Structure and diversity of intestinal methanogens in grass carp and black carp

Chengxing Long
Hunan University of Humanities Science and Technology
Jieqi Wu
Loudi Fisheries Science Research Institute
Jialin Liu
Hunan University of Humanities Science and Technology
Zhoujin Tan (tanzhjin@sohu.com)
Hunan University of Chinese Medicine https://orcid.org/0000-0003-3193-073X
Wenge Li
Hunan Institute of Nuclear Agricultural Science and Space Breeding

Research Article

Keywords: grass carp, black carp, high-throughput sequencing, methanogens, microbiota characteristics

Posted Date: July 8th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1755081/v1

Abstract

The current study aimed to explore the community characteristics of methanogens in the intestinal tract of grass carp and black carp, and their correlation with methanogens in cultured water. Samples of grass carp, black carp and cultured water in a pond were collected in Spring 2021. Based on LIlumina HiSeq 2500 high-throughput sequencing platform, the metagenomic mcrA gene sequences of grass carp, black carp and cultured water samples were determined and analyzed. The results showed that the richness and diversity of methanogens in the intestinal tract of grass carp and black carp were highly correlated with the cultured water. A total of 5 bacteria genera were detected in the three groups of samples, Methanosarcina, Methanocorpusculum, Methanospirillum, Methanobacterium and Methanofollis, in which Methanosarcina and Methanocorpusculum were the dominant genera. In addition, Methanosarcina had the highest abundance in CY and Methanocorpusculum had the highest abundance in QY. In conclusion, Methanosarcina and Methanocorpusculum were the main methanogens in the intestinal tract of grass carp and black carp and culture water, and hydrolytic fermentative bacteria were its main metabolic substrate, hydrotrophic was its main metabolic pathway. The results will provide reference for the relationship between intestinal methanogens and aquaculture and greenhouse effect.

Introduction

Methanogens, also known as Methanogenic archaea, are a class of archaea microorganisms that can produce methane gas in an obligate anaerobic environment using hydrogen, carbon dioxide, acetic acid, formic acid, methanol and methylamine, etc., which are widespread in nature (Gibson et al. 1988, Ferry 1999, Shima et al. 2002, Karakashev et al. 2006). Methanogens are abundant and diverse. Methanogens have developed into 4 classes (Methanobacteria, Methanococci, Methanomicrobia, Methanopyri) and 5 orders (Methanococcales, Methanosarcinales, Methanopyrales, Methanomicrobiales and Methanobacteriales) by 2016 according to the Handbook of Berger Bacteria Identification (ninth edition) (Cui 2007). Methanogens mainly generate methane through H_2/CO_2 reduction pathway and acetic acid fermentation pathway, while methyl trophication pathway mainly occurs in specific environments such as river and pond sediments (Le Mer & Roger 2001, Ben et al. 2018).

Methane (CH_4) is a greenhouse gas produced by methanogens decomposing organic matter under anaerobic conditions, and its contribution to global climate warming is second only to that of carbon dioxide CO_2 (Xing et al. 2005, Pangala et al. 2010). Many CH_4 accumulated on the earth come from the action of microorganisms. Because methanogens play an important role in the natural carbon cycle, and methane is the second largest greenhouse gas contributing to global warming, methanogens and the mechanism of methane production have attracted much attention from researchers. Especially in recent years, researchers have conducted in-depth discussions on the living habits and metabolic mechanism of methanogens (Liu et al. 2012, Chaudhary et al. 2018, Lyautery et al. 2021). The results showed that the methanogens from sediments and animals were distinct, and the methanogens from fresh water and sea water were also different. Methanogens from the same sediments or animals have a high similarity,

reflecting the close correlation between the ecological environment and the distribution of methanogens (Yan et al. 2017).

CH_4 emission from lakes and ponds is an important source of atmospheric methane (Schmid et al. 2007, Bastviken et al. 2011, Bussmann et al. 2012). Methanogens, as the main role of methane generation, are usually characterized by the diversity or abundance (Pester et al. 2004, Earl et al. 2005, Schwarz et al. 2008). Some scholars have studied the seasonal dynamics of the abundance, structure and diversity of methanogens and methanotrophic bacteria in lake sediments, and determined that the diversity of methanotrophic bacteria was dominated by methylobacter in the deep part and methylococcus in the shallow part, and organic matter was the main environmental parameter controlling methanogenes (Lyautey et al. 2021). In this study, we collected the samples of grass carp, black carp and cultured water from the same pond, and analyzed their methanogens community structure and diversity based on two different kinds of predatory fish intestine and their cultured water. The results will help to deepen the understanding of methanogens in the ecological functions of matter and energy circulation and transformation in the rural aquaculture pond ecosystem, and provide reference information for the ecological diversity of rural aquaculture ponds and the possible impact of aquaculture on the greenhouse effect.

Materials And Methods

Sample collection and preservation

Samples of black carp (QY), grass carp (CY) and cultured water (SY) were collected from the same pond of Loudi Fishery Science Research Institute (N27°43'47", E112°0'6"), Hunan Provence, China. The pond has a depth of 2.0 m and an area of 1.5 hm^2. The sampling time is 08:00 a.m. on January 19, 2021, with water temperature 17.8 ℃, PH 8.10–8.56 and dissolved oxygen > 4.35 mg/L. Five grass carps (1431.34 ± 33.25 g) and five black carps 2627.3 ± 42.69 g) of the same size without disease symptoms were randomly selected from the fish caught in the net and brought back to the laboratory together with water samples, and the others were put back into the pond.

In order to reduce pollution, the fish surface was washed with sterile water and 70% ethanol successively before dissection. The content samples of grass carp (no. CY1-CY5) and black carp (No. QY1-QY5), and water samples (No. SY1-SY5) were collected in the sterile operating trays, and then placed in a sterile centrifuge tube and refrigerate at -20 ℃ for later use.

