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Application of Homemade Organic Fertilizer for Improving Quality of Apple Fruit, Soil Physicochemical Characteristics, and Microbial Diversity

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Abstract: Application of the organic fertilizers can improve soil properties and agricultural product quality, while the in-depth effect of organic fertilizer needs further exploration. In this study, the apple fruit (*Malus pumila* Mill.) quality, soil physicochemical characteristics, and microbial community under different fertilizers including control without fertilizer (CK), composted manure (CM), biogas slurry (BS), and chemical fertilizer (CF) were systematically investigated, and each treatment was repeated three times in completely random block plots with equal NPK content of fertilizer applied in each treatment. The CM treated apple fruit were observed with the best vertical diameter (78.72 ± 4.32 mm), transverse diameter (87.51 ± 1.3 mm), and color index (L* = 55.48 ± 5.48, b* = 18.96 ± 1.86). Meanwhile, the CM and BS treated apple fruit had higher flesh firmness than that of CK and CF treatment, implying that the organic fertilization can improve the storability and crispy taste apple fruit. Furthermore, the total sugars, essential amino acid, crude protein, total acids, and soluble solid contents of apple fruit were also significantly improved by using CM and BS fertilizer due to the resulting higher N, P, and organics content in soil. The application of organic fertilizer also dramatically enhanced the bacterial and fungi diversity, which may contribute to better soil respiration. The results obtained in this study reveals the insight effect of organic fertilizer on improvement of fruit quality and soil health and can supply technical assistance to organic cultivation of apple.

Keywords: organic fertilizer; composted manure; apple fruit quality; soil fertility; soil microbial diversity

Citation: Wang, X.; Bao, Q.; Sun, G.; Li, J. Application of Homemade Organic Fertilizer for Improving Quality of Apple Fruit, Soil Physicochemical Characteristics, and Microbial Diversity. *Agronomy* **2022**, *12*, 2055. https://doi.org/10.3390/agronomy12092055

Academic Editor: Masoud Hashemi

Received: 13 August 2022
Accepted: 25 August 2022
Published: 29 August 2022

1. Introduction

China possesses the largest apple orchard planting area of 2.04 million hectares with apple fruit production of 42.42 million tons in the world [1]. As the largest apple fruit producing region of China, Shaanxi Province produced 26.7% of total apple fruit yield in China [1]. Due to the high altitude origin of Loess Plateau and abundant solar resources, the apple fruit that originated from Shaanxi Province has been accepted for its various health benefits and fantastic texture [2,3]. However, the apple fruit production in Shaanxi Province was also confronted with a situation in which apple fruit quality regarding nutritional value and flesh firmness evidently decreased due to the constant application of chemical fertilizer [4]. In order to improve the current difficulties in apple fruit production, exploring better fertilization strategy to improve apple fruit quality and soil properties has become the focus of research.

Various fertilization methods have been studied to improve crop yield, fruit quality, and nutritional value [5]. Chemical fertilizer has been extensively used to maintain soil fertility and improve crop yields, especially in China. However, their long-term use has also led to soil deterioration because the chemical fertilizer builds up inside the soil and eventually exceeds what the soil can actually handle. The deterioration of soil environment may adversely affect soil fertility, soil microbial diversity, and then the nutrient quality of the agricultural product [6,7]. In order to avoid further deterioration of the soil environment, the animal manure derived fertilizers such as composted manure and biogas slurry have been widely used in farmland instead of chemical fertilizer. The composted manure is produced by aerobic microorganisms under artificial controlled conditions [8], and the application of it as part of the green agriculture concept has been widely recognized because of its potential benefits [9,10]. As a byproduct of anaerobic fermentation, biogas slurry contains large quantities of trace elements and amino acids which can increase crop yield and improve soil physicochemical characteristics, which have been broadly utilized as pesticides and organic fertilizer additives [11].

The intensive studies by researchers have reported that using organic fertilizer instead of chemical fertilizer can improve soil microbial community and physicochemical characteristics, and enrich the plant production quality. For example, Safaei Khorram et al. concluded that organic fertilizer could improve soil nutrient content and reduce soil bulk density [12]. Li et al. discovered that organic fertilizer could improve apple fruit output in the Loess Plateau area and increase soil organic carbon [13]. Chen et al. demonstrated that addition of pig manure compost could improve bacterial richness on rice yield [14]. Milošević et al. indicated that fertilization could significantly increase apple fruit weight and firmness, but none of the fertilizer treatments showed significant difference on fruit size and size ratio [2]. Leff et al. discovered that excessive application of nitrogen fertilizer would acidify soil, thus reducing soil pH value [15]. Different fertilization conditions and crop types had different effects on the structure, quantity, and activity of soil microbial communities. In turn, different soil microbial communities have different effects on soil physical and chemical properties as well as crop growth, yield, and quality [16]. However, it was found that there are many studies on the effects of fertilizer on crop quality, soil physicochemical properties, and soil microbial diversity, but effects of different fertilizers on the combination of apple fruit quality, soil physicochemical properties, and soil microbial community in apple orchards were rarely reported. For example, Wang et al. illustrated that application of bioorganic fertilizer could increase apple fruit yield and soil organic matter content, and changed the relative abundance of dominant bacteria, but there was no further study on apple fruit quality [17]. Similarly, Milošević & Milošević only studied the effect of fertilizer on apple fruit quality, but did not elaborate on soil fertility and soil microbial community [3]. Furthermore, to the best of our knowledge, there have been many studies investigating the influence of organic fertilizer on apple fruit quality, but very few studies were done to elucidate the effect of organic fertilizer on soil microbial community and the co-relationship between soil condition and fruit quality.

In order to alter the situation of apple orchard regarding the soil degradation and decreased apple fruit quality, the insight study of the organic fertilizer on soil microbial community might be the breakthrough point. Therefore, this study aimed to systematically investigate the influence of different fertilizer treatments on apple fruit quality, soil fertility, and soil microbial community, in order to provide theoretical reference for nutrient management and sustainable development of apple orchards.

