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RESEARCH ARTICLE

Effects of straw mulching on predatory myxobacterial communities in different soil aggregates under wheat-corn rotation

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Abstract

Crop straw mulching is an important organic supplement in sustainable agriculture; however, the effect of increased organic matter on the diversity of micropredators such as *myxobacteria* and the correlation between *myxobacteria* and microorganisms have been little explored. In the current investigation, high-throughput sequencing was performed to analyze the myxobacterial community composition in a wheat-corn rotation experimental field with 6-year straw mulching and fertilization treatments. The results reveal no significant influence of straw mulch application on myxobacterial α-diversity ($P < 0.05$). NMDS (nonmetric multidimensional scaling) and perMANOVA results indicate the significant influence of straw mulching application on myxobacterial community composition ($P < 0.05$), and several groups, including *Haliangiaceae*, *Polyangiaceae*, and *Archangiaceae*, also varied in soil aggregates. RDA (redundancy analysis) results show that TOC (total organic carbon) was the most important factor affecting the *myxobacterial* community structure. In addition, RDA and random forest analysis results show the contribution of myxobacterial community structure to soil bacterial community α- and β-diversity, especially in the 0.25–1 mm and < 0.25 mm soil aggregate fractions. In conclusion, we suggest that the variation in myxobacterial community structure may be a driver of bacterial α- and β-diversity in soil microhabitats and might be a cause of soil microbial community changes. Our results are fruitful for finding more efficient ways to use straw from waste for the betterment of sustainable agriculture by analyzing changes in myxobacterial community structure.

Keywords Crop straw mulching · Soil aggregates · Myxobacterial community · Fertilization · Wheat · Corn

Introduction

Crop straw mulching is an important application in sustainable agriculture. The method has great potential to improve soil fertility in agricultural ecosystems (Singh et al. 2007), and it is one of the important sources of soil organic matter supply. Soil bacteria, as the main soil organic matter decomposers, are crucial to the carbon cycle (Nielsen et al. 2011; Zhang and Lueders 2017). These microorganisms can introduce organic carbon into the soil food web through complex interactions between different decomposers (Handa et al. 2014). Although the interaction of microbial communities is very important to the soil carbon cycle, the details of microbial population characteristics and interactions under exogenous organic carbon (such as straw mulching) in the soil ecosystem remain unclear, including the correlation between soil microorganisms and their direct predators. *Myxobacteria*, the order Myxococcales in domain bacteria with complex life strategies (Zhou et al. 2014), are the most important micropredators in the soil ecosystem (Mohr 2018). *Myxobacteria* are widely distributed in a variety of environments. They comprise a broad spectrum of predatory bacteria and are essential in soil (Muñoz-Dorado et al. 2016). In general, *myxobacteria* mainly include bacteriolytic

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Key points
- Straw mulching application can affect soil myxobacterial community.
- TOC was found to be the most important factor affecting the myxobacterial community.
- *Myxobacteria* was found to be the most important potential driver of soil bacterial diversity.

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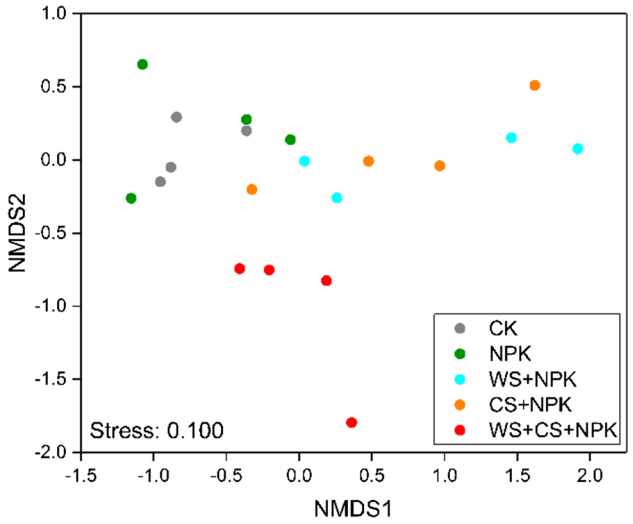


Fig. 1 Ordering of the myxobacterial community composition by non-metric multidimensional scaling (NMDS) based on the Bray–Curtis distance index

communities and cellulosic decomposing communities; among these, the former includes the most culturable *myxobacteria*, which can effectively degrade other microbial cells, while the latter degrades dead microbial cells instead of feeding directly on living bacteria. In soil ecosystems, these bacteriolytic groups are predatory consumers that feed on various soil microbes (Remis et al. 2014; Thakur et al. 2018), which are closely related to soil microbial community structure and function as an important evolutionary ecological factor (Pasternak et al. 2013). However, the effect of straw mulching on *myxobacteria* in agricultural ecosystems is often overlooked, and information on how straw mulching systems affect the soil myxobacterial community is limited even though straw mulching significantly affects soil microbial communities (Li et al. 2019; Zhou et al. 2019; Yang et al. 2020). Furthermore, the actual activity of micropredators represented by *myxobacteria* in complex farmland soil systems is still undetermined.

