

Growth-year-associated variation in the bulb metabolome, bulb-associated microbiota, and root transcriptome of wild *Fritillaria cirrhosa*

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Research Article

Keywords: medicinal geophyte, plant development, untargeted metabolomics, fungal community, 16S rRNA gene, ITS, RNA sequencing

Posted Date: August 5th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10453246/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background: Wild *Fritillaria cirrhosa* is a slow-growing alpine medicinal geophyte for which harvest age is assumed to influence bulb quality, yet it is not known whether consecutive growth years are accompanied by concordant changes in bulb chemistry, bulb-associated microbiota, and root transcription. We profiled 1- to 4-year-old wild plants by untargeted bulb LC-MS, bacterial 16S and fungal ITS amplicon sequencing of bulb-adhering soil, and root RNA sequencing, using three biological replicates per growth year and data layer.

Results: Of 2,117 retained LC-MS features, 11 (0.5%) were associated with growth year after false-discovery-rate correction and application of an absolute Spearman threshold of $|p| \geq 0.60$. Bacterial richness, Shannon diversity, and Bray-Curtis composition did not differ significantly among years (PERMANOVA $R^2 = 0.294$, $P = 0.339$). Fungal alpha diversity was also nonsignificant, whereas fungal community composition differed among years ($R^2 = 0.401$, $P = 0.002$). Adjacent-year root transcriptome contrasts contained 157, 157, and 318 differentially expressed genes for years 1-2, 2-3, and 3-4, respectively; protein processing in the endoplasmic reticulum was the only KEGG pathway that remained significant after multiple-testing correction, specifically in the year 3-4 contrast (adjusted $P = 1.98 \times 10^{-4}$).

Conclusions: Growth-year responses were layer specific rather than synchronous. Fungal community turnover and the late root transcriptional response enriched for endoplasmic-reticulum protein processing were the strongest signals, complemented by 11 age-associated bulb metabolite features. Together, these results define the year 3-4 interval as a priority developmental window and provide focused molecular, chemical, and microbial candidates for validation across independent *Fritillaria* populations.

Background

Fritillaria cirrhosa D. Don is an alpine perennial geophyte whose dried bulbs are used as the traditional medicinal material *Chuanbeimu*. Demand still relies partly on wild collection, while slow growth, restricted alpine habitats, and intensive harvesting challenge both resource sustainability and consistency of medicinal-material quality[1-3]. The bulbs contain steroidal and isosteroidal alkaloids with documented biological activity[4, 5]. Nucleosides and other constituents also vary with cultivation conditions[6, 7]. Because the harvested organ develops below ground over several years, growth year may integrate plant ontogeny with cumulative environmental exposure.

Plant ontogeny changes carbon allocation, root exudation, nutrient demand, and immune status[8-10]. These processes can reshape metabolite production and microbial community assembly[11, 12]. Age-associated microbiome variation has been reported in wild perennial plants, whereas longitudinal evidence from perennial *Arabis* indicates that soil residence time can also alter root communities independently of flowering[13, 14]. Root-derived metabolites provide one route by which host development may influence fungal assemblages[15], but soil remains the principal microbial source

pool[16-18]. Fungal partners may be especially relevant to perennial geophytes because nutrient exchange and colonization depend on host developmental status, carbon supply, and environmental conditions[19, 20]. Bacterial and fungal communities should therefore be evaluated separately rather than assumed to follow the same age-related trajectory.

Untargeted metabolomics, amplicon sequencing, and transcriptomics capture complementary dimensions of age-associated plant variation. LC-MS maps chemical feature space, amplicon sequencing resolves bacterial and fungal community composition, and RNA sequencing reveals tissue-specific transcriptional programmes[21-23]. Applying layer-appropriate statistics to each data type allows the biological information in each layer to be retained while their temporal patterns are compared across the same growth-year gradient.

Research on *Fritillaria* has addressed wild-resource availability, conservation genetics, phytochemistry, and cultivation-dependent variation in bulb constituents[1-7]. However, consecutive growth years have not been evaluated simultaneously across bulb chemistry, bulb-associated bacterial and fungal communities, and root transcription in one field design. We therefore tested whether these layers contain reproducible growth-year signals and whether their strongest changes occur in the same developmental interval. The analysis combined replicate-based testing, false-discovery-rate control, community-level statistics, and pathway enrichment to identify the biological layers most responsive to growth year.

The aim of this study was to identify the strongest reproducible changes across 1- to 4-year-old wild *F. cirrhosa* using a common growth-year design with three biological replicates per data layer. We assessed bulb metabolomic variation, bacterial and fungal community structure, and adjacent-year root transcriptional transitions, then compared the strength and timing of evidence across layers. We hypothesized that the different biological layers would show distinct response magnitudes and that the strongest host and microbial changes would converge within a defined growth-year interval.

Methods

Sample collection and experimental design

Wild *F. cirrhosa* plants representing consecutive growth years were collected in September 2021 from their natural habitat near Daofu County, Ganzi Tibetan Autonomous Prefecture, Sichuan Province, China (30°30'28.5948" N, 101°27'20.1852" E; 3376 m above sea level). Four growth-year classes (years 1-4) were included, with three biological replicates available for each year and data layer. During our field sampling process, we strictly adhered to the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. The specific sampling method is referred to in the published literature[24]. The botanical identification was confirmed by a botanist, Prof. Zhuyun Yan. A voucher specimen was deposited at the Herbarium of the State Key Laboratory of Southwest Characteristic Traditional Chinese Medicine Resources. Bulbs were used for untargeted LC-MS profiling, soil adhering to harvested bulbs was used for bacterial 16S rRNA

gene and fungal internal transcribed spacer (ITS) amplicon profiling, and roots were used for RNA sequencing. The principal analysis was limited to years 1-4 because all four data layers were available over this interval (Table 1). Year-5 metabolome and amplicon data were retained as a late-stage extension because root RNA sequencing was unavailable for that year (see Additional file 1: Fig. S1).

