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Soil microbial biodiversity supports the delivery of multiple ecosystem functions under elevated CO_2 and warming

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The contribution of the soil microbes to agroecosystem multifunctionality under global change remains poorly understood. Here, based on data from a field experiment involving elevated carbon dioxide (CO_2) and warming in a rice-wheat agroecosystem, we found that soil microbes influence the impact of climate change on agroecosystem functions. The stability of food production during the rice season increased under elevated CO_2 but decreased under warming, with no significant changes in the wheat season. The interactive influences of elevated CO_2 and warming on agroecosystem multifunctionality were found to be minimal. The abundance of soil fungi and nematode was associated with agroecosystem stability during the rice and wheat seasons, respectively. Soil archaeal diversity and bacterial abundance were linked to agroecosystem multifunctionality in the rice and wheat seasons, respectively. Our work proves the positive effects of soil microbes on agroecosystem functions and highlights the implications of maintaining microbial diversity for agroecosystem health under climate change.

Agroecosystems play a pivotal role in providing multiple functions (multifunctionality) like food production, food security, carbon sequestration, and nutrient cycling[1–3]. However, these functions are highly vulnerable to climate change[4]. Ecosystem stability, defined as the ratio of the temporal mean of plant production to its standard deviation, represents a fundamental property that remains largely unexplored under ongoing climate change[5,6]. Numerous studies have consistently demonstrated that climate change impacts plant production, soil microbial communities, and subsequently, ecosystem functioning and stability[7–9]. For instance, elevated temperature can reduce crop production by shortening the crop growth periods thought to promote nutrient cycling with increased soil microbial activity[10–12]. Elevated carbon dioxide (CO_2), on the other hand, positively influences food production by improving crop photosynthesis[13]. However, tradeoffs that were often noted between elevated CO_2 and climate warming may not account for the adverse influences of climatic change[11]. Numerous studies reported that elevated CO_2 may not fully counterbalance the adverse

impacts of elevated temperature on food productivity and ecosystem stability[8,14,15]. To date, the effects of elevated temperature and CO_2, either alone or in combination, on ecosystem stability and multifunctionality in agroecosystems remain poorly understood.

The diversity of soil microbial communities is recognized as crucial catalysts for regulating ecosystem multifunctionality[5,16]. More recently, it has been found that microbial biomass and community composition were closely related to changes in ecosystem stability and functionality[3,17]. The abundance, diversity, and community composition of soil microbial guilds are normally assumed sensitive to ongoing climate change[18]. It is generally accepted that elevated CO_2 could benefit soil microbial growth and enrichment due to stimulated root biomass and exudation[19,20]. By contrast, elevated temperature can have directly adverse effects on the fluidity of the cell membrane and enzyme reactions, ultimately affecting cellular synthesis and microbial community[21–23]. Warming may also indirectly affect soil microbial growth by reducing carbon inputs into the

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soil[8,24,25]. The interplay between soil microbes and climate change suggests that shifts in the abundance, diversity, and community composition of soil microbes can modulate their roles in driving ecosystem stability and multifunctionality[5,23,25–27]. To date, the tradeoffs between CO_2 and warming on soil microbial communities and their effects on ecosystem stability and functionality remain largely unknown in agroecosystems.

Soil microbial communities, including archaea, bacteria, fungi, and fauna, play important roles in agroecosystems[28,29]. When studying the influence of climatic change on ecosystem functioning, most research has predominantly emphasized the role of soil bacteria and fungi while overlooking the essential contributions of soil microfauna and archaea[3]. Archaea, as prominent members of soil ecosystems, are highly sensitive to climate change and vital for mediating carbon, nitrogen, and sulfur cycles in terrestrial ecosystems[25,30]. Soil nematodes, important components of soil microfauna, include bacterivores, fungivores, herbivores, and omnivores-predators[31], that play more pivotal roles than other microbial communities in influencing both ecosystem stability and functions[3,32]. Yet, there is presently a dearth of experimental observations regarding the effects of soil microbes on ecosystem stability and multifunctionality under climate change[17,28]. Therefore, there is an urgent need to elucidate the role of soil microbial communities as regulators of ecosystem stability and multifunctionality in the context of ongoing climate change.

To address these knowledge gaps, we further investigated results from a field experiment including elevated CO_2 and temperature[8] to explore how agroecosystem stability and multifunctionality respond to climate change within a rice-wheat rotation system. We hypothesize that elevated CO_2 and temperature will have opposing effects on ecosystem stability and multifunctionality, potentially due to their contrasting impacts on crop biomass and soil microbial communities (H1). Additionally, we anticipate that soil microbial communities will have a strong impact on agroecosystem stability and multifunctionality (H2), as elevated CO_2 and temperature altered the activity of soil bacterial, fungal, archaeal, and nematode communities, which have been identified as key predictors of agroecosystem multifunctionality under climate change[3]. Lastly, we hypothesize that climate change impacts on the stability and multifunctionality of the ecosystem may differ between wheat and rice systems (H3). This hypothesis is based on previous observations suggesting that the influence of elevated CO_2 and climate warming on food productivity and quality was more pronounced for wheat than rice[11].