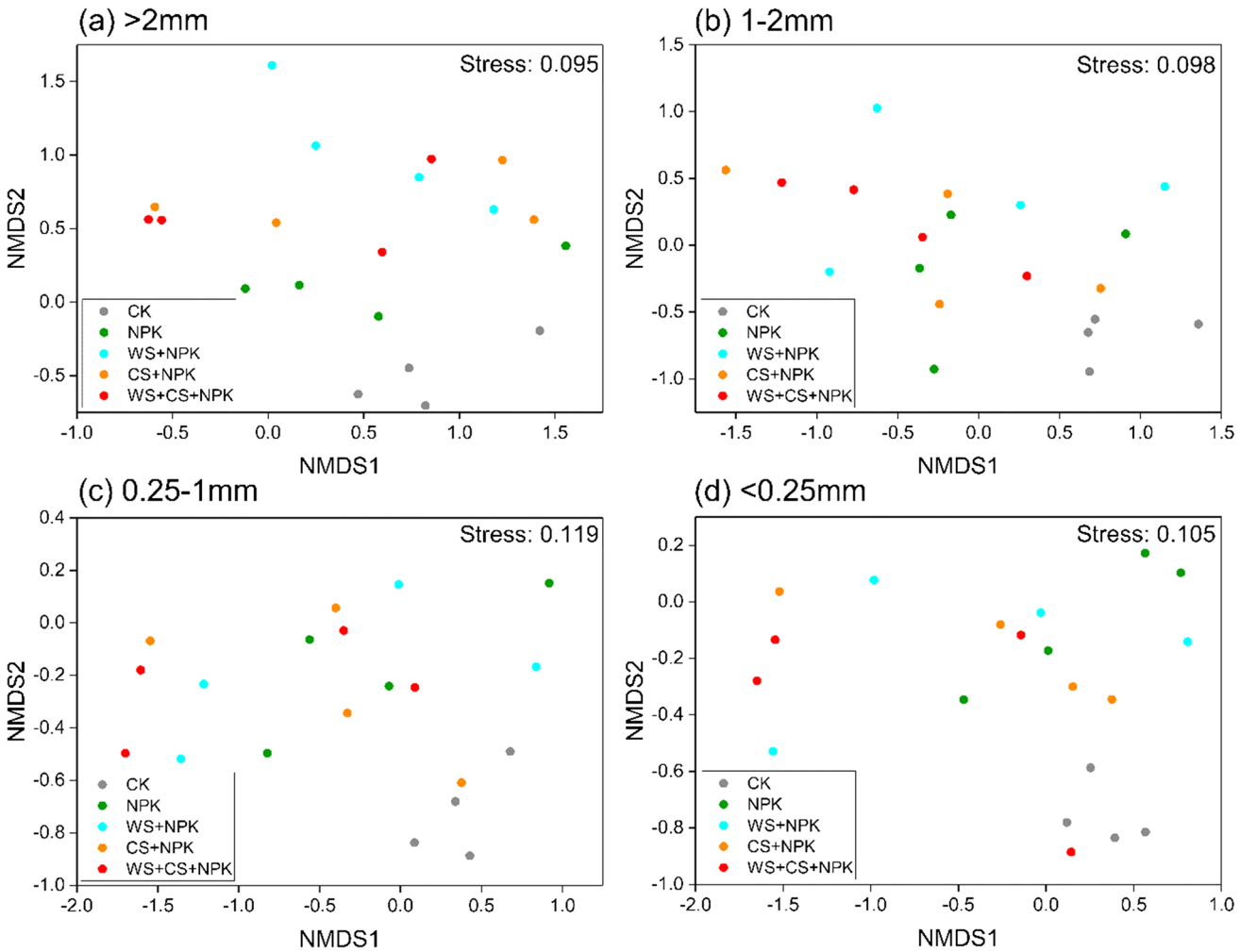


Fig. 2 Ordering of the myxobacterial community composition by NMDS in different soil aggregates

Table 1 Relative abundance ($\times 10^{-4}$) of order myxococcales with its 8 known families in the different field treatments

	CK	NPK	WS + NPK	CS + NPK	WS + CS + NPK
Myxococcales	169.95 ± 8.70a	161.37 ± 27.50a	183.48 ± 38.45a	178.65 ± 17.95a	194.40 ± 30.86a
Haliangiaceae	46.24 ± 3.20b	42.18 ± 7.25b	56.41 ± 9.98ab	53.37 ± 13.79ab	66.34 ± 10.95a
Polyangiaceae	15.06 ± 5.54a	18.21 ± 4.36a	28.06 ± 18.66a	19.89 ± 3.21a	22.57 ± 10.39a
Sandaracinaceae	12.50 ± 3.16a	11.86 ± 6.28a	7.91 ± 1.45a	14.39 ± 5.39a	9.66 ± 5.19a
Archangiaceae	11.45 ± 3.66a	9.70 ± 3.58a	8.97 ± 4.66a	6.24 ± 2.73a	7.76 ± 4.02a
Nannocystaceae	5.77 ± 1.76ab	5.34 ± 0.85ab	7.74 ± 3.43a	5.00 ± 2.03ab	3.89 ± 1.15b
Phaselicystidaceae	2.55 ± 0.29a	3.07 ± 1.12a	2.67 ± 0.69a	5.96 ± 5.02a	6.41 ± 0.95a
Myxococcaceae	1.53 ± 0.93a	1.33 ± 0.52a	1.62 ± 0.88a	1.36 ± 0.77a	1.37 ± 0.49a
Vulgatibacteraceae	1.14 ± 0.56a	1.21 ± 0.61a	0.82 ± 0.42ab	0.36 ± 0.41b	1.05 ± 0.27ab

Different lowercases in the same column indicate a significant difference ($P < 0.05$, $n = 4$). Treatments: *CK*, soil without fertilizer and straw returning; *NPK*, fertilizer without straw; *WS*eturning and fertilizer

Soil aggregates are of different sizes and can serve as microenvironments for the soil microbial community (Zheng et al. 2018; Wang et al. 2019). The variation in nutrient distribution results in differences of microbial community structure and composition in different soil aggregates (Datta et al. 2018; Luo et al. 2018). In addition, the application of straw mulch and fertilizer affects the diffusion of soil gas and water through the fraction of soil aggregates in agricultural ecosystems (Naveed et al. 2014) as well as the composition and function of bacterial communities (Lin et al. 2019). In addition, the distribution of nutrients in each soil aggregate and the diffusion of gas and water are different (Wan et al. 2020), leading to differences in the diversity of potential micropredators. However, the effect of straw mulch on the composition of micropredators represented by *myxobacteria* in soil aggregates is not yet clear.

The myxobacterial community plays a vital role in the soil ecology of agricultural systems. However, limited research has been conducted on myxobacterial diversity from straw mulching in agricultural soil. Previous studies (Wang et al. 2020) have used universal 16S rRNA gene primers to study a mass of bacterial groups. In addition, increasing the sequencing depth can produce a sufficient number of myxobacterial sequences. Therefore, high-throughput sequencing using 16S rRNA gene primers is still the most effective way to analyze the myxobacterial community. In this study, a six-year field experiment of wheat-corn rotations at the Suzhou Agricultural Research Institute was conducted to investigate the effect of straw mulching on myxobacterial diversity via 16S rRNA gene high-throughput sequencing. The aim of the study was to explain how the variation in factors, particularly of straw mulching, affects soil myxobacterial diversity. Myxobacterial diversity variation in soil aggregates, myxobacterial diversity, and soil total bacterial community relationships is also explored. The present research formulates an optimal way to transform straw for the sustainable agricultural utilization of resources from waste to treasure.