Plants were assigned to consecutive growth-year classes according to the field sampling criteria used by the collection team. Within each class, three independently collected biological replicates were analysed for every available data layer. The datasets were aligned by growth-year group, and annual group means were used for the descriptive cross-layer summaries.

LC-MS feature processing and growth-year association analysis

Positive- and negative-ion peak-area matrices from the untargeted LC-MS analysis were processed separately. Features without a valid compound identifier, features with insufficient finite observations, and zero-variance features were excluded. Missing values within retained features were replaced by the feature median. The final matrix contained 1,212 positive-ion and 905 negative-ion features. Peak areas were transformed as $\log_2(x + 1)$, the two ion-mode matrices were concatenated by sample, and features were standardized to unit variance before unsupervised principal component analysis (PCA).

For each LC-MS feature, differences among the four growth years were tested by one-way analysis of variance, and P values were adjusted using the Benjamini-Hochberg procedure[25]. Spearman rank correlation was calculated between feature abundance and growth year. A feature was classified as growth-year associated when the adjusted P value was < 0.05 and $|p|$ was ≥ 0.60 . Adjacent-year contrast tables were screened at nominal $P < 0.05$ and $|\log_2 \text{ fold change}| > 0.5$ and summarized separately from the across-year FDR analysis. For visualization, all retained features were plotted by Spearman ρ and $-\log_{10}(\text{FDR } q)$, the 11 supported features were summarized by effect-size lollipop plots, and their annual mean abundances were standardized as z scores. Metabolite names were reported at library-annotation confidence level according to accepted metabolite-identification terminology.[21, 22]

Bacterial 16S rRNA gene sequencing and community analysis

Microbial DNA was extracted with the Zymo BIOMICS DNA Microprep Kit (D4301). The V4 region of the bacterial 16S rRNA gene was amplified using primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR was conducted with KOD-Plus-Neo polymerase on an ABI 9700 instrument using an initial denaturation at 94 °C for 1 min; 25-30 cycles of 94 °C for 20 s, 54 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min. Three technical PCRs were pooled for each sample. Amplicons were checked on 2% agarose gels, purified with a Zymoclean Gel DNA Recovery Kit

(D4008), quantified using a Qubit 2.0 fluorometer, and prepared with the NEBNext Ultra II library kit for paired-end 250-bp Illumina sequencing.

Paired reads were merged with FLASH and processed using QIIME 1.9.[26] Reads were retained when the average quality score was ≥ 25 , sequence length was ≥ 200 bp, and no more than two ambiguous bases were present. Chimeric sequences were screened against the gold database with UCHIME. Operational taxonomic units were generated at 97% sequence identity with UPARSE in USEARCH 10[27], and taxonomy was assigned with UCLUST against SILVA release 132.[28] The rarefied alpha-diversity table, Bray-Curtis distance matrix, principal-coordinate analysis (PCoA) coordinates, and genus-level relative-abundance table were used for statistical analysis.

Fungal ITS sequencing and community analysis

Fungal communities were profiled using the ITS1F/ITS2R primer pair and paired-end 300-bp sequencing. The processed dataset contained sample-level observed richness and Shannon diversity, PCoA coordinates, a community distance matrix, and genus-level taxonomic profiles. For years 1-4, these outputs were analysed using the same diversity and community-level statistical framework as the bacterial data.

Root transcriptome and pathway-enrichment analysis

Root RNA-seq data were available for three biological replicates in each growth year. The analysis used the expression matrix, within-year replicate-correlation summaries, adjacent-year differential-expression tables, and KEGG enrichment outputs. Transcripts with an adjusted P value < 0.05 in the contrast tables were classified as differentially expressed genes (DEGs). KEGG terms were considered statistically supported only when the FDR-adjusted enrichment P value was < 0.05 [29].

Diversity, community, and cross-layer statistics

Observed richness and Shannon diversity were compared among growth years using Kruskal-Wallis tests because each group contained three biological replicates and several distributions were heterogeneous. The global tests determined whether pairwise follow-up comparisons were warranted. Community-level differences were evaluated from Bray-Curtis dissimilarities using permutational multivariate analysis of variance (PERMANOVA) with 999 label permutations[30]. Principal-coordinate analysis (PCoA) was used to visualize sample relationships, and PERMANOVA provided the primary community-level inferential test.

For the cross-layer overview, the numbers of age-associated LC-MS features and differentially expressed genes were displayed together with microbial PERMANOVA R^2 values multiplied by 100. Selected annual means were standardized within each metric as z scores and plotted across growth years. These

predefined integration outputs compared the timing and relative trajectories of the measured layers. Statistical analyses were conducted in Python using NumPy, SciPy, and scikit-learn, and final figures were produced in R from the exported analysis tables. Unless otherwise stated, two-sided $P < 0.05$ or FDR-adjusted $P < 0.05$ was used as the significance threshold.

Results

Study design and data coverage

The primary analysis comprised four consecutive growth years, three biological replicates per year, and four complementary data layers: bulb LC-MS, bacterial 16S, fungal ITS, and root RNA sequencing (Table 1). Restricting the integrated comparison to years 1-4 preserved equivalent data coverage across layers and yielded 12 observations per data type. Year 5 was evaluated only as a supplementary metabolome and microbial-richness extension because matched root RNA-seq data were unavailable (see Additional file 1: Fig. S1).

Growth year identified a focused set of bulb metabolomic changes

After filtering, 2,117 nonconstant LC-MS features were retained, including 1,212 positive-ion and 905 negative-ion features. The first two principal components explained 22.7% and 15.4% of the standardized variance, respectively (Fig. 1A). Samples occupied partially distinct regions of the PCA space, with the greatest divergence apparent among selected year groups and measurable variation retained within each year (Fig. 1A).