Abundance detection of methanogens

The Tguide S96 kit was used to extract DNA from 15 samples, and the universal primers MLf and MLr were used to amplify methanogenic mcrA gene. After qualified PCR products were detected by electrophoresis, the target fragment was recovered, and the library was sequenced by Illumina HiSeq 2500. The primer, reaction system and amplification conditions are as follows. Primer synthesis and sequencing were completed by Beijing Biomarker Technologies Co., Ltd (Beijing, China).

Amplification primer: MLf (5'-GGTGGTGTMGGATTCACACARTAYGCWA CAGC-3'), MLr (5'-TTCATTGCRTAGTTWGGRTAGTT-3'). The amplification reaction was performed as follows: 5 μL KOD FX Neo Buffer, 0.3 μL (10 μM) of each forward primer and reverse primer, 2 μL (2 mM) of dNTPs, 0.2 μL KOD FX Neo, and 5–50 ng of DNA Template, ddH2O supplement to 10 μL.Reaction conditions: Initial denaturation at 95 ℃ for 5 min, followed by 25 cycles consisting of denaturation at 95 ℃ for 30 s, annealing at 50 ℃ for 30 s, and extension at 72℃ for 40 s, with a final extension of 7 min at 72℃.

Diversity analysis of methanogens

The original data obtained by sequencing were filtered by quality control (Trimmomatic, V0.33) (Bolger et al. 2014), and the identification and removal of primer sequences (Cutadapt, V1.9.1) (Martin 2011). Double-terminated sequence splicing (Usearch, V10) (Magoč & Salzberg 2011) and removal of chimeras (UCHIME, V4.2) (Edgar et al. 2011) obtained high-quality sequences for subsequent analysis. Usearch software can cluster reads with 97% similarity to obtain OUT (Edgar 2013). QIIME2 software (https://qiime2.org/) was used to calculate alpha and beta diversity in the samples to comprehensively assess the overall diversity and reveal differences between samples. Alpha diversity reflected the richness and diversity of species in individual samples, including Chao1, Ace, Shannon and Simpson. Chao1 and Ace indices measure species abundance, which is how many species there are. Shannon and Simpson indices measure species diversity and are affected by species abundance and community evenness in sample communities. Beta diversity analysis is used to judge the similarity in composition of different samples, including Principal Component Analysis (PCA), Principal Coordinat Analysis (PcoA), Nonmetric Multidimensional Scaling (NMDS) and Unweighted Pair-group Method with Arithmetic Mean (UPGMA), and calculate the distance between samples based on four different algorithms to obtain the beta value between samples.

Line Discriminant Analysis Effect Size (LEfSe) (Segata et al. 2011) uses linear discriminant analysis (LDA) to estimate the impact of each species' abundance on the different effect size, and searched for species with significant differences among groups.

Statistical Analysis

SPSS 24.0 statistical software (IBM Corp., Armonk, NY, USA) was used for data statistics, the measurement data were represented by means ± standard deviations, and independent sample T test was used for pair comparison. $P < 0.05$ indicated statistically significant difference. The mcrA gene sequences obtained in this study were uploaded to the NCBI sequence read archive (accession number is PRJNA 838802 https://www.ncbi.nlm.nih.gov/).

Experimental Results

Sequencing characteristics and OTU distribution of samples

The community characteristics of methanogens in grass carp, black carp and cultured water samples were analyzed using Illumina high-throughput sequencing technology. A total of 1512019 high-quality

sequences were obtained from 15 samples of the three groups, and the average effective sequence of each sample was 90.99%, with the average sequence length concentrated in 421–435 bp. Through quality control, filtering and chimerism removal, a total of 25 OTUs were obtained based on 97% sequence similarity clustering. There were 21 OTU in CY samples. There were 21 OTU in QY samples. There were 22 OTU in SY samples. There were 16 identical OTU numbers in the three groups of samples. The results showed that there was no difference in the richness and diversity of methanogens among black carp, black carp and water samples (Fig. 1).

Comparison of alpha diversity of methanogens in grass carp, black carp and water samples

To illustrate the diversity and richness of methanogens in the intestinal tract of grass carp, black carp and cultured water, we used Mothur software to evaluate the alpha diversity index of samples. From the perspective of richness index, Chao1 index and Ace index in cultured water samples were the highest, and Chao1 index and Ace index in intestinal samples of grass carp and black carp were similar. From the perspective of diversity index, Simpson index and Shannon index of species in aquaculture water samples were the highest, which were similar to those in the intestinal samples of black carp, while Simpson index and Shannon index were the lowest in the intestinal samples of grass carp. The richness index in the intestinal samples of black carp and grass carp was significantly different from that in the cultured water samples ($P < 0.05$ or $P < 0.01$). The diversity index in the intestinal samples of black carp and water samples was similar, and the diversity index in the intestinal samples of grass carp was the lowest (Table 1, Fig. 2). These results indicated that the richness and diversity of methanogens in cultured water were the highest. The richness of methanogens in the intestinal tract of grass carp and black carp was similar to that of water samples, which was significantly different from that of water samples. The diversity of the intestinal tract samples of black carp was similar to that of water samples, but there was no significant difference.