2. Materials and Methods

2.1. Experimental Conditions

The experimental site is located in Yanchuan County, Shanxi Province, China (36°52′ N latitude and 110°42′ E longitude). This region has a northern warm temperate zone continental semi-arid monsoon climate, with an average annual temperature of 10.4 °C, average annual frost-free period of 170 days, and a mean annual precipitation of 564 mm. The soil type was identified to be a typical calcareous soil in beige color formed from Malan loess. The soil texture in selected experimental plot is featured with high porosity and uniform particles. The pH of the soil is in the range of 8.2–8.3. The experiment was conducted in a plot of appropriate-density (3 m distance between trees and 4 m between rows), fruit trees grow strong, and there is medium tree potential and planted varieties of red Fuji (*Malus pumila* Mill). The soil of 0–20 cm depth layer had the characteristics including total organic matter of 7.5 g kg^{-1}, total nitrogen of 0.64 g kg^{-1}, available nitrogen of 38.71 mg kg^{-1}, available phosphorus of 2.33 mg kg^{-1}, and available potassium of 1.82 g kg^{-1}. The soil of the 20–40 cm depth layer had the characteristics including total organic matter of 4.44 g kg^{-1}, total nitrogen of 0.31 g kg^{-1}, available nitrogen of 23.15 mg kg^{-1}, and available phosphorus of 0.43 mg kg^{-1}, and available potassium of 1.21 mg kg^{-1}.

Tested fertilizers include composted manure (CM), biogas slurry (BS) and chemical fertilizer (CF). The CM was prepared by homemade composting of pig manure and apple sawdust with wood vinegar (1%) and biochar (5%) as coupling additives. The physicochemical characteristics of CM were analyzed and contained organics of 61.02%, total nitrogen of 1.78%, total phosphorus of 2.23%, and total potassium of 1.89%. The BS were collected from the biogas plant (Liangjiahe) managed by CGN New Energy Co. Ltd (Beijing, China). with organics of 0.53%, total N of 0.11%, total P of 0.02%, and total K of 0.13%. CF is purchased from the Yanchuan Run Sheng Agricultural Science and Technology Co., Ltd (Yanchuan, China). with a mixture of urea (N 46%), superphosphate (P_2O_5 16%) and potassium sulfate (K_2SO_4 50%).

On beginning of October 2017, the experiment was established as a completely randomized block design with four treatments and three replicates for each treatment. The experiment was conducted under four treatments including control group with no fertilizer application (CK), chemical fertilizer (CF), composted manure (CM), and biogas slurry (BS). Taking 100 square meters as a treatment plot, each plot selected 9 apple fruit trees with basically the same growth trend and more than 7 years of age as experimental fruit trees. Therefore, the total 1200 square meters of apple fruit orchard were selected for the fertilization experiment. Apple orchard management measures (irrigation, weeding, pruning, pest control, spraying, bagging, harvesting, etc.) are consistent with previous years. CF, CM, and BS were applied based on the consistency of total nitrogen content and supplemented with superphosphate and potassium sulfate as needed to achieve the same total nitrogen, phosphorus, and potassium content applied at all stages. Fertilizer application rates of different treatments are presented in Table 1. The base fertilizers were buried in a ditch (1.2 m length, 40 cm width, 35 cm depth) 1 m from the trunk of each tree in October 2017. The topdressing fertilizer was applied in a circle ditches (20 cm width and 20 cm depth) with 3 m distance to the tree trunk in March and July 2018. The soil sample and fruit sample were collected at the same time when the apple fruit ripen in October 2018.

Table 1. Fertilizer application procedure of different treatments.

Treatment	Fertilization Time	N ($kg\ ha^{-1}$)	P_2O_5 ($kg\ ha^{-1}$)	K_2O ($kg\ ha^{-1}$)
CK	October 2017 (Base fertilize)			
	March 2018 (Topdressing)			
	July 2018 (Topdressing)			
CF	October 2017 (Base fertilize)	150	188	188
	March 2018 (Topdressing)	150	188	188
	July 2018 (Topdressing)	75		135
CM	October 2017 (Base fertilize)	C150	C188	C159 + 29
	March 2018 (Topdressing)	C150	C188	C159 + 29
	July 2018 (Topdressing)	75		135
BS	October 2017 (Base fertilize)	C150	C29 + 159	C188
	March 2018 (Topdressing)	C150	C29 + 159	C188
	July 2018 (Topdressing)	75		135

C stands for the corresponding content of N, P_2O_5 and K_2O present in biogas slurry and composted manure CK: Control Group, CF: Chemical Fertilizer, CM: Composted Manure, BS: Biogas Slurry.

2.2. Sampling and Determination of Apples

After apple fruit ripening (October 2018), 30 fruits were randomly selected from each treatment to determine the fruit quality. Apple fruit size (vertical and transverse diameter, both in mm) was determined with caliper gauge Sttarret 727 (Athol, MA, USA). The weight of apple fruit samples was measured using electronic scales. Fruit firmness was determined with Gy-1 fruit durometer (Jiangsu, China), and the color index (L*, a*, b*) of apple fruit skin coordinates was determined using Minolta chromomete (Shenzhen, China) [18]. Soluble solid content was measured with a Carl Zeiss 32-G hand refractometer (Carl Zeiss, Germany) at 25 °C. The total acidity (TA, %) was measured by using an acid–based titration method. Fruit juice (1 mL) and distilled water (50 mL) were added to a conical flask and titrated with aqueous sodium hydroxide to pH 8.1 by using a Metrohm 719S titrimeter (Titrino, Switzerland). The total acid content was calculated as the equivalent of citric acid, and the mean values were recorded in triplicate [19]. After harvesting, apples were homogenized in a blender, and total sugar (TS, %) was determined by the Lane–Eynon's method. The apple homogenate sample was placed in a 250 mL volumetric flask and extracted with ethanol. The extracted solution was titrated with Fehling reagent using methylene blue as an indicator until the blue solution disappeared. Total sugar/total acid ratio (TS/TA) and vertical diameter/ transverse diameter ratio (fruit shape index) were calculated. The crude protein content of apple fruit was determined by using the Method of Kelvin [20]. The amino acid content in apple fruit was determined by automatic amino acid analyzer (SYKAM S-433D, Germany) after acidifying the apple fruit with hydrochloric acid (6 mol/L).

2.3. Soil Physicochemical Analysis

Soil samples were collected at 0–20 cm and 20–40 cm depth using a five-point method [21]. The sampling sites were selected to avoid the fertilization ditches. The soil samples at the same depth were mixed together and sieved (2 mm) to remove debris such as gravel and plant roots, sample quartering. The soil samples were divided into two groups, in which one group was air-dried for soil physical and chemical analysis, and the other one was stored at −80 °C for DNA extraction and microbial community analysis.