To test these hypotheses, we conducted annual evaluations of ecosystem stability and multifunctionality over five consecutive years in a rice and wheat rotation system. Wheat and rice are global primary staple crops[33]. China plays a crucial role as one of the major grain-producing countries in the world, with rice-wheat cropping systems serving as a critical component for food security[34]. However, the rice-wheat rotation system has been demonstrated to be susceptible to the impacts of climate change[8,35]. Consequently, evaluating the potential effect of climate change on the stability and multifunctionality of the rice and wheat rotation system could provide important insights into enhancing human well-being and combatting climate change in the future. They can also be informative about the factors influencing agroecosystem stability and multifunctionality under climate change in the future.

Results
Agroecosystem stability
Agroecosystem stability exhibited higher values in rice than in wheat, with a 33.9% increase under elevated CO_2 ($P = 0.010$), but a 28.4% decrease under canopy warming ($P = 0.005$) in rice. However, elevated CO_2 and canopy warming had no significant effects on agroecosystem stability in wheat (Fig. 1 and Table 1).

Agroecosystem multifunctionality
The responses of multifunctionality relied on both crop types and climate change factors. Elevated CO_2 positively affected food production in rice (60.0%, $P < 0.001$) and wheat (90.3%, $P = 0.003$) (Table 1). Similarly,

elevated CO_2 led to increases in the SOM decomposition in rice (105.7%, $P = 0.001$) and wheat (51.7%, $P = 0.056$), as well as carbon pools in wheat (39.0%, $P = 0.046$). However, elevated CO_2 had negative impacts on food quality in both rice (−49.5%, $P < 0.001$) and wheat (−55.8%, $P < 0.001$). By contrast, canopy warming negatively affected the function of food production in rice (−56.3%, $P < 0.001$) and wheat (−51.1%, $P = 0.002$). However, canopy warming increased the functions of food quality in rice (113.33%, $P < 0.001$) and wheat (46.4%, $P = 0.002$), and that of SOM decomposition in rice (69.3%, $P = 0.007$) and wheat (59.2%, $P = 0.038$). The decrement of food quality under eT was lower under ambient conditions than under eCO$_2$ (Supplementary Fig. S1). The PCoA ordinations and PERMANOVA analysis revealed significant differences between control and treatment groups for both crops ($P = 0.001$) (Fig. 2).

Agroecosystem stability and multifunctionality associated with soil biodiversity
The results of Pearson correlation analysis and multiple regression models highlighted the critical association between soil microbial communities and agroecosystem stability and functions under elevated CO_2 and canopy warming (Figs. 3, 4). Specifically, during the rice season, fungal diversity and structure were the best predictors of ecosystem stability (Fig. 3). In the wheat season, ecosystem stability was strongly influenced by the abundance of soil archaea and nematodes, as well as fungal diversity (Fig. 4).

Fungal diversity and structure displayed positive impacts on food production and negative impacts on food quality. Additionally, soil archaeal diversity emerged as the primary predictor for nutrient cycling, SOM decomposition, and multifunctionality during the rice season (Fig. 3). In the wheat season, soil bacterial diversity was the most influential predictor for food production. Notably, food quality exhibited a negative association with soil archaeal abundance but a positive correlation with archaeal diversity. Soil nematode and fungal structure respectively associated with nutrient cycling and SOM decomposition. Furthermore, soil bacterial abundance and nematode structure were identified as the primary predictors for multifunctionality (Fig. 4).

Discussion
Agroecosystems are critical to support food security and provide multiple ecosystem services that are of importance due to the increases in world populations and climate change. Our field experimental data indicate that the responses of agroecosystem multifunctionality are climate change dependent, and largely influenced by crop types. Warming led to the main negative effects, while elevated CO_2 acted as a plant nutrient supporting function. We further showed that soil microbiomes influence the responses of multifunctionality to future climate change. This knowledge is critical to support the conservation of soil organisms in ecosystems, and anticipate details about their future functioning.

Effects on agroecosystem stability
Agroecosystem stability appeared somewhat higher in the rice season compared to the wheat season (Fig. 1a), aligning with previous research indicating greater inter-annual variability in wheat production than in rice[36]. Our findings support this observation, as wheat yield and biomass displayed a higher inter-annual variability than rice[8].

Agroecosystem stability was affected by elevated CO_2 and temperature in rice seasons. Climate change may be responsible for roughly one-third of the variability in global crop yields[37]. Elevated CO_2 and temperature have been identified as direct regulators of agroecosystem stability[24,38]. Our data highlights that canopy warming is the primary driver of the decline in ecosystem stability, likely due to substantial crop yield losses[39]. Shi et al.[40] reported that warming affects ecosystem stability by influencing both mean and variation in plant production. Climate warming can restrict crop growth and food production by shortening the growth period and reducing the grain weights for both rice and wheat[10,11]. However, elevated CO_2 has been observed to enhance leaf photosynthesis, increase crop yields, promote plant growth, and contribute to ecosystem stability[38,41]. Nevertheless,

elevated CO_2 may not entirely mitigate the adverse effects of elevated temperature on crop yield and ecosystem stability, consistent with findings from previous studies[42]. Additionally, our previous research emphasized the influence of inter-annual temperature variation during crucial growth stages on crop production under elevated CO_2 and temperature[8].

