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Environmental stress stimulates microbial activities as indicated by cyclopropane fatty acid enhancement

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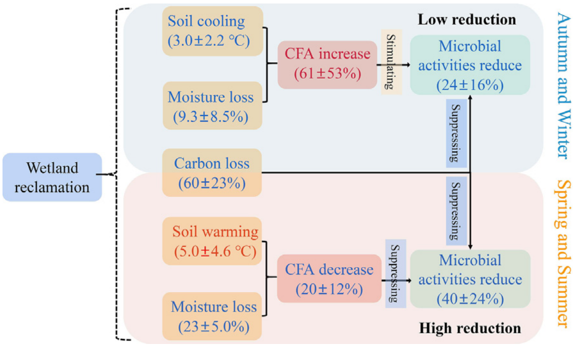
HIGHLIGHTS

- CFA is used to study microbial ecological suitability during wetland reclamation.
- Seasonal CFA stimulates microbes to better adapt to environmental stress.
- Soil nutrients dominate the differentiation in CFA-produced microbes.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil microbial responses to environmental stress remain a critical question in microbial ecology. The content of cyclopropane fatty acid (CFA) in cytomembrane has been widely used to evaluate environmental stress on microorganisms. Here, we used CFA to investigate the ecological suitability of microbial communities and found a stimulating impact of CFA on microbial activities during wetland reclamation in Sanjiang Plain, Northeastern China. The seasonality of environmental stress resulted in the fluctuation of CFA content in the soil, which suppressed microbial activities due to nutrient loss upon wetland reclamation. After land conversion, the aggravation of temperature stress to microbes increased the CFA content by 5 % (autumn) to 163 % (winter), which led to the suppression of microbial activities by 7 %–47 %. By contrast, the warmer soil temperature and permeability decreased the CFA content by 3 % to 41 % and consequently aggravated the microbial reduction by 15 %–72 % in spring and summer. Complex microbial communities of 1300 CFA-produced species were identified using a sequencing approach, suggesting that soil nutrients dominated the differentiation in these microbial community structures. Further analysis with structural equation modeling highlighted the important function of CFA content to environmental stress and the stimulating influence of CFA induced by environmental stress on microbial activities. Our study shows the biological mechanisms of seasonal CFA content for microbial adaption to environmental stress under wetland reclamation. It advances our knowledge of microbial physiology affecting soil element cycling caused by anthropogenic activities.

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1. Introduction

Cyclopropane fatty acid (CFA) primarily biosynthesized in hostile conditions, has been widely used to indicate environmental stress to microorganisms (Jiang et al., 2019; Fierer et al., 2003; Zhu et al., 2021). Investigating the temporal dynamic of phospholipid fatty acids (PLFA) compositions at a given resource, Cronan and Luk (2022) and Kim et al. (2021) argued that CFA accumulation in microbial membranes predominantly occurred in the stationary phase under laboratory conditions. In addition, the aggravation of environmental stresses, such as poor oxygen supply, low pH, and extreme temperature (Choi et al., 2020; Poger and Mark, 2015; Chen and Gänzle, 2016), has been proved to accelerate the conversion of unsaturated fatty acids to CFA (Hari et al., 2018; Bossio and Scow, 1998). Therefore, numerous studies (To et al., 2011; Paulucci et al., 2011; Li et al., 2019; Fierer et al., 2003; Zhu et al., 2021) used the ratio of CFA to its precursors to evaluate the environmental stress to microbes because the ratio can be shifted by meteorologic and edaphic factors (Zhu et al., 2022; Fierer et al., 2003). An increasing proportion of CFA to cyclopropyl precursors indicates a reduction of microbial growth and an aggravated environmental limitation, and vice versa (Zhu et al., 2021; Li et al., 2019). Due to the complexity of ecosystems, temporal and spatial dynamics in the soil environment exist, such as nutrient, hydrothermal conditions, and temperature, can form environmental stresses to microbes (Fierer et al., 2003; Zhu et al., 2021; Hernandez et al., 2021). For example, Fierer et al. (2003) reported that environmental stress increased with soil depth attributed to nutrients decreasing, and Zhu et al. (2022) indicated the different impacts of soil temperature across seasons. Till now, fewer studies have focused on investigating the key factors controlling the change of CFA content in multivariate environments, especially across seasons and along soil profiles.

The protective effects of CFA on microbial membranes, and the survival and activity of microbes have been widely substantiated in previous studies (Choi et al., 2020; Velly et al., 2015; Xu et al., 2018; Bae et al., 2021). As an essential component of cytomembrane in certain microorganisms (Cronan and Luk, 2022; Chen and Gänzle, 2016), CFA is necessary to participate in physiological activities and thrive in a wide range of environments (Guillot et al., 2000; Zhang and Rock, 2008). Specifically, CFA can change the biophysical properties of the membrane lipid bilayer, mainly by rearranging fatty chains and controlling the accumulation of lipids in the cell membrane to adapt to environmental changes (Loffhagen et al., 2007; Poger and Mark, 2015), so the regulation of the biosynthesis of CFA in membrane lipid can improve cell survival (Pini et al., 2009; Bae et al., 2021). On the other hand, increased CFA content results in improvements in the membrane fluidity and stability (Yang et al., 2021; Kolbeck et al., 2021; Zhang and Rock, 2008), promotes the exchanges of the substrate and terminal electron acceptors, including Fe^{3+}, NO_3^- and SO_4^{2-}, with the external environment (Kolbeck et al., 2021; Maier and Gentry, 2015), which is vital for microbial activities, such as microbial respiration (MR). Considering the critical roles of CFA in maintaining cell membrane functions and survival (Chen and Gänzle, 2016; Kolbeck et al., 2021), the microbial activities influenced by substance-driven exchange would be enhanced by increasing CFA. However, there was no direct experimental evidence or studies supporting this hypothesis.

Wetland reclamation, a typical anthropogenic perturbation, has been widely utilized to expand farmland or industrial areas in many regions of the world (Verhoeven and Setter, 2010). The conversion from wetland to cropland leads to notable substantial and long-lasting changes in soil environment and microbial properties (Edwards et al., 2015; Song et al., 2012), threatening natural ecosystem functions (Hassan et al., 2005). For instance, previous studies have demonstrated the dramatic loss of soil carbon (C) and microbial biomass (Zhu et al., 2021; Huang et al., 2008) and the differentiation in microbial community structure resulting from soil management practices occurred (Ceja-Navarro et al., 2010). In addition, the divergence of wetland reclamation impacts on soil properties (e.g., nutrient contents, temperature and moisture), and environmental stress to microbes along soil profiles and over seasons were observed (Zhu et al., 2021, 2022). Therefore, discrepancies in soil and meteorological properties with soil depth, season and land use type might induce the change in CFA content, influencing soil MR. However, the impact patterns and mechanisms explaining environmental stress stimulates soil MR indirectly by promoting CFA biosynthesis remains under-investigated.

