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Dynamics of microbial community composition on grass surfaces in response to glucose and sucrose spraying for enhanced silage quality

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Article

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Abstract

This study was to investigate the effects of glucose and sucrose spraying on the microbial community on the surface of Italian ryegrass (*Lolium multiflorum*) and alfalfa (*Medicago sativa*) and thereby provide a theoretical foundation for the preparation of high-quality silage. To assess the changes in microbial profile, 16S rRNA amplicon sequencing technology was employed. Results showed that a total of eighty-oneoperational taxonomic units (OTUs), encompassing four phyla, eight classes, fifteen orders, twenty-one families, and thirty-one genera, colonized the surface of Italian ryegrass and alfalfa. At the phylum level, both plant materials were abundant in *Proteobacteria*(Italian ryegrass 87.73% and alfalfa 95.28%) and *Bacteroidetes* (Itlian ryegrass 10.22% and alfalfa 2.23%). At the genus level, Italian ryegrass was enriched with *Methylobacterium* (22.83%), *Pseudomonas* (16.32%), *Rhizobium* (13.12%), and *Sphingomonas* (12.63%), whereas alfalfa mainly included *Pseudomonas* (23.14%), *Brucella* (17.24%), and *Methylobacterium* (15.10%). Sucrose spraying enriched *Sphingomonas* and *Xanthomonas* in Italian ryegrass, while glucose spraying increased the abundance of *Rhizobium* and *Pedobacter*in Italian ryegrass. In alfalfa, *Duganella* and *Erwinia* were enriched by sucrose spraying. Overall, glucose and sucrose spraying led to change microorganism alpha diversity and a significant LefSe difference. Furthermore, statistical variations in microorganisms were observed within seven days after spraying sucrose and glucose, and the changes varied across the grasses.

Significance and Impact of Study: Microorganisms are very important for silage fermentation. This study reported the effects of spraying different sugars on the quantity and distribution of microorganisms on grass surface.

Introduction

Silage is an essential feed source for ruminants, especially the animal husbandry in developed countries. Microbial community is a critical factor affecting the fermentation quality of silage (Chen et al., 2017b). Lactic acid bacteria (LAB) play a crucial role in the fermentation process, which involves the conversion of water-soluble carbohydrates (WSC) into organic acids, mainly lactic acid. Insufficient LAB in silage raw materials often results in the decline of pH and inhibition of undesirable microorganisms (Gavrilova et al., 2019; Wei et al., 2021). Traditionally, silage's lactic acid fermentation depends on the normal metabolic activities of microorganisms that naturally exist on the surface of forage crops (Wang et al., 2021). However, silage often exhibits poor fermentation due to insufficient LAB in the raw materials (Yang et al., 2022). The number of LAB in the silage raw material should exceed 10^5 CFU/g (fresh weight), the LAB become the dominant bacteria in the early stage of silage fermentation (Silva et al., 2016). Additive addition was widely used to improve silage quality, and many commercial LAB additives for silage are available in the market (Şanlier et al., 2019). However, their effects are not always stable, and competitive inhibition between exogenous LAB and epiphytic LAB in the early stage of fermentation may contribute to this instability. Therefore, the epiphytic microbiota (especially LAB) of fresh forages plays a critical role in silage fermentation. However, its size varies among different crops (Pontonio et al., 2018). To date, information about the epiphytic LAB grass is limited. Therefore, research on how to increase the species and number of LAB attached to the forage itself has realistic meaning.

Understanding the microbial community, function and fermentation metabolism regulation network of silage is crucial for in-depth analysis of the biological process of silage fermentation. Additionally, this understanding can provide a scientific basis for the fermentation and modulation of safe and high-quality silage (Wang et al., 2022b). However, the traditional detection methods have low accuracy and cannot deeply analyze the microbial community structure in silage (Dankwa et al., 2021). High-throughput sequencing is the most effective method for analyzing population structure (Parola et al., 2018). The 16S rRNA sequence of prokaryotes contains ten highly conserved regions and nine hypervariable regions (Watts et al., 2017; Johnson et al., 2019). Water-soluble carbohydrates in crops, as the fermentation substrate of microorganisms, play a critical role in the production of high-quality silage. Some studies have indicated that the silage quality is better when LAB on the surface reaches a certain number and that LAB are selective in the utilization of different carbohydrate substances as carbon sources (Yang et al., 2017; çabuk et al., 2018). This study aims to investigate changes in the exogenous epiphytic microbiota profile on Italian ryegrass and alfalfa surfaces sprayed with different sugars before harvest using the 16S rRNA amplicon sequencing method.