Interestingly, elevated CO_2 and temperature had a negligible impact on ecosystem stability in the wheat season (Table 1). These results emphasize the distinct influence of plant types on agroecosystem stability under climate change. Consistent with our findings, several prior studies have noted that climate change effects on agroecosystem processes are contingent on plant varieties[3,43]. Specifically, the positive influence of elevated CO_2 and the adverse effect of increasing temperature on ecosystem stability were only observed in the rice season, indicating a high vulnerability of agroecosystem stability during this crop. Therefore, enhancing rice paddy resistance and mitigating the adverse influence of future climate change on ecosystem stability represent important challenges.
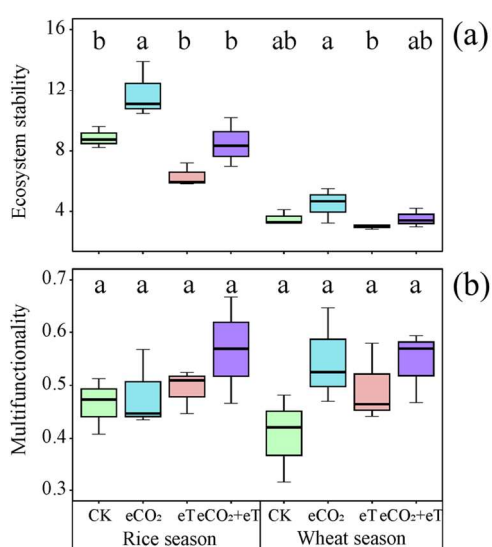

Fig. 1 | Changes in agroecosystem stability and multifunctionality under climate change. a Changes in agroecosystem stability under elevated CO_2 and temperature. **b** Changes in agroecosystem multifunctionality under elevated CO_2 and temperature. Different letters indicate significant differences between treatments at $P < 0.05$. CK, ambient conditions; eCO_2, elevated CO_2 (500 µmol mol^{-1}); alone; eT, elevated canopy temperature by 2°C alone; eCO_2+eT, simultaneous elevation of CO_2 and temperature.

Effects on multifunctionality
Results partially support our initial hypothesis. Multiple functions were positively influenced by elevated CO_2 in both crops. These positive effects can be attributed to the well-known CO_2 capacity to enhance leaf photosynthesis and soil microbial activity through a fertilization effect[8,18]. Importantly, elevated CO_2 improved carbon pools during the wheat season, primarily due to increased carbon allocation belowground[20]. However, the positive influence of elevated CO_2 on food productivity was accompanied by changes in food quality[44,45], with a significant reduction observed in this study.

On the other hand, canopy warming had adverse effects on food production, but showed positive impacts on food quality, as well as SOM decomposition, in rice. Furthermore, canopy warming led to a reduction in SOM decomposition during the wheat season. Previous research has identified canopy warming as a driver of grain yield declines in wheat and rice seasons[8], potentially contributing to a relative reduction in food production. However, the relative changes in other functions did not align with those observed in food production. For example, canopy warming increased food quality and SOM decomposition but had minimal effects on nutrient cycling and carbon pools in both rice and wheat seasons. This increase in food quality may result from a concentration effect of crop yield loss under canopy warming. In practice, the trade-off between grain quality and crop yield may not entirely mitigate the adverse effects of warming on food quality[11].

Surprisingly, our findings indicate that these changes did not consistently alter ecosystem multifunctionality under the conditions of elevated CO_2 and canopy warming (Table 1 and Fig. 1). This aligned with previous studies that found negligible effects of climate change on multifunctionality[3]. The limited responsiveness of multifunctionality to elevated CO_2 and temperature could be attributed to the short-term duration of the treatments[46], suggesting that the duration of simulated climatic change played a pivotal role in shaping agroecosystem multifunctionality. Moreover, the adverse influence of elevated CO_2 on food quality was mitigated by canopy warming, indicating a notable interaction between CO_2 and temperature. Therefore, trade-offs between individual functions may counteract the effects of elevated CO_2 and temperature on agroecosystem multifunctionality.

Soil microbial biodiversity and ecosystem stability
Our findings emphasize the positive relationship between soil fungal communities and ecosystem stability during the rice season under elevated CO_2 and temperature (Fig. 3a). Soil fungal communities are crucial for global terrestrial biomass and diversity, playing essential roles in nutrient

Table 1 | Summary of GLM for agroecosystem stability and functions under elevated CO_2 and temperature

