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Changes in Foxtail Millet (*Setaria italica* L.) Yield, Quality, and Soil Microbiome after Replacing Chemical Nitrogen Fertilizers with Organic Fertilizers

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Abstract: Foxtail millet (*Setaria italica* L.) is one of the most economically valuable drought-resistant crops in arid and semi-arid regions as a nutrition health crop, which has garnered considerable research attention. We evaluated the effects of replacing chemical nitrogen fertilizers with organic fertilizers on two primary plant accessions of foxtail millet (Dungu and Jinfen no. 2). Nitrogen in a standard fertilizer was replaced with organic fertilizer at application levels of 0, 25, 50, 75, and 100%, with effects on crop yield, quality (appearance, taste, and nutritional value), and soil microbiome, assessed using field cultivation experiments. Our results indicate that partial replacement of conventional fertilizers with organic fertilizers improved both yield and quality. Specifically, the 75% replacement significantly improved the appearance (yellow pigment content and grain diameter) and taste (amylose content and soluble sugar content) of foxtail millet, while the 50% replacement significantly improved the taste (gel consistency) and nutritional qualities (crude protein content and seven amino acids' content). The 50% replacement of organic fertilizer regulated amino acid content more significantly than starch content. Increased ratios of organic fertilizer significantly reduced the soil pH by 0.03–0.36 and increased the relative abundance of *Chloroflexi* as well as that of Basidiomycota and Cercozoa in the soil microbiome. Our findings provide a solid theoretical foundation for subsequent studies on fertilizer use for foxtail millet and contribute to developing functional nutritional foods in the foxtail millet industry.

Keywords: foxtail millet; appearance quality; taste quality; nutritional quality; organic fertilizer; soil microbiome

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1. Introduction

Foxtail millet (*Setaria italica* L.), of the genus *Setaria* [1], is one of the most economically valuable drought-resistant crops in arid and semi-arid regions, owing to its short reproductive period, high adaptability, low fertilizer and water requirements, wide applicability of grain and grass, storage resistance, and high water-utilization rates [2]. In terms of production volume, foxtail millet is ranked sixth among cereal crops globally [3]. Foxtail millet is good in high-quality amino acids, resistant starch, dietary fiber, proteins, folic acid, vitamin E, carotenoids, selenium, and antioxidants [4,5], with nitric acid accounting for 65% of total fatty acids, and it may help prevent diabetes [6]. Therefore, foxtail millet has received considerable research and investment attention.

Dietary health is of global concern in the 21st century. Owing to its high nutritional value, there is an increasing demand for high-quality foxtail millet [7]. The quality of foxtail millet directly affects sales volume and pricing [8]; therefore, the methods used for breeding and cultivating high-quality foxtail millet accessions are essential. The yield and quality of foxtail millet are affected by genotype, cultivation measures, and/or the growth environment [9].

Fertilizer application is important to regulate foxtail millet yield and quality. Nitrogen, specifically, is the most basic and important nutrient for crop growth, as well as amino acid and protein synthesis [10]. Although synthetic nitrogen fertilizers are widely used in crop cultivation, overuse thereof leads to a deterioration in soil characteristics, decreases in crop quality, and wastage of fertilizers, in addition to causing serious environmental issues, such as greenhouse gas (N_2O) emissions and eutrophication [11,12]. The appropriate use of organic fertilizers as alternatives to chemical nitrogen fertilizers can reduce both greenhouse gas emissions and chemical nitrogen application rates in crop production systems [13]. The use of organic fertilizers has been reported to improve yield in tomatoes [14], while the 70% replacement of synthetic nitrogen with organic manure has led to improved wheat and maize yields and enhanced soil fertility [15].

Accession, cultivation site, and crop management are important factors affecting the growth and quality of millet. A study by Liu et al. concluded that 20% of inorganic N was substituted by commercial organic fertilizer compared with local conventional chemical fertilizer application increased yield by 3.05% [11]. Moreover, Oliveira et al. [16] reported that the content of soluble solids and vitamin C increased by 57 and 55%, respectively, with the application of an organic fertilizer. However, according to a seven-year study conducted by Das et al., the favorable effects of organic fertilizers on yield may vary with crop type [17]. Specifically, the majority of quality indicators in tomatoes and carrots improved under organic cultivation conditions compared with those under inorganic conditions, whereas the yields of potato and French beans were higher under inorganic fertilizer treatment than under organic fertilizer treatment [17].

The use of organic fertilizers can decrease nitrous oxide, carbon dioxide, and other greenhouse gas emissions, thereby reducing global warming [15]. Long-term organic fertilizer application can also improve soil fertility by considerably altering the physicochemical and biological characteristics of soil [18]. Therefore, switching to organic fertilizers can alter crop yield and quality, depending on crop type and nitrogen fertilizer replacement ratio; however, only a few studies have comprehensively evaluated these effects. Previously, grain yield under organic production conditions has been found to be 26.00% lower than that under chemical fertilization conditions, possibly owing to insufficient nitrogen supply and difficulties in phosphorous management at peak growth [19]. Specifically, the yield achieved with organic fertilizers was 19.00–21.00% lower than that with mineral fertilizers, and the protein content was 22.31% lower under organic fertilization conditions [20]. A long-term organic fertilizer-substitution study showed that partial organic fertilizer substitution reduced N_2O emission by 31.18–46.18% in the first year, with no significant difference observed in the following three years, but an increase of 44.02–53.72% in the fourth year [21]. To date, the effects of organic fertilizers on crop quality have primarily been studied in rice, with a few studies focusing on wheat [13,15,21]. Relevant studies on foxtail millet are scarce.

In this study, we selected two plant accessions with different crude protein contents to investigate the effects of organic fertilizers on both foxtail millet quality and the soil microbiome, and to identify the best application ratio. Thus, our study provides a theoretical basis for efficient fertilizer use to enable the cultivation of high-quality foxtail millet, which supports the development of functional nutritional foods in the foxtail millet industry. We hypothesize that (i) different organic fertilizer-replacement ratios could affect the yield and quality of foxtail millet as well as affect the soil microbiome, and (ii) these effects would vary among different foxtail millet varieties.

2. Materials and Methods

2.1. Growth Conditions and Plant Materials

In the summer of 2018, during the foxtail millet growth season, organic fertilizer-substitution field tests were performed at the Dongshijing (XT; N35′38″, E117′35″) and Zhoulin experimental stations (DP; N36′00″, E116′30″). These sites have a warm temperate continental monsoon climate. All accessions were planted in randomized block designs with three replicates; each plot was 40 m^2 (5.0 m × 8.0 m), with a 1.0 m buffer zone separating the plots. In XT, the soil was identified as cinnamon soil (Alfisol), with good irrigation and drainage conditions, plain topography features, and an average annual temperature of 13.6 °C, average annual precipitation of 730.2 mm, and average frost-free period of 198.0 d. At this site, foxtail millet was sown on 21 June 2018 and harvested on 20 September 2018. The basic characteristics of the 0–20-cm soil layer were as follows: soil organic carbon, 9.30 g kg^{-1}; total nitrogen, 0.81 g kg^{-1}; available phosphorous (Olsen-P), 21.8 mg kg^{-1}; available potassium, 66.1 mg kg^{-1}; and soil pH (H_2O), 6.74. At the DP experimental station, the soil was identified as brown soil, with poor irrigation and drainage conditions, mountainous topography features, and an average annual temperature of 13 °C, average annual precipitation of 697 mm, and average frost-free period of 190.0 d. The basic characteristics of the 0–20 cm soil layer were as follows: soil organic carbon, 9.96 g kg^{-1}; total nitrogen, 0.85 g kg^{-1}; available phosphorous (Olsen-P), 9.57 mg kg^{-1}; available potassium, 173.03 mg kg^{-1}; and soil pH (H_2O), 7.03. At this site, foxtail millet was sown on 22 June 2018 and harvested on 25 September 2018.