Materials and methods

Plant materials and sample collection

Italian ryegrass (*Lolium multiflorum*; R) and alfalfa (*Medicago sativa*; A) were planted in Quantou Experimental Base, Jin'an District, Fuzhou City, Fujian Province, China. All the plant materials in this study were obtained from the Plant Science Affiliation of Fujian Agriculture and Forestry University (NO. 2019 – 01007). The experimental field was located at the altitude of 601 m, latitude of 26.13' N, and longitude of 119.34' E. The average annual rainfall in this area is 1342 mm, and the average annual temperature is 19.6 °C. The experimental plants were planted in plots of 3.0 m × 4.0 m, and community distribution was designed with random zoning (Chen et al., 2017b). Three treatments were designated for each grass: (1) CK, control – spraying distilled water, (2) G, spraying glucose, and (3) S, spraying sucrose. Sugar solutions were prepared by mixing 1.5 g sugar with 50 mL sterilized distilled water. Each treatment was sprayed with 50 mL of sugar solution on the leaf surface twice (at 9 a.m. and 4 p.m.), and plants in each treatment were sampled four times (1, 2, 3 and 4 represented sampling time after 24 h, 48 h, 72 h and one week, respectively). The samples were collected and cut with sterile scissors at the distance of 10 cm from the ground. Then, the samples were put in sealed sterile bags, and the bags were put into an ice box for refrigeration. The box was brought back to the laboratory for analysis. In this study all plants material and methods were carried out in accordance with IUCN Policy Statement guidelines and Convention on International Trade in Endangered Species of Wild Fauna and Flora.

Bacterial genomic DNA extraction

High-efficiency and high-fidelity PCR enzymes, Phusion® High-Fidelity PCR Master Mix with GC Buffer, and TruSeq® DNA PCR-Free Sample Preparation Kit were purchased from New England Biolabs (UK) Ltd. (BIOHUB international trade Co., LTD., Shanghai, China), and the gel recovery kit was purchased from Qiagen (BIOHUB international trade Co., LTD., Shanghai, China). Under sterile conditions, about 0.5 g epibiotic microorganism was suspended in 1 mL lysate.

According to the improved chemical lysis method (Baoguo et al., 2008), total microorganism DNA was directly extracted, and the purity and concentration of the obtained DNA samples were detected by agarose gel electrophoresis.

Amplification of bacterial 16S rRNA V3 hypervariable region

First, we amplified full-length bacterial 16S rRNA fragments using universal primers with the primer sequence 27f: 5'-AACTGA AGAGTTTGATCCTGGCTC-3'; 1492r: 5'-TACGGCTACCTT GTTACGACT-3'. The primers were synthesized by Shanghai Yingjun Biotechnology Co, Ltd. (Shanghai, China), and amplification was performed on the Biometra PCR system (California, USA). Using the PCR products amplified in the first step as templates, the forward direction Lac1 (5'-AGCAGTAGGGAATCTTCCA-3') and the reverse direction GC-Lac2 primers (5' -CGCCCGGGGCGCGCCCCGGGCGGCCCGGGGGCAC-CGGGGGATTYCACCGCTACACATG-3') were used to amplify the V3 region for the second time (Kim et al., 2009). The PCR sequence bands with the size of 360 bp were selected, and the target bands were cut, recovered, and purified using a gel recovery kit (Qiagen, Valencia, California, USA).

Library construction, sequencing, and bioinformatics analysis

The library was constructed using the TruSeq® DNA PCR-Free Sample Preparation Library Construction Kit (Illumina, San Diego, CA, USA). The constructed library was quantified by Qubit and Q-PCR, then the Illumina HiSeq2500 PE250 sequencing system (Illumina, San Diego, CA, USA) was used for sequencing.

