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Effects of Irrigation Patterns on Soil Microbial Network Structure and Methanogenic Pathways in Subtropical Paddy Fields

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Abstract Paddy fields in subtropical regions are constantly submerged in water, which can easily lead to a strong reducing environment, making methane-producing bacteria active and increasing methane emissions. However, in the context of increasingly scarce water resources and the continuous emphasis on the "dual carbon" goals, people have begun to pay more attention to alternate wetting and drying (AWD) irrigation, which is believed to save water and potentially reduce emissions. However, the situation is not that simple: some studies combining field experiments, meta-analyses, and multi-omics results have found that the microbial community, key functional genes, and network structure all change under different irrigation patterns. Generally speaking, compared with continuous flooding, AWD can significantly reduce methane emissions and lower the overall warming potential, but sometimes N_2O emissions increase, and the effect is also influenced by factors such as temperature, precipitation, soil organic carbon, and pH. In field practice, the often-mentioned "safe AWD", such as refilling water when the water level drops to about -15 cm, can generally ensure yield while saving water and reducing methane emissions. From a microbial perspective, the periodic changes in water can alter soil Eh and substrate supply, causing the methane-producing related groups and their connections to readjust. These changes often correspond to the variations in gas fluxes and also provide some references for paddy field water management.

Keywords Alternate wetting and drying (AWD); Subtropical paddy fields; Microbial co-occurrence network; mcrA/pmoA; Methane production pathway

1 Introduction

Rice paddies can actually be regarded as a kind of long-term managed wetland. Once watered, the oxygen in the soil is quickly consumed, and the environment gradually becomes anoxic. Various anaerobic decomposition processes become active, and methane becomes one of the important end products. However, the internal environment of the rice paddy is not entirely uniform. Early microbiological studies have found that flooded rice paddies are more like systems divided into several small "compartments": the surface layer often has a little oxygen, the lower layer is mostly anaerobic, and the rhizosphere and rhizoplane of rice form special micro-zones. Oxygen, nitrate, and methane often show obvious micro-scale gradients in these places. Because of this, methane production and methane oxidation often coexist and are significantly influenced by environmental conditions (Kögel-Knabner et al., 2021). From this perspective, rice paddies are not only like a continuously operating biogeochemical reaction field but are also often used to observe the relationship between microbial community structure and ecological function. For subtropical rice-growing areas, the climate is hot and humid, the multiple cropping index is high, and organic matter input and farming activities are relatively frequent. Therefore, methane emissions from rice paddies are not only related to climate change but also to regional ecological security and the transformation of agriculture towards a green approach (Li et al., 2022).

Rice production has always been inseparable from irrigation. The traditional approach is continuous flooding (CF), which indeed helps suppress weeds and ensure stable yields, but the cost is also obvious: it consumes a large amount of water and the long-term waterlogging makes the soil more oxygen-deficient, increasing the risk of methane emissions (Zhang et al., 2019). In recent years, under the dual pressure of water resource constraints and emission reduction requirements, alternate wetting and drying (AWD) irrigation has gradually been promoted. Simply put, it involves allowing the field to periodically dry to a certain water level before re-flooding. The

International Rice Research Institute commonly uses a reference line of about 15 cm below the field surface and monitors water level changes by inserting perforated tubes. Many field studies have summarized data from 1990 to 2024, and the general results are relatively consistent: compared with continuous flooding, AWD can significantly reduce methane emissions and also lower the combined warming potential of CH_4 and N_2O (Jiang et al., 2022). However, the situation is not entirely uniform; in some areas, N_2O emissions may increase, and the effect is also influenced by many factors, such as soil dryness, the frequency of wetting and drying, local precipitation and temperature, soil organic carbon, pH, and nitrogen application levels. Precisely because of this, when discussing irrigation methods now, the focus has gradually shifted from "whether to dry" to more detailed questions, such as to what water level to dry, at which growth stage to do it, and how to coordinate with nutrient management.

In many past studies on greenhouse gas emissions from paddy fields, attention was often focused solely on the changes in the abundance of a certain type of functional microorganism, using it to explain the gas flux. However, in a system like soil where multiple processes occur simultaneously, this perspective is actually a bit simplistic: whether different groups change together and what resources or electron flows they might be linked through are also worthy of attention. Thus, the method of co-occurrence networks has gradually been introduced. Researchers first used high-throughput sequencing data to attempt to reconstruct the association relationships between different groups or genes, and then used indicators such as node degree, clustering coefficient, and modularity to describe the network structure. However, the amplification data itself has a relative abundance limitation, which can easily lead to false correlations. Therefore, some more robust inference methods have been developed later, such as SparCC for compositional data and SPIEC-EASI based on conditional independence relationships. With this network framework, people can not only see the influence of environmental selection but also discuss potential interactions and the position of key groups in the functional process, making paddy field methane research no longer rely solely on scattered indicators but closer to an understanding of the overall structure.

2 Theoretical Foundation and Research Hypotheses

2.1 Microbiological mechanism of methane generation and oxidation in paddy soil

The amount of methane emitted from paddy fields is not determined by a single process. It can be roughly regarded as the cumulative result of "generation, oxidation and transport". The lower layer of the soil is anaerobic, where methanogenic archaea produce methane; but in the surface layer or near the rice roots, there is oxygen, and some methane will be consumed by methane-oxidizing bacteria. When these processes occur simultaneously, the remaining methane will enter the atmosphere through the rice ventilation structure, bubbling or diffusion (Nazaries et al., 2017). The stratified structure formed after flooding - the surface layer prefers oxygen, the lower part prefers anaerobic conditions, and the rhizosphere micro-region - precisely allows these processes to occur simultaneously at different locations.

In research, some molecular markers are commonly used to track related microorganisms. For example, the mcrA gene encodes the alpha subunit of methanocoumarin reductase and is a key enzyme in the final step of methane production, and is usually regarded as a functional marker for methanogenic archaea; while pmoA is often used to describe the phylogeny and potential functions of aerobic methane-oxidizing bacteria. Some studies have proposed that the abundance of mcrA and pmoA, as well as their ratio, often provide clues for determining the source and sink relationship of soil methane (Tveit et al., 2019). However, it should be noted that simply looking at the gene quantity cannot directly indicate the flux size; it is necessary to combine environmental conditions and actual processes for understanding.

2.2 Mechanism of soil redox environment regulation by irrigation modes

Many discussions will mention that when the irrigation method changes, the methane process also changes. The key lies in the fact that the soil's redox state is re-adjusted. During continuous flooding, the soil's Eh often remains at a relatively low level, and there are fewer available electron acceptors. As a result, the anaerobic processes such as fermentation, nutrient synthesis, and methane production tend to be more dominant (Conrad et al., 2020). However, if alternate wetting and drying irrigation is adopted, the situation is quite different: after the field surface dries, oxygen begins to diffuse in, and some reduced states are re-oxidized. The Eh gradually increases, and nitrification also intensifies. Sometimes, processes such as iron reduction may be more active than methane production for a period of time. This often delays or suppresses methane generation. Field observations can also show similar changes. For example, in some typical paddy fields in Hunan, the Eh is usually relatively low during the long irrigation stage after transplanting, but once the field is left dry or exposed to the sun, the Eh rises quickly (Yang et al., 2019). For farmers, there is a relatively intuitive reference method for operation: insert a perforated pipe in the field to observe the water level. When the water level drops to about −15 cm below the field surface and then re-irrigate. This relatively gentle drying method is considered to be neither likely to cause obvious water stress nor conducive to balancing yield, water saving, and emission reduction.