Seasons	Source	Ecosystem stability	Food production	Food quality	Nutrient cycling	SOM decomposition	Carbon pools	Multifunctionality
Rice season	CO_2 effect (%)[a]	33.87	59.95	−49.50	5.89	105.72	−13.73	9.68
	Warming effect (%)[b]	−28.42	−56.27	113.33	15.94	69.27	2.08	11.97
	CO_2	0.010	<0.001	<0.001	0.619	0.001	0.304	0.247
	Warming	0.005	<0.001	<0.001	0.208	0.007	0.868	0.194
	CO_2×Warming	0.615	0.748	0.222	0.083	0.531	0.417	0.524
Wheat season	CO_2 effect (%)[a]	22.41	90.27	−55.81	41.38	51.67	38.99	21.13
	Warming effect (%)[b]	−18.26	−51.06	46.40	16.26	59.21	9.56	8.90
	CO_2	0.106	0.003	<0.001	0.130	0.056	0.046	0.072
	Warming	0.107	0.002	<0.001	0.481	0.038	0.528	0.383
	CO_2×Warming	0.633	0.274	0.006	0.152	0.345	0.051	0.339

[a]Main effects of elevated CO_2 calculated as ((elevated CO_2)/(ambient)−1))×100.
[b]Main effects of warming calculated as ((warming)/(ambient)−1)×100.

Fig. 2 | The structure of multiple ecosystem functions under climate change. a The structure of multiple ecosystem functions was analyzed through PCoA during rice seasons, considering the effects of elevated CO_2 and temperature. **b** The structure of multiple ecosystem functions was analyzed through PCoA during wheat seasons, considering the effects of elevated CO_2 and temperature. CK, ambient conditions; eCO_2, elevated CO_2 (500 µmol mol⁻¹) alone; eT, elevated canopy temperature by 2°C alone; eCO_2 +eT, simultaneous elevation of CO_2 and temperature.
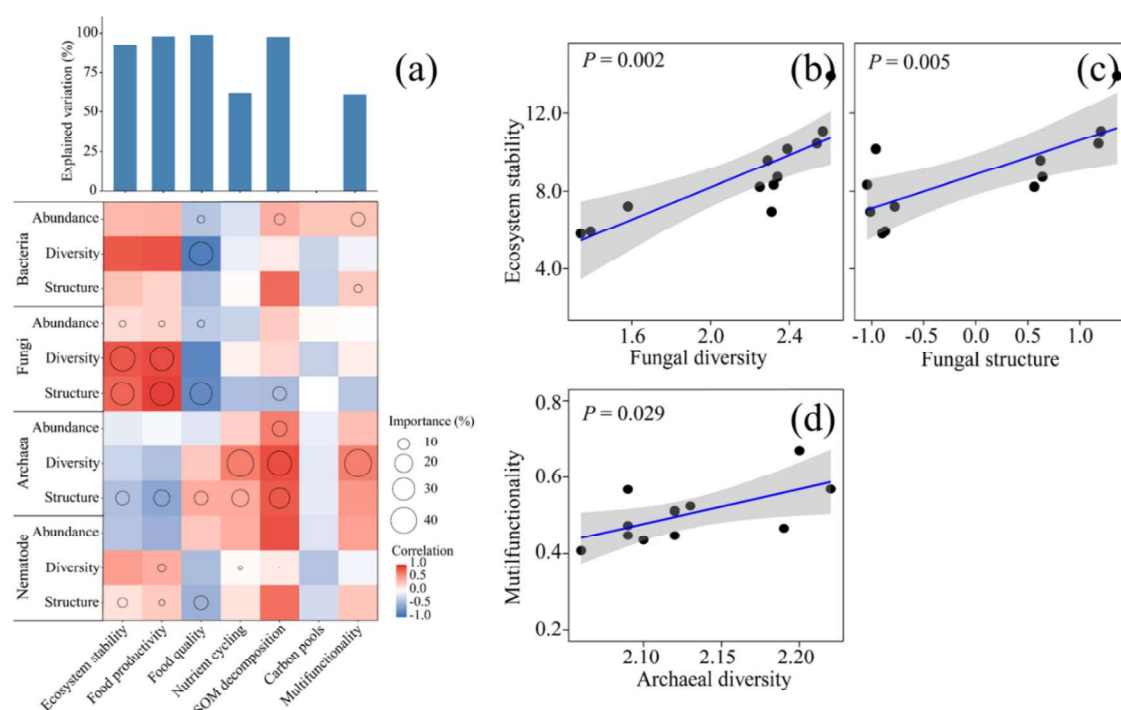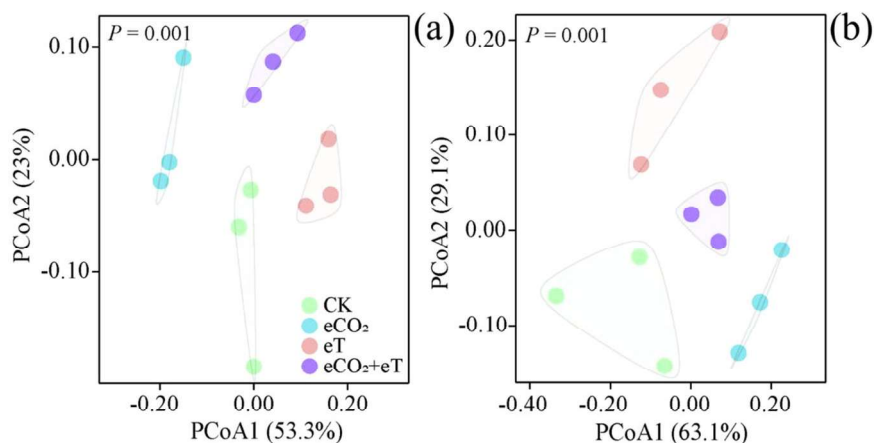
Fig. 3 | Effects of soil microbial communities on the agroecosystem stability and functions under elevated CO_2 and temperature in rice season. a A multiple regression model for assessing the relationships between soil microbial communities and ecosystem functions. **b** The linear relationship between ecosystem stability and soil fungal diversity. **c** The linear relationship between ecosystem stability and soil fungal structure. **d** The linear relationship between ecosystem multifunctionality and soil archaeal structure. The colors in the figure represent Pearson's correlation, where blue and red indicate negative and positive connections, respectively.