Paired-end reads were merged by FLASH (Segata et al., 2011; T and SL, 2011). The reads were assigned to each sample according to unique barcodes. Diversity analyses, including Observed Species and Shannon indexes, were performed using QIIME (Wang et al., 2022). First, the reads were filtered by QIIME quality filters. Then, UPARSE64 (http://www.drive5.com/uparse/) was used to select operational taxonomic units (OTUs) and obtain the OTUs table. Sequences with ≥ 97% similarity were assigned to the same OTUs. SILVA and RDP classifier were used to obtained taxonomic information for each representative sequence (Lu and Salzberg, 2020). OTUs abundance information were normalized using a standard of sequence number corresponding to the sample with the least sequences. Principal component analysis (PCA) and LefSe analysis (http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=lefse_upload) were performed based on the normalized data output, and figures were drawn using the ggplot2 package.

Results

Appropriate sequencing depth and key OTUs

The 24 samples produced a total of 1,705,857 tags, which were combined from PE 250 Reads by Flash software. These tags were clustered with 97% similarity to, yield 666 operational taxonomic units (OTUs). Among them, a total of 660 OTUs were annotated. According to the rarefaction curve (Fig. 1), when the tag number was above 45,000, the curve tended to be flat, which reflected the rationality of sequencing data size. Furthermore, 81 OTUs were presented in all 24 samples (Fig. 2), covering four phyla, eight classes, 15 orders, 21 families, and 31 genera (Table 1).

Table 1
Core operational taxonomic units (OTUs) identified and their taxonomical annotation

Rank	Number	Taxonomy
Phylum	4	Proteobacteria; Bacteroidetes; Actinobacteria; Cyanobacteria
Class	8	Gammaproteobacteria; Alphaproteobacteria; Cytophagia; Betaproteobacteria; Sphingobacteriia; unidentified_Actinobacteria; Chloroplast; Flavobacteriia
Order	15	*Rhizobiales; Enterobacteriales; Burkholderiales; Cytophagales; Sphingomonadales*; Pseudomonadales; Sphingobacteriales; Xanthomonadales; Micrococcales; unidentified_Chloroplast; Flavobacteriales; Caulobacterales; Rickettsiales; Frankiales; Rhodobacterales
Family	21	Enterobacteriaceae; Cytophagaceae; Sphingomonadaceae; Pseudomonadaceae; Oxalobacteraceae; Sphingobacteriaceae; Methylobacteriaceae; Comamonadaceae; Microbacteriaceae; Xanthomonadaceae; unidentified_Chloroplast; Rhizobiaceae; Aurantimonadaceae; Flavobacteriaceae; Brucellaceae; Mitochondria; Rhodobacteraceae; Alcaligenaceae; Geodermatophilaceae; Hyphomicrobiaceae; Caulobacteraceae
Genus	31	*Hymenobacter; Pseudomonas; Sphingomonas; Pedobacter; Methylobacterium*; *unidentified_Chloroplast*; *Stenotrophomonas*; *Rhizobium*; *Novosphingobium*; *Erwinia*; *Massilia*; *Pantoea*; *Aureimonas*; *Ochrobactrum*; *Variovorax*; *Xanthomonas*; *Modestobacter*, *Devosia*; *Brevundimonas*; *Paracocccus*; *Luteibacter*, *Acidovorax*; *Buchnera*; *unidentified_Mitochondria*; *Lysinimonas*; *Microbacterium*; *Epilithonimonas*; *Hafnia*; *Rathayibacter*, *Achromobacter*, *Duganella*

Changes in microorganism diversity

Non-metric multi-dimensional scaling (NMDS) based on the relative abundance of the OTUs indicated that sugar spraying (either glucose or sucrose) changed the microorganism composition and structure of Italian ryegrass and alfalfa, compared with those of the CK (Figs. 3 and 4). When Stress was less than 0.2, it indicated that NMDS accurately reflected the differences between samples. In addition, the Shannon index increased after glucose or sucrose spraying, indicating that sugar addition improved microorganism diversity of Italian ryegrass and alfalfa (Fig. 5 **and** Fig. 6).

Microbial abundance at the phylum and genus level

The microbial composition analysis revealed that the Italian ryegrass and alfalfa were similar at the phylum level, but different at the genus level based on Barplot analysis (Fig. 7). The genera enriched in Italian ryegrass were *Methylobacterium* (22.83%), *Pseudomonas* (16.32%), *Rhizobium* (13.12%), and *Sphingomonas* (12.63%), and the genera the enriched in alfalfa were *Pseudomonas* (23.14%), *Buchnera* (average 17.24%), and *Methylobacterium* (average

15.10%). At the phylum level, both grasses were abundant in Proteobacteria (average 95.28% in A, 85.73% in R) and Bacteroidetes (average 2.23% in A, 10.22% in R). Moreover, LefSe analysis (Fig. 8) indicated that Bacteroidetes were enriched in the R.G, and Proteobacteria were enriched in the A.S at the phylum level. *Sphingomonas* and *Xanthomonas* were enriched in the R.S, *Rhizobium* and *Pedobacter* were enriched in the R.G, and *Duganella* and *Erwinia* were enriched in the A.S at the genus level.