2.3 Complexity of microbial networks and ecological stability hypothesis

The relationship between "complexity" and "stability" in ecology has been debated for a long time. The model proposed by May in the early days already pointed out a problem: if the interactions in the system are random, then an increase in scale and connectivity does not necessarily mean greater stability. Therefore, it is necessary to be cautious when simply equating "more complex networks" with "more stable systems". When looking at the soil microbial network from this perspective, under disturbances such as changes in the water cycle, such as alternate wetting and drying irrigation, the network structure is likely to be re-adjusted (Hernandez et al., 2021). Some strictly anaerobic nodes, such as groups related to methane production, may have reduced connections or even withdraw, while nodes related to oxidation processes, nitrogen cycling, or iron cycling may become more prominent. As a result, the system sometimes shows more obvious modules and key nodes may become more concentrated or more dispersed. Based on this idea, a testable hypothesis can be proposed: In subtropical paddy fields, compared to continuous flooding, alternate wetting and drying irrigation may weaken the connectivity of the co-occurrence network centered on methane-producing groups, while increasing the degree of modularization and functional differentiation, making the system more structurally resilient under water disturbance, and this change often shows a consistent trend with the decrease in methane flux (Figure 1) (Banerjee et al., 2019).

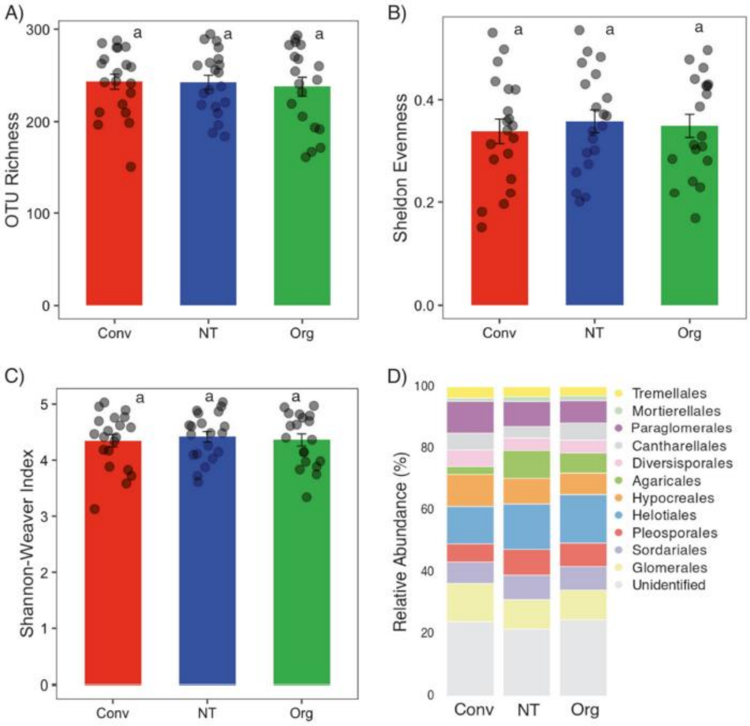

Figure 1 Alpha diversity indices and community composition of root fungal communities across conventional (Conv), no-till (NT), and organic (Org) farming systems. OTU richness (a), Sheldon evenness (b), and Shannon–Weaver index (c) were calculated from the rarefied fungal OTU table. Same lowercase letter indicates no statistically significant ($P < 0.05$) difference between farming systems. d Stacked bar chart showing the relative abundance of various orders of wheat root fungal communities (Adopted from Banerjee et al., 2019)

3 Overview of the Study Area and Experimental Design

3.1 Natural conditions and soil types in the study area

The main areas of subtropical rice cultivation in China are distributed in the middle and lower reaches of the Yangtze River to the south of the country. These regions generally have hot and humid climates with long frost-free periods, and double-cropping is quite common, such as double rice crops or rice-crop rotation with other crops (Chen et al., 2020). Taking the Changsha Agricultural Environmental Observation Research Station of the China Flux Observation Research Alliance as an example, this site is often used to represent the typical agricultural ecological environment of the mid-subtropical hilly areas. The local area has a humid monsoon climate, with an average annual temperature of approximately 17.5 ℃ and an annual rainfall of about 1330 mm, and the frost-free period is close to 280 days. The soil in the paddy fields at this site is typical paddy soil, with a plough layer thickness of approximately 0.2 m. It also records basic physical and chemical indicators such as organic matter, total nitrogen, and particle composition, and is therefore often used as a reference background for the study of the microenvironment of subtropical paddy fields. From the perspective of soil origin, many paddy fields in the south originally evolved from red soil or red-yellow soil parent materials. After long-term cultivation and repeated flooding, they gradually form characteristic layers of paddy soil, where iron and manganese patches and pH gradients are quite common (Huang et al., 2018). These properties often affect the redox cycle and are closely related to the iron reduction process and methane production.

3.2 Irrigation treatment setup and field experiment layout

In field studies, if one wants to compare the effects of different irrigation methods, a continuous flooding (CF or FI) treatment is usually set as a control first, and then several different intensity or frequency dry-wet alternating irrigation treatments are arranged. A "safe AWD" experiment was conducted in Guangzhou, South China, which can serve as an example (Lampayan et al., 2015). The experiment was carried out in two seasons in 2014: in the early season, a randomized block design was used to compare AWD15, AWD30 and continuous flooding; in the late season, a split-plot design was adopted, with AWD15, AWD30, CF and the commonly used irrigation method by farmers (FP) as the main plots, and different rice varieties were also included. Throughout the process, the field water level and soil water potential were continuously recorded, and methane emissions were monitored at fixed time intervals. At the same time, crop growth, yield and water productivity were observed. Regarding the irrigation threshold, "safe AWD" usually considers a depth of about 15 cm below the soil surface as a relatively safe re-irrigation level. However, during the sensitive period from heading to flowering, a shallow water layer is generally required to avoid obvious water stress, and this point is often mentioned in many reviews and management recommendations (Carrijo et al., 2017).

3.3 Sample collection and data acquisition methods

The measurement of greenhouse gas fluxes in paddy fields typically involves two approaches: one is the static chamber or automatic chamber method, and the other is the eddy correlation method. The static chamber method is more common, where a chamber is placed in the field plot and the gas inside the chamber is sampled at regular intervals, and then the concentration changes are measured using gas chromatography, and the flux is calculated through regression (Figure 2) (Butterbach-Bahl et al., 2016). In contrast, the eddy correlation method can continuously monitor the gas exchange at the entire field scale, but it requires high equipment standards, and has stricter requirements for terrain conditions and data processing. Regarding the manual chamber method, some operational guidelines specifically emphasize the arrangement of sampling times, the airtightness of the chamber, and the standardization of flux calculation methods; there have also been studies specifically comparing the scale differences between the static chamber and eddy correlation methods in paddy field methane monitoring (Dengel et al., 2019). As for the acquisition of microbial data, the common practice is to collect surface or rhizosphere soil samples, first using 16S rRNA amplicon sequencing to analyze the community structure, and then detecting the abundance of mcrA and pmoA through qPCR. If a deeper understanding of functional changes is needed, combined macro- or transcriptomic analysis can be conducted, which allows for the simultaneous explanation of microbial communities, functional genes, and gas fluxes within the same framework.