Table 1
Difference analysis of alpha diversity index.

group	Chao1	Ace	Simpson	Shannon
CY	11.30 ± 2.9069*	12.8623 ± 2.3313*	0.3187 ± 0.3345*	0.9594 ± 1.0360
QY	11.44 ± 5.6007*	11.9343 ± 5.5446**	0.6105 ± 0.1340	1.6332 ± 0.7127
SY	18.15 ± 1.1673	19.1511 ± 1.2232	0.6339 ± 0.1111	1.7513 ± 0.4562

Note: CY stands for grass carp, QY stands for black carp; SY stands for water sample; Compared with water sample *stands for $p < 0.05$,**stands for $p < 0.01$

Characterization of methanogens in grass carp, black carp and water samples

There were 3 classes, 4 orders, 5 families and 5 genera of methanogens were identified from 15 samples collected from CY, QY and SY groups. The genera detected were Methanosarcina, Methanocorpusculum, Methanospirillum, Methanobacterium and Methanofollis. Methanosarcina and Methanocorpusculum were the dominant genera, accounting for 91.15%, 89.36% and 69.17% of CY, QY and SY, respectively (Fig. 3, Table 2). Compared with SY samples, Methanosarcina and Methanospirillum increased in CY and QY samples to varying degrees. Methanobacterium and Methanofollis have decreased in CY and QY samples to varying degrees. Methanocorpusculum increased in QY samples, but decreased in CY samples. There were no significant differences among all genera.

Table 2
Relative abundance of methanogens at genus level

genus	CY	QY	SY
Methanosarcina	0.6014 ± 0.4409	0.4555 ± 0.1186	0.3020 ± 0.1841
Methanocorpusculum	0.3101 ± 0.4052	0.4380 ± 0.1498	0.3898 ± 0.1904
Methanospirillum	0.0628 ± 0.1391	0.0493 ± 0.1101	0.0002 ± 0.0001
Methanobacterium	0.0117 ± 0.0180	0.0571 ± 0.1271	0.2584 ± 0.2888
Methanofollis	0.0095 ± 0.0212	0.0001 ± 0.0000	0.0497 ± 0.0951
Note: CY stands for grass carp, QY stands for black carp; SY stands for water sample; Compared with water sample *stands for $p < 0.05$,**stands for $p < 0.01$			

Wighted Unifrac distance matrix was used to construct a cluster analysis of fifteen samples known as methanobacteria using the unweighted pair-group method with UPGMA. The results showed that the similarity of species composition among each group was relatively high (Fig. 4A). According to LefSe analysis results, *Methanobacterium*_sp and *Methanofollis*_ethanolicus are markers with statistical differences in SY samples (Fig. 4B).

Discussion

Understanding changes in the abundance and diversity of microbial communities is a necessary condition for evaluating the role of microorganisms in the environment (Lyautey et al. 2021). Methanogens, as common microorganisms, are widely distributed in various environments, including soil (Kim et al. 2017, Heděnec et al. 2018), water sediments (McKay et al. 2017, Wang 2019) and animal digestive tracts (Moraes et al. 2014, van Lingen et al. 2017). Ponds and lakes are important natural emission sources of methane, and methane generation is closely related to methanogens community. At present, three main metabolic pathways have been described for methane production, namely hydrogenotrophic (convert H_2 plus CO_2 to CH_4), aceticlastic (convert acetate to CH_4 and CO_2) and methylotrophic (generate CH_4 by methanol, methylamine, dimethylamine and other mechanisms), which involve the diversity of methanogens (Conrad 2007, Özbayram 2012, Evans et al. 2019). In freshwater sediments, methane production is regulated by different environmental factors, such as hypoxia (Lehours

et al. 2005), quality and quantity of organic matter (Bastviken et al. 2008, Schwarz et al. 2008), temperature (Duc et al. 2010), etc. Temperature variation is likely to be one of the factors affecting CH_4 production capacity in the shallowest areas of deep lakes or shallow lakes (Fuchs et al. 2016). In a certain range, the increase of temperature has an obvious promotion effect on the metabolic capacity of microorganisms, which is beneficial to improve the rate of gas production. In addition, the production capacity of CH_4 is closely related to the community abundance of fermentation microorganisms (Yang 2017). Therefore, it is helpful to clarify the relationship between aquaculture and greenhouse effect to study the community characteristics of methanogens in aquaculture water and intestinal tract of aquatic animals.

In this study, we applied high-throughput sequencing technology to methanogens in aquaculture water and aquatic animal intestines. Our results showed that a total of 5 genera were identified from methanogens, among which *Methanosarcina*, *Methanocorpusculum* and *Methanobacterium* were the three genera with the highest relative abundance. *Methanosarcina* is hydrogen and acetic acid mixotrophic methanogens, *Methanocorpusculum* and *Methanobacterium* are hydrogenotrophic methanogens (Yang 2017). The results showed that CH_4 was produced by H_2 reduction of CO_2 and acetic acid degradation, and mainly by hydrogen reduction of CO_2. In SY sample, *Methanocorpusculum*, *Methanosarcina* and *Methanobacterium* were the dominant bacteria, which is consistent with the characteristics of methanogenic bacteria community in wetland (Parkes et al. 2012, Zhang et al. 2020). In QY and CY samples, *Methanosarcina* and *Methanocorpusculum* were the dominant bacteria genera. In addition, *Methanosarcina* has the highest abundance in CY and *Methanocorpusculum* has the highest abundance in QY, which may be related to the feeding habits of grass carp and black carp.

Grass carp is an herbivorous freshwater fish, which feeds on the stems and leaves of aquatic plants, and its food riched in cellulose and polysaccharide. Black carp is a carnivorous freshwater fish, which feeds on snails, clams and other mollusks, and its food riched in protein and fat. The intestinal bacteria of grass carp and black carp are mainly Firmicutes (69% vs 37.5%), Proteobacteria (6.9% vs 37.5%) and Actinobacteria (6.9% vs 16.7%), which are highly similar to the bacterial community in cultured water (Wu et al. 2012, Zhang et al. 2013, Ding et al. 2021). Firmicutes, Proteobacteria and Actinomycetes belong to hydrolytic fermentation bacteria (Zhang et al. 2014). Among them, Clostridium in Firmicutes is a typical cellulose-decomposing bacteria with the function of fermenting monosaccharides to produce organic acids, while Streptococcus in Firmicutes is a typical protein-decomposing bacteria (Lawson 2016, Whitman 2015). Vibrio in Proteobacteria is the dominant lipopolysis bacteria (Zhang et al. 2004). In addition, the intestinal tract of grass carp is rich in amylase and cellulase (Wu et al. 2020), and the intestinal tract of black carp is rich in protease and lipase (Zhou et al. 2021). These facts indicate that metabolic matrix of methanogens in ponds and lakes mainly comes from hydrolytic fermentative bacteria, and methanogens can effectively use the H_2 and CO_2 generated by these hydrolytic fermentative bacteria to produce CH_4, which also fully demonstrates that ponds and lakes are important natural emission sources of methane, and are dominated by hydrogenotrophic methanogens.

