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Ozone reduces the fruit decay of postharvest winter jujube by altering the microbial community structure on fruit surface

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ABSTRACT

Microbial community structure on fruit surface plays an important role in fruit decay during postharvest storage, although the underlying mechanism has not been fully elucidated. Winter jujube (*Ziziphus jujuba* Miller cv. Dongzao) is a unique fruit resource with high edible and commercial value in China, while postharvest decay has always been a severe problem leading to short shelf life and poor quality of fruit. Ozone treatment is regarded as one of the most effective means to control decay and extend shelf life because of its cost-effective and eco-friendly properties. In the present study, three concentrations of ozone (2.5, 5 and 10 μL L^{-1}) were found to reduce significantly postharvest decay of winter jujube on days 10 and 15, which were produced from Huanghua City, Hebei, China. High-throughput sequencing revealed significant changes in the bacterial and fungal communities in response to the application of ozone treatment, while *Didymella*, *Rhizopus*, *Alternaria*, *Phialemoniopsis* and *Mycosphaerella* were found to be the most abundant in fungi, and *Methylobacterium*, *Pseudomonas*, *Pantoea*, *Sphingomonas* and *Gluconobacter* being the most abundant in bacteria. Results of linear discriminant analysis (LDA) effect size (LEfSe) indicated that ozone treatments considerably reduced the abundance of *Rhizopus* and *Gluconobacter* on the surface of winter jujube fruit. Furthermore, Pearson correlation analysis showed that *Rhizopus* was positively correlated with *Gluconobacter* ($r = 0.97$) while negatively correlated with *Didymella* ($r = -0.96$). By predicting the metabolic function, ozone may inhibit metabolic pathways including nucleoside and nucleotide biosynthesis, amino acid biosynthesis, fatty acid and lipid degradation, respiration, and electron transfer, thereby reducing the incidence of fruit decay and maintaining the firmness of winter jujube fruit.

1. Introduction

Winter jujube (*Ziziphus jujuba* Miller cv. Dongzao) is a Chinese native that is prized for its high nutritional value and good table quality (Li et al., 2007; Liu et al., 2020). However, winter jujube often faces great challenges due to the fruit decay caused by various postharvest pathogens during storage (Luo et al., 2009; Li et al., 2012). Therefore, controlling postharvest disease and decay is of great significance to storage and preservation in winter jujube.

Ozone, which comprises three singlet oxygen atoms, is a powerful active oxidant that may be used to disinfect food surfaces, food production equipment, packaging materials, and minimize pests and diseases in storage (Sujayasree et al., 2021). Among several postharvest applications, ozone treatment is considered a cost-effective and eco-friendly food-processing technology to preserve the fruit quality, with a higher oxidation potential than sanitizers used in the fresh produce industry (Fan, 2021). The use of gaseous ozone has been shown to have higher efficiency and practical benefits than using aqueous solutions (Prabha et al., 2015). Numerous studies have been reported on the application of gaseous ozone to fresh and fresh-cut fruit. Among them, research on pathogenic resistance of ozone has made significant progress in recent years (Fan, 2021). For example, ozone treatment of papaya fruit with 1.6 ppm ozone for 96 h delayed the onset of anthracnose disease, and simultaneously decreased its disease incidence (Ong et al., 2013). In addition, ozone has been used to control fruit decay infected with *Penicillium digitatum* or *P. italicum* during citrus storage (Palou et al., 2007), decrease the incidence of gray mold rot in strawberry fruit (Zhang et al., 2009), and limit the spread of *Botrytis cinerea* in grapes

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(Palou et al., 2002). Ozone sterilization is a necessary technology in the storage practice of winter jujube in Huanghua City, Hebei, China (Li et al., 2012). However, the surface microbial disturbance caused by ozone treatment of winter jujube fruit has not been fully investigated, which prompted us to carry out this study.

High-throughput sequencing and omics tools have provided a deeper understanding of fruit- or vegetable-microbiome interactions during postharvest storage, as well as greatly contributed to the development of postharvest treatments (Peter et al., 2020). Recent research has shown that the microbiota on fruit surfaces affect the occurrence of fruit decay, rather than just the pathogens working alone (Wassermann et al., 2019; Zhang et al., 2021a). It has been found that packhouse treatments significantly impact the resident fruit microbiome of mandarin fruit (Kumar et al., 2021). Results suggested that the initial drenching treatment had a lesser impact on the peel microbiome, while hot chemical drenching and waxing treatments significantly altered the fruit peel microbial diversity. Microbiome studies have been considered to provide information that will result in a fundamental paradigm shift in how we think about biocontrol strategies, biocontrol products, and postharvest biology (Droby and Wisniewski, 2018). However, the microbiome structure on fruit surface of winter jujube and its alteration under ozone treatment during fruit storage are not well understood.

In this study, winter jujube fruit were exposed to four different ozone concentrations, and the disease index, microbial community on fruit surface and fruit firmness were assessed during postharvest storage. The objective of this study was to investigate the effect of ozone on the microbial diversity of winter jujube fruit produced from Huanghua City, Hebei, China, and to provide a reference for ozone as an effective means to reduce postharvest fruit decay.

2. Materials and methods

2.1. Winter jujube preparation and ozone treatment

The winter jujube (*Ziziphus jujuba* Miller) fruit were harvested on October 14, 2020 from an orchard in Huanghua City (117.229589 E, 38.634471 N), Hebei Province, China. Fruit with similar size and maturity (half red color on fruit surface) were randomly divided into four groups, each with three replicates. After washing with sterilized distilled water, the fruit of the four groups were treated with 0, 2.5, 5 and 10 μL L^{-1} ozone in airtight containers for one hour per day, respectively, and stored in sterile plastic wrap at 25 ± 0.5 °C. The treatments on day 0 were marked as D0_0, D0_2.5, D0_5, D0_10, representing the samples from the ozone-treated fruit surface at 0, 2.5, 5 and 10 μL L^{-1}, respectively. The same naming rules were also applied to other treatments including D5_0, D5_2.5, D5_5, D5_10 on day 5; D10_0, D10_2.5, D10_5, D10_10 on day 10, and D15_0, D15_2.5, D15_5, D15_10 on day 15. A total of 240 fruit were used in this experiment.

2.2. Calculation of disease index

Fruit decay was graded on a scale of 0–6 according to the severity of disease where 0 = no observable disease symptoms and 6 = whole fruit decay (Merz et al., 2012). The disease index is calculated according to the following formula: Disease index = ∑ (Number of diseased fruit at each grade × Representative value of corresponding grade) / (Total number of investigated fruit × Highest representative value).

2.3. Microbial DNA extraction and Illumina sequencing

Microbial samples on the fruit surface were collected by filtering after soaking in a buffer solution. Briefly, the fruit of winter jujube were collected after ozone treatment on day 0, 5, 10 and 15, then soaked in PBS buffer (10 mmol/L Phosphate buffer, 137 mmol/L NaCl, 2.7 mmol/L KCl, pH 7.4) and shaken at 200 r/min for 1 h. The fruit were discarded, and the PBS solution was filtered through two layers of sterile gauze to remove the large particles. The resulting suspension was then filtered by a 0.22 μm nitrocellulose membrane and frozen with liquid nitrogen for 15 min, and stored at − 80 ℃.

Genomic DNA of the microbial community was extracted from the nitrocellulose membrane with three distict repeats of the experimental activities of washing, gauze filtration, and vacuum filtration. The V3-V4 region of 16 S rRNA gene was amplified with the primers 308 F (5′-ACT CCT ACG GGA GGC AGC A-3′) and 806 R (5′-CGG ACT ACH VGG GTW TCT AAT-3′) combined with adapter and barcode sequences (Zhang et al., 2021a). The ITS1 region of the fungal community was amplified with the primers ITS5 (5′-GGA AGT AAA AGT CGT AAC AAG G −3′) and ITS2 (5′-GCT GCG TTC TTC ATC GAT GC-3′) combined with adapter and barcode sequences (Zhang et al., 2021a). Reagents for PCR amplification (25 μL) were added as follows: 5 μL 5 × reaction buffer (Q5 Reaction Buffer), 5 μL 5 × GC buffer, 2 μL dNTP (2.5 mM), 1 μL forward primer (10 μM), 1 μL reverse primer (10 μM), 2 μL DNA template, 8.75 μL ddH_2O, 0.25 μL Q5 DNA Polymerase (M0491L, NEB, USA). ABI 2720 PCR cycler machine (Thermo, USA) was used for PCR and the thermal cycling condition was set as follows: 98 ℃ for 2 min; 30 cycles of 98 ℃ for 15 s, 55 ℃ for 30 s, 72 ℃ for 30 s; 72 ℃ for 5 min, 10 ℃ to hold. The PCR products were sequenced using Illumina MiSeq/NovaSeq platform at Personal Biotechnology, Shanghai, China.