Microbial abundance variations over time

The application of sugar through spraying was found to induce significant changes in the dynamics of microorganisms over time, as compared to the control group. Our results identified four distinct patterns of variations in genus abundance following the application of glucose and sucrose (Table 2). *Pseudomonas*, *Methylobacterium*, *Sphingomonas*, *Chryseobacterium*, *Variovorax*, and *Nubsella* showed continuously increased abundance after glucose spraying, and *Methylobacterium*, *Rathayibacter*, *Microbacterium*, *Bradyrhizobium*, *Nocardioides*, *Phycicoccus* showed similar trend after sucrose spraying, both in alfalfa and Italian ryegrass. The same trend was observed for *Novosphingobium*, *Achromobacter*, *Aliihoeflea*, *Candidatus_Captivus*, *Rhodococcus*, and *Patulibacter* after glucose spraying, and *Bdellovibrio*, *Spirosoma*, and *Tatumella* after sucrose spraying in Italian ryegrass. *Massilia* showed continuously decreased abundance after sucrose spraying in alfalfa and after glucose and sucrose spraying in Italian ryegrass. In alfalfa, *Rhizobium* and *Xanthomonas* abundance decreased at first and then increased after glucose and sucrose spraying, and the same trend was observed for *Rhizobium*, *Sphingomonas*, and *Roseomonas* in Italian ryegrass. After the application of glucose and sucrose, a trend of initial increase followed by a subsequent decrease in abundance was observed in *Brevundimonas*, *Paracocccus*, *Limnobacter*, and *providencia* in alfalfa, as well as in unidentified_*Mitochondria*, *Limnobacter*, *Comamonas*, *Acinetobacter*, *Arthrobacter*, *Modestobacter*, *Providencia*, and *Aeromicrobium* in Italian ryegrass.

Table 2
Four types of variations of genus abundance after sugar spraying

Genus Variation	alfalfa		Italian ryegrass	
	Glucose	Sucrose	Glucose	Sucros
Continuous increase	*Pseudomonas, Methylobacterium, Sphingomonas, Chryseobacterium, Variovorax, Nubsella*	*Methylobacterium, Rathayibacter, Microbacterium, Bradyrhizobium, Nocardioides, Phycicoccus*	*Novosphingobium, Achromobacter, Aliihoeflea, Candidatus_Captivus,Rhodococcus, Patulibacter*	*Bdellov*
Continuous decrease	*unidentified_Mitochondria, Luteibacter*	*Massilia,Hafnia*	*Massilia,Duganella*	*Massili Stenotr Ochrob Herbas*
Decrease-increase	*Rhizobium, Xanthomonas, Aureimonas, Bacillus, Devosia, Acinetobacter, Bradyrhizobium,Caulobacter, Patulibacter*	*Rhizobium, Xanthomonas, Luteibacter, Serratia*	*Methylobacterium, Rhizobium, Sphingomonas, Stenotrophomonas, Sphingobacterium, Roseomonas, Serratia, Bosea, Agaricicola, Leptothrix*	*Rhizob Novosp Brevun Sphing Aureim Peredib Candid Dyadob Candid Pigmer Caulob Fibrella*
Increase - decrease	*Pantoea, Duganella, Hafnia, Stenotrophomonas, Acidovorax, Brevundimonas, Enterococcus, Sphingobacterium, Paracocccus, Limnobacter, Arthrobacter, Providencia,Serratia, Kineococcus, Pigmentiphaga, Streptococcus*	*Buchnera,Hymenobacter,Brevundimonas,Paracocccus, Limnobacter,Aureimonas,Variovorax,Tatumella,Providencia, Falsirhodobacter,Clostridium_sensu_stricto_1,Kaistia*	*Pedobacter, Erwinia, unidentified_Mitochondria, Hymenobacter, Brevundimonas, Limnobacter, Bdellovibrio, Spirosoma, Comamonas, Devosia,Acinetobacter, Arthrobacter, Modestobacter, Dyadobacter, Providencia,Paucimonas, Exiguobacterium, Caulobacter,Salana, Phycicoccus,Bacteroides, Cellulomonas, Coxiella, Parabacteroides, Aeromicrobium, Quadrisphaera, Siphonobacter*	*unident Limnob Acinetc Modest Rubelli Aeromi*

