineral complexes. Thus, the effects of DRW events on plant litter decomposition and SOC formation may depend not only on direct effects on microbial activity and growth, but also on Fe redox cycling mediated by soil O_2 fluctuations.

Oxisols are widely distributed in tropical and subtropical zones of the world. In addition to their globally-significant C stocks under forests and pastures, food production from these soils supports a large and increasing proportion of the world population (Huang et al., 2010). Due to intensive weathering, these soils are typically rich in Fe oxides. Thus, Fe redox processes may be especially important for SOC dynamics in Oxisols (Yang and Liptzin, 2015, Hall et al., 2015). In this study, we conducted a laboratory incubation experiment to assess how DRW cycles affect the partitioning of residue C among particulate and mineral-associated fractions in an Oxisol soil under long-term bare fallow. To assess impacts of fluctuating redox conditions that may accompany DRW cycling, an O_2 fluctuation experiment was also conducted to assess impacts of O_2 independent of fluctuating moisture. We hypothesized that DRW cycles would increase residue decomposition due to both the "Birch effect" and Fe redox cycling, while O_2 fluctuations alone would also sustain similar residue decomposition as the aerobic controls due to Fe redox cycling. However, both DRW cycles and O_2 fluctuations would decrease mineral-associated C formation due to microbial growth suppression, regardless of increased SRO Fe.

2. Materials and methods

2.1. Soil and residue characterization

Soil (0–20 cm depth) was collected from the Red Soil Ecological Experimental Station of the Chinese Academy of Sciences, located in Yingtan County, Jiangxi Province (28° 15′ 20″ N, 116° 55′ 30″ E). The site has a subtropical monsoon climate,

with mean annual temperature of 17.6 °C and precipitation of 1795 mm. The soil is derived from Quaternary red clay and classified as an Udic Ferralsol in Chinese Soil Taxonomy or an Oxisol in the USDA soil taxonomy with a clay texture. The clay, silt and sand contents were 57.9 %, 28.5 % and 13.6 %, respectively. In order to better track the impact of DRW cycling on mineral-associated C formation and reduce the impact of potentially confounding factors and spatial heterogeneity of plant C inputs that occur in agroecosystems, soils were sampled from a long-term bare fallow soil (ca. 10 years). Before the fallow management, maize was the dominant crop in this field. This soil could be considered as a degraded Oxisol because of its low fertility and organic matter, and had very low particulate C, allowing us to readily assess impacts of treatments on particulate and mineral-associated C at the end of the experiment. After roots and rocks were removed, the field moist soil was passed through a 2 mm mesh sieve and thoroughly mixed. Then, the fresh soil was stored at 4 °C prior to incubation. The residue we used was maize leaves which were collected at the grain-filling stage. In the laboratory, the plant material was dried at 60 °C to constant weight and cut into ~5 mm pieces with scissors. The C and N contents of soil and residue were measured using a CN analyzer (Elementar Vario Macro Cube, Germany). Initial soil water content was determined by drying subsamples at 105 °C for 24 h. The water content of soil at 100% water holding capacity was determined from the gravimetric water content of soil that was saturated and then allowed to drain for 2 h in a filter funnel (Karhu et al., 2014). The basic properties of soil and residue were summarized in Table 1.

2.2. Experimental design

We filled 250 mL glass jars (sealed with butyl rubber stoppers and aluminum caps) with 50 g soil (oven dry mass equivalent) at 60 % water holding capacity (WHC). All jars were then equilibrated for one week at 25 °C. Following the initial equilibration, 0.5 g residue was mixed into the soil (1 % w/w), which was slightly higher than the average amount of straw incorporation in the fields (Liu et al., 2014). Soils were immediately exposed to different moisture treatments to simulate different field water conditions. The treatments consisted of: (i) intermediate moisture (60 % WHC, approximately −0.043 MPa); (ii) saturation moisture (100 % WHC, approximately −0.003 MPa); (iii) DRW cyclings. Each DRW cycle started at 120 % WHC (approximately −0.001 MPa), followed by a 15 day dry-down period to 30 % WHC (approximately −1.99 MPa). Then soils were rewetted by slowly adding deionized water into the jar using a syringe until the soils reached 120 % WHC. There were thirty-six replicates for each treatment so that soils could be destructively sampled in quadruplicate on days 2, 6, 13, 33, 37, 42, 78, 82 and 89 for determination of soil microbial and physicochemical parameters (Fig. 1a). All jars were incubated in the dark up to 90 days at 25 °C. To maintain the water content in the constant moisture treatments, we weighed each jar and moistened the soil as needed every two days.

To better characterize the effects of O_2 fluctuations on the decomposition of plant residues and formation of mineral-associated C, the remaining jars received three headspace treatments: (i) continuous lab air (constantly aerobic treatment); (ii) N_2 (constantly hypoxic treatment); (iii) four days of aerobic conditions followed by five

days of hypoxia (fluctuating aerobic/hypoxic treatment). The periodicity of O_2 fluctuations employed in this experiment was adapted from Hall et al. (2015). Note that in situ periods of fluctuation can vary widely because of the uncertainty of the precipitation. All soils were maintained at 60 % WHC. In this study, the incubation condition of soils in the 60 % WHC treatment (described above) and the constantly aerobic treatments were the same, but these were destructively sampled at different times to provide controls for both the DRW and O_2 fluctuation experiments. To maintain water content in the constantly hypoxic treatment, additional N_2-flushed water was added as necessary. For all treatments, jars were flushed with the appropriate gas as described above to purge the jar headspace every day. Soils were destructively sampled in quadruplicate on days 4, 9, 22, 27, 40, 45, 67, 72, 112 and 117 (Fig. 1b).

2.3. Analysis of CO_2, methane, microbial biomass C and dissolved organic C

For the DRW experiment, CO_2 and methane (CH_4) production was measured on days 1, 5, 10, 14 after soil rewetting for each DRW cycle. The intermediate and saturation moisture treatments were sampled at the same time as the soils in the DRW cycling treatment. For the fluctuating O_2 experiment, CO_2 and CH_4 production was measured at the end of each four-day aerobic period and at the end of each five-day hypoxic period. The constantly aerobic and hypoxic treatments were sampled at the same time as the soils in the fluctuating O_2 treatment. Jar headspace gas was sampled using a gas-tight syringe and measured using a gas chromatograph (Agilent 7890A, USA). At each destructive sampling date, soil microbial biomass C (MBC) was estimated using

the fumigation-extraction method (Vance et al., 1987). Briefly, 12.5 g of fresh soil was fumigated with ethanol-free chloroform for 48 h. Both fumigated and non-fumigated soils were extracted with 0.5 M K_2SO_4 solution and the extracts were measured on a TOC analyzer (Elementar Vario Micro Cube, Germany) to determine organic C concentrations. The measured organic C was converted to MBC using conversion factor k_{ec} = 0.33 (Mo et al., 2008). The organic C in the non-fumigated extracts was used as dissolved organic C (DOC).