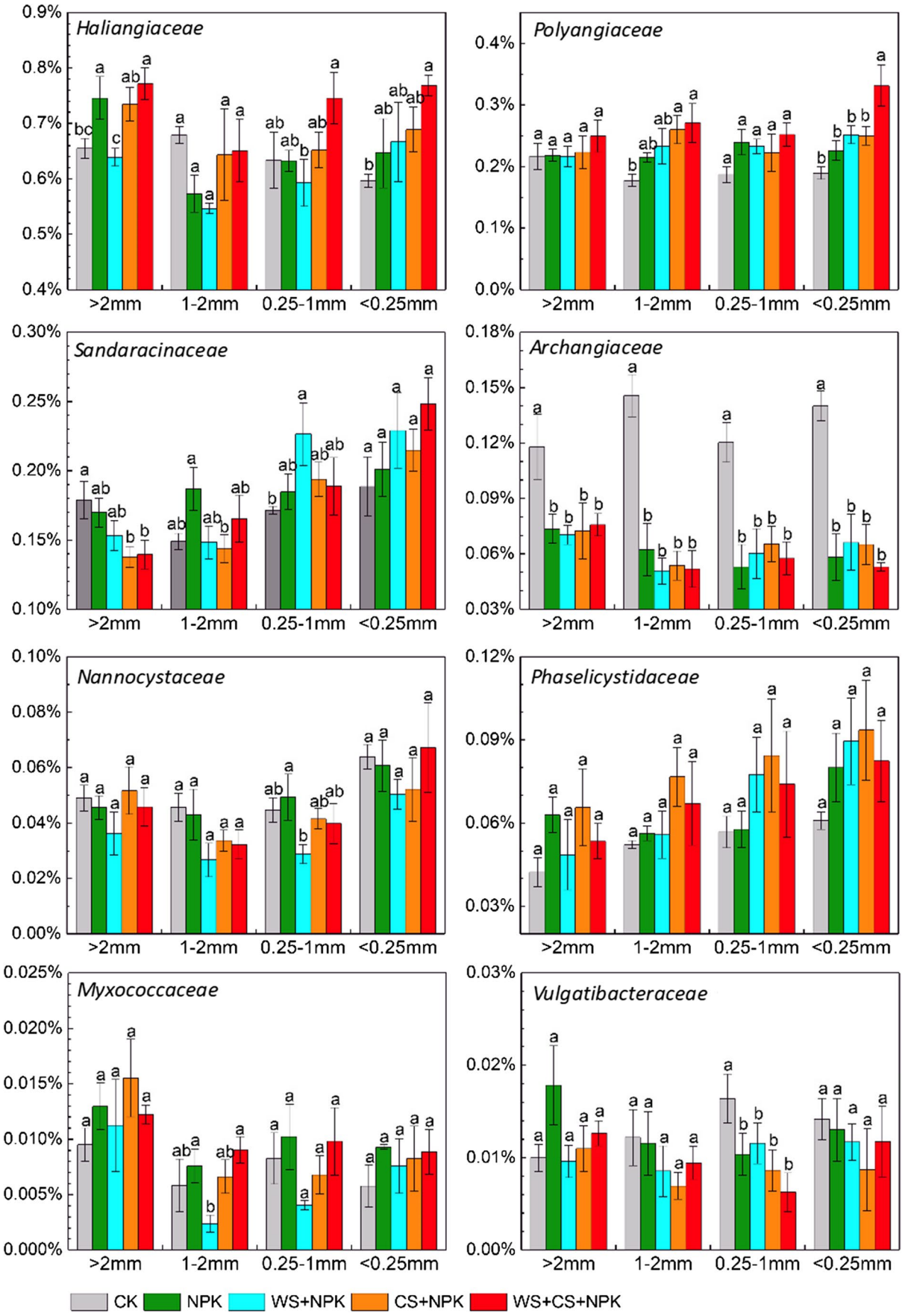
Materials and methods

Experimental design

The experimental study spanned 6 years (from Oct. 2013 to June 2019) and was performed in fields of the Suzhou Agricultural Research Institute, Suzhou, Anhui Province, China (33° 38′ E, 117° 04′ N).

The soil is classified as a Eutric Vertisol according to the FAO soil classification system, originating from Quaternary semihydromorphic soil on fluvio-lacustrine sediments. Wheat-corn (wheat: *Triticum aestivum* L. Su 553; corn: *Zea mays* L. Longping 206) rotations with five treatments of straw mulch in four replications were performed in the field. The field experiment involved twenty experimental cells (each subplot was 15 m × 8 m × 0.6 m). Wheat plant rows were spaced 20 cm apart, and the sowing quantity was 13 kg/mu. The row spacing of sown corn was set to 60 cm, and the sowing quantity was 3 kg/mu. The wheat–maize rotation period for maize ran from mid-June to the end of September, and for wheat, it ran from October to June.

The five treatments of the experiment were as follows: treatment 1: control (CK): no fertilizer or straw application; treatment 2:(NPK) nitrogen phosphorus and potassium chemical fertilizer application with urea 39 kg/mu, superphosphate 50 kg/mu, and potassium chloride 10 kg/mu; treatment 3: (WS + NPK) wheat straw (500 kg/mu) application to the soil in June of each year with NPK; treatment 4: (CS + NPK) corn straw (500 kg/mu) application to soil in October of each year with NPK; and treatment 5: (WS + CS + NPK) wheat straw (500 kg/mu) application to the soil in June of each year and corn straw (500 kg/mu) application to the soil in October of each year with NPK. To return the straw to the experimental field, crop straw was crushed in situ after the harvest and then carried out via rotary tillage. In contrast, the straw was removed from the experimental field after harvest.


Haliangiaceae
Polyangiaceae
Sandaracinaceae
Archangiaceae
Nannocystaceae
Phaselicystidaceae
Myxococcaceae
Vulgatibacteraceae
>2mm
1-2mm
0.25-1mm
<0.25mm
CK
NPK
WS+NPK
CS+NPK
WS+CS+NPK

◂**Fig. 3** Relative abundance (percentage) of the main identified myxobacterial taxonomic groups, i.e. families *Haliangiaceae*, *Polyangiaceae*,*Sandaracinaceae*, *Archangiaceae*, *Nannocystaceae*,*Phaselicystidaceae*, *Myxococcaceae*, and *Vulgatibacteraceae*, in different soil aggregates among treatments. For each sample, the relative abundance of the sequences assigned to a given taxonomic unit was calculated for each of the four subsamples, and the average value was then used to represent the relative abundance of each sample. The error bars show the standard deviation of relative abundance of the four subsamples for each sample. Different letters indicate that values are significantly different from each other in each soil aggregate fraction ($P < 0.05$, LSD)