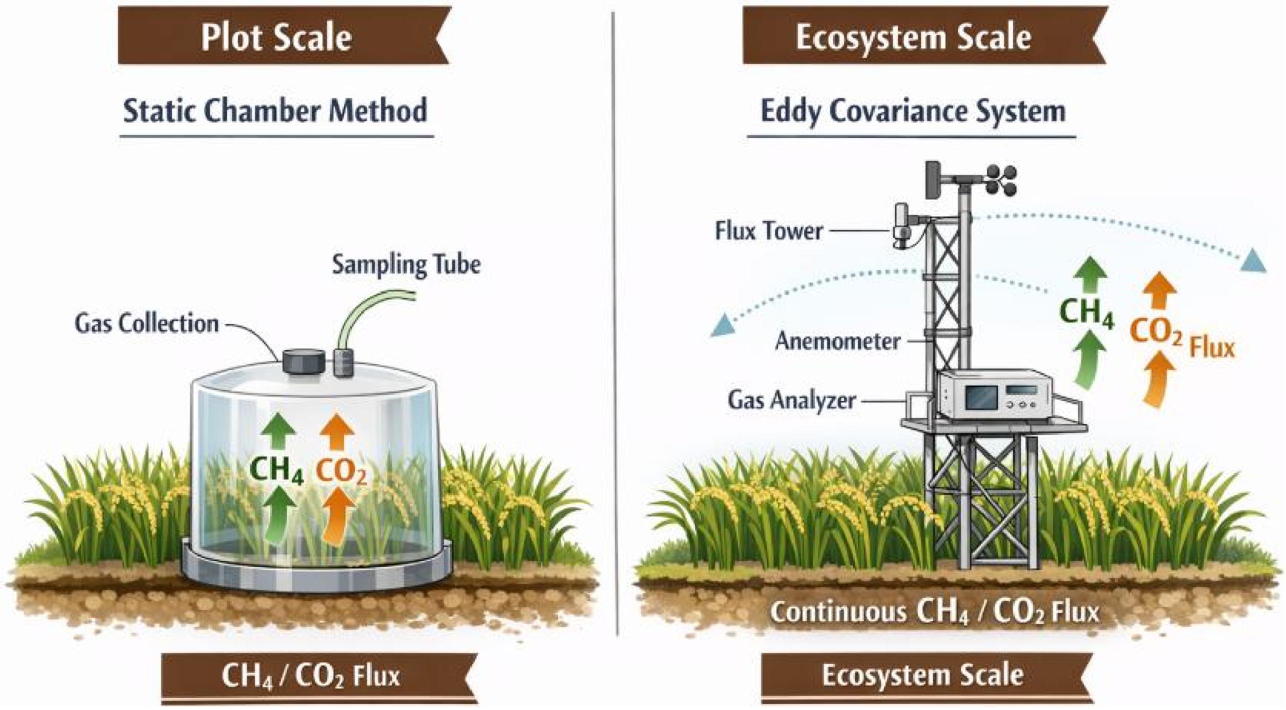

Figure 2 Conceptual comparison between the static chamber method and the eddy covariance method for measuring greenhouse gas fluxes in paddy fields. The static chamber method measures gas accumulation at the plot scale, whereas the eddy covariance method captures continuous ecosystem-scale gas exchange (Adopted from Butterbach-Bahl et al., 2016)

4 Microbial Community Structure and Functional Gene Analysis

4.1 High-throughput sequencing and community diversity analysis

When studying microbial communities in paddy fields, amplicon sequencing has become a common tool, such as 16S rRNA for bacteria and archaea, and ITS for fungi. Through this method, changes in community composition under different irrigation conditions can be observed at a high throughput (Knight et al., 2018). During the analysis, the α diversity indicators, such as Shannon or Chao1, are usually examined first, to roughly reflect the species richness and evenness; if one wants to compare the differences in communities between different treatments, β diversity and ordination methods, such as PCoA or NMDS based on Bray-Curtis distance, are often used. However, the research focus has been changing in recent years. Often, it is not only about "what microorganisms are present", but also about what they might be doing.

Therefore, metagenomic shotgun sequencing has gradually been used to analyze functional potential and metabolic pathways, especially when discussing carbon cycling, nitrogen cycling, and methane-related genes. Some studies have compared three irrigation patterns - continuous flooding (FI), alternate wetting and drying (AI, also known as AWD), and another intermittent irrigation (RI) - together. The results showed that WGS not only could reveal the differences in taxonomic composition but also simultaneously present changes in functional pathways (Zhang et al., 2021). The resulting results often provide a clearer functional background for subsequent network analysis or the identification of key functional groups.

4.2 Determination of functional gene abundance and expression levels

When studying the methane cycle in paddy fields, mcrA and pmoA are often regarded as two key functional gene indicators. The former corresponds to the key enzyme in the final step of methane production, while the latter is related to the components of methane-oxidizing enzymes. Therefore, many studies use the abundance or expression levels of these genes to roughly assess the potential activity of methane production and methane oxidation (Yang et al., 2019). Some reviews suggest that the ratio of mcrA/pmoA under certain conditions can indicate whether methane is more likely to be "produced" or "consumed". However, this explanation is not reliable in all cases. For example, the sampling location (whether in the surface soil or rhizosphere), the soil moisture condition, and the availability of substrates can all affect the results. Therefore, the more common practice nowadays is to analyze the gene abundance obtained by qPCR together with transcriptional level information, while monitoring environmental factors such as Eh, DOC, NH_4^+, and NO_3^- (Deng et al., 2021). This makes it easier to establish a complete explanatory framework. One important point to note is that the number of genes and the actual gas flux do not simply correspond. Therefore, it is often necessary to combine process measurements and use statistical methods such as structural equation or mediation analysis to more clearly define the relationship between "genes - processes - fluxes".

4.3 The impact of irrigation modes on the composition of functional microbial communities

Numerous field studies have shown that when the irrigation method changes, the microbial community structure in paddy fields also adjusts accordingly. The key factor is often the reconfiguration of the redox environment: after changes in water management, the dominance relationships of methanogenic archaea, methane-oxidizing bacteria, and the fermentation and synthesis bacteria that provide substrates for them all change (Liu et al., 2019). Comparative studies using metagenomic data have demonstrated that compared to long-term flooding irrigation (FI), water-saving irrigation methods such as alternate wetting and drying or intermittent irrigation often lead to a decrease in the relative abundance of methanogenic groups of archaea; at the functional level, the enrichment of genes related to methane metabolism also decreases, while pathways related to carbohydrate decomposition and nitrification become more active (Wang et al., 2021).

These changes suggest that AWD not only may inhibit methane production but also may cause the nitrogen cycle to develop in a more oxidized direction. However, in some field experiments, another situation has also occurred: although water-saving irrigation significantly reduces CH_4 emissions and affects the comprehensive warming potential of greenhouse gases, the changes in community abundance at the 16S level are not significant. That is to say, the community structure may only have undergone slight adjustments, while the functional expression has changed significantly. This is also the reason why many subsequent studies have introduced network analysis and multi-omics methods.

5 Structure Characteristics of Soil Microbial Co-occurrence Network

5.1 Network construction methods and topological parameters

There is a common problem with the amplicon data from paddy fields, which is that the "constitutive" features are quite obvious. If a direct correlation analysis is conducted, it is easy to obtain some false correlations. To solve this problem, some specialized methods have been developed later, such as SparCC, which is based on the logarithmic ratio concept and estimates the correlation structure between variables under the sparse assumption; and SPIEC-EASI, which combines the composition data transformation and sparse graph model to infer the potential association network through conditional independence relationships (Kurtz et al., 2015).