The crop yield, grain quality, and soil microbiome of the foxtail millet crop partially treated with organic fertilizers were compared with those obtained using only chemical fertilizers. Two foxtail varieties were planted, namely Dungu (DG) with a low protein content and Jinfen no. 2 (JF) with a high protein content (Figure 1). Five treatments were applied as follows: 100% chemical nitrogen fertilizer (CK), 25% organic nitrogen fertilizer + 75% chemical nitrogen fertilizer (OC1), 50% organic nitrogen fertilizer + 50% chemical nitrogen fertilizer (OC2), 75% organic nitrogen fertilizer + 25% chemical nitrogen fertilizer (OC3), and 100% organic nitrogen fertilizer (OM). Under all treatments, 225 kg N ha^{-1}, 90 kg P_2O_5 ha^{-1}, and 150 kg K_2O ha^{-1} were applied. Urea (46% nitrogen), diammonium phosphate (18% nitrogen, 42% phosphorus pentoxide), and potassium sulfate (50% potassium oxide) were used as the chemical fertilizers, while a commercial straw pelletized organic fertilizer (2.0% nitrogen, 1.0% phosphorus pentoxide, 1.5% potassium oxide) was used. The fertilizers were applied evenly to the soil, followed by rotary tillage. After foxtail millet sowing, the special herbicide for foxtail millet was uniformly sprayed, and bird nets were built during the heading stage to prevent birds from eating, and no pests were observed during the foxtail millet growth.

2.2. Measurements

At maturity, plant height (PH), leaf length (LL), and spike length (SL) of foxtail millet were measured using a ruler, and leaf width (LW), stem diameter (SD), and spike width (SW) were measured using Vernier calipers. Once mature, the crops in all plots were harvested by artificial picking. The samples were collected in an S-shaped manner at a depth of 0–20 cm (plough layer soil). One square meter of foxtail millets was randomly selected from each plot and threshed and weighed to calculate grain yield. The grain was then dried, threshed, and shelled using a rice dehuller (JLGJ4.5; Taizhou Food Instrument Factory, Taizhou, China). Thousand-kernel weight (TKW), grain diameter (GD), yellow pigment content (YPC), amylose content (AC), gel consistency (GC), crude protein content (CPC), and essential amino acid content were determined according to the methods described by Sun et al. [8]. Grain diameter and round degrees were measured using a seed appearance analysis system (SC-E, WSeen, Guangzhou, China) and essential amino acid content was measured using an automatic amino acid analyzer (L-8900; Hitachi, Tokyo, Japan).

Figure 1. Photographs of the Dongshijing (XT) and Zhoulin (DP) experimental stations.

Soluble sugar content (SSC) was measured according to the method described by Liu et al. [22]. The sample was placed in a scaled test tube with 5 mL of distilled water. After incubation for 30 min in a boiling water bath, the sugars were extracted twice and the extract collected in a 25 mL bottle. Thereafter, 0.5 mL of the extract was placed in a 20 mL scaled test tube with 1.5 mL of distilled water and 0.5 mL of ethyl acetate. Next, 5 mL of thick sulfuric acid was added to the sample to apply shock. The test tube was immediately placed in a boiling water bath and the temperature was maintained for 1 min before removing and allowing it to naturally cool to 25 °C. Soluble sugar content was determined at 630 nm.

2.3. DNA Extraction

The total microbial genomic DNA was extracted from 0.20 g of fresh soil using the DNeasy PowerSoil Pro Kit (QIAGEN, Inc., Venlo, The Netherlands). After extraction, the quantity and quality of DNA were assessed using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and 0.7% agarose gel electrophoresis, respectively. The DNA samples were stored at −20 °C until further analysis.

2.4. Polymerase Chain Reaction Amplification and Illumina MiSeq-Sequencing

Polymerase chain reaction (PCR) amplification of the bacterial 16S rRNA gene V3-V4 region was performed using the positive quotation 338F (5′-ACTCGGGGGAGGCAGCA-3′) and the reverse lead 806R (5′-GGACTACHVGGGTTCTAAT-3′). The positive lead ITS5F (5′-GGAAGTAAAAGTTAACAAGG-3′) and the reverse lead ITS1R (5′-GCTGCTTCTTCAT CATATGC-3′) were selected to amplify the fungal sequence. Sample-specific 7-bp barcodes were integrated into the quotation for multiple sequencing.

The reaction mixture consisted of 4 μL of FastPfu Buffer (5×), 2 μL of 2.5 mM dNTPs, 0.8 μL each of the 5 μM forward primer and reverse primer, 0.4 μL of FastPfu polymerase, 0.2 μL of bovine serum albumin, and 10 ng of the template DNA; the volume was made up to 20 μL with ddH$_2$O. The bacterial PCR conditions were as follows: 95 °C for 3 min, 27 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, and final extension for 10 min at 72 °C. The reaction mixture comprised the following: 2 μL of buffer (10×), 2 μL of

2.5 mM dNTPs, 0.8 μL of forward primer (5 μM), 0.8 μL of reverse primer (5 μM), 0.2 μL of Taq polymerase, 0.2 μL of bovine serum albumin, and 10 ng of the template DNA; the volume was made up to 20 μL with ddH_2O. The fungal PCR conditions were as follows: 95 °C for 3 min, 35 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, and final extension for 10 min at 72 °C. The PCR products were purified using the AxyPrep DNA Gel Extraction Kit; the products were quantified and eventually sequenced using the Illumina MiSeq platform.

2.5. Statistical Analysis

Statistical analyses were performed using SPSS (version 21.0, IBM, Armonk, NY, USA) and data were plotted using Origin 2021b (OriginLab Corporation, Northampton, MA, USA). Significant differences in the use of organic fertilizers for different crop varieties in the experimental sites ($p < 0.05$) were examined using a one-factor analysis of variance (ANOVA) in conjunction with Duncan's multiple range test. A three-way ANOVA was used when analyzing both sites and accessions. Residuals were checked for normality and homogeneity using Shapiro–Wilk and Levene's tests, respectively.

3. Results

3.1. Crop Yields and Agronomic Traits

Crop yields and agronomic traits were found to be affected by the level of organic fertilizer application, accession, cultivation site, and the interaction between these factors (Table S1). Yield was significantly influenced by accession, cultivation site, organic fertilizer-application level, and the interaction between accession and cultivation site ($p < 0.01$). Furthermore, our results indicated that pH was significantly influenced by accession ($p < 0.01$), cultivation site, and organic fertilizer-application level ($p < 0.05$), while SD was significantly influenced by accession ($p < 0.05$), cultivation site, organic fertilizer-application level, and the interaction between these factors ($p < 0.01$). Leaf length was significantly influenced by cultivation site and the interaction between accession and organic fertilizer-application level ($p < 0.05$), while LW, SL, and SW were significantly influenced by organic fertilizer-application level ($p < 0.01$). The effect of organic fertilizer substitution on foxtail millet yield was primarily related to variety and substitution ratio (Figure 2). Compared with the 100% chemical fertilizer treatment (CK), the yield of variety DG under the 50% organic 50% chemical fertilizer treatment (OC2) increased significantly by 15.58%, while the yield of variety JF at site DP also increased significantly by 14.39%. However, the effect on the yield of variety JF at site XT was not significant. The 100% organic fertilizer (OM) treatment significantly reduced the yield of variety DG at site XT (decreased by 20.74%). The effect of organic fertilizer substitution on the agronomic traits of foxtail millet was primarily reflected in the leaf size and PH. The difference in the effect on SL and SW was not significant (Table 1). Compared with CK, the OC2 treatment significantly increased the pH at site JF (10.31%), with a corresponding increase of 25.30% in SW. However, under the OM treatment, the growth of variety JF at site XT was inhibited and compared with OC2, the pH, SD, and LW decreased by 13.06, 23.13, and 28.28%, respectively.