2.4. Determination of Fe(II), Fe(III) and short-range-ordered Fe oxides

Reactive Fe phases were also determined at each destructive sampling date. Briefly, 5 g (oven dry mass equivalent) of fresh soil was extracted with 50 mL 0.5 M hydrochloric acid (HCl) solution and the supernatant was syringe-filtered through a 0.2 μm nylon membrane filter. The HCl extract was analyzed for Fe(III) and Fe(II) concentrations by using a modified ferrozine assay (Viollier et al., 2000; Liptzin and Silver, 2009), which we refer to as $Fe(II)_{HCl}$ and $Fe(III)_{HCl}$. A separate sample was extracted by 0.2 M ammonium oxalate at pH 3 with a soil:solution ratio of 1:50 and the mixtures were shaken for 4 hours in the dark, and Fe was measured using an Agilent 5100 ICP-OES. We refer to oxalate-extractable Fe as SRO Fe.

2.5. Soil fractionations

Physical fractionation of soil samples was performed at the end of the incubation following a procedure adapted from Cambardella and Elliott (1992) and Wang et al. (2016). Briefly, a 20 g air-dried soil sample was placed in a centrifuge tube with 50 mL of sodium hexametaphosphate solution (5 g L^{-1}) and shaken on a reciprocating

shaker (180 rpm) for 18 h. The mixture was then successively sieved through 250 μm and 53 μm mesh. Organic material retained on the 250 μm sieve was defined as coarse particulate organic C (POC) and on the 53 μm sieve as fine POC. In general, POC is composed of free organic debris or material released by dispersion of soil aggregates. Material in the chemically dispersed < 53 μm soil fraction has often been defined as mineral-associated (Cambardella and Elliott, 1992, Procter et al., 2015, Wang et al., 2016). Nevertheless, this fraction not only includes organic C bound to silt and clay-size particles, but also includes any micro-aggregates that may have resisted dispersion and potential contributions from very fine free particulate C. However, fine soil size fractions show markedly different chemical composition and turnover times than coarser fractions (von Lützow et al., 2007), supporting the general utility of the < 53 μm fraction as a liberal proxy for mineral-associated organic C (Cambardella and Elliott, 1992). The separated soil fractions were dried at 60 °C, weighed, and ground to homogenized fine powders for organic C analysis using the CN analyzer. Carbonates were negligible in this soil given the strongly acidic pH value.

2.6. Statistical analyses

First, we performed a repeated measures analysis to examine the effects of treatment and incubation time on microbial respiration, MBC, DOC, Fe(II), Fe(III) and SRO Fe oxides. Second, one-way ANOVA with Duncan's multiple-range tests was performed to compare the effects of treatments on these parameters at the end of incubation. All statistical analyses were performed using the R software version 3.1.2 (R

Development Core Team, 2014).

3. Results

3.1. Microbial respiration, MBC and DOC

Microbial respiration rate in the intermediate and saturation moisture treatments gradually declined over the first 75 days to relatively stable levels, while DRW cycling consistently induced temporal variation of respiration rate with stimulated respiration occurring after soil rewetting (Fig. 2a). The total amount of CO_2 respired was significantly higher in the DRW soils than in the intermediate and saturation moisture soils, while there was no significant difference in cumulative respiration between the two constant moisture treatments ($P < 0.05$; Fig. 2c). Fluctuating O_2 also induced variation of respiration rate similar to DRW cycling, with the magnitude declining over time (Fig. 2b). Soils under the fluctuating O_2 treatment respired significantly less CO_2 than the aerobic soil and the hypoxic soil respired the least CO_2 ($P < 0.001$, Fig. 2d).

Significant CH_4 production was not detected in the DRW experiment throughout the incubation. In the fluctuating O_2 experiment, the maximum rate of CH_4 production was 1.2 μg CH_4-C kg^{-1} soil h^{-1} in the hypoxic treatment. So, the percent contribution of CH_4 to total C mineralization was negligible.

Microbial biomass C in the the intermediate and saturation moisture treatments generally decreased throughout the incubation (Fig. 3a). In the DRW treatment, MBC fluctuated with changes of soil moisture, with the decrease occurring when soil drying

and increase when soil rewetting (Fig. 3a). At the end of incubation, MBC was significantly higher in the intermediate moisture treatment than in the saturation moisture and DRW treatments (Fig. 3a).

The dynamics of MBC in the aerobic treatment exhibited a similar pattern to those of the intermediate moisture treatment (Fig. 3b). Oxygen fluctuations induced variability of MBC over the first 45 days, with the increase occurring in the aerobic phase of the fluctuating treatment and decrease in the hypoxic phase of the fluctuating treatment (Fig. 3b). The hypoxic soils had significantly lower MBC than the aerobic and fluctuating O_2 soils, and the content of MBC in the hypoxic treatment decreased through the incubation ($P < 0.05$; Fig. 3b). At the end of incubation, MBC followed a decreasing order of aerobic > fluctuating > hypoxic treatment ($P < 0.05$; Fig. 3b).

Dissolved organic C in the the intermediate and saturation moisture treatments decreased throughout the incubation (Fig. 3c). In the DRW treatment, DOC was affected by moisture fluctuations, decreasing with soil drying and then increasing following rewetting conditions (Fig. 3c). At the end of incubation, saturation moisture soil had the highest DOC, followed by the DRW and intermediate moisture treatments (Fig. 3c).

In all the treatments of the O_2 fluctuation experiment, DOC peaked after 9 days and decreased throughout the rest of the incubation (Fig. 3d). During the first 27 days, the aerobic phase of the fluctuating O_2 treatment decreased DOC, then an increase in DOC occurred following the hypoxic phase of the fluctuating O_2 treatment (Fig. 3d). At the end of incubation, the hypoxic soil had significantly higher DOC than the

fluctuating O_2 and aerobic soils ($P < 0.05$; Fig. 3d).

3.2. $Fe(II)_{HCl}$, $Fe(III)_{HCl}$ and SRO Fe oxides

Soil extractable $Fe(II)_{HCl}$ responded strongly to the different moisture and O_2 regimes. In the saturation moisture treatment, $Fe(II)_{HCl}$ concentrations increased over time and peaked at the end of incubation, while concentrations of $Fe(II)_{HCl}$ in the intermediate moisture and DRW cycling treatments were relatively consistent throughout the incubation (Fig. 4a). At the end of incubation, saturation moisture soil had significantly higher $Fe(II)_{HCl}$ than the DRW and intermediate moisture soils ($P < 0.05$; Fig. 4a).

The trend of $Fe(II)_{HCl}$ concentrations in the hypoxic treatment was similar to the saturation moisture treatment ($P < 0.05$; Fig. 4b). Concentrations of $Fe(II)_{HCl}$ in the aerobic treatment were consistent throughout the incubation, while concentrations in the fluctuating O_2 treatment varied with O_2 availability (Fig. 4b). At the end of incubation, $Fe(II)_{HCl}$ concentrations followed a decreasing order of hypoxic > fluctuating > aerobic treatment ($P < 0.05$; Fig. 4b).

Concentrations of $Fe(III)_{HCl}$ in the intermediate and saturation moisture treatments initially increased and then declined until the end of the incubation, while DRW treatment produced variability of $Fe(III)_{HCl}$ concentrations, with $Fe(III)_{HCl}$ increasing with initial wetting, but decreasing with soil drying (Fig. 4c). At the end of the incubation, $Fe(III)_{HCl}$ concentrations in the DRW cycling soil were significantly less than in the saturation and intermediate moisture soils ($P < 0.05$; Fig. 4c).