uptake, organic matter decomposition, and plant production[47–49]. These communities are likely to enhance ecosystem stability by fortifying the resilience and resistance of plant productivity in the face of climate change[50,51]. Fungal decomposers, for example, play a critical role in breaking down plant litter, providing a constant source of accessible nutrients that contribute to stable plant production[52]. Recent studies have emphasized the vital contribution of fungal diversity to sustaining consistent plant productivity in global ecosystems over time and mitigating the effects of extreme climate events[5]. Notably, soil fungal diversity and structure positively explained variations in agroecosystem stability in this study (Fig. 3b, c).

We also found a consistent negative correlation between agroecosystem stability and soil nematode communities, particularly soil nematode abundance during the wheat season (Fig. 4a, b). Nematode communities occupy a pivotal role within the soil micro-food web[31], engaging in various

ecological processes such as litter decomposition, plant health, and plant stability. Nematode abundance has been demonstrated to be susceptible to disturbances caused by elevated CO_2 and climate warming[53], potentially posing threats to ecosystem stability.

Soil microbial biodiversity and ecosystem functions
Soil archaeal diversity emerged as the most important indicator of multifunctionality during the rice season (Fig. 3a, d). Archaea, as prominent members of terrestrial ecosystems, are ubiquitously present in the soil, accounting for a substantial carbon mass and exhibiting distinctive physiologies[54,55]. Soil archaeal communities play pivotal roles in global carbon, nitrogen, and sulfur cycles, including ammonia-oxidizing and methanogenic taxa[20,56]. Recent studies have demonstrated that climate change-induced changes in the archaeal community affect ecosystem functionality[25]. Indeed, soil archaeal diversity was identified as the primary
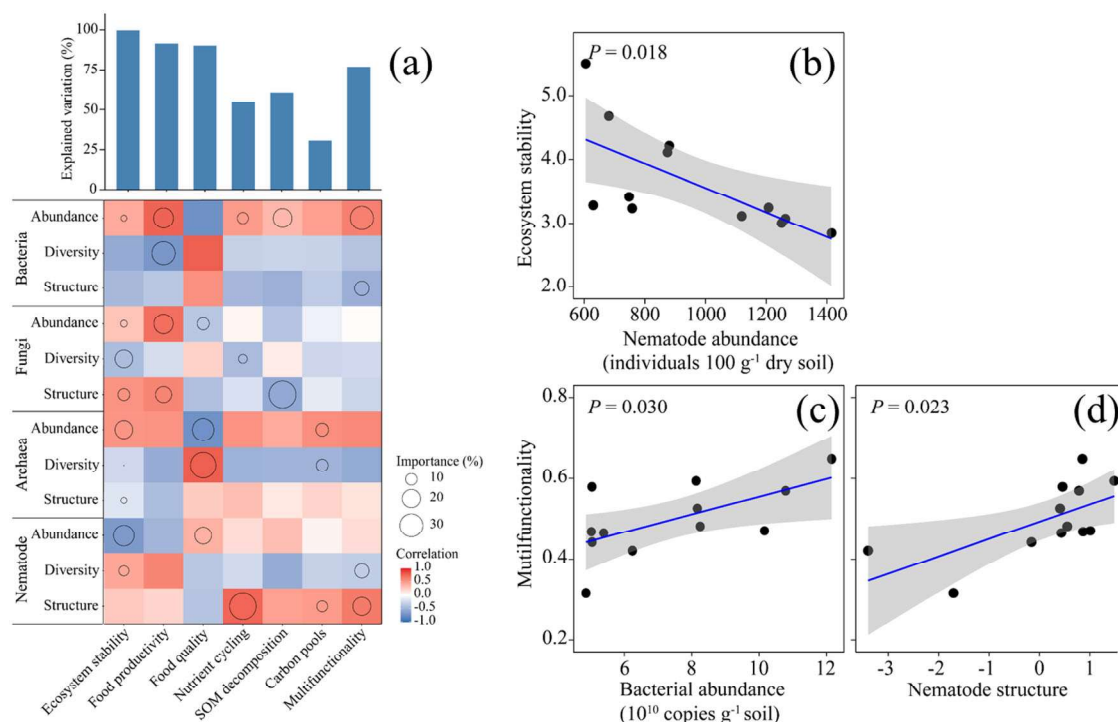
Fig. 4 | Effects of soil microbial communities on the agroecosystem stability and functions under elevated CO₂ and temperature in wheat season. a A multiple regression model for assessing the relationship between soil microbial communities and ecosystem functions. **b** The linear relationship between ecosystem stability and soil nematode abundance. **c** The linear relationship between ecosystem multifunctionality and soil bacterial abundance. **d** The linear relationship between ecosystem multifunctionality and soil nematode structure. The colors in the figure represent Pearson's correlation, where blue and red indicate negative and positive connections, respectively.

predictor for nutrient cycling, SOM decomposition, and multifunctionality during the rice season.