Generally, when constructing such a network, there is a set of basic steps: first, filter out low-abundance nodes, then perform data normalization or transformation, then select an appropriate inference algorithm, and conduct significance tests and multiple comparison corrections; if one wants to compare the networks between different treatments, it is necessary to ensure comparability, such as using the same node set or a unified sparsity threshold. The network structure is usually described by some topological indicators, such as the number of nodes and edges, average degree, clustering coefficient, average path length, modularity, and the proportion of positive and negative associations. Among them, modularity is often used to observe possible functional partitions, while average degree and clustering coefficient reflect whether the network connections are tight. However, it should be noted that the co-occurrence network only shows statistical correlations and cannot directly indicate real interaction relationships. Therefore, it is usually necessary to combine environmental factors and functional evidence for analysis (Figure 3) (Faust and Raes, 2016).

5.2 Comparison of network complexity under different irrigation modes

When comparing microbial networks under different irrigation conditions, the main intention is to see if the changes in water content will cause adjustments to the overall organizational structure of the system. Some macro-genomic studies in the field have found that between continuous flooding (FI) and the two water-saving irrigation methods (AI/AWD, RI), not only will the microbial composition be significantly separated, but the structure of the co-occurrence network will also show differences (Zhou et al., 2019). This indicates that the irrigation changes are not just the increase or decrease of certain species, but the original cooperative or mutual response relationships between species may also be rearranged. Cross-regional studies have also shown that some topological features of the methane-producing archaea network are closely related to the methane generation process, and the network structure itself can even help explain the flux differences (Shi et al., 2020). In other words, when comparing different irrigation modes, if only looking at single-point indicators such as mcrA or pmoA, the information may not be sufficient; treating network complexity as a response feature at the system level and combining it with environmental factors such as Eh, DOC, and nitrogen forms for analysis often helps to better understand the underlying ecological mechanisms.

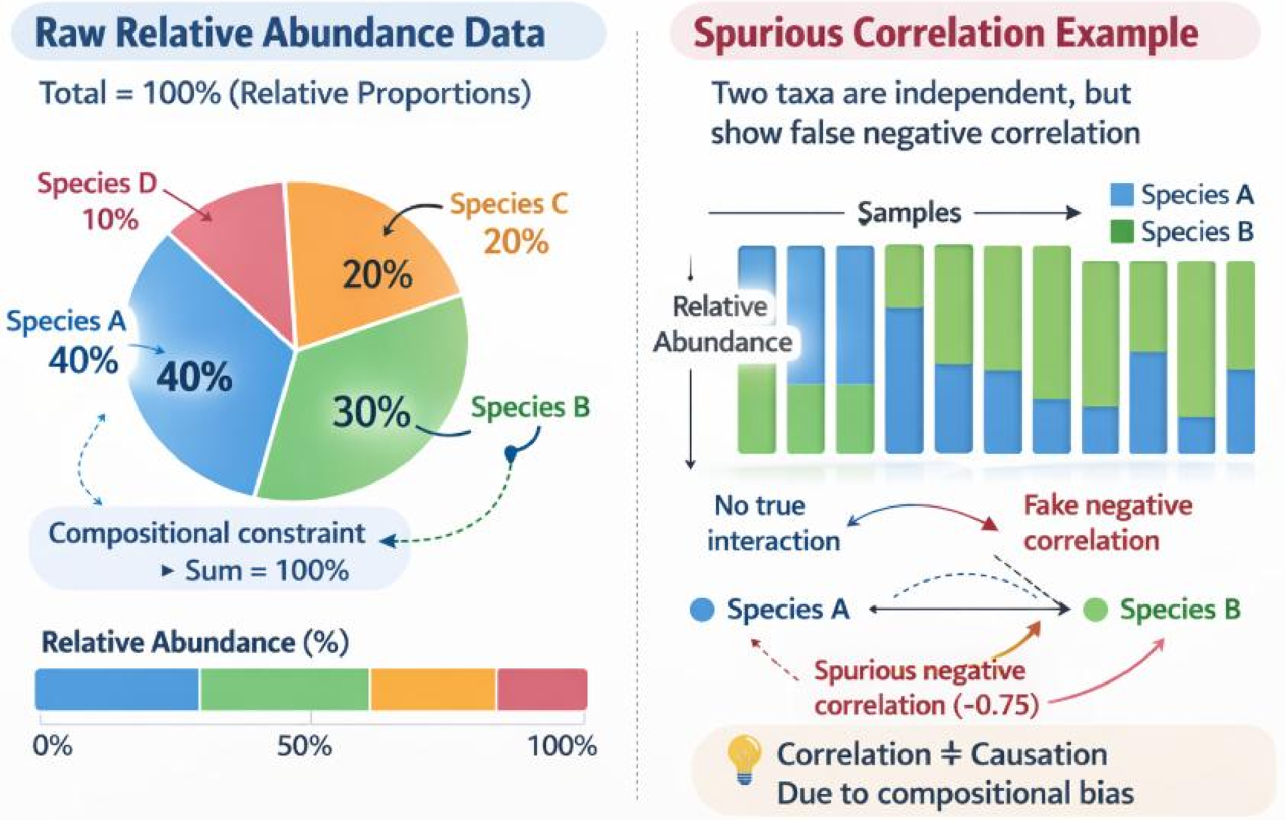

Figure 3 Illustration of compositional bias in amplicon sequencing data. Because microbial abundance data are constrained to relative proportions, direct correlation analysis may generate spurious associations among taxa (Adapted from Faust & Raes, 2016)

5.3 Identification of key groups and ecological function analysis

In microbial network analysis, the identification of "key groups" often involves referring to some centrality indicators, such as degree centrality, betweenness centrality, or determining which nodes may be the "functional pivots" of the system based on whether they belong to module hubs or connectors (Banerjee et al., 2018). Some studies across Asian paddy fields have identified multiple potential key genera in the methane-producing archaea-related networks, and found that the co-occurrence connections of these groups and the contribution to methane production show quantifiable differences. This also reminds us that key nodes are not necessarily important just because they have many connections; sometimes they are exactly at the critical links in the substrate transformation chain. However, it should be noted that changes in key nodes in the network do not necessarily mean that the interactions themselves have become stronger or weaker. For example, under water-saving irrigation conditions, the groups related to methane production may have fewer connections, while the groups related to nitrification or iron cycling are more active, seemingly indicating a shift in the "functional center" of the network (Xue et al., 2022). Therefore, when interpreting key groups, it is usually necessary to analyze together with functional gene information (such as mcrA, pmoA) or metabolic pathway enrichment results, rather than making overly strong causal conclusions based solely on centrality indicators.

6 Regulation Mechanism of Methane Production Pathways by Irrigation Modes

6.1 Changes in acetic acid-type and hydrogen-nutrient-type methane production pathways

The methane production in paddy fields is generally classified into two main pathways: one is the acetic acid decomposition type, and the other is the hydrogen-nutrient type through CO_2 reduction. However, these two pathways do not remain in a fixed proportion all the time. They often adjust constantly in response to changes in substrate supply, pH, water conditions, and available electron acceptors. Existing studies have found that the methane production pathway itself in paddy fields shows obvious temporal variations, and is often related to DOC, acetic acid concentration, water content, and NH_4^+, NO_3^-, etc., indicating that the pathway ratio is actually the result of the combined effect of "substrate - environment - community" (Conrad, 2020).

For alternate wetting and drying irrigation, re-oxidation during the dry period usually reduces the available substrates under anaerobic conditions and makes some alternative electron acceptor processes more active, thus overall suppressing methane production. However, as for which pathway is more dominant and which is less, the results often vary depending on the context, and need to be determined by combining stable isotopes, key enzyme gene typing, and process measurements (Figure 4) (Angle et al., 2017). Based on this, a hypothesis that requires further verification can be proposed: AWD may cause systematic changes in the ratio of methane production pathways. Therefore, conducting quantitative studies on different growth stages and soil layers in subtropical paddy fields would be more appropriate, and it can also avoid using single-season or single-point data to infer the entire process.