3.2. Appearance

Foxtail millet quality was found to be affected by the level of organic fertilizer application, accession, cultivation site, and the interactions among these factors (Table S1). Specifically, YPC was significantly influenced by accession ($p < 0.01$), cultivation site ($p < 0.05$), and organic fertilizer-application level ($p < 0.01$). Yellow pigment content was generally higher at DP than at XT (3.69–11.70% for the DG variety and 2.54–5.26% for the JF variety). Under conventional fertilizer application, the YPC of DG was 17.44 and 18.81 mg kg^{-1} at DP and XT, respectively. Compared with the CK treatment, the OM treatment significantly improved the YPC at DG (9.92–14.34%), with no significant differences under the remaining organic fertilizer-replacement treatments. The OC3 and OM treatments significantly improved the YPC at both DG and JF (23.71% and 23.50% at DG and 20.99% and 16.94% at JF, respectively). Grain diameter was significantly influenced by accession ($p < 0.05$) and cultivation site ($p < 0.01$). Organic fertilizer application increased the GD at DG by 1.39–5.16% and 1.41–4.02% in two cultivation sites, respectively, compared with conventional fertilization. The highest GD was recorded under treatments OC2 and OC3 at DP and XT, respectively (Figure 3). The GD at JF did not change significantly with an increase in the amount of organic fertilizer. Thousand-kernel weight was significantly influenced by accession ($p < 0.05$) and organic fertilizer-application level ($p < 0.01$). Furthermore, compared with CK, TKW was relatively stable, while at DP, the TKW of variety DG under treatment OC2 increased by 11.54%, with no significant change observed under the remaining treatments.

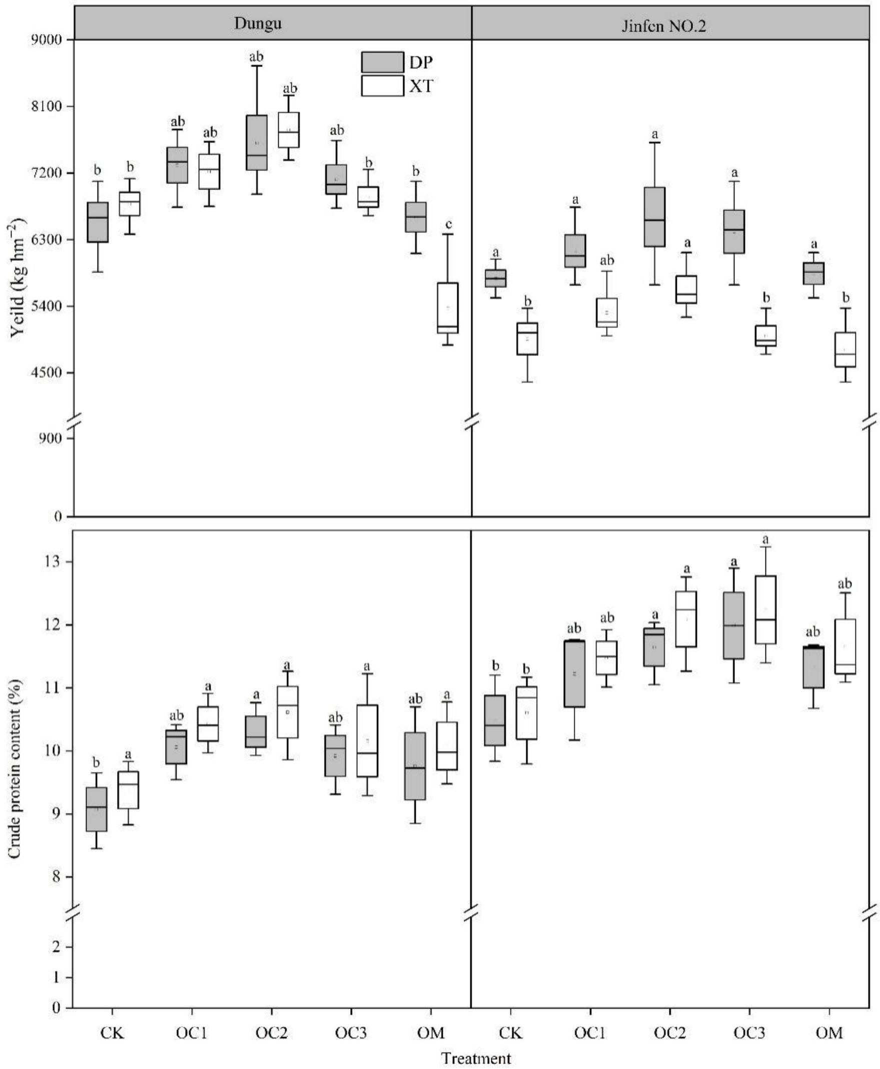

Figure 2. Yield and crude protein content in foxtail millet under different treatments including 100% chemical nitrogen fertilizer (CK), 25% organic nitrogen fertilizer + 75% chemical nitrogen fertilizer (OC1), 50% organic nitrogen fertilizer + 50% chemical nitrogen fertilizer (OC2), 75% organic nitrogen fertilizer + 25% chemical nitrogen fertilizer (OC3), and 100% organic nitrogen fertilizer (OM) at Dongshijing (XT) and Zhoulin (DP) experimental stations. Means with the same lowercase letter indicate no significant difference ($p < 0.05$).

Table 1. Agronomic traits of foxtail millet under different treatments including 100% chemical nitrogen fertilizer (CK), 25% organic nitrogen fertilizer + 75% chemical nitrogen fertilizer (OC1), 50% organic nitrogen fertilizer + 50% chemical nitrogen fertilizer (OC2), 75% organic nitrogen fertilizer + 25% chemical nitrogen fertilizer (OC3), and 100% organic nitrogen fertilizer (OM) for Dungu (DG) and Jinfen no. 2 (JF) foxtail millet varieties. Plant height (PH), leaf length (LL), and spike length (SL), leaf width (LW), stem diameter (SD), and spike width (SW). Data are presented as the mean ± standard deviation. Means with the same lowercase letter indicate that there is no significant difference ($p < 0.05$).