Eleven of 2,117 features (0.5%) satisfied both the across-year FDR criterion and the prespecified correlation threshold (adjusted $P < 0.05$ and $|p| \geq 0.60$; Fig. 1B,C; Table S1 in Additional file 1). Seven library-annotated features decreased with growth year: PC(18:1/18:2), PC(17:0/17:1), LPE 20:5, DL-stachydrine, L-cystine, L-ornithine, and nootkatone. Four features increased: tracheloside, antiarol, emetine, and 4-methoxycinnamaldehyde (Fig. 1C,D). Their standardized annual profiles diverged most clearly by year 4 (Fig. 1D), defining a compact and reproducible age-associated metabolomic signature.

Adjacent-year contrasts contained 67 differential features between years 1 and 2 and 82 between years 2 and 3 (Fig. 2A-C). In the year 1-2 comparison, 26 features were higher and 41 were lower in the later year; the corresponding counts for year 2-3 were 34 and 48, respectively (Fig. 2C). The bidirectional response indicates chemical remodelling rather than uniform accumulation. Representative database-annotated features with large absolute fold changes included phenolic, amino-acid-related, and other specialized-metabolite candidates (Fig. 2D), providing a prioritized set for targeted chemical analysis.

Bacterial communities showed substantial within-year variation and no global growth-year separation

Bacterial observed richness was lowest numerically in year 2 (427.7 ± 331.3) and highest in year 3 ($1,156.0 \pm 267.7$), whereas Shannon diversity ranged from 2.72 ± 0.72 in year 2 to 4.93 ± 0.59 in year 3 (Fig. 3A; Table S2 in Additional file 1). Neither observed richness (Kruskal-Wallis $P = 0.192$) nor Shannon diversity ($P = 0.270$) differed significantly among years (Fig. 3A).

The first two Bray-Curtis PCoA axes explained 57.4% and 17.3% of the variation, and bacterial community composition did not differ significantly among growth years (PERMANOVA $R^2 = 0.294$, $P = 0.339$; Fig. 3B). *Pseudomonas*, *Janthinobacterium*, Caulobacteraceae, *Ralstonia*, *Duganella*, *Sphingobium*, R7C24, *Variovorax*, and related rhizobial assignments were among the dominant taxa displayed (Fig. 3C). Their year-specific relative-abundance patterns occurred against high replicate dispersion and therefore represented a weaker growth-year signal than that observed for the fungal community.

Fungal community composition differed among growth years despite nonsignificant alpha diversity

Fungal observed richness increased numerically from 24.7 ± 14.0 in year 1 to 240.3 ± 185.4 in year 3 and 264.3 ± 91.2 in year 4 (Fig. 4A; Table S2 in Additional file 1). Shannon diversity was highest in year 3 (3.49 ± 0.49) and lower in year 4 (2.53 ± 0.51 ; Fig. 4A). Neither observed richness (Kruskal-Wallis $P = 0.082$) nor Shannon diversity ($P = 0.248$) differed significantly among years (Fig. 4A).

Community composition provided stronger evidence than alpha diversity. The first two fungal PCoA axes explained 19.4% and 15.3% of the variation, and growth year explained a significant fraction of Bray-Curtis community composition (PERMANOVA $R^2 = 0.401$, $P = 0.002$; Fig. 4B). Glomeraceae, *Ilyonectria*, *Cladosporium*, *Tetracladium*, Pezizaceae, Ceratobasidiaceae, *Aspergillus*, *Hyphoderma*, *Cadophora*, and *Trametes* were prominent in the displayed profiles (Fig. 4C). The significant separation identifies fungal compositional turnover as the clearest microbial signature of the growth-year gradient.

The year 3-4 transition produced the strongest root transcriptional response

Adjacent-year contrasts identified 157 DEGs between years 1 and 2 (69 upregulated and 88 downregulated), 157 between years 2 and 3 (66 upregulated and 91 downregulated), and 318 between years 3 and 4 (202 upregulated and 116 downregulated; Fig. 5A; Table S3 in Additional file 1). The year 3-4 transition therefore contained approximately twice as many DEGs as either earlier interval and was biased toward upregulation. The $\log_2$ fold-change distribution also shifted rightward in the year 3-4

comparison (mean +3.08) relative to the year 1-2 and year 2-3 comparisons (means -1.70 and -1.74, respectively; Fig. 5C).

No KEGG pathway remained significant after multiple-testing correction in the first two contrasts. Glycolysis/gluconeogenesis and starch and sucrose metabolism in the year 1-2 contrast each had an adjusted P value of 0.512, whereas protein processing in the endoplasmic reticulum in the year 2-3 contrast approached but did not reach significance (adjusted P = 0.059; Fig. 5B; Table S3 in Additional file 1). In the year 3-4 contrast, protein processing in the endoplasmic reticulum was significantly enriched (23 input transcripts; adjusted P = 1.98×10^{-4} ; Fig. 5B; Table S3 in Additional file 1). Mean within-year replicate correlations ranged from 0.676 to 0.837, and 17,986-19,005 transcripts showed nonzero expression per year (Fig. 5D).

Cross-layer synthesis identified fungal turnover and the year 3-4 root response as the dominant signals

The cross-layer evidence summary placed age-associated metabolite features, adjacent-year DEG counts, and microbial community effect sizes in a common visual overview (Fig. 6A). Fungal beta diversity and the year 3-4 root transcriptomic response were the strongest growth-year-associated signals. The metabolome contributed 11 FDR-supported features and two early pairwise feature sets, while bacterial communities showed greater within-year variability and no significant global separation.

The standardized annual profiles followed distinct but temporally informative trajectories (Fig. 6B). Fungal Shannon diversity, bacterial richness, and Glomeraceae relative abundance were highest in year 3, whereas the age-increasing metabolite score and the number of expressed root transcripts were highest in year 4. This sequence places the strongest community-level change and the strongest host transcriptional response within the year 3-4 interval and supports this period as the principal developmental window captured by the current dataset.

