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# Effects of cropland and cultivars on phyllosphere bacterial communities in forage crops in Southwest China

Shunqiang Yang[1,2,3] | Yongzhi Zhang[1] | Siqi Liu[1] | Qing Lang[1] | Liuyuan Bao[1] | Chengcui Yang[1] | Liuxing Xu[1]

[1]College of Agronomy and Life Sciences, Zhaotong University, Zhaotong, China

[2]Yunnan Key Laboratory of Gastrodia Elata and Fungal Symbiotic Biology, College of Agronomy and Life Sciences, Zhaotong University, Zhaotong, China

[3]Yunnan Engineering Research Center of Green Planting and Processing of Gastrodia Elata, Zhaotong University, Zhaotong, China

**Correspondence**
Liuxing Xu, College of Agronomy and Life Sciences, Zhaotong University, Zhaotong 657000, China.
Email: 331405719@qq.com

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## Abstract

Rotation between rice (*Oryza sativa*) or corn (*Zea mays*) (summer) and forage crop (winter) is common in Southwest China. However, the impact of different ecological environments on phyllosphere bacterial communities and the factors influencing the survival and distribution of undesirable bacteria remain unclear. Therefore, we aimed to investigate the effects of cropland and cultivars on the abundance and diversity of phyllosphere bacteria in forage crops. Field experiments were conducted on croplands (dryland, DL; paddy field, PF) and three cultivars (forage pea, FP [*Lathyrus sativus*]; Italian ryegrass, IR [*Lolium multiflorum*]; smooth vetch, SV [*Vicia villosa*]). The results revealed 87 shared operational taxonomic units (OTUs) between the PF and DL bacterial communities, while FP, IR and SV shared 58 OTUs. *Pantoea*, *Plesiomonas* and *Bryobacter* were the three most abundant genera between the two cropland types. The relative abundances levels of *Rahnella1*, *Aeromonas*, *Cetobacterium*, *Flavobacterium* and *Vibrio* were significantly different among the three cultivars ($p < 0.05$). The physico-biochemical properties and microorganism numbers in SV and FP exhibited a high degree of overlap; however, they were distinct from those of IR (95% confidence interval). Physiological properties did not directly contribute to bacterial abundance but rather explained the differences in bacterial abundance among samples, likely by indirectly shaping the phyllosphere microenvironment. In summary, compared with PF, DL indirectly influenced microbial numbers indirectly by regulating leaf physiological properties, while both exhibited similar effects on bacterial diversity. Among the different forage crops, IR suppressed phyllosphere moisture and nutrient exchange owing to its lower stomatal density, resulting in a lower bacterial relative abundance than those of FP and SV. This finding elucidates bacterial community dynamics, particularly during the initial establishment phase of crop rotation systems, providing a valuable perspective for optimizing forage crop rotation strategies.

**KEYWORDS**
forage crop, phyllosphere bacterial communities, physico-biochemical properties, rotation

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## 1 | INTRODUCTION

Rice (*Oryza sativa*) (Yang et al., 2014) and corn (*Zea mays*) (Zhu et al., 2011) are the primary food crops in Southwest China. Sowing forage crops (to increase forage supply) (Xu et al., 2021) or green manure (to improve the quality and health of soil and crop yield) (Chen et al., 2022) following the autumn rice or corn harvest is common practice in this region. This stable rotation pattern promotes the formation of a stable micro-ecosystem within the phyllosphere margins of food and forage crops (Vorholt, 2012). This micro-ecosystem can enhance soil health (Chen et al., 2022), promote plant growth (Mehlferber et al., 2023) and decrease the occurrence of pests and diseases (Deng et al., 2021). Therefore, a stable rotation pattern is crucial for sustainable agricultural production.

The phyllosphere, the aboveground part of plants and the air boundary layer, is often inhabited by a wide range of bacteria, including epiphytes and endophytes (Armitage et al., 2023). These bacteria play a vital role in promoting plant growth (Afordoanyi et al., 2023) and biological control (Ahmed et al., 2022). In forage crops, these phyllosphere bacteria may affect later silage utilization. For instance, *Lactobacillus* inhibits forage spoilage owing to its lactic acid-producing capabilities in silage (Yan et al., 2019). Conversely, *Pseudomonas* produces biogenic amines, leading to protein degradation in silage (Dunière et al., 2013). The primary function of *Proteobacteria* is organic nitrogen fixation (Welbaum et al., 2004). Bacteria sustain themselves in the phyllosphere environment by competing for limited resources (e.g., space, nutrients). This competition may be deterministic but may also exhibit different patterns depending on the spatial scale (Armitage et al., 2023); these factors influencing bacterial competition include climate (Guan et al., 2018), fertilizer management (Li et al., 2016), planting techniques (Xu et al., 2023), phyllosphere age (Muller & Ruppel, 2014), plant nutrition (Chen et al., 2021), location, surface structure and physico-biochemical properties (Tang et al., 2023). However, endophytic bacteria are less susceptible to environmental disturbances (Bacon & White, 2015) microorganism abundance. Moreover, resident and transient bacteria on the phyllosphere surface are crucial for plant health (Whipps et al., 2008). These bacteria engage in diverse interactions with plants, contributing to their nutrition, growth and disease resistance. For instance, certain bacteria produce plant growth hormones (phytohormones) that promote plant growth and development, while others produce antimicrobial agents that protect plants against pathogens (Tang et al., 2023). Understanding the diversity, relative abundance and activity of phyllosphere-associated bacteria, as well as their interactions with crops, is crucial to improving crop health and productivity. Therefore, exploring the utilization of these bacteria in promoting plant growth and disease resistance will provide novel strategies and technologies for agricultural applications.

Current research on the identification and quantification of bacteria adhering to forage crops remains scarce. Previous studies have identified key factors influencing the bacteria attached to forage surfaces (Tang et al., 2022), which directly impact microbial migration, growth and mortality. Paddy fields (PF, planting rice in summer, winter fallow or planting forage crops or green manure) and drylands (DL, planting corn in summer, winter fallow or planting forage crops or green manure) are exposed to markedly different ecological environments. These differences encompass variations in soil physico-chemical properties and bacterial community structure. However, the impact of these environments on the resonance of phyllosphere bacteria is unclear. Moreover, little is known about the factors that alter the survival and distribution of unwanted phyllosphere bacteria. In general, colonization by phyllosphere bacteria relies on phyllosphere nutrients, water and space, which are often influenced by the growing environment.

Therefore, this study aimed to investigate the effects of cropland and cultivars on the abundance and diversity of phyllosphere bacteria associated with forage crops. To this end, we hypothesized that (1) PF improves bacterial species and abundance through increased phyllosphere nutrients in forage crops compared with DL and (2) forage peas (FP, *Lathyrus sativus*) increase the number and relative abundance of bacteria compared with smooth vetch (*Vicia villosa*, SV) and Italian ryegrass (IR, *Lolium multiflorum*) by regulating inter-phyllosphere nutrients and water partitioning through higher stomatal density. The results of this study could provide predictive information for plant health.

## 2 | MATERIALS AND METHODS

### 2.1 | Experimental site

The field experiment was conducted between November 21, 2022, and April 28, 2023, in Hongnizha Village (27.28° N, 103.60° E, at an altitude of 1989 m), Zhaotong, Yunnan, China. In this region, rice and corn are only grown once a year, and rotation with forage has been practiced for decades. Forage crops are mainly used to feed ruminant animals (to alleviate the insufficient supply of forage), rather than being returned to the fields. During the crop growing period, the following meteorological data were recorded (Nov. 2022–Apr. 2023): total rainfall 159 mm, mean temperature 8.85°C (Table 1). Soil data were not collected.

FP, IR and SV, the three forage species selected for this study, are typically cultivated in winter fallow fields and during fallow periods in southwestern China, exhibiting distinct functional characteristics. These plants showed differences in leaf nutritional composition and structural features, which directly influence the colonization process of phyllosphere microorganisms. As leguminous species, FP and SV alter leaf nutrient status through rhizobial symbiotic nitrogen fixation, thereby affecting the microbial community composition on leaf surfaces. In contrast, IR, as a fast-growing gramineous forage, creates a distinct phyllosphere microenvironment through its unique carbon input patterns.