		DG					JF				
		CK	**OC1**	**OC2**	**OC3**	**OM**	**CK**	**OC1**	**OC2**	**OC3**	**OM**
DP	PH (cm)	88.69 ± 9.08a	98.56 ± 4.55a	104.00 ± 3.95a	102.81 ± 4.16a	102.50 ± 10.29a	121.88 ± 6.06b	134.5 ± 1.96a	135.81 ± 2.49a	129.13 ± 1.32a	130.31 ± 11.02a
	SD (mm)	6.32 ± 0.78a	6.86 ± 0.9a	7.23 ± 0.38a	7.53 ± 0.9a	6.30 ± 0.59a	5.19 ± 0.65b	6.96 ± 0.10a	6.55 ± 0.36a	5.41 ± 0.25ab	6.04 ± 0.79ab
	LL (cm)	34.44 ± 3.68a	39.88 ± 3.35a	41.31 ± 1.63a	36.44 ± 1.99a	36.5 ± 2.81a	31.13 ± 2.93b	40.25 ± 2.32a	36.56 ± 0.45a	34.19 ± 3.05ab	34.19 ± 1.89ab
	LW (mm)	17.74 ± 1.61a	21.86 ± 2.3a	20.22 ± 1.70a	19.80 ± 0.78a	18.80 ± 0.80a	19.53 ± 1.29a	20.52 ± 1.12a	20.66 ± 0.92a	18.38 ± 1.48a	19.39 ± 1.24a
	SL (cm)	13.15 ± 0.87a	14.60 ± 0.36a	15.00 ± 1.04a	15.45 ± 0.90a	13.65 ± 1.2a	13.75 ± 0.28a	12.7 ± 0.49a	14.35 ± 0.78a	13.35 ± 0.90a	13.40 ± 1.29a
	SW (mm)	18.33 ± 0.54a	18.33 ± 0.91a	20.52 ± 0.68a	19.25 ± 1.20a	17.43 ± 1.38a	19.13 ± 0.91a	19.01 ± 0.50a	20.41 ± 0.67a	19.36 ± 0.80a	20.32 ± 2.02a
XT	PH (cm)	98.50 ± 4.31ab	104.25 ± 1.83ab	108.13 ± 3.88a	95.38 ± 3.48b	105.50 ± 1.7ab	133.25 ± 3.80bc	138 ± 3.86ab	145.50 ± 3.65a	128.00 ± 2.79bc	126.5 ± 3.39c
	SD (mm)	8.41 ± 0.71ab	8.40 ± 0.65ab	9.30 ± 0.65a	7.50 ± 0.53ab	6.69 ± 0.43b	7.09 ± 0.20b	8.63 ± 0.14a	8.82 ± 0.70a	8.07 ± 0.54ab	6.78 ± 0.13b
	LL (cm)	36.00 ± 1.17ab	35.25 ± 1.05ab	38.13 ± 1.09a	33.25 ± 0.97b	33.38 ± 1.31b	34.50 ± 0.54b	40.63 ± 0.75a	37.38 ± 1.01ab	38.13 ± 0.77ab	35.38 ± 2.07b
	LW (mm)	1.84 ± 0.08a	2.16 ± 0.13a	2.03 ± 0.13a	1.86 ± 0.06a	1.54 ± 0.07b	1.88 ± 0.07c	2.32 ± 0.15ab	2.44 ± 0.09a	2.04 ± 0.13bc	1.75 ± 0.06c
	SL (cm)	19.6 ± 0.94a	18.07 ± 1.38a	20.33 ± 1.03a	19.60 ± 0.11a	20.00 ± 0.80a	18.53 ± 1.61a	20.73 ± 1.56a	19.73 ± 0.29a	18.20 ± 1.38a	17.07 ± 1.04a
	SW (mm)	23.42 ± 0.76a	22.29 ± 1.85a	23.25 ± 1.52a	21.13 ± 0.90a	23.35 ± 2.25a	21.81 ± 1.34a	22.39 ± 0.46a	22.7 ± 1.17a	21.57 ± 0.54a	21.44 ± 0.79a

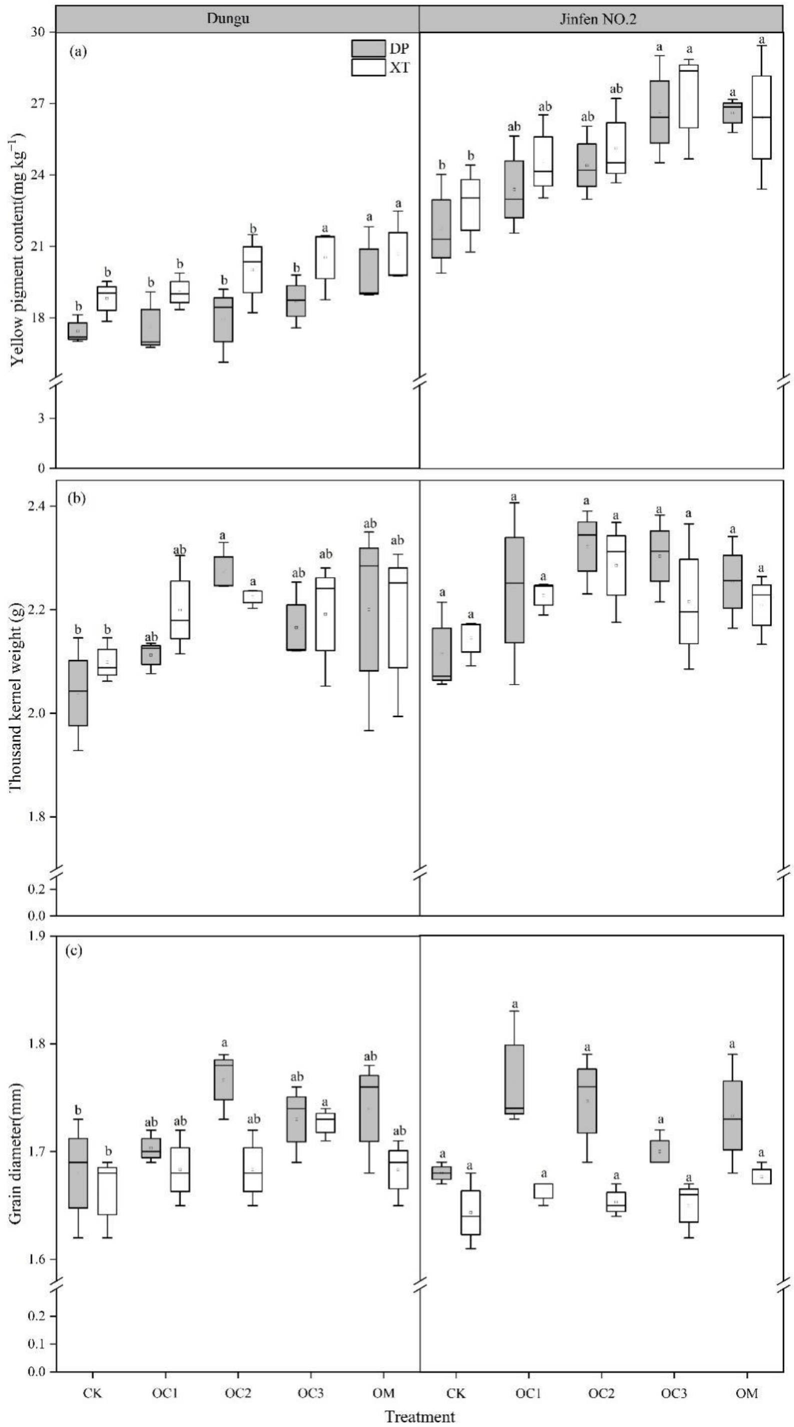

Figure 3. Variation in appearance of foxtail millet under different treatments including (**a**) yellow pigment content (YPC), (**b**) 1000-kernel weight (TKW), and (**c**) grain diameter (GD). Treatments include 100% chemical nitrogen fertilizer (CK), 25% organic nitrogen fertilizer + 75% chemical nitrogen fertilizer (OC1), 50% organic nitrogen fertilizer + 50% chemical nitrogen fertilizer (OC2), 75% organic nitrogen fertilizer + 25% chemical nitrogen fertilizer (OC3), and 100% organic nitrogen fertilizer (OM) at Dongshijing (XT) and Zhoulin (DP) experimental stations. Means containing the same lowercase letter indicate no significant difference ($p < 0.05$).

