

Faster cycling but lower efficiency: A microbial metabolic perspective on carbon loss after wetland conversion to cropland

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ABSTRACT

Natural wetlands store 20–30% of soil organic carbon (C) in terrestrial ecosystems. Wetlands conversion to croplands has caused a massive loss of soil organic C; however, the microbial contribution to this C loss remains elusive. In this study, we took 24 soil cores from a natural wetland and a 23-year cultivated cropland over four seasons to compare the abundance of functional genes encoding 138 enzymes for ten C-cycling pathways classified as organic C degradation, catabolic and anabolic processes. We found that land use-induced C loss was primarily driven by enhanced organic C degradation (~24% increase) but suppressed microbial C assimilation efficiency, represented by shifted catabolism and declined anabolism (~22% decrease). The enhanced organic C degradation was advocated by the enriched genes encoding organic C degradation enzymes; the suppressed microbial C assimilation efficiency was upheld by the shifted catabolic processes and inhibited anabolic processes. Compared with natural wetlands, the croplands showed substantial shifts in catabolic processes - 22% suppression of the Embden-Meyerhof-Parnas pathway, 27% decline in the Citrate Cycle, 22% increase in Entner-Doudoroff, and 25% stimulation in pentose phosphate pathways. Meanwhile, the anabolic process, especially the Wood-Ljungdahl pathway, was significantly suppressed (~55%) in the cropland. The shifts in organic C degradation, catabolism, and anabolism were stronger in spring and summer than in winter and autumn. At the ecosystem level, microbial metabolic quotient was suppressed by 44%, while microbial biomass carbon was boosted by 5% after wetland conversion to cropland, indicating a faster microbial growth rate but lower C use efficiency. Differentiating microbial metabolic processes explained the C loss after wetland cultivation, indicating an urgency to represent microbial metabolism in soil C models.

1. Introduction

Natural wetlands store approximately 20–30% of the soil organic carbon (C), although they only occupy 5–8% of the land surface (Mitsch et al., 2012); a minor C loss in wetlands may yield a substantial impact on the global C cycling (Were et al., 2019; Poulter et al., 2021). Over 50% of wetlands were lost between 1700 and 2020, and 61.7% of total loss was caused by wetland drainage for upland croplands (Hu et al.,

2017; Fluet-Chouinard et al., 2023), leading to a massive wetlands C loss across the globe (Zedler and Kercher, 2005). Soil microorganisms, the hub for C cycling through their vital roles in mineralizing soil organic matter and stabilizing soil organic C via entombing effects (Bardgett et al., 2008; Benner, 2011; Schimel and Schaeffer, 2012; Liang, 2020), are the decisive factor of C stability in soils (Gougoulas et al., 2014).

Soil microorganisms mineralize soil organic C primarily through three processes: organic C degradation through extracellular enzymes,

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catabolic process, and anabolic process, with the last two being endogenous metabolic process (Liang et al., 2017) (Fig. 1). Microbes produce extracellular enzymes to degrade plant residues and soil organic matter to form bioavailable C, readily for microbial assimilation (Schimel and Schaeffer, 2012; Xu et al., 2014). Through this process, a large fraction of C is released as carbon dioxide (CO_2); the relatively small molecules are broken down *in vivo* by microorganisms, which is defined as the catabolic process, producing energy to fuel microbial growth or biosynthesis (Chubukov et al., 2014) (Fig. 1). Anabolism is the energy-consuming process to synthesize building blocks and macromolecules (such as mono and oligomers of fatty acids, amino acids, amino sugars, nucleotides, etc.) in cells (Drotz et al., 2010; Gunina et al., 2014). Those macromolecules will be used for cell formation and growth, maintenance, or extracellular enzyme secretions (Fig. 1). The dynamic balance between catabolic and anabolic processes results in community-level assimilation efficiency and growth rate (Gonzalez-Cabaleiro et al., 2015; Geyer et al., 2016), and further affect the formation of relatively stable soil C by microbial necromass (Liang et al., 2017; Cai et al., 2022).

Though the organic C degradation, catabolic, and anabolic processes jointly determine soil organic C sequestration (Benner, 2011; Fan and Liang, 2015; Kallenbach et al., 2016), it remains elusive how these three processes mediate C cycling after land use change (Pagel et al., 2020). Microbial catabolism and anabolism are essential in cycling accessible organic compounds and producing microbial necromass (Liang et al., 2017; Zhu et al., 2021). The catabolic process is usually estimated as soil microbial respiration, without separating from organic C degradation, as both organic C degradation and catabolic processes release CO_2 (Degens et al., 2001; Drotz et al., 2010). Higher CO_2 release from reclaimed croplands vs. wetlands observed in a meta-data analysis (Knox et al., 2015) would result from enhancement of either organic C degradation or catabolic processes or both. Only a few studies have explored the anabolic capacity of microorganisms by isotopic labeling (Tang et al., 2009; Drotz et al., 2010). Enhanced soil organic C degradation needs more extracellular enzyme secretions, resulting in less synthesis in

anabolic processes in cell formation and growth and, thereby lower C use efficiency (CUE). The functional genes for the organic C degradation process have been widely used to prove the vital roles of microbial metabolisms in soil C sequestration and stabilization (Cheng et al., 2021), while genes in the other two processes have not yet been estimated in C cycling studies. Metagenomic technology provides a powerful tool for a comprehensive quantification of the characteristics of those processes, allowing us to explore the three processes during wetland reclamation and address C preservation and stabilization from a microbial metabolism perspective.

In light of organic C degradation and catabolic processes releasing CO_2 , and anabolic process in cell growth and entombing effect, we hypothesized that wetland cultivation stimulates C degradation while suppressing microbial CUE mediated by catabolic and anabolism processes, resulting in soil C loss. To address this, we investigated the abundance of functional genes encoding organic C degradation, catabolic, and anabolic processes along 0–100 cm soil profiles in four seasons in a pristine wetland and reclaimed cropland. The ecosystem functions, including soil total C storage (TC), microbial biomass C (MBC), respiration potential, and microbial metabolic quotient, were also measured.