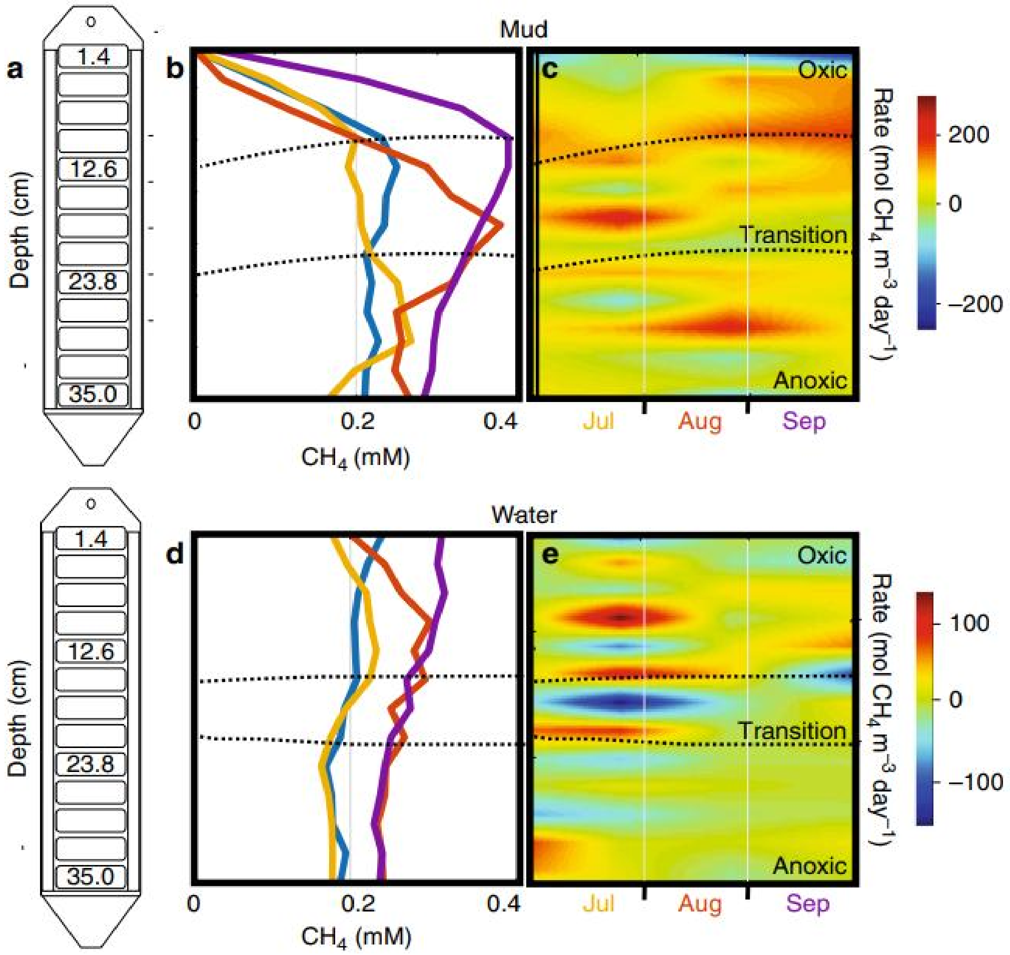

Figure 4 Methane concentrations and production rates across soil depths. a Porewater dialysis peepers provide 2.8 cm resolved depth methane measurements. b, d Monthly in situ porewater dissolved methane concentrations in mud and water-covered soils with data collected from June (blue), July (yellow), August (red), and September (purple). Black dashed lines depict the 95% confidence interval for location of the oxic to anoxic transition. c, e. The calculated net methane volumetric fluxes in soils columns from mud and water ecosites show seasonal methane production (orange and red) in oxic soils (Adopted from Angle et al., 2017)

6.2 Oxidation-reduction gradient and electron acceptor competition mechanism

When explaining "why increasing oxygen reduces methane", the competition among electron acceptors is often regarded as a key factor. In many paddy soil environments, the duration of the iron reduction stage can affect when methane production begins. If the soil is rich in iron, acidic, and has sufficient oxidants, the methane production process may be suppressed for a long time (Fan et al., 2018). On the other hand, the role of electron acceptors is not only to compete with methane production for substrates, but they may also participate in the formation of new methane "consumption pathways". For example, some studies have found that anaerobic methane oxidation (AOM) can occur with the participation of electron acceptors such as trivalent iron or nitrate (Ettwig et al., 2016). Such processes can to some extent reduce methane emissions from paddy fields and also identify active microbial groups related to electron acceptor reduction. If this mechanism is viewed in the context of alternate wetting and drying irrigation, it becomes easier to understand: the fields repeatedly change between drying and re-flooding, and the oxidation-reduction gradient is constantly being re-adjusted. On the one hand, this will inhibit the activity of methane-producing bacteria, while on the other hand, it provides phased opportunities for the iron cycle, nitrogen cycle, and AOM. Eventually, this often manifests as a decrease in net methane flux (Figure 5).