### 2.2 | Experimental treatment

The experiment included two croplands (PF and DL) and three cultivars (FP, IR and SV). All three cultivars were grown in the two

**TABLE 1** Meteorological data during growing period of forage crops at the experimental fields.

| Month | Mean rainfall (mm) | Mean temperature (°C) | Extreme high temperature (°C) | Extreme low temperature (°C) |
|---|---|---|---|---|
| Nov. 2022 | 0 | 12.1 | 26 | 0 |
| Dec. 2022 | 0 | 3.8 | 17 | −5 |
| Jan. 2023 | 1.7 | 3.1 | 25 | −7 |
| Feb. 2023 | 14.8 | 8.3 | 22 | −2 |
| Mar. 2023 | 74.2 | 11.1 | 26 | 0 |
| Apr. 2023 | 68.1 | 14.7 | 31 | 4 |

**TABLE 2** Agricultural operations dates of crops.

| Cropland | Cultivars | Transplanting density or seeding rate | Sowing date | First fertilization date | Second fertilization date | Harvesting date | Crop growth time (d) |
|---|---|---|---|---|---|---|---|
| Paddy field | Rice | $13.8 \times 10^4$ plants/ha | May 18, 2022 | Jul. 10, 2022 | Aug. 28, 2022 | Dec. 1, 2022 | 167 |
| | Forage pea | 100 kg/ha | Dec. 8, 2022 | Jan. 6, 2023 | Feb. 26, 2023 | May 2, 2023 | 175 |
| | Italian ryegrass | 30 kg/ha | Dec. 8, 2022 | Jan. 6, 2023 | Feb. 26, 2023 | May 2, 2023 | 175 |
| | Smooth vetch | 80 kg/ha | Dec. 8, 2022 | Jan. 6, 2023 | Feb. 26, 2023 | May 2, 2023 | 175 |
| Dryland | Corn | $7.5 \times 10^4$ plants/ha | May 24, 2022 | Jun. 22, 2022 | Aug. 17, 2022 | Oct. 9, 2022 | 138 |
| | Forage pea | 100 kg/ha | Dec. 8, 2022 | Jan. 6, 2023 | Feb. 26, 2023 | May 2, 2023 | 175 |
| | Italian ryegrass | 30 kg/ha | Dec. 8, 2022 | Jan. 6, 2023 | Feb. 26, 2023 | May 2, 2023 | 175 |
| | Smooth vetch | 80 kg/ha | Dec. 8, 2022 | Jan. 6, 2023 | Feb. 26, 2023 | May 2, 2023 | 175 |

cropland areas. The three cultivars were positioned in a random block pattern, with three duplicates for each plant variety. Each plot covered an area of 40 $m^2$ (5 × 8 m), and the distance between the plots was 30 cm. Three forage crops were sown on December 8, 2022, and harvested on May 2, 2023 (Table 2). The forage crops were collected after 170 days. Only one irrigation session of 600 $m^3/hm^2$ was conducted during the crop growth period (December 27, 2022). After the rice or corn harvest in October, PF or DL was used for FP, IR and SV planting (for harvesting in the next spring season). Subsequently, rice or corn were planted again. After tilling the PF and DL soil, manual seeding was performed, and the seeds were covered with soil using a hoe (seeding depth of 1 cm). The FP, IR and SV cultivars were sown at 100, 30 and 80 kg/ha, respectively. All cultivars were treated with 270 kg/ha compound fertilizer (N: $P_2O_5$:$K_2O$ = 15:15:15) in two distinct applications: 40% of the total was applied 31 days after sowing, and 60% of the total was 82 days after sowing. Table 2 presents detailed agronomic management measures and dates. No insecticides and fungicides were used during the forage crops growing periods.

## 2.3 | Field sampling

We collected 300 g of healthy, fully expanded, mature leaves from each plot. Subsequently, six fully expanded and mature leaves were collected from the central region of each marked plant, with three randomly selected individuals per cultivar. This resulted in a total of 18 leaves per cultivar. Leaves were cut into 2 cm leaf segments and thoroughly mixed to ensure representative subsampling. The materials were divided into three parts and placed in separate sterile bags. One bag was placed in a foam box with ice bags and immediately stored in a freezer at −20°C to determine the microbial counts and biochemical properties. The second bag was also placed in a foam box with ice bags, quickly transported to the laboratory and used to determine the physiological properties of the cultivars at room temperature (18°C–22°C). The third bag was stored in liquid nitrogen and sent to a sequencing company (Majorbio, Shanghai, China) for bacterial community analysis. At 9 am on days with <30% cloud cover, photosynthetic parameters of the leaves were directly measured in the field. The bacteria measured were whole-leaf microorganisms, including both endophytic and epiphytic communities in this study. To avoid interference from dew, all leaf samples were collected under dew-free conditions, and photosynthetic parameters were measured on the same day as sample collection.

## 2.4 | Sequencing of the bacterial community

Total DNA from microbial communities was extracted using the E.Z.N.A.®Plant DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's instructions. Subsequently, the V3–V4

region of the 16S rDNA was amplified using the specific barcoded primers 341F (CCTACGGGNGGCWGCAG) and 806R (GGACTACHVGGGTATCTAAT) (Guo et al., 2017). The amplified products were purified and linked with sequencing adapters to create a sequencing library. Sequencing was performed on an Illumina PE250 platform. We pooled the PCR-products from the same sample and recovered them using 2% agarose gel. The recovered products were then purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions. We evaluated the quantity of the purified products using 2% agarose gel electrophoresis followed by a Quantus™ Fluorometer (Promega, USA). Sequencing data were processed using Uparse software (available at http://drive5.com/uparse/). Raw FASTQ files were de-multiplexed using an in-house perl script, quality-filtered using fastp version 0.19.6 (Chen et al., 2018) and merged using FLASH version 1.2.7 (Magoc & Salzberg, 2011) with the following criteria: (i) The reads were truncated at any site receiving an average quality score of <20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp and those containing ambiguous characters were discarded; (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of the overlap region was 0.2 and reads that could not be assembled were discarded; (iii) samples were distinguished according to the barcode and primers, and the sequence direction was adjusted, exact barcode matching, 2 nucleotide mismatch in primer matching. Subsequently, the optimized sequences were clustered into operational taxonomic units (OTUs) using UPARSE 7.1 with a 97% sequence similarity level (Edgar, 2013). The most abundant sequence for each OTU was selected as a representative sequence. Each sequence was aligned with the Silva database (version 138) using the RDP classifier (available at http://rdp.cme.msu.edu/), and species classification annotations were obtained using an alignment threshold of 70%. All bacterial relative abundance and species were analyzed only at the genus level.

## 2.5 | Physiological and biochemical properties of leaves

All items were measured in triplicate. The moisture retention capacities of leaves were determined using their fresh and dry weights (Ni et al., 2015). Specifically, the moisture retention capacity (%) was calculated using the formula: (FW2 − DM) × 100/(FW1 − DM) according to the weight of fresh leaves (FM1), natural conditions of 5 h water loss (FM2) and drying at 75°C to a constant weight (DM). Cuticular permeability was determined by inducing stomatal closure under dark/light conditions (Bi et al., 2017), with minor modifications. The leaves were subjected to dark treatment for 0.5 h (to induce stomatal closure while preventing water loss), weighed and subsequently placed in an open environment at 25°C for natural water loss for 10 h (with weight measured once every 1 h). Finally, the leaves were incubated at 37°C for 72 h, and the weight was recorded as the dry weight of the leaves. Vitamin C was extracted using 6% trichloroacetic acid, and use of boric acid-sodium acetate solution was used as an additive solution (fluorescence method, ascorbic acid was used to create the standard curve) (Stevens et al., 2006). Liquid nitrogen was used to grind 1 g of plant leaves into a fine powder. Then, 3 mL of pre-cooled 6% trichloroacetic acid solution was added, and the mixture was homogenized in an ice bath. The homogenate was centrifuged at 12,000 × *g* and 4°C for 15 min, and the supernatant was collected as the crude extract of vitamin C. Boric acid-sodium acetate solution was used to stabilize the ascorbic acid and enhance the fluorescence signal. Chlorophyll was extracted by grinding the leaves with the addition of *N*,*N*-dimethylformamide (Porra et al., 1989). The leaf samples were cut into small pieces and placed in a centrifuge tube, and then 5 mL of *N*,*N*-dimethylformamide was added. The tissue was ground in an ice bath using a mortar until completely disrupted. The homogenate was centrifuged at 12,000 × *g* and 4°C for 10 min in the dark, and the supernatant was collected. Chlorophyll a and chlorophyll b contents were then measured at 664 and 647 nm, respectively. Stomatal density on the adaxial and abaxial leaves was evaluated using imprints comprising transparent nail polish (Leroy et al., 2008). Specifically, transparent nail polish was evenly applied to the targeted leaf epidermis area and left to dry for 5–10 min to form a thin film. Once fully dried, the film was peeled off using transparent adhesive tape and mounted onto a microscope slide. Under a light microscope (at 100–400× magnification), five fields of view were randomly selected, and the number of stomata within each field was counted. The transpiration rate, stomatal conductance and total water vapor conductance of the three cultivars (selected mature leaves, avoiding the main vein of the leaf) were determined using an LI-6800 portable photosynthesis system analyzer (LI-COR, Lincoln, NE, USA). The system parameters were set as follows: flow rate, 500 μmol/s; relative humidity, 50%; $CO_2$ concentration, 400 ppm; rotation speed, 10,000 rpm; and light intensity, 1500 μmol/$m^2$/s.