2. Materials and methods

2.1. Study area and site description

The Sanjiang Plain is located in the eastern Heilongjiang Province, Northeastern China, and inhabits the largest freshwater wetlands (approximately 10400 km^2) in China (Song et al., 2009). However, this region has experienced approximately 80% loss of natural wetlands since the 1950s due to drainage and land conversion to farmlands, such as paddies and uplands (Wang et al., 2023). This area is characterized by temperate humid and subhumid continental monsoon climates. The mean annual temperature is 2.5 °C, and the mean annual precipitation ranges from 500 to 600 mm (Wang et al., 2010). The study sites are

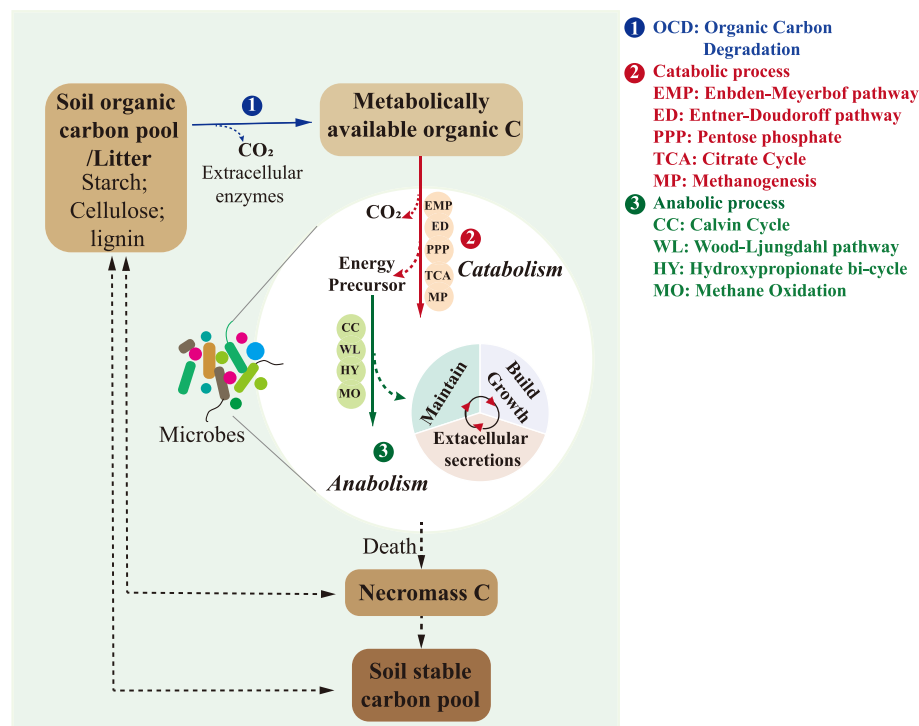


Fig. 1. Schematic diagram of soil microbial C metabolism in terrestrial ecosystems. Soil microbial C metabolism includes three processes: soil organic C degradation (in blue), catabolic process, and anabolic process. Microbial catabolic metabolism (in red) includes: EMP, ED, PPP, TCA, and MP pathways, and anabolic mechanisms include (in green): CC, WL, HY, and MO pathways.

located in the Sanjiang Mire Wetland Experimental Station, Chinese Academy of Sciences, Tongjiang City (56 m a.s.l.). Natural freshwater wetlands and adjacent cultivated soybean cropland were selected as our sampling sites. The wetland site (133°30'E, 47°35'N) was dominated by *Carex meyeriana*, *Carex lasiocarpa*, and *Deyeuxia angustifolia* communities, and a water layer of 30–35 cm depth covers the surface of the natural wetlands throughout the year. The cropland site (133°30'E, 47°36'N) was converted from a wetland in 1996 and has been kept cultivated with soybean (*Glycine max* [L.] Merr.) from May to October yearly. The soil parent material was sediment originating from the Quaternary period, and the soils were classified as Albaquic Paleudalfs with a silty clay texture.

2.2. Experiment design and sample collection

We chose three sampling sites in wetland and cultivated cropland, respectively. The soil samples were collected in four seasons: Autumn (October 2019), Winter (January 2020), Spring (May 2020), and Summer (July 2020). Three soil cores were randomly excavated from each sampling site. Every soil core was 100 cm deep and divided into ten sections of 10 cm each. Three soil cores in each sampling site were mixed in layers. Finally, 24 soil cores and 240 soil samples were collected from wetlands and cropland across four seasons. Soils were packed in polyethylene bags immediately after collection, cooled with ice packs, and returned to the laboratory for further analysis.

2.3. Determination of soil total C, microbial biomass C, and respiration

To compare C composition and cycling between natural wetlands and cultivated cropland, soil total C (TC), MBC, and anaerobic respiration were estimated for every soil sample. Soil TC was determined by Multi N/C 2100 TOC analyzer (Analytikjena, Germany) with high temperature (1100 °C) combustion method using air-dried soil samples. The MBC was measured using the fumigation extraction method (Vance et al., 1987). Microbial respiration was determined with an incubation experiment. Specifically, a fresh soil of ~20 g dry weight from wetlands was sealed in a bottle with 100 ml distilled H₂O. Then, nitrogen gas was used to fill the bottle to maintain an anaerobic environment. For soil samplings from croplands, fresh soil without additional water and nitrogen gas was used in the incubation experiments. Every sample had three replicates. All bottles were put into an incubator at 30 °C for 5 h. Before and after incubation, 10 ml gas was extracted using injectors and measured by chromatography (Agilent 7800, USA).

2.4. Metagenomic sequence analyses

Soil total DNA was extracted using the fastDNA® Spin Kit (M.P. Biomedicals, Inc., CA, USA). We successfully obtained DNA from 214 samples. Metagenomic sequencing was performed at Illumina NovaSeq 6000 (Illumina Inc., San Diego, CA, USA) using NovaSeq Reagent Kits at Majorbio Bio-Pharm Technology Co. Ltd (Shanghai, China). Sequence quality and downstream processing, including removing adaptor sequences, trimming, and removing low-quality reads, were evaluated with FastQC (<https://github.com/OpenGene/fastp>, version 0.20.0) (Chen et al., 2018). High-quality reads obtained were then used to assemble contigs using MEGAHIT (parameters: *kmer_min* = 47, *kmer_max* = 97, *step* = 10. <https://github.com/voutcn/megahit>, version 1.1.2) (Li et al., 2015). Contigs lengths over 300 bp were used to identify open reading frames (ORFs) using MetaGene (<http://metagene.cb.k.u-tokyo.ac.jp/>) (Noguchi et al., 2006), and the predicted ORFs with lengths being or over 100 bp were retrieved and translated into amino acid sequences using the NCBI translation table (<http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi?chapter=tgencodes#SG1>). A non-redundant gene catalog was conducted using CD-HIT (<http://www.bioinformatics.org/cd-hit/>, version 4.6.1) with 90% sequence identity and 90% coverage. Then reads after quality

control were mapped to the non-redundant gene catalog with 95% identity using SOAPaligner (<http://soap.genomics.org.cn/>, version 2.21) (Li et al., 2008) and were evaluated for gene abundance in each sample. Representative sequences of non-redundant gene catalog were annotated based on the NCBI NR database using blastp as implemented in DIAMOND version 0.9.19 with an e-value cutoff of $1e^{-5}$ using Diamond (<http://www.diamondsearch.org/index.php>, version 0.8.35) for taxonomic annotations. The genes annotations were conducted using Diamond (Buchfink et al., 2015) and HMMER against the multiple protein databases (Kyoto Encyclopedia of Genes and Genomes, KEGG; Cluster of orthologous groups of proteins, COG; and CAZy). Enzyme Nomenclature (EC number system) was used to identify the KEGG enzyme.