In the fluctuating O_2 experiment, $Fe(III)_{HCl}$ concentrations initially increased in

all treatments and then decreased over time (Fig. 4d). At the end of the incubation, $Fe(III)_{HCl}$ concentration significantly differed among treatments and followed a decreasing order of hypoxic > fluctuating > aerobic treatment ($P < 0.05$; Fig. 4d).

Concentrations of SRO Fe in the intermediate and saturation moisture treatments generally decreased over incubation time, while SRO Fe concentrations in the DRW treatment increased with initial wetting, but decreased with soil drying (Fig. 4e). At the end of the incubation, SRO Fe concentrations in all the treatments showed no significant differences ($P > 0.05$; Fig. 4e).

In the fluctuating O_2 experiment, SRO Fe concentrations under all treatments decreased over time (Fig. 4f). At the end of the incubation, SRO Fe concentrations in the hypoxic treatment were significantly higher than in the aerobic and fluctuating treatments ($P < 0.05$; Fig. 4f).

3.3. Soil organic C in different fractions

Both soil DRW cycling and O_2 fluctuations significantly affected the conversion of plant litter to mineral-associated C. Although mineral-associated C was increased by 42-64% in all treatments at the end of the experiment relative to initial values, it was significantly lower in both DRW and O_2 fluctuation treatments compared with the control (60 % water holding capacity under constantly aerobic condition) ($P < 0.05$; Fig. 5e, f). Correspondingly, DRW cycling and O_2 fluctuation soils had significantly higher fine POC than the control ($P < 0.05$; Fig. 5c, d). The hypoxic and saturation moisture treatments of the respective experiments had the highest coarse and fine POC but the lowest mineral-associated C ($P < 0.05$; Fig. 5).

4. Discussion

We hypothesized that DRW cycles would stimulate and O_2 fluctuations would sustain residue decomposition. Our results showed that DRW cycles increased but O_2 fluctuations decreased CO_2 emissions relative to their corresponding soils, maintained under constant moisture or aerobic conditions, respectively (Fig. 2c, d). Increased C mineralization as a result of DRW was likely due to increased turnover of microbial biomass and altered microbial physiology. Decreased microbial metabolism during the hypoxic periods can explain the reduction in total C mineralization in O_2 fluctuating soil. We also hypothesized that both DRW cycles and O_2 fluctuations would reduce mineral-associated C formation. Our results supported this hypothesis and the decreased mineral-associated C was mainly attributed to limited microbial transformation of plant litter (Fig. 5e, f). Together, these findings suggest that short-term weather variability and its resulting impacts on moisture and O_2 availability may significantly impact plant litter decomposition and the formation of mineral-associated C.

4.1. Divergent effects of DRW cycles and O_2 fluctuations on C mineralization

Consistent with our hypothesis, results from our study showed that soil DRW cycles significantly increased cumulative CO_2 emissions, as compared to CO_2 emissions from the control soils maintained at a constant soil moisture (Fig. 2c). Many previous

studies have reported DRW-enhancement of C mineralization (e.g. Miller et al., 2005; Xiang et al., 2008) and largely attributed it to rewetting-induced increases in substrate supply (Fierer and Schimel, 2003; Borken and Matzner, 2009). In our study, a large but representative supply of crop residue C was incorporated into bare soil with very low particulate organic C content (Table 1), suggesting that CO_2 emissions were mainly derived from residue decomposition. However, we observed higher fine particulate organic C in the DRW treatment relative to the control treatment (Fig. 5c), implying that degradation of the added residue was suppressed by DRW cycling. We also observed dissolved organic C flushes immediately after rewetting and subsequently rapid decline during the drying periods (Fig. 3c), indicating that rewetting likely caused lysis of microbial cells and then fast mineralization of microbial biomass and microbial osmoregulatory compounds (Fierer and Schimel, 2003; Yemadje et al., 2017). Thus, increased turnover of microbial biomass and microbial products with rewetting may partly explain the enhanced C mineralization in the DRW soil, despite the partial suppression of particulate organic matter decomposition noted above.

Microbial carbon use efficiency (CUE) determines the fate of C in soils by partitioning C between microbial biomass and CO_2 production (Frey et al., 2013). The increased CO_2 production and decreased microbial biomass in the DRW treatment indicated that low CUE appeared to be another reason for the increased C mineralization. Schimel et al. (2007) proposed that microbes would invest more resources to survival than they invest to growth in stressful environments. A recent

study supported this view and showed that rewetting dry soils strongly stimulated soil respiration but slowly increased microbial growth (Meisner et al., 2017). Similarly, DRW consistently suppressed microbial biomass C throughout the incubation in our study (Fig. 3a), suggesting that DRW-induced enhancement of CO_2 emissions could also be due to altered microbial physiology that resulted in low microbial CUE. In addition, the extent of drying can also determine the amount of C that is mineralized. A meta-analysis showed that microbial activity ceased at a water potential of about −14 MPa in mineral soils (Manzoni et al., 2012). In our study, soils were dried to −1.99 MPa and the respiration rate at the driest time of DRW treatment was even higher relative to corresponding intermediate moisture treatment over the first 60 days (Fig. 2a), thus suggesting that dried soil could also maintain relatively high microbial activity in our study. Although we observed DRW-induced Fe redox cycling, Fe(II) concentrations (ca. 1~4 mg kg^{-1}) in the DRW treatment were far below the values (ca. 80~800 mg kg^{-1}) in the previous studies (Hall et al., 2015; Winkler et al., 2018). Thus, Fe redox cycling likely contributed little to the enhanced CO_2 emissions in the DRW soil.

Contrary to our hypothesis, we observed that fluctuating O_2 availability significantly suppressed cumulative CO_2 production (Fig. 2d). Similar to the results of our DRW experiment, we also observed low content of Fe(II) under fluctuating O_2 conditions (Fig. 4b). The reduced C mineralization and low rates of Fe reduction and oxidation indicated that Fe redox cycling played little impact on residue decomposition in the O_2 fluctuating soil. Given the frequent reducing conditions

favorable for Fe reduction in our study, the low Fe reduction potential might be attributed to several factors shown to control Fe reduction in other humid tropical soils: limited availability of low-molecular-weight organic compounds, low microbial availability of extant SRO Fe (i.e., due to complexation), and/or low abundance of Fe(III)-reducing bacteria (Hall et al., 2016).

Although CO_2 production increased immediately after soils were exposed to O_2, this did not compensate for the low CO_2 production from plant residues during the hypoxic periods, which explained why fluctuating O_2 availability resulted in significantly lower CO_2 emission relative to aerobic treatment. The decrease in the mineralization of residue during the hypoxic periods indicated constraints on microbial metabolism induced by O_2 limitations (Keiluweit et al., 2016), as evidenced by decreased microbial biomass and increased DOC during periods of O_2 limitation (Fig. 3b, d). Together, these findings showed that alternation between hypoxic and aerobic conditions suppressed plant litter decomposition in these soils.

4.2. Convergent effects of DRW cycles and O_2 fluctuations on mineral-associated C

Our results showed that both repeated DRW cycles and O_2 fluctuations reduced the proportion of residue C transferred to the fine fraction (mineral-associated C) pools (Fig. 5e, f), indicating that the variability of soil moisture and O_2 may critically affect the efficiency of C retention in this degraded Oxisol. Although some studies have reported the effects of DRW cycles on SOC dynamics in soils with residue amendment, they focused on C and N mineralization (Gao et al., 2016; Yemadje et al.,

2017), not on the formation of mineral-associated C. To link litter decomposition with SOC stabilization in the mineral soil matrix, Cotrufo et al. (2013) developed the Microbial Efficiency-Matrix Stabilization framework which proposed that most mineral-associated C is of microbial origin. Short-range-ordered Fe oxides also function as an important regulator for the formation of mineral-associated C in Fe-rich soils (Kleber et al., 2005). Thus, the dynamics of mineral-associated C in Oxisols could depend critically on the contents of both microbial residues and SRO Fe (Hall et al. 2018).