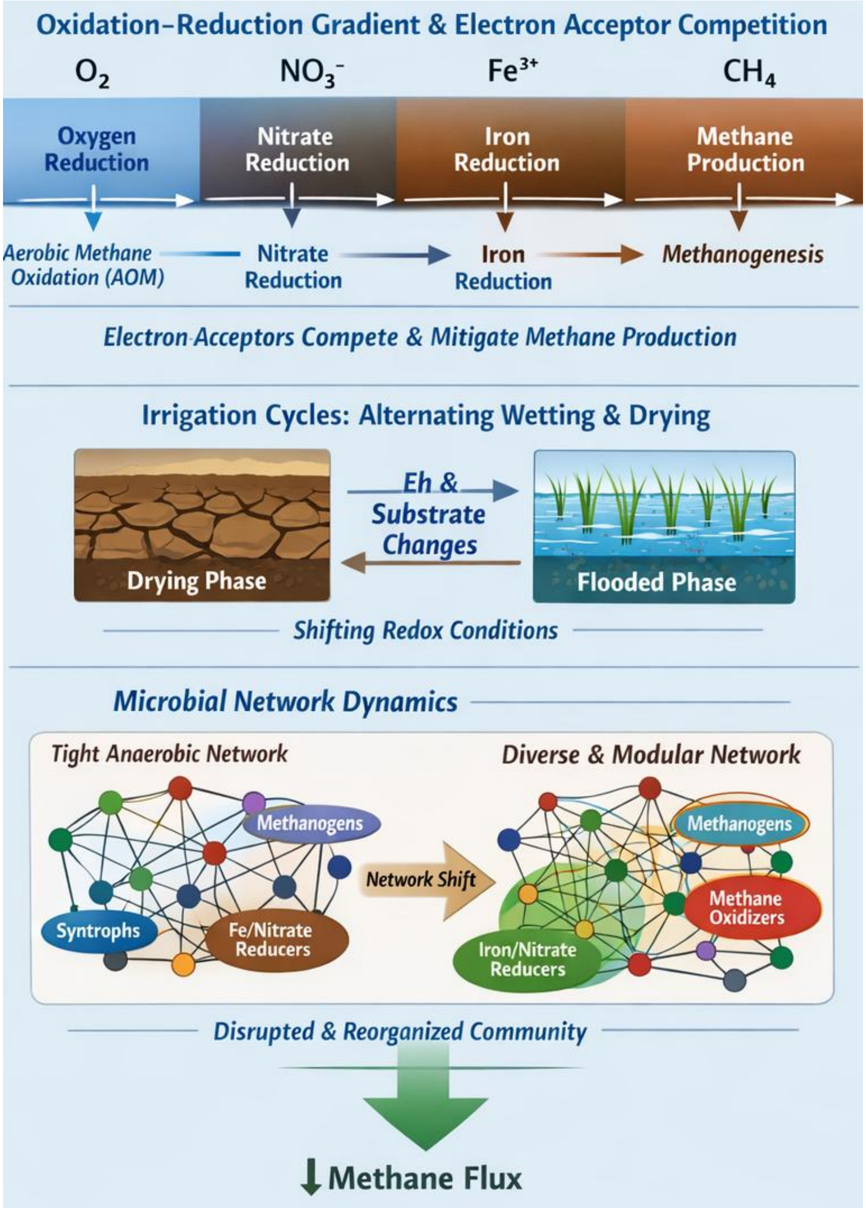

Figure 5 Analysis of mechanisms for methane emission reduction in rice paddies

6.3 Coupling relationship between microbial network structure and methane production flux

When discussing the network structure and flux together, the value lies not merely in answering "are these microorganisms present", but rather it is closer to "how are they combined and together pushing the process forward". Some empirical studies across Asian paddy fields have depicted the co-occurrence relationships among methanogenic archaea as network links. The results revealed that many topological features were closely related to methane emissions, and they also had a prominent explanatory power among a variety of influencing factors (Zhou et al., 2019). Therefore, the network itself can be regarded as a kind of system-level "process intensity signal". If placed within the AWD framework, this link can be described more straightforwardly: Irrigation first alters Eh and substrate supply, and subsequently, methanogenic archaea, synthetic nutrient bacteria, methane-oxidizing bacteria, and iron/nitrate reduction-related groups change their relative positions; the previously "tight anaerobic chain" structure may be disrupted, and the network is more like multiple processes running simultaneously and with clearer modules, and finally, the net CH_4 flux decreases (Banerjee et al., 2018). Co-occurrence is not equivalent to actual interaction, but if the changes in the network and functional genes, metabolic pathways, and flux changes align in direction, it can still be used for mechanism explanations and even as a connection point for predictive modeling.

7 Case Study: Empirical Analysis of the AWD Model in Typical Subtropical Rice Fields

7.1 Background of the case area and irrigation management practices

This article takes a field trial of the "safe AWD" in the subtropical rice area of South China as the case background. The trial was conducted at the Agricultural Science Research Institute Experimental Station in Guangzhou, Guangdong Province, with the location roughly at 113°20′E, 23°08′N. The area has a typical subtropical humid monsoon climate. The study was carried out in both the early and late seasons, comparing treatments such as AWD15, AWD30, continuous flooding, and the commonly used irrigation methods by farmers (Carrijo et al., 2017). Water levels and water potentials in the fields were continuously recorded, and methane fluxes were regularly monitored. Crop yields and water productivity were also evaluated. In terms of management practices, the "safe AWD" approach is actually quite intuitive: a perforated pipe is inserted in the field to observe the water level. When the water level drops to about −15 cm below the field surface, water is then pumped to the shallow water layer. This threshold is basically consistent with the "15 cm rule" promoted by IRRI, aiming to help farmers make repeatable irrigation decisions using a simple method (Lampayan et al., 2015). The significance of this case lies in that it not only compares different irrigation methods, but also focuses on water conservation, yield, and methane emission reduction, providing a real field background for further discussion of microbial mechanisms.

7.2 Reconstruction of microbial network and changes in methane emission

From the results of methane emissions, this experiment in Guangzhou provided a relatively clear conclusion: With the production remaining basically unchanged or with very little variation, the AWD treatment significantly reduced CH_4 emissions, while the water productivity also improved (Carrijo et al., 2017). The most notable result was that AWD15 and AWD30 tended to be in a lower methane emission range compared to the common irrigation methods used by farmers (FP), and the water-saving effect and improvement in water utilization efficiency were also more significant. Regarding the microbial mechanism, another type of evidence comes from comparative studies of different water management methods. For example, a macro-genomic study comparing continuous flooding (FI), alternate wetting and drying (AI, i.e. AWD), and another intermittent irrigation (RI) found that under water-saving irrigation conditions, the relative abundance of groups related to methane production would decrease, and the co-occurrence network structure would also undergo significant changes; at the functional level, the enrichment degree of genes related to methane metabolism decreased, while the pathways related to nitrification and carbohydrate decomposition were enhanced (Figure 6) (Zhang et al., 2021). When the field flux results and these multi-omics evidence are considered together, a relatively consistent explanation can be obtained: The re-oxidation process brought by AWD would weaken the network connections centered on the methane-producing food web, and at the same time, more modules related to the oxidation process would become active, reducing the overall intensity of methane generation and emission from the system structure.

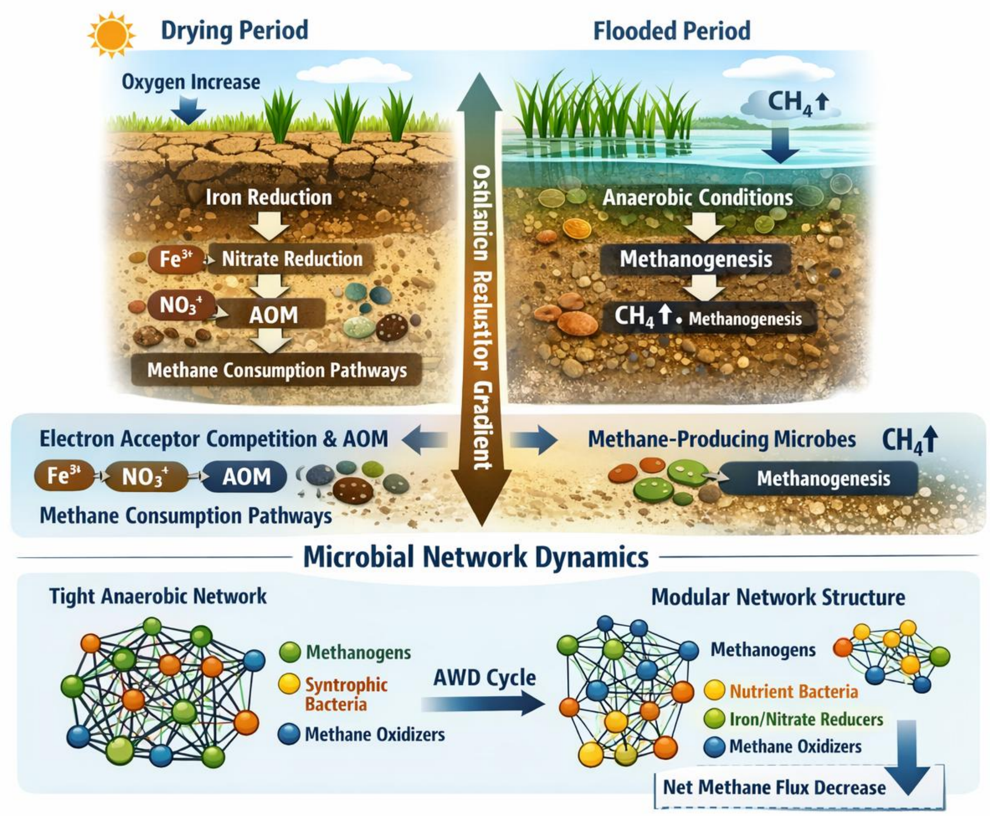

Figure 6 Mechanisms of methane reduction under alternate wetting and drying (AWD) in paddy soils.