Discussion

Many microorganisms were distributed on the surface of fresh plants, and usually the proportion of harmful microorganisms, negatively influencing the fermentation process, was higher richness than the proportion of beneficial microorganisms. This led to a loss of nutrients, particularly in the initial stages of ensiling when air is still present in the silage material (Langston and Bouma, 1960; Ennahar et al., 2003). Therefore, studying changed microorganism composition in forage crops would help to control the silage fermentation (Li et al., 2015). Previous studies have used the Illumina MiSeq sequencing method targeting the 16S rRNA gene V3-V4 variable region to investigate bacterial community structure and pathogenic bacterial characteristics in hay (Giello et al., 2017). In the present study, the utilized Illumina MiSeq sequencing technology to analyze the microbial community present on the surfaces of Italian ryegrass and alfalfa treated with various sugars. The resulting data provided a wealth of comprehensive and in-depth information regarding the bacterial community. For instance, the result showed that a total number of 15,416 OTUs were obtained from hay samples under the similarity level of 97%, including 29 phyla, 87 classes, 144 orders, 219 families, and 323 genera. Our study was found that 81 OTUs which covered four phyla, eight classes, 15 orders, 21 families, and 31

genera were presented in all 24 samples. At the phylum level, the two forage grasses had the highest abundance of *Proteobacteria*, followed by *Bacteroidetes*. *Methylobacter*, *Pseudomonas*, *Rhizobium* and *Sphingomonas* were most abundant in ryegrass, while *Brucella*, *Pseudomonas*, *Methylobacter* and *Sphingomonas* were the most abundant in alfalfa. It indicated that these microorganisms played an important role in the microbial communities of the two forages, and the microbial communities of the two forages had its similar and specific.

Proteobacteria and *Bacteroides* were the most common phyla in both Italian ryegrass and alfalfa, and *Lactobacillus* accounted for less than 1% of total CAt the genus level, Italian ryegrass was abundant in *Methylobacterium, Pseudomonas, Rhizobium, Sheath*, and *Aminomonas*, whereas alfalfa was abundant in *Brucella, Pseudomonas, Methylobacterium*, and *Sphingomonas*. These were similar to the results of previous studies (Dutta et al., 2014; Giello et al., 2017). Dutta et al. found that *Pseudomonas*, *Exiguobacterium*, *Massilla*, and *Planococcus* were the main epiphytic flora on alfalfa wilt, while the relative content of *Lactobacillus* in microorganisms is only 2%. Giello et al. investigated the microbial composition and the characteristics of pathogenic bacteria in six different hay samples, resulted that *Lactobacillus* also less than 1%. The study showed that the microorganisms attached to the surface of alfalfa raw materials mainly included *Pantoea*, *Lactobacillus*, *Enterobacter*, *Streptococcus* and so on (Canaviri Paz et al., 2020). The reasons for these differences were closely related to the growth environment and geographical location of the samples. Wang et al. found that the microbial diversity of wilted alfalfa silage was significantly lower than that of unwilted alfalfa silage in tropical regions (Wang et al., 2022).

After spraying sugar, the number of *Methylobacter* on the surface of ryegrass decreased significantly, the number of pathogenic bacteria *Brucella* and *Sphingomonas* on the surface of alfalfa decreased, and the number of *Pseudomonas* increased. *Proteobacteria* was the dominant bacteria attached to the surface of crops (Tkalec et al., 2022). It was the largest phylum of bacteria, including many pathogenic bacteria. In the core OTUs of the 24 samples this time, *γ-Proteobacteria*, which contain more pathogenic bacteria, were detected. *Enterobacteriaceae*, *Paleobacter*, *Burkholderia*, *Pseudomonas* and other pathogenic bacteria. The study revealed a significant decrease in the abundance of *Proteobacteria* and a significant increase in lactic acid bacteria (LAB) after the silage fermentation process. This suggests that silage fermentation can effectively mitigate the population of harmful bacteria and promote the proliferation of beneficial bacteria, potentially reducing the impact of pathogenic microorganisms on livestock and mitigating potential health risks, ultimately improving animal health (Suong et al., 2022).