The phyllosphere sample (10 g) was ground and thoroughly mixed with liquid nitrogen. The soluble sugar concentration was determined at 620 nm using anthrone colorimetry (Dubois et al., 1956). Specifically, the ground powder was transferred into a 50-mL centrifuge tube. Then, 20 mL of 80% ethanol was added, and the mixture was extracted in an 80°C water bath for 30 min, with vortex mixing 2–3 times during the process. The extract was centrifuged at 10,000 × *g* and 4°C for 15 min. The supernatant was collected, the residue was re-extracted once and the two supernatants were combined. A standard curve was prepared using glucose standard solutions. The phosphorus concentration was determined using the ammonium molybdate method (Knowles & Plaxton, 2013). Next, 1 g of fresh sample was added to 0.5 M $NaHCO_3$ buffer (pH 8.5) and extracted at a 1:10 (w/v) ratio, with shaking for 30 min. Subsequently, the sample was centrifuged at 10,000 × *g* for 10 min, and the supernatant was collected. A 1 mL aliquot of the supernatant was placed in a test tube, followed by the addition of 2 mL of 2.5% ammonium molybdate and 0.5 mL of 10% ascorbic acid. After mixing thoroughly, the mixture was heated in a boiling water bath for 10 min. The absorbance was measured at a wavelength of 700 nm using a spectrophotometer. The soluble protein concentration was determined using the Coomassie

brilliant blue method (Bradford, 1976). Subsequently, 1 g of fresh sample was rapidly frozen in liquid nitrogen and ground into a fine powder. The pre-chilled protein extraction buffer (50 mM Tris–HCl, pH 7.5) was added, and the mixture was centrifuged at 12,000 × *g* and 4°C for 15 min. The supernatant was collected. A 100 μL aliquot of the sample to be measured was added to 5 mL of Bradford reagent. The mixture was vortexed and allowed to stand at room temperature for 10 min The absorbance was measured at 595 nm using a spectrophotometer. The concentration of free amino acids was determined by the addition of phosphate buffer (pH 8.0), as described by Wang (1998). One gram of fresh sample was taken and added to 50 mM pH 8.0 phosphate buffer. The mixture was subjected to an ice-bath ultrasonic extraction for 15 min, followed by centrifugation at 12,000 × *g* and 4°C for 15 min. The supernatant was collected, and the absorbance at 570 nm was measured using a spectrophotometer. The wax concentration was determined by soaking leaves in chloroform for 30 s (Bewick et al., 1993). The leaves were immersed in 10 mL of pre-chilled chloroform (4°C) and gently shaken for 30 s (to avoid tissue damage). The leaves were then immediately removed, and the residual solvent on the surface was evaporated under a gentle stream of nitrogen gas. The concentration of reducing sugars was determined by titration using methylene blue as the indicator (Bicudo de Almeida-Muradian et al., 2020). Subsequently, 1 g of fresh sample was collected and mixed with 10 mL of 80% ethanol. The mixture was extracted in an 80°C water bath for 30 min and centrifuged at 4000 × *g* for 10 min, and the supernatant was collected. After the sample was brought to a boil, two drops of methylene blue indicator were added to determine the reducing sugar content.

### 2.6 | Microbial count composition of leaves

The number of microorganisms in the phyllosphere was determined using the culture medium cultivation method (Zuo et al., 2014). Specifically, 10 g of material was immersed in 90 mL of sterile water under sterile conditions and shaken for 5 min on a shaking table. Serial dilutions were prepared and separately plated on selective media to quantify microbial populations on the leaf surfaces. Nutrient agar was used for aerobic bacteria; potato dextrose agar supplemented with 0.04% Chloromycetin was used for yeasts and molds; de Man, Rogosa, Sharpe agar was used to estimate the colony forming unit (CFU) counts of lactic acid bacteria; Luria-Bertani was used for *Escherichia coli*; and Gauze No. 1 medium was used to determine actinomycetes. All media were obtained from Guangdong HuanKai Microbial Sci. & Tech. Co., Ltd. (Guangzhou, China).

### 2.7 | Statistical analysis

Analysis of variance (ANOVA) was conducted to assess the effects of cropland and cultivars, as well as their interaction, on physiological and biochemical properties, microbial abundance and the relative abundance of bacterial taxa. Levene's test was used to assess homogeneity. If the associated *p*-value for these tests was <0.05, the null hypothesis was rejected, indicating that the variances between the treatments were not equal. Pearson's correlation (first testing for normality and homogeneity of Pearson residuals) was used to determine the relationship between physiological and biochemical properties, microbial abundance and relative abundance using SPSS 26.0 for Windows (SPSS, Chicago, IL, USA). All microbial species and their relative abundances were analyzed at the genus level. This equation was used for analysis: $Y = \mu + T_i + \varepsilon_{ij}$, where Y was the response variable, $\mu$ is the overall mean, $T_i$ is the effect of treatment and $\varepsilon_{ij}$ is the residual error (Li et al., 2018).

Two datasets were used to comprehensively evaluate the relationships between various indicators (all variables that we measured): (1) the physiological and biochemical properties and microbial counts (cropland or cultivar treatments) and (2) the relationship between microbial relative abundance and physiological and biochemical properties (cropland or cultivar treatments). Furthermore, the Bray–Curtis distance matrices were calculated to assess dissimilarities among samples based on species composition and environmental factor abundances, and the Mantel test was used to determine the correlation between the two distance matrices (OriginPro®2022b software, OriginLab Corp., WA, USA).

## 3 | RESULTS

### 3.1 | Phyllosphere bacteria among different forage crops and cropping systems

The four most abundant bacteria at the genus level were *Pantoea*, *Erwinia*, *Acinetobacter* and *Pseudomonas* (Figure 1a). Between the PF and DL bacterial communities, 87 OTUs were identified (Figure 1b). However, compared with PF, the relative abundances of genera such as *Pseudomonas*, *Plesiomonas*, *Gemmatimonas*, *Edwardsiella*, *Vibrio*, *Staphylococcus*, *Sphingomonas*, *Serratia* and *Cetobacterium* were higher in DL (Figure S1a). In the two croplands (Figure 1d), *Pantoea* ($p = 0.013$), *Plesiomonas* ($p = 0.033$) and *Bryobacter* ($p = 0.041$) were the three most differentially abundant genera ($p < 0.05$). The Venn diagram shows that 58 OTUs were shared among FP, IR and SV (Figure 1e). Among the three cultivars, the relative abundance of five bacterial genera, namely, *Rahnella1*, *Aeromonas*, *Cetobacterium*, *Flavobacterium* and *Vibrio* differed significantly ($p < 0.05$) (Figure 1f). However, compared those in IR and SV, the relative abundance of genera such as *Plesiomonas*, *Cetobacterium*, *Serratia*, *Acinetobacter* and *Gemmatimonas* was also higher in FP (Figure S1b).

Alpha diversity analysis revealed that the average values of the Sob, Chao1, Shannon and Simpson indices for DL and PF were 442 versus 420, 690 versus 675, 1.45 versus 1.89 and 0.29 versus 0.51, respectively. The alpha diversity revealed that the average values of the Sob, Chao1, Shannon and Simpson indices for FP, IR and SV were 433 versus 406 versus 535, 672 versus 631 versus 798 ($p = 0.019$), 1.67 versus 1.30 versus 1.63 and 0.44 versus 0.35 versus 0.38, respectively (Figure 2).