Studies have shown that redox fluctuations could lead to an increase in SRO Fe due to reductive dissolution of Fe oxides coupled with the rapid oxidative precipitation of mainly SRO Fe (Vogelsang et al., 2016; Winkler et al., 2018). In our study, neither DRW cycles nor O_2 fluctuations increased SRO Fe relative to the controls at the end of incubation (Fig. 4e, f). However, we found that residue addition increased the SRO Fe pool in all treatments at the beginning of incubation (Fig. 4e, f), suggesting that organic material input induced a change in Fe phase composition. Besides redox environment, weathering of Fe oxides by organic acids and protonation as mediated by microorganisms can also promote formation of SRO Fe (Colombo et al., 2014). Thus, the increase in SRO Fe could be attributed to dissolution of organic acids from litter and/or increased microbial activity induced by residue inputs. The initial flushes of SRO Fe and microbial biomass also explained why residue addition could rapidly increase mineral-associated C in our degraded Oxisol. We also observed that the contents of SRO Fe decreased over time, consistent with the results of Liptzin

and Silver (2009), indicating that SRO Fe could ripen to more crystalline phases and/or that these phases that were less vulnerable to oxalate extraction due to protection within co-precipitates over time.

The decreased mineral-associated C in the DRW and fluctuating O_2 treatments was likely due to the reduced microbial biomass observed in these treatments, given the lack of difference in extractable SRO Fe pools and weak potential for the reduction of Fe(III) in our soils (Fig. 4). Furthermore, the increased particulate organic C in these treatments relative to the controls suggested that both soil moisture and O_2 fluctuations limited microbial decomposition and transformation of plant residues. Together, the increase in respiration but decrease in microbial biomass in the DRW treatment indicate that DRW-induced low microbial substrate use efficiency may be an important factor limiting the transformation of plant residues into mineral-associated C (Fig. 6a), while decreases in both respiration and microbial biomass in O_2 fluctuation treatment indicate that O_2 limitation of organic matter transformations could play an important role in mineral-associated C formation (Fig. 6b).

5. Conclusions

Results from our experiment showed that plant litter incorporation into degraded Oxisols could rapidly increase mineral-associated C content in the short term. This finding is important given the increasing attention placed on C sequestration in degraded agricultural soils in the context of global climate change policy (Paustian et al., 2016). However, both soil DRW cycles and O_2 fluctuations decreased the transfer

of residue C into the mineral-associated C pool. Soil DRW cycles and O_2 fluctuations showed the potential to promote Fe redox cycling but rates were very low and did not significantly increase short-range-ordered Fe phases. The greater C mineralization in the DRW treatment and lower C mineralization in the O_2 fluctuation treatment may have been due to lower microbial CUE and O_2 deficiency, respectively, which were not counteracted by an increase in substrate availability. The decline in mineral-associated C formation could thus be explained by a lower amount of plant substrates incorporated into microbial biomass during residue decomposition under fluctuating moisture and O_2 conditions (Fig. 6). These findings suggest that conversion of litter to microbial biomass under moist but aerobic conditions may allow the efficient formation of mineral-associated organic matter in degraded, clay-rich soils such as those examined here, but that drastic changes in soil moisture and O_2 may suppress this process.

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References

Birch, H.F., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant Soil 10, 9−31.

Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Glob. Change Biol. 15, 808−824.

Cambardella, C.A., Elliott, E.T., 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Sci. Soc. Am. J. 56, 777−783.

Colombo, C., Palumbo, G., He, J.Z., Pinton, R., Cesco, S., 2014. Review on iron availability in soil: interaction of Fe minerals, plants, and microbes. J. Soils Sediments 14, 538−548.

Cosentino, D., Chenu, C., Le Bissonnais, Y., 2006. Aggregate stability and microbial community dynamics under drying–wetting cycles in a silt loam soil. Soil Biol. Biochem. 38, 2053−2062.

Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, A.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nat. Geosci. 8, 776−779.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Glob. Change Biol. 19, 988−995.

Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of dry–wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biol.

Biochem. 33, 1599−1611.

Fierer, N., Schimel, J.P., 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Sci. Soc. Am. J. 67, 798−805.

Frey, S.D., Lee, J., Melillo, J.M., Six, J., 2013. The temperature response of soil microbial efficiency and its feedback to climate. Nat. Clim. Change 3, 395−398.

Gao, J., Feng, J., Zhang, X., Yu, F.H., Xu, X., Kuzyakov, Y., 2016. Drying-rewetting cycles alter carbon and nitrogen mineralization in litter-amended alpine wetland soil. Catena 145, 285−290.

Hall, S.J., Berhe, A.A., Thompson, A., 2018. Order from disorder: do soil organic matter composition and turnover co-vary with iron phase crystallinity? 140, 93−110.

Hall, S.J., Liptzin, D., Buss, H.L., DeAngelis, K., Silver, W.L., 2016. Drivers and patterns of iron redox cycling from surface to bedrock in a deep tropical forest soil: a new conceptual model. Biogeochemistry 130, 177−190.

Hall, S.J., Silver, W.L., Timokhin, V.I., Hammel, K.E., 2015. Lignin decomposition is sustained under fluctuating redox conditions in humid tropical forest soils. Glob. Change Biol. 21, 2818−2828.

Halverson, L.J., Jones, T.M., Firestone, M.K., 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Sci. Soc. Am. J. 64, 1630−1637.

Huang, S., Peng, X.X., Huang, Q.R., Zhang, W.J., 2010. Soil aggregation and organic

carbon fractions affected by long-term fertilization in a red soil of subtropical China. Geoderma 154, 364−369.

IPCC, 2007. Climate Change 2007: The physical science basis. Summary for policymakers. Contribution of working group I to the fourth assessment report. The Intergovernmental Panel on Climate Change.

Karhu, K., Auffret, M.D., Dungait, J.A.J., Hopkins, D.W., Prosser, J.I., Singh, B.K., Subke, J.A., Wookey, P.A., Ågren, G.I., Sebastià, M.T., Gouriveau, F., Bergkvist, G., Meir, P., Nottingham, A.T., Salinas N., Hartley I.P., 2014. Temperature sensitivity of soil respiration rates enhanced by microbial community response. Nature, 513, 81−84.

Keiluweit, M., Nico, P.S., Kleber, M., Fendorf, S., 2016. Are oxygen limitations under recognized regulators of organic carbon turnover in upland soils? Biogeochemistry 127, 157−171.

Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R., Nico, P.S., 2015. Mineral–organic associations: formation, properties, and relevance in soil environments. In *Advances in agronomy*. Academic Press.

Kleber, M., Mikutta, R., Torn ,M.S., Jahn, R., 2005. Poorly crystalline mineral phases protect organic matter in acid subsoil horizons. Eur. J. Soil Sci. 56, 717−25.