Microorganisms play a vital role in the ensiling process, and the distribution of microorganisms on forage plants is affected by the substances secreted by the plants (Chen et al., 2017a). The differences in microorganism distribution between two grasses could be explained by different substances on the surfaces of two grasses including sugars, amino acids, and organic acids secreted from grasses (Mao et al., 2022). Our findings were consistent with previous studies that have reported that the substances secreted by plants can affect the distribution of microorganisms on their surfaces. As important carbon sources for microorganisms on the grasses, these secreted substances accelerate the growth of microbes, resulting in plant-specific microorganism distribution (Mao et al., 2022). The genus *Enterococcus* is one of an important LAB for silage development, and it reached the highest level three days after spraying with glucose. The present study investigated the differences in microorganism distribution between Italian ryegrass and alfalfa, and how these distributions were affected by the spraying of glucose or sucrose. Our findings revealed that the substances secreted by the two grass species led to a different distribution of microorganisms. The results showed that the genus Enterococcus, which is important for silage development, reached its highest level three days after glucose spraying. Additionally, the observed variations in microorganism composition at different stages of the grass after sugar spraying indicated that different microorganisms dominated at different stages. This information can be used for future silage improvement research... Therefore, forage mowing at three days after spraying glucose could improve the quality of the silage.

Conclusion

In the current study, we employed 16S rRNA to assess the microbial makeup on the external surfaces of two forage plants. Divergent from prior investigations, our emphasis was placed on the microbial composition and fluctuations throughout the silage development process, thereby establishing a groundwork for forthcoming research aimed at improving silage. It contributed to the understanding of the impact of different sugars on the microbial community and highlights the potential of using specific sugars to enhance the beneficial microbial population while reducing the harmful microbial population during the silage fermentation process. Moreover, our study relevant for improving the quality of silage, reducing nutrient loss, and enhancing the health of livestock.

Declarations

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Data Availability Statement

The raw data of current study had been submitted to NCBI Sequence Read Archive (SRA) with accession no: PRJNA980709.

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Figures

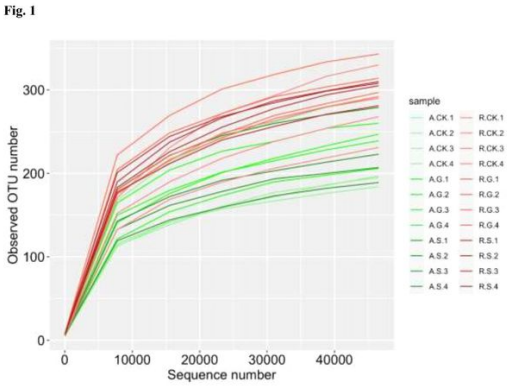


Fig. 1 Rarefaction curves of plant samples. OTU, operational taxonomic unit; A, alfalfa; R, Italian ryegrass; CK, control; G, spraying glucose; S, spraying sucrose.

Figure 1

See image above for figure legend.

Fig. 2

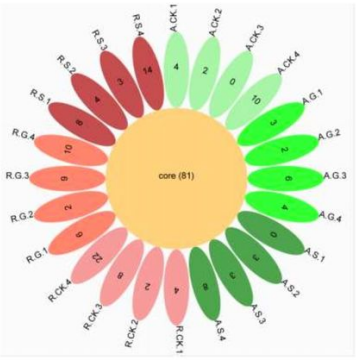


Fig. 2 81 Core operational taxonomic units (OTUs) and special OTUs in 24 plant samples. A, alfalfa; R, Italian ryegrass; CK, control; G, spraying glucose; S, spraying sucrose.

Figure 2

See image above for figure legend.

Fig. 3

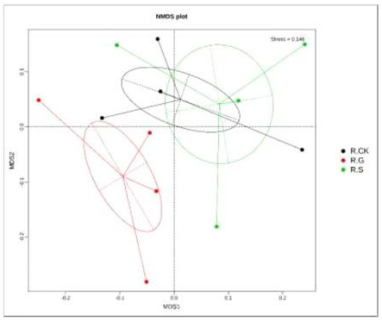


Fig. 3 Non-metric multi-dimensional scaling (NMDS) analysis of Italian ryegrass (R).