This study highlights the close association between bacterial abundance and ecosystem multifunctionality during the wheat season under elevated CO₂ and climate warming. Soil bacteria play a vital role in terrestrial ecosystems[57]. Previous research has underscored that soil functionality was primarily explained by soil microbial abundance rather than other microbial traits under climate change[22]. Additionally, climate change influenced soil bacterial communities[58]. Therefore, changes in bacterial abundance induced by elevated CO₂ and temperature may be associated with variations in ecosystem functions, such as food production and multifunctionality during the wheat season (Fig. 4).

Not all soil microbial communities serve as the predominant biological factors governing changes in ecosystem stability and functions. Data show that the impact of soil microbial communities on ecosystem stability and functionality under elevated CO₂ and temperature varied depending on crop types. For instance, soil nematode abundance was significantly and negatively associated with agroecosystem stability during the wheat season, but it showed no significant association in the rice season. Furthermore, soil archaeal diversity was positively and significantly associated with agroecosystem multifunctionality in the rice season, but had a negative relationship during the wheat season. These results indicated that different ecosystem functions may not be simply predicted and assessed based on a single microbial community[59]. Consistent with our findings, several studies have emphasized that a single microbial community may not adequately represent ecosystem stability and multifunctionality, as certain groups can counterbalance the impacts of other groups[28]. Therefore, future research on ecosystem stability and functionality should comprehensively consider various soil microbial communities to better address global climate change challenges.

In summary, we provide evidence on the influence of elevated CO₂ and climate warming on agroecosystem stability and multifunctionality. The results indicated the close association among ecosystem stability, functions, and soil microbial communities, and highlighted the positive effects of soil microbial traits on ecosystem stability and multifunctionality under climate change[60,61]. Moreover, we anticipate that soil microbial communities would be capable of mitigating climate change and maintaining ecosystem stability and functioning in the future. However, it should be acknowledged that these findings are based on observations from a single experimental site, and further research is encouraged to generalize the effects of elevated CO₂ and temperature on agroecosystem stability and multifunctionality.

Conclusion

Agroecosystem stability enhanced under elevated CO₂ but reduced under canopy warming in the rice season, while no significant shifts were found in the wheat season. Elevated CO₂ and temperature had limited influence on agroecosystem multifunctionality in both crops. Nevertheless, soil microbial communities were closely associated with agroecosystem stability and functions. Fungal communities and nematode abundance were primary factors influencing agroecosystem stability during the rice and wheat seasons, respectively. Additionally, soil archaeal diversity and bacterial abundance were crucial predictors of multifunctionality in rice and wheat seasons, respectively. Our findings underscore the vulnerability of agroecosystem stability to climate change, particularly warming, and highlight the importance of incorporating multiple soil microbial communities, including soil food webs, into studies on ecosystem stability and multifunctionality.

Materials and methods
Experimental design
The field experiment was carried out between 2011 and 2015 at an open-air field located in Kangbo Village, Jiangsu Province (31°30′N, 120°33′E), China. The study area features a subtropical monsoon climate with an average annual temperature of 16 °C and an annual precipitation range of

1100–1200 mm. We conducted our experiment using a winter wheat-summer rice rotation[8].

Our experiment comprises a factorial design involving two CO_2 concentrations and two canopy temperatures, specifically elevated CO_2 (500 µmol mol⁻¹) alone (eCO₂), elevated canopy temperature by 2 °C alone (eT), simultaneous elevation of CO_2 and temperature (eCO₂+eT), and ambient CO_2 and temperature (CK). During the crop-growing period, elevated CO_2 and temperature were kept throughout the day. In this study, treatments were conducted in octagonal ring plots with an 8.0 m diameter (approximately 50 m² area). The treatments were organized into three blocks, each encompassing all four treatment conditions, and were consistently maintained throughout the respective growing periods. To mitigate potential cross-contamination of CO_2 and temperature within the treatment areas, we established open fields as buffers, maintaining a distance of approximately 28 meters between them and the experimental rings.

For elevated CO_2 treatments, pure CO_2 (purity 99.99%) was injected into the ring plot via a liquid tank connected to perforated pipes surrounding the ring plots. The release of CO_2 was automatically regulated based on air CO_2 concentration, wind direction and speed. Seventeen Li-COR CO_2 sensors were strategically positioned both over the canopy and around the ring to ensure the precise control of CO_2 injection. The elevated temperature treatments involved the use of infrared heaters suspended over the plot. Each ring plot was equipped with twelve ceramic infrared heaters, providing 140 W/m² of infrared radiation. These infrared lamps emitted radiation that elevated the canopy air temperature during the growing season. To ensure precise control, five infrared detector thermometers were installed over the canopy and around the ring to provide feedback to the air heating systems.