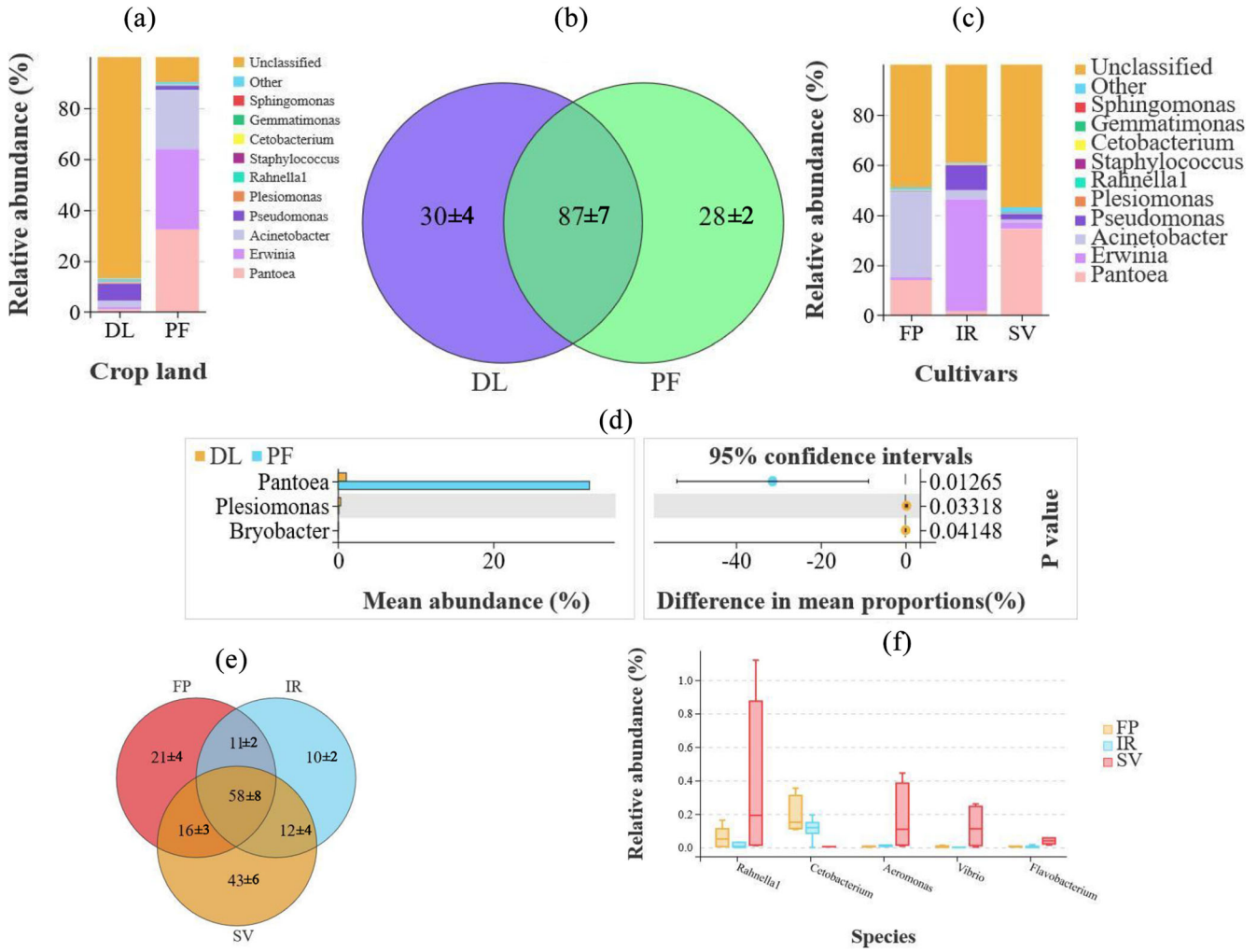



**FIGURE 1** Effects of cropland and cultivars on bacterial communities. DL, dryland; FP, forage pea; IR, Italian ryegrass; PF, paddy field; SV, smooth vetch. The different colors correspond to different genera (a, c, d and f) or cropland (b) or cultivars (e) treatments. The x-axis indicates the cropland or cultivars treatments, and the y-axis indicates the relative abundances of bacterial genera (a and c).

The principal component analysis (PCA) of bacterial diversity revealed that the total cumulative variance contribution rate of the first and second axes was 53.97% (Figure 3). Although the overlap between the 95% confidence intervals for DL and PF was high (Figure 3a, $p = 0.057$), a clear separation was observed at the 95% confidence intervals among the three cultivars (Figure 3b, $p = 0.004$).

## 3.2 | Phyllosphere physiological and biochemical properties among different crops and environments

Although cropland had no effect on almost physico-biochemical indicators ($p > 0.05$) (Tables 3 and 4 and Figure 4a,b), DL exhibited a higher transpiration rate, stomatal conductance, total conductance of water vapor and chlorophyll, reducing sugar, phosphorus and free amino acid contents but lower soluble sugar and soluble protein contents than those of PF ($p < 0.05$).

The three crops showed considerable differences in the physiological and biochemical properties closely related to the dense survival and reproduction of bacteria. The SV exhibited a higher moisture retention capacity and cuticular permeability, than FP and IR ($p < 0.05$) (Table 3). The SV exhibited the highest chlorophyll (5.35 mg/g) and vitamin C (3.25 mg/g) contents, while it had the lowest soluble sugar content (22.5 mg/g) (Tables 3 and 4), compared to FP and IR. The soluble protein and free amino acid contents in FP and SV exhibited no differences ($p > 0.05$). The stomatal density on the upper and lower surfaces of FP was significantly higher than that of IR ($p < 0.05$) (Figure 4c,d). In most cases, the water loss of SV was lower than that of FP and IR (Figure 5f).

## 3.3 | Phyllosphere microbial counts among different crops and environments

The counts of aerobic bacteria, lactic acid bacteria, *Escherichia coli* and actinomycetes in DL leaves increased by 0.04, 0.44, 0.74 and 0.89 lg cfu/g FM (where cfu means colony forming units), respectively, compared with those in PF ($p < 0.05$). Apart from aerobic and lactic acid

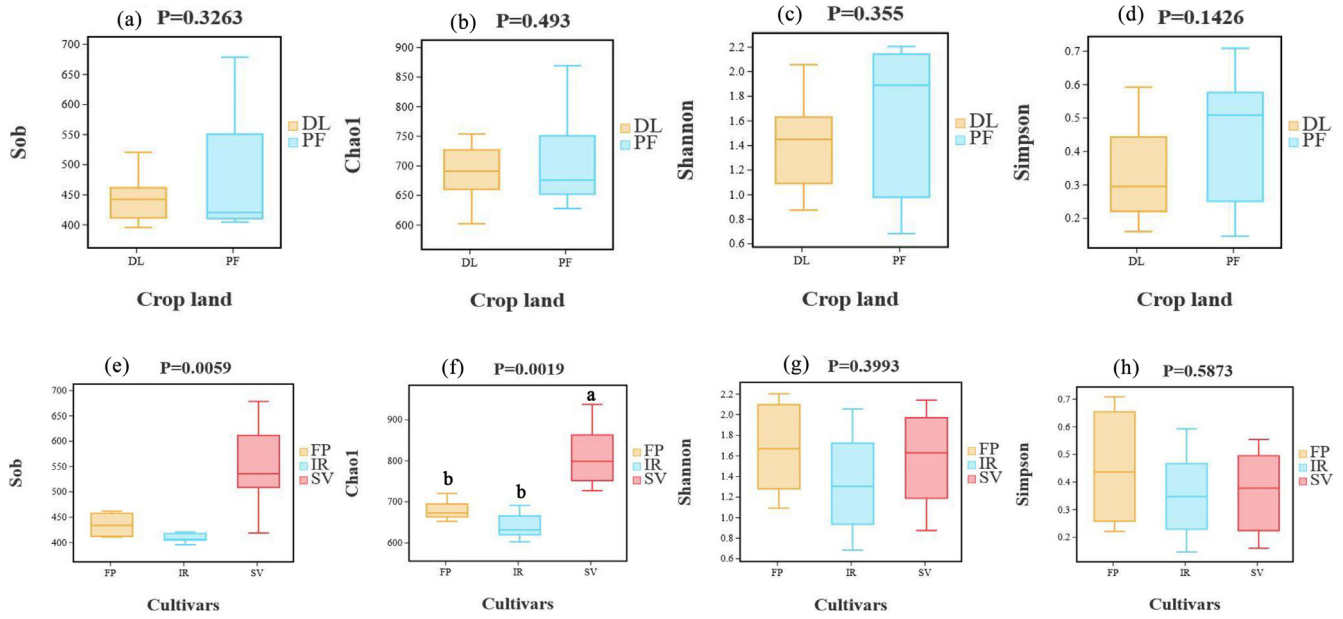



**FIGURE 2** Influence of cropland and cultivars on bacterial alpha diversity. DL, dryland; FP, forage pea; IR, Italian ryegrass; PF, paddy field; SV, smooth vetch. The abscissa shows the cropland or cultivars treatments, and the ordinate shows the alpha diversity indexes and their units. *p* values were calculated by *t*-tests.