2.4. Sequence analysis

Sequences were quality filtered, denoised, merged and chimera removed using QIIME2 DADA2 (Bolyen et al., 2019). The DADA2 program attempts to rebuild the exact biological sequences found in the sample, known as Amplicon Sequence Variants (ASVs). In order to analyze all samples at the same sequencing depth level in the subsequent analysis, a certain number of sequences were randomly selected from each sample to reach an even depth by using the Rarefaction method (Kenneth et al., 1975; Kemp and Aller, 2004). The number of reads to normalize the samples was set as 95% of the sequences for the minimum sample. Therefore, the normalized sampling depths were 50,719 (53, 389 ×95%) reads per sample for fungi and 46,542 (48,992 ×95%) reads per sample for bacteria.

Shannon-Wiener diversity, a represented index of alpha diversity, was shown to be the species richness, diversity, and evenness within homogeneous habitats (Shannon, 1948). The beta diversity matrix was visualized using principal coordinate analysis (PCoA) to reduce the dimension of multidimensional microbial data and show the trend of changes through the distribution of samples on the coordinate axis (Ramette, 2007). The Bray-Curtis distance was used to calculate the difference distance between samples (Bray and Curtis, 1957). The differences between treatments were analyzed by PerMANOVA using Adonis function of vegan package (Version 2.5-7, https://github.com/vegandevs/vegan/) within the R (Version 4.0.5) environment.

Taxonomic trees in packed circles were plotted by using the circle packing charts to draw the classification tree of microorganisms, while the abundance of each ASV group was added to the diagram as a pie chart. The largest circle represents phylum level, and the decreasing circle represents class, order, family, genus, and species, respectively. Linear discriminant analysis (LDA) effect size (LEfSe) was conducted to identify potential biomarkers that caused significant differences in taxonomic abundance amongst various groups (LDA score > 4.0, $P < 0.05$).

Pearson correlation coefficient ($P < 0.05$) was performed to analyze the correlations among microorganisms, which was plotted by the genescloud tools, a free online platform for data analysis (https://www.genescloud.cn). The relationship among fruit firmness, disease index and microbial community diversity were analyzed by using redundancy analysis (RDA) and the results were visualized using the genescloud tools. The metagenomeSeq method was used to compare ozone treatment (5 μL L^{-1}) with control (0 μL L^{-1}), and the results of metagenomeSeq analysis were further demonstrated by Manhattan Plot.

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) was used to analyze the potential function of microbial communities, and the MetaCyc database (https://metacyc.org/) was used to predict fungal metabolic pathways.

2.5. Fruit firmness

The fruit firmness was determined after peeling about 1 mm thick at the equator with a handheld firmness meter (GY-4, Tuopu, China). Moreover, redundancy analysis was used to investigate the link among disease index, fruit firmness and microbial community following ozone

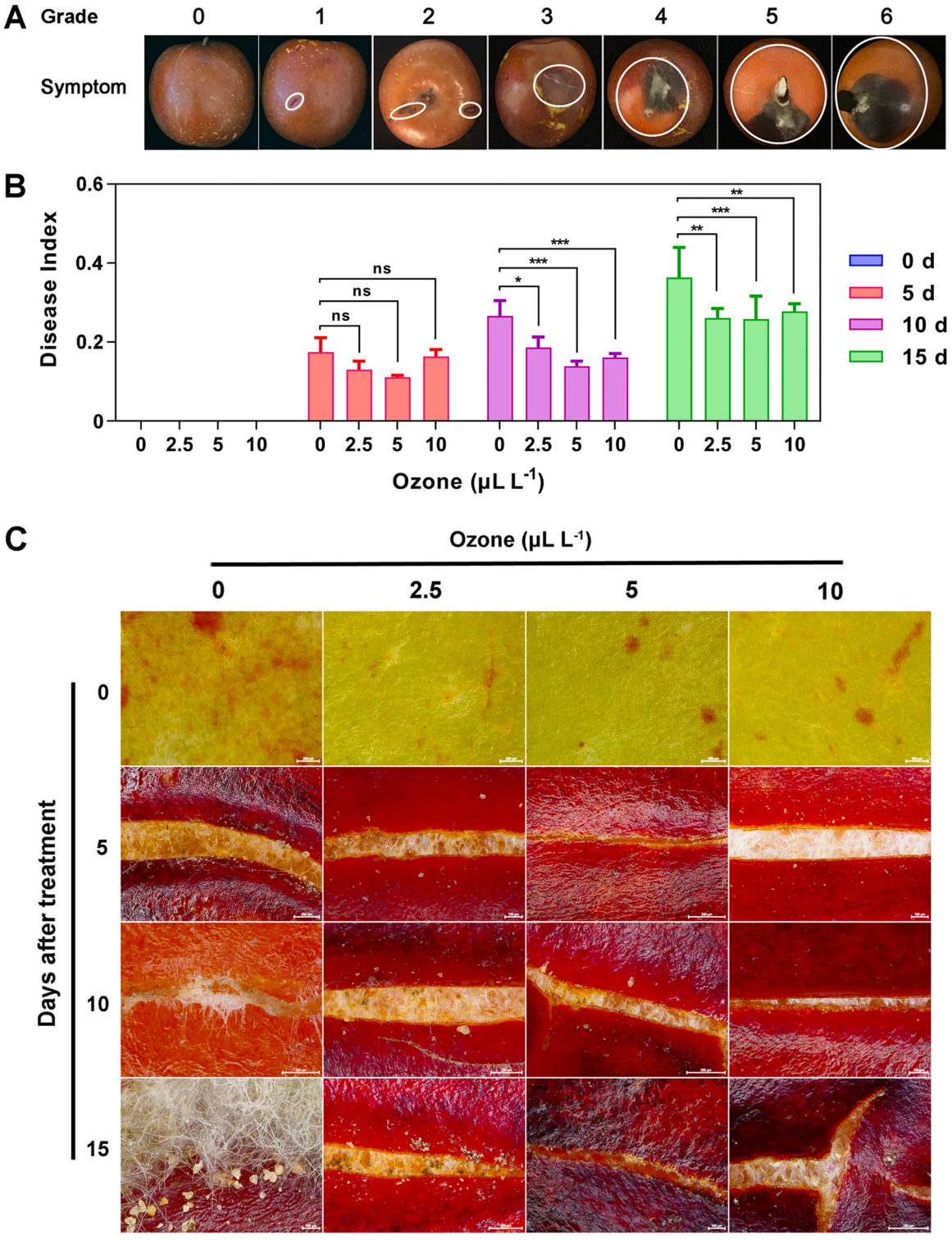

Fig. 1. Fruit decay of jujube (*Ziziphus jujuba* Miller cv. Dongzao) was reduced by ozone at 2.5 μL L^{-1}, 5 μL L^{-1} and 10 μL L^{-1} during storage. A: Symptoms of the grades based on the degree of disease, which were marked by the spare circles. B: Disease index of jujube fruit treated with ozone at different concentrations. Significant differences were analyzed by two-way analysis of variance (ANOVA) and indicated by 'ns' for $P > 0.05$, '*' for $P < 0.05$, '**' for $P < 0.01$ and '***' for $P < 0.001$, respectively; C: Observation of fungal hypha on fruit surface wounds, indicating by the arrows under ultra-depth of field microscope.

treatments at 0 and 5 μL L^{-1} on day 15.

2.6. Statistical analysis

The figures of disease index and fruit firmness were plotted by GraphPad Prism 8 Software (GraphPad Inc., CA, USA). Two-way analysis of variance (ANOVA) was used to test the significance of different groups. Figures of alpha diversity, taxonomic tree in packed circles, LEfSe, Pearson correlation, RDA, and metagenomeSeq analysis were created by using the genescloud tools (https://www.genescloud.cn) and modified by Adobe Illustrator CC 2018 (Adobe, USA). PerMANOVA was performed by vegan package (Version 2.5-7, https://github.com/vegandevs/vegan/) using Adonis function within the R (Version 4.0.5) environment.

3. Results

3.1. Ozone reduced jujube fruit decay

There was no significant difference in disease index among treatments within five days after ozone treatment (Fig. 1A, B). Indeed, the disease indexes of D5_0, D5_2.5, D5_5 and D5_10 were 0.175, 0.131, 0.111 and 0.164, respectively. Afterwards, compared with the control group (0 μL L^{-1}), ozone treatment at different concentrations significantly inhibited fruit decay at days 10 and 15. The disease indexes of D10_0, D10_2.5, D10_5 and D10_10 were 0.267, 0.186, 0.139 and 0.161, respectively, while the disease indexes of D15_0, D15_2.5, D15_5 and D15_10 were 0.364, 0.261, 0.258 and 0.278, respectively.

With the increase of storage time, more and more mycelia were observed in the wounds of the control group (Fig. 1C). The growth of fungi was largely inhibited by ozone with concentrations being higher than 2.5 μL L^{-1}, indicating that ozone could reduce the fruit decay of jujube by inhibiting fungal proliferation on fruit surface.

3.2. Comparison of alpha and beta diversity in response to ozone treatment

Bacterial 16 S rRNA gene and fungal internal transcription spacer (ITS) region were amplified and sequenced to identify microbial communities in various treatments of jujube fruit. After sequence denoising, there were 5,456,575 reads in fungi, which were assigned to 720 ASVs, and 5,933,854 reads in bacteria, which were assigned to 19,783 ASVs. In order to facilitate the comparative analysis of all samples, the data were normalized at an even sampling depth, with 50,719 sequences per sample for fungi and 46,542 sequences per sample for bacteria.