Soil collection and analysis

The soil samples were collected from each subplot (depth of 0–20 cm) of five random locations in June 2019. Soil samples were mixed and homogenized by removing aboveground roots, visible residues, and stones; placed in an icebox; and transported to the laboratory. The soil samples were stored at 4 °C for soil physical and chemical analysis and at 80 °C for bacterial analysis. Soil samples served as the original sample. For soil aggregate separation, a dry-sieving procedure was implemented (Bach and Hofmockel 2014), and different aggregate fractions, including > 2 mm, 1–2 mm, 0.25–1 mm, and < 0.25 mm, were selected (Wang et al. 2018). Soil chemical properties and bacterial communities were evaluated in all soil aggregate fractions. Soil microbial biomass C (MBC) was determined by the chloroform fumigation extraction method (Huang et al. 2017). Total phosphorus (TP) was extracted via HF–$HClO_4$ digestion and determined using molybdenum blue colorimetric analysis with a visible spectrophotometer (UV-1200, Shanghai, China) (Bowman 1988). Available phosphorus (AP) content was extracted by a 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5) solution and analyzed using the same methods used for TP (Bowman 1988; Huang et al. 2017). The soil total organic carbon (TOC) content in different aggregates was measured through $K_2Cr_2O_7$ oxidation and $FeSO_4$ titration (Huang et al. 2017). Total nitrogen (TN) was determined by the Kjeldahl nitrogen determination method (Huang et al. 2017). Alkaline hydrolysis nitrogen (AN) was determined by the alkali-hydrolyzed diffusion method (Huang et al. 2017). Soil total potassium (TK) was determined by hydrofluoric acid-perchloric acid digestion and flame spectrophotometry (Huang et al. 2017). The available potassium (AK) content was extracted by 1 mol L^{-1} NH_4Ac (pH 7.0) and measured using the same method as that applied for TK (Huang et al. 2017). Soil pH was recorded with a glass electrode (1:5 wt/vol) (Li et al. 2019).

DNA extraction and microbial community analysis

Total DNA was extracted from each sample (0.5 g) with the FastDNA® SPIN Kit for soil (MP Biomedicals, Cleveland, OH, USA) according to the manufacturer's instructions. The bacterial and archaeal communities in all samples were analyzed by pyrosequencing the 16S rRNA genes from samples. The 515F/907R (515F: 5′-GTG CCA GCM GCC GCG G-3′; 907R: 5′-CCG TCA ATT CMT TTR AGT TT-3′) primer pair was used for the amplification of the V4-V5 regions of the bacterial 16S rRNA gene (Fan et al. 2020). Amplification parameter: initial denaturation 98℃ 2 min, denaturation 98℃ 15 s, annealing 55℃ 30 s, extension 72℃ 30 s, final extension 72℃ 5 min, 10℃ hold, 25–30 cycles.

Primers were tagged with unique barcodes for each sample. Negative controls using sterilized water instead of soil DNA extract were included to check for primer or sample DNA contamination. The qualities and concentrations of the purified barcoded PCR products were determined using a NanoDrop spectrophotometer. High-throughput sequencing was conducted with Illumina MiSeq PE300 developed by Hangzhou Lianchuan Biotechnology Co., Ltd. (Zhejiang, China).

The read merging and quality filtering of the raw sequences were performed by QIIME Pipline. Operational taxonomic units (OTUs) were clustered at 97% similarity, and OTU picking and taxonomic assignment were performed according to the SILVA reference data (version 128) (Quast et al. 2013). The reads that did not align with the anticipated region of the reference alignment were removed as chimeras. Reads classified as "chloroplasts," "mitochondria," or "unassigned" were removed.

Myxobacterial community analysis

The α-diversity indices, including Chao1 and Shannon, of the myxobacterial community were measured by R software (version 3.6.2) with the 'vegan' package. The phylogenetic tree of the top 30 dominant myxobacterial OTUs was constructed using MEGA 7.0. The heatmap of these 30 myxobacterial OTUs was generated with the 'pheatmap' package in the R software. Based on the 446 myxobacterial OTUs, nonmetric multidimensional scaling (NMDS) with Bray–Curtis distance matrices, a permutational multivariate analysis of variance (perMANOVA), and redundancy analysis (RDA) with Monte Carlo permutations were performed in the R software. Linear regression analysis was used to analyze the relationships between the soil bacterial/myxobacterial indices and predictor variables. Random forest (RF) analysis was used to measure the main predictors of the soil bacterial communities (Jiao et al. 2018). Sequence Read Archive (SRA) submission: SUB8976216. BioProject Accession: PRJNA700379 (TaxID: 410,658).