Discussion

Across the four-year field gradient, the data reveal a staged, layer-specific pattern rather than uniform multi-omics change. Fungal community composition and the year 3-4 root transcriptome showed the clearest statistically supported changes, whereas metabolomic variation was concentrated in a small set of features and bacterial community structure was comparatively stable. Such heterogeneity is expected because plant ontogeny affects organs, microbial kingdoms, and ecological compartments through different processes [8, 13, 14]. The present design therefore resolves where growth-year information is expressed most strongly: fungal community turnover and late root proteostasis-related transcription.

The bulb metabolome showed selective chemical remodelling. Eleven features passed both FDR control and the prespecified age-correlation threshold, and their annual trajectories separated most clearly by

year 4 (Fig. 1). *Fritillaria* bulbs are valued principally for steroidal and isosteroidal alkaloids[4, 5]. Nucleosides and other constituents also vary with cultivation conditions[6, 7]. The seven decreasing and four increasing library-annotated features indicate coordinated redistribution among lipid-related, amino-acid-related, and specialized-metabolite signals rather than simple accumulation with age. Their agreement across the four-year gradient, together with the bidirectional pairwise contrasts, provides a focused chemical signature for targeted quantification of age-responsive constituents.

Growth-year-associated microbiome patterns in wild perennial plants are expected to integrate host ontogeny with cumulative rhizosphere exposure. Soil provides the principal microbial source pool, whereas root architecture, exudation, immune status, and nutrient demand act as host filters[16-18]. Host age and genotype contribute to microbiome variation in wild perennials[13], and longitudinal work in perennial *Arabis* shows that residence time in soil can restructure root communities[14]. In *F. cirrhosa*, the year classes therefore represent successive host-environment states along a natural alpine field gradient. This perspective explains why fungal and bacterial communities need not respond in parallel and why marked compositional turnover can occur without a monotonic change in alpha diversity.

The fungal data support compositional turnover rather than a simple increase in diversity. Bray-Curtis composition differed significantly among years, whereas observed richness and Shannon diversity did not (Fig. 4). Root-derived compounds can alter fungal assemblages [15], and perennial root communities change as host development and soil history accumulate [14]. Functional studies show that root-associated fungi can improve phosphorus acquisition and plant performance under defined nutrient conditions[31, 32]. Arbuscular mycorrhizal exchange also depends on host carbon supply and fungal physiological state[33, 34]. The shifting prominence of Glomeraceae, *Ilyonectria*, *Tetracladium*, Pezizaceae, and other taxa is therefore consistent with dynamic reorganization of the fungal assemblage across growth years. Among the microbial layers examined, this turnover was most closely aligned with the plant age gradient.

The bacterial results provide a complementary ecological contrast. Richness and Shannon diversity reached their highest numerical values in year 3, but replicate dispersion was substantial and the global Bray-Curtis test was nonsignificant (Fig. 3). Soil is the major reservoir for root-associated bacteria, and host compartment effects are superimposed on environmental and spatial variation[35-38]. The weaker bacterial response in the present field gradient suggests that bacterial assembly was more variable among individual plants or less tightly coupled to growth year than fungal assembly. The contrast between the two kingdoms is itself informative, showing that host age does not impose a uniform filter on the bulb-associated microbiota.

The year 3-4 root contrast provided the clearest molecular evidence of a late growth-year transition. It contained 318 DEGs, twice the number detected in either earlier interval, and was biased toward upregulation (Fig. 5A,C). Protein processing in the endoplasmic reticulum was the only KEGG pathway that remained significant after FDR correction (Fig. 5B). This pathway integrates nascent-protein folding, secretion, ER-associated degradation, and the unfolded-protein response[39, 40]. Plant ER-stress

signalling contributes to development, immunity, and adaptation to abiotic stress[41, 42]. The IRE1-*bZIP60* and *bZIP28* branches coordinate major components of this response[43-45]. The enrichment therefore supports increased demand for protein folding, membrane trafficking, protein quality control, and stress acclimation in older roots. The near-significant enrichment of the same pathway in year 2-3 further suggests that this response may begin before the pronounced year 3-4 transition.

Cross-layer comparison places the fungal and root responses within a coherent temporal sequence. Fungal Shannon diversity, bacterial richness, and Glomeraceae relative abundance peaked in year 3, followed by the highest age-increasing metabolite score and expressed-root-transcript count in year 4 (Fig. 6B). Plant phosphate status can restructure root microbiota[46], and defined fungal isolates can improve phosphorus nutrition under specific host and nutrient conditions[31, 32]. Although the present layers were measured in different materials, their aligned timing nominates the year 3-4 interval for mechanistic work linking soil conditions, fungal assemblages, root physiology, and bulb chemistry.

Two limitations should be considered. First, the cross-sectional wild-field design used three biological replicates per year, so growth year was measured together with microsite and cumulative soil-history variation; multi-site replication with matched soil physicochemical measurements will be needed to test the generality of the observed patterns. Second, LC-MS identities were based on library annotation and amplicon sequencing measured relative community composition; targeted compound quantification together with absolute and functional microbial measurements will be needed to connect the identified signatures directly to medicinal quality and microbial activity. These points define the next validation steps while leaving the significant fungal separation and late root transcriptional response intact.

A focused validation strategy follows directly from these findings. The 11 age-associated LC-MS features can be remeasured alongside absolute UHPLC-MS/MS quantification of characteristic *Fritillaria* alkaloids[4, 5, 7]. Amplicon sequencing can be paired with 16S and ITS copy numbers, functional-gene assays, and root-colonization measurements, while soil pH, water content, organic carbon, mineral nitrogen, available phosphorus, and available potassium can be measured for each plant. Matching bulb, root, microbial, and soil measurements at the individual-plant level would permit mixed-effects modelling and direct tests of the year 3-4 transition.