Liptzin, D., Silver, W.L., 2009. Effects of carbon additions on iron reduction and phosphorus availability in a humid tropical forest soil. Soil Biol. Biochem. 41, 1696−1702.

Liu, S., Huang, D., Chen, A., Wei, W., Brookes, P.C., Li, Y., Wu, J., 2014. Differential

responses of crop yields and soil organic carbon stock to fertilization and rice straw incorporation in three cropping systems in the subtropics. Agr. Ecosyst. Environ. 184, 51−58.

Lovley, D.R., Phillips, E.J., 1986. Organic matter mineralization with reduction of ferric iron in anaerobic sediments. Appl. Environ. Microb. 51, 683−689.

Lund, V., Goksøyr, J., 1980. Effects of water fluctuations on microbial mass and activity in soil. Microb. Ecol. 6 115−123.

Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93, 930−938.

Meisner, A., Leizeaga, A., Rousk, J., Bååth, E., 2017. Partial drying accelerates bacterial growth recovery to rewetting. Soil Biol. Biochem. 112, 269−276.

Mikha, M.M., Rice, C.W., Milliken, G.A., 2005. Carbon and nitrogen mineralization as affected by drying and wetting cycles. Soil Biol. Biochem. 37, 339−347.

Miller, A.E., Schimel, J.P., Meixner, T., Sickman, J.O., Melack, J.M., 2005. Episodic rewetting enhances carbon and nitrogen release from chaparral soils. Soil Biol. Biochem. 37, 2195−2204.

Mo, J., Zhang, W., Zhu, W., Gundersen, P., Fang, Y., Li, D., Wang, H., 2008. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Glob. Change Biol., 14, 403−412.

O'Connell, C.S., Ruan, L., Silver, W.L., 2018. Drought drives rapid shifts in tropical rainforest soil biogeochemistry and greenhouse gas emissions. Nat. Commun. 9, 1348.

Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G.P., Smith, P., 2016. Climate-smart soils. Nature, 532, 49−57.

Procter, A.C., Gill, R.A., Fay, P.A., Polley, H.W., Jackson, R.B., 2015. Soil carbon responses to past and future CO_2 in three Texas prairie soils. Soil Biol. Biochem. 83, 66−75.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386−1394.

Shi, A., Yan, N. and Marschner, P., 2015. Cumulative respiration in two drying and rewetting cycles depends on the number and distribution of moist days. Geoderma 243, 168−174.

Silver, W.L., Lugo, A.E., Keller, M., 1999. Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils. Biogeochemistry 44, 301−328.

Utomo, W.H., Dexter, A.R., 1982. Changes in soil aggregate water stability induced by wetting and drying cycles in non-saturated soil. Eur. J. Soil Sci. 33, 623−637.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703−707.

Viollier, E., Inglett, P.W., Hunter, K., Roychoudhury, A.N., Van Cappellen, P., 2000. The ferrozine method revisited: Fe (II)/Fe (III) determination in natural waters. Appl. Geochem. 15, 785−790.

Vogelsang, V., Kaiser, K., Wagner, F.E., Jahn, R., Fiedler, S., 2016. Transformation of clay-sized minerals in soils exposed to prolonged regular alternation of redox

conditions. Geoderma 278, 40−48.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. Soil Biol. Biochem. 39, 2183−2207.

Wang, F., Zhu, W., Chen, H., 2016. Changes of soil C stocks and stability after 70-year afforestation in the Northeast USA. Plant Soil 401, 319−329.

Wen, Y., Xiao, J., Liu, F., Goodman, B.A., Li, W., Jia, Z., Yu, G., 2018. Contrasting effects of inorganic and organic fertilisation regimes on shifts in Fe redox bacterial communities in red soils. Soil Biol. Biochem. 117, 56−67.

Winkler, P., Kaiser, K., Thompson, A., Kalbitz, K., Fiedler, S., Jahn, R., 2018. Contrasting evolution of iron phase composition in soils exposed to redox fluctuations. Geochim. Cosmochim. Acta 235, 89−102.

Wu, J., Brookes, P.C., 2005. The proportional mineralisation of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. Soil Biol. Biochem. 37, 507−515.

Xiang, S.R., Doyle, A., Holden, P.A., Schimel, J.P., 2008. Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. Soil Biol. Biochem. 40, 2281−2289.

Yang, W.H., Liptzin, D., 2015. High potential for iron reduction in upland soils. Ecology 96, 2015−2020.

Yemadje, P.L., Chevallier, T., Guibert, H., Bertrand, I. and Bernoux, M., 2017.

Wetting-drying cycles do not increase organic carbon and nitrogen mineralization in soils with straw amendment. Geoderma 304, 68−75.

Zhao, B., Chen, J., Zhang, J., Qin, S., 2010. Soil microbial biomass and activity response to repeated drying–rewetting cycles along a soil fertility gradient modified by long-term fertilization management practices. Geoderma 160, 218−224.

Table 1. Basic properties of soil and plant residues used in the incubation experiment

	Soil	Residue	Soil + residue
Total organic C (g kg^{-1})	4.8	495.7	9.8
Total N (g kg^{-1})	0.5	31.1	0.8
C/N	9.4	15.9	9.5
Coarse POC (g kg^{-1})	0.6	-	5.5
Fine POC (g kg^{-1})	0.3	-	0.3
Mineral-associated C (g kg^{-1})	3.9	-	3.9
DOC (g kg^{-1})	0.14	-	-
Soil pH	4.3	-	-
Fe_{DCB} (g kg^{-1})	31.3	-	-
Fe_{OX} (g kg^{-1})	2.9	-	-
Al_{DCB} (g kg^{-1})	8.7	-	-
Al_{OX} (g kg^{-1})	0.8	-	-

Figure Captions:

Figure 1 A schematic diagram of the experimental design showing the dry/wet experiment (a) and fluctuating aerobic/hypoxic experiment (b). The circles on the line indicate the time of destructive sampling of soil samples.

Figure 2 Responses of respiration rate and cumulative CO_2 production to variable water (a, c) and O_2 regimes (b, d). Data represent means ± SE (n = 4).

Figure 3 Responses of microbial biomass C and dissolved organic C to variable water (a, c) and O_2 regimes (b, d). Data represent means ± SE (n = 4). The inset graphs show the data at the end of incubation and the horizontal dotted lines indicate initial values before incubation.

Figure 4 Responses of $Fe(II)_{HCl}$, $Fe(III)_{HCl}$ and short-range-ordered Fe (Fe_o) to variable water (a, c, e) and O_2 regimes (b, d, f). Data represent means ± SE (n = 4). The inset graphs show the data at the end of incubation and the horizontal dotted lines indicate initial values before incubation.

Figure 5 Responses of organic C in different fractions to variable water (a, b, c) and O_2 regimes (d, e, f) at the end of the incubation. Data represent means ± SE (n = 4). The horizontal dotted lines indicate initial values before incubation.

Figure 6 A schematic overview of the response patterns of litter decomposition and mineral-associated C formation to drying–rewetting (DRW) cycles and O_2 fluctuations in this study. (a) Soil DRW cycles increased microbial respiration but reduced microbial biomass likely due to increased turnover of microbial biomass and low microbial carbon use efficiency, resulting in decreased mineral-associated organic

C (MAOC). Although DRW cycles promoted iron (Fe) redox cycling, they did not increase short-range-ordered (SRO) Fe production in this study. (b) Oxygen fluctuations reduced both microbial respiration and microbial biomass, likely due to O_2 deficiency, resulting in decreased MAOC. Oxygen fluctuations also promoted Fe redox cycling but did not increase SRO Fe production.The dotted arrows indicate little or no effect. The positive and negative signs indicate positive and negative effect, respectively.