In conclusion, we revealed the community structure and richness characteristics of methanogens in the intestinal tract of black carp and grass carp and aquaculture water for the first time, and clarified the relationship between intestinal methanogens and aquaculture and greenhouse effect. These results will provide reference for the relationship between intestinal methanogens and aquaculture and greenhouse effect.

Declarations

Authors' Contributions: Zhou-jin Tan designed the study; Jie-qi Wu collected the data; Jialin Liu analyzed the data; Cheng-xing Long wrote the manuscript and Zhoujin Tan and Wenge Li reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Scientific Research Fund of Hunan Provincial Education Department, grant number 19A256 and the Ministry of Education, Humanities and Social Sciences Research Projects, grant number 19YJC7900766.

Data Availability Statement: The data in this study have been uploaded to the NCBI sequence read archive (accession number is PRJNA 838802)

Acknowledgments: We thank the editors and the reviewers of this paper for their constructive

Conflicts of Interest: The authors declare that there are no conflicts of interest regarding the publicationof this article.

Compliance with Ethical Standards All animal work was carried out in accordance within the guidelines of the Institutional Animal Care and Use Committee of Hunan University of Chinese Medicine (NO.20171202). All authors knew and approved of this animal experiment.

References

1. Bastviken D, Cole JJ, Pace ML, Van de Bogert MC (2008) Fates of methane from different lake habitats: connecting whole-lake budgets and CH_4 emissions. *J Geophys Res-Biogeo.*113,G02024. https://doi.org/10.1029/2007JG000608
2. Bastviken D, Tranvik LJ, Downing JA, Crill PM, Enrich-Prast A (2011) Freshwater methane emissions offset the continental carbon sink. *Science*. 331,50. https://doi.org/10.1126/science.1196808.
3. Ben-Abdallah M, Karray F, Kallel N, Armougom F, Mhiri N, Quéméneur M, Cayol JL, Erauso G, Sayadi S (2018) Abundance and diversity of prokaryotes in ephemeral hypersaline lake Chott El Jerid using Illumina Miseq sequencing, DGGE and qPCR assays. *Extremophiles*. 22,811-823. https://doi.org/10.1007/s00792-018-1040-9.
4. Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*.30,2114-2120. https://doi.org/10.1093/bioinformatics/btu170.

5. Bussmann I, Damm E, Schlüter M, Wessels M (2013) Fate of methane bubbles released by pockmarks in Lake Constance. *Biogeochemistry*. 112:613-623. https://doi.org/10.1007/s10533-012-9752-x
6. Chaudhary PP, Conway PL, Schlundt J (2018) Methanogens in humans: potentially beneficial or harmful for health. *Appl Microbiol Biotechnol*. 102,3095-3104. https://doi.org/10.1007/s00253-018-8871-2.
7. Conrad R (2007) Microbial ecology of methanogens and methanotrophs. *Adv Agron*. 96:1-63. https://doi.org/10.1016/S0065-2113(07)96005-8.
8. Cui XG (2007) Isolation and identification of methanogenic bacteria from digester and its distribution analysis. Dissertation: Dalian University of Technology.
9. Ding HX, Li ZY, Liu J, Sha XM, Zhang L, Tu ZC (2021) Comparison of intestinal microbiome composition and community characteristics of grass carp from different habitats. *Acta Microbiologica Sinica*. 61,729-739. https://doi.org/10.13343/j.cnki.wsxb.20200439.
10. Duc NT, Crill P, Bastviken D (2010) Implications of temperature and sediment characteristics on methane formation and oxidation in lake sediments. *Biogeochemistry*. 100,185-196. https://doi.org/10.1007/s10533-010-9415-8.
11. Earl J, Pickup RW, Ritchie DA, Edwards C (2005) Development of temporal temperature gradient electrophoresis for characterising methanogen diversity. *MicrobEcol*. 50,327-36. https://doi.org/10.1007/s00248-005-0192-1.
12. Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods*. 10,996-998. https://doi.org/10.1038/nmeth.2604.
13. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*.27,2194-2200. https://doi.org/10.1093/bioinformatics/btr381.
14. Evans PN, Boyd JA, Leu AO, Woodcroft BJ, Parks DH, Hugenholtz P, Tyson GW (2019) An evolving view of methane metabolism in the Archaea. *Nat Rev Microbiol*. 17,219-232. https://doi.org/10.1038/s41579-018-0136-7.
15. Ferry JG (1999) Enzymology of one-carbon metabolism in methanogenic pathways. *FEMS Microbiol Rev*.23,13-38. https://doi.org/10.1111/j.1574-6976.1999.tb00390.x.
16. Fuchs A, Lyautey E, Montuelle B, Casper P (2016) Effects of increasing temperatures on methane concentrations and methanogenesis during experimental incubation of sediments from oligotrophic and mesotrophic lakes. *J Geophys Res-Biogeo.* 121, 1394-1406. https://doi.org/10.1002/2016JG003328
17. Gibson GR, Cummings JH, Macfarlane GT. Competition for hydrogen between sulphate-reducing bacteria and methanogenic bacteria from the human large intestine. *J Appl Bacteriol*.**1988**,65,241-247. https://doi.org/10.1128/AEM.00489-06.
18. Heděnec P, Rui JP, Lin Q, Yao MJ, Li JB, Li H, Frouz J, Li X (2018) Functional and phylogenetic response of soil prokaryotic community under an artificial moisture gradient. *Appl Soil Ecol*, 124,372-378. https://doi.org/10.1016/j.apsoil.2017.12.009