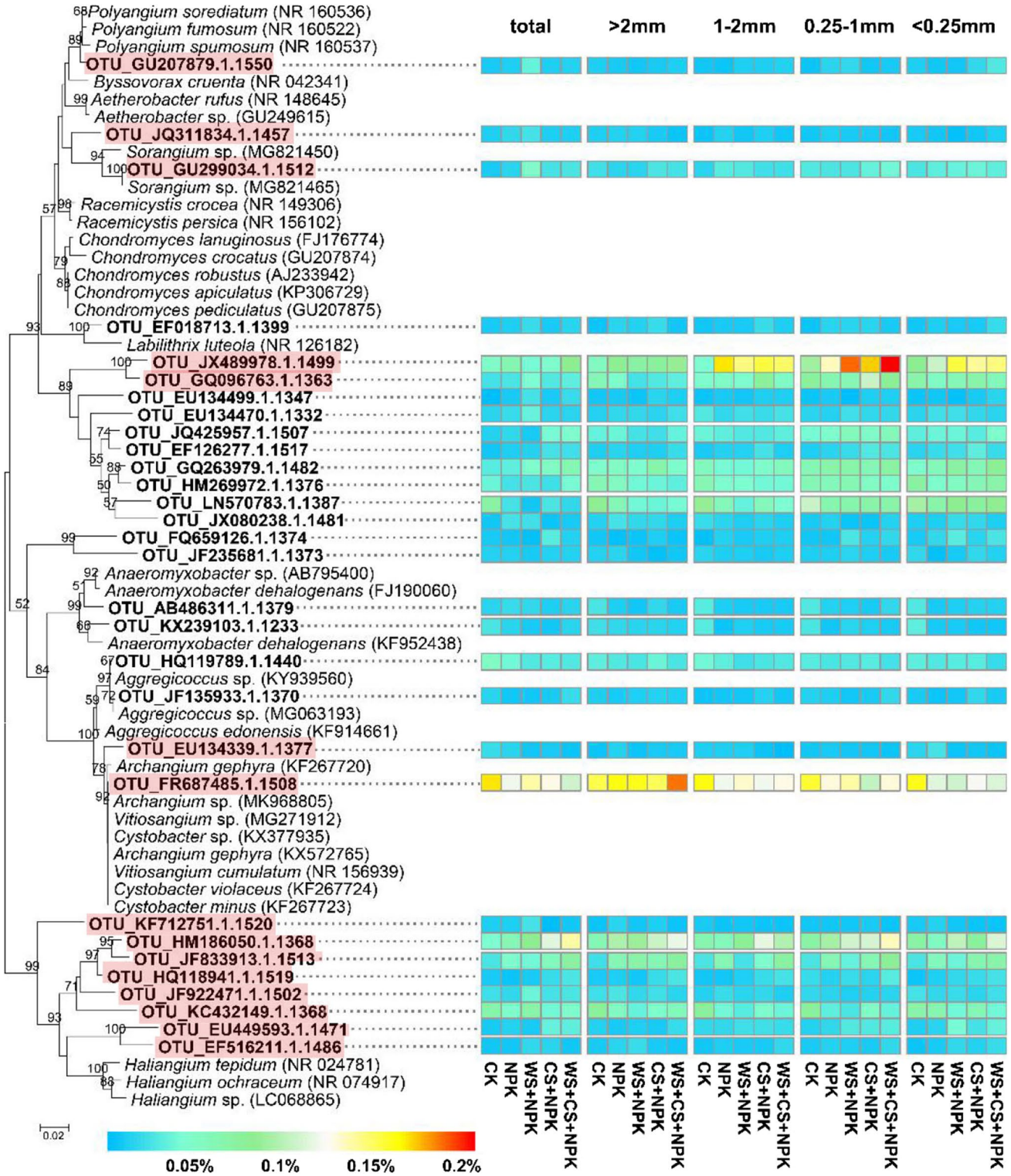


Fig. 4 Phylogenetic tree of the top 30 myxobacterial OTUs sequences in samples. The mean of the relative frequencies (%) are marked in the heatmap. Bootstrap values > 50% are indicated at branch points

Results

Effects of straw application on the bacterial communities and structure

In terms of bacterial α-diversity, fertilization and straw mulching had no effect on the bacterial Chao1 index and Shannon index in soils but affected the bacterial Chao1 index and Shannon index in < 2 mm soil aggregate fractions (Table S1).

PerMANOVA results show that the bacterial community was influenced by the fertilization and straw mulching treatments of soils (Table S2), but fertilization only affected the bacterial community in the 0.25–1 mm and < 0.25 mm soil aggregate fractions. Fertilization and straw mulch application also influenced bacterial community composition (Fig. S1).

Effects of straw application on myxobacterial diversity

A total of 16,944 myxobacterial sequences ranging from 513 to 1,242 were obtained from the soil samples, and a total of 71,142 myxobacterial sequences ranging from 580 to 1,344 were obtained from the soil aggregate samples. In terms of myxobacterial α-diversity, the fertilization and straw mulching treatments had no effect on the myxobacterial Chao1 index and Shannon index (Table S3) in soils and different soil aggregates. Sample ordering by NMDS according to myxobacterial Bray–Curtis dissimilarity (Figs. 1 and 2) shows the separation of the myxobacterial community structures (both in total and different soil aggregates) among the treatments. The PerMANOVA with pairwise comparison (Table S4) shows that the myxobacterial community was influenced by the fertilization and straw application treatments of soils. There was no difference in myxobacterial community similarity distance between CK and NPK or between fertilization and straw applications in soil aggregates except for the < 0.25 mm fraction. A difference in myxobacterial community similarity distance between NPK and WS + CS + NPK was found in the < 0.25 mm soil aggregate fraction.

Effects of straw application on the myxobacterial community composition

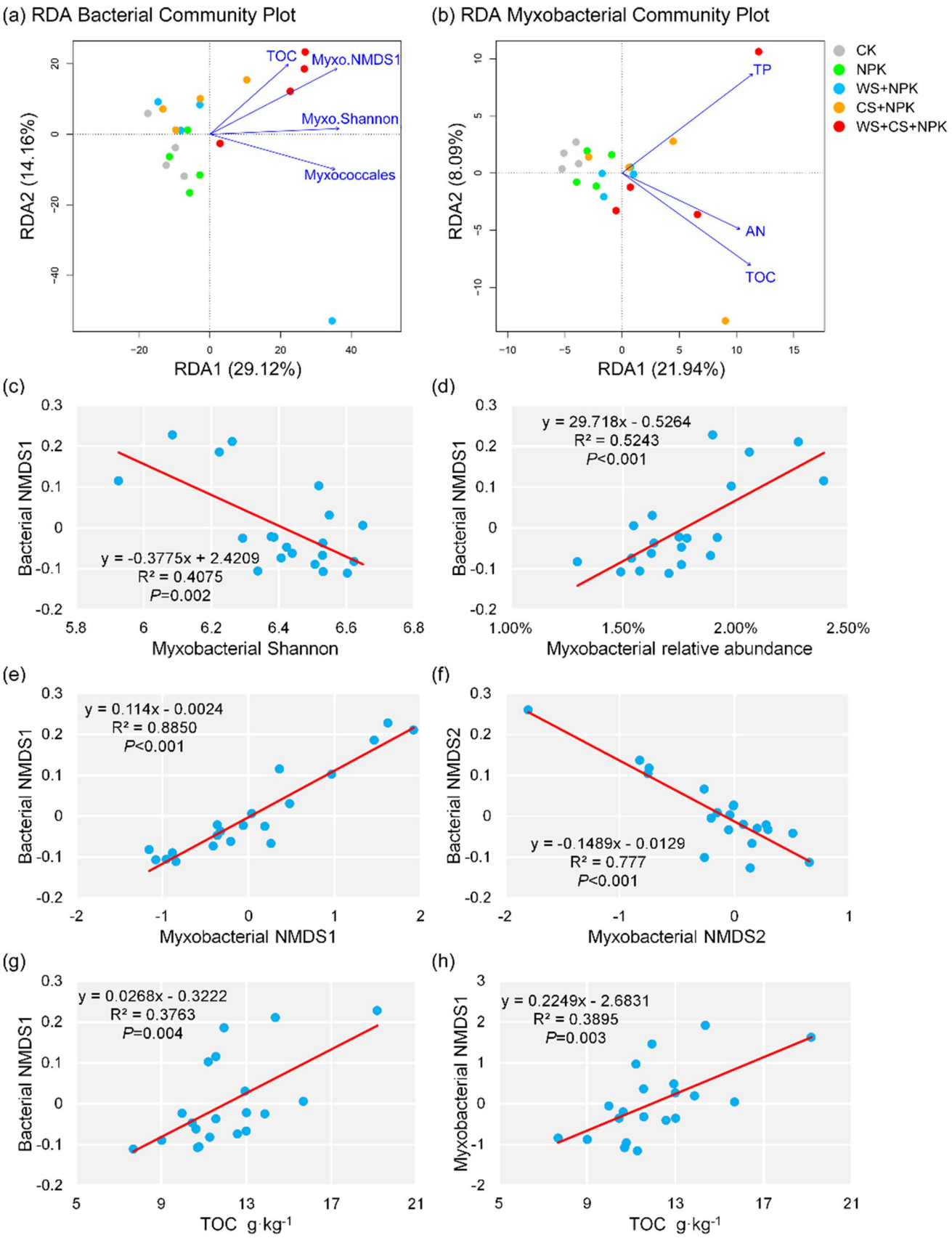
A total of 446 myxobacterial OTUs were assigned to order *Myxococcales*, which accounted for 1.30–2.39% of all bacteria (Table S5). All 446 myxobacterial OTUs were divided into 8 known families, including unclassified *myxobacteria* (138 OTUs, accounting for 40.78%, *Haliangiaceae* (131, 29.63%), *Polyangiaceae* (53, 11.40%), *Sandaracinaceae* (49, 6.36%), *Archangiaceae* (27, 5.03%), *Nannocystaceae* (20, 3.17%), *Phaselicystidaceae* (18, 2.29%), *Myxococcaceae* (5, 0.81%), and *Vulgatibacteraceae* (5, 0.53%) (Table 1). Straw mulch application in the field had no significant effect on *myxobacteria* (Table S5) but significantly increased the relative abundance of *Haliangiaceae* (Table S5). In addition, the family *Haliangiaceae* was significantly positively correlated with soil TOC, AN (NH_4NO_3), and AK (available potassium) (Table S6, Pearson's correlation, $P < 0.05$).