Overall, this study establishes a growth-year profile in which fungal compositional turnover and a late root protein-processing response are the dominant biological signals. The metabolome adds a compact set of age-responsive chemical features, while bacterial community structure provides a comparatively stable background. The convergence of fungal, root-transcriptomic, and selected metabolomic changes around years 3-4 identifies a biologically meaningful interval for quality-oriented and mechanistic investigation in wild *F. cirrhosa*. These results provide specific chemical features, microbial taxa, and host pathways that can now be tested across independent alpine populations.

Conclusion

Across 1- to 4-year-old wild *Fritillaria cirrhosa*, growth year was most strongly expressed in fungal community composition and the year 3-4 root transcriptomic response. The bulb metabolome contained 11 FDR-supported age-associated features and showed bidirectional chemical remodelling between adjacent years, whereas bacterial communities exhibited greater within-year variability and no significant global separation. The year 3-4 root contrast contained 318 DEGs and significant enrichment of protein processing in the endoplasmic reticulum, defining a late interval of intensified protein folding, trafficking, degradation, and stress-response activity. Together, these findings establish a layer-specific developmental profile in which fungal turnover and root proteostasis-related transcription are the principal signals. The identified metabolite features, fungal taxa, and root pathways provide a focused basis for targeted alkaloid quantification and matched soil-microbe-host validation across independent field populations.

Abbreviations

ANOVA, analysis of variance; DEG, differentially expressed gene; FDR, false discovery rate; ITS, internal transcribed spacer; KEGG, Kyoto Encyclopedia of Genes and Genomes; LC-MS, liquid chromatography-mass spectrometry; OTU, operational taxonomic unit; PCA, principal component analysis; PCoA, principal-coordinate analysis; PERMANOVA, permutational multivariate analysis of variance; RNA-seq, RNA sequencing.

Declarations

Ethics approval and consent to participate

This study does not involve any ethically related experiments.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request. The data will be made available upon request.

Competing interests

The authors confirm that there are no conflicts of interest.

Funding

This study were supported by the National Key R&D Program (2018YFC1706101) and the basic scientific research operating expenses of Sichuan Provincial research institutes 2026JDKY0028

Authors' contributions

Can Zhao: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Chunyu Li:** Determination and Calibration of Research Methods. **Mian Xiang** and **Yuxia Yang:** Sample and Data Processing. **Zhuyun Yan** and **Zhengcheng Zhang** : Writing – review & editing, Project administration, Investigation, Conceptualization

Acknowledgements

Thanks to Mr. Zhu who served as our guide during the field sampling process. We would like to express our gratitude to Shanghai Meiji Biomedical Technology Co., Ltd. and Chengdu Luoning Biotechnology Co., Ltd. for providing the sequencing services.

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Table

Table 1. Primary 1- to 4-year study design and available data layers.

Year	Bulb LC-MS	Bacterial 16S	Fungal ITS	Root RNA-seq	n
1	Available	Available	Available	Available	3
2	Available	Available	Available	Available	3
3	Available	Available	Available	Available	3
4	Available	Available	Available	Available	3

Note: Year 5 was excluded from the primary multi-omics design because root RNA-seq was unavailable.