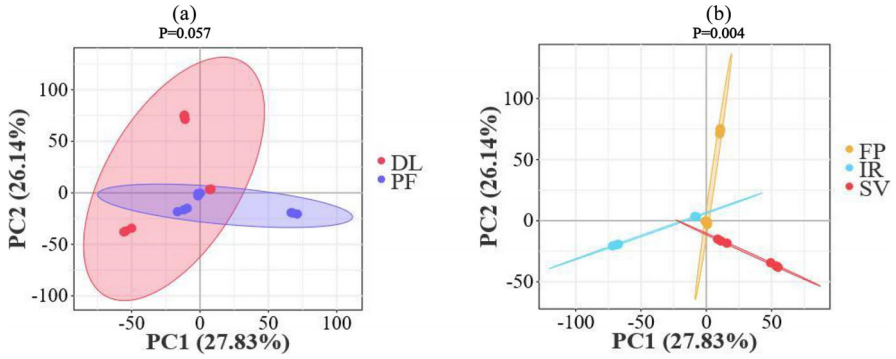



**FIGURE 3** Principal coordinate analysis of microbial composition at different cropland (a) and cultivars (b). DL, dryland; FP, forage pea; IR, Italian ryegrass; PF, paddy field; SV, smooth vetch. The first axis accounts for 27.83% of the total variance and the second for 26.14%. The original attributes, with their vectors intersecting at (0, 0), are also inserted. The length of each attribute vector is proportional to its contribution to the principal component axis. The ellipse indicates 95% confidence. In (a), red and purple colors indicate DL and PF, respectively. In (b), yellow, light blue and red colors indicate FP, IR and SV, respectively.

bacteria, the numbers of microorganisms in the leaves of the different cultivars varied significantly ($p < 0.05$). Mold exhibited the lowest number among all the microorganisms (Table 3). A lower number of yeast was identified in the phyllosphere of IR (1.12 lg cfu/g FM), which was significantly lower than those in FP (1.48 lg cfu/g FM) and SV (1.60 lg cfu/g FM) ($p < 0.05$), whereas no significant differences were identified between FP and SV ($p > 0.05$).

## 3.4 | Correlation among all variables and the microbial communities

The numbers of aerobic bacteria, lactic acid bacteria and actinomycetes were significantly positively correlated with the transpiration rate, stomatal conductance, total conductance of water vapor and chlorophyll content ($p < 0.05$) (Table 5). However, stomatal density (upper and lower surfaces) and cuticular permeability were negatively correlated with most microbial numbers. Wax, reducing sugar and phosphorus contents were positively correlated with the abundance of aerobic and lactic acid bacteria ($p < 0.05$).

Regarding phyllosphere properties and microbial counts, a PCA was performed to decrease the number of dimensions in the dataset. The initial dataset comprised 21 variables. Following PCA, these variables were summarized into two principal components, which captured a substantial proportion of the variability originally present across the variables. The first and second principal components collectively explained 54.0% of the total cumulative variance (Figure 5a,b), suggesting that these two components encompassed a notable

**TABLE 3** Effects of cropland and cultivars on physical properties and microorganism compositions of leaves ($n = 18$).

| Time and treatments | | Physical properties | | | | | | Microorganism compositions ($log_{10}$ cfu/g FM) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Tr (mmol/m$^2$/s) | Sc (mmol/m$^2$/s) | Tcwv (mmol/m$^2$/s) | MRC (%) | Cp (g/h/g DM) | Ch (mg/g) | AB | Yeasts | LAB | Molds | *Escherichia coli* | Actinomycetes |
| Cropland (CL) | Dryland | 7.83 ± 0.54a | 465 ± 41.3a | 419 ± 34.1a | 36.1 ± 3.12 | 2.84 ± 0.09 | 5.46 ± 0.27a | 4.86 ± 0.02a | 1.35 ± 0.16 | 1.65 ± 0.11a | 0.54 ± 0.11 | 5.01 ± 0.15a | 2.91 ± 0.21a |
| | Paddy field | 3.20 ± 0.46b | 161 ± 26.8b | 154 ± 24.7b | 37.2 ± 3.44 | 3.02 ± 0.05 | 3.23 ± 0.20b | 4.70 ± 0.02b | 1.43 ± 0.03 | 1.21 ± 0.07b | 0.42 ± 0.04 | 4.27 ± 0.21b | 2.02 ± 0.10b |
| Cultivars (C) | Forage pea | 5.96 ± 1.37 | 370 ± 92.3 | 328 ± 79.7 | 43.2 ± 3.64a | 3.04 ± 0.07a | 3.95 ± 0.50b | 4.74 ± 0.05 | 1.48 ± 0.05a | 1.26 ± 0.13 | 0.57 ± 0.05a | 3.67 ± 0.20c | 1.72 ± 0.16b |
| | Italian ryegrass | 6.30 ± 0.44 | 357 ± 29.9 | 331 ± 25.7 | 37.9 ± 2.88ab | 3.01 ± 0.05a | 3.74 ± 0.23b | 4.78 ± 0.01 | 1.12 ± 0.11b | 1.24 ± 0.01 | 0.58 ± 0.08a | 4.50 ± 0.15b | 2.69 ± 0.18a |
| | Smooth vetch | 4.28 ± 0.57 | 213 ± 33.1 | 201 ± 30.1 | 28.7 ± 4.32b | 2.74 ± 0.11b | 5.35 ± 0.40a | 4.73 ± 0.03 | 1.60 ± 0.02a | 1.57 ± 0.16 | 0.22 ± 0.04b | 5.37 ± 0.03a | 2.52 ± 017a |
| *p* value | CL | 0.000 | 0.000 | 0.000 | 0.822 | 0.078 | 0.000 | 0.000 | 0.511 | 0.002 | 0.210 | 0.025 | 0.000 |
| | C | 0.247 | 0.130 | 0.143 | 0.027 | 0.022 | 0.012 | 0.525 | 0.000 | 0.111 | 0.000 | 0.000 | 0.001 |
| | CL × C | 0.000 | 0.000 | 0.000 | 0.320 | 0.320 | 0.000 | 0.000 | 0.000 | 0.032 | 0.003 | 0.000 | 0.076 |

*Note*: Different lowercase letters in the same column represent significant difference among cropland or cultivars treatments ($p < 0.05$).

Abbreviations: AB, aerobic bacteria; Ch, chlorophyll; Cp, cuticular permeability; LAB, lactic acid bacteria; MRC, moisture retention capacity; Sc, stomatal conductance; Tcwv, total conductance of water vapor; Tr, transpiration rate.

amount of information from the original dataset. The PF and DL were clearly separated at the 95% confidence intervals on the positive sides of the first and second axes, respectively (Figure 5a). This separation was observed because DL exhibited a higher abundance of microorganisms distributed along the first axis, resulting in a significant divergence from PF. In the PCA analysis of the three cultivars, the IR indicators were positioned on the negative side of the horizontal axis (which represents one of the principal components derived from the PCA), whereas FP was positioned on the positive side. The significant separation between the IR and FP along this axis (Figure 5b) was attributed to the underlying differences in microbial population and physiological properties between these two cultivars.