2.5. Statistical analysis

In this study, 138 ECs participating in C cycling were selected, which involved in organic C degradation, catabolism, and anabolism processes and could be sorted into 9 pathways, including Embden-Meyerhof-Parnas pathway (EMP), Entner-Doudoroff pathway (ED), Pentose phosphate pathway (PPP), Citrate Cycle (TCA), Methanogenesis (MP), Calvin Cycle (CC), Wood-Ljungdahl pathway (WL), Hydroxypropionate bi-cycle (HY), and Methane oxidation (MO) (Fig. 1, Table S1). The gene abundances were rarefied to 1 million observations per sample, which is parts per million (ppm) (Wang et al., 2023). Then the effect size was calculated using the standardized mean difference metric “Hedges’ *d*” to evaluate the effect of wetland cultivation on functional genes (Response Ratios: RRs) (Wu et al., 2020). The equation is: $Hedges' d = \frac{\bar{X}_w - \bar{X}_c}{S} J$, where $\bar{X}_w$ and $\bar{X}_c$ denote the mean value of functional genes abundances for wetland and cropland, respectively. *S* is the pooled SD based on the SDs per pair of functional genes (Han and Zhu, 2020). *J* is a weighting factor to screen the small-sample-size bias of the standardized difference of means (Dai et al., 2018). It is calculated as: $J = 1 - \left[\frac{3}{4(N_w + N_c - 2) - 1} \right]$, where *N_w* and *N_c* are the samples size of each functional gene for wetland and cropland, respectively.

Soil C loss by respiration was estimated by respiration rate per unit C, and microbial metabolic quotient was calculated by respiration per MBC (Zhu et al., 2021). The microbial CUE is negatively correlated with the microbial metabolic quotient (Geyer et al., 2016; Xu et al., 2017). Structural equation models (SEMs) were constructed using “lavaan” package (Smith et al., 2021), and 225 independent observations were included to estimate the influence of three C metabolism processes on soil C, MBC, soil C loss, and microbial metabolic quotient under land use change. Principal component analysis was used to reduce dimensionality (Wang et al., 2022). The potential relative importance of C metabolism pathways to soil TC, MBC, anaerobic respiration per unit C, and anaerobic respiration per MBC in wetland and cultivated cropland were estimated based on random forest (RF) analyses using the “randomForest” package in R (version 4.2.0). In RF models, C cycling pathways were predictors for anaerobic respiration potential, TC, and MBC after dimensionality reduction based on factor analyses. To estimate the importance of each metabolic pathway, the percentage increases in the MSE (mean squared error) of pathways were calculated: higher MSE% values imply more importance of pathways (Jiao et al., 2018). The significance of models and cross-validated *R*² were estimated with 1000 permutations of the response variable by using the “A3” package in R (version 4.2.0). The significance of each predictor pathway was assessed with the “rfrPermute” package. The differences of all genes and respiration, respiration per unit C, and microbial metabolic quotient were estimated by Kruskal-Wallis. The functional genes and other parameters across seasons and along soil profiles were compared by using ANOVA test, and logarithm transformation was used to achieve normality. All statistical analysis and graph production were performed in R (version 4.2.0 on Windows 10).

3. Results

3.1. Carbon content, microbial biomass, and respired C in wetland vs. cropland soils

Wetland cultivation significantly altered TC, MBC, respiration potential per unit TC, and microbial metabolic quotient, and the impacts varied among seasons. Specifically, soil TC in the wetland was significantly higher than in cropland across four seasons (Fig. 2a, $P < 0.05$). However, soil MBC in the wetland was significantly lower than that in cropland annually (Fig. 2b, $P = 0.034$), but the difference between wetland and cropland was insignificant in each season ($P > 0.05$). The soil anaerobic respiration potential per unit soil organic C content was higher in cropland than in wetlands (Fig. 2c, $P = 0.025$), which was more remarkable in spring and summer ($P < 0.001$). In contrast, the soil microbial metabolic quotient was significantly higher in the wetland than in the cropland (Fig. 2d).

Soil MBC, respiration per unit soil TC, and microbial metabolic quotient showed clear seasonality (Fig. 2). Soil MBC reached its lowest value in winter but highest in autumn in both wetland and cropland (Fig. 2b, $P < 0.05$). The seasonal ranking of the respiration rate per unit C and microbial metabolic quotient were similar in wetland and cropland: summer > spring > autumn > winter (Fig. 2, $P < 0.05$).

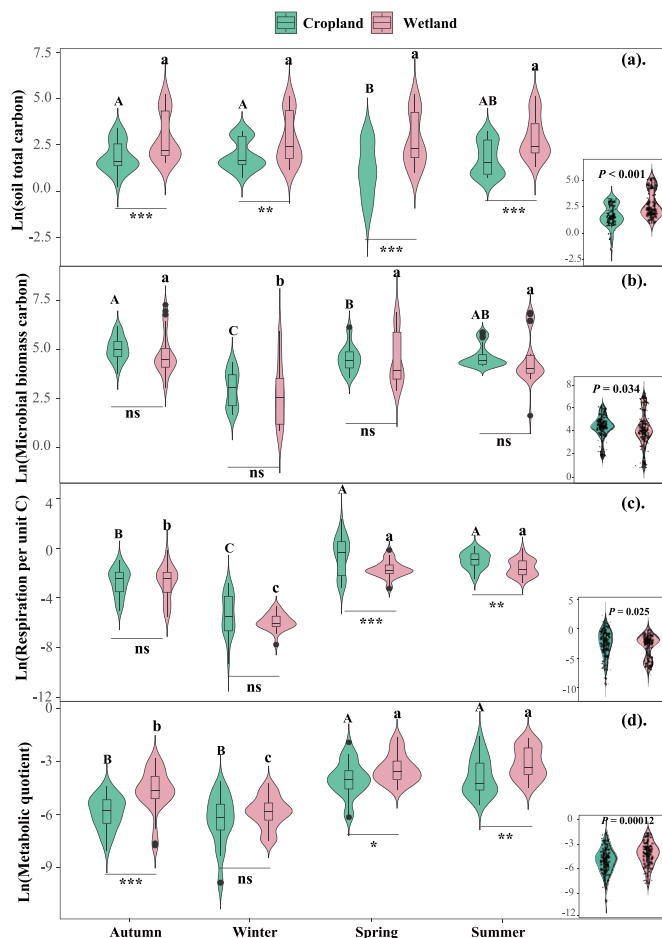


Fig. 2. Cultivation impacts on soil total C (TC), microbial biomass C (MBC), anaerobic potential respiration rate per unit C, and microbial metabolic quotient across seasons. “***”, “**”, and “*” indicates the differences between wetland and cropland are significant at the level of $P = 0.05$, $P = 0.01$, $P = 0.001$, respectively. The same letters indicate indifference across seasons at the significance level of $P = 0.05$. The insets illustrate mean annual values for key parameters.