CK, control; G, spraying glucose; S, spraying sucrose.

Figure 3

See image above for figure legend.

Fig. 4

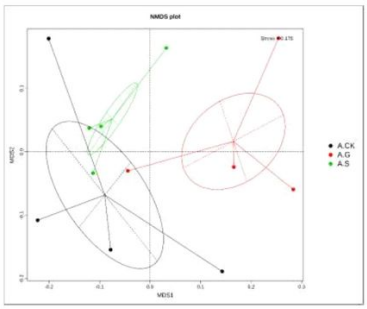


Fig. 4 Non-metric multi-dimensional scaling (NMDS) analysis of alfalfa (A). CK, control; G, spraying glucose; S, spraying sucrose.

Figure 4

See image above for figure legend.

Fig. 5

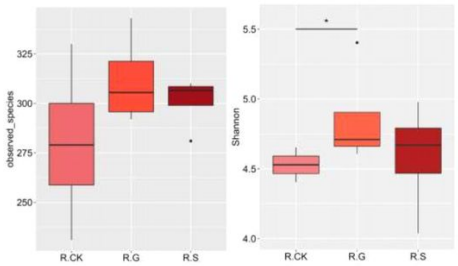


Fig. 5 Observed Species and Shannon boxplots for Italian ryegrass (R). CK, control; G, spraying glucose; S, spraying sucrose.

Figure 5

See image above for figure legend.

Fig. 6

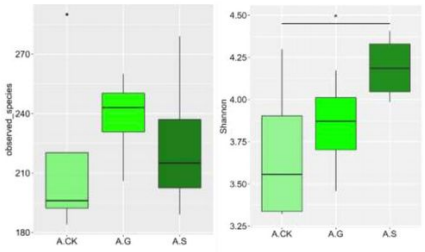


Fig. 6 Observed Species and Shannon boxplots for alfalfa (A). CK, control; G, spraying glucose; S, spraying sucrose.

Figure 6

See image above for figure legend.

Fig. 7

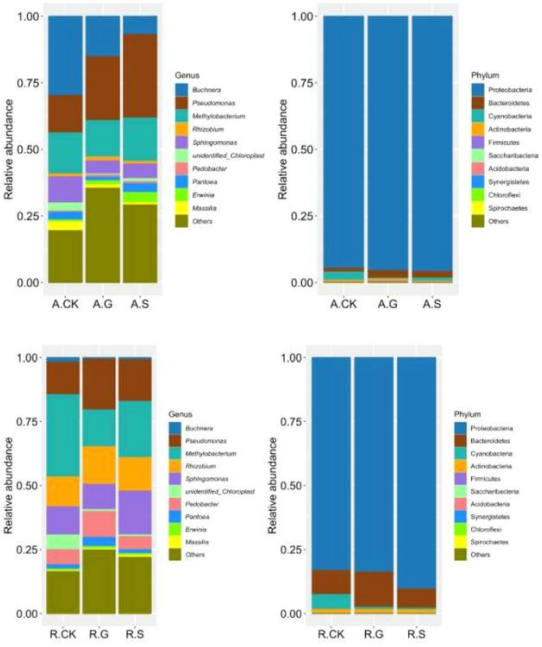


Fig. 7 Composition of bacterial communities at the genus and phylum levels. A, alfalfa; R, Italian ryegrass; CK, control; G, spraying glucose; S, spraying sucrose.

Figure 7

See image above for figure legend.

Fig. 8

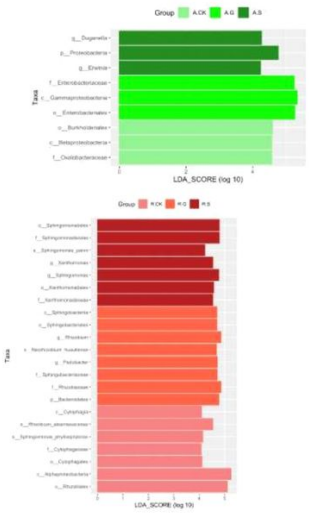


Fig. 8 Significance tests of relative abundance among groups by LefSe. A, alfalfa; R, Italian ryegrass; CK, control; G, spraying glucose; S, spraying sucrose; LDA, linear discriminant analysis.

Figure 8

See image above for figure legend.