19. Karakashev D, Batstone DJ, Trably E, Angelidaki I (2006) Acetate oxidation is the dominant methanogenic pathway from acetate in the absence of Methanosaetaceae. *Appl Environ Microbiol*.72,5138-5141. https://doi.org/10.1128/AEM.00489-06.
20. Kim J, Yoo G, Kim D, Ding W, Kang H (2017) Combined application of biochar and slow-release fertilizer reduces methane emission but enhances rice yield by different mechanisms. *Appl Soil Ecol*, 117-118,57-62. https://doi.org/10.1016/j.apsoil.2017.05.006
21. Lawson PA. The taxonomy of the genus Clostridium: current status and future perspectives.*Microbiol China*.**2016**,43,1070-1074.
22. Lehours AC, Bardot C, Thenot A, Debroas D, Fonty G. Anaerobic microbial communities in Lake Pavin, a unique meromictic lake in France. *Appl EnvironMicrobiol*.**2005**,71,7389-7400.
23. Le-Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: a review. *Eur J Soil Biol*. 37,25-50. https://doi.org/10.1016/S1164-5563(01)01067-6.
24. Liu YC, Beer LL, Whitman WB (2012) Methanogens: a window into ancient sulfur metabolism. *Trends Microbiol*. 20,251-258. https://doi.org/10.1016/j.tim.2012.02.002.
25. Lyautey E, Billard E, Tissot N, Jacquet S, Domaizon I (2021) Seasonal Dynamics of Abundance, Structure, and Diversity of Methanogens and Methanotrophs in Lake Sediments. *Microb Ecol*. 82,559-571. https://doi.org/10.1007/s00248-021-01689-9.
26. Magoč T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*. 27(21):2957-2963. https://doi.org/10.1093/bioinformatics/btr507.
27. Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *Embnet J*, 17,10-12. https://doi.org/10.14806/ej.17.1.200.
28. McKay LJ, Hatzenpichler R, Inskeep WP, Fields MW (2017) Occurrence and expression of novel methyl-coenzyme M reductase gene (mcrA) variants in hot spring sediments. *SciRep*. 7,7252. https://doi.org/10.1038/s41598-017-07354-x.
29. Moraes LE, Strathe AB, Fadel JG, Casper DP, Kebreab E (2014) Prediction of enteric methane emissions from cattle. *Global Change Biol*. 20,2140-2148.
30. https://doi.org/10.1111/gcb.12471.
31. Özbayram, E.G (2012) Determination of the synergistic acute effects of antibiotics on methanogenic pathwany. Dissertation: Istanbul Technical University.
32. Pangala SR, Reay DS, Heal KV (2010) Mitigation of methane emissions from constructed farm wetlands. *Chemosphere*. 78:493-499. https://doi.org/10.1016/j.chemosphere.2009.11.042.
33. Parkes RJ, Brock F, Banning N, Hornibrook ERC, Roussel EG, Weightman AJ, Fry JC (2012) Changes in methanogenic substrate utilization and communities with depth in a salt-marsh, creek sediment in southern England. *Estuar CoastShelf S*. 96,170-178. https://doi.org/10.1016/j.ecss,2011.10.025.
34. Pester M, Friedrich MW, Schink B, Brune A (2004) pmoA-based analysis of methanotrophs in a littoral lake sediment reveals a diverse and stable community in a dynamic environment. *Appl Environ Microb*. 70,3138-3142.https://doi.org/10.1128/AEM.70.5.3138-3142.2004.

35. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C (2011) Metagenomic biomarker discovery and explanation. *Genome Biol*. 12, R60. https://doi.org/10.1186/gb-2011-12-6-r60.
36. Schmid M, De-Batist M, Granin NG, Kapitanov VA, McGinnis DF, Mizandrontsev IB, Obzhirov AI, Wüest A (2007) Sources and sinks of methane in Lake Baikal: A synthesis of measurements and modeling. *Limnol Oceanog*. 52,1824-1837. https://doi.org/10.4319/lo.2007.52.5.1824
37. Schwarz JIK, Eckert W, Conrad R (2008) Response of the methanogenic microbial community of a profundal lake sediment (Lake Kinneret, Israel) to algal deposition. *Limnol Oceanogr*. 53,113–121. https://doi.org/10.4319/lo.2008.53.1.0113
38. Shima S, Warkentin E, Thauer RK, Ermler U (2002) Structure and function of enzymes involved in the methanogenic pathway utilizing carbon dioxide and molecular hydrogen. *J Biosci Bioeng*. 93,519-530. https://doi.org/10.1016/s1389-1723(02)80232-8.
39. Wang, B.C (2019) Electrochemical activity of iron reducing bacteria and diversity of methanogens in sediments of Bohai sea and its coastal rivers. Dissertation, University of Chinese Academy of Sciences.
40. Whitman W (2015) Bergey's manual of systematics of archaea and bacteria. New York: John Wiley & Sons, Inc.
41. Wu JQ, Zeng FJ, Lei P, Gong P, Hu D, Zhou YH, Zeng A, Gao SF (2020) Preliminary study on intestinal microbial population and enzyme activity of fish in Loudi area. *Hunan Agr Sci*, 55-57. https://doi.org/10.16498/j.cnki.hnnykx.2020.004.015.
42. Wu SD, Wang GT, Angert ER, Wang WW, Li WX, Zou H (2012) Composition, diversity, and origin of the bacterial community in grass carp intestine. *PLoS One*. 7,e30440. https://doi.org/10.1371/journal.pone.0030440.
43. van-Lingen HJ, Edwards JE, Vaidya JD, van-Gastelen S, Saccenti E, van-den-Bogert B, Bannink A, Smidt H, Plugge CM, Dijkstra J (2017) Diurnal Dynamics of Gaseous and Dissolved Metabolites and Microbiota Composition in the Bovine Rumen. *Front Microbiol*. 8,425. https://doi.org/10.3389/fmicb.2017.00425.
44. Xing YP, Xie P, Yang H, Ni LY, Wang YS, Rong KW (2005) Methane and carbon dioxide fluxes from a shallow hypereutrophic subtropical Lake in China. *Atmos Environ*. 39, 5532-5540. https://doi.org/10.1016/j.atmosenv.2005.06.010.
45. Yan L, Lang CP, Wang WD, Wang YJ (2017) Geographical distribution characteristics of methanogenic bacteria based on the analytics of 16S rRNA in genbank. *Heilongjiang Bayi Agr Univ*. 29,59-65. https://doi.org/10.3969/j.issn.1002-2090.2017.05.015.
46. Yang B (2017) Community structures and diversity of bacteria and archaea in low temperature biogas systems. Dissertation,Yunnan Normal University.
47. Zhang H, Zhou T, Wang Y (2013) Bacterial composition in the intestine of freshwater pearlmussel and co-cultured fishes in an integrated culture pond. *Acta Microbiologica Sinica*. 37,824-835. https://doi.org/10.7541/2013.105.