Crop cultivation

We sowed wheat seedlings (cv. Yangmai No.14) to a density of 250 seedlings per square meter, maintaining a row spacing of 20 cm. Rice, specifically the Changyou No.5 cultivar, was transplanted at a density of 26 hills per square meter, with each hill containing three seedlings. We used urea fertilizer (containing 46% N) as a basal application at a rate of 188 kg per hectare. Additionally, a topdressing fertilizer was used at a rate of 150 kg per hectare, twice during the rice seasons and once during the wheat seasons. We applied a compound fertilizer with an N: P_2O_5: K_2O ratio of 15:15:15 at a rate of 375 kg per hectare as topdressing after the heading stage for both wheat and rice crops. The basal fertilizer was evenly spread and integrated into the soil through plowing to a depth of 10 cm prior to planting, while the top-dressed fertilizer was evenly distributed on the soil surface without incorporation. Water management, weed control, and insecticide application followed local practices, with winter wheat relying on rain-fed irrigation and summer rice being flooded until approximately 15 days before harvest.

Soil and plant samples

During the harvest stage, five rhizosphere soil samples were collected randomly from each ring plot. After removing plant residues, the soil particles adhering to the roots were meticulously extracted. These soil particles were combined, homogenized, and formed into composite samples representing each plot. To maintain sample integrity, the composite samples were stored in an icebox within two days of collection.

During the harvest stage, the entire aboveground biomass, including both shoots and grains, was collected and weighed to measure grain yield and biomass production for each treatment plot. To ascertain the water content of both straws and grains, three sub-samples (approximately 500 g each) were extracted from each ring plot. These sub-samples were subsequently subjected to initial oven-drying for 30 min at 105 °C, followed by further drying at 70 °C until a constant weight was achieved.

Sample analysis

The methods for the analysis of soil dissolved organic carbon (DOC), microbial biomass carbon (MBC), and soil enzyme activity (urease, invertase, and phosphatase) were described by Liu et al. [18]. Soil NO_3^--N and

NH_4^+-N were determined by a flow analysis instrument (Skalar, Netherlands). Soil available P was extracted using $NaHCO_3$ and quantified using a spectrophotometer (TU-1810, China). Soil available K was extracted using ammonium acetate (NH_4OAc) and quantified using a flame photometer (FP6410, China).

Plant samples underwent pretreatment with H_2SO_4–H_2O_2, and the resulting mixture was subsequently heated on an electric heating plate at 550 °C for a duration of 4 h. Afterward, the N and P content were respectively determined by the Kjeldahl digestion method and spectrophotometry (TU-1810, China), while plant K content was analyzed by a flame photometer (FP6410, China). Grain essential amino acids were analyzed following the method described by Wang et al. [11]. Grain protein was calculated by applying conversion factors of 5.95 for rice and 5.83 for wheat to the grain N concentration.

Soil biodiversity

The analysis of soil nematode communities was conducted using a modified Baermann method, with 100 g of fresh soil as the sample[19]. Subsequently, under a Motic microscope (40× and 400×), 150 randomly chosen individuals per soil sample were utilized for the taxonomic classification of nematode genera. Soil nematode abundance was expressed as the total number of nematode individuals per 100 g dry soils. The Shannon–Wiener diversity index was employed to assess the diversity of soil nematode using the following approaches: Shannon–Weaver diversity $H' = -\sum Pi(\ln Pi)$, where Pi is the proportion of nematode individuals in the i-th taxon.

The analysis of fungal, bacterial, and archaeal communities followed the procedures outlined by Liu et al. [18]. Soil DNA samples were extracted from 0.35 g of fresh soil with a PowerSoil™ DNA isolation kit (MoBio, CA, USA) according to the manufacturer's instructions. The quality of soil DNA was evaluated via agarose gel electrophoresis, and the DNA quantity was measured with a Nanodrop spectrophotometer (Thermoscientific, DA, USA).

The primer pairs (338/518 for bacteria, NS1-F/FungR for fungi, and Parch519f/Arch915r for archaea) were employed to quantify archaeal, fungal, and bacterial abundances[62–64]. Briefly, quantitative polymerase chain reaction (qPCR) analysis of soil bacteria, fungi and archaea was performed using a 7500 real-time PCR system (Applied Biosystems, Germany). PCR was performed in 25 µL reaction volume consisting of 12.5 µL of SYBR premix EX Taq TM (Takara Shuzo, Shinga, Japan), 1 µL of each primer (10 mM), 1 µL ROX Reference Dye II (50×), 1 µL diluted DNA template, and 9.5 µL sterile water. The PCR conditions for bacterial 16S rRNA were as follows: 3 min at 95 °C; 40 cycles of 95 °C for 1 min, 56 °C for 1 min, and 72 °C for 1 min. The PCR conditions for archaeal 16S rRNA were as follows: 3 min at 95 °C; 40 cycles of 95 °C for 1 min, 57 °C for 1 min, and 72 °C for 1 min. The PCR conditions for fungal 18S rRNA were as follows: 3 min at 95 °C; 40 cycles of 95 °C for 15 s, 53 °C for 30 s, and 72 °C for 1 min. The standards for quantifying bacterial 16S rRNA, archaeal 16S rRNA and fungal 18S rRNA gene abundance were developed using cloned inserts specific to each rRNA gene. The specificity of qPCR products was evaluated through melting curves and gel electrophoresis. The inhibitory effect of qPCR amplification was assessed using a series of DNA template dilutions. The R^2 of both standard curves was greater than 0.99, and the amplification efficiency for the genes tested was between 90% and 100%[18].