To explore the indicative role of CFA content on environmental stress and the mechanisms explaining how CFA influence soil MR during the wetland reclamation along soil profiles and over seasons, we measured the PLFAs, microbial community structure, CFA gens, relative microbial abundance and MR from a natural boreal wetland and an adjacent 25-year cultivated cropland in Sanjiang Plain, northeastern China in autumn, winter, spring, and summer. We hypothesized that: 1) the significant variation in soil properties along soil profiles led to the differentiation in CFA with soil depth; 2) seasonal solid responses of meteorological factors to wetland reclamation led to the seasonal variation of environmental stress (Zhu et al., 2022; Brown et al., 1997), which altered the CFA content during wetland reclamation; 3) the increase of CFA content induced by the environmental stress enhanced the microbial activities.

2. Material and methods

2.1. Study site and soil sampling

This study was conducted at the Sanjiang Experimental Station of Wetland Ecology (133°31′ E, 47°35′ N), locating at Sanjiang Plain in northeastern China. The site has a coexisting subhumid continental monsoon and temperate humid climate (Zhu et al., 2021). Mean annual precipitation is 500–650 mm and mean annual temperature ranges from 1.4 to 4.3 °C, with January and July being the coldest (−18 °C) and warmest months (22 °C), respectively (Ding et al., 2004; Fu et al., 2020). Natural wetlands at this study site were utilized to cultivate soybean (*Glycine* max [L.] Merr.) since 1996. The soybean grew from May to October as an annual crop species. For nearly 25 years, the soybean field has been unfertilized but tilled annually. Furthermore, there is an approximately 35 cm water layer covered on its surface, and its vegetation is dominated by *Carex meyeriana*, *Carex lasiocarpa*, and *Deyeuxia angustifolia* communities in undisturbed natural wetlands. Along the soil profile, based on WRB classification, 0–70 cm is dominated by meadow soil, while 70–100 cm is white territory soil (Wang et al., 2003). Both in wetland and cropland, the soil texture is a silty loam based on USDA Classification System (Zhu et al., 2021; Wang et al., 2017). The soil in the study site is black soil containing voluminous humus and unusual microbial community due to the frigid climatic conditions in winter (Sui et al., 2019).

Soil cores were collected from the natural wetland (133°30′ E, 47°35′ N) and soybean field (133°30′ E, 47°36′ N) during October 10–12, 2019, December 27–29, 2019, May 13–15, 2020 and July 19–21, 2020, representing autumn, winter, spring, and summer, respectively. For each ecosystem, three soil cores were randomly excavated per sampling season as replicates. To lower the effect of spatial heterogeneity, the sampling site spacing is about 50-200 m for each ecosystem. Each soil core was divided into 10 sections by 0–10, 10–20, 20–30, 30–40, 40–50, 50–60, 60–70, 70–80, 80–90, and 90–100 cm. Finally, we totally collected 120 samples of 10 layers and three replicates in four seasons for each ecosystem. All the visible roots, stone and residues were removed. Then the soil samples were immediately packed into polyethylene bags to be cooled with ice packs and brought back to the laboratory for further analysis. Half of the soil samples were stored at 4 °C to measure the soil total carbon (TC), total nitrogen (TN), total phosphorus (TP), total sulfur (TS), pH, moisture, microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), microbial biomass phosphorus (MBP), and microbial biomass sulfur (MBS) and soil MR. The other soils were stored at −80 °C for PLFA analysis, DNA extraction and sequencing. The soils of 0–30 cm were defined as topsoil in the study (Xu et al., 2013, 2017; He et al., 2020). Due to the maximum frozen depth in soybean fields can reach 70 cm in winter (Zhu et al., 2021), we considered 30–70 cm as the middle soil layer and 70–100 cm as the deep soil layer.

2.2. Measurements of soil and microbial properties

TC was measured by Multi N/C 2100 TOC analyzer (Analytikjena, Germany) using high temperature (1100 °C) combustion method. TN, TP and TS were determined using a heating digestion method with concentrated sulfuric acid. Soil pH was measured using pHs-25 (Shanghai, China) at a ratio of 1:10 for soil to water. Microbial biomass elements including C, N, P and S were calculated as the difference in the amounts of elements between fresh soils fumigated with $CHCl_3$ and unfumigated soils (Wu et al., 1990; Brookes et al., 1982, 1985).

The measurements of PLFA and CFAs were followed by Frostegard et al. (1993) and Wardle and Ghani (1995). Specifically, a mixture of chloroform-methanol-citrate buffer (1:2:0.8) was used to extract 8 g freeze-dried soil sample, and silicic acid column was utilized to separate and collect phospholipids in extracting solution. Then we esterified lyophilized lipid sample with an alkaline mixture of methanol and toluene (1:1), gas chromatography (Agilent 7820, USA) was used to separate fatty acid methyl esters by a 50-m HP-5 (phenylmethyl silicone) capillary column. We used nitrogen as a carrier gas and added methyl nonadecanoate fatty acid (19:0) liquid as an internal standard to quantify peak areas. The concentration of each PLFA was calculated based on the ratio of the peak area of this PLFA to the area of the internal standard by MIDI peak identification software (MIDI, Inc., Newark, DE). Totally, 146 PLFAs including 2 types of CFA (Cy17:0 and Cy19:0) and their precursors (16:1ω7c and 18:1ω7c) were detected in our study; the sum of Cy17:0 and Cy19:0 indicated the CFA, the sum of 16:1ω7c and 18:1ω7c represented CFA precursors, and the sum of PLFAs indicated microbial biomass.

2.3. Soil DNA extraction and sequencing

DNA was extracted from each soil sample using fast-DNA® Spin Kit (MP Biomedicals, Inc., USA). TBS-380 (Yuanpinghao Biotechnology CO.LTD, China) and NanoDrop2000 (Thermo Fisher Scientific, USA) were used to identify the concentration and purity of extracted DNA, respectively. After the detection by 1 % agarose gel electrophoresis, DNA was fragmented to an average size of ~400 bp by Covaris M220 (Gene Company Limited, China) and constructed a paired-end library using NEXTflexTM Rapid DNA-Seq (Bioo Scientific, Austin, TX, USA). We ligated the adapters containing the hybridization site of the complete sequence primer to the blunt end of fragments. Paired-end sequencing was implemented on NovaSeq 6000 (Illumina Inc., USA) by NovaSeq Reagent Kits from Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

Finally, we obtained 948,156 reads annotated to the CFA biosynthetic pathway (pathway id: K00574; KEGG_Enzyme: 2.1.1.79). Modules and enzymes table were rarefied to 1 million observations per sample, that is parts per million (PPM):

$$PPM_i = R_i * \frac{10^6}{\Sigma_1^n (R_i)}. \tag{1}$$

In which R_i represents reads number in the ith sample; n is the total sample size. The relative abundance of CFA gene (R) was calculated by the equation as below:

$$R = R_c / R_t * 100\% \tag{2}$$

where R_c is the read number annotated to the CFA biosynthetic pathway and R_t is total gene reads number. Microbial groups including the CFA gene were the CFA producers.