3.2. Cultivation impacts on functional genes for C cycling

We investigated a total of 138 ECs involved in the C cycling (Table S1), including 15 ECs for the organic C degradation process (Fig. 3a), 80 ECs for the C catabolic process (Figs. 3c), and 48 ECs for the anabolic process (Fig. 4), and 5 ECs shared in multiple pathways. Based on the weighted RRs for the functional genes in wetland and cultivated cropland, the cultivation significantly stimulated functional genes of enzymes that catalyzed both labile and recalcitrant C degradation (Fig. 3a) except chitinases such as EC 3.2.1.96. Another exception was EC. 3.2.135, which encodes glucosylhydrolase and was lower in cropland than in natural wetlands (Fig. 3a).

The response ratios of different pathways in the catabolic process varied; cultivation enhanced ED and PPP while suppressed EMP, TCA, and MP pathways (Fig. 3b and c). More specifically, there were 23 ECs detected in the EMP pathway, 9 ECs were suppressed, while 4 ECs were enhanced including aldose 1-epimerase (5.1.3.3), glucokinase (2.7.1.2), phosphohexose isomerase (5.3.1.9), and hexose diphosphatase (3.1.3.11) (Fig. 3b and c, Table S2). Moreover, all 4 functional genes in the ED pathway and 5 of 8 ECs in the PPP pathway were significantly more abundant in cultivated cropland than in natural wetlands (Fig. 3b and c, Table S2). A total of 16 ECs were measured for the TCA pathway, 7 ECs decreased significantly, 9 ECs remained unchanged, and only one (1.1.1.41, encoding isocitric dehydrogenase) increased. For the MP pathway, 14 ECs were estimated, 11 ECs decreased, 1 EC increased, and 2 ECs remained unchanged (Fig. 3b and c, Table S2).

The cultivation effects on functional genes varied among different anabolic pathways. Specifically, 11, 6, 27, and 4 ECs were measured for CC, WL, HY, and MO pathways, respectively (Fig. 4). For the CC pathway, EC.3.1.3.11 and EC.1.2.1.13 that encoded D-fructose-1,6-bisphosphate 1-phosphohydrolase and D-glyceraldehyde-3-phosphate oxidoreductase, respectively, enriched; while EC 4.1.1.39, EC.1.2.1.59, and EC.4.1.2.13 were suppressed. The other 6 ECs remained unchanged after cultivation (Fig. 4a, e, Table S1, Table S2). Cultivation decreased 3 ECs for the WL pathway, including EC.2.3.1.169, EC.6.3.4.3, and EC.2.1.1.258 that encoded CO-methylating acetyl-CoA synthase, tetrahydrofolate ligase, and Co-methyltransferase, respectively, and 3 others remained unchanged (Fig. 4b, e, Table S1, Table S2). Most ECs (19 of 27) for the HY pathway remained unchanged between wetland and cropland, with 5 ECs decreased and 3 ECs increased after wetland cultivation (Fig. 4c, e, Table S1, Table S2). However, 2 ECs for the MO pathway were enhanced after the wetland cultivation, including EC.1.14.18.3 (encoding particulate methane monooxygenase) and EC.1.1.3.13 (encoding alcohol oxidase) (Fig. 4d and e, Table S1, Table S2).

3.3. Seasonality of functional genes along soil profiles in wetland and cropland

The abundance of functional genes for C cycling showed an apparent seasonality in both wetland and cropland. For the organic C degradation process, seasonality for 3 ECs was consistent between wetland and cropland, including EC.3.2.1.3, EC.3.2.1.41, and EC.1.11.1.7. The other 12 ECs were higher in spring, summer, and autumn but lower in winter (Fig. S1, Table S2). For the catabolic process, 8 ECs in the EMP pathway did not show seasonality, while the other 15 ECs yielded seasonality (Fig. S1, Table S2). One of 4 ECs for ED, 5 of 16 ECs for TCA, one of 8 ECs for PPP, and 4 of 14 ECs for the MP pathway did not show seasonality, but most other ECs for those pathways were higher in spring, summer, and autumn than in winter (Fig. S1).

For the anabolic process, only 1 of 11 ECs for the CC pathway, 1 of 6 ECs for the WL pathway, and 2 of 27 ECs for the HY pathway did not show seasonality, while all other ECs for these three pathways and all ECs in MO pathway fluctuated over seasons – the highest in spring or summer and the lowest in winter (Fig. S2, Table S2). Moreover, the magnitudes of the seasonality of organic C degradation, catabolic, and anabolic processes were more remarkable in natural wetlands than in