To determine the levels of available nitrogen, the dried soil samples were extracted with 50 mL 1 mol/L potassium chloride solution (1:10 (*w*/*v*)) and analyzed using a segmented flow analyzer (Technicon Auto-analyzer II System, Norderstedt, Germany). The available phosphorus was analyzed using a segmented flow analyzer (Technicon Auto-analyzer II System, Germany) after extracting the soil samples with 50 mL 0.5 mol/L Sodium Bicarbonate solution (1:20 (*w*/*v*)). The available potassium was determined by a flame photometer (M410 blue notes, City, UK) after the extraction with 50 mL ammonium acetate solution (1 mol/L) [22]. The Kjeldahl method was utilized for total nitrogen (TN) estimation [23]. Mo-Sb Colorimetry was used for total phosphorus (TP) detection, and total potassium (TK) was determined with flame photometry [24]. Soil organic matter was measured using an SSM-5000A total organic carbon analyzer (Shimadzu, Kyoto, Japan).

The soil samples collected from different soil depth and fertilization treatment were used for total genomic DNA extraction, which was conducted using an E.Z.N.A.® Soil DNA Kit (D5625-01, Omega BIO-TEK, USA). The obtained DNA samples were amplified using 16S rRNA universal primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), and fungi ITS primers ITS1F (5′- CTTGGTCATTTAGA GGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′). The amplified DNA samples were then analyzed using an Illumina MiSeq platform (Biozeron, Shanghai, China). The obtained pyrosequencing data were analyzed by USEARCH (version 7.0, Robert C. Edgar, CA, USA http://drive5.com/uparse, accessed on 19 November 2018).

2.4. Statistical Analysis

Significant differences between fertilization treatments were determined with a one-way (ANOVA) to compare the least significance difference (LSD) at $p = 0.05$ using SPSS software version 20.0 (Armonk, USA).

3. Results

3.1. Physical Properties of the Fruit

As shown in Table 2, compared to the CK treatment, the vertical diameter of BS, CM, and CF treatment increased by 3.94%, 8.12%, and 2.84%, respectively, and the transverse diameters also increased by 2.39%, 7.35%, and 0.01%, separately. Meanwhile, CM treatment obtained maximum transverse (87.51 mm) and vertical diameters (78.72 mm) with significant difference from CK treatment. Moreover, it should be noted that fertilization treatments can change the vertical and transverse dimensions of apples, but there were no significant differences in the fruit shape index among different fertilizer treatments. The weight of single apple fruit in the treatments of the CF, CM, and BS treatments were raised in contrast to CK, increased significantly ($p < 0.05$) by 8.42%, 14.47%, and 8.66%, respectively. In addition, the maximum value was observed in CM treatment. Meanwhile, the application of BS could improve the size and weight of apples, but the effect was less than that of CM treatment.

Table 2. Physical properties of apple fruit response to different fertilizers application.

Fertilizer Treatment	Shape			Weight of Single Fruit (g)	Color			Flesh Firmness (kg cm^{-2})
	Vertical Diameter (mm)	Transverse Diameter (mm)	Fruit Shape Index		L*	a*	b*	
CK	72.81 ± 1.79 b	81.33 ± 2.07 b	0.89 ± 0.04 a	246.16 ± 9.18 c	54.20 ± 1.55 ab	32.52 ± 1.06 ab	17.72 ± 0.53 b	6.86 ± 0.24 b
CF	74.88 ± 4.09 ab	81.38 ± 1.52 b	0.92 ± 0.05 a	267.48 ± 13.98 b	50.93 ± 4.56 b	33.97 ± 5.56 a	17.24 ± 2.07 b	6.71 ± 0.23 b
CM	78.72 ± 4.32 a	87.51 ± 1.3 a	0.90 ± 0.04 a	281.79 ± 19.3 a	55.48 ± 5.48 a	29.70 ± 6.42 c	18.96 ± 1.86 a	7.43 ± 0.32 a
BS	75.68 ± 3.62 ab	83.37 ± 1.35 ab	0.89 ± 0.03 a	266.88 ± 17.63 b	52.20 ± 2.2 b	31.98 ± 3.03 b	17.50 ± 0.65 b	7.51 ± 0.34 a

Different letters in the same column indicate significant difference at ($p < 0.05$), L*: brightness; a*: red–green axis; b*: yellow–blue axis. CK: Control Group, CF: Chemical Fertilizer, CM: Composted Manure, BS: Biogas Slurry.

Compared with the CK treatment, the 'L*' value of representing color brightness of apple fruit outer epidermis was significantly decreased by applying chemical fertilizer, but the 'L*' and 'b*' value of organic fertilizer was increased. Meanwhile, the lowest 'a*' value was obtained by CM treatment. Compared with the CK and CF treatments, CM and BS treatments could significantly increase the flesh firmness of apple fruit by 8.31% and 9.48%, respectively (Table 2).

3.2. Chemical Properties of the Fruit

As shown in Table 3, the BS and CM treatment significantly ($p < 0.05$) increased the total acid of apple fruit compared to the CK treatment, and CM treat apple fruit had highest total acid (0.35%), followed by BS (0.30%), CF (0.245%), and CK (0.25%). In this study, the application of organic fertilizer evidently reduced the TS/TA ratio with the minimum TS/TA ratio (37.9) by CM treatment. Among all treatments, the lowest soluble solid content was observed in the CK treatment (11.63 °Brix), whereas BS treated apple fruit showed maximum soluble solid (13.25 °Brix) followed by CM (13.15 °Brix) and CF (12.44 °Brix). Compared to the CK treatment, the vitamin C content of CF, CM, and BS treatment clearly ($p < 0.05$) increased by 78.0%, 59.1%, and, 99%, respectively. However, the vitamin C content of CM treatment was lower than that of CF and BS treatment. Meanwhile, the content of crude protein in apple fruit treated with BS and CM was significantly increased compared with the CK and CF treatment.

Table 3. Effects of different fertilizers on chemical properties of apple fruit.