In soil aggregates, eight different known groups at the family level among straw mulching application treatments were found (Fig. 3). The relative abundance of *Polyangiaceae* was the highest in the WS + CS + NPK treatment in all of the soil aggregates, whereas the relative abundance of *Archangiaceae* decreased with fertilizer and straw mulch application. Most of the highest observed relative abundances of Haliangiaceae were of WS + CS + NPK in the soil aggregates except for the 1–2 mm fraction. In addition, *Sandaracinaceae* exhibited the highest relative abundance in the 0.25–1 mm and < 0.25 mm soil aggregate fractions.

The top 30 myxobacterial OTUs were selected to build a phylogenetic tree (Fig. 4). These OTUs accounted for the majority (51.28%) of the myxobacterial abundance. The top 30 OTUs covered 4 known myxobacterial families, including 8 *Haliangiaceae*, 3 *Polyangiaceae*, 2 *Archangiaceae*, and 2 *Sandaracinaceae* OTUs. Among them, only OTU_HQ119789.1.1440 (uncultured *myxobacteria*), OTU_LN570783.1.1387 (uncultured *myxobacteria*), OTU_EF126277.1.1517 (uncultured *myxobacteria*), OTU_EU134499.1.1347 (uncultured *myxobacteria*), OTU_HM186050.1.1368 (*Haliangiaceae*), and OTU_KF712751.1.1520 (*Haliangiaceae*) showed a significant difference among treatments. However, in different soil aggregates, the top 30 myxobacterial OTUs were mostly affected by fertilizer and straw mulch applications (Table S7). For example, most OTUs assigned to unclassified *myxobacteria* increased by fertilization and straw mulching in all of the soil aggregates. In addition, most OTUs assigned to *Haliangiaceae* presented higher numbers in the fertilization and straw mulching application treatments.

Factors affecting the soil bacterial and myxobacterial community

A redundancy analysis (RDA) was used to analyze the OTU-level bacterial and myxobacterial communities (Fig. 5a, b). All of the parameters, including myxobacterial diversity indices, order *Myxococcales* abundance, myxobacterial NMDS axes, and soil chemical properties, were selected according to variance inflation factor analysis. *Myxobacteria* (including the myxobacterial Shannon index, order *Myxococcales* abundance and the first NMDS axis of the myxobacterial community) were an important factor in regulating soil bacterial


(a) RDA Bacterial Community Plot
TOC
Myxo.NMDS1
Myxo.Shannon
Myxococcales
RDA2 (14.16%)
RDA1 (29.12%)
(b) RDA Myxobacterial Community Plot
TP
AN
TOC
RDA2 (8.09%)
RDA1 (21.94%)
CK
NPK
WS+NPK
CS+NPK
WS+CS+NPK
(c)
y = -0.3775x + 2.4209
R² = 0.4075
P=0.002
Bacterial NMDS1
Myxobacterial Shannon
(d)
y = 29.718x - 0.5264
R² = 0.5243
P<0.001
Bacterial NMDS1
Myxobacterial relative abundance
(e)
y = 0.114x - 0.0024
R² = 0.8850
P<0.001
Bacterial NMDS1
Myxobacterial NMDS1
(f)
y = -0.1489x - 0.0129
R² = 0.777
P<0.001
Bacterial NMDS2
Myxobacterial NMDS2
(g)
y = 0.0268x - 0.3222
R² = 0.3763
P=0.004
Bacterial NMDS1
TOC g·kg-1
(h)
y = 0.2249x - 2.6831
R² = 0.3895
P=0.003
Myxobacterial NMDS1
TOC g·kg-1

◂**Fig. 5** Redundancy analysis (RDA) for bacterial (**a**) and myxobacterial (**b**) community with forward selection of predictor variables followed by Monte Carlo permutations (999 permutations); **c–e** relationships between bacterial NMDS1 and myxobacterial community indices including myxobacterial Shannon index, myxobacterial abundance, and myxobacterial NMDS1; **f** relationships between bacterial NMDS2 and myxobacterial NMDS2; **g** relationships between bacterial NMDS1 and TOC; **h** relationships between myxobacterial NMDS1 and TOC. Myxo.NMDS1: Myxobacterial NMDS1, *Myxococcales*: the relative abundance of *Myxococcales* in total bacteria, TOC: total organic C, TN: total N, TP: total P, TK: total K, AN: available N, AP: available P, AK: available K, MBC: microbial biomass C

structure. TOC, AN, and TP were also the prime parameters influencing the myxobacterial community. Similarly, the two NMDS axes of the myxobacterial community, TOC and MBC, affected the soil bacterial structure in different soil aggregates (Fig. S2), and the order *Myxococcales* abundance and myxobacterial Shannon index affected the soil bacterial structure in the 0.25–1 mm and < 0.25 mm soil aggregate fractions. TOC was the main soil property influencing the myxobacterial community in different soil aggregates.