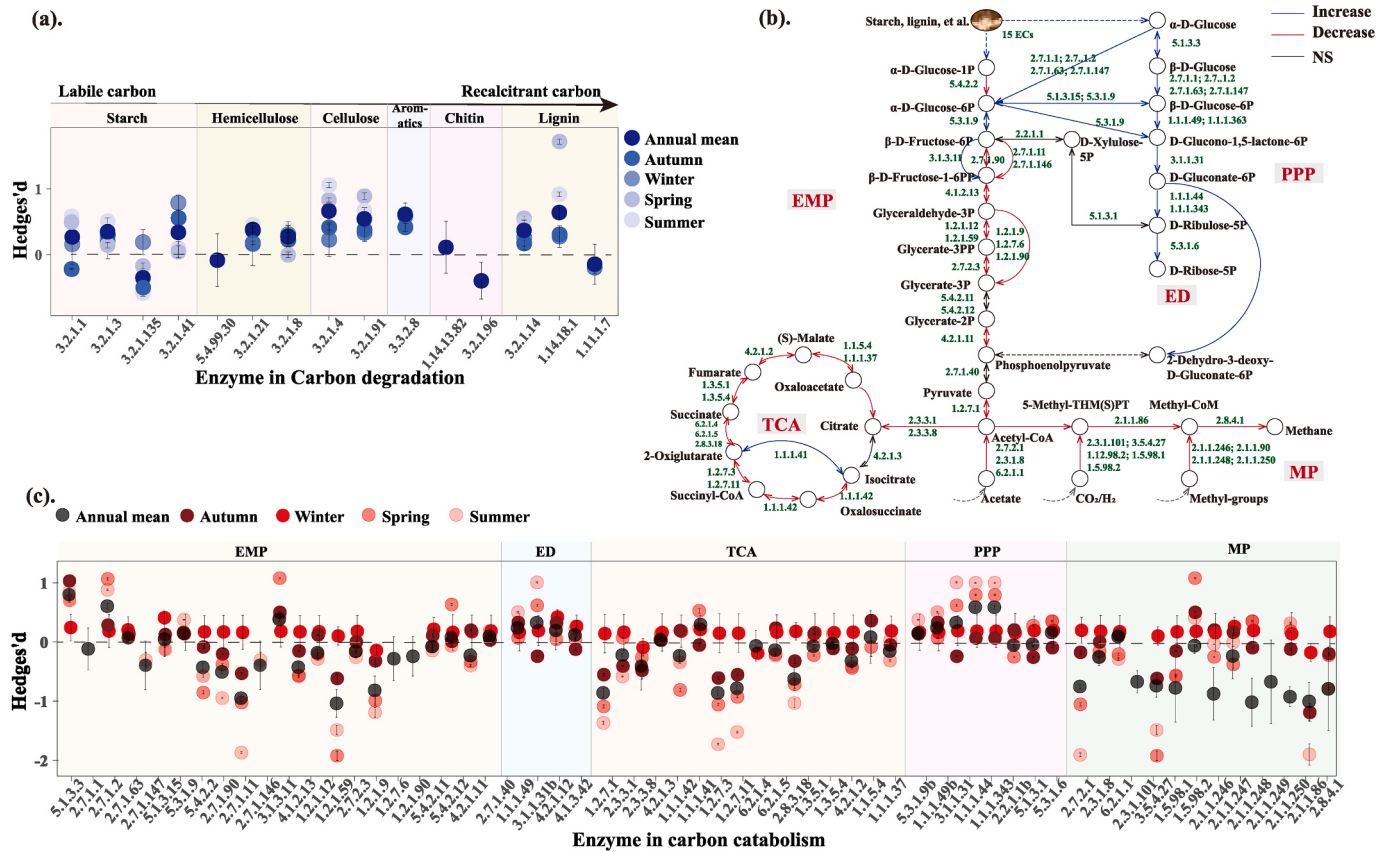


Fig. 3. Responses of C degradation and catabolic genes to wetland cultivation. (a). Effects of wetland cultivation on ECs for C degradation process. (b). Catabolic pathways of C cycling and wetland cultivation impacts. (c). Effects of wetland cultivation on ECs for the catabolic processes. The blue lines indicate promotion, the red lines indicate inhibition, and NS (black line) indicates insignificance at the significance level of $P = 0.05$.

cropland (Fig. S2).

The functional genes for C cycling varied along soil layers (Table S2, Fig. S1, Fig. S2). For the organic C degradation process, all but 2 ECs (EC.3.2.1.92 and EC.1.14.18.1) significantly decreased along soil profiles (Table S2, Fig. S1, $P < 0.05$). For the catabolic process, 17 ECs in EMP, 2 ECs in ED, 11 ECs in TCA, 7 ECs in PPP, and 7 ECs in MP significantly changed along soil profiles (Table S2, Fig. S1, $P < 0.05$), and except ECs of MP in cropland and EC. 1.5.88.2 in both wetland and cropland, all varied ECs reduced along soil profiles. In the anabolic process, 7 ECs in CC, 2 ECs in WL, 21 ECs in HY, and all ECs in MO significantly decreased along soil profiles (Table S2, Fig. S1, $P < 0.05$).

3.4. Metabolic pathways affecting soil C content and anaerobic respiration

The SEMs showed significant impact of wetland cultivation on ECs in organic C degradation, catabolism, and anabolism processes, as well as soil TC, MBC, soil C loss, and microbial metabolic quotient (Fig. 5). Cultivation had positive effect on organic C degradation (Fig. 5, path coefficient = 0.45, $P < 0.001$) and anabolic processes (Figs. 5 and 0.17, $P < 0.01$), while has significant negative effect on catabolic processes (Figs. 5, -0.17 , $P < 0.01$). Soil C loss due to cultivation was directly associated with higher ECs in organic C degradation (Figs. 5a and 0.18, $P < 0.01$), lower ECs in catabolic process (Figs. 5a and 0.32, $P < 0.001$), and higher ECs in anabolic process (Figs. 5b, -0.21 , $P < 0.001$). Soil C loss was also indirectly enhanced by lower CUE, which impact by catabolic and anabolic processes (Fig. 5a). Consistent with this, wetland cultivation reduced soil C content directly (Figs. 5b, -0.24 , $P < 0.001$) and also affected by ECs in catabolic process (Figs. 5b and 0.12, $P < 0.05$), and ECs in anabolic process (Figs. 5b, -0.17 , $P < 0.001$). Soil C

content was also positively related to MBC, which was affected by ECs in organic C degradation (Figs. 5b and 0.19, $P < 0.01$) and catabolic processes (Figs. 5b and 0.54, $P < 0.001$), while was impacted by ECs in the anabolic process (Figs. 5b, -0.29 , $P < 0.001$) negatively.

The fractional contribution of the individual pathway to soil TC, MBC, and anaerobic respiration rate per unit C and microbial metabolic quotient differed between wetland and cropland. The RF models could explain 54.15% and 59.17% of variance related to soil TC in wetland and cropland, respectively (Fig. S3). In the wetland, EMP was potentially the most critical pathway to TC, with a 23.7% increase in the MSE. WL (12.5%), organic C degradation (10.2%), CC (9.2%), MP (8.9%), TCA (7.4%), and PPP (5.1%) also significantly contributed to TC in the wetland (Fig. S3a). In cropland, MP (33.5) contributed the most to TC, followed by the EMP (29.1%), PPP (18.8%), TCA (17.6%), CD (15.3%), HY (10.3%), CC (10.0%), and WL (7.9%) (Fig. S3b). The explanations of pathways to MBC were 49.17% in wetlands and 53.06% in cropland (Fig. S3). Ranked by the importance value of pathways for MBC in the wetland, the most important pathway was EMP (23.5%), followed by MP (14.5%), CC (13.8%), WL (12.5%), TCA (8.4%), ED (6.0%), and MO (3.3%) (Fig. S3). In cropland, the most important pathway was MP, with MSE reaching 21.5%. Ranked by pathways, the pathways of EMP (19.9%), HY (13.6%), PPP (12.3%), MO (11.5%), TCA (9.4%), and WL (8.9%) significantly contributed to MBC (Fig. S3).