Treatment	Total Sugars (%)	Total Acid (%)	TS/TA Ratio	Vitamin C mg/100 g	Soluble Solid (°Brix)	Crude Protein (%)
CK	12.70 b	0.25 c	53.0 a	1.81 d	11.63 c	0.24 b
CF	12.30 b	0.24 c	51.3 a	3.22 b	12.44 b	0.19 c
CM	13.25 ab	0.35 a	37.9 c	2.88 c	13.15 a	0.29 a
BS	13.85 a	0.30 b	46.2 b	3.61 a	13.25 a	0.27 a

Different letters in the same column indicate significant difference at ($p < 0.05$). TS: total sugars, TA: total acid. CK: Control Group, CF: Chemical Fertilizer, CM: Composted Manure, BS: Biogas Slurry.

As shown in Table 4, compared with the CK, BS, and CM treatments significantly increased the total amino acid concentration by 22.93% and 10.24%, respectively. As part of the total amino acids, the essential amino acids in BS and CM treated apple fruit were increased by 8.89% and 17.18%, and medicinal amino acids were increased by 7.64% and 24.84%, respectively.

Table 4. The amino acids content (%) of in apple fruit under different fertilizer treatments.

Amino Acid	CK	CF	CM	BS
Thr *	0.008	0.008	0.009	0.01
Vla *	0.007	0.006	0.008	0.007
Met *#	0.001	0	0	0
Ile *#	0.006	0.006	0.007	0.007
Leu *#	0.009	0.009	0.011	0.010
Phe *#	0.003	0.005	0.006	0.004
Lys *#	0.011	0.01	0.012	0.011
His	0.003	0.003	0.005	0.004
Arg #	0.009	0.004	0.005	0.004
Pro	0.01	0.011	0.013	0.013
Gly #	0.007	0.006	0.007	0.007
Ala	0.008	0.008	0.009	0.009
Cys	0	0	0	0
Asp #	0.093	0.081	0.099	0.134
Ser	0.009	0.008	0.01	0.01
Glu #	0.018	0.017	0.022	0.019
Tyr	0.003	0.003	0.003	0.003
E	0.045 c	0.044 c	0.053 a	0.049 b
N	0.16 c	0.141 d	0.173 b	0.203 a
T	0.205 c	0.185 d	0.226 b	0.252 a
M	0.157 c	0.138 d	0.169 b	0.196 a

T: Total amino acid, E: Total essential amino acid, N: Total nonessential amino acid, M: Medicinal amino acid, * represents essential amino acid, # represent medicinal amino acid. Different letters in the same column indicate significant difference at ($p < 0.05$). CK: Control Group, CF: Chemical Fertilizer, CM: Composted Manure, BS: Biogas Slurry.

3.3. Soil Physicochemical Characteristics

In this work, the utilization of different fertilizers resulted in the variation of soil physicochemical properties. As shown in Table 5, the addition of fertilizer significantly ($p < 0.05$) improved the total nitrogen content in the 0–20 cm soil layer, and CM treatment achieved the maximum total nitrogen (1.12 g kg^{-1}). Furthermore, compared to the CM treatment, the total nitrogen content of BS treatment decreased significantly. The fertilizer application also increased soil total P content, and the CM treatment acquires the highest total P content of 0.84 g kg^{-1} and 0.61 g kg^{-1} in 0–20 cm soil depth and 20–40 cm soil depth, respectively. Notably, the total K content (0–20 cm soil layer) in BS treated soil had no significant difference to the CK treatment. In addition, total N and total P decreased with increasing soil depth. Meanwhile, the CM treatment presented the highest available P, N, and K concentrations at different depths, while the minimum available P content came from CK treatment. Furthermore, available P and available K decreased with the increase of soil depth. Meanwhile, the fertilization can also increase soil organic matter content at different depths, and CM treated soil achieved the best results. In addition, the soil organic matter content decreased with increasing soil depth.

Table 5. Soil chemical properties under different fertilization treatments.

Soil Depth (cm)	Fertilizers	Total N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})	Organics (%)
0–20	CK	0.60 d	0.52 d	23.25 a	50.19 b	2.31 d	167 c	0.74 d
	CF	0.67 c	0.57 c	21.39 b	51.92 b	2.65 c	196 b	1.06 c
	CM	1.12 a	0.84 a	23.20 a	58.84 a	26.93 a	487 a	1.64 a
	BS	0.84 b	0.68 b	23.17 a	51.92 b	8.60 b	188 b	1.24 b
20–40	CK	0.32 c	0.57 b	19.27 c	46.72 b	1.39 c	116 b	0.45 c
	CF	0.34 c	0.54 c	23.07 ab	41.53 c	1.27 d	114 b	0.45 c
	CM	0.70 a	0.61 a	24.08 a	82.12 a	3.82 a	358 a	0.92 a
	BS	0.47 b	0.56 b	22.40 b	38.07 d	1.55 b	101 c	0.63 b

Different letters in the same column indicate significant difference at ($p < 0.05$). CK: Control Group, CF: Chemical Fertilizer, CM: Composted Manure, BS: Biogas Slurry.

3.4. Diversity of the Microbial Community

Table 6 shows the microbial diversity and abundance indices of all treatments. The coverage estimator of all samples was greater than 0.98, indicating that these libraries well represented the true situation of microbes in the samples. For bacterial community, compared with CK treatment, Chao 1 index of CF treatment in a 0–20 cm depth layer decreased significantly ($p < 0.05$). Inversely, an increase of Chao 1 and Shannon index was observed in the BS and CM treatments. For the fungal community, compared with CK treatment, the Shannon index of BS and CM treatment increased significantly ($p < 0.05$) and Simpson index decreased significantly ($p < 0.05$). Compared with CK treatment, the CF treatment could significantly ($p < 0.05$) decrease the Chao 1 index of bacteria in 0–20 cm depth layer and could increase the Shannon index of fungi.

Table 6. Species diversity and abundance index at the different treatment and soil depth.