In parallel, the first NMDS axis of the bacterial community was significantly correlated with TOC, *Myxococcales* abundance, and the myxobacterial Shannon diversity index (Fig. 5c–d, g, Table S8). Meanwhile, the first and second NMDS axes of the myxobacterial community exhibited a significant correlation with that of the bacterial community (Fig. 5e–f, Table S8). In addition, most myxobacterial families were related to the first NMDS axes of the bacterial community. In parallel, the first NMDS axes of the myxobacterial community showed significant correlations with TOC, AN, TP, AP, and AK (Fig. 5h, Table S9). In soil aggregates, the NMDS axes of the bacterial community were also related to different chemical indices and myxobacterial groups (Table S10-13).

The main predictors of the soil bacterial community structure were confirmed by RF analysis (Fig. 6). TOC was the most important variable explaining the soil bacterial structure as proven by the Shannon index and the first NMDS axis of the myxobacterial community. In addition, myxobacterial NMDS1 was the most important variable explaining bacterial NMDS1 in all the soil aggregates and bacterial α-diversity in the 0.25–1 mm and < 0.25 mm fractions. The myxobacterial Shannon index, the relative abundance of order *Myxococcales* and TOC were also the prime factors contributing to bacterial α-diversity in the 0.25–1 mm and < 0.25 mm soil aggregate fractions.

Effects of straw reintroduction and fertilization methods on soil physical and chemical properties

Compared to the control, fertilization, wheat, and corn straw reintroduction significantly reduced soil pH. TOC levels increased by 18.55–44.89%, and there was a significant difference in the maize straw reintroduction area (Table S14). TN levels increased by 32.42–73.75%, and there was a significant difference between the single fertilization and mixed straw reintroduction results. The variation trend of AN was basically the same as that of TP. Levels increased by 15.80–39.63%, TP levels increased by 11.77–44.11%, and AK levels increased by 13.06–21.19%, for which the mixed reintroduction condition showed significant differences. MBC levels significantly increased by 19.53–71.27%, and the effect of wheat straw was the most significant.

Discussion

Myxobacteria are important micropredators in the food web, and numerous studies (Zhou et al. 2014; Mohr 2018; Wang et al. 2020) have shown that such organisms are widely distributed in soil ecosystems and control the soil microbial population. However, there has been little research on the role of *myxobacteria* in regulating the diversity and structure of soil microbial communities in agricultural soils.

Straw mulching can affect the soil myxobacterial community. We found straw mulching for six years to have no effect on the diversity and relative abundance of *myxobacteria* in a wheat-corn rotational field. Similarly, fertilization did not affect the diversity of myxobacteria. A previous work suggests a possible decrease in myxobacterial diversity, volume, and cell density in soil due to fertilization (Wang et al. 2020). Long-term fertilization in the corresponding experimental field resulted in a decrease in soil pH, while *myxobacteria* preferred to live in high-organic soils with pH values ranging from 6 to 8 (Mohr 2018), and acidic conditions caused by fertilization directly led to a decrease in myxobacterial populations. The current work suggests that straw mulching and fertilization for six years shows a positive correlation with maintaining soil pH, supporting the efficient growth of *myxobacteria*. Such treatment also makes myxobacterial abundance relatively more stable (Table S14). Similarly, Wang et al. (2016) suggested that organic fertilizers could significantly increase myxobacterial abundance. In this study, soil organic carbon content increased significantly after 6 years of treatment (Table S14), but the difference in the organic carbon fractions caused by direct straw crushing and mulching in the field (Dhaliwal et al. 2020) might be the cause of an absence of significant differences in myxobacterial abundance among treatments.

The effect of straw mulch application on *myxobacteria* was mainly reflected in its community structure and specific group. These effects were not related to the species of straw crop returned to the field but were mainly