Soil bacterial, fungal, and archaeal communities were assessed using Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis[18]. Briefly, PCR was performed on all samples using specific primers to amplify the 16S small subunit rRNA region for bacteria (27 F and 1401 R)[65] and archaea (A20f and AR927r)[66], as well as the nuclear ITS regions for fungi (ITS1f and ITS4)[67]. Each forward primer in the pairs was labeled with a fluorescent tag at the 5' end (6- FAM for 27 F, A20f, and ITS1f). PCR products were purified using PCR Fragment Purification Kit (Takara). Purified PCR products were used to restriction digestion using HhaI (Takara, Dalian, China) for bacterial and fungal genes, and AfaI (Takara, Dalian, China) for archaeal genes following the manufacturer's guidelines.

Fragment analysis was performed using capillary electrophoresis on an ABI 3100 Genetic Analyzer (Applied Biosystems, CA), with a GeneScan ROX-labeled GS500 internal size standard. T-RFLP patterns were generated using GeneMapper software (Applied Biosystems). Peaks ranging from 50 to 550 bp were selected to exclude T-RFs resulting from primer-dimers. The relative abundance of a true T-RF within each T-RFLP pattern was determined based on the ratio of its peak height. Peaks with heights <2% of the total peak height were excluded from further analysis.

The diversity of soil microbial communities was assessed using the Shannon–Wiener diversity index and the following approaches: Shannon–Weaver diversity $H' = -\sum Pi(\ln Pi)$, where Pi is the relative abundance of each T-RF compared to all T-RFs in the sample. Finally, principal component analysis (PCA) of the T-RFLP profiles was employed to evaluate the structure of soil microbial communities using the 'vegan' package in R4.2. For each microbial community, the first principal component axis values of PCA were used to represent the structure of soil microbial community.

Agroecosystem stability

Changes in grain yield were employed to illustrate ecosystem stability in this study. We computed the temporal stability as the ratio of the mean crop yield from 2012 to 2015 to the standard deviation of the annual crop yield within that time frame[5]. Therefore, the ecosystem stability was computed using the following formula:

$$\text{Ecosystem stability} = \frac{\text{Mean}}{\text{Standard deviation}} \quad (1)$$

Multifunctionality

We collected information on five groups of ecosystem functions including food production, food quality, nutrient cycle, SOM (soil organic matter) decomposition, and carbon pools (Supplementary Table S1). Grain yield was used to indicate food production. Grain essential amino acids, protein, iron, and zinc were measured as an index of food quality. Total plant and soil nutrient contents were measured to determine the nutrient cycle. Soil DOC and MBC were employed to evaluate the function of carbon pools. The function of SOM decomposition was evaluated by soil enzyme activities. In each of these functional classes, standardized ecosystem variables transformed individual functions through the utilization of Z-scores. Subsequently, the normalized individual functions were subjected to averaging, leading to the computation of an overall multifunctionality score[3].

Statistical analysis

We investigated the impacts of elevated CO_2 and temperature on ecosystem stability and functions. An analysis employing a one-way ANOVA was followed by Duncan's test. Evaluating the primary impacts of CO_2 and temperature on ecosystem stability and functions was achieved through general linear model analysis (GLM). Additionally, the distribution of various ecosystem functions was illustrated using PCoA (principal co-ordinates analysis). We employed the PERMANOVA (permutational multivariate analysis of variance) with the Adonis function to assess differences in ecosystem functions under elevated CO_2 and temperature. We utilized Spearman correlation analysis to examine the relationship between soil microbial communities and agroecosystem stability and functions. We used the 'relaimpo' package in R to carry out a multiple regression model for assessing how soil microbial communities influence variations in ecosystem stability and functions[3].

Data availability

Data are available from DRYAD https://datadryad.org/stash/share/7Y666scbBovTVPuvNRJcueGMVS51RdGb5r_wgmR759w.

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Author contributions

J.W. contributed ideas to the study, collected and analyzed the data, and drafted the manuscript. M. D. B., and X.S. contributed ideas to the study, and improved the manuscript. J.P., and G.Z. improved the manuscript. Y.L. and L.C. collected the data. J.M. analyzed the data. G.P. provided experimental design and supervision, and carried out the management. All authors discussed the analyzes and the results, contributed to the writing of the article, and approved the submitted version.

Competing interests

The authors declare no competing interests.

Additional information

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