2.4. Potential of soil microbial activities

The MR potential, the maximum rate of soil CO_2 production, was measured by laboratory incubation experiment and was used to present the soil microbial activities for quantifying the impacts of CFA content and wetland reclamation (Stout et al., 2014). Since pre-experiment showed there was no significant difference in MR potential between aerobic with anaerobic conditions (Table S5), in the study we only measured the soil respiration under anaerobic conditions, and most soil samples in situ were also under anaerobic conditions. Specifically, three replicates of fresh soil samples, equivalent of 20 g dry soil per sample, were sealed in bottles. Each bottle was added with 100 ml ddH_2O and was filled with nitrogen gas to maintain anaerobic conditions and the same moisture of each treatment. Each bottle was put in a 30 °C incubator for 5 h to maintain the optimal temperature for the microbes (Barcenas-Moreno et al., 2009). Before and after the incubation, CO_2 concentration was measured by gas chromatography (Agilent 7820, USA); and the MR rate was calculated as below:

$$MR = \frac{(C - C_0)(V - 100) \times 273 \times 48}{1000 \times 22.4 \times (273 + T) \times m \times t} \tag{3}$$

where C (ppm) is the CO_2 concentration after incubation; C_0 (ppm) is the CO_2 concentration at the beginning of incubation; V (cm^3) is the volume of the bottle; T (K) is the temperature of incubation; m (g) is the dry soil weight in each bottle and t (h)is the time of incubation.

2.5. Statistical analyses

Statistical analyses were conducted using R version 4.1.2. Duncan's multiple range test was applied to compare the variables, such as soil pH, TC, TN, TP, TS, MBC, MBN, MBP, MBS, PLFA, CFA, CFA producers, CFA gene, and soil MR, among soil layers in wetland and cropland, respectively, followed by the least significant difference (LSD) test at the 0.05 significance level. One factor ANOVA test was used to estimate the seasonality of these parameters. The *t*-test was performed on comparing each independent variable in wetland against cropland among soil layers and seasons. The independent variable of each layer in each season was measured by three replicates. The PLFA ratio, cyclopropane fatty acids: cyclopropane precursors (CF: CP), was used to indicate the environmental stress on microbial groups (Fierer et al., 2003). Data were expressed as the mean (± SE) of three replicates.

Bray Cluster analysis was used to evaluate the effects of soil depth, season, and land conversion on the community structure of CFA producers in the taxonomic level of phylum. Redundancy analysis (RDA) was implemented to investigate the contributions of meteorological and soil factors, such as temperature, pH, and substrate content, to the variation of the CFA producer composition. Prior to Cluster analysis and RDA, the relative abundances of CFA producer groups soils were calculated as the average of the corresponding replicates at the surface, middle, and deep layers. According to the results of Bray Cluster analysis (see Section 3.3), we simplified season classification from four to two for the RDA and following analysis: cold seasons (autumn and winter) and warm seasons (spring and summer). All data were normalized using zero-mean normalization.

To investigate the controlling factors on the biosynthesis of CFA over seasons, Pearson's correlation was used to analyze the covariation between soil chemical variables and microbes. Mantel test was conducted to evaluate the associations between CFA concentration, CFA producers, and meteorological and edaphic properties. To quantify the effects of season and land cover type on the relative abundances of CFA and CFA producers, the wetland was replaced with a numerical entity of 1 and the cropland as 2, and soil temperature as the surrogate of seasons. The log-transformation was applied to ensure the normality of data before correlation analyses.

We used structural equation modeling (SEM) to quantify multiple variables and their interactive controls on soil and microbial properties, as well as CFA and MR seasonal variations along the soil depths. First, we developed a priori model, which allows a hypothesized causal interpretation of the linkages among soil depth, season, the variation in temperature, soil nutrients, soil pH, soil moisture, CFA, CFA producers, and MR for the wetland and cropland. Then conceptual models were established, and the optimal model was determined by the closest fitting. In SEM, the soil temperature represents seasons; the soil TC represents soil element components. To quantify CFA and MR variations in wetland reclamation and cultivation, we standardized temperature, soil nutrients, soil pH, soil moisture, CFA, CFA producers, and MR at each soil layer in the wetland and cropland with the equation:

$$V = |V_w - V_c| \tag{4}$$

where V is the variation, such as soil or microbial properties, CFAs, CFAs producers or soil MR; V_w is the parameter in wetland and V_c is the parameter in cropland. Because the relative abundance of CFA producers was highly consistent with CFA gene (Fig. S1), thus we only kept the relative abundance of CFA producers for the Mantel test and SEM.

3. Results

3.1. Environmental stress at seasonal scale

There were substantial seasonal discrepancies in the responses of environmental stress to wetland reclamation (Table S1). In autumn and winter, wetland reclamation intensified environmental stress on microbes; conversely, in spring and summer, the conversion of wetland to cropland alleviated the environmental stress caused by microbes. After the wetland reclamation, CF: CP significantly increased from 0.26–1.89 in the wetland to 0.59–26.65 in cropland along all soil profiles in autumn-winter ($p < 0.05$); in spring-summer the CF: CP largely decreased from 0.19–1.23 in the wetland to 0.69–0.88 in the cropland ($p > 0.05$).

3.2. Wetland reclamation impacts on CFA and microbial activities

Wetlan reclamation had strong impact on the seasonal variations of relative abundances, genes and producers of CFA (Fig. 1; Table 1). After wetland reclamation, the relative abundance of CFA increased by 5 % to 163 % in autumn and winter; and was suppressed by 3 % to 41 % ($p < 0.05$) in spring and summer (Tables 2 and S4). Along the soil profiles, tillage increased the CFA content from 2.63 (0.44)–5.54 (0.35) nmol/g in wetland to 4.67 (0.66)–11.07 (1.72) nmol/g in cropland in autumn, and from 6.12 (1.02)–11.88 (0.81) to 6.03 (0.54)–14.19 (0.22) nmol/g in winter. In spring the CFA content reduced from 6.9 (0.12)–15.47 (0.11) to 5.44 (1.7)–10.61 (1.84) nmol/g, and 2.42 (0.62)–6.29 (0.63) to 2.44 (0.5)–5.23 (0.62) nmol/g in summer (Table S2). Similarly, land conversion significantly promoted the relative abundance of CFA producers by 9 % to 29 % in autumn and winter, respectively, and depressed by 6 % to 40 % in spring and summer, respectively ($p < 0.05$; Tables 2 and S4; Fig. 1). Meanwhile, wetland conversion significantly promoted the relative abundance of the CFA gene by 11 % to 104 % in autumn and winter but depressed by 10 % to 65 % in spring and summer ($p < 0.05$; Tables 2 and S4; Fig. 1).