In the canonical correspondence analysis, environmental factors impacted the relative abundance of bacteria and showed a clear separation between DL and PF (Figure S1c, $p = 0.521$). Although SV and FP exhibited a high degree of overlap, both were clearly separated from IR (Figure S1d, $p = 0.006$). Negatively significant correlations were observed between the relative abundances of *Pantoea*, *Acinetobacter* and *Rahnellal* and the transpiration rate, stomatal conductance and total conductance (Figure 5c). However, the transpiration rate, stomatal conductance and total conductance were positively correlated with the relative abundances of *Cetobacterium*, *Gemmatimonas* and *Bryobacter* ($p < 0.05$). Significant positive correlations were observed between the relative abundances of *Cetobacterium*, *Gemmatimonas* and *Bryobacter* and moisture retention capacity; however, moisture retention capacity was negatively correlated with *Flavobacterium* ($p < 0.05$). Wax, reducing sugar and free amino acid contents were positively correlated with the relative abundance of *Bryobacter* ($p < 0.05$). The soluble sugar content was negatively correlated with the relative abundances of *Staphylococcus* and *Sphingomonas* ($p < 0.05$). Physiological properties ($r = 0.531$) had a greater impact on relative bacterial abundance compared with biochemical properties ($r = 0.250$) (Figure 5d,e).

## 4 | DISCUSSION

### 4.1 | Effects of cropland and cultivars on the bacterial community

In contrast with the stable soil microbial communities in PF and DL (Su et al., 2024), the species and relative abundance of the phyllosphere bacterial communities were prone to change, which was primarily attributed to the fragile growth environment that was susceptible to perturbations caused by variations in light and nutrient availability (Tang et al., 2023). Only three bacterial species (*Pantoea*, *Plesiomonas* and *Bryobacter*) showed significant differences in relative abundance between PF and DL in this study, owing to the difference in soil nutrients in the two growing environments. However, this could be explained by the difference in the soil moisture contents of PF (50.5%) and DL (26.8%), respectively (Table S1). The waterlogged stress in PF may have favored *Pantoea*, which can improve plant tolerance (Chen et al., 2017; Gond et al., 2015). In the PF soil, the relative

**TABLE 4** Effects of cropland and cultivars on biochemical properties of leaves ($n = 18$).

| Time and treatments | | Wax (mg/g) | Vitamin C (mg/g) | Reducing sugar (mg/g) | Soluble sugar (mg/g) | Soluble protein (mg/g) | Phosphorus (mg/g) | Free amino acids (mg/g) |
|---|---|---|---|---|---|---|---|---|
| Cropland (CL) | Dryland | 49.6 ± 9.02 | 1.72 ± 0.50 | 5.62 ± 0.42a | 28.5 ± 1.89b | 2.84 ± 0.37b | 0.28 ± 0.02a | 4.06 ± 0.51a |
| | Paddy field | 51.0 ± 4.4 | 1.64 ± 0.41 | 3.48 ± 0.13b | 46.1 ± 3.29a | 6.12 ± 0.75a | 0.12 ± 0.04b | 2.18 ± 0.29b |
| Cultivars (C) | Forage pea | 51.8 ± 9.14 | 0.80 ± 0.08b | 4.98 ± 0.70 | 41.8 ± 4.05a | 6.53 ± 0.79a | 0.12 ± 0.03 | 4.42 ± 0.48a |
| | Italian ryegrass | 44.7 ± 7.03 | 1.01 ± 0.07b | 4.18 ± 0.39 | 44.4 ± 3.78a | 2.39 ± 0.71b | 0.18 ± 0.05 | 1.09 ± 0.19b |
| | Smooth vetch | 54.5 ± 9.86 | 3.25 ± 0.78a | 4.50 ± 0.29 | 25.8 ± 2.29b | 4.52 ± 0.67a | 0.29 ± 0.06 | 3.85 ± 0.44a |
| *p* value | CL | 0.890 | 0.905 | 0.000 | 0.000 | 0.000 | 0.004 | 0.003 |
| | C | 0.716 | 0.001 | 0.524 | 0.001 | 0.001 | 0.052 | 0.000 |
| | CL × C | 0.325 | 0.988 | 0.044 | 0.019 | 0.168 | 0.046 | 0.116 |

*Note*: Different lowercase letters in the same column represent significant difference among cropland or cultivars treatments ($p < 0.05$).

abundances of *Erwinia* (Abbas et al., 2022) and *Acinetobacter* (Li et al., 2019) were high. The species attached to the plants mainly originated from the soil (Wang et al., 2021), which also explained the higher relative abundances of *Erwinia* and *Acinetobacter* attached to the cultivars planted in PF. The soil in PF underwent a dry (plant forage)/wet (plant rice) cycle, leading to reductions in bacterial diversity compared with DL and the transition of microorganisms from the soil to plants. This is similar to previously reported results (Qi et al., 2021). Furthermore, the relative abundances of *Erwinia* and *Pantoea* in the cultivars were high owing to their close relationship (Zhang & Qiu, 2015). This correlated with a previous report indicating the presence of *Erwinia* in IR (Li et al., 2021). Unfortunately, studies on the influence of crop cultivation sites on phyllosphere bacterial diversity were still limited, resulting in a lack of sufficient references for the present research.

The predominant epiphytic bacterial communities on silage maize (*Zea mays*) leaves include aerobic genera such as *Leuconostoc*, *Weissella*, *Sphingobacterium* and *Stenotrophomonas* (Wang et al., 2021), while the dominant bacterial communities on the phyllosphere of forage wheat (*Triticum aestivum*) are mainly composed of *Pantoea*, *Weissella*, *Pseudomonas*, *Exiguobacterium* and *Paenibacillus* (Keshri et al., 2019). In this study, the dominant bacteria include *Plesiomonas*, *Pseudomonas*, *Acinetobacter*, *Erwinia* and *Pantoea*, indicating that there were considerable differences in phyllosphere bacterial communities among different crops. The relative abundance of species in IR was lower than those in FP and SV by 15 and 38 species, respectively, indicating that IR is unsuitable for increasing species diversity. The significant changes and bacterial alpha diversity (Figure 2) in the relative microbial abundance also confirmed this finding. In general, ryegrass stimulates the growth of *Flavobacterium* (Miao et al., 2020). However, *Flavobacterium* had the lowest relative abundance in IR. This may be related to the lower nutrients and stomatal density in IR. In summary, fluctuating phyllosphere microbial communities reflect environmental conditions that can be leveraged to improve crop growth. For example, waterlogged stress in PF favored beneficial microbes such as *Pantoea* and *Acinetobacter*, which enhance plant tolerance. Understanding these microbial dynamics can guide management practices to optimize plant health and growth. By promoting beneficial microbes and managing soil moisture, agricultural sustainability can be improved. Future research will further refine these strategies for more effective crop management.

## 4.2 | Relationships between physiological and biochemical properties of the phyllosphere and the abundance of microorganisms

The population of microorganisms in DL was higher than that of PF (except for yeast). This observation was closely related to the higher transpiration rate, stomatal conductance and total water vapor conductance in DL. Generally, excessively low stomatal conductance may hinder the entry of $CO_2$ into the leaves and the conductance of water vapor in plants (Lawson & Blatt, 2014), thereby affecting the permeability of water vapor. However, an increase in the transpiration rate could improve the wetting performance of the grass surface by providing sufficient water or humidity for microorganisms to colonize the plant (including the phyllosphere surface). The PF faced mild waterlogged stress that could inhibit the physiological properties of grass, including the transpiration rate, stomatal conductance and total conductance of water vapor. The DL had higher phosphorus and free amino acid contents that were positively correlated with the abundance of lactic acid bacteria compared to PF because phosphorus is involved in various plant metabolic activities that facilitate the reproduction of lactic acid bacteria. For instance, these metabolic activities may enhance the amino acid content, which serves as an important nutritional source for lactic acid bacteria. This observation aligned with previous reports (Tang et al., 2022). The microbial numbers in this study were smaller than those previously reported (Chen et al., 2021). This may be because the forage primarily grows in winter and spring, seasons