Soil Death (cm)	Treatment	Simpson	Chao1	Shannon	Coverage
	Bacteria				
0–20	CK	0.009 b	2079 b	6.25 a	0.99 a
	CF	0.030 a	1849 c	5.50 b	0.99 a
	CM	0.006 d	2239 a	6.48 a	0.99 a
	BS	0.008 c	2091 b	6.32 a	0.98 a
20–40	CK	0.046 a	1921 b	4.98 b	0.99 a
	CF	0.006 c	2121 a	6.34 a	0.99 a
	CM	0.007 b	2204 a	6.36 a	0.99 a
	BS	0.004 d	2110 a	6.42 a	0.99 a
	Fungal				
0–20	CK	0.107 a	297 c	3.31 d	1 a
	CF	0.061 b	256 d	3.68 c	1 a
	CM	0.036 c	412 b	3.98 b	1 a
	BS	0.025 d	464 a	4.38 a	1 a
20–40	CK	0.173 a	266 c	2.54 c	1 a
	CF	0.045 b	407 a	3.97 b	1 a
	CM	0.025 c	340 b	4.23 a	1 a
	BS	0.044 b	354 b	4.1 ab	1 a

Different letters in the same column indicate the significant difference at ($p < 0.05$). CK: Control Group, CF: Chemical Fertilizer, CM: Composted Manure, BS: Biogas Slurry.

The similarity and overlap of OTUs number composition in different treatments samples can be intuitively expressed via Venn diagram. As shown in Figure 1a, more than two-thirds of the common bacterial OTUs were present in all treatment. The data distinctly indicated that 174 fungal OUTs were common for different treatment, and more than one third of fungal OTUs exists only in specific treatments (Figure 1b).

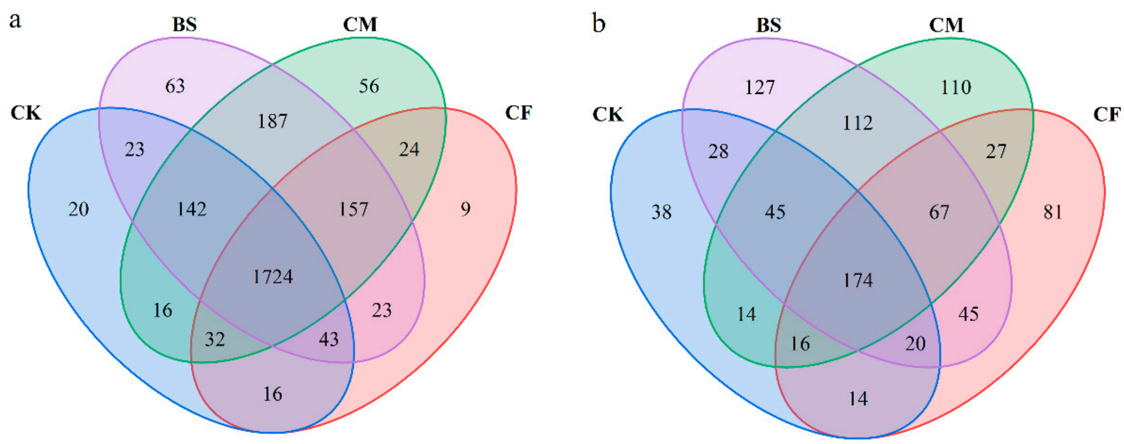

Figure 1. Venn diagram for bacterial (**a**) and fungal (**b**) operational taxonomic units (OTUs) found under different fertilization treatments. CK: Control Group, CF: Chemical Fertilizer, CM: Composted Manure, BS: Biogas Slurry.

3.5. Microbial Community Structures

In this study, a total of five fungi phyla were detected among all samples (Figure 2a). The preponderant phyla were Ascomycota (91.8–56.8%), followed by Mucoromycota (41.09–1.21%), Basidiomycota (5.6–0.8%), Unclassified (7.3–1.3%), and Chytridiomycota (2.4–0%). The fungi species Chytridiomycota with the function for lignocellulose degradation was only detected in CM and BS treated soil. Notably, the relative abundance of Basidiomycota of fertilizer treated soil increased compared with the CK. Furthermore, the diversity of fungi varied greatly at different soil depths. The relative abundance of Ascomycota and Basidiomycota decreases with increasing soil depth.

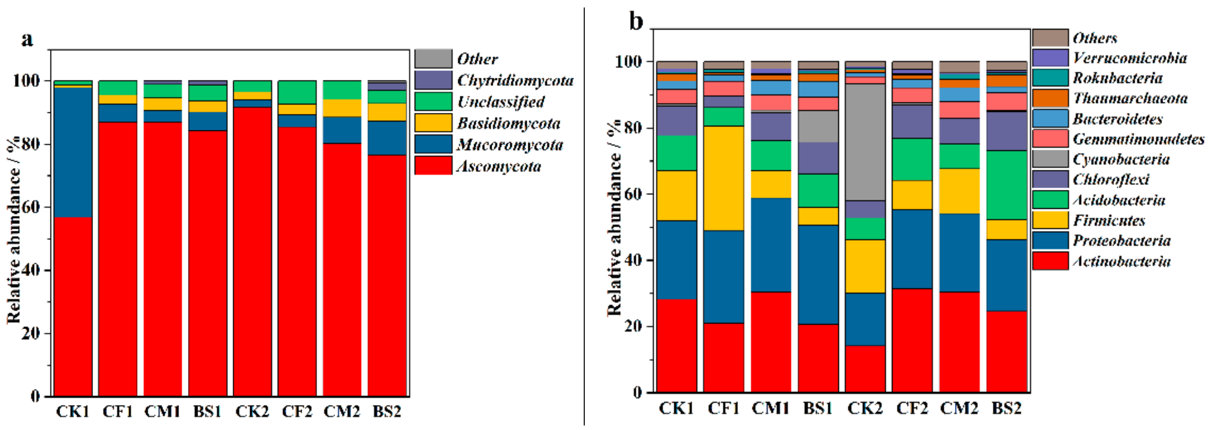

Figure 2. The soil microbial community composition under different fertilization treatments in phylum levels of fungi (**a**) and bacteria (**b**). 1: 0–20 cm depth layer; 2: 20–40 cm depth layer. CK: Control Group, CF: Chemical Fertilizer, CM: Composted Manure, BS: Biogas Slurry.