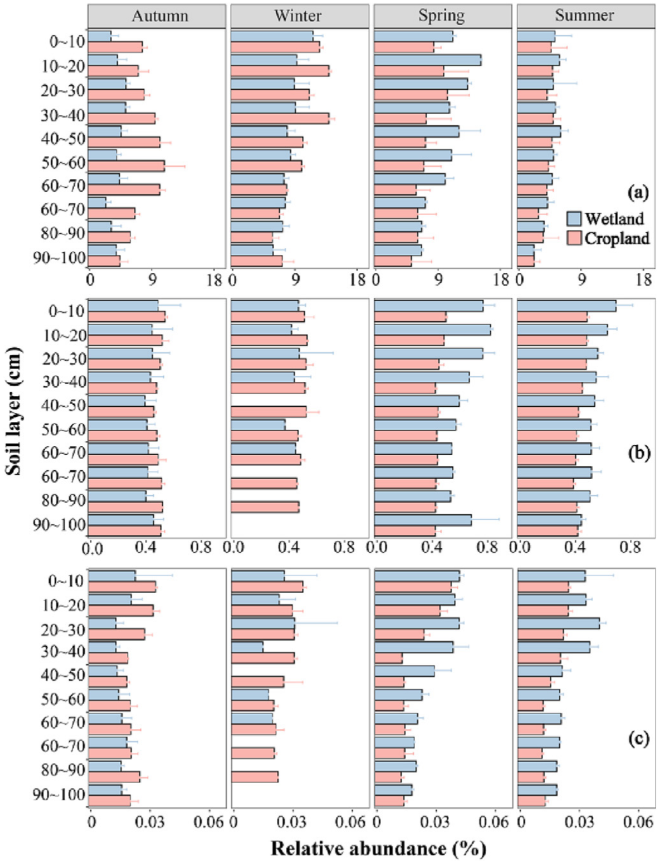

Fig. 1. The relative abundances of a) cyclopropane fatty acids (CFA), b) CFA producers, c) CFA gene in autumn, winter, spring, and summer for wetland and cropland along soil profiles.

Table 1
The results of the one factor ANOVA test for the seasonal comparisons for the relative abundances of cyclopropane fatty acid (CFA), CFA producers, CFA genes and soil microbial respiration (MR) in wetland and cropland. Bold fonts indicate the p values < 0.05.

Land use	CFA	CFA producer	CFA genes	MR
Cropland	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Wetland	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Land conversion has obvious seasonal effects on microbial activities along soil profiles. Soil MR generally decreased with soil depth (Fig. 2; Table S2) and significantly varied among seasons (Table 1). The reason of land conversion reduction was mainly caused by surface soils with seasonal variation; in autumn-winter, the reduction was lower (7 %–47 %), and a higher depression was observed in spring-summer (15 %–72 %) (Table S4). Wetland reclamation decreased soil microbial activities, from 0.68 (0.28)–2.64 (0.14) μg/g*h to 0.91 (0.04)–2.12 (0.86) μg/g*h in autumn; from 0.17 (0.07)–0.44 (0.03) μg/g*h to 0.12 (0.02)–0.26 (0.02) μg/g*h in winter; from 0.68 (0.08)–1.65 (0.3) μg/g*h to 0.67 (0.01)–0.99 (0.05) μg/g*h in spring; from 0.45 (0.01)–2.65 (0.22) μg/g*h to 0.43 (0.03)–0.76 (0.02) μg/g*h in summer ($p < 0.05$; Table 1), respectively.

3.3. Change in community structure of CFA producers caused by wetland reclamation

In the study, 1300 species and 35 phylum of microbial groups containing CFA gene were identified, and the CFA producers in the phylum with the highest relative abundance were *Proteobacteria* and *Actinobacteria* (Fig. 3a). Cluster analysis showed that both land type and soil depth had an intensive influence on the community composition of CFA producers (Fig. 3a). Specifically, CFA producers of the wetland group and cropland group were differentiated, and the surface soil group was separated from the middle and deep soil groups.

There were obvious distinctions in the controls of CFA producer composition in different seasons (Fig. 3b–c). Most of the differences can be explained by PC1 (76.3 %) and PC2 (17.1 %) in autumn-winter, and by PC1 (92.0 %) in spring-summer, with PC1 functioning as *Proteobacteria* and PC2 representing *Actinobacteria*. In autumn-winter, the dominant factors

Table 2
The results of the *t*-test for the comparisons of land use types for the relative abundances of cyclopropane fatty acid (CFA), CFA producers, CFA genes and soil microbial respiration (MR) in autumn, winter, spring and summer. Bold fonts indicate the p values < 0.05.

Season	CFA	CFA producer	CFA genes	MR
Autumn	**<0.0001**	**0.000146**	**<0.0001**	0.362
Winter	**0.0369**	**0.0361**	**0.0424**	0.156
Spring	**<0.0001**	**<0.0001**	**0.000229**	**0.017**
Summer	**0.0101**	**<0.0001**	**<0.0001**	**0.0118**

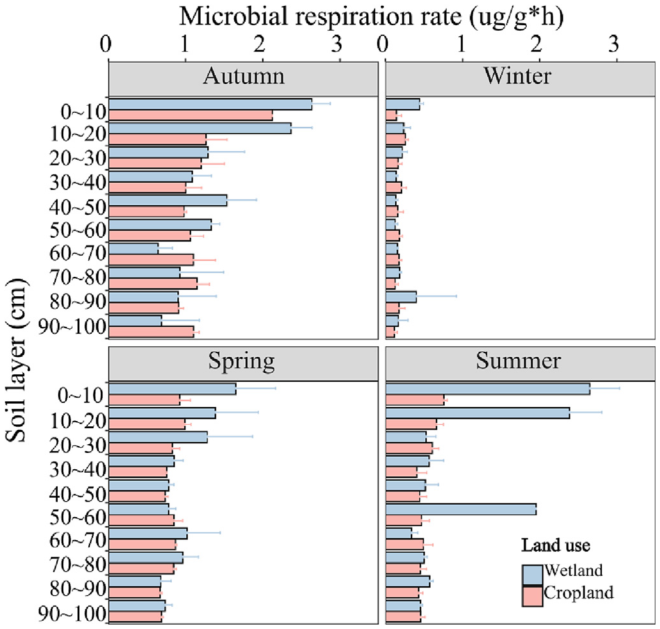

Fig. 2. Microbial respiration rates in autumn, winter, spring, and summer for wetland and cropland along soil profiles.

controlling the differentiation in CFA producer composition were substrate contents, such as TC, TS, and soil temperature. *Proteobacteria* was correlated to substrate contents and temperature, and *Actinobacteria* was related to soil moisture (Fig. 3b). However, in spring-summer, the dominant factors were TC, TS, pH, and moisture, with *Proteobacteria* negatively correlated to TC, TS, and moisture and positively related to pH (Fig. 3c).

3.4. Controls of CFA by edaphic factors

The associations among the relative abundances of CFA, CFA producers, soil, and microbial properties varied greatly over seasons (Fig. 4). In autumn-winter, the relative abundances of CFA and CFA producers were significantly positively related to soil depth, but negatively related to soil temperature ($p < 0.05$). In addition, the relative abundance of CFA was significantly positively correlated to TN, MBS and pH, while the relative abundances of CFA producers were significantly positively correlated to TP, MBN and land use ($p < 0.05$). However, in spring-summer, the relative abundances of CFA and CFA producers were significantly positively related to total PLFA and moisture but negatively related to land use ($p < 0.05$). Furthermore, the relative abundance of CFA was significantly positively correlated to TS and MBN, but inversely correlated to MBP and temperature. The relative abundances of CFA producers were significantly positively correlated to TC, TN, TS, MBC, MBN, MBP, MBS, and pH, but negatively correlated to soil depth ($p < 0.05$).