3.3. Eating Quality

Amylose content was significantly influenced by accession ($p < 0.01$), cultivation site ($p < 0.01$), and their interactions ($p < 0.01$), as well as organic fertilizer-application level

($p < 0.01$; Table S1). Compared with CK, treatments OC3 and OM significantly increased the AC of the DG variety (5.00–7.17% and 6.19–6.89%, respectively; Figure 4), while OC3 also significantly increased the AC of the JF variety (7.03–10.42%). Gel consistency and SSC were significantly influenced by the level of organic fertilizer application ($p < 0.01$). Planting site had no significant effect on GC and SSC. Compared with CK, the OC2 treatment significantly improved the GC of both DG and JF varieties (11.15–11.82% and 3.91–8.92%, respectively), while OC3 also significantly improved the SSC of the DG variety (9.64–10.15%). In addition, OC3 and OM significantly improved the SSC of the JF variety (10.14–10.97% and 10.53–11.20%, respectively).

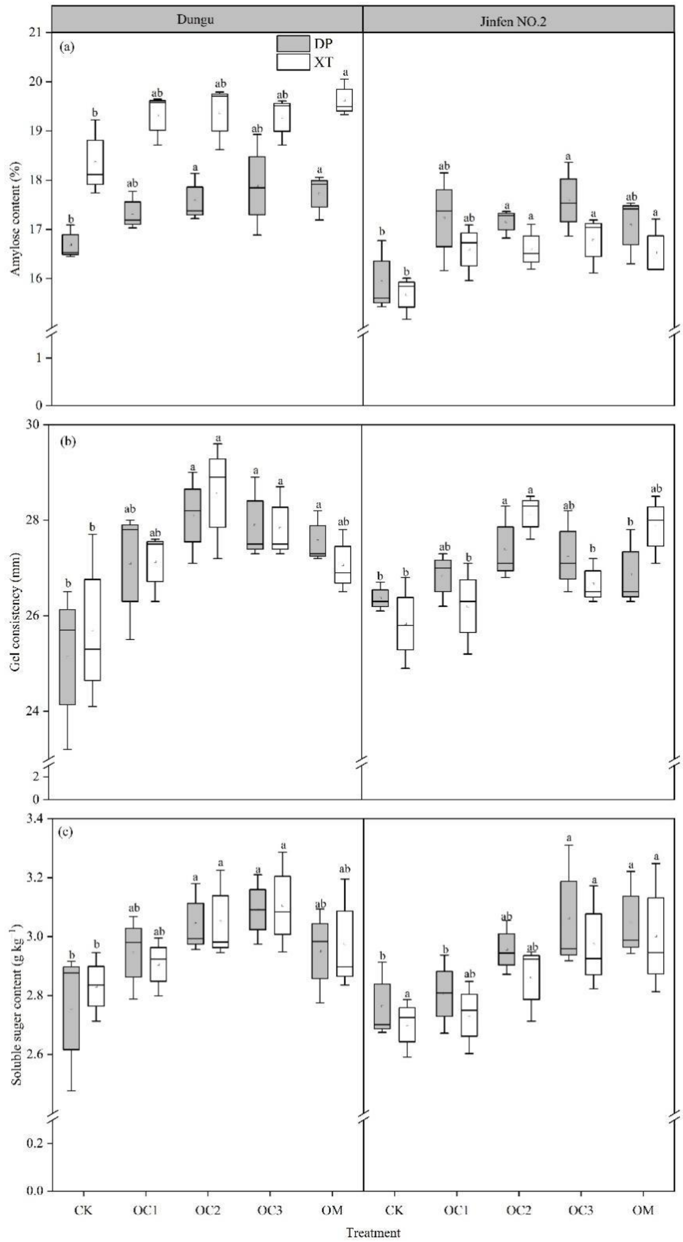

Figure 4. Eating quality of foxtail millet under different treatments including (**a**) amylose content, (**b**) gel consistency, and (**c**) soluble sugar content. Treatments include 100% chemical nitrogen fertilizer (CK), 25% organic nitrogen fertilizer + 75% chemical nitrogen fertilizer (OC1), 50% organic nitrogen fertilizer + 50% chemical nitrogen fertilizer (OC2), 75% organic nitrogen fertilizer + 25% chemical nitrogen fertilizer (OC3), and 100% organic nitrogen fertilizer (OM) at Dongshijing (XT) and Zhoulin (DP) experimental stations. Means containing the same lowercase letter indicate no significant difference ($p < 0.05$).

3.4. Nutritional Quality

Crude protein content was significantly influenced by accession ($p < 0.1$) and the level of organic fertilizer application ($p < 0.01$; Table 1). Amino acids were significantly influenced by accession and cultivation sites ($p < 0.05$). The contents of Thr and Val were significantly influenced by the three-way interaction between accession, level of organic fertilizer application, and cultivation site ($p < 0.05$). Under CK, a CPC of 9.22 and 10.54% was recorded for DG and JF, respectively (Figure 2). Compared with CK, the OC2 treatment significantly improved the CPC of variety DG at DP (13.60%), whereas OC2 and OC3 significantly improved the CPC of variety JF (11.12–14.06% and 14.39–15.45%, respectively). Similarly, organic fertilizer use significantly increased the amino acid content in grains (Table 2). The Thr, Val, Ile, Leu, Phe, Lys, and Pro contents of DG increased by 5.46–28.82%, 1.96–68.57%, 2.60–36.36%, 4.56–51.81%, 3.28–38.65%, 1.64–36.49%, and 2.18–33.76%, respectively, under the various organic fertilizer-replacement treatments. The Thr, Val, Ile, Leu, Phe, Lys, and Pro content of JF increased by 4.79–37.16%, 17.31–46.15%, 1.20–27.23%, 9.05–34.52%, 5.35–40.99%, 8.00–30.29%, and 3.44–38.30%, respectively, under the various organic fertilizer-replacement treatments.

3.5. Comprehensive Evaluation of Foxtail Millet Quality

Our results indicate that PH, LL, SL, AC, and YPC were positively correlated with organic fertilizer use in foxtail millet production, regardless of cultivation site and accession (Figure 5). In addition, GC was positively correlated with accession and cultivation site and negatively correlated with organic fertilizer application. However, Lys, Val, Phe, Thr, Ile, TKW, GD, and SSC were negatively correlated with organic fertilizer use, cultivation site, and accession. The clustering analysis between treatments showed the CK and OC1 treatments tended to be clustered in the same class under fertilizer treatments, whereas OC3 and OM treatments were clustered in the same category. This indicates that the effect of low levels of organic fertilizer substitution on foxtail millet quality was not significant and, furthermore, that the effect was only significant at an organic fertilizer-replacement ratio of >50%.