Figures

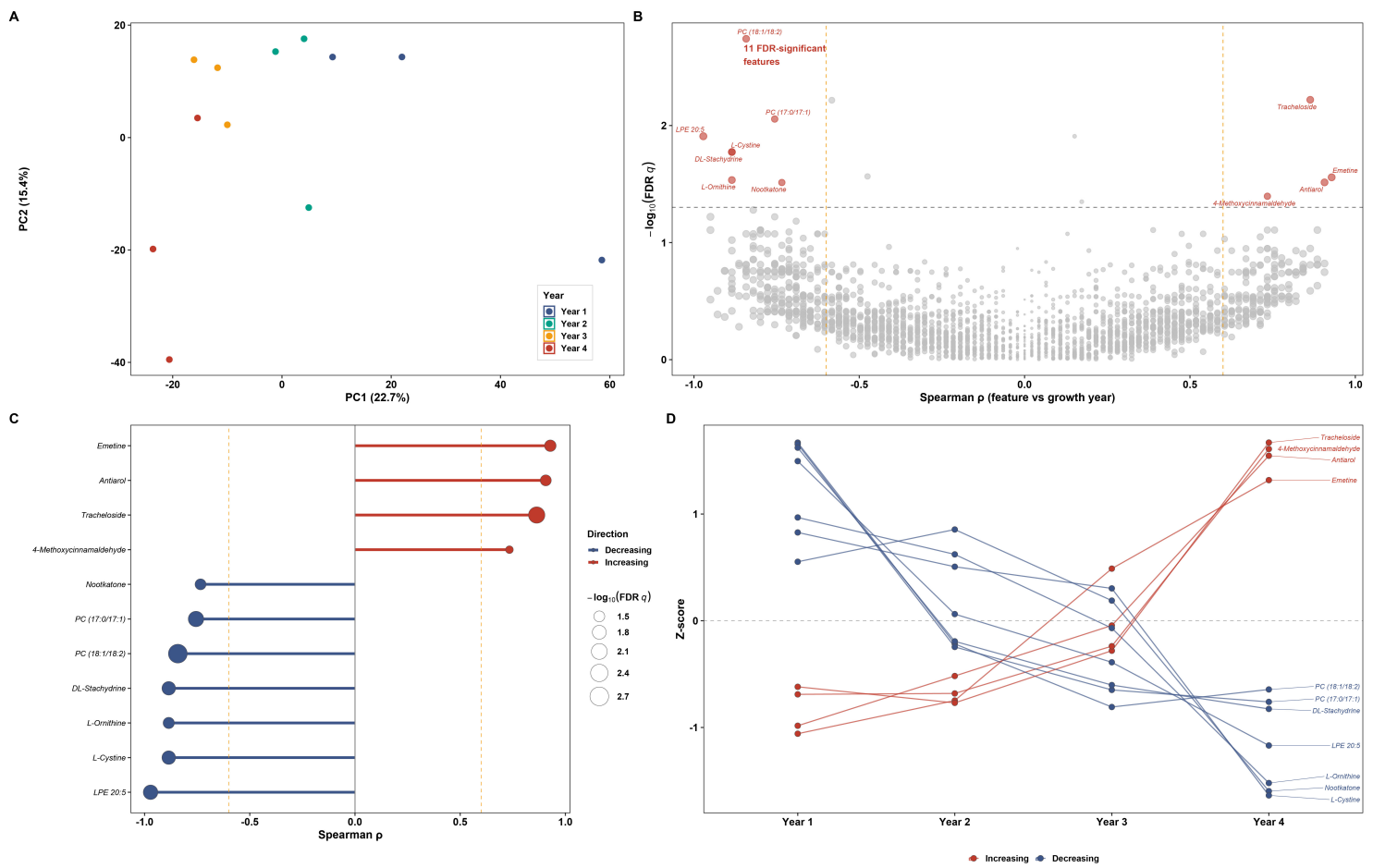


Figure 1

Growth-year patterns in the bulb metabolome. (A) Unsupervised principal component analysis of 2,117 filtered LC-MS features from positive- and negative-ion modes. Points represent biological replicates (n = 3 per growth year), and colours denote growth year. (B) Feature-level association plot for all 2,117 features. The x-axis shows Spearman ρ between abundance and growth year, and the y-axis shows $-\log_{10}(\text{FDR } q)$. Red points denote the 11 features meeting $\text{FDR } q < 0.05$ and $|\rho| \geq 0.60$; dashed lines indicate these thresholds. (C) Effect-size lollipop plot of the 11 supported features. Segment direction

and length represent Spearman ρ , point size represents $-\log_{10}(\text{FDR } q)$, blue denotes decreasing abundance, and red denotes increasing abundance. (D) Standardized annual profiles of the same features. Lines show annual mean z scores, colours denote direction, and labels identify features at year 4. Metabolite names are putative library annotations.

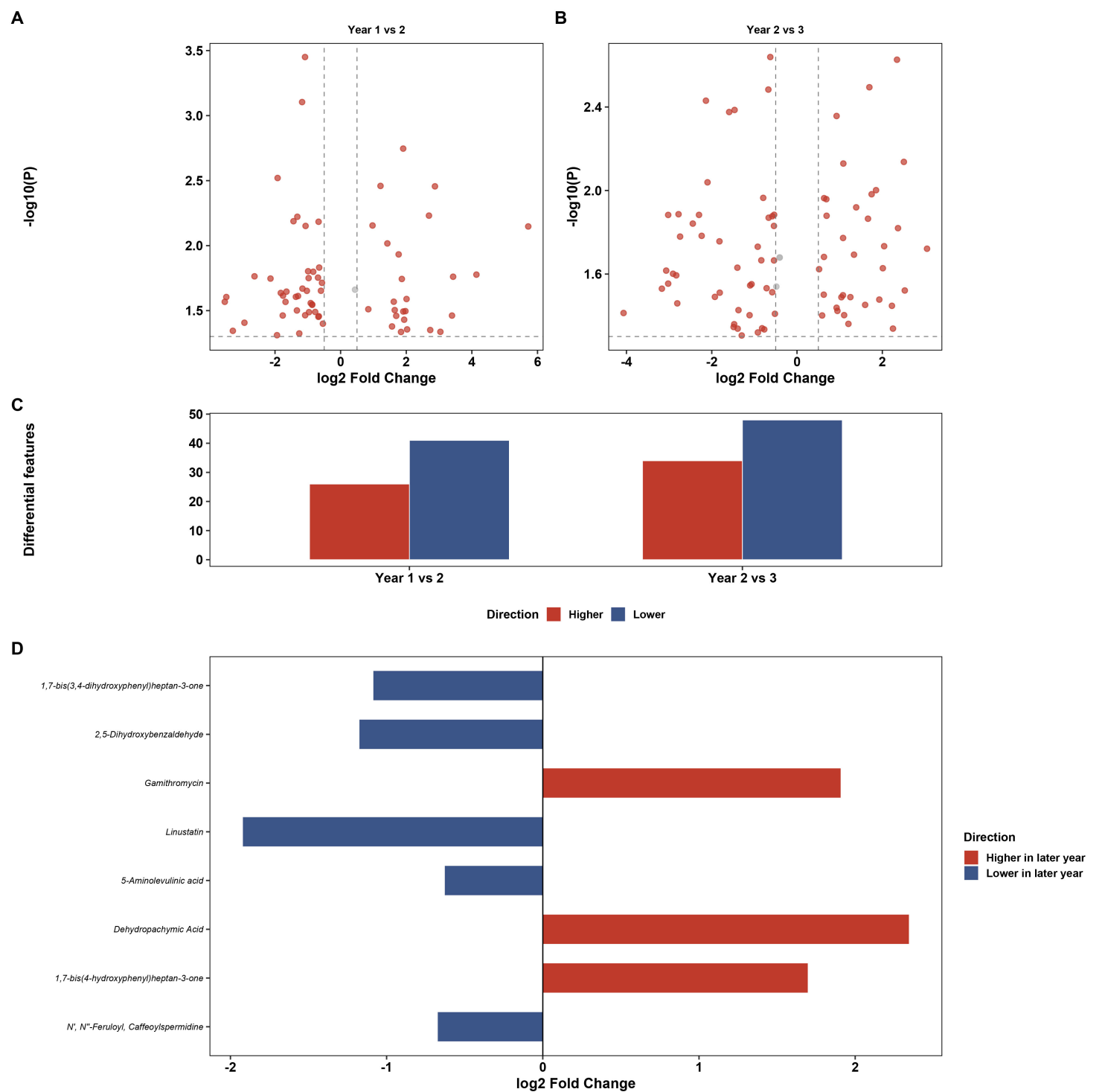


Figure 2

Pairwise metabolomic changes between adjacent growth years. (A,B) Volcano plots for the year 1-2 and year 2-3 comparisons, respectively. Vertical dashed lines indicate $|\log_2 \text{ fold change}| = 0.5$ and the

horizontal dashed line indicates $P = 0.05$; grey points fall within the fold-change interval. (C) Numbers of differential features that were higher or lower in the later year. (D) Representative database-annotated features with large absolute pairwise fold changes. Red denotes higher abundance in the later year and blue denotes lower abundance. The year 4-5 comparison is shown in Additional file 1: Fig. S1.