7.3 Mitigation potential and implications for sustainable agriculture

From the results of broader studies, many meta-analyses have reached similar conclusions: Compared with continuous flooding (CF), alternate wetting and drying irrigation (AWD) generally can significantly reduce CH_4 emissions and also reduce the overall warming potential (Linquist et al., 2015). However, the situation is not entirely uniform. Some studies have also found that N_2O emissions may increase accordingly, and the mitigation effect is also affected by factors such as dryness level, climate conditions, soil organic carbon, and pH (Zhang et al., 2019). For subtropical rice-growing areas, these results generally bring several implications. First, when promoting AWD, a "moderate" or "safe threshold" approach is more suitable, such as lowering the water level to about −15 cm below the field surface before irrigating. This can both ensure emission reduction and more easily guarantee yields. Secondly, AWD is not suitable to be regarded as a single technology. It often needs to be considered together with nitrogen application methods, the time of straw return, and variety selection. Otherwise, problems such as N_2O rebound or unstable substrate supply may occur. Finally, in carbon accounting or policy formulation, regionalized emission factors and standardized monitoring methods should be relied upon, rather than simply promoting the results of a single experimental site. The IPCC also lists "improving rice management" as an important option in agricultural emission reduction measures, which to some extent indicates that rice field water management has both policy significance and methodological value.

8 Discussion and Conclusion

From the perspective of ecological processes, alternate wetting and drying irrigation actually seems to be constantly "applying pulses" to the anaerobic system of the paddy field. Each time the field dries out and then is irrigated again, there is a cycle of oxygen entering and then re-oxidizing. The intensity of environmental selection and the resources and electron flow channels also change accordingly. As a result, the microbial organizational pattern originally dominated by a strict anaerobic chain may be disrupted, and the network structure gradually shows more obvious modularization and functional differentiation. However, the complexity of the network does not necessarily mean greater stability. May has already pointed out this point. Therefore, in the study of paddy field networks, it is not advisable to simply compare the complexity, but rather to specifically examine which connections have changed, which modules are more stable, whether key nodes have become more concentrated, and whether the system is more resilient to node absence. Considering some cross-regional research results, the topological characteristics related to methane production in the network are often closely related to methane generation. This means that changes in the network structure are not only descriptive phenomena but may also become important clues for understanding and even predicting flux changes.

Based on the existing evidence, the emission reduction strategies for subtropical paddy fields can take "water control" as the main line, while also considering the synergy of other processes. Firstly, in terms of water management, the AWD approach with clear thresholds is more suitable, such as using perforated pipes to monitor water levels, and irrigating when the water level under the field surface drops to approximately −15 cm; however, during the relatively sensitive period from grain filling to flowering, a shallow water layer should generally be maintained to reduce the impact on yield. Secondly, considering that AWD sometimes leads to an increase in N_2O, nitrogen fertilizer management should be adjusted together, such as optimizing the timing of nitrogen application and fertilizer form, avoiding stages with high soil moisture and high NO_3^- levels, and it is more suitable to evaluate the effect by looking at the comprehensive warming potential rather than just a single gas. Furthermore, the timing of organic matter input such as straw should also be noted, as many studies have found that there is a significant interaction between water regime and organic input, and adjusting the time of land application often changes the methane emission level. Finally, in terms of carbon accounting and technology promotion, regionalized emission factors and model tools need to be established as support. The climate conditions, soil texture, and original water management methods in different regions will affect the final emission estimation.

At present, there are still some obvious limitations in this type of research. Firstly, network inference itself is prone to be affected by compositional effects and sampling design. Even when using methods such as SparCC or SPIEC-EASI, the connections in the network can only be regarded as statistically correlated and cannot directly indicate the real interaction relationships. To confirm key connections, it often requires cultivation experiments, isotope tracing, or more rigorous causal inference designs. Secondly, studies that simultaneously obtain "gas flux - multi-omics - network structure" data are not numerous, and many lack high temporal resolution sequences; while AWD's core lies precisely in the water pulse process, therefore, more continuous sampling by crop growth stages and soil layers is needed. Thirdly, in terms of monitoring methods, static chambers are commonly used, but they have limitations in representativeness and continuity; eddy correlation can provide continuous observations, but it is more complex in scale interpretation and attribution. A more suitable direction in the future might be the combination of multiple methods and gradually forming a more unified operational process. Finally, at the mechanism level, it is necessary to more clearly evaluate the role of electron acceptor competition and anaerobic methane oxidation in paddy fields, and at the same time, place the iron cycle, nitrogen cycle, and methane process in the same network framework for discussion, so as to more reliably explain and predict the effects of emission reduction measures.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Reference

Angle J.C., Morin T.H., Solden L.M., Narrowe A.B., Smith G.J., Borton M.A., Rey-Sanchez C., Daly R.A., Mirfenderesgi G., Hoyt D.W., Riley W.J., Miller C.S., Bohrer G., and Wrighton K.C., 2017, Methanogenesis in oxygenated soils is a substantial fraction of wetland methane emissions, Nature Communications, 8: 1567.
https://doi.org/10.1038/s41467-017-01753-4

Banerjee S., Schlaeppi K., and van der Heijden M.G.A., 2018, Keystone taxa as drivers of microbiome structure and functioning, Nature Reviews Microbiology, 16(9): 567-576.
https://doi.org/10.1038/s41579-018-0024-1

Banerjee S., Walder F., Büchi L., Meyer M., Held A.Y., Gattinger A., Keller T., Charles R., and van der Heijden M.G.A., 2019, Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots, The ISME Journal, 13(7): 1722-1736. https://doi.org/10.1038/s41396-019-0383-2

Butterbach-Bahl K., Baggs E.M., Dannenmann M., Kiese R., and Zechmeister-Boltenstern S., 2016, Nitrous oxide emissions from soils: how well do we understand the processes and their controls?, Philosophical Transactions of the Royal Society B: Biological Sciences, 368(1621): 20130122. https://doi.org/10.1098/rstb.2013.0122

Carrijo D.R., Lundy M.E., and Linquist B.A., 2017, Rice yields and water use under alternate wetting and drying irrigation: a meta-analysis, Field Crops Research, 203: 173-180. https://doi.org/10.1016/j.fcr.2016.12.002

Chen H., Zou J., Cui J., Nie Y., and Fang C., 2020, Carbon and nitrogen dynamics in paddy soils under double rice cropping systems in subtropical China, Agriculture, Ecosystems & Environment, 295: 106883. https://doi.org/10.1016/j.agee.2020.106883

Conrad R., 2020, Importance of hydrogenotrophic, aceticlastic and methylotrophic methanogenesis for methane production in terrestrial, aquatic and other anoxic environments: a mini review, Pedosphere, 30(1): 25-39. https://doi.org/10.1016/S1002-0160(18)60052-9

Conrad R., Klose M., Claus P., and Enrich-Prast A., 2020, Methanogenic pathways and processes in rice field soils: microbial ecology and biogeochemistry, FEMS Microbiology Ecology, 96(3): fiaa020. https://doi.org/10.1093/femsec/fiaa020

Deng Y., Jiang Y., Yang Y., He Z., Luo F., Zhou J., and Liu L., 2021, Molecular ecological network analyses reveal environmental drivers of microbial community structure and function in soils, The ISME Journal, 15(1): 1-12. https://doi.org/10.1038/s41396-020-00741-9