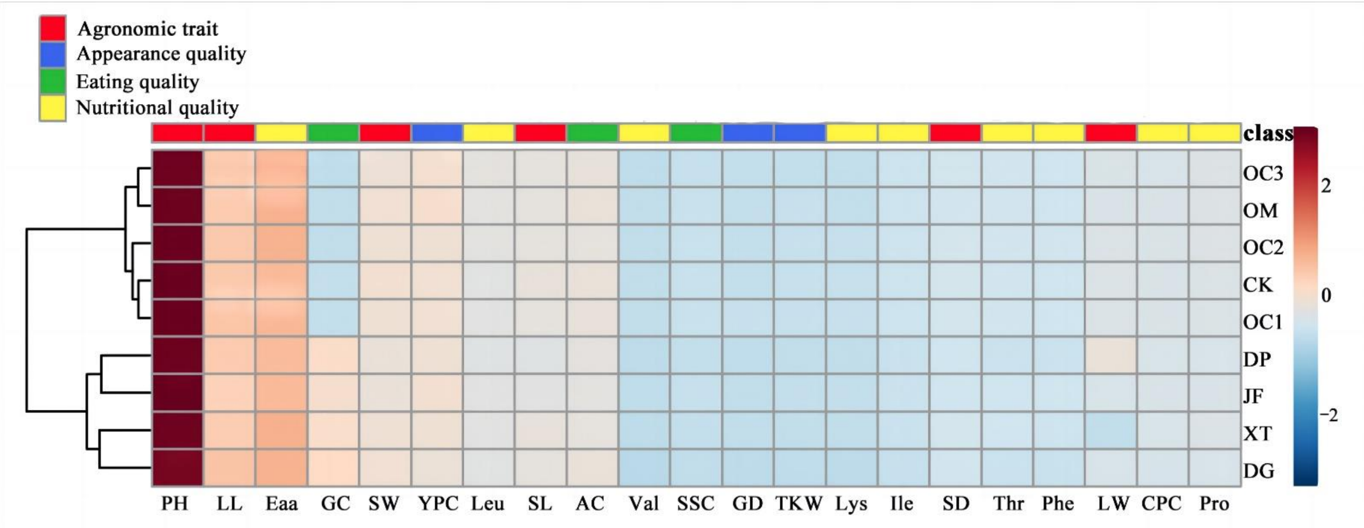

Figure 5. Heatmap of Pearson's correlation coefficients between foxtail millet quality traits including plant height (PH), leaf length (LL), spike length (SL), leaf width (LW), stem diameter (SD), spike width (SW), yellow pigment content (YPC), amylose content (AC), gel consistency (GC), crude protein content (CPC), soluble sugar content (SSC), grain diameter (GD), and thousand-kernel weight (TKW).

Table 2. Amino acid content of foxtail millet under different treatments including 100% chemical nitrogen fertilizer (CK), 25% organic nitrogen fertilizer + 75% chemical nitrogen fertilizer (OC1), 50% organic nitrogen fertilizer + 50% chemical nitrogen fertilizer (OC2), 75% organic nitrogen fertilizer + 25% chemical nitrogen fertilizer (OC3), and 100% organic nitrogen fertilizer (OM) for Dungu (DG) and Jinfen no. 2 (JF) foxtail millet varieties. Lysine (Lys), Phenylalanine (Phe), ThreonineI (Thr), Leucine (Leu), Valine (Val), Isoleucin (Ile), and Proline (pro). Data are presented as the mean ± standard deviation. Means containing the same lowercase letter indicate that there is no significant difference ($p < 0.05$).

		DG					JF				
		CK	**OC1**	**OC2**	**OC3**	**OM**	**CK**	**OC1**	**OC2**	**OC3**	**OM**
DP	Thr	6.26 ± 0.48b	6.72 ± 0.71ab	7.17 ± 0.3a	6.87 ± 0.12ab	7.48 ± 0.33a	6.54 ± 0.62a	6.3 ± 0.37a	7.06 ± 0.78a	6.49 ± 0.19a	6.63 ± 0.33a
	Val	0.51 ± 0.15a	0.52 ± 0.23a	0.7 ± 0.06a	0.65 ± 0.03a	0.65 ± 0.04a	0.56 ± 0.07a	0.58 ± 0.05a	0.65 ± 0.06a	0.6 ± 0.01a	0.65 ± 0.03a
	Ile	3.85 ± 1.11a	3.95 ± 1.5a	4.98 ± 0.21a	4.76 ± 0.11a	5.25 ± 0.26a	4.71 ± 0.47a	4.74 ± 0.24a	4.91 ± 0.31a	4.79 ± 0.19a	4.96 ± 0.09a
	Leu	12.93 ± 2.07a	13.52 ± 0.81a	17.23 ± 0.56a	16.41 ± 0.51a	17.95 ± 0.77a	15.55 ± 1.95a	15.89 ± 0.88a	16.71 ± 1.63a	16.12 ± 0.5a	16.28 ± 0.47a
	Phe	5.19 ± 1.55a	5.36 ± 2.12a	6.7 ± 0.46a	6.38 ± 0.07a	6.82 ± 0.45a	6.01 ± 0.78a	6.21 ± 0.39a	6.36 ± 0.33a	6.23 ± 0.34a	6.55 ± 0.3a
	Lys	1.83 ± 0.61a	1.86 ± 0.69a	2.34 ± 0.09a	2.19 ± 0.12a	2.3 ± 0.28a	1.97 ± 0.26a	2.04 ± 0.03a	2.13 ± 0.18a	1.97 ± 0.1a	1.95 ± 0.11a
	Pro	10.1 ± 2.81a	10.32 ± 4.08a	13.3 ± 0.52a	12.6 ± 0.63a	13.51 ± 0.51a	11.69 ± 1.18a	11.89 ± 0.68a	12.95 ± 1.19a	12.27 ± 0.56a	12.12 ± 0.85a
XT	Thr	4.58 ± 0.34c	5.51 ± 0.07ab	4.96 ± 0.1bc	5.9 ± 0.49a	4.83 ± 0.43c	5.22 ± 0.56b	5.47 ± 0.7b	7.16 ± 0.35a	7.02 ± 0.7a	5.98 ± 0.7ab
	Val	0.35 ± 0.04b	0.49 ± 0.04ab	0.38 ± 0.09ab	0.59 ± 0.21a	0.42 ± 0.06ab	0.52 ± 0.07b	0.66 ± 0.04ab	0.74 ± 0.08a	0.76 ± 0.11a	0.61 ± 0.09ab
	Ile	3.08 ± 0.29b	3.72 ± 0.2ab	3.62 ± 0.05ab	4.11 ± 0.93a	3.72 ± 0.39ab	4.15 ± 1.02a	4.2 ± 0.3a	5.28 ± 0.23a	5.16 ± 0.58a	4.46 ± 0.56a
	Leu	9.38 ± 0.81b	12.75 ± 0.46ab	12.11 ± 0.21ab	14.24 ± 3.75a	11.81 ± 1.38ab	13.15 ± 0.72b	14.34 ± 0.66b	17.67 ± 0.89a	17.69 ± 2.33a	15.01 ± 2.08ab
	Phe	3.7 ± 1.01b	5.13 ± 0.07a	4.64 ± 0.08ab	4.61 ± 0.5ab	4.57 ± 0.49ab	5.05 ± 0.48c	5.32 ± 0.73c	7.12 ± 0.31a	6.73 ± 0.74ab	5.89 ± 0.78bc
	Lys	1.48 ± 0.26c	2.02 ± 0.12a	1.57 ± 0.05bc	1.86 ± 0.26ab	1.66 ± 0.06bc	1.75 ± 0.22c	1.89 ± 0.09bc	2.28 ± 0.11a	2.14 ± 0.1ab	1.99 ± 0.19bc
	Pro	8.99 ± 1.62a	10.83 ± 0.37a	9.51 ± 0.21a	9.94 ± 1.37a	9.31 ± 0.99a	10.47 ± 1.07c	10.83 ± 1.22c	14.48 ± 0.55a	13.99 ± 1.69ab	11.98 ± 1.61bc

We conducted the main component analysis of 14 quality index indicators at two sites of two accessions. Three primary components were derived, with a cumulative contribution rate of 88.88% (Table S2). As the three new comprehensive indicators can replace the original 14 quality indicators to comprehensively analyze foxtail millet quality, we used PC1 as a nutritional factor (contribution rate of 57.069%), PC2 as a taste factor (contribution rate of 18.482%), and PC3 as an appearance factor (contribution rate of 13.328%). The value of the membership function was calculated according to the main component analysis of the factor coefficients of each indicator in each main component. This enabled us to calculate the comprehensive score of the different fertilizer treatments in the two sites of the two accessions (Table S3). Our results show that organic fertilizer treatments can improve grain quality and that different organic fertilizers can be used instead of nitrogen fertilizers. Specifically, the OC1 treatment showed the lowest comprehensive score, with the change in the comprehensive score of the DG variety initially increasing and then decreasing. The OC2 treatment showed the best results with respect to grain quality, and the JF variety had the highest combined scores for OC3 and OM treatments. The average comprehensive score of JF was higher than that of DG.