The contributions of C cycling pathways to the potential respiration rate per unit C differed between wetland and cropland, explaining 44.71% and 48.31% of variance related to anaerobic respiration, respectively ($P < 0.01$) (Fig. S3). In the wetland, EMP (20.4%) and CC (17.0%) contributed the most to anaerobic respiration rate per unit C in wetland, followed by MP (14.2%), PPP (6.9%), HY (6.9%), TCA (6.9%), and WL (6.8%) (Fig. S3a). While in cropland, the most important

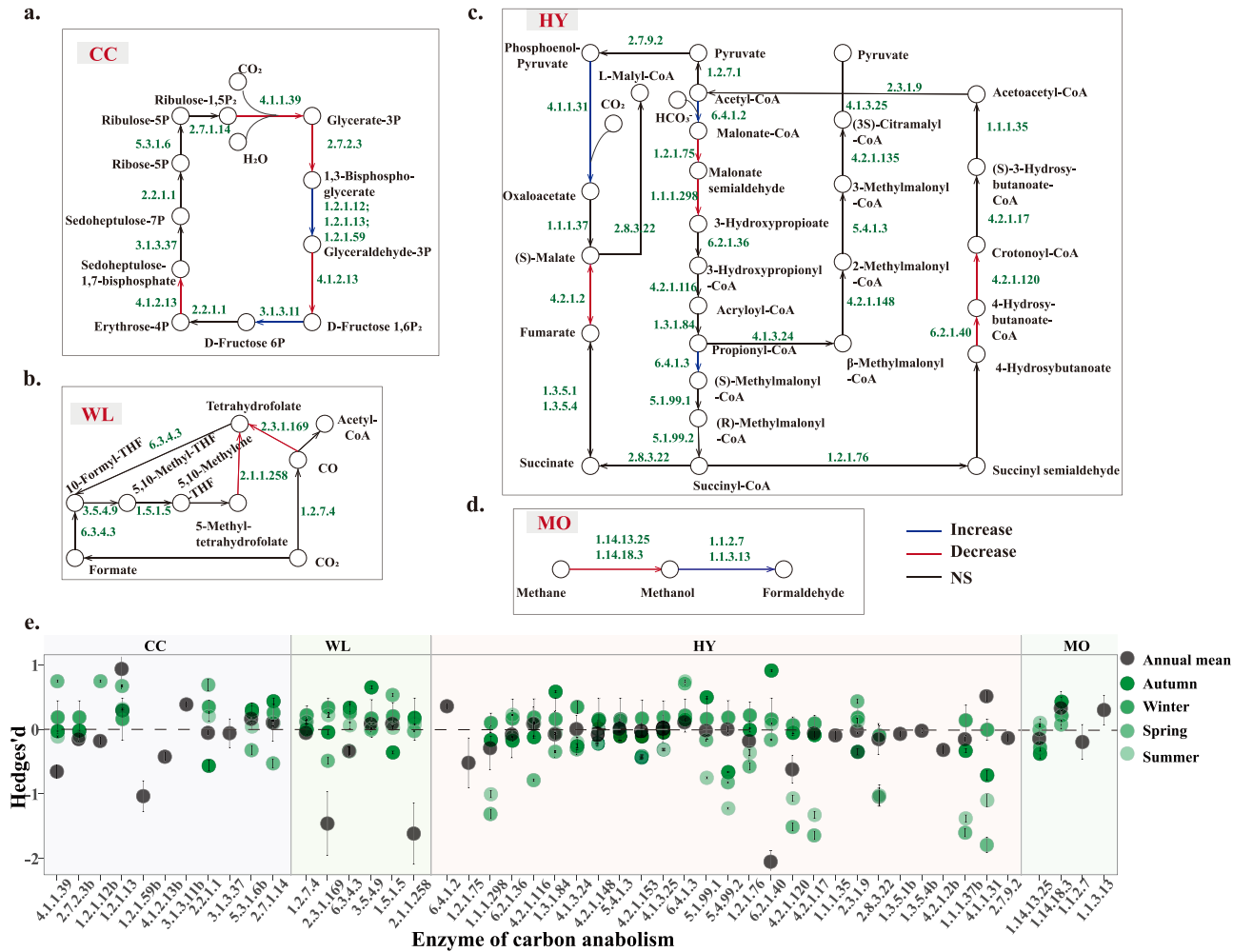


Fig. 4. Responses of C anabolic genes to wetland cultivation. (a). CC pathway; (b). WL pathway; (c). HY pathway; (d). MO pathway; (e). Response of ECs for anabolic processes to wetland cultivation. The blue lines indicate promotion, the red lines indicate inhibition, and NS (black line) indicates insignificance at the significance level of $P = 0.05$.

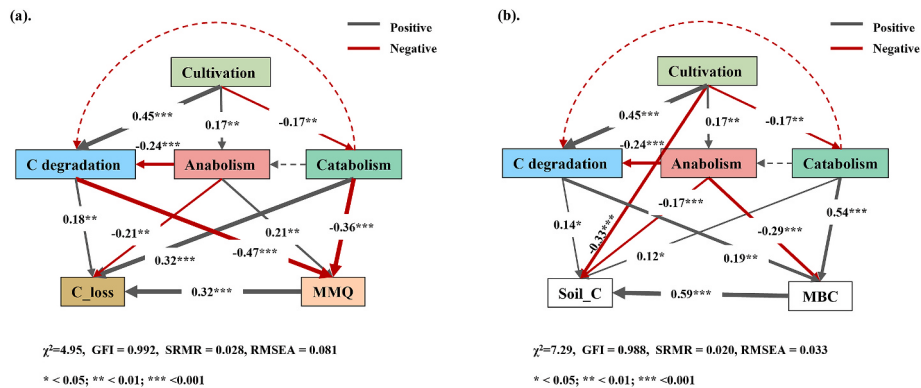


Fig. 5. Results of structural equation model (SEM). (a). The path diagram shows the relationships of three processes with C loss and microbial metabolic quotient (MMQ). (b). The path diagram shows the relationships of three processes with soil C content and MBC. Line thickness corresponds to the strength of the relationship based on standardized path coefficients. Dark grey lines indicate a positive relationship and red lines are negative. * indicates the significance level of path is $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P < 0.001$.

pathway was MP, reaching 29.0%. Additionally, EMP (10.1%), HY (9.1%), organic C degradation (9.0%), WL (8.8%), PPP (7.4%), and CC (6.6%) also significantly contributed to respiration per unit C in cropland (Fig. S3b). The explanations of pathways to microbial metabolic quotient were 32.9% in wetlands and 61.28% in croplands (Fig. S3). The

EMP was the most important potential pathway to the microbial metabolic quotient, with a 21.1% increase in the MSE. Ranked other pathways, the pathways of CC (8.1%), ED (7.6%), WL (7.4%), TCA (6.7%), MP (6.6%), and organic C degradation (5.4%) also significantly contributed to microbial metabolic quotient (Fig. S3a). In cropland, the

most important pathway was EMP (30.2%), followed by ED (13.3%), TCA (12.5%), MP (10.2%), MO (9.3%), CD (6.1) and HY (6.1%) (Fig. S3b).