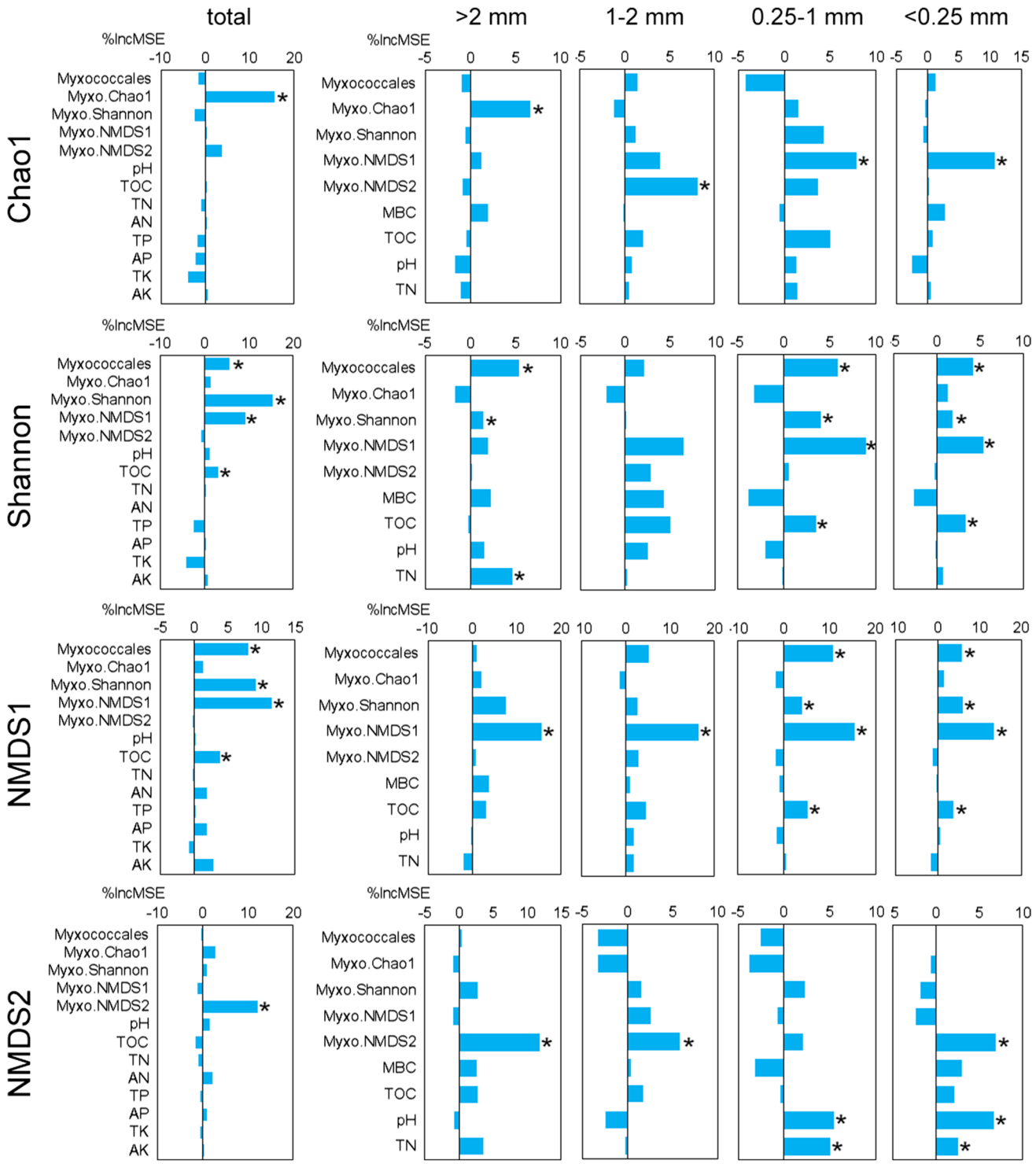


Fig. 6 RF mean predictor importance (percentage increase in MSE) of *myxobacteria* and soil properties as drivers of the soil bacteria. The accuracy importance measure was computed for each tree and averaged over the forest (500 trees). Percentage increases in the mean square error (MSE%) values imply the importance of these predictors. The asterisk indicates significant predictors ($P < 0.05$)

related to the total amount of straw crop used or the content of organic carbon supply. In parallel, this effect had similar results in different soil aggregates. Correlation analysis and RDA were conducted to explore the relationship between oil properties and myxobacterial community composition in selected soils and different soil

aggregates. Our results show soil organic carbon content to be an important factor influencing the myxobacterial community structure. Crop straw could provide additional prey bacteria and organic materials (Wang et al. 2020), which proved to be a stimulus in changing the structure of the myxobacterial community. A previous study also reported that straw mulch application could increase soil organic carbon fractions (Chen et al. 2016). Straw mulching treatment could improve soil aggregate stability and has been shown to have positive effects on soil SOC and MBC (Zhang et al. 2014). Additionally, the diffusion of SOC and MBC in soil aggregates is affected by organic treatments (Dhaliwal et al. 2020) such as straw mulching (Fig. S3). Therefore, higher carbon availability (Chen et al. 2015) caused by straw mulching was the most important reason for the difference found in the myxobacterial community. However, the increase in TOC had no significant effect on the myxobacterial α-diversity index or on relative abundance. Numerous studies have also shown that other factors, such as pH and nutrients (Zhou et al. 2014; Bahram et al. 2018; Li et al. 2019; Wang et al. 2020), might be more important to microbial diversity and abundance than soil organic carbon.

Unculturable microorganisms might play a vital role in soil ecosystems (Cotton 2000); however, based on current culture research methods, approximately 4,000 16S rRNA sequences called "uncultured *myxobacteria*" (Tian et al. 2009) were retained in the NCBI database. This has led to neglect of the importance of the large numbers of these groups in the soil food web, allowing us to only surmise the true extent of uncultured *myxobacteria*. In this study, the known groups affected by straw mulching application mainly included *Haliangiaceae*, *Polyangiaceae*, and *Archangiaceae*. These *myxobacteria* contain a large number of genes that form adhesives, proteases, and specific metabolic proteins (Gerth et al. 2003) that can be used to adsorb, process, and digest assistant bacteria (Pasternak et al. 2013; Berleman et al. 2014).

Myxobacteria can affect the soil bacterial community. In this study, the complex changes in soil properties observed were mainly due to the application of straw mulching and fertilization in the experimental field. These changes could explain the change in the soil microbial community (Zhou et al. 2014). As micropredators, bacteriolytic *myxobacteria* are predatory consumers that feed on various soil microbes (Remis et al. 2014) and are active in soil microbial food webs (Zhou et al. 2014; Thakur et al. 2018). Therefore, previous studies have suggested that the number and cell density of *myxobacteria* are important factors in restricting the abundance of the soil bacterial community and functional genes (Wang et al. 2020). However, the relationship between the myxobacterial community structure and bacterial community structure is still unknown. This study directly shows the myxobacterial community structure to be the most important factor that directly restricts the bacterial community structure. This may be related to the various feeding strategies of *myxobacteria*. To date, two distinct patterns of predation have been identified, namely, frontal and wolf-pack attacks (Pérez et al. 2014), but why and how they adopt different predation strategies is still unknown. Different myxobacterial community structures inevitably lead to different feeding strategies that affect the soil bacterial community structure. In addition, the contribution of the myxobacterial community structure to the change in the bacterial community in the < 1 mm soil aggregate fractions was greater than that in other soil aggregates. In such soil microaggregates, myxobacterial community structure could affect the α- and β-diversity of the bacterial community in soils. Previous studies have suggested that soil microhabitats have the highest levels of microbial diversity because of the lower carbon and nitrogen levels and lower C:N ratios of microaggregates, reflecting more microbially processed SOM (Hofmockel et al. 2011; Bach et al. 2018). The higher SOM availability of microaggregates could induce changes in the diversity of the soil bacterial community (Liu et al. 2014; Zhou et al. 2015). Furthermore, microaggregates have complex organics such as phenols, and alkyl groups exert a strong selective force for microorganisms (Davinic et al. 2012). In this study, TOC was also found to be an important influencing factor, but myxobacterial community structure made a strong contribution to the change in soil microbial diversity in different soil aggregates. There could be many reasons for these results, including factors related to mineral components, the diffusion of soil gas and water through soil aggregate fractionation (Kremen et al. 2005; Naveed et al. 2014), the superficial area of microaggregates, and the interactions between microorganisms and organic matter (Ruamps et al. 2013; Rabbi et al. 2016).

Conclusion

In conclusion, our report is the first to describe the effect of straw mulching in the field on *myxobacterial micropredators*. The effect of straw mulch application on *myxobacteria* was mainly reflected in its community structure and specific group but indicated no significant effect on the α-diversity index, regardless of which straw crop species was returned to the soil. The increased organic carbon content resulting from straw mulch application proved to be a key factor influencing the myxobacterial community structure. In addition, RDA and RF analysis results show myxobacterial community structure to be the most important potential driver of soil microbial diversity, especially in soil microaggregates. *Myxobacteria* are considered micropredators in microbial communities in soil and play a key role in the turnover of

carbon in soil ecosystems. *Myxobacteria* can promote the carbon decomposition of straw, and organic carbon can in turn affect the community structure of *myxobacteria*, which complement each other. This study provides partial positive support for the sustainable development method of straw reintroduction to fields.

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Author contribution QYS and YL conceived and designed research. ZJW, HC, and JXR conducted experiments. YL contributed new reagents or analytical tools. ZJW wrote the manuscript. All authors read and approved the manuscript.

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Data availability The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

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