3.5. A conceptual framework for the wetland reclamation impacts on microbial activities by CFA

Seasonality and soil depth strongly affected the variations of edaphic factors, and the effect of wetland reclamation on the CFA content further altered the reduction of microbial activities (Fig. 5). First, the variations of soil nutrients (−0.758) and soil moisture (−0.419) significantly related to soil depth; and season significantly affected the differences of soil pH (−0.301) and soil temperature (−0.358) with land conversion. Then, the

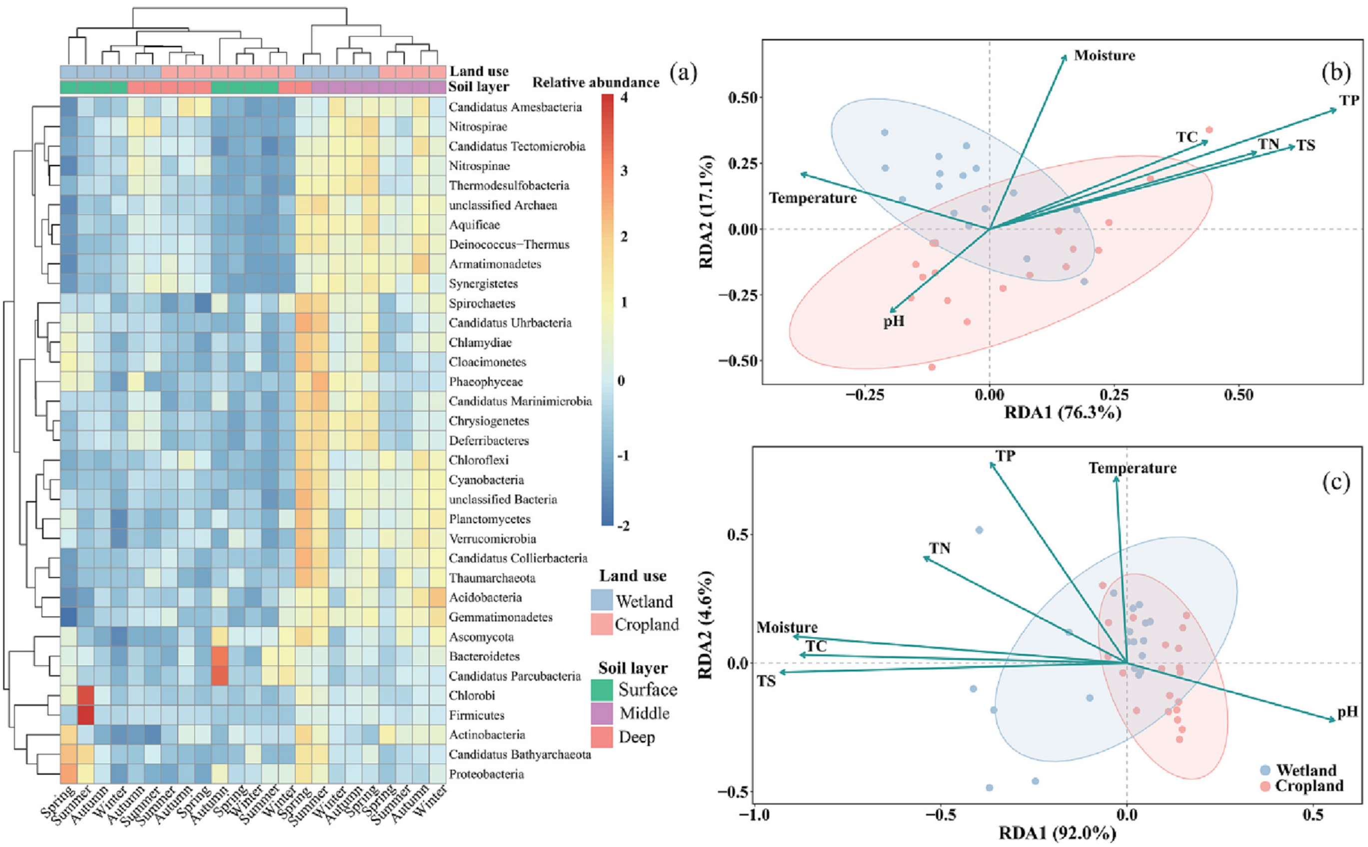

Fig. 3. a) Clustering analysis of the community structure of cyclopropane fatty acid (CFA) producers in the phylum level according to different soil layers (surface, middle, and deep soils), seasons (autumn, winter, spring, and winter), and land use types (wetland and cropland). Redundancy analysis (RDA) for the contributions of edaphic factors to the variation of composition of CFA producer are shown for b) cold seasons (autumn and winter), and c) warm seasons (spring and summer). Surface soil: 0–30 cm; middle soil: 30–70 cm; deep soil: 70–100 cm.

variations of soil pH (0.308), soil temperature (0.550), and soil moisture (0.729) jointly determined the variation of the abundances of CFA producers, affecting the variation of CFA content (0.483). Finally, the changes in CFA content (0.207) and soil nutrients (0.378) together determined the soil MR.

Based on our field measurements, incubation experiment, and SEM analysis (Fig. 5), we propose a conceptual framework summarizing how the seasonality of wetland reclamation impacts CFA content and the possible mechanisms explaining the stimulating impacts of CFA on microbial activities with wetland reclamation (Fig. 6). Generally, the change in habitat alters environmental stress and then CFA content (Fierer et al., 2003). The seasonality of human impacts on soil temperature and moisture during land conversion leads to the fluctuation of environmental stress to microbes. Decreased soil temperature caused the increase of CFA in autumn-winter but warming and permeability enhancement due to wetland reclamation decreased CFA in spring-summer. The increasing CFA content induced by enhanced environmental stress would have positive effects on membrane stability and fluidity, and then stimulate the activity of soil microbes, such as MR. Consequently, fluctuation in the impacts of wetland reclamation on CFA content results in seasonal variation in the reduction of microbial activities.

4. Discussion

4.1. Identification of CFA producers

Genomic sequence analysis can potentially be used to identify the microbial groups synthesizing CFA, for example, the amino acid sequences of enzymes catalyzing CFA have been validated to be highly conserved (Grogan and Cronan, 1997). In this study, a total of 504 genus of CFA producers were detected, including microorganisms previously reported, such as *Escherichia* (Law et al., 1963), *Arthrobacter* (Grogan and Cronan, 1997) and *Vibrio* (Guckert et al., 1986), which indicates that CFA producers were widely distributed in various microbial populations of the wetland and cropland (Ma et al., 2021; Grogan and Cronan, 1997; Lechivalier, 1977; Gottesman, 1996; Guckert et al., 1986). However, we found that few microbial species above had the ability or potential to produce CFA as well. For instance, 19 microbes belonging to *Escherichia* were detected in the study, but only *Escherichia coli* can possess CFA gene and synthesize CFA (Law et al., 1963). In the future, identification of CFA producers at the taxonomic level of specie using more precise methods during laboratory domestication will be helpful for a better understanding of the biosynthesis of membranes.