4. Discussion

4.1. Cultivation's promoting impact on soil C degradation

The first stage (organic C degradation) involves the breakdown of high-molecular-weight C. We identified 15 ECs in this degradation process, and our results showed that 10 out of 15 ECs were enhanced by wetland cultivation (Fig. 3a), especially in spring and summer (Table S2). Those enriched genes encode enzymes involved in the degradation of starch, cellulose, hemicellulose, and lignin. Cellulose, hemicellulose, and lignin comprise the largest proportion of plant-derived polysaccharides (Coûteaux et al., 1995). It implies that wetland cultivation stimulates plant-derived C degradation. Moreover, the stimulation of degradation potential is not only on labile (cellulose, hemicellulose) but also on recalcitrant (lignin) C. Consistent with the molecular data, previous studies also found that wetland cultivation could decrease soil labile C pool (Zhang et al., 2006; Song et al., 2012). Meanwhile, the heavy fraction of organic C, which would be more recalcitrant for degradation, decreased by about 62% after wetland conversion to dry land (Zhao et al., 2020). The enriched functional genes confirmed the C loss by stimulating microbial decomposition on plant-derived C, both labile and recalcitrant components, after wetland cultivation.

Additionally, genes involved in chitin degradation were depressed in cultivated cropland (Fig. 3). Chitin is a component of the cell walls of soil fungi and the external skeletons of invertebrates (Vorob'ev et al., 2007). Thus, the depressing of genes involved in chitin degradation indicates that the degradation of fungi-derived C would be less in cropland than in wetlands. A previous study found a depression of fungi in cropland vs. wetland at the same location by quantitative measurement of the phospholipid fatty acids analysis (Zhu et al., 2022). We argue that the suppression of chitin decomposition might result from less fungi-derived C in cropland soil, which provide less substrate for microbes preferencing chitin. Together, the changes in gene abundance in organic C degradation processes suggest that the decay of plant-derived C was enhanced in cropland, regardless of labile or recalcitrant. In contrast, the decomposition of microbial-derived C might be depressed due to less fungi-derived C accumulation in cultivated cropland.

4.2. Cultivation impacts on catabolic vs. anabolic processes

The genes in catabolic processes transferred from EMP and TCA dominant to ED and PPP dominant after wetland cultivation, accompanied by MP decreasing significantly (Fig. 3b and c). In contrast with EMP and TCA pathways, the ED or PPP pathway can be viewed as an alternative to the EMP glycolytic pathway, which is more straightforward and energetically less efficient while better designed for biosynthesis (Conway, 1992; Bender, 2013). The conversion of the catabolic process from EMP and TCA pathways to ED and PPP pathways refers to less ATP that could be accessed from the catabolic process in cropland. In addition, the EMP pathway uses several folds more protein than ED to metabolize the same amount of glucose per second, which would limit the growth of microbes (Dekel and Alon, 2005; Molenaar et al., 2009; Scott et al., 2010; Schuetz et al., 2012). Weaker enzyme synthesis by using the ED pathway could allow cells to increase their growth rates, which is confirmed by a slight but significant increase of MBC in cropland (Fig. 2). It would also allow microbial cells to save protein for the synthesis of more extracellular enzymes for achieving more C. The cost of this conversion was that the C assimilation efficiency would decline in wetland cultivation, which was also proved by a higher respiration rate per unit C (Fig. 2). The anaerobic-aerobic transition from wetland to cropland also verifies this conversion of the catabolic pathway,

consistent with a previous kinetic analysis that reported the reliance of energy-deprived anaerobes upon the higher ATP yield of the EMP pathway (Bar-Even et al., 2012). In contrast, the ED pathway is common among facultative anaerobes and even more common in the aerobes (Flamholz et al., 2013).

In anabolic processes, CC and HY remained unchanged after wetland cultivation, while WL declined (Fig. 4), which further suppressed the efficiency of *in vivo* turnover, implying the anabolic re-synthesis of microbes might be depressed. *In vivo* transformation is the critical process to drive mineral-associated organic C formation and has also been found to be stabilized more than plant-derived C (Ma et al., 2018), thus benefits long-term C sequestration in soil (Liang et al., 2017; Lavalley et al., 2020). The WL pathway, or the reductive pathway acetyl-CoA, is also the most extensive C fixation pathway in the anaerobic condition (de Souza and Rosado, 2019). Less abundant genes in the WL process indicated a decrease in microbial-derived mineral-associated C, leading to lower C sequestration in cropland. Accompanied by the enhancement of the ED pathway, anaerobic-aerobic transfer after wetland conversion to cropland might be the predominant reason that declines C sequestration by changing the microbial catabolic and anabolic processes. Furthermore, the suppression of anabolic *in vivo* is indirectly proved by the microbial community results, in which the fungi depression is more remarkable than bacteria in cropland vs. wetland (Zhu et al., 2022). The fungi produce more chemically recalcitrant compounds, yielding a higher C-use efficiency than bacteria (Jackson et al., 2017; Frey, 2019). Thus, microbial-derived stabilized C decreased by suppressing fungi in cropland, corresponding to the suppression of genes encoding enzymes in chitin degradation in cropland.

4.3. A conceptual diagram for the microbial metabolism of soil C cycling after cultivation

A number of studies demonstrated that wetland cultivation leads to C loss (Ding et al., 2019; Xu et al., 2019; Niu et al., 2021), which has been advocated in this study (Fig. 2). Furthermore, we also proved the C loss was mediated by two mechanisms. Firstly, C decomposition in cropland was stimulated. It is proved by the declines of most ECs in the organic C degradation process (Fig. 3a), the enhancement of declined organic C degradation on soil C loss (Fig. 5a), as well as the significant contribution of the organic C degradation to potential respiration rate per unit C in croplands rather than in wetlands (Fig. S3). The decomposition was enhanced in cultivated croplands agreed with part of our hypothesis and also supported by enzyme (Song et al., 2012) and delta C ($\delta^{13}\text{C}$) analysis (Malo et al., 2005).