3.6. Soil pH and Soil Microbiome Composition

Our results indicate that organic fertilizer utilization significantly reduced soil pH (0.03–0.36; Table S4). Notably, the dominant bacterial phyla in our soil samples were *Actinobacteria* (35.02–49.99%), *Proteobacteria* (22.60–37.23%), *Chloroflexi* (7.80–14.81%), and *Acidobacteria* (6.19–11.58%) (Figure 6a). At site XT, upon application of organic fertilizer, the relative abundance of *Actinobacteria*, *Chloroflexi*, and *Acidobacteria* increased, whereas that of *Proteobacteria* decreased. At site DP, the relative abundance of *Proteobacteria*, *Chloroflexi*, and *Acidobacteria* increased with organic fertilizer use, whereas that of *Actinobacteria* decreased. In particular, the relative abundance of *Actinobacteria* increased with an increase in the organic fertilizer-replacement ratio, which was the highest under the OC3 treatment. The relative abundance of *Actinobacteria* increased by 4.59–28.71% under OC3, compared with that of the CK treatment (35.22–36.73%), and attained 44.11–47.11% at site XT. However, the relative abundance of *Actinobacteria* decreased from 49.75–49.99% (CK) to 35.31–35.36% (OC3) at site DP with organic fertilizer replacement. The relative abundance of *Chloroflexi* increased with an increase in the organic fertilizer-replacement ratio. The relative abundance of *Chloroflexi* increased by 2.64–50.84% with organic fertilizer replacement compared with that under the CK treatment.

Ascomycota (70.36–93.42%), Basidiomycota (0.92–22.45%), and Zygomycota (0.12–24.90%) were the dominant fungi observed after organic fertilizer substitution (Figure 6b). While the relative abundance of Basidiomycota, Cercozoa, and Chytridiomycota increased with the use of organic fertilizers (with the exception of the OM treatment), the relative abundance of Ascomycota decreased. Specifically, the relative abundance of Basidiomycota increased from 1.28–12.65% under CK to 7.13–15.12% under OM, while that of Ascomycota decreased from 78.01–92.30% under CK to 74.64–89.12% under OM. The relative abundance of Basidiomycota increased with an increase in the organic fertilizer-replacement ratio (by 0.12–629.66% compared with that under CK). The relative abundance of Ascomycota decreased with an increase in the organic fertilizer-replacement ratio and decreased by 10.65–42.48% compared with that under the CK treatment.

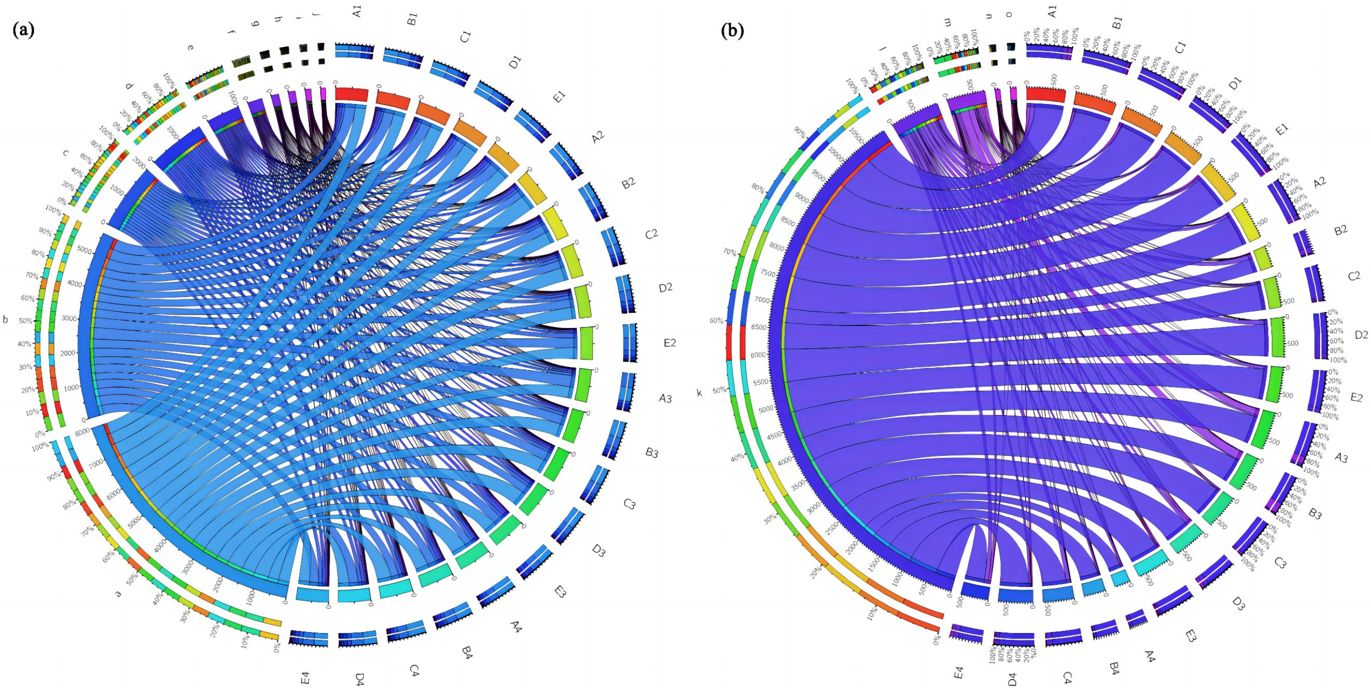

Figure 6. Composition of (**a**) prevalent bacterial phyla and (**b**) fungal phyla detected under different treatments. A = XT DG, B = XT JF, D = DP JF; 1 = CK, 2 = OC1, 3 = OC2, 4 = OC3, 5 = OM. a = *Actinobacteria*, b = *Proteobacteria*, c = *Chloroflexi*, d = *Acidobacteria*, e = *Gemmatimonadetes*, f = *Bacteroidetes*, g = Saccharibacteria, h = *Nitrospirae*, i = *Cyanobacteria*, j = *Firmicutes*, k = Ascomycota, l = Basidiomycota, m = Zygomycota, *n* = Chytridiomycota, o = Cercozoa.

4. Discussion

4.1. Effects of Organic Fertilizer on Agronomic Traits and Foxtail Millet Quality

Crops promote the growth and development of aboveground parts by absorbing beneficial substances, such as minerals and trace elements, from the soil and fertilizers. In this study, 50% organic fertilizer replacement significantly increased foxtail millet yield and improved certain agronomic traits (PH, SD, and LL). Similar results have been observed in two mint species, where significant increases in PH and essential oil production were observed under the combined application of chemical and organic fertilizers [23]. In this study, we showed that agronomic characteristics (PH, SD, LW) and millet yield were significantly reduced when chemical fertilizer was completely replaced with organic fertilizer. Hasan and Solaiman [24] also reported that the use of organic fertilizers reduced the PH of cabbage. This could potentially be caused by slow nutrient release from organic fertilizers, leading to decreased PH linked to insufficient nutrient availability.