4.2. The indicative role of CFA on environmental factors under wetland reclamation

The seasonal effects of land conversion on the CFA and CFA producer contents and their responses to soil temperature were significantly correlated (Figs. 4–6), which further indicated that CFA had an indicative function on temperature decrease. Winter temperatures below freezing could lead to the solidification of ice crystals with cell membrane rupture (Methe et al., 2005), being potentially fatal for microbial growth (Bossio and Scow, 1998). In response, microbial groups attempt to change their physiological strategies, such as promoting CFA synthesis (Methe et al., 2005; Zhu et al., 2022). Biosynthesis of CFA strengthened the cell membranes' stability (Chen and Gänzle, 2016), which further improved the ability of microbes to adapt to repeated freeze-thaw cycles (Grogan and Cronan, 1986; Zavaglia et al., 2000), and increased the survival rate of microbe below freezing (Muñoz-Rojas et al., 2006). Finally, the increased temperature stress generated by wetland reclamation led to increases in the CFA, CFA producers, and CFA gene content in autumn and winter. However, the acclimatization of improving freeze-tolerance inevitably induces substantial losses of microbes, including CFA producers (Tables S2–3; Methe et al., 2005), which means that freezing temperatures will be detrimental to CFA producers. Therefore, compared with autumn, spring, and summer, microbial biomass decreased, and CFA relative content increased significantly in winter, ($p < 0.05$; Tables S2–3), suggesting that CFA relative content was a strong indication of environmental stress, and CFA could improve microbial survival.

The increase of soil temperature and the decrease of CFA content (Fig. 6) in spring-summer suggested the indication of CFA to environmental stress as well. The higher the soil temperature, the more suitable environment for microbial communities (Barcenas-Moreno et al., 2009), especially in our study, as the optimal temperature for microbial growth was higher than the in-situ soil temperature (Table S2). According to the competitor/stress-tolerator/ruderal (CSR) theory (Grime and Pierce, 2012; Wood et al., 2018), CFA producers are theoretically classified as low-temperature stress-tolerant traits because of their higher survival rates in winter than other microbes, whereas their growth rates are lower than other microbes (Kerr et al., 2002). The increase in temperature generated by wetland reclamation improved the growth of other microbes rather than CFA producers (Table S2), resulting in lower contents of CFA, CFA producers, and CFA gene (Kerr et al., 2002). Therefore, the temperature stress of cropland CFA in spring-summer was lower than that of wetland, which results in the suppression of soil MR (Chen and Gänzle, 2016).

The significant relationships between CFA content and soil moisture suggested the strong indication of CFA to anaerobic stress to microorganisms (Figs. 4–5). High soil moisture leads to low O_2 level in cropland soils (Rubol et al., 2013) and water cover induced anoxic for wetland soil microbes, both of which threaten microbial community and promote CFA biosynthesis (Bossio and Scow, 1998; Fierer et al., 2003; Knivett and Cullen, 1965). Meanwhile, CFA production enhances the membrane fluidity and soil gas exchange, thus improving the survival of CFA producers in anaerobic environments (Poger and Mark, 2015). In our study, seasonal fluctuation of CFA content in cropland was consistent with the soil moisture seasonality (Fig. 6). Higher CFA content indicated more limitation of O_2 level, and vice versa. Anthropogenic tillage decreased soil moisture and increased soil O_2 levels along soil profiles (Table S2), suppressing the relative abundances of CFA, CFA producers, and CFA genes (Thomas and Batt, 1969). The decreases of the relative abundances of CFA, CFA producers and CFA gene in spring and summer were the results of the synergistic effect of wetland reclamation to increase temperature and decrease moisture (Fig. 6). However, in autumn and winter, although land conversion reduced soil moisture, the impact of soil moisture on CFA content seemed to be slight compared with spring and summer. Three mechanisms explain this in cropland: (1) lower temperature and productivity lead to reduced evapotranspiration, resulting in higher soil moisture in autumn and winter (Hargreaves and Samani, 1985; Guo et al., 2021;Fig. S2); (2) as the most important input of fresh carbon resource in autumn, crop residue activated the quick growth of certain microbial groups that specializes in metabolizing fresh organic carbon (Zhu et al., 2022; Fontaine et al., 2010) in cropland, and the process could consume a large amount of O_2 (Griffiths et al., 1999), which decreases soil O_2 level partially; (3) in winter, snow cover provides an anoxic environment for microbes. The maximum frozen depth of soil can reach 70 cm at the site.

Despite the strong facilitation for CFA biosynthesis of nutrient deprivation was widely recognized (Geske et al., 2012; Poger and Mark, 2015), the positive relation between TC to CFA, CFA producers and CFA gen contents along soil profiles was observed (Fig. 6; Tables S2–3). Unlike laboratory experiments, the in-situ measurements, in our study, focus on the original characteristics of microbial composition depending on the long-term adaption for microorganisms to environmental (Fierer et al., 2003; Insam, 2001). Microbial groups producing CFA have stronger competitiveness both in cropland frequently distributed by agricultural activities and wetland that is anaerobic (Loffhagen et al., 2007; Härtig et al., 2005). Therefore, CFA producers grew faster in surface soils with high nutrients level, increasing the relative abundance of CFA, CFA producers and CFA gene contents in both cropland and wetland (Ghoul and Mitri, 2016), which led to a decrease in CFA contents with soil depth. In addition, soil nutrient variations had a minor contribution to the responses of CFA, CFA producers, and CFA gene content in wetland reclamation (Fig. 5). Thus, our