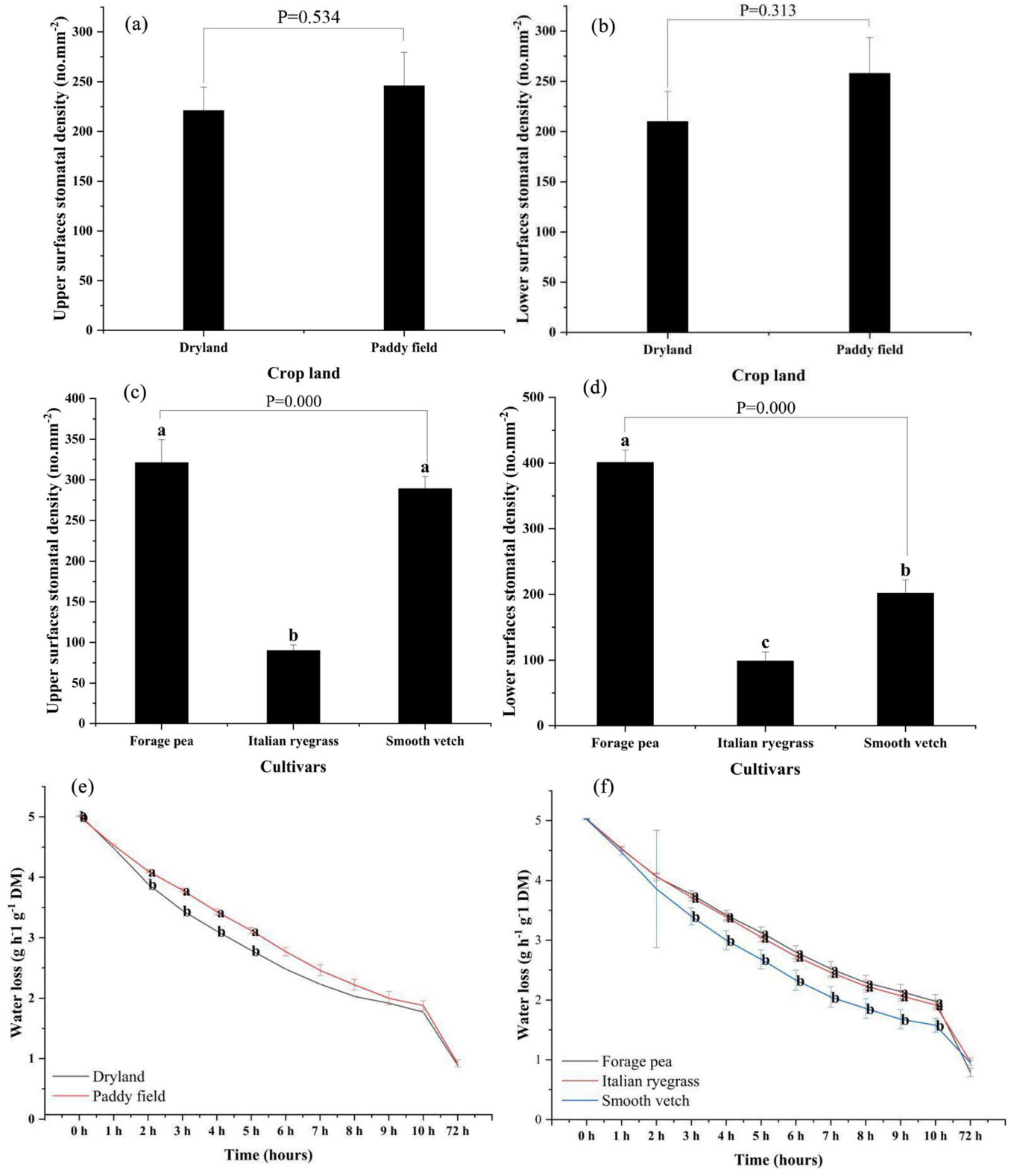



**FIGURE 4** Stomatal density (a, b, c and d) and cuticular permeability (e and f) of leaf for different cropland or cultivars. Error bars represent standard errors. Different lowercase letters at the top of the columns show significant differences on the upper or lower leaf surfaces among treatment, respectively.

characterized by limited rainfall and low temperatures. In addition, the high local altitude further reduces the environmental temperature, which is not conducive to the increase in microbial abundance. The soluble protein content and microorganism number were negatively correlated (except for yeast and molds). This finding provides a rationale for the higher soluble sugar and protein contents in PF

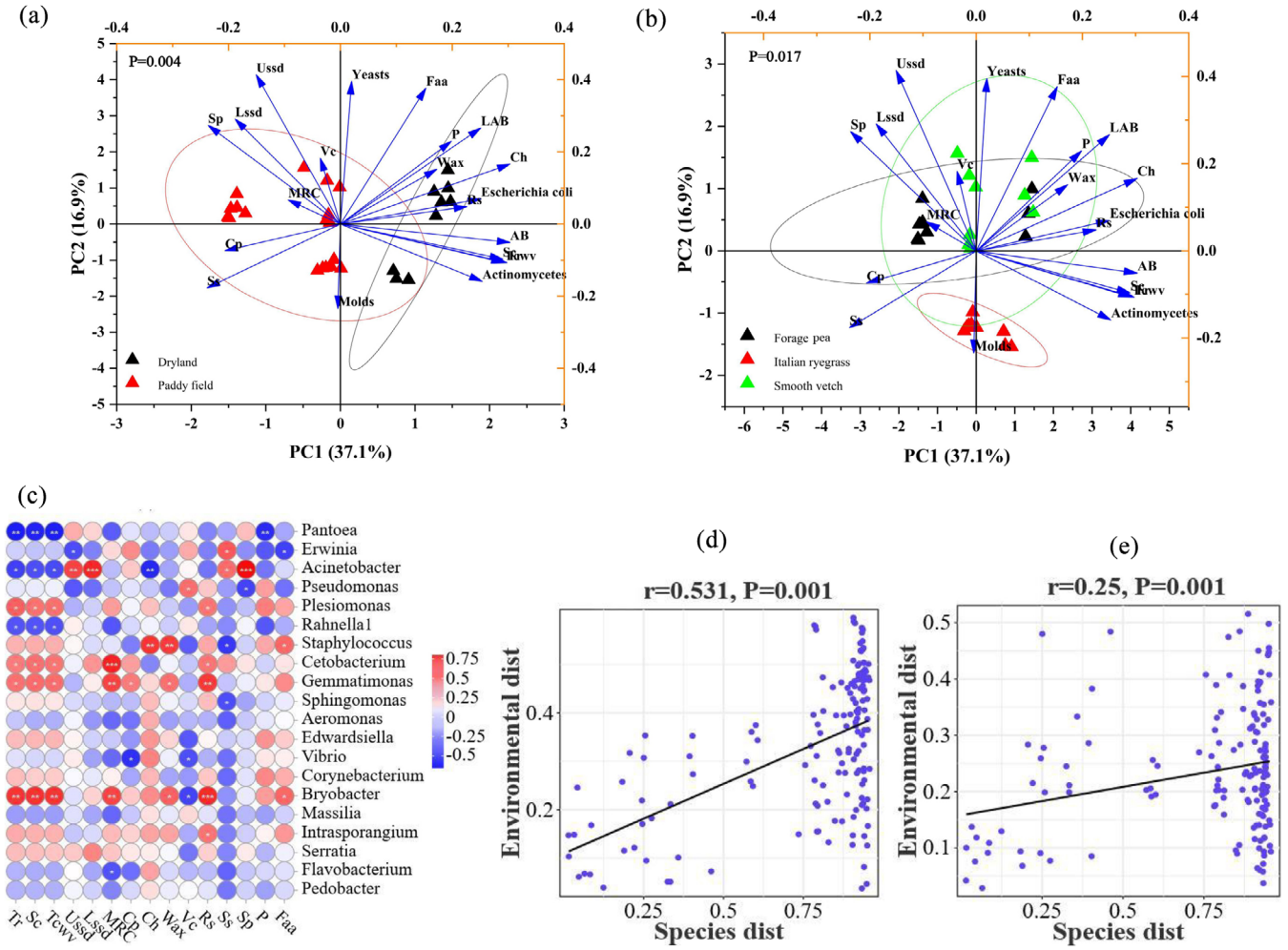



**FIGURE 5** Influence of environmental factors on bacterial communities. (a) Crop land; (b) cultivars. The first axis accounts for 37.1% of the total variance and the second for 16.9%. The original attributes, with their vectors intersecting at (0, 0), are also inserted. The length of each attribute vector is proportional to its contribution to the principal component axis. The ellipse indicates 95% confidence. (c) The horizontal axis represents environmental factors, the vertical axis represents species and the color represents the strength of correlation, and *, ** and *** represent $p < 0.05$, 0.01 and 0.001, respectively. (d and e) The horizontal axis represents the sample distance corresponding to species, and the vertical axis represents the sample distance corresponding to environmental factors, points represent pairs of samples, lines represent fitted correlation lines, *p*-values represent significance of correlation and *r* values represent correlation. The (d) and (e) represent the relationships among physiological factors and chemical factors with species abundance, respectively. AB, aerobic bacteria; Ch, chlorophyll; Cp, cuticular permeability; Faa, free amino acids; LAB, lactic acid bacteria; Lssd, lower surface stomatal density; MRC, moisture retention capacity; P, phosphorus; Rs, reducing sugar; Sc, stomatal conductance; Sp, soluble protein; Ss, soluble sugar; Tcwv, total conductance of water vapor; Tr, transpiration rate; Ussd, upper surface stomatal density; Vc, vitamin C.

compared with DL (as these indicators tend to increase in adverse environments).