Figure 1

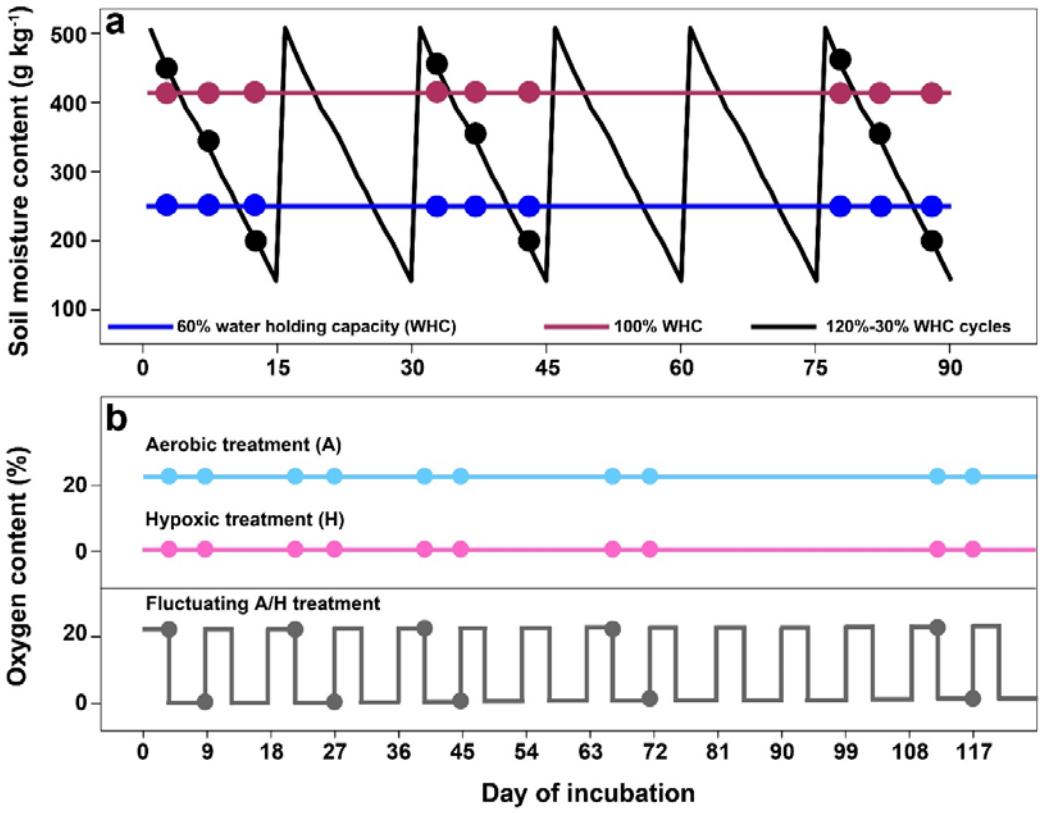

a
Soil moisture content (g kg-1)
500
400
300
200
100
60% water holding capacity (WHC)
100% WHC
120%-30% WHC cycles
0
15
30
45
60
75
90
b
Oxygen content (%)
Aerobic treatment (A)
Hypoxic treatment (H)
Fluctuating A/H treatment
20
0
20
0
0
9
18
27
36
45
54
63
72
81
90
99
108
117
Day of incubation

Figure 2

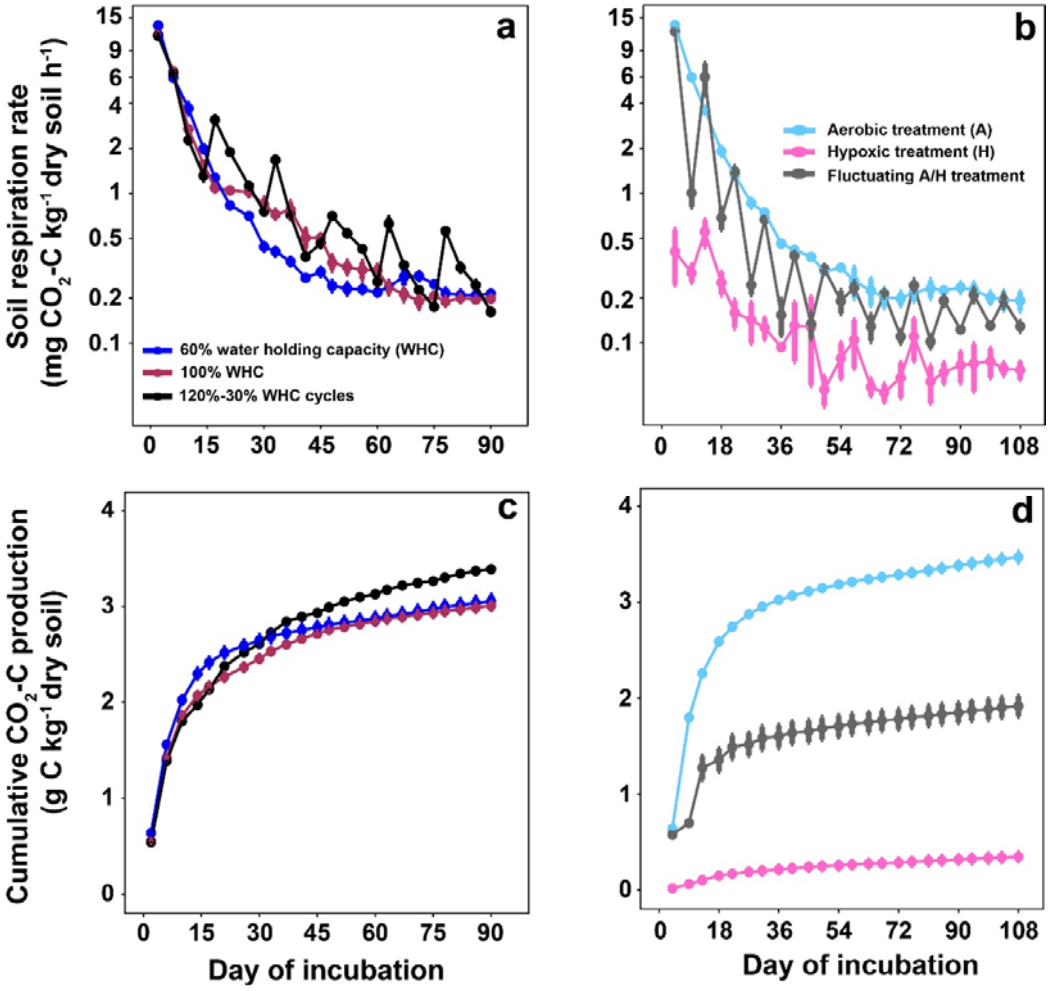

a
b
c
d
Soil respiration rate
(mg CO_2-C kg^{-1} dry soil h^{-1})
60% water holding capacity (WHC)
100% WHC
120%-30% WHC cycles
Aerobic treatment (A)
Hypoxic treatment (H)
Fluctuating A/H treatment
Cumulative CO_2-C production
(g C kg^{-1} dry soil)
Day of incubation
Day of incubation