Alpha and beta diversity indices were used to characterize the intra-

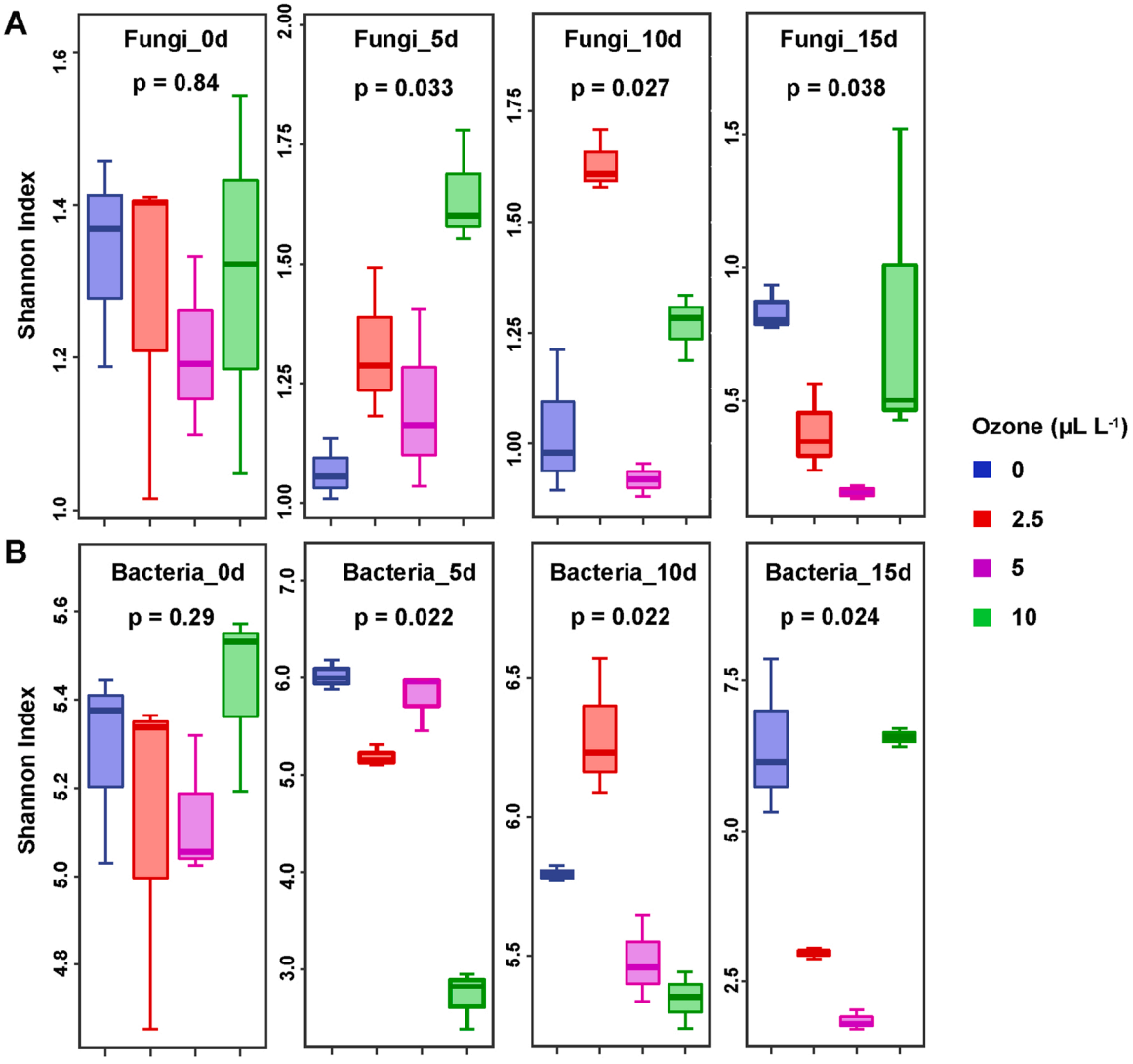

Fig. 2. Box plots showing the alpha diversity (Shannon Index) variation in fungal (A) and bacterial (B) communities of winter jujube fruit treated with various ozone concentrations for 0, 5, 10, and 15 days. Significance of the difference was verified by rank sum test by Kruskal-Wallis.

and inter-habitat diversity of species, respectively. The Shannon index indicates the richness and evenness of microbial community within the group, which is a representative index of alpha diversity. According to Fig. 2, no significant difference ($P > 0.05$) was observed among the samples in both fungi and bacteria on day 0. When treated with ozone for 5, 10 and 15 days, the Shannon index showed significant differences ($P < 0.05$) among the samples in both fungi and bacteria. Our results suggested that ozone treatment at 2.5 μL L^{-1}, 5 μL L^{-1} and 10 μL L^{-1} significantly changed the microbial diversity of jujube fruit surface, indicating both fungi and bacteria are sensitive to the ozone.

Principal coordinates analysis (PCoA) was drawn further to analyze the differences in beta diversity between different habitats. PCoA analysis based on Bray-Curtis dissimilarities was computed and visualized at individual sampling times in both fungal (Fig. 3A) and bacterial communities (Fig. 3B). The distance between the two points projected on the coordinate axis indicates the similarity of community composition of the two samples in corresponding dimensions, and the differences between treatments were analyzed by PerMANOVA using Adonis function of vegan package. As shown in Fig. 3, it is apparent that significant differences were observed between treatments after 5 days of ozone treatments and increased over time in both fungi and bacteria. The control group (0 μL L^{-1}) was significantly different from the ozone treatments with various concentrations on day 15, indicating that ozone treatment disturbed severely the microbial diversity of jujube fruit surface.

3.3. Microbial composition at all taxonomic levels

Taxonomic tree in packed circles illustrating the overall abundance of taxa at different levels revealed distinct differences in the abundance and distribution of taxa between ozone treatment and control (Fig. 4). *Didymella* was found to be the most abundant fungal genus, followed by *Rhizopus*, *Alternaria*, *Phialemoniopsis*, *Mycosphaerella*, *Filobasidium*, *Buckleyzyma*, *Erythrobasidium*, *Symmetrospora* and *Hannaella* (Fig. 4A). However, the relative abundance of *Didymella* was only 12.96% in D15_0, while *Rhizopus* was found to have the highest relative abundance of 84.50% (Fig. 4B). The fungal genus *Rhizopus* might be ozone-sensitive, as evidenced by its decreased abundance in the ozone-treated groups. For bacteria, *Methylobacterium* was superior in all samples at the genus level, followed by *Pseudomonas*, *Pantoea*, *Sphingomonas*, *Gluconobacter*, *Novosphingobium*, *Curtobacterium*, *Acinetobacter*, *Aureimonas* and *Hymenobacter* (Fig. 4C). However, in the group with the most severe disease occurrence (D15_0), the relative abundance of *Methylobacterium* decreased to 13.05%, while the bacterium with the highest abundance was replaced by *Gluconobacter* with a relative abundance of 31.52% (Fig. 4D). These results suggest that *Gluconobacter* may be closely related to fruit decay.

3.4. Biomarkers identification by linear discriminant analysis effect size

The differences in fungal and bacterial community with ozone treatment and incubation time were analyzed using linear discriminant analysis (LDA) effect size (LEfSe) to uncover biomarkers among various treatment groups (Fig. 5, Fig. S1). LDA value distribution showed that 3 fungal genera and 10 bacterial genera were significantly enriched in several groups (LDA score > 4.0, $P < 0.05$, Fig. 5A, Fig. S1A). Specifically, *Rhizopus* was differentially enriched in the control group on day 15 (D15_0), while *Verticillium* and *Didymella* were differentially abundant in the ozone-treated group at 5 μL L^{-1} on day 10 (D10_5) and 15 (D15_5), respectively. For bacteria, *Gluconobacter*, *Aeromonas*, *Acinetobacter*, and *Muribaculaceae* were differentially enriched in control group on day 15 (D15_0) (Fig. 5B, Fig. S1B). The abundances of *Methylobacterium* and *Novosphingobium* were significantly enriched in the ozone-treated group at 2.5 μL L^{-1} on day 10 (D10_2.5). On the other hand, the genera *Pantoea* and *Sphingomonas* were considerably abundant in the ozone-treated group at 10 μL L^{-1} on day 10 (D10_10). Differences in the abundance of *Pseudomonas* separated the ozone-treated group at 10 μL L^{-1} on day 5 (D5_10) from other groups, and *Curtobacterium* was identified as differentially abundant in the ozone-treated group at 2.5 μL L^{-1} on day 15 (D15_2.5).