48. Zhang L, Liang JF, Cui WW, Du LZ, Gao WX, Feng XM, Schnürer A (2014) Characteristics of microbial communities in full-scale biogas digesters with straw as substrate. *J Agro-Environ Sci*. 33,584-592. https://doi.org/10.11654/jaes.2014.03.027.
49. Zhang N, Bao H, Zuo DZ, Cui BL, Chen KL (2021) Community characteristic of methanogenic bacteria in different types of alpine wetland in Qinghai Lake. *Chin J Appl Environ Biol*. https://kns.cnki.net/kcms/detail/51.1482.q.20210126.0955.002.html
50. Zhang WD, Song HC, Yin F, Li JC (2004) Biogas fermentation and comprehensive utilization. Kunming: Yunnan Science and Technology Press, 36-146.
51. Zhou FL, Tao LZ, Wang AQ, Mao SQ, Xu XY, Li JL, Shen YB. Evaluation of tissue status of cultured balck carp and distribution characteristics of digestive enzyme and antioxidant enzyme in the intestine. *J Shanghai Ocean U*. 2021,30, 205-213. https://doi.org/10.12024/jsou.20200403006.

Figures

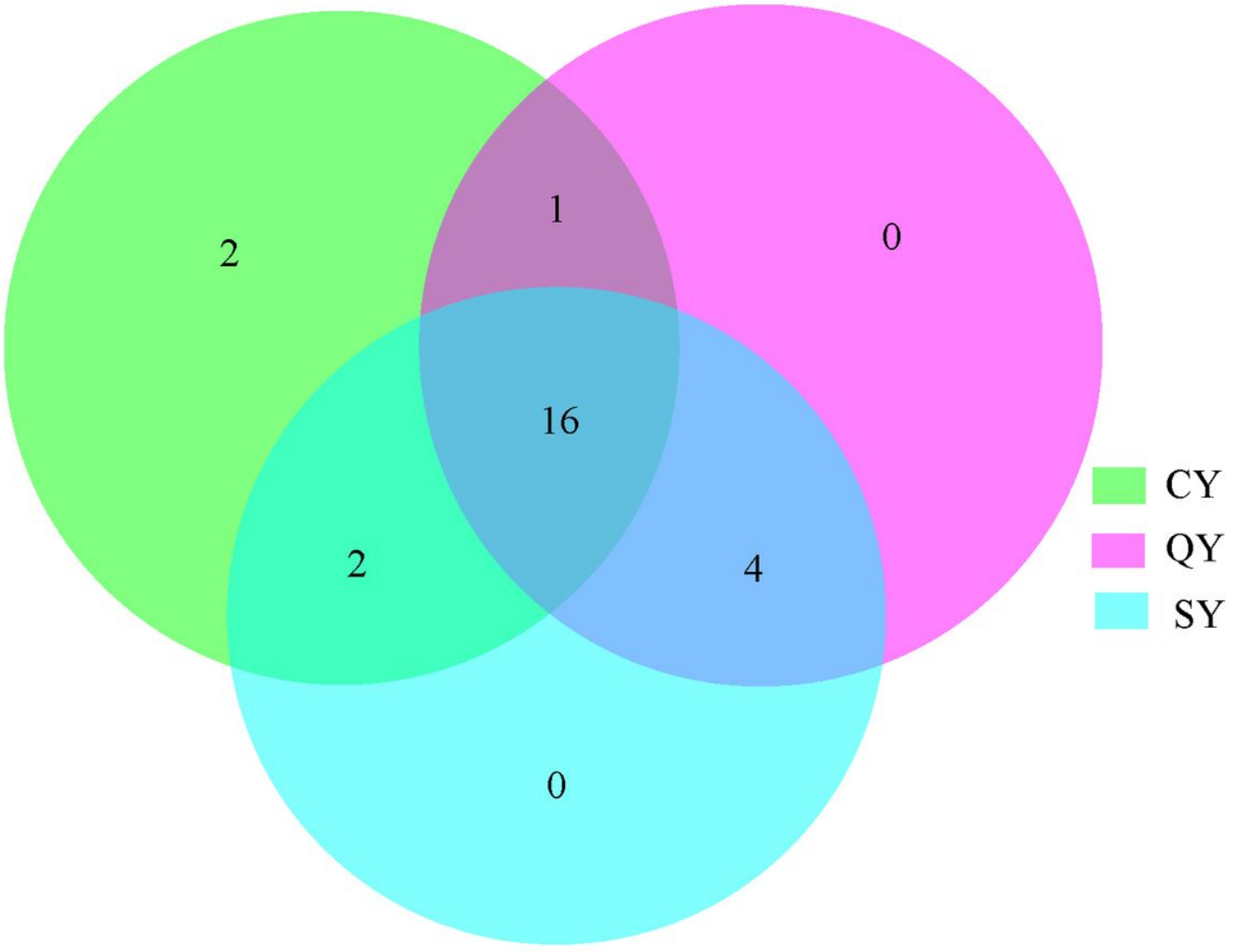

Figure 1

Methanogens Venn diagram in different test groups. Note: CY stands for grass carp, QY stands for black carp; SY stands for water sample.

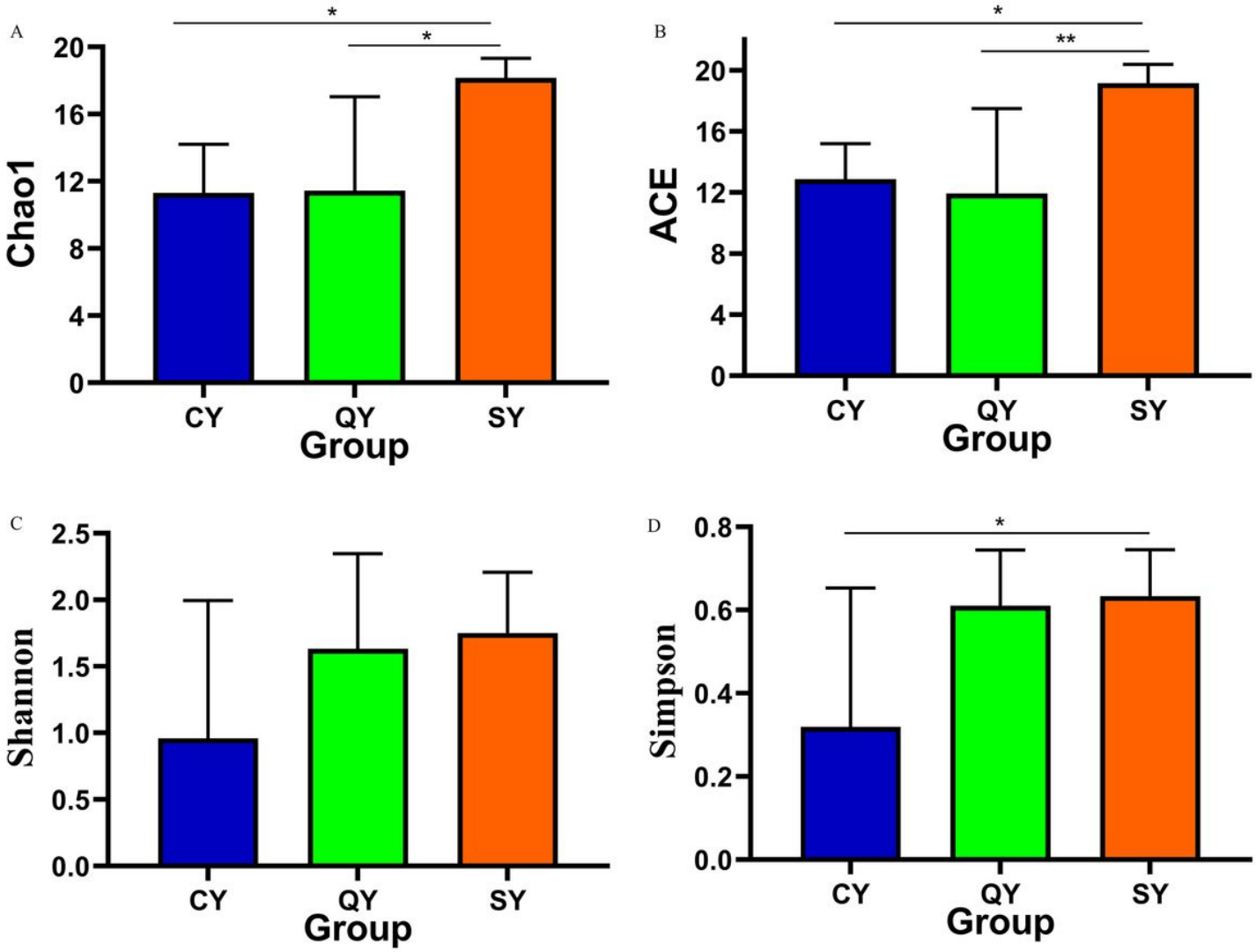

Figure 2

Difference analysis of alpha diversity index. Note: CY stands for grass carp, QY stands for black carp; SY stands for water sample.