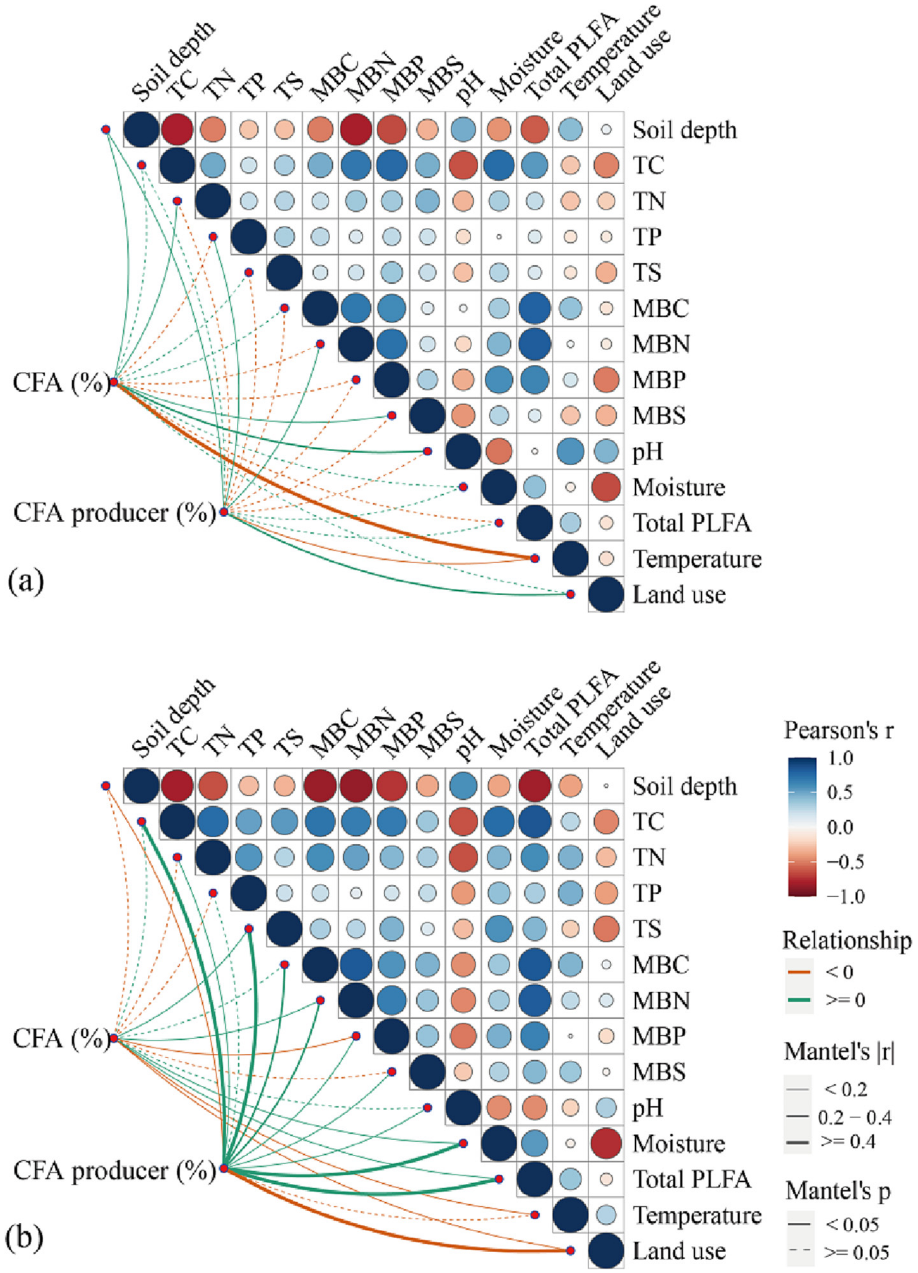

Fig. 4. Mantel's test for the correlations between soil chemical parameters and the relative abundances of cyclopropane fatty acid (CFA) and CFA producers in a) cold seasons (autumn and winter) and b) warm seasons (spring and summer). The circle size indicates the correlation coefficients between soil physio-chemical parameters, i.e., small circles indicate weak correlation and vice versa. The correlations between the relative abundances of CFA and CFA producer and soil parameters are associated with the line thickness; solid line indicates a significant correlation at a significance level of $P = 0.05$, and dotted line indicates an insignificant correlation; red color indicates positive relation; green color indicates negative relation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

study could not use CFA content as a starvation stress indicator. Edaphic nutrients stimulate or suppress the growth of different CFA producers because the results of DNA extraction and sequencing detected the taxonomic variations in CFA producers in response to the nutrient limitation (Fig. 3). Specifically, some CFA producers belong to copiotrophs, such as *Proteobacteria* and *Bacteroidetes*, which preferentially consume labile soil organic C pools and have high nutritional requirements (Fierer et al., 2007). When resources are abundant, copiotrophs can exhibit high growth rates (Reznick et al., 2002; Fierer et al., 2007). While other microbes, such as *Acidobacteria*, exhibit slower growth rates and are likely to outcompete copiotrophs under conditions of low resource availability due to their high affinities with substrates (Meyer, 1994; Tate, 2000). The increase of soil depth impoverished soils (Zhu et al., 2021; Table S2) and increased the percentage of oligotrophs growing slowly (Fierer et al., 2007). Therefore, the relative abundance of CFA producers and CFA gene decreased with soil depth (Fig. 1). Overall, the seasonality of the impacts on meteorological factors altered the variations in CFA content over seasons and there are strong indicative functions of CFA to environmental stress, thus the first and second hypotheses were verified.

4.3. The stimulation of CFA to microbial activities

There were significant relationships between the variations of CFA content and microbial activities (Figs. 5–6), lending weight to the predominant mechanism explaining the regulation of CFA content to membrane function and stimulating microbial activities (Kolbeck et al., 2021). As two main characteristics of cell membranes, fluidity and permeability primarily depend on the content of different fatty acids (Zhang and Rock, 2008), and

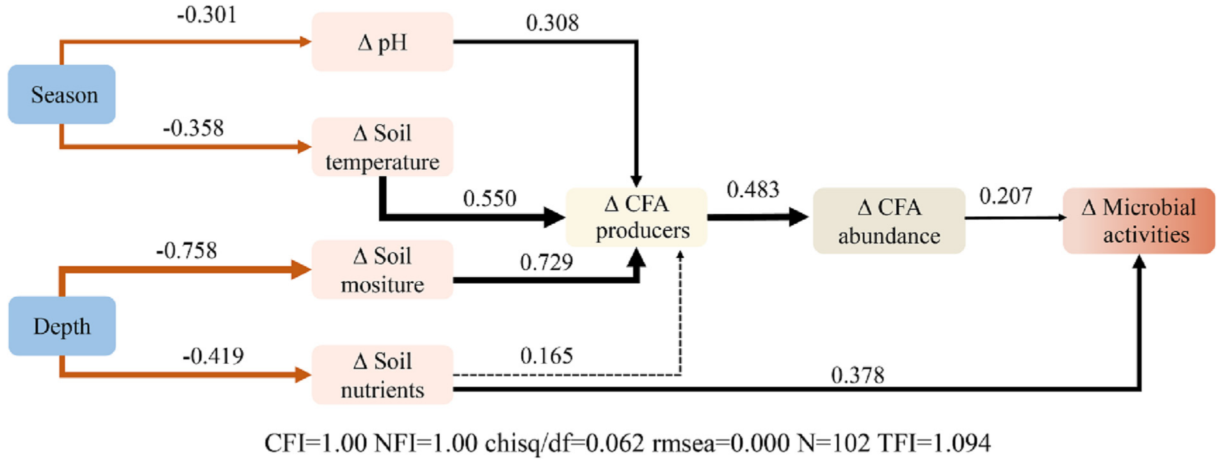

Fig. 5. Structural equation modeling (SEM) of the impacts of environmental changes on the alterations of cyclopropane fatty acid (CFA) and respiration (soil depth, season, and variations of soil nutrients (soil TC), soil temperature, soil moisture, soil pH, CFA producer, CFA abundance and microbial activities) black and orange solid arrows represent significant positive and negative effects, respectively. Solid line indicates the relationship was statistically significant ($p < 0.05$). Values associated with the arrows represent standardized coefficients. The information of the best-fitting regression models was: comparative fit index (CFI) = 1.000, normed fit index (NFI) = 1.000, Chisqare/df (chisq/df) = 0.062, root mean square error of approximation (RMSEA) = 0.000, and n = 102.

determine microbial physiological functions by controlling the transportation of matter (Velly et al., 2015). The modifications to existing fatty acids structure as well as regulation of new fatty acids biosynthesis, such as CFA, alter membrane fluidity and permeability, leading to regulation of transport processes, protein-protein interactions, and passive permeability of hydrophobic molecules (Kolbeck et al., 2021; Zhang and Rock, 2008; Cronan and Gelmann, 1975). CFA biosynthesis led to the enhancements of the fluidity, permeability and stability of the membrane lipid bilayer (Loffhagen et al., 2007; Moss et al., 1992), which improved the efficiency of carrier-mediated transport (Velly et al., 2015). The increase of CFA enhanced the active transportation and facilitated diffusion of inorganic ion (e.g. Fe^{3+}, NO_3^-) as electron acceptors and organic matters (e.g. glucose) as electron donors in the anaerobic environment (Chen and Gänzle, 2016; Spector and Yorek, 1985). CO_2 was produced by anaerobic respiratory, and the improved transport of these substrates contribute directly to MR, because the process fundamentally occurs in the cytoplasm rather than mitochondria (Zumft, 1997; Chen and Gänzle, 2016). In this study, CFA biosynthesis may improve membrane fluidity, permeability and stability, thereby improving the transportation efficiency of respiratory substrate and providing more favorable conditions for microbial activities.