3.5. Correlations of fungi and bacteria

Pearson correlation was used to reveal the relationship between the core fungal and bacterial genera of jujube fruit microbiome in response to ozone treatment (Fig. 6). The synergetic or antagonistic functions between microorganisms were observed by calculating the positive or negative correlations with their abundances. Fungal genus *Rhizopus* was positively correlated with bacterial genera *Gluconobacter* (r = 0.97) while negatively correlated with fungal genus *Didymella* (r = −0.96). The abundance of *Sphingomonas* had significant positive correlations with the abundances of *Novosphingobium* (r = 0.93), *Aureimonas* (r = 0.87), *Methylobacterium* (r = 0.86) and *Hymenobacter* (r = 0.85). *Filobasidium* was positively correlated with *Symmetrospora* (r = 0.84), *Erythrobasidium* (r = 0.55), *Hannaella* (r = 0.71), *Alternaria* (r = 0.43), *Mycosphaerella* (r = 0.80), *Buckleyzyma* (r = 0.66) and *Phialemoniopsis*

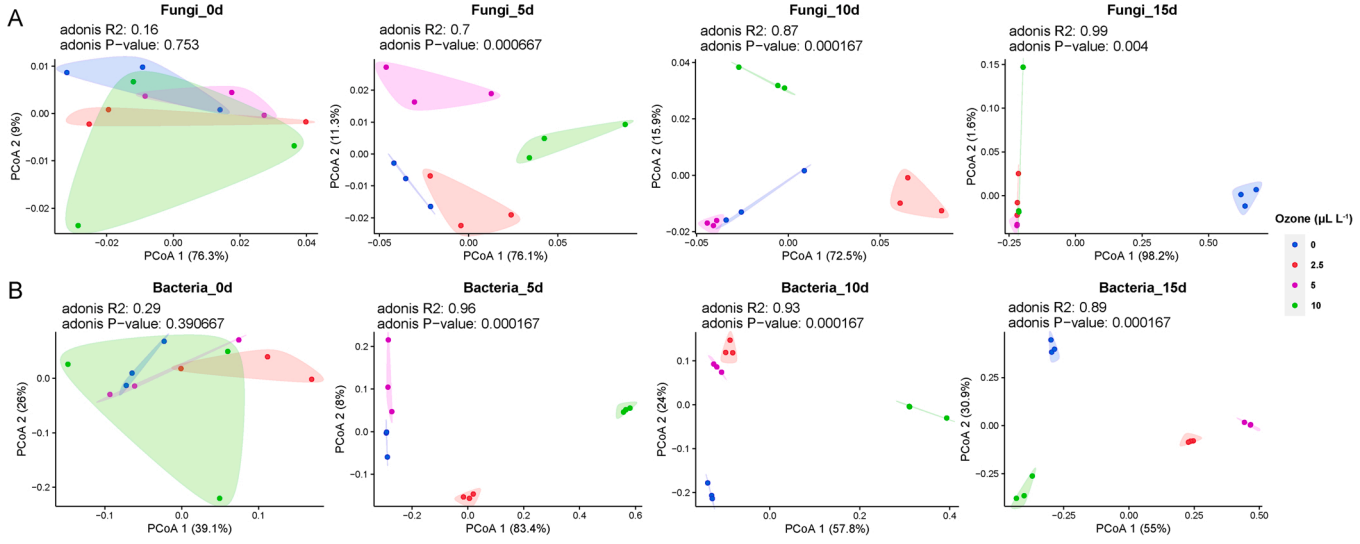

Fig. 3. Principal coordinates analysis (PCoA) plots of Bray-Curtis dissimilarities in the fungal (A) and bacterial (B) communities of winter jujube fruit treated with various ozone concentrations for 0, 5, 10, and 15 days. PerMANOVA was performed by vegan package using Adonis function.

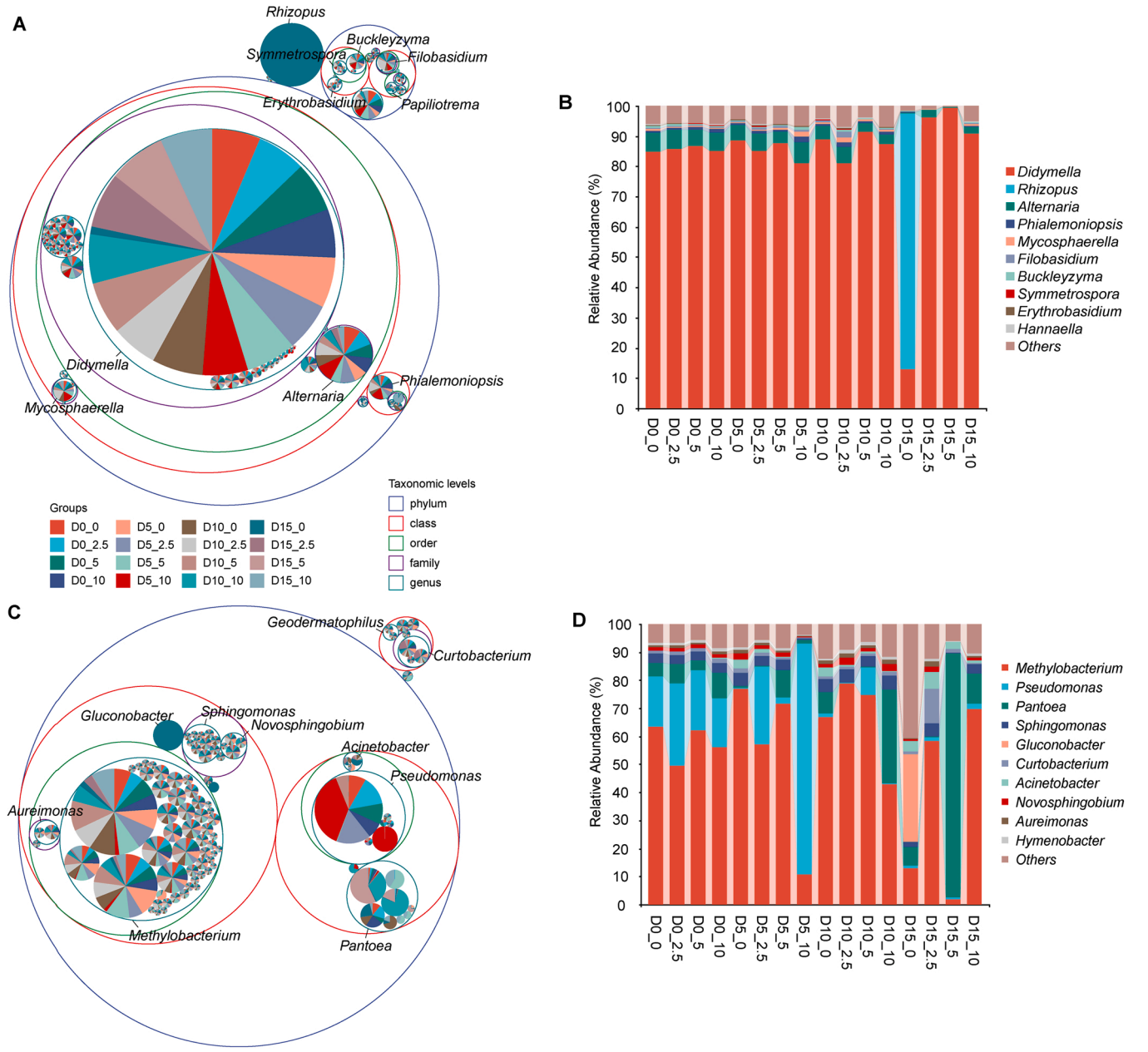

Fig. 4. Microbiological composition at all taxonomic levels in packed circles and at genus level in histogram of fungal (A, B) and bacterial (C, D) communities on winter jujube fruit surface. For the taxonomic tree, the largest circle represents the phylum level, and the progressively smaller circles symbolize the level of class, order, family, genus, and species in gradient order with different colors. The abundance of taxa corresponding to the circle is indicated by the area of the origin within the circle. Each amplicon sequence variant (ASV) is displayed as a pie chart, showing the proportion of the ASVs in each group.

(r = 0.62).

3.6. Relationship between fruit firmness, disease index and microbial community

Fruit firmness is one of the most important factors of fruit quality because it is related to fruit ripeness. As shown in Fig. 7A, fruit firmness loss was significantly delayed by ozone at 2.5, 5 and 10 μL L^{-1} compared to the control group (0 μL L^{-1}), and the most significant effect was found by 5 μL L^{-1}. Redundancy analysis showed the relationship among disease index, firmness and microbial community following ozone treatment at 0 and 5 μL L^{-1} (Fig. 7B). The abundance of *Rhizopus* was positively correlated with disease index but negatively correlated with firmness, indicating ozone treatment might affect the growth of *Rhizopus*.

3.7. Ozone affects fruit quality by altering the abundance of fungi with specific functions

In order to determine the features that are differentially abundant between ozone treatment at 0 and 5 μL L^{-1}, we further analyzed the differences between the two groups using metagenome Seq and demonstrated the differential ASVs and taxonomic annotations with Manhattan plot. Compared with ozone treatment at 5 μL L^{-1} for 15 days, ASV2 of *Rhizopus* was significantly up-regulated in control, while ASV1 of *Didymella* was significantly down-regulated (Fig. 8A). Taken together, these results suggest that ozone at 5 μL L^{-1} significantly inhibited the growth of *Rhizopus*, reduced fruit decay and maintained higher firmness.