Figure 2b is showing the relative abundance of bacteria at phylum level. The common dominant bacterial phyla, including Cyanobacteria (35.3–0.4%), Actinobacteria (31.4–14.1%), Proteobacteria (29.8–15.8%), Firmicutes (31.6–5.3%), and Acidobacteria (20.9–5.6%), were diffusely reported to present in orchard soil, and plays an important role in promoting plant growth, inhibiting disease, and improving carbon availability [16]. Notably, compared with CK treatment, the addition of fertilizer not only changed the relative abundances of the main bacterial phylum, but also changed the composition of the main bacterial phylum. The relative abundance of Proteobacteria significantly increased from 23.7% in CK treatment to 28.0% in CF treatment and 28.3% in CM treatment in the 0–20 cm depth layer. The similar results were also obtained in 20–40 cm soil layer. In the 0–20 cm depth layer, the most dominant bacterial phylum in CK treatment were Actinobacteria (28.2%), followed by Proteobacteria (23.7%) and Firmicutes (15.3%). In contrast, Acidobacteria became predominant species for CM and BS treated soil with relative abundance of 9.2% and 10.1%, respectively.

4. Discussion

The attractiveness of apples to consumers is impacted by visual factors that contain shape, color, size, weight, firmness, etc. These appearance attributes of apple fruit are not only influenced by inheritance, but also related to environmental growth conditions [4]. Fruit diameter and fruit weight are important parameters affecting the commercial rate of fruit and determining the market price of apple [25]. In our study, fertilizer application can increase apple size and individual fruit weight compared with CK treatment. The results about variations of the fruit size to fertilizer treatment were consistent with findings by Milošević and Mladenović, in which the effect of different fertilizers treatments on apple fruit quality of 'Idared' was studied [2]. Other studies revealed that nitrogen and potassium fertilizers were important factors affecting the weight and size of apple fruits, and fruit weight can be increased with the increase of N application [26,27]. As confirmed by our data, the available nitrogen and potassium components of the soil treated with CM were significantly higher than those of the other treatments (Table 5), and the CM treatment achieved the largest apple size and individual fruit weight, showing good commercial characteristics. This may be due to the CM contains rich organic materials to promote nitrogen transformation, and microporous structure of biochar in CM to reduce soil nitrogen loss and improve plant nitrogen uptake [28]. Möller and Müller found that the nutrients in BS could be easily absorbed by plants, but most nitrogen in BS would be lost in the form of ammonia, indicating that the application of BS only had a significant effect on crop yield and quality in a short period after application [29]. On the whole, the soil treated with organic fertilizer can accelerate the transformation between soil mineralization and plant absorption, improve the absorption efficiency of plant nutrients, and make organic fertilizer treatment have better fruit weight and size. Meanwhile, there was no significant difference in the fruit shape index between different fertilization treatments, possibly due to the fact that fruit shape index was determined by the genetic level [30].

Color is one of the most important quality parameters for evaluating apple fruit, and the color of apples can be attributed to many factors, including ripeness, ecology, genotype, soil type, climate, etc. [31]. The results showed that, compared with CK treatment, the application of chemical fertilizer and biogas slurry reduced the brightness (L*) color of fruit epidermis, but there was no significant difference in CM treatment. This may be related to the higher concentration of organic matter and potassium in the soil treated with CM. Nava et al. and Kilic et al. found that the soil with high organic matter and potassium level was more conducive to the development of fruit color [18,32]. Meanwhile, the 'a*' value of color index indicates the intensity of fruit color. The lower 'a*' value indicates the lower red intensity [18]. CM treatment showed the lowest 'a*' value, representing the lowest red intensity. This can be attributed to fact that CM treated apple tree absorbed high nitrogen content and then resulted in a decrease in anthocyanin involved in red pigment formation [33]. The variations in color 'a*' value and weight of single fruit were similar to the findings by Amiri and Fallahi, who used animal manure to treat 'Golden Delicious' apple [34]. In addition, the synthesis of nitrogen and anthocyanins in red Fuji apple fruits can be regulated by adding exogenous abscisic acid in order to improve the problem of poor pigmentation caused by excessive nitrogen content in the fruit at the later stage of apple development [35].

The storage ability of the same apple fruit variety depends not only on maturity and storage conditions, but also on firmness of the fruit [36]. As shown in Table 2, CM and BS treatments could significantly increase the flesh firmness of apple fruit. This finding is also in good agreement with the previous studies by Milošević et al. and Lin et al. [2,37]. This phenomenon can be explained by reason that the organic fertilizer such as BS and CM contain Ca, Mg, and other trace elements, which provide the foundation for cell wall and membrane nutrient formation to improve the flesh firmness [38]. Küçükyumuk and Erdal reported that spraying $CaCl_2$ solution on apple fruits could improve fruit weight, size, firmness, and delay fruit ripening under the condition of soil calcium deficiency [39]. In addition, the decrease in fruit firmness may also be related to excess potassium fertilizer. Potassium increases fruit size, while fruit growth is usually caused by the expansion of the cell wall, which leads to a decrease in fruit density and a decrease in fruit firmness [32]. This may be the reason why the apple hardness of CM treatment was slightly lower than that of BS treatment because the soil potassium concentration of BS treatment was lower than that of CM treatment.

A PLS-DA multivariate statistical method was conducted to display the differences in various physical properties of apple fruit under different fertilization treatments. Figure 3a shows the PLS-DA score plot with the first principal component (Component 1) and the second principal component (Component 2) accounting for 70.9% and 5.2% of the total variability, respectively. As shown in Figure 3a, there is slight separation among all the groups. The variable importance in projection VIP scores of different traits was calculated based on the PLS-DA mode. The traits with VIP scores were displayed in Figure 3b. Furthermore, using a cutoff of p-value < 0.05 and VIP score > 1, physical traits of apple fruit including vertical diameter, 'a*' value, and firmness were determined as the variables that are most influenced by different fertilizer treatments.