The lowest moisture retention capacity and cuticular permeability in the IR treatment were related to a lower stomatal density. In general, lower stomatal density can inhibit phyllosphere moisture loss. Compared to IR, FP and SV exhibited higher yeast numbers, due to their higher soluble protein content. Soluble proteins serve as an important source of organic nitrogen that is a crucial nutritional source for yeast. Yeast can reduce organic nitrogen in grass leaves (Nix-Stohr et al., 2008). Notably, the soluble protein content was also negatively associated with microbial number, likely owing to the selective nature of different microorganisms, such as aerobic bacteria and lactic acid bacteria, which prefer nutrient sources with high sugar content (Chen et al., 2021). Although no difference in wax content was observed among the three crops, wax content was positively correlated with the number of lactic acid and aerobic bacteria. This finding is inconsistent with a previous finding (Tang et al., 2023), which may be because wax can hinder the transfer of phyllosphere nutrients from the mesophyll tissue to the phyllosphere surface. In this study, we found that microorganisms could utilize higher nutrient concentrations within the plant body. Notably, the number of Actinomycetes on the leaves was negatively correlated with stomatal density. Stomata are important channels for plants to exchange gases and nutrients with the outside environment, whereas Actinomycetes are anaerobic

**TABLE 5** Correlation coefficients between chemical and physical properties and population sizes of epiphytes ($n = 18$).

| Items | | Aerobic bacteria | Yeasts | Lactic acid bacteria | Molds | *Escherichia coli* | Actinomycetes |
|---|---|---|---|---|---|---|---|
| Physical properties | Transpiration rate | 0.885** | 0.038 | 0.508** | 0.237 | 0.302 | 0.427* |
| | Stomatal conductance | 0.877** | 0.023 | 0.491** | 0.294 | 0.250 | 0.390* |
| | Total conductance of water vapor | 0.880** | 0.008 | 0.492** | 0.287 | 0.263 | 0.411* |
| | Upper surfaces stomatal density | −0.388* | 0.413* | 0.010 | −0.205 | −0.301 | −0.543** |
| | Lower surfaces stomatal density | −0.368 | 0.240 | −0.187 | 0.187 | −0.581** | −0.707** |
| | Moisture retention capacity | 0.005 | 0.205 | 0.161 | 0.277 | −0.363 | −0.235 |
| | Cuticular permeability | −0.313 | −0.099 | −0.302 | 0.272 | −0.525** | −0.482* |
| | Chlorophyll | 0.541** | 0.325 | 0.644** | −0.320 | 0.719** | 0.557** |
| Biochemical properties | Wax | 0.391* | 0.339 | 0.400* | −0.107 | 0.183 | 0.080 |
| | Vitamin C | −0.095 | 0.150 | 0.354 | −0.264 | 0.365 | −0.006 |
| | Reducing sugar | 0.634** | −0.022 | 0.410* | 0.454* | 0.254 | 0.210 |
| | Soluble sugar | −0.347 | −0.126 | −0.423* | 0.154 | −0.758** | −0.416* |
| | Soluble protein | −0.508** | 0.386* | −0.157 | 0.013 | −0.534** | −0.692** |
| | Phosphorus | 0.464* | 0.110 | 0.811** | −0.120 | 0.646** | 0.533** |
| | Free amino acids | 0.340 | 0.463* | 0.589** | −0.078 | 0.120 | −0.013 |

*Note*: Coefficients of non-parametric Spearman's correlation.
*Significant correlation at $p < 0.05$.
**Significant correlation at $p < 0.01$.

bacteria. Therefore, an excessive stomatal density is not conducive to anaerobic bacteria survival.

In summary, environmental factors such as transpiration rate, stomatal conductance and nutrient availability (e.g., phosphorus and amino acids) can enhance microbial colonization, boosting plant health and productivity. For example, higher nutrient concentrations and water availability in certain stages favor the growth of lactic acid bacteria, promoting beneficial microbial communities. Additionally, understanding the relationships between stomatal density and microbial populations can help modify environmental conditions to enhance specific microbial groups, ensuring better growth and resilience. By applying these insights, sustainable agricultural practices can be developed that optimize both crop and microbial health.

## 4.3 | Factors influencing bacterial community distribution in cropland or cultivars

CCA revealed that environmental factors exhibited different effects on bacterial communities between croplands and cultivars. This result suggests that the regulations of the physiological and biochemical properties of crops grown on different croplands vary significantly. The PF and DL have distinct management systems, leading to differences in microbial species and their selectivity for the environment. However, the nutritional and physiological properties of the three forage varieties are diverse. The changes in microbial species associated with forage plants under environmental conditions, such as those applied in this study, have not been reported previously, whereas changes in soil microorganisms from similar environments have been reported (Li et al., 2019). Additionally, resource availability is the primary factor that determines the number of microorganisms attached on plants, particularly those colonizing the plant surface (Tang et al., 2023). For example, *Gemmatimonas* tend to utilize organic matter such as humus and lignin as a carbon source (Liu et al., 2022), while *Staphylococcus* prefers to utilize amino acids (Prince et al., 2023). Notably, the physiological properties of leaves exhibited a higher coefficient of determination for relative bacterial abundance compared with the biochemical properties. Furthermore, the reduction in β-diversity was interpreted as a reduction in the stochastic processes of bacterial community assembly (Qi et al., 2021).

The reduction of bacterial diversity in IR may be closely related to poorer biochemical properties. Studies have revealed that phyllosphere microorganisms originate from soil or rhizosphere microorganisms (Chen et al., 2022). This study suggested that the phyllosphere microorganisms colonizing the phyllosphere were influenced by local environmental conditions and external factors, such as soil-borne microorganisms. Therefore, understanding the factors influencing the composition and activity of phyllosphere microorganisms is crucial for sustainable agriculture and ecosystem management. However, we did not collect data on soil microorganisms. Therefore, future studies should collect comprehensive soil and phyllosphere microorganism data to elucidate their interactions and contributions to plant growth and health.

The observed short-term fluctuations in phyllosphere bacterial communities were more likely to instantly reflect environmental condition variations compared with the complex adaptations associated with long-term cropland and cultivar interactions. Nevertheless, this finding elucidated the bacterial community dynamics, particularly during the initial establishment phase of crop rotation systems, providing a valuable perspective for optimizing forage crop rotation strategies. To comprehensively determine the evolution trajectory of microbial communities, future studies should focus on longer rotation cycles and more diverse ecological conditions.

## 5 | CONCLUSION

Although the paddy field soil was inherently rich in nutrients, excessively high moisture content hindered the healthy growth and characteristic improvement of forage crops. This imposed nutritional and environmental constraints on the microorganisms that rely on crop nutrient distribution and physiological structure enhancement, including their habitat environment. In the phyllosphere bacterial communities of forage crops, aerobic bacteria dominated. Furthermore, forage pea effectively regulated the distribution of nutrients and humidity between the plant interior and surface through its unique physiological mechanism of higher stomatal density, creating a more favorable microenvironment that significantly promotes the colonization and growth of phyllosphere bacteria. This discovery not only reveals the key factors influencing the composition of phyllosphere bacterial communities but also underscores the important role of forage peas in promoting the establishment of phyllosphere microbial ecosystems.

### CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

### DATA AVAILABILITY STATEMENT

Data were deposited in the National Microbiology Data Center (NMDC) with accession numbers NMDC10018678 (https://nmdc.cn/resource/genomics/sra/detail/NMDC40051558-40051575).

### ORCID

*Liuxing Xu* https://orcid.org/0000-0002-9704-2594

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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