Additionally, the metabolic pathways of fungi were predicted by PICRUSt2 in MetaCyc database, and the differences in the abundance of metabolic pathways with ozone (5 μL L^{-1}) or without ozone treatment

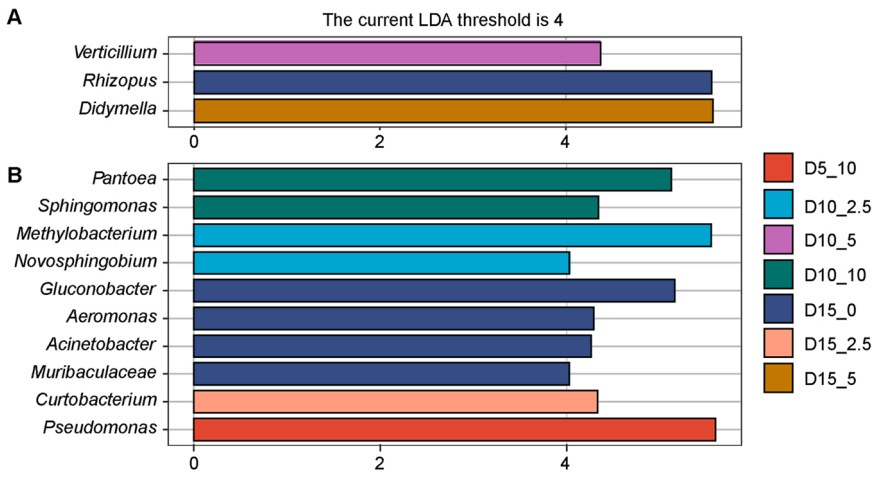

Fig. 5. Linear discriminant analysis (LDA) effect size (LEfSe) of fungi (A) and bacteria (B) on winter jujube fruit surface.

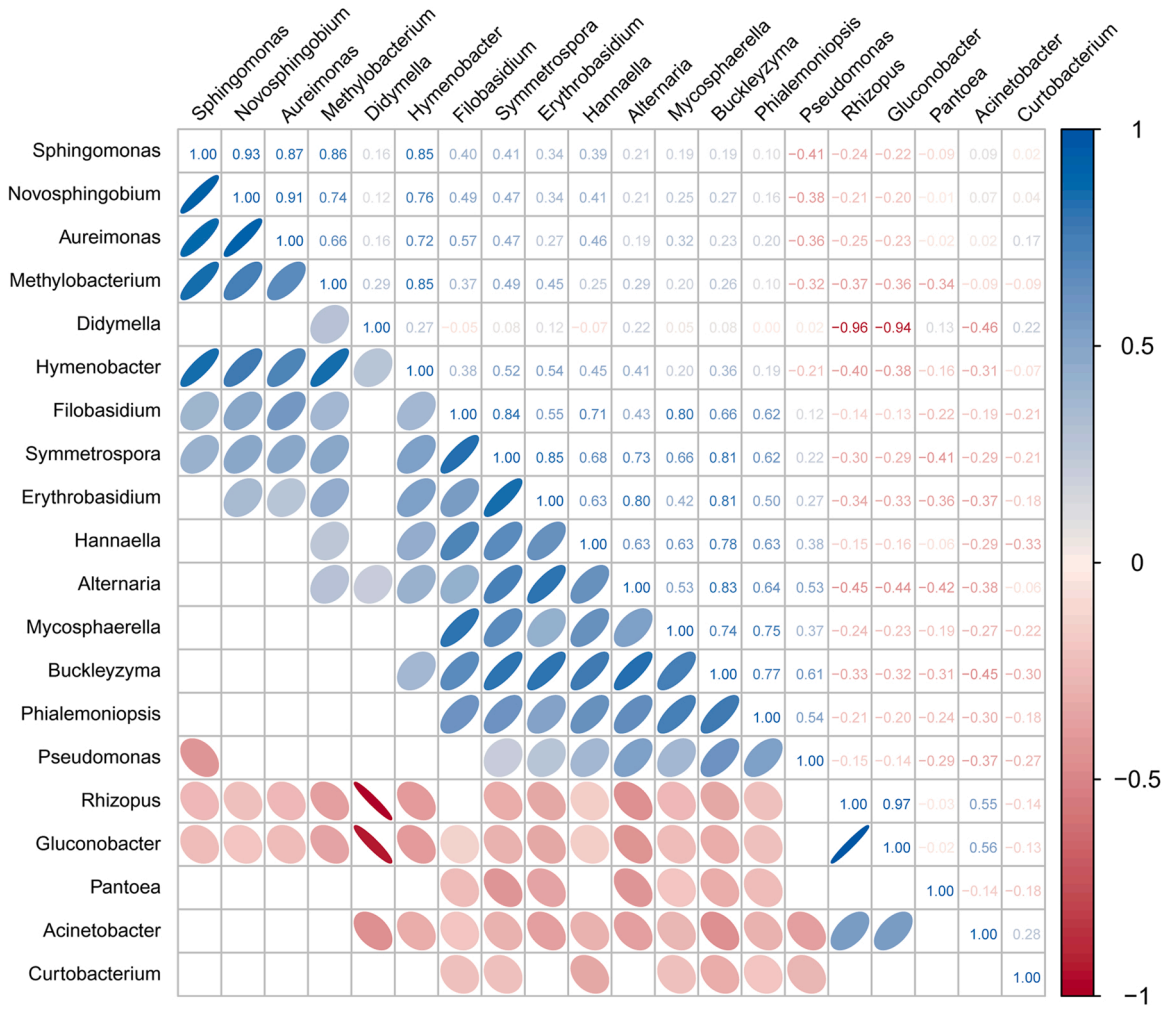

Fig. 6. Correlations of fungal and bacterial genera identified in winter jujube fruit microbiome in response to ozone treatment. Pearson correlation coefficient was analyzed ($P < 0.05$). The lower triangle used ellipses to show correlations, positive correlations were shown in blue, negative correlations shown in red, and nonsignificant correlations not shown, while the upper triangle showed correlation values.

are shown in Fig. 8B. It is notable that ozone treatment significantly reduced the metabolic pathways of nucleoside and nucleotide biosynthesis, amino acid biosynthesis, fatty acid and lipid degradation, respiration and electron transfer of fungi, suggesting that ozone may have reshaped the microbial community on the surface of jujube fruit by changing the abundance of fungi through these pathways.

4. Discussion

A thorough understanding of microbial composition on fruit surface

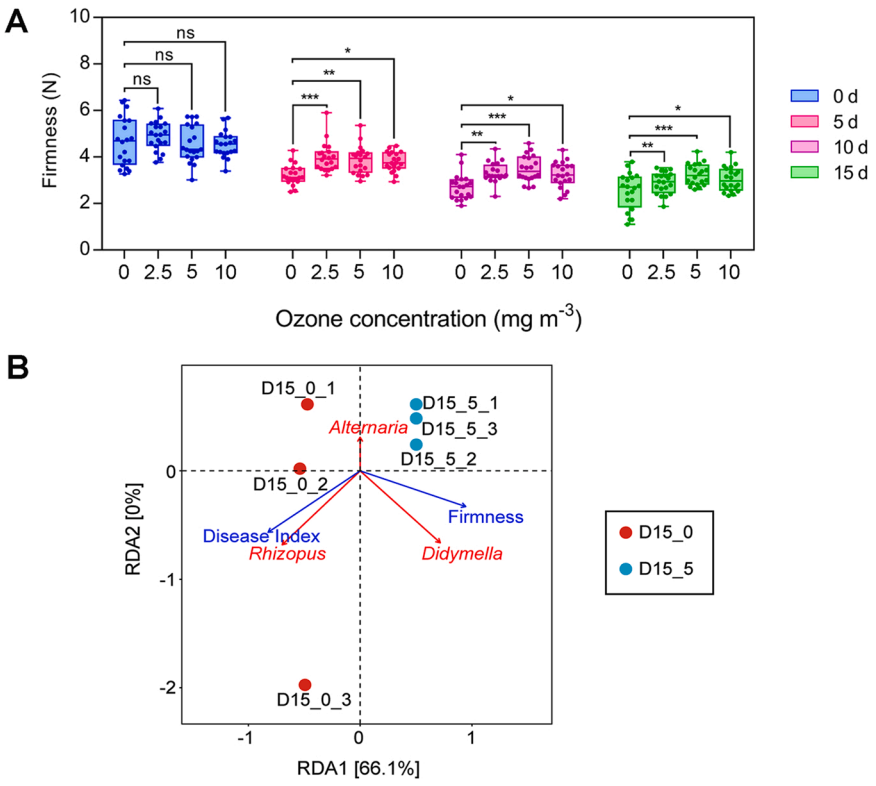

Fig. 7. The firmness and its relation to disease index, fungal community in response to ozone treatment in winter jujube fruit. A: Changes in fruit firmness with or without ozone treatment (ns: no significance, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$); B: Redundancy analysis (RDA) for fruit firmness, disease index, fungal community and ozone treatment at 0 μL L^{-1} and 5 μL L^{-1} on day 15. The acute angle indicated a positive correlation between two factors, while the obtuse angle indicated a negative correlation, and right angles suggested no correlation.

and the fruit quality during postharvest storage will help to develop and improve the preservation technology. There were very few studies on the alterations of microbial community after ozone treatment in fruit. For cantaloupes, ozone treatment at 15.008 mg m^{-3} provided optimal postharvest results, inhibiting most of the bacteria and fungi that lead to spoilage of fruit and human pathogenicity (Chen et al., 2020). For grapes, ozone reduced the total number of fungi and the fungal diversity on the grapefruit surface, as well as the occurrence of diseases (Gao et al., 2020). The changes of bacterial and fungal communities on the surface of winter jujube fruit under various concentrations of ozone treatments during postharvest storage have been reported here. Previously, it has been shown that environmental factors influence the microbial diversity in different habitats (Pereira et al., 2020). This study focused on comparing the effects of ozone treatment on surface microorganisms of winter jujube rather than on different producing areas, so jujube fruit were collected in only one orchard. There may be differences in microbial communities of winter jujube from different producing areas, and their response to ozone may be different from this result, which needs and deserves further study.