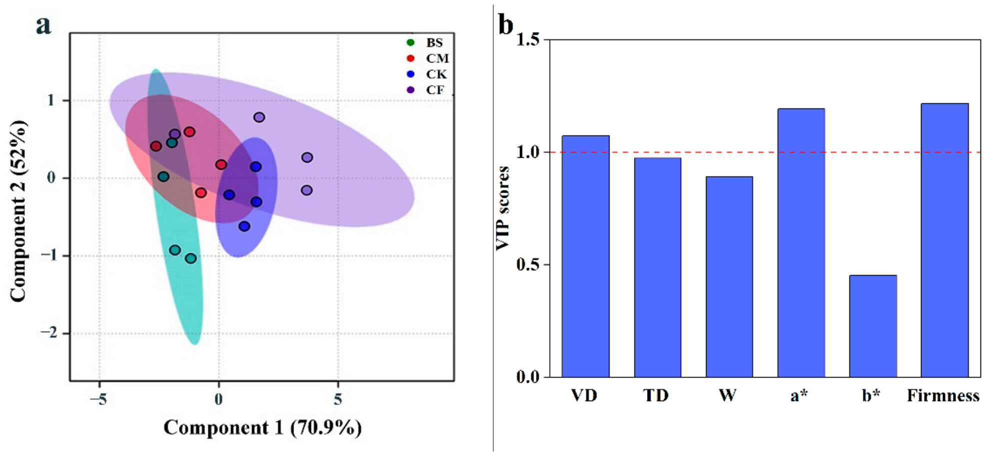

Figure 3. PLS-DA score plot (**a**) and VIP plot of the potential physicochemical markers (**b**) for the apples under different fertilizer treatments. VD: Vertical diameter, TD: Transverse diameter, W: Weight of single fruit, a*: red–green axis, b*: yellow–blue axis. CK: Control Group, CF: Chemical Fertilizer, CM: Composted Manure, BS: Biogas Slurry.

Total acid is a major index for predicting apple fruit taste because the consumers generally have different preferences for sour or sweet apples [4]. We found that organic fertilizer treatment can increase the total acid content of apple fruit, which was similar to the results presented by Kai and Adhikari that the organic fertilizer can improve the total acid content in contrast to CF [40]. As for the effect of fertilizers on acid content in apple fruits, however, a previous literature work by Ernani et al. reported the organic fertilizer reduced the content of total acid in apple (Royal Gala) fruit [41]. The effect of organic fertilizer on total acid may be related to the difference in acid metabolism caused by the specific knowledge of fertilizers, as well as other reasons such as different agricultural climate conditions, quality factors, planting methods, tree age, etc. [3]. Meanwhile, some studies reported that fruit acidity was not significantly affected by nitrogen fertilizer, and was positively and linearly correlated with soil K content. The titratable acidity of fruit juice increased linearly with the increase of potassium application rate [31]. In addition, TS/TA is another key factor to evaluate fruit taste, which can be further applied as the basis for apple fruit classification [2]. Specifically, apple fruit with a TS/TA ratio lower than 20 are appropriate for by-product processing and cider generation, while fruit varieties with a TA/TS ratio higher than 20 are suitable for direct consumption. In this study, the results proved that the CM and BS treatment could improve the acidity of apples, suggesting that the CM and BS treated apples can be used for direct consumption and be more suitable for consumers who prefer sour taste.

Soluble solids refer to all compounds dissolved in water in liquid or liquid food, including sugars, acids, vitamins, proteins, minerals, etc., which are also important indicators to evaluate the quality of apple fruits [40]. Compared to the CK treatment, the addition of fertilizer could increase the soluble solid of apple fruit, possibly due to the fertilizers containing a lot of nutrients that promote the formation of soluble solid [33]. Furthermore, the soluble solid content of CM and BS treatment was higher than that of CF treatment, likely due to the high amount of organic matter that could supply balanced nutrient to soil respiration and further improve the nutrient transformation [42]. This finding is in good agreement with the published literature by Milošević et al. in exploring the effects of organic and mineral fertilizer on soil biochemical characteristics and fruit nutrient content in apple orchards attributing this phenomenon to the fact that organic fertilizer can provide various nutrients to apple trees, improve soil pH value, and stimulate soil microbial activity [2]. The effect of different fertilization on fruit vitamin C content was similar to that found by Zhao et al., who explored the effects of different proportions of organic fertilizer on apple fruit quality [43]. The results indicated that the increase of the proportion of organic fertilizer resulted in the vitamin C content showing a trend of first increasing and then decreasing, to the point lower than that of CF. Meanwhile, compared with CK and CF treatment, organic fertilizer treatment has higher crude protein. This indicated that organic fertilizer treatment could further promote nitrogen uptake by fruits and then result in higher nutritional value, which may be because application of organic fertilizer could promote the transformation of nitrogen by increasing the content of nitrate nitrogen, thus improving the activity of nitrate reductase and improving the protein content of fruits.

Fertilization is one of the main factors affecting soil nutrient content in apple orchards [17]. Long-term fertilization can improve soil fertility of apple orchard, but excessive application of N, P, and K fertilizer will cause problems such as soil settling and acidification [44]. In our study, both organic matter and total nitrogen in the soil treated with fertilizer were increased, and the increase of organic matter and total N concentration in the soil treated with CM was greater than that in the soil treated with BS and CK treatment; Zhu et al. and Zhong et al. reported the similar conclusions [16,45]. Over the past decades, the consistent application of organic fertilizers to apple orchards has improved soil fertility and soil microbial community diversity. Meanwhile, long-term application of N fertilizer could increase total N and available N in 0–20 cm soil depth of apple orchards [44]. Meanwhile, our study found that the soil nutrient reserve of BS treatment was lower than that of CM treatment, likely due to the fact that nitrogen in BS is easily lost and volatilized in the form of ammonia [46]. The total amount of nutrient content level can reflect the reserve of soil nutrients and the available nutrient content level can reflect the dynamic balance between soil mineralization and plant absorption [17]. Our results indicated that CM treatment can effectively promote the increase of soil available nutrients, and the CM treatment was better than BS and CF treatment. The results reported above were quite similar to that of Wen et al., who attributes this to the fact that the application of organic fertilizers increases the content of organic matter in the soil, thus speeding up the nutrient cycling between the soil and the plants [6]. However, some scholars have found that the content of available nitrogen, phosphorus, and potassium in fertilization experiments utilizing CM and BS is lower than that of CF treatment [14], possibly due to the different raw materials of aerobic compost and soil acidity and alkalinity.

Soil organic matter plays an important role in plant growth and is considered an important indicator of soil health and productivity, and high levels of soil organic matter not only increase plant productivity but also improve soil microbial community diversity [40,47]. Some studies have shown that the application of biochar and organic fertilizer is conducive to the winding and condensation of soil particles in the topsoil, and promotes the formation of organic carbon [48]. The same results were obtained in our data, and the soil organic matter content of CM treatment was higher than that of BS treatment. At the same time, the application of chemical fertilizers also increased soil organic matter level, which may be related to the promotion of plant root growth and the increase of organic matter input in the rhizosphere [49]. In addition, the level of most soil properties decreased with the increase of soil depth, and some studies have shown that 0–20 cm is an effective depth system for simultaneous detection of soil fertility changes during apple production [44].