The quality of appearance of foxtail millet was found to be strongly related to the YPC content of the grain. Yellow pigment content can be used as an important index to measure foxtail millet quality [25]. The YPC of foxtail millet is 1.91–28.54 mg kg^{-1}, significantly higher than that of wheat (1.5–2 mg kg^{-1}) and sorghum (1.8–2.3 mg kg^{-1}) [26]. In this study, the YPC of foxtail millet was higher with the application of organic fertilizer compared to that observed with the application of chemical fertilizer (16.94–23.71%). However, this difference was not significant when chemical fertilizer was replaced with low amounts of organic fertilizer. The application of organic fertilizer improved the GD compared with the application of 100% chemical fertilizer (0.61–5.16%). The GD and TKW are yield traits and our findings support those of Iqbal et al. [27], who showed that a combination of organic fertilizer and chemical fertilizer can significantly improve the yield and yield traits of rice.

Over-fertilization with artificial nitrogen adversely affects soil quality, environmental health, and crop production, which can lead to seed-growth problems [28]. In this study, with an increase in the replacement ratio of organic fertilizer to chemical nitrogen fertilizer, the SSC and AC of foxtail millet gradually increased (10.14–12.02% and 5.00–10.42%, respectively). These two indicators presented the highest values at the highest organic fertilizer-substitution ratio (>75%), with the effect of organic fertilizer on SSC being more significant than on AC. Combining organic and inorganic fertilizers facilitates the absorption of large amounts of trace nutrients by plants for various metabolic activities, such as the synthesis of chlorophyll, proteins, and carbohydrates, for normal growth and development [29]. In our study, the CPC and partial amino acid content in foxtail millet grain increased with an increase in organic fertilizer replacement at a ratio of up to 75%. Nitrogen is an important element in protein formation, with high organic fertilizer-replacement ratios indicating an insufficient supply of quick-acting nitrogen, causing foxtail millet CPC to decrease. In this study, replacing 50% of the chemical fertilizer with organic fertilizer yielded the best-quality foxtail millet; however, the effects may vary among regional varieties. This is consistent with the findings of Das et al. [17], who showed that the best-quality carrots and tomatoes were grown with a replacement of 50% of inorganic fertilizer with organic fertilizer. However, Lv et al. [15] demonstrated that the replacement of 70% of conventional fertilizer with organic fertilizer resulted in the highest yields and nitrogen-utilization rates. This may be because nitrogen from chemical fertilizers is retained in the soil in an easily available form only for a short period after entering the soil, whereas organic fertilizers provide nitrogen without any interference [30,31]. The nutrients in organic fertilizers are released slowly and steadily, whereas those in chemical fertilizers are quick acting.

In previous studies, accession, cultivation site, and crop management were shown to be important factors affecting the growth and quality of millet [32]. In this study, accession played a dominant role in the appearance and nutritional quality of foxtail millet, but the interaction between accession and cultivation site was also important for amino acid content. The amino acid content and yield of foxtail millet differed significantly between the two study sites that had different soil types and organic carbon contents. Fertilizer use is the dominant factor influencing agronomic traits and taste. Flagella [32] reported that grain yield and protein content were significantly affected by the soil clay and organic carbon content, while Keshavarz et al. [23] reported that accession significantly affected oil percentage, and fertilizer treatment significantly affected both agronomic traits and oil quality. There was no significant difference in the yield in DP between the two accessions with the increase in organic fertilizer substitution, while in XT, the yield was the highest with 50% replacement, and then the yield decreased significantly. For foxtail millet quality, the 75% replacement significantly improved the YPC, GD, AC, and SSC of foxtail millet, while the 50% replacement significantly improved the GC, CPC, and amino acids. This study showed that organic fertilizer significantly increased the amino acid content of the two accessions in XT. The results of this study are consistent with those of Tang et al. [33], indicating that organic fertilizer (biogas slurry) plays a role in balancing carbon and nitrogen metabolism, thereby increasing grain amino acid content.

4.2. Effects of Organic Fertilizers on the Soil Microbiome

Organic fertilizer replacement can affect the accumulation of organic carbon in the soil by serving as an external carbon source and promoting biological microbial nitrogen fixation [34]. In addition, organic fertilizer replacement can improve soil characteristics, such as soil structure, water-holding capacity, and microbial activity, thereby improving crop quality [12]. In this study, organic fertilizer replacement and soil type had significant effects on the soil microbiome, whereas foxtail millet accession had no significant effects on soil microorganisms. Organic fertilizer use reduced the soil pH by 0.03–0.36 units, while an increase in the replacement ratio of organic fertilizer increased the relative abundance of *Chloroflexi* and that of *Basidiomycota* and Cercozoa among bacteria and fungi, respectively. Notably, some members of *Chloroflexi* may contribute to nitrification [35]. Moreover, studies by Rao et al. [36] showed that *Chloroflexi* is involved in the biogeochemical cycles of N and can absorb a variety of organic acids from both biotic and abiotic sources in the assimilating environment. Organic fertilizer can promote microbial activity and enhance the synergy within the soil microbiome to increase plant biomass. In this study, the relative abundance of *Chloroflexi* increased under organic fertilizer treatment. Therefore, organic fertilizer substitution may affect the soil nitrogen cycle and affect the nitrogen absorption and utilization of foxtail millet, thus, promoting photosynthesis and the growth of foxtail millet, affecting the quality and increasing the content of crude protein and some amino acids. Tan et al. [37] showed that the abundance of *Basidiomycota* increased under the application of organic fertilizer (panda feces). The application of straw organic fertilizer in this experiment also increased the relative abundance of *Basidiomycota*. Wang et al. [38] indicated that the use of organic fertilizer increased the pH of red soil by approximately 0.04 units. This phenomenon may be attributed to different soil types and soil nutrients. Rao et al. [36] reported that pH is a major determinant factor of microbial community composition. In this study, the experimental sites consisted of brown and cinnamon soil types with a soil pH > 7.0, which differs from the pH of red soil (pH = 4.3). A study on the macrogenomics of soil from the acidic peat bog forests in southern Thailand reported that *Lactobacilli* have many genes that encode cellulose and hemicellulose [39], indicating that *Lactobacilli* play an important role in degrading plant residues. Furthermore, in this study, *Actinobacteria* and *Proteobacteria* were the dominant bacterial communities in the control group, with *Ascomycota* being the dominant fungal community. The relative abundance of *Ascomycota* in the organic fertilizer treatments was lower than that in the CK treatment (10.65–63.45%). Our results support those of a previous study showing that *Acidobacteria* members are more abundant in nutrient-poor soils [40]. Moreover, Li et al. [41] reported that *Ascomycota* can decompose organic matter, thus, playing an important role in nutrient cycling decomposition of organic matter can increase the availability of rhizosphere soil nutrients.

5. Conclusions

In summary, our results show that organic fertilizer replacement affects foxtail millet yield and quality. The 50% organic fertilizer replacement significantly increased the yield, taste, and nutritional qualities of foxtail millet, and it was the optimal treatment to obtain high-quality foxtail millet. Accession was observed to be a dominant factor related to qualities of appearance and nutritional content, and fertilizer use predominantly influenced agronomic traits and taste. Foxtail millet accession was the leading factor affecting crude protein, yellow pigment, and amino acid contents. With an increase in the replacement ratio of organic fertilizers, the relative abundance of *Chloroflexi* and *Acidobacteria*, as well as that of Basidiomycota and Cercozoa, increased. This study provides a theoretical basis for efficient fertilizer use to promote favorable yield factors in foxtail millet, which will, in turn, support the development of functional nutritional foods in the foxtail millet industry.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su142416412/s1, Supplementary Table S1: Combined analysis of variances for quality traits of two foxtail millet accessions in two sites. Supplementary Table S2: Principal component analysis of quality traits across different treatments. Supplementary Table S3: Comprehensive scores of foxtail millet under different treatments. Supplementary Table S4: Soil pH under different treatments.

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