Winter jujube spoils rapidly due to its sensitivity to postharvest pathogenic infections, which strongly limits shelf life and affects the quality attributes (Wang et al., 2011). An increasing number of pathogenic fungi and bacteria have been found to cause jujube fruit decay. However, the prevalence of certain fungi and bacteria associated with decay were also found in healthy samples (Diskin et al., 2017; Yurgel et al., 2018; Wu et al., 2019). *Didymella* has been reported to cause many vegetable diseases, including tomatoes (Phillips, 1961), peppers (Duong and Lee, 2016), bitter gourd (Singh et al., 2013) and some species of cucurbits (Keinath et al., 1995). However, its role in postharvest jujube fruit decay has rarely been documented. In this study, a large amount of *Didymella* was detected on the surface of both healthy and decayed fruit during the storage of winter jujube, indicating that it might be able to coloniz on the fruit surface of winter jujube and cause the latent infection, although its precise role in decay needs to be further studied. Similarly, *Alternaria* was also confirmed to be abundant on the surface of winter jujube fruit, suggesting that it might be one of the primary sources causing fruit decay. In 2017, severe fruit rot disease of jujube caused by *Alternaria alternat* was observed in several markets in Pakistan, with disease incidence ranging from 15% to 26% (Alam et al., 2018). Recently, *A. alternat* was identified as the major pathogen of winter jujube with a high isolation rate in Hebei province, China (Song et al., 2016). However, *Rhizopus* was the most prevalent in the ozone-free group with severe jujube fruit decay, and *Didymella* and *Alternaria* were crowded out with the proliferation of *Rhizopus* in the later stage. Likewise, *Rhizopus* has also been identified as a prominent postharvest pathogen, causing postharvest losses in a wide variety of fruits (Kwon et al., 2012, 2011; Pang et al., 2021). Researchers have investigated a variety of methods for combating this difficult-to-eradicate fungus, including gaseous ozone treatment (Fan, 2021). Interestingly, this study found that ozone treatment can significantly reduce the abundance of *Rhizopus*, indicating that ozone is an effective means to control *Rhizopus* reproduction.

Methylobacterium was the most abundant bacterial genus in this study, yet there have been no reports of fruit decay caused by bacterial species in this genus. *Methylobacterium oryzae* CBMB20 enhanced photosynthesis and decreased volatile emissions in salt-stressed rice plants via delaying ethylene-dependent responses and activating vacuolar H^+-ATPase (Chatterjee et al., 2019). Therefore, it may play a probiotic role during the storage of postharvest winter jujube. *Gluconobacter*, a Gram-negative bacterium, has been found abundant during the decay of many kinds of fruits, including pear (Zhang et al., 2021b), apple (Keer et al., 1981) and peach (Burgos et al., 2017), etc. However, it is mostly known as an industrial bacterium (Kommanee et al., 2011), and some species in this genus have antagonistic effects against plant pathogenic fungi (Martina et al., 2013). *Gluconobacter* was identified as the most abundant bacteria in the ozone-free jujube fruit after 15 days of storage. Fruit firmness is closely related to fruit quality and can be used as an important index of fruit senescence. It has been reported that the ozone-treated kiwifruit retained higher fruit firmness and titratable acidity, and the defense-related enzyme activity was

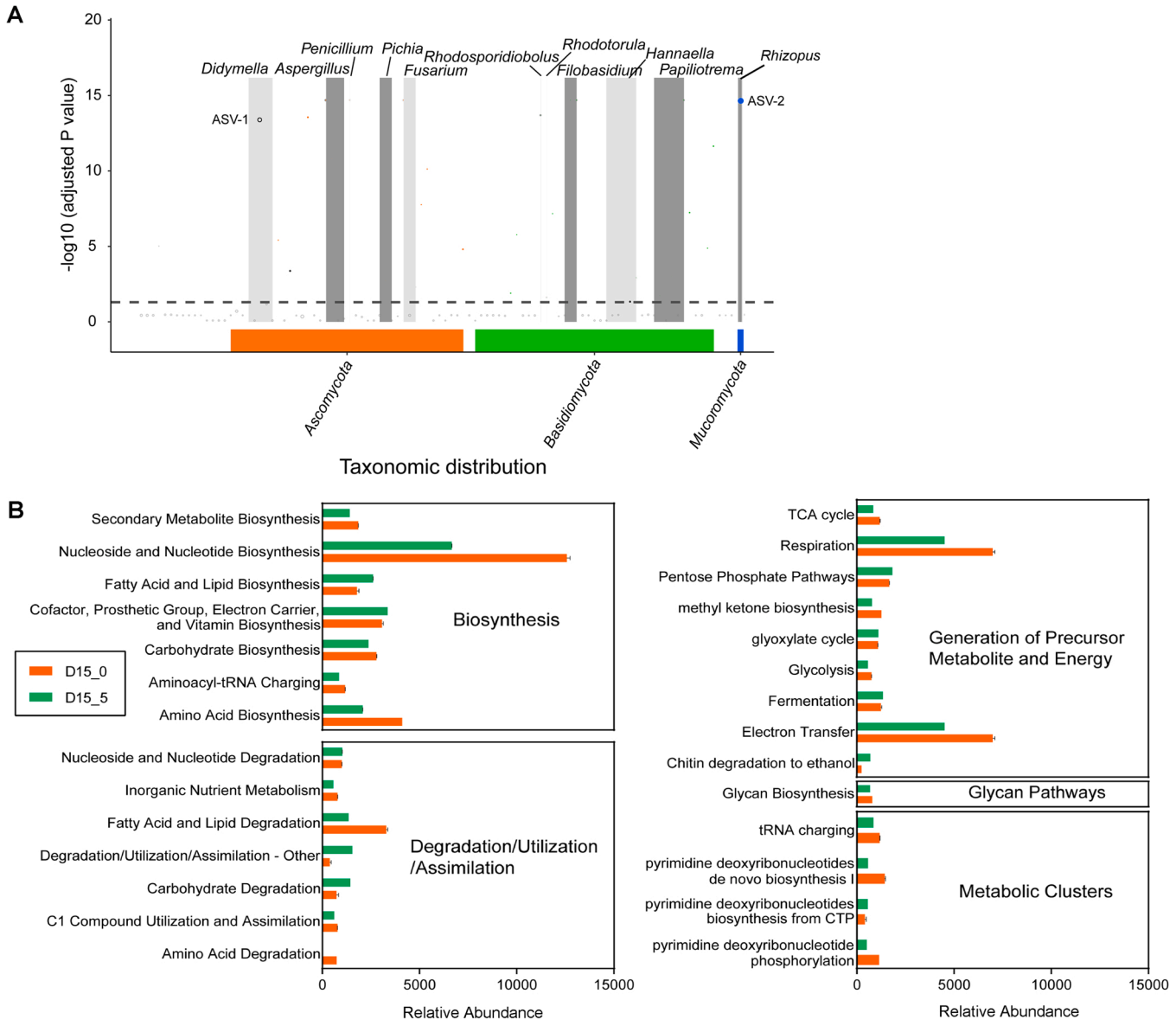

Fig. 8. Alteration of microbial diversity and metabolic pathways on fruit surface of winter jujube in response to ozone treatment. A: MetagenomeSeq analysis between ozone treatment at 0 and 5 μL L^{-1}. The horizontal coordinate showed the information from phylum to species, while the vertical coordinate showed the significant difference value with -log10 (adjusted p value). Each ASV was represented as a point or circle in the coordinate system, and its size denoted its relative abundance in log2(CPM/n) (CPM: Copy per million, n: Sample number). The points with significant difference were marked by colored dots or rings, the insignificant points were marked by gray rings; B: Relative abundance of metabolic pathways predicted by PICRUSt2 within MetaCyc categories for ozone (green bar) and without treatment (orange bar).

incredibly increased (Luo et al., 2019). Many studies have claimed that an appropriate ozone concentration could significantly improve fruit quality at the proper temperature and humidity in many fruit (Selma et al., 2008; Salvador et al., 2010; Anibal et al., 2015; Glow Ac Z and Rees, 2016). Ozone significantly maintained higher fruit firmness of winter jujube, which may be one of the reasons for maintaining the higher fruit quality in the ozone-treated group than control.