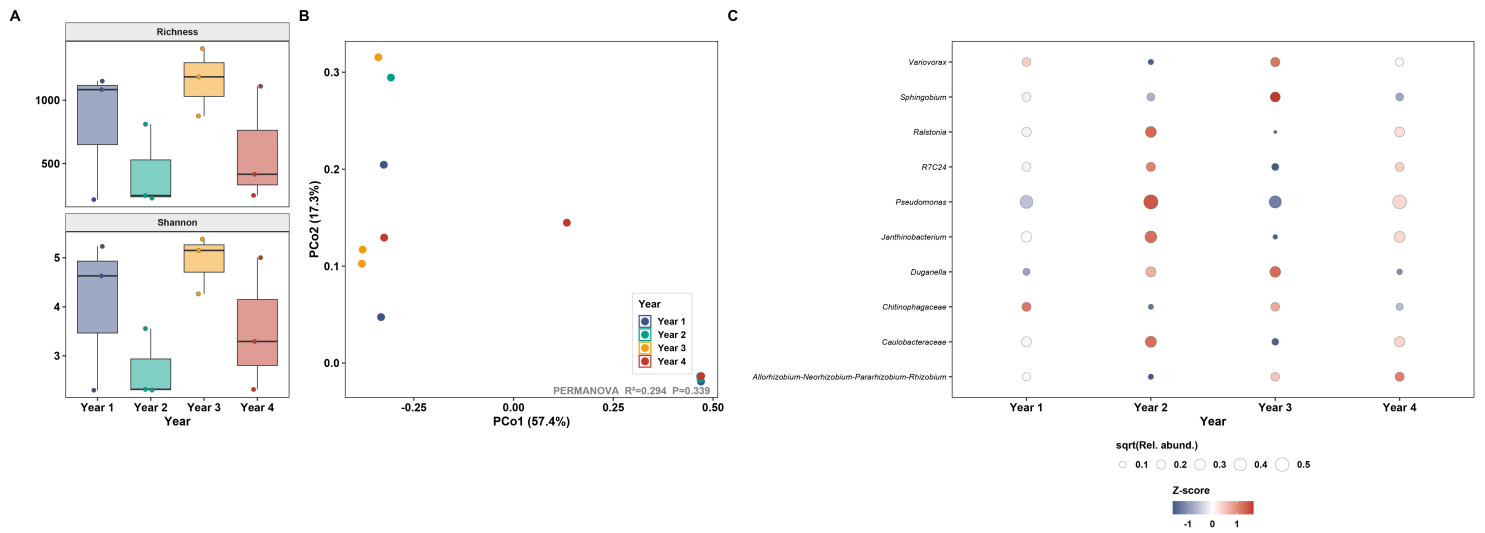


Figure 3

Bacterial diversity and community composition across growth years. (A) Observed richness and Shannon diversity. Boxes show interquartile ranges, centre lines show medians, and points represent biological replicates ($n = 3$). (B) Bray-Curtis principal-coordinate analysis with the global PERMANOVA result. (C) Bubble plot of dominant bacterial taxa. Bubble area represents the square root of mean relative abundance, and fill colour denotes the within-taxon z score across years. Alpha-diversity and community-level tests were not significant.