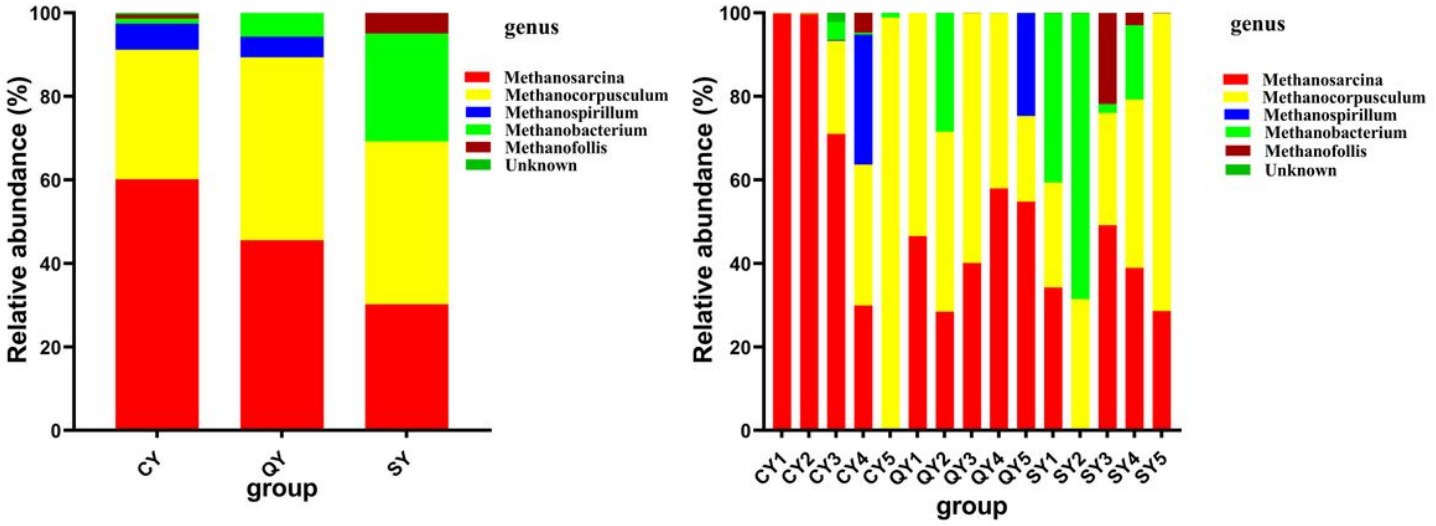

Figure 3

Relative abundance of methanogens at genus level. Note: CY stands for grass carp, QY stands for black carp; SY stands for water sample.

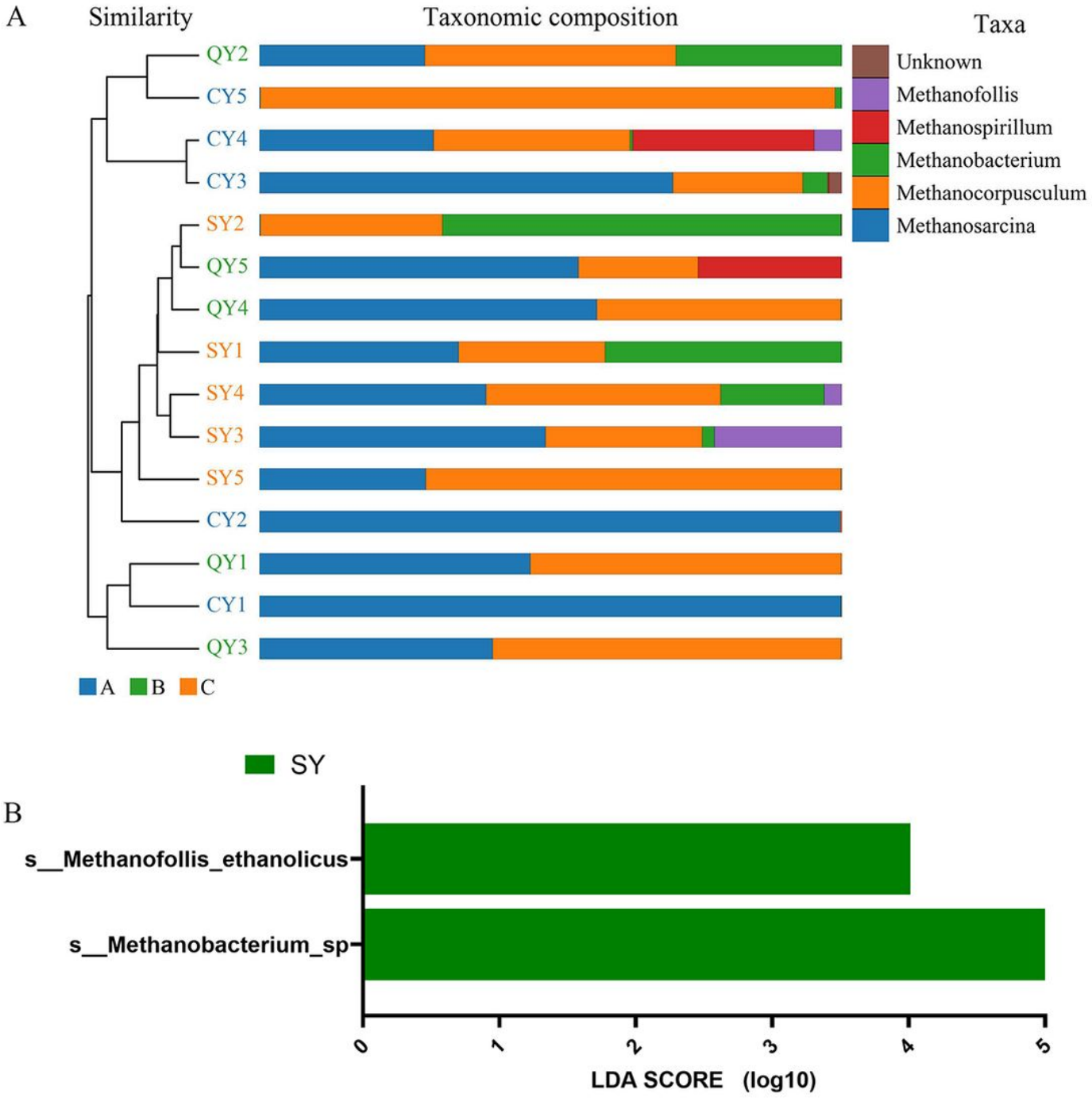

Figure 4

Similarity and difference analysis of methanogens in each group. A: Uphma analysis, B: LefSe analysis.