Furthermore, we found that ozone significantly inhibited the metabolism pathways which are closely related to fungal growth and host invasion. In the present study, pathway of nucleoside and nucleotide biosynthesis was found to be largely suppressed by ozone, which has been reported to be used as a target for the antifungal activity of amphotericin B (Dithi et al., 2014). Antifungal chemotherapy normally targets enzymes catalyzing certain steps of amino acid production (Jastrzebowska and Gabriel, 2015), which have also been shown to be suppresed by ozone treatment in this study. The L-glutamine biosynthetic enzymes of *Aspergillus niger* have been proposed as antifungal targets by inhibiting fungal growth or changes in mycelial morphology (Choudhury and Punekar, 2007; Noor and Punekar, 2005). The respiratory chain has been determined to be inhibited by ozone in this study, which was proposed as a target to develop new methods to control fungal pathogens. For instance, anthracene-9-carboxylic acid (A9C) and niflumic acid (NFA) have been found to significantly decrease the growth and respiration of mycelium by inhibiting growth, energy metabolism and anionic current of fungus *Phycomyces blakesleeanus* (Stanić et al., 2017). Therefore, ozone may have similar patterns of inhibition on the pathogens of winter jujube, however, the underlying mechanism needs further study.

5. Conclusions

In conclusion, the research has shown that ozone treatment

significantly changed the microbial diversity of fruit surface and reduced the decay of winter jujube. *Didymella*, *Rhizopus*, *Alternaria*, *Phialemoniopsis* and *Mycosphaerella* in fungi and *Methylobacterium*, *Pseudomonas*, *Pantoea*, *Sphingomonas* and *Gluconobacter* in bacteria were found to be dominant genera on "Huanghua" winter jujube fruit surface during postharvest storage. Ozone treatments reduced the abundance of *Rhizopus* and *Gluconobacter* considerably and maintained the firmness of winter jujube fruit. Metabolic pathways including nucleoside and nucleotide biosynthesis, amino acid biosynthesis, fatty acid and lipid degradation, respiration, and electron transfer may involve in the reduction of fruit decay in response to ozone treatments. These findings have significant implications for us to understand how ozone reduces fruit decay by the altering the microbial community structure on fruit surface of winter jujube.

CRediT authorship contribution statement

Yang Zhang: Conceptualization, Methodology, Writing – original draft, Funding acquisition. **Md. Mahidul Islam Masum**: Software, Writing – review & editing. **Congcong Gao**: Methodology, Validation. **Yudou Cheng**: Validation, Resources. **Junfeng Guan**: Conceptualization, Methodology, Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors would like to declare that there is no conflict of interest in this paper.

Data availability

Data of ITS and 16S amplicon sequencing of the samples were submitted to NCBI under the Bioproject Id PRJNA795762.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.micres.2022.127110.

References

Alam, M.W., Rehman, A., Malik, A.U., Khan, S.M., Ali, S., Aslam, S., Hameed, A., Sarfraz, S., 2018. First report of *Alternaria alternata* causing postharvest fruit rot of jujube in Pakistan. Plant Dis. 102, 452–453. https://doi.org/10.1094/pdis-09-17-1430-pdn.

Anibal, C.M., Eifert, J.D., Williams, R.C., Marcy, J.E., Welbaum, G.E., 2015. Shelf life determination of fresh blueberries (*Vaccinium corymbosum*) stored under controlled atmosphere and ozone. Int. J. Food Sci. 2015, 164143 https://doi.org/10.1155/2015/164143.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K. C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27, 325–349. https://doi.org/10.2307/1942268.

Burgos, M., Aguayo, M., Pulido, R.P., Gálvez, A., Lucas, R., 2017. Analysis of the bacterial biodiversity of peaches under refrigerated storage after treatment by high hydrostatic pressure. Food Bioprod. Process. 102, 55–61. https://doi.org/10.1016/j.fbp.2016.12.003.

Chatterjee, P., Kanagendran, A., Samaddar, S., Pazouki, L., Sa, T.-M., Niinemets, Ü., 2019. *Methylobacterium oryzae* CBMB20 influences photosynthetic traits, volatile emission and ethylene metabolism in *Oryza sativa* genotypes grown in salt stress conditions. Planta 249, 1903–1919. https://doi.org/10.1007/s00425-019-03139-w.

Chen, C., Zhang, H., Zhang, X., Dong, C., Xue, W., Xu, W., 2020. The effect of different doses of ozone treatments on the postharvest quality and biodiversity of cantaloupes. Postharvest Biol. Technol. 163, 111124 https://doi.org/10.1016/j.postharvbio.2020.111124.

Choudhury, R., Punekar, N.S., 2007. Competitive inhibition of glutamate dehydrogenase reaction. Febs Lett. 581, 2733–2736. https://doi.org/10.1016/j.febslet.2007.05.032.

Diskin, S., Feygenberg, O.O., Maurer, D., Droby, S., Alkan, N., 2017. Microbiome alterations are correlated with occurrence of postharvest stem-end rot in mango fruit. Phytobiomes J. 1, 117–127. https://doi.org/10.1094/Pbiomes-05-17-0022-R.

Dithi, B., Lauren, B., Panepinto, J.C., Joy, S., 2014. Inhibition of nucleotide biosynthesis potentiates the antifungal activity of Amphotericin B. PLoS One 9, e87246. https://doi.org/10.1371/journal.pone.0087246.

Droby, S., Wisniewski, M., 2018. The fruit microbiome: a new frontier for postharvest biocontrol and postharvest biology. Postharvest Biol. Technol. 140, 107–112. https://doi.org/10.1016/j.postharvbio.2018.03.004.

Duong, T.T., Lee, H.B., 2016. A new record of Didymella pinodella isolated from a fruit of red pepper in Korea. International Meeting of the Microbiological Society of Korea 4, 5–5.

Fan, X., 2021. Gaseous ozone to preserve quality and enhance microbial safety of fresh produce: recent developments and research needs. Compr. Rev. Food Sci. Food Saf. 20, 4993–5014. https://doi.org/10.1111/1541-4337.12796.

Gao, C., Lin, Q., Dong, C., Ji, H., Yu, J., Chen, C., Zhu, Z., Ban, Z., Zhang, N., Bao, Y., 2020. Effects of ozone concentration on the postharvest quality and microbial diversity of *Muscat Hamburg* grapes. Rsc Adv. 10, 9037–9045. https://doi.org/10.1039/c9ra10479h.

Glow Ac Z, M., Rees, D., 2016. Exposure to ozone reduces postharvest quality loss in red and green chilli peppers. Food Chem. 210, 305–310. https://doi.org/10.1016/j.foodchem.2016.04.119.

Jastrzebowska, K., Gabriel, I., 2015. Inhibitors of amino acids biosynthesis as antifungal agents. Amino Acids 47, 227–249. https://doi.org/10.1007/s00726-014-1873-1.

Keer, C.V., Abeele, P., Swings, J., Gosselé, F., Ley, J.D., 1981. Acetic acid bacteria as causal agents of browning and rot of apples and pears. Zent. Bakteriol. Mikrobiol. Hyg. Abt. Orig. C Allg. Angew. ökologische Mikrobiol. 2, 197–204. https://doi.org/10.1016/S0721-9571(81)80040-3.

Keinath, A.P., Farnham, M.W., Zitter, T.A., 1995. Morphological, pathological, and genetic differentiation of *Didymella bryoniae* and *Phoma* spp. isolated from cucurbits. Phytopathology 85, 364–369. https://doi.org/10.1094/Phyto-85-364.

Kemp, P.F., Aller, J.Y., 2004. Bacterial diversity in aquatic and other environments: what 16S rDNA libraries can tell us. FEMS Microbiol. Ecol. 161–177. https://doi.org/10.1016/S0168-6496(03)00257-5.

Kenneth, L., Heck Jr., Gerald, van, Belle, Daniel, Simberloff, 1975. Explicit calculation of the rarefaction diversity measurement and the determination of sufficient sample size. Ecology 56, 1459–1461. https://doi.org/10.2307/1934716.

Kommanee, J., Yukphan, P., Moonmangmee, D., Thongchul, N., Yamada, Y., 2011. Identification and oxidation products of *Gluconobacter* strains isolated from fruits and flowers in Thailand. Int. J. Biol. 4, 69. https://doi.org/10.5539/ijb.v4n1p69.

Kumar, A., Zhimo, V.Y., Biasi, A., Feygenberg, O., Salim, S., Wisniewski, M., Droby, S., 2021. Impact of packhouse treatments on the peel microbiome of mandarin fruit (cv. Orr). Postharvest Biol. Technol. 176, 111519 https://doi.org/10.1016/j.postharvbio.2021.111519.

Kwon, J.-H., Kim, J., Kim, W.-I., 2011. First report of *Rhizopus oryzae* as a postharvest pathogen of apple in Korea. Mycobiology 39, 140–142. https://doi.org/10.4489/MYCO.2011.39.2.140.

Kwon, J.-H., Ryu, J.-S., Chi, T.T.P., Shen, S.-S., Choi, O., 2012. Soft rot of *Rhizopus oryzae* as a postharvest pathogen of banana fruit in Korea. Mycobiology 40, 214–216. https://doi.org/10.5941/MYCO.2012.40.3.214.