Figure 3

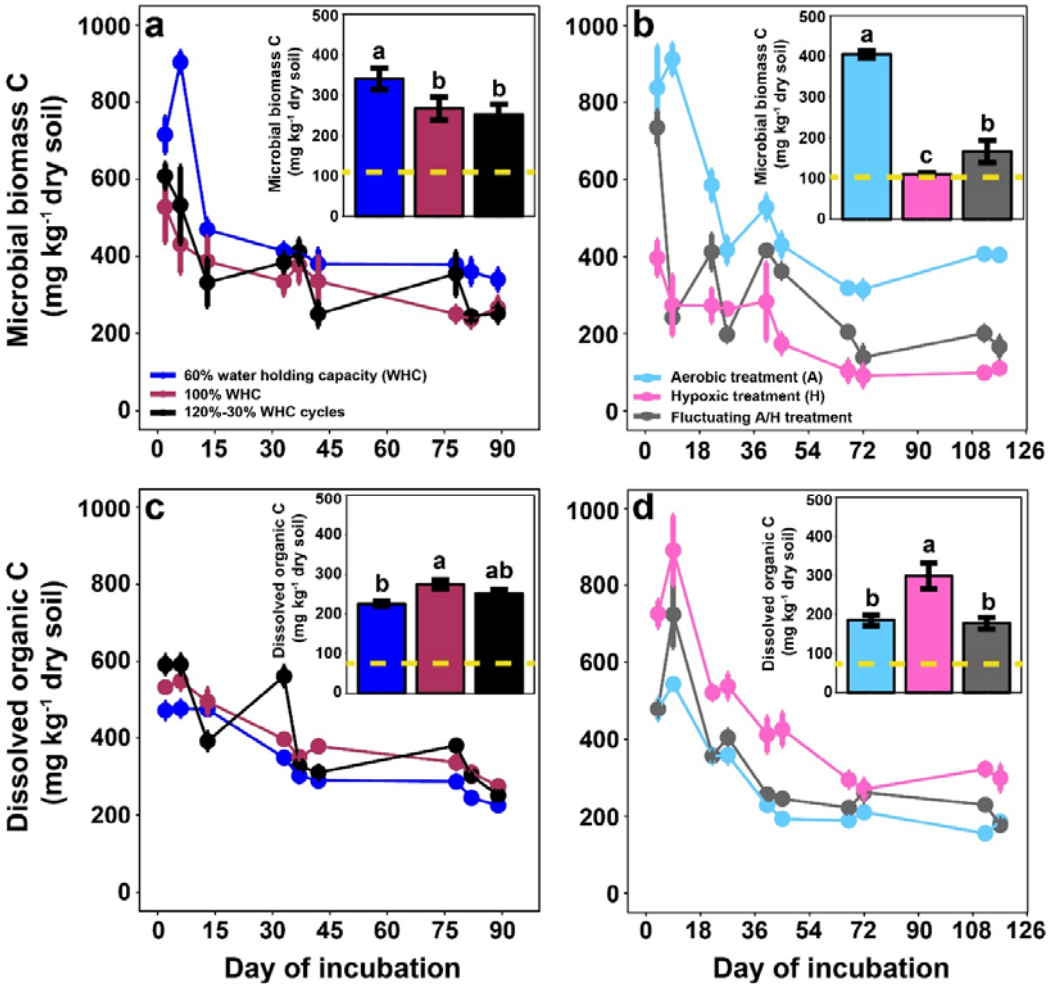

a
b
c
d
Microbial biomass C (mg kg-1 dry soil)
Dissolved organic C (mg kg-1 dry soil)
Day of incubation
60% water holding capacity (WHC)
100% WHC
120%-30% WHC cycles
Aerobic treatment (A)
Hypoxic treatment (H)
Fluctuating A/H treatment

Figure 4

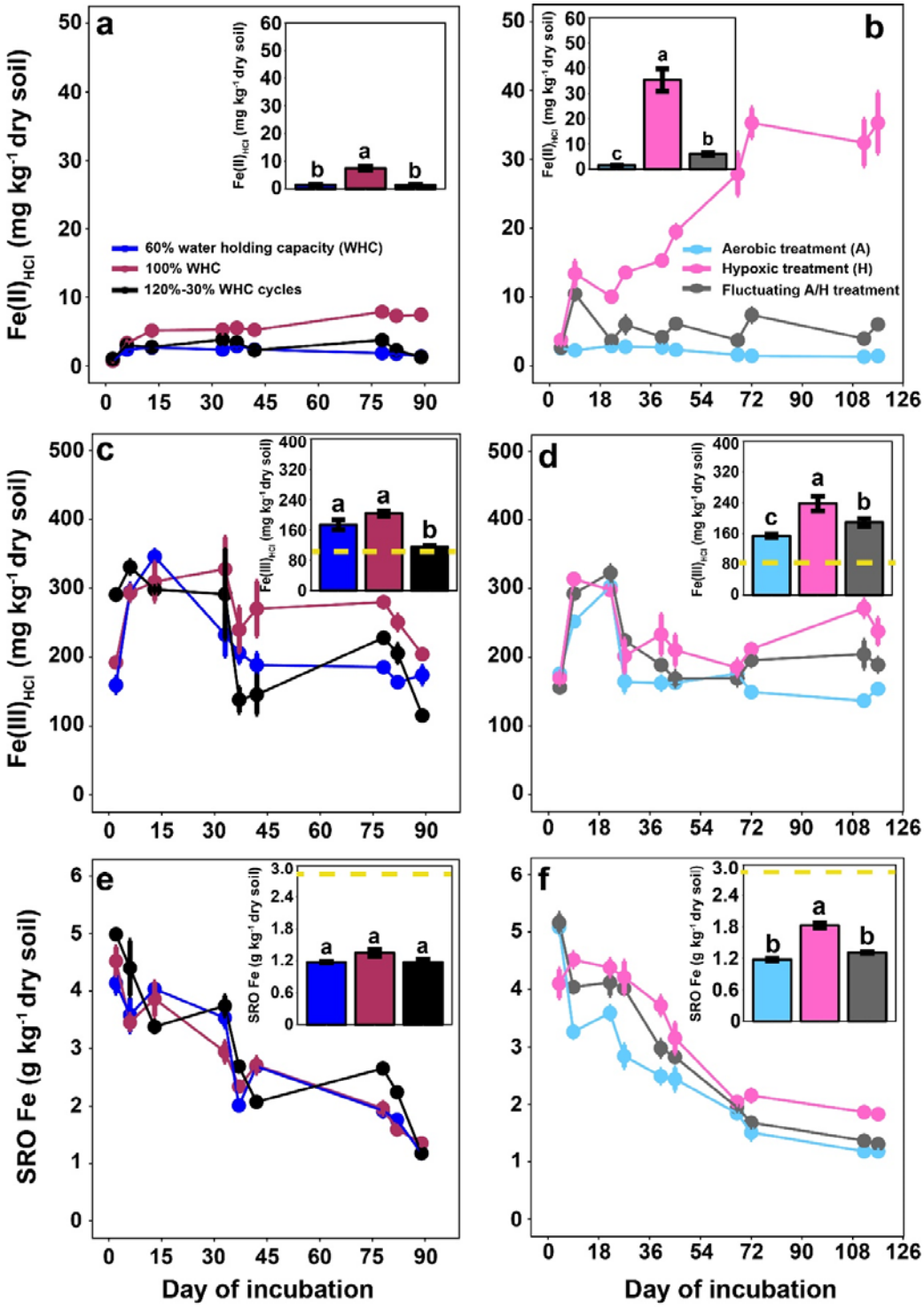

a
b
c
d
e
f
60% water holding capacity (WHC)
100% WHC
120%-30% WHC cycles
Aerobic treatment (A)
Hypoxic treatment (H)
Fluctuating A/H treatment
Fe(II)HCl (mg kg-1 dry soil)
Fe(III)HCl (mg kg-1 dry soil)
SRO Fe (g kg-1 dry soil)
Day of incubation