Dengel S., Levy P.E., Grace J., Skiba U., and Sutton M.A., 2019, Methane emissions from rice paddies: comparison of eddy covariance and chamber methods, Agricultural and Forest Meteorology, 265: 324-338. https://doi.org/10.1016/j.agrformet.2018.11.021

Ettwig K.F., Zhu B., Speth D., Keltjens J.T., Jetten M.S.M., and Kartal B., 2016, Archaea catalyze iron-dependent anaerobic oxidation of methane, Proceedings of the National Academy of Sciences, 113(45): 12792-12796. https://doi.org/10.1073/pnas.1609534113

Fan X., Yu H., Wu Q., Ma J., Xu H., Yang J., and Zhuang Y., 2018, Iron reduction suppresses methane production in paddy soils and influences microbial community structure, Geoderma, 327: 142-150. https://doi.org/10.1016/j.geoderma.2018.04.031

Faust K., and Raes J., 2016, CoNet app: inference of biological association networks using Cytoscape, F1000Research, 5: 1519. https://doi.org/10.12688/f1000research.9050.2

Hernandez D.J., David A.S., Menges E.S., Searcy C.A., and Afkhami M.E., 2021, Environmental stress destabilizes microbial networks and drives functional changes in soil ecosystems, Ecology Letters, 24(11): 2252-2263. https://doi.org/10.1111/ele.13820

Huang S., Zeng Y., Wu J., Shi Q., and Pan X., 2018, Effect of soil properties on methane emission from paddy fields in southern China, Journal of Soils and Sediments, 18(4): 1482-1493. https://doi.org/10.1007/s11368-017-1863-0

Jiang Y., Carrijo D., Huang S., and Linquist B., 2022, Water management for mitigating greenhouse gas emissions in rice systems: a global perspective, Field Crops Research, 279: 108464. https://doi.org/10.1016/j.fcr.2022.108464

Knight R., Vrbanac A., Taylor B.C., Aksenov A., Callewaert C., Debelius J., Gonzalez A., Kosciolek T., McCall L.I., McDonald D., Melnik A.V., Morton J.T., Navas-Molina J.A., Quinn R.A., Sanders J.G., Swafford A.D., Thompson L.R., Tripathi A., Xu Z.Z., Zaneveld J.R., Zhu Q., and Caporaso J.G., 2018, Best practices for analysing microbiomes, Nature Reviews Microbiology, 16(7): 410-422. https://doi.org/10.1038/s41579-018-0029-9

Kögel-Knabner I., Amelung W., Cao Z., Fiedler S., Frenzel P., Jahn R., Kalbitz K., Kölbl A., and Schloter M., 2021, Biogeochemistry of paddy soils: environmental drivers and microbial processes regulating methane dynamics, Journal of Plant Nutrition and Soil Science, 184(1): 14-34. https://doi.org/10.1002/jpln.202000366

Kurtz Z.D., Müller C.L., Miraldi E.R., Littman D.R., Blaser M.J., and Bonneau R.A., 2015, Sparse and compositionally robust inference of microbial ecological networks, PLoS Computational Biology, 11(5): e1004226. https://doi.org/10.1371/journal.pcbi.1004226

Lampayan R.M., Rejesus R.M., Singleton G.R., and Bouman B.A.M., 2015, Adoption and economics of alternate wetting and drying water management for irrigated lowland rice, Field Crops Research, 170: 95-108. https://doi.org/10.1016/j.fcr.2014.10.013

Lampayan R.M., Rejesus R.M., Singleton G.R., and Bouman B.A.M., 2015, Adoption and economics of alternate wetting and drying water management for irrigated lowland rice, Field Crops Research, 170: 95-108. https://doi.org/10.1016/j.fcr.2014.10.013

Li S., Chen Y., Yu F., Zhang Y., Liu K., Zhuo X., Qiu Y., Zhang H., Gu J., Wang W., Yang J., and Liu L., 2022, Reducing methane emission by promoting its oxidation in rhizosphere through nitrogen-induced root growth in paddy fields, Plant and Soil, 475(1-2): 391-406.
https://doi.org/10.1007/s11104-022-05360-1

Linquist B.A., van Groenigen K.J., Adviento-Borbe M.A., Pittelkow C., and van Kessel C., 2015, An agronomic assessment of greenhouse gas emissions from major cereal crops, Global Change Biology, 21(8): 265-277.
https://doi.org/10.1111/gcb.12788

Liu X., Wang H., Zhou J., Hu F., Zhu D., and Chen Z., 2019, Water management alters microbial community structure and methane cycling in rice paddies, Applied Soil Ecology, 138: 123-132.
https://doi.org/10.1016/j.apsoil.2019.02.012

Nazaries L., Murrell J.C., Millard P., Baggs L., and Singh B.K., 2017, Methane, microbes and models: fundamental understanding of the soil methane cycle for future predictions, Environmental Microbiology, 19(7): 2395-2417.
https://doi.org/10.1111/1462-2920.13715

Shi Y., Delgado-Baquerizo M., Li Y., Yang Y., Zhu Y.G., Peñuelas J., and Chu H., 2020, Abundance of kinless hubs within soil microbial networks are associated with high functional potential in agricultural ecosystems, Environmental Microbiology, 22(12): 5110-5124.
https://doi.org/10.1111/1462-2920.15179

Tveit A.T., Urich T., Frenzel P., and Svenning M.M., 2019, Metabolic and trophic interactions modulate methane production by Arctic peat microbiota in response to warming, Proceedings of the National Academy of Sciences, 116(10): 4095-4100.
https://doi.org/10.1073/pnas.1816812116

Wang W., Lai D.Y.F., Sardans J., Wang C., Zeng C., and Peñuelas J., 2021, Water management affects microbial functional genes and greenhouse gas emissions in paddy soils revealed by metagenomic analysis, Science of the Total Environment, 780: 146590.
https://doi.org/10.1016/j.scitotenv.2021.146590

Xue Y., Chen H., Yang J., Liu M., Huang B., and Yang Y., 2022, Irrigation management shapes soil microbial co-occurrence networks and methane cycling in paddy fields, Science of the Total Environment, 806: 150541.
https://doi.org/10.1016/j.scitotenv.2021.150541

Yang J., Zhou S., Sun Y., Li Y., and Gu J., 2019, Effects of alternate wetting and drying irrigation on soil redox conditions and greenhouse gas emissions in paddy fields, Agricultural Water Management, 223: 105717.
https://doi.org/10.1016/j.agwat.2019.105717

Yang S., Liebner S., Alawi M., Ebenhöh O., and Wagner D., 2019, Taxonomic database and cut-off value for processing mcrA gene 454 pyrosequencing data by MOTHUR, Journal of Microbiological Methods, 103: 3-5.
https://doi.org/10.1016/j.mimet.2014.05.005

Zhang H., Ding W., He X., Yu H., Fan J., and Liu D., 2021, Functional shifts of soil microbial communities in paddy fields under different irrigation regimes revealed by metagenomic analysis, Science of the Total Environment, 780: 146628.
https://doi.org/10.1016/j.scitotenv.2021.146628

Zhang Y., Chen H., Zhu Q., Peng C., and Li W., 2019, Water management practices influence methane and nitrous oxide emissions from rice paddies: a global meta-analysis, Global Change Biology, 25(9): 3219-3232.
https://doi.org/10.1111/gcb.14722

Zhou J., Deng Y., Luo F., He Z., Tu Q., and Zhi X., 2019, Functional molecular ecological networks, mBio, 10(4): e00169-19.
https://doi.org/10.1128/mBio.00169-19