Li, J., Fan, L., Ding, S., Ding, X., 2007. Nutritional composition of five cultivars of Chinese jujube. Food Chem. 103, 454–460. https://doi.org/10.1016/j.foodchem.2006.08.016.

Li, M., Wen, X., Wang, Y., Wei, F., Ge, Y., Yang, L., 2012. Study on ozone infection techniques of winter-jujube fruit. Chin. Agric. Sci. Bull. 28, 169–173. https://doi.org/10.3969/j.issn.1000-6850.2012.28.031.

Liu, M., Wang, J., Wang, L., Liu, P., Zhao, J., Zhao, Z., Yao, S., Stănică, F., Liu, Z., Wang, L., Ao, C., Dai, L., Li, X., Zhao, X., Jia, C., 2020. The historical and current research progress on jujube-a superfruit for the future. Hortic. Res. 7, 119. https://doi.org/10.1038/s41438-020-00346-5.

Luo, A., Bai, J., Li, R., Fang, Y., Li, L., Wang, D., Zhang, L., Liang, J., Huang, T., Kou, L., 2019. Effects of ozone treatment on the quality of kiwifruit during postharvest

storage affected by *Botrytis cinerea* and *Penicillium expansum*. J. Phytopathol. 167, 470–478. https://doi.org/10.1111/jph.12819.
Luo, J., He, W., Kuang, H.-l, Han, H.-z, Jiang, K.-n, 2009. Study on the infection and incidence time of jujube fruit diseases. In: Ecol. Sci., 28, pp. 158–163. https://doi.org/10.3969/j.issn.1008-8873.2009.02.012.
Martina, Bevardi, Jadranka, Frece, Dragana, Mesarek, Jasna, Bonir, Jasna, Mrvi, 2013. Antifungal and antipatulin activity of *Gluconobacter oxydans* isolated from apple surface. Arch. Ind. Hyg. Toxicol. 64, 279–284. https://doi.org/10.2478/10004-1254-64-2013-2308.
Merz, U., Lees, A.K., Sullivan, L., Schwärzel, R., Hebeisen, T., Kirk, H.G., Bouchek-Mechiche, K., Hofferbert, H.R., 2012. Powdery scab resistance in *Solanum tuberosum*: an assessment of cultivar × environment effect. Plant Pathol. 61, 29–36. https://doi.org/10.1111/j.1365-3059.2011.02489.x.
Noor, S., Punekar, N.S., 2005. Allosteric NADP-glutamate dehydrogenase from aspergilli: purification, characterization and implications for metabolic regulation at the carbon-nitrogen interface. Microbiology 151, 1409–1419. https://doi.org/10.1099/mic.0.27751-0.
Ong, M.K., Kazi, F.K., Forney, C.F., Ali, A., 2013. Effect of gaseous ozone on papaya anthracnose. Food Bioprocess Technol. 6, 2996–3005. https://doi.org/10.1007/s11947-012-1013-4.
Palou, L., Crisosto, C.H., Smilanick, J.L., Adaskaveg, J.E., Zoffoli, J.P., 2002. Effects of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage. Postharvest Biol. Technol. 24, 39–48. https://doi.org/10.1016/S0925-5214(01)00118-1.
Palou, L., Smilanick, J.L., Crisosto, C.H., Mansour, M., 2007. Effect of gaseous ozone exposure on the development of green and blue molds on cold stored citrus fruit. Plant Dis. 85, 632–638. https://doi.org/10.1094/PDIS.2001.85.6.632.
Pang, L., Adeel, M., Shakoor, N., Guo, K., Ma, D., Ahmad, M.A., Lu, G., Zhao, M., Li, S., Rui, Y., 2021. Engineered nanomaterials suppress the soft rot disease (*Rhizopus stolonifer*) and slow down the loss of nutrient in sweet potato. Nanomaterials 11, 2572. https://doi.org/10.3390/NANO11102572.
Pereira, L.L., Veloso, T., Silva, M., Kasuya, M., Guaroni, R.C., 2020. Effects of environmental factors on microbiota of fruits and soil of *Coffea arabica* in Brazil. Sci. Rep. 10, 14692. https://doi.org/10.1038/s41598-020-71309-y.
Peter, K., Tomislav, C., Ahmed, A., Jarishma, G., Lise, K., Gabriele, B., 2020. Microbiome approaches provide the key to biologically control postharvest pathogens and storability of fruits and vegetables. FEMS Microbiol. Ecol. 96, fiaa119 https://doi.org/10.1093/femsec/fiaa119.
Phillips, D.H., 1961. *Didymella* stem and fruit rot on outdoor Tomatoes I. Studies on sources of infection and their elimination. Ann. Appl. Biol. 48, 245–258. https://doi.org/10.1111/j.1744-7348.1960.tb03521.x.
Prabha, V., Barma, D., Singh, R., Madan, A., 2015. Ozone technology in food processing: a review. Trends Biosci. 8, 4031–4047.
Ramette, A., 2007. Multivariate analyses in microbial ecology. FEMS Microbiol. Ecol. 62, 142–160. https://doi.org/10.1111/j.1574-6941.2007.00375.x.
Salvador, A., Abad, I., Arnal, L., Martínez-Jávega, J.M., 2010. Effect of ozone on postharvest quality of persimmon. J. Food Sci. 71, 443–446. https://doi.org/10.1111/j.1750-3841.2006.00059.x.
Selma, M.V., Ibanez, A.M., Cantwell, M., Suslow, T., 2008. Reduction by gaseous ozone of *Salmonella* and microbial flora associated with fresh-cut cantaloupe. Food Microbiol. 25, 558–565. https://doi.org/10.1016/j.fm.2008.02.006.
Shannon, C.E., 1948. A mathematical theory of communication. Bell Syst. Tech. J. 27, 379–423. https://doi.org/10.1002/j.1538-7305.1948.tb01338.x.
Singh, K., Kpv, S., Singh, K., 2013. *Didymella* black fruit rot of bittergourd. Indian Phytopathol. 49, 294–296.
Song, C., Huang, Y., Xie, C., Jia, Z., Song, S., 2016. Isolation and identification of the pathogen causing black spot disease in dongzao fruits and study on the biocontrol. J. Microbiol. 36, 85–89 https://doi.org/CSCD:5851885.
Stanić, M., Križak, S., Jovanović, M., Pajić, T., Ćirić, A., Žižić, M., Zakrzewska, J., Antić, T.C., Todorović, N., Živić, M., 2017. Growth inhibition of fungus *Phycomyces blakesleeanus* by anion channel inhibitors anthracene-9-carboxylic and niflumic acid attained through decrease in cellular respiration and energy metabolites. Microbiology 163, 364–372. https://doi.org/10.1099/mic.0.000429.
Sujayasree, O.J., Chaitanya, A.K., Bhoite, R., Pandiselvam, R., Khaneghah, A.M., 2021. Ozone: an advanced oxidation technology to enhance sustainable food consumption through mycotoxin degradation. Ozone Sci. Eng. 2021, 1–21. https://doi.org/10.1080/01919512.2021.1948388.
Wang, Y., Tang, F., Xia, J., Yu, T., Wang, J., Azhati, R., Zheng, X.D., 2011. A combination of marine yeast and food additive enhances preventive effects on postharvest decay of jujubes (*Zizyphus* jujuba). Food Chem. 125, 835–840. https://doi.org/10.1016/j.foodchem.2010.09.032.
Wassermann, B., Kusstatscher, P., Berg, G., 2019. Microbiome response to hot water treatment and potential synergy with biological control on stored apples. Front. Microbiol. 10, 2502. https://doi.org/10.3389/fmicb.2019.02502.
Wu, W., Lei, J., Hussain, M., Cao, S., Wang, R., 2019. Structure and function of the fruit microbiome in healthy and diseased kiwifruit. Pak. J. Agric. Sci. 56, 577–585. https://doi.org/10.21162/PAKJAS/19.8820.
Yurgel, S.N., Abbey, L., Loomer, N., Gillis-Madden, R., Mammoliti, M., 2018. Microbial communities associated with storage onion. Phytobiomes 2, 35–41. https://doi.org/10.1094/Pbiomes-12-17-0052-R.
Zhang, H., Ma, L., Jiang, S., Dong, Y., Zheng, X., 2009. Effect of ozone in combination with antagonistic yeast on postharvest gray mold rot incidence in strawberry fruits. Trans. Chin. Soc. Agric. Eng. 25, 258–263. https://doi.org/10.1360/972009-754.
Zhang, Q., Shi, W.C., Zhou, B., Du, H.Y., Xi, L.Q., Zou, M., Zou, H., Xin, L., Gao, Z., Chen, Y.L., 2021a. Variable characteristics of microbial communities on the surface of sweet cherries under different storage conditions. Postharvest Biol. Technol. 173, 111408 https://doi.org/10.1016/j.postharvbio.2020.111408.
Zhang, Y., Gao, C.C., Masum, M.M.I., Cheng, Y.D., Wei, C.Q., Guan, Y.Q., Guan, J.F., 2021b. Dynamic microbiome changes reveal the effect of 1-methylcyclopropene treatment on reducing post-harvest fruit decay in “Doyenne du Comice” pear. Front. Microbiol. 12, 729014 https://doi.org/10.3389/fmicb.2021.729014.