Secondly, the microbial-derived C contributing to a stable carbon pool might decline by changing two endogenous metabolic processes: the shifting of the catabolic process and the declining of the anabolic process. For instance, the dominant catabolic pathway converted from EMP and ATC to ED and PPP (Fig. 3) (Geyer et al., 2016), the energetically less efficient pathways. The conversion of catabolic processes would mediate the anabolic process changes to allocate more energy and precursor to energy obtaining, which led to lower C assimilation efficiency (Fig. 5). Meanwhile, the growth rate of soil microorganisms is faster (higher MBC), but C use efficiency (higher microbial metabolic quotient) is lower in cropland (Fig. 2), reflected by faster growth rate and higher functional abundance in organic C degradation (Figs. 5 and 6) (Geyer et al., 2016). Moreover, the faster growth while lower C use efficiency community would also enhance the microbial necromass input into soil organic C while reducing its maintenance in a stable C pool (Kallenbach et al., 2015). It was also proved by our previous results that lower fungi/bacteria ratio in cultivated cropland vs wetland (Zhu et al., 2022). These changes would stimulate declining the microbe-derived C input to stable C pool, and thus disadvantage for soil C sequestration and stabilization (Fig. 6). Overall, the enhancement of the organic C degradation process, accompanied by the conversion of the catabolic process and decline of the anabolic process for synthesize

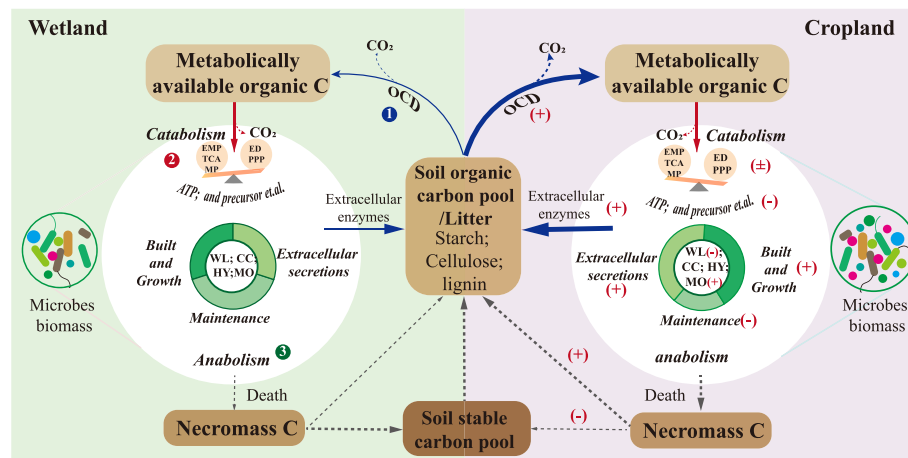


Fig. 6. C loss driven by discrepancy microbial C metabolism in cropland vs. wetland. (1) Organic C degradation process. (2) Catabolic process. This process is a set of pathways, including EMP, ED, PPP, TCA, MP et al., that break down large molecules into smaller ones and produce energy. (3) Anabolic process. Anabolism is an energy-dependent biosynthetic process through WL, CC, HY, and MO pathways. In cultivated cropland, (+) means enhancement, (-) means suppression, and (±) means insignificant effects.

building blocks, would thus stimulate soil C processing and loss while suppressing microbial-derived C stabilizing in soil carbon pool in cultivated croplands (Fig. 6).

In this study, we confirmed the roles of soil microorganisms in both *ex vivo* degradation and endogenous metabolism and further stated the changes in those processes after wetland conversion. In *ex vivo* degradation, the processes of soil organic C decomposition were enhanced after cultivation, stated by a higher abundance of functional genes encoding enzymes involved in organic C degradation processes (Figs. 3a and 6). Those decomposition enhancement modifications could decline the soil organic C pool. In endogenous metabolism, the catabolic processes were transferred from higher energy yield pathways (EMP and TCA) to energetically less efficient pathways (ED and PPP) (Fig. 3b and c, Fig. 6), accompanied by anabolic strategy changes to obtain more energy by growth faster and synthesis more C degradation enzymes (Fig. 6). This growth strategy (faster growth rate while lower C assimilation efficiency) might be in cost of lower relative stable microbial-derived C input to stable C pool. In summary, soil microbial community metabolism mediates the C loss after wetland cultivation by stimulating soil degradation and microbial C turnover while declining C assimilation efficiency. To our current knowledge, it is the first time to provide a comprehensive understanding of functional genes for soil C cycling for land conversion. These insightful analyses provide microbial metabolic evidence of microbial roles in massive C loss after wetland conversion to cropland.

4.4. Limitations and prospects

In the study, the soil microbial C metabolism was estimated and proved for the distinguishment of microbial C metabolism in wetland and cropland, which led to the C loss after cultivation. We identified a few limitations on insufficient data and measurement technology that deserve further investigation. First, both the C loss and soil microbial metabolism changes depending on the types of natural wetland and the duration of the cultivation (Goss-Souza et al., 2017; Xie et al., 2017). Sampling in both more paired wetland-converted cropland continentally/globally and a list of sites over time series or a chrono-sequence gradient of cultivation will provide more robust evidence in addressing microbial metabolism during wetland conversion; more insightful seasonality analysis may also advance the microbial roles in soil C loss over seasons (Zhao et al., 2022). Second, microbial necromass C links microbes to the soil C pool, but it is not fully addressed (Fan et al., 2021). The multiple C pools, such as aggregate C, mineral-associated C, and microbial necromass C, could provide a full

sketch of the soil C stabilization (Bai and Cotrufo, 2022). Third, the soil C turnover is hard to trace in real-time nature accompanied by microbial metabolic processes because of high measurement cost and technical limitations (St James et al., 2021). Therefore, multiple techniques and more efficient methods would accelerate the exploration of microbes in regulating soil C cycling (Bay et al., 2021). Fourth, it remains controversial that functional genes as a reliable proxy for biogeochemical processes due to conflicting observations in the literature (Prosser, 2015; Trivedi et al., 2016). Thus, more data are needed to answer whether functional abundance can be used to assess the processes of C cycling and other ecosystem processes (Yang, 2021).

Our work addressed organic C degradation, catabolic and anabolic processes through 10 pathways and 138 enzymes. It proved that wetland cultivation changed microbial C metabolism by enhancing the C degradation, changing catabolism to the less efficient, and suppressing anabolism. The detailed analysis of functional genes for microbial C turnover processes provides insights into microbial metabolism affected by land use change. It also provided a new perspective and probability to explore the role of microbes in C turnover, both in the view of decomposition and stabilization. This kind of work takes a step forward not only in mechanistic understanding of soil C cycling but also in improving the C prediction.

5. Conclusions

In this study, we explored three major processes of C cycling (organic C degradation, catabolism, and anabolism) carried out by soil microbes after wetland cultivation to cropland. We found that wetland cultivation significantly promoted C loss by enhancing organic C degradation but suppressing microbial endogenous assimilation efficiency, advocated by the changes in functional genes. Moreover, changes in all those functional genes were more remarkable in spring and summer than in autumn and winter. This study provided empirical evidence that changes in soil C catabolic and anabolic genes directly affect soil C cycling after wetland conversion to cropland. This study suggested that wetland cultivation promoted soil microbial activity but suppressed its efficiency, leading to a massive C loss, indicating a need to represent microbial metabolisms when modeling microbes in soil C cycling. The seasonality of microbial roles in C cycling deserves further analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2023.109260>.

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