Figure 5

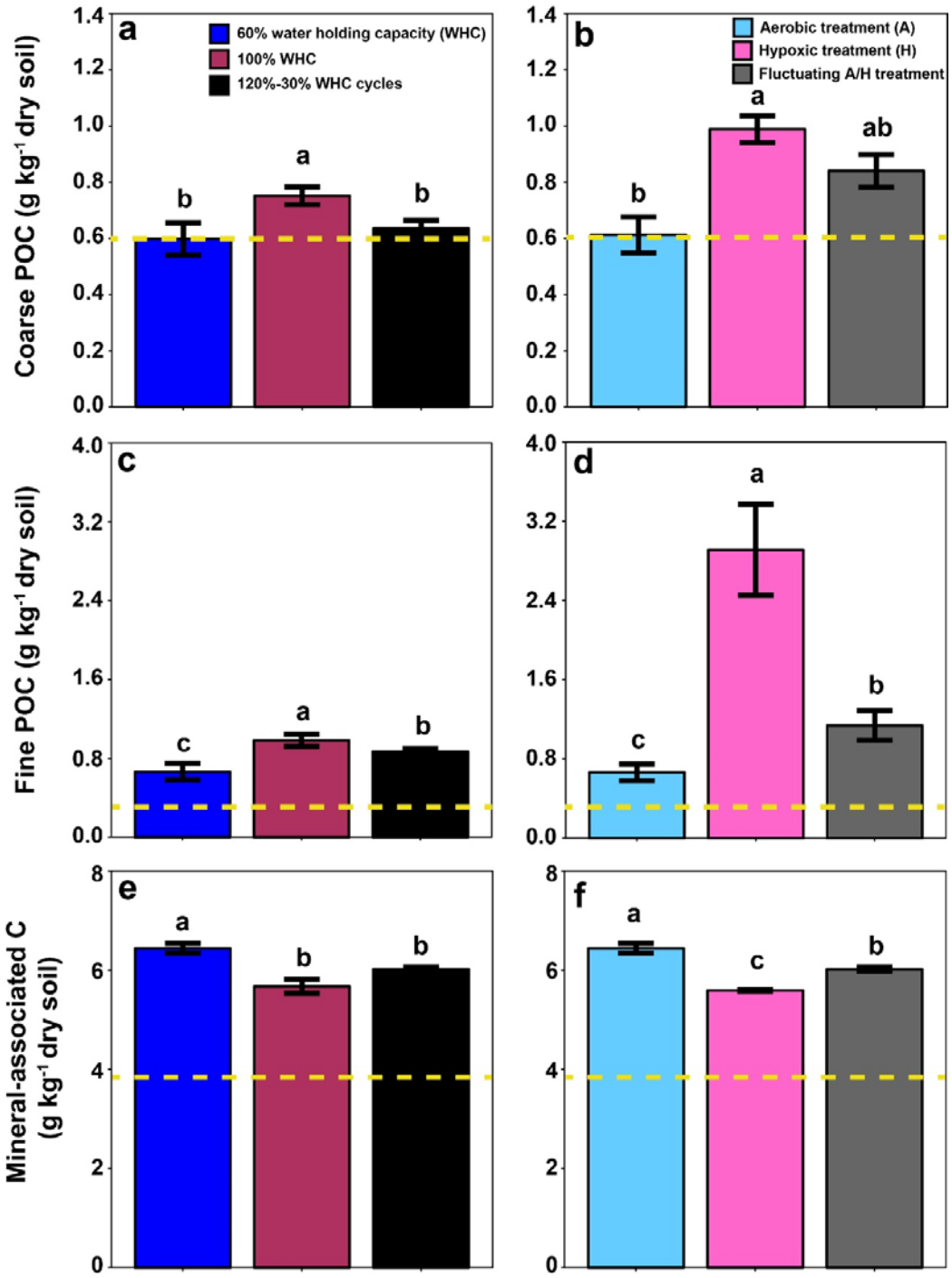
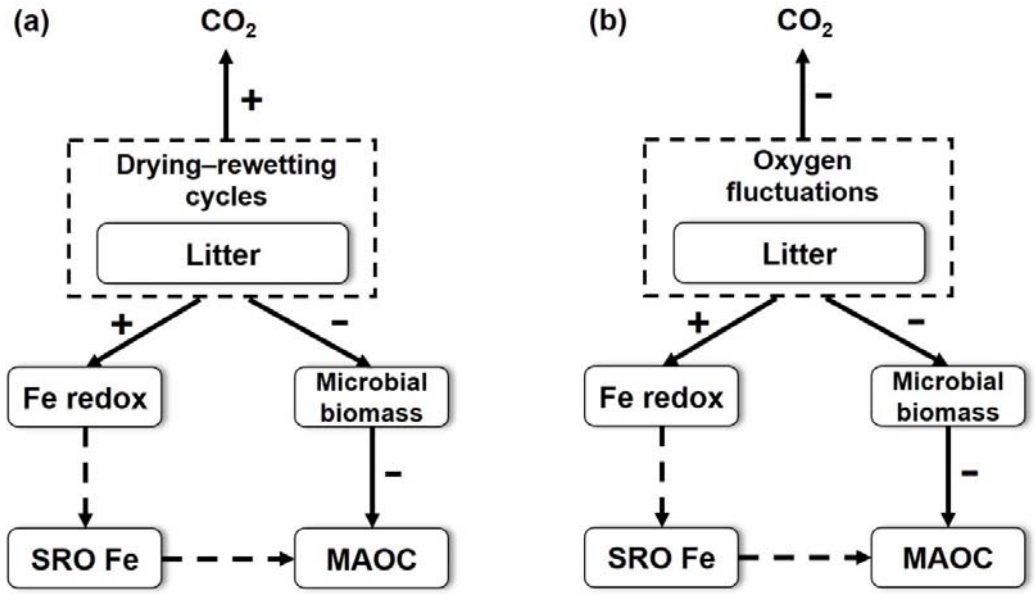

a
60% water holding capacity (WHC)
100% WHC
120%-30% WHC cycles
b
Aerobic treatment (A)
Hypoxic treatment (H)
Fluctuating A/H treatment
c
d
e
f
Coarse POC (g kg-1 dry soil)
Fine POC (g kg-1 dry soil)
Mineral-associated C (g kg-1 dry soil)

Figure 6


(a)
CO_2
+
Drying–rewetting cycles
Litter
+
–
Fe redox
Microbial biomass
–
SRO Fe
MAOC
(b)
CO_2
–
Oxygen fluctuations
Litter
+
–
Fe redox
Microbial biomass
–
SRO Fe
MAOC