The seasonal response of microbial activities to wetland reclamation supported the hypothesis that wetland reclamation stimulates the CFA content of microbial activities (Figs. 5–6). Although the variations of edaphic nutrients significantly influenced microbial activities, edaphic nutrients were not the determining factors controlling microbial activities over seasons. Our previous studies confirmed that wetland reclamation significantly and substantially reduced the concentrations of elements in surface soils, and this decreasing tendency was consistent over seasons (Zhu et al., 2021, 2022). Microbial activities were strongly suppressed by soil matrix loss (He et al., 2021), and the significant associations between microbial activities and edaphic nutrients were reflected in soil profile rather than in season. On the other hand, the increase of CFA content caused by environmental stress aggravation stimulated microbial activities in autumn and winter. The higher reduction of microbial activities in spring-summer was the synergistic consequence of element loss and the environmental stress intensification, while the smaller reduction of microbial activities in autumn-winter was the antagonistic effect between substrate loss and environmental stress alleviation. The energy produced by anaerobic respiratory is much less than aerobic respiration (Moodie and Ingledew, 1990), but the pattern of nutrient metabolism pattern plays an essential

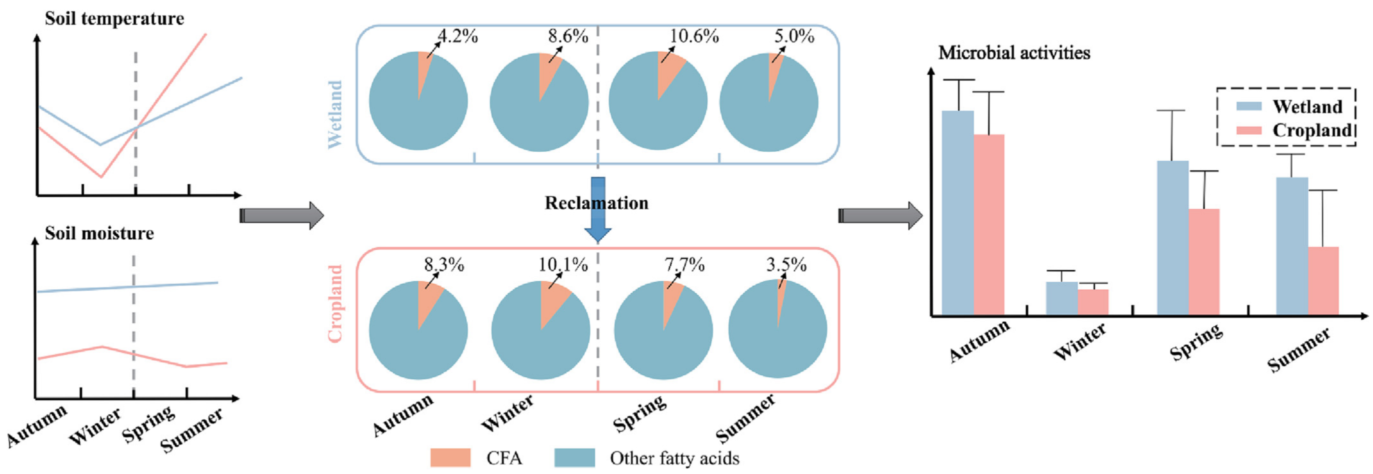

Fig. 6. Conceptual diagram for the impacts of wetland conversion to cropland on cyclopropane fatty acid (CFA) and microbial respiration rate in four seasons. The different responses of soil temperature and moisture to wetland reclamation caused various implications for CFA and controlled soil microbial activities.

role in maintaining other physiological activities, especially in a low-level O_2 environment (Dassonville and Renault, 2002). Therefore, the changing biosynthesis of CFA is likely a survival strategy for microbes to adapt to environmental stress, and CFA production plays a significant role in maintaining normal physiological activities.

4.4. Limitations and further work

This study illustrated the indication of CFA content to environmental stress and the strong stimulation of microbial activities induced by CFA due to environmental stress. However, the current study still had a few limitations, which need to be addressed in future work. First, it was challenging to measure other microbial functions such as nutrient cycling to estimate CFA influence. Secondly, this study is restricted to comparing two ecosystems (i.e, cropland, and wetland). Further studies would help to better understand the indication mechanisms of CFA content to environmental stress: 1) RNA-based techniques such as QPCR, may be implemented to detect the absolute biomass of CFA producer, so further investigation of the response of CFA producer to wetland reclamation need to be conducted; 2) future studies of soil microbial community and CFA under different tillage periods will be helpful to understand the process that land conversion gradually influences microbial activities; and 3) more measurements of soil physiochemical parameters, such as soil bulk density, dissolved organic carbon, ammonium, and metal ion, and microbial functions, will help to better understand the microbial mechanisms of CFA biosynthesis. The investigation of the factors controlling CFA production can also strengthen our understanding of global carbon cycling under a microbial macroecology framework (Xu et al., 2020; Guo et al., 2020), and provide new perspectives to evaluate the impacts of anthropogenic activities on microorganisms.

5. Conclusions

This study evaluated the response of CFA content to environmental stress induced by wetland reclamation and examined the stimulating effect of environmental stress-induced CFA on microbial activity. Wetland reclamation altered seasonal variations of soil temperature, moisture, and environmental stress on microbes. The content of CFA varied consistently with the fluctuation of soil temperature. Although soil nutrients played a vital role in the differentiation of CFA producer community structure, edaphic elements had little influence on the CFA biosynthesis in natural conditions. Increased CFA alleviated the reduction of microbial respiration caused by wetland reclamation, suggesting a strong stimulation effect of CFA content on microbial activity. This study proved the indication of CFA content of environmental stress and provided a mechanism framework of CFA stimulating microbial activities. It showed that the seasonal impacts of wetland reclamation on environmental factors led to the fluctuation of the CFA variations, then altered the reduction of microbial activities. The framework provides a potential insight for explaining the feedback of microbial communities to environmental change during the anthropogenic disturbance. It can help us better understand and predict the spatiotemporal distributions and dynamic changes of microbial and soil element cycling.

CRediT authorship contribution statement

The authors confirm contribution to the paper as follows: F.Y., and L.Z. conceived and designed the project. X.Z., N.W, J.L and Y.Z carried out soil sampling. K.L. and N.W. carried out lab experiments. X.Z carried out data analysis and interpretation with Z.G., Y.S., and C.G. X.Z. prepared the manuscript with assistance from F.Y., L.Z, and X.X. X.X. and C.S. supervised the project.

Data availability

The genome and gene data presented in this study can be found from the NCBI database repository at https://dataview.ncbi.nlm.nih.gov/object/PRJNA853804.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162338.

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