Soil microorganisms are important drivers of agro-ecosystem function, and their diversity and biomass are critical to soil quality and health [50]. In our experiment, the application of different types of fertilizers in apple orchards not only greatly changed the physical and chemical properties of soil, but also affected the composition and structure of bacterial communities. Generally, both Chao 1 index and Shannon index can reflect the diversity and richness of microbial community, respectively. The higher Shannon index represents a more complex microbial community, and the higher Chan 1 index represents a more species richness [51]. Compared with the CK, the addition of CF clearly decreased species richness in the 0–20 cm soil layer, likely due to the fact that the soil were acidified because of long-term application of fertilizer [16,52]. Inversely, the BS or CM could improve the richness and diversity of soil bacteria [17], suggesting that a possible benefit of organic fertilizer application can increase abundance and activity of soil biota [53]. Compared with CK, the diversity and abundance of fungi in BS and CK soil were increased, possibly due to the fact that CM and BS contain more balanced nutrients [16]. Some studies have shown that soil with balanced nutrients contains more soil microorganisms, and available nitrogen and potassium are the key factors for the growth of soil microorganisms [54]. Meanwhile, compared with CK treatment, the CF treatment could significantly ($p < 0.05$) decrease the Chao 1 index of bacteria in the 0–20 cm depth layer and could increase the Shannon index of fungi, which explained that the utilization of CF showed a positive effect on fungal growth, but a negative effect on soil bacteria abundance [50]. The results showed that the bacterial community variations were mainly reflected by ratio, while the fungal community differences were mainly reflected by species variety. Meanwhile, according to the similarity and overlap of OTU number composition of different processed samples in Figure 1, our study found that the bacterial community variations were mainly reflected by ratio, while the fungal community differences were mainly reflected by species variety.

Fertilization alters the distribution of major phyla of bacteria and fungi. In our study, Chytridiomycota was only detected in soil treated with CM and BS. It is reposted that Chytridiomycota can be used for plant absorption and nutrient cycling by converting organic sources of nitrogen and phosphorus into inorganic forms such as nitrate and ammonia phosphate, and can also help degrade cellulose and chitin in materials [55,56]. These results indicated that the soil treated by CM and BS may be more beneficial to the growth of apple trees and the improvement of apple fruit quality. Compared with the CK treatment, the relative abundance of Basidiomycetes increased under fertilization, probably because soil organic matter provided energy for Basidiomycetes reproduction. Kjøller and Rosendahl discovered that Basidiomycota plays a significant role in decomposition of soil organic matter, and they can decompose complex organic matter under the action of enzymes [57]. Furthermore, the relative abundance of Ascomycota and Basidiomycota decreases with increasing soil depth, indicating that the change of microbial community also has a relationship with soil depth. This phenomenon can be attributed to the fact that the different fertilization resulted in the variation of root exudates, which are the main nutrients of the rhizosphere microbes and may be affected by plant nutrient status [58].

The results of our study indicated that fertilization resulted in significant changes in bacterial community structure. Previous literature has showed that Proteobacteria are Gram-negative bacteria with an effective function of nitrogen fixation in symbiotic legumes [17]. Our study found that fertilizer application could increase the relative abundance of Proteobacteria, possibly due to the fact that nutrient-rich soil is beneficial to the growth of Proteobacteria [59]. The *Bacteroideds* play an important role in the transformation of lipids, proteins, and other organic materials. The *Actinobacteria* was participated in cellulose and chitin degrading for organic material active turnover [60]. The phylum Acidobacteria is an oligotrophic bacteria and favors the conditions with lower soil organic C, their members are adapted to malnourished soils and are associated with turnover of soil organic carbon and nutrient availability [17]. Firmicutes are the most dynamic group that is associated with disease inhibition [17]. Compared with the CK, the relative abundance of Firmicutes in BS and CM treatment was relatively low, which may be because the addition of BS and CM could supply a healthy soil environment for the growth of the root system. Furthermore, the bacterial community composition varies with the changes of soil depth due to the difference of the physicochemical characteristics (nutrients and soil organics) in various soil layers as shown in Table 5. On the whole, CM treatment can significantly improve the diversity and community structure of the soil microbial community, which is beneficial for improving the ability of plant organic matter degradation, plant growth, disease resistance, and nitrogen fixation.

5. Conclusions

In this study, the influence of chemical fertilizer (CF), biogas slurry (BS) and composted manure (CM) on the quality of apple fruits, soil nutrients, and soil microbial diversity were evaluated. The results showed that fertilizers played an important role in the physicochemical properties of apple fruit and soil. Compared with the control group, the levels of vertical diameter, transverse diameter, weight of single fruit, and soluble solid increased by 8.42–14.47%, 0.01–7.35%, 8.84–14.47%, and 6.96–13.93%, respectively, for CF, CM, and BS treatments. At these levels, CM treatment obtains the best results. Meanwhile, the CM and BS treatments could significantly increase fruit hardness, essential amino acid, and total acid compared to CK and CF treatments. Among all treatments, the application of CM significantly increased the accumulation of soil organic matter and total nitrogen, accelerated the nutrient transformation between plants and soil, and improved the functional diversity of soil communities, thus ensuring more stable and higher apple quality. The results obtained in this study reveals the insight effect of organic fertilizer on improvement of fruit quality and soil health and could potentially promote organic cultivation of apple fruit and green agricultural development. However, the further investigation of organic fertilization affection on root nutrient metabolism and the activities of rhizospheric microorganism is needed in future study.

Author Contributions: Conceptualization, X.W. and G.S.; software, X.W.; investigation, G.S.; resources, G.S. and J.L.; data curation, X.W. and Q.B.; writing—original draft preparation, X.W.; writing—review and editing, X.W., J.L. and G.S.; project administration, J.L. and G.S. All authors have read and agreed to the published version of the manuscript.

Funding: The current work is supported by the Key R&D Program of Shaanxi Province (2022ZDLNY02-09, 2019TSLNY01-03), China Agriculture Research System (CARS-23-C-05), and the Post-doctoral Foundation of the Shaanxi Province (2018BSHEDZZ20).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The dataset analyzed in this article is available upon request to the corresponding authors.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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