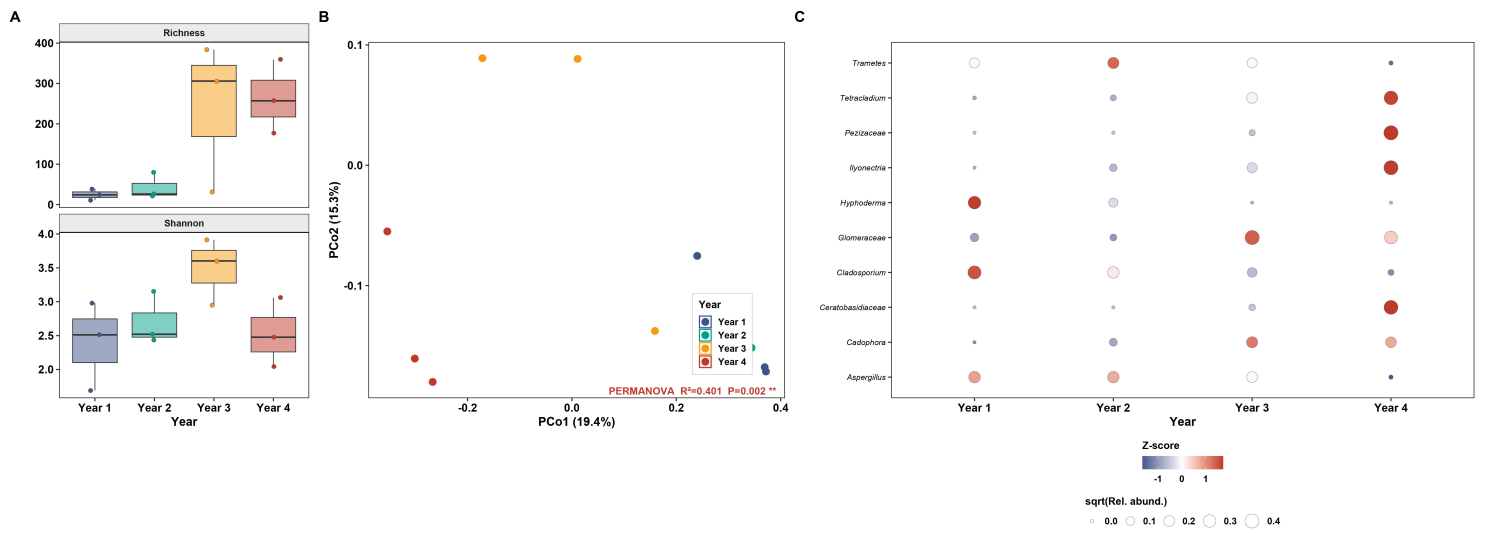


Figure 4

Fungal diversity and community composition across growth years. (A) Observed richness and Shannon diversity. Boxes show interquartile ranges, centre lines show medians, and points represent biological replicates ($n = 3$). (B) Bray-Curtis principal-coordinate analysis with the global PERMANOVA result. (C) Bubble plot of dominant fungal genus- or family-level taxa. Bubble area represents the square root of mean relative abundance, and fill colour denotes the within-taxon z score across years. Fungal community composition differed among growth years, whereas alpha-diversity tests were not significant.

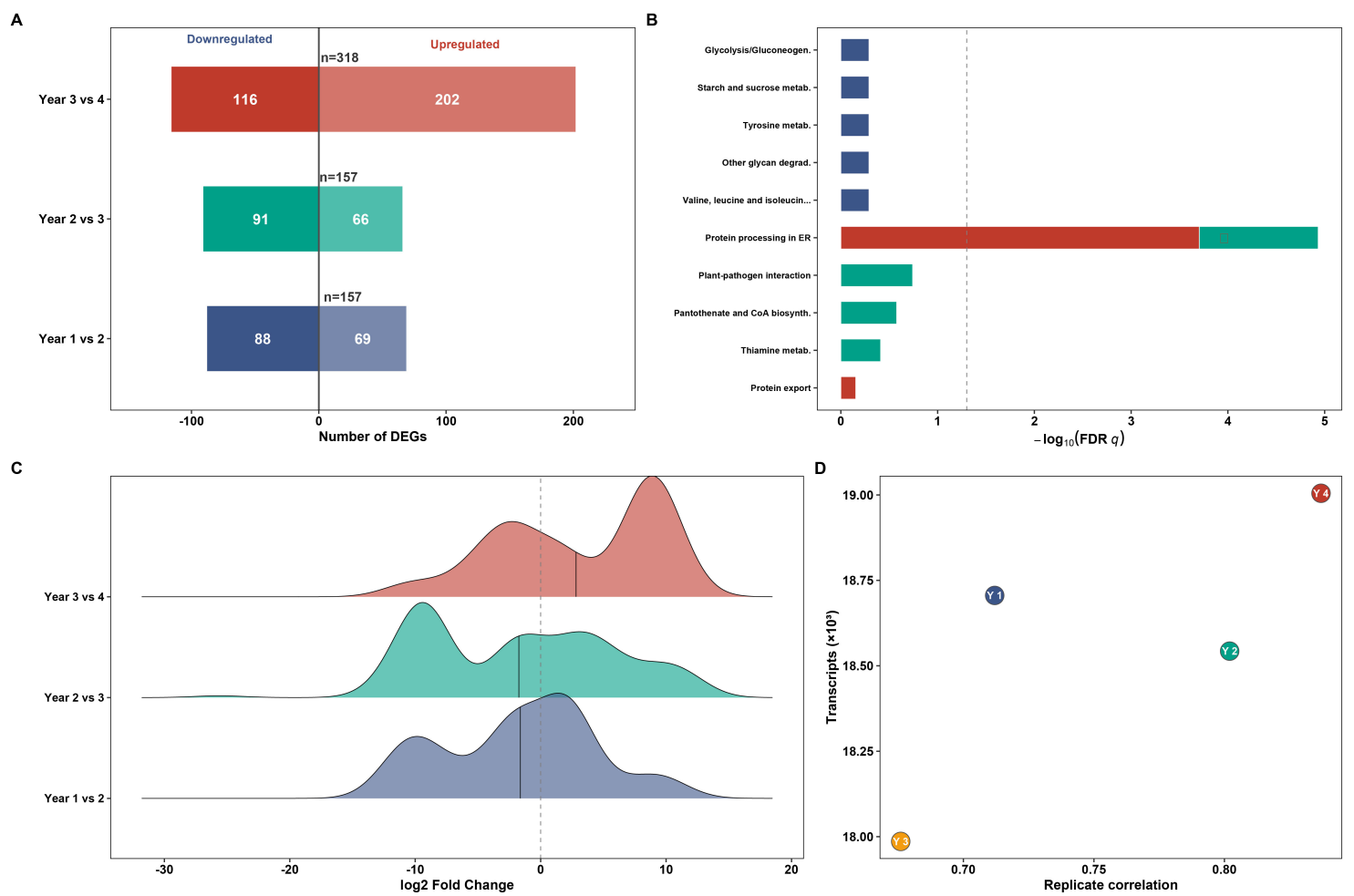


Figure 5

Adjacent-year changes in the root transcriptome. (A) Diverging bars show downregulated and upregulated differentially expressed genes (DEGs) in each contrast; values within bars indicate direction-specific counts and n indicates total DEGs. (B) Selected KEGG enrichment results plotted as $-\log_{10}(\text{FDR } q)$; the dashed line corresponds to $q = 0.05$, and colours denote adjacent-year contrasts. Protein processing in the endoplasmic reticulum in the year 3-4 contrast was the only pathway above the FDR threshold. (C) Density distributions of DEG $\log_2$ fold changes, with vertical lines indicating the mean for each contrast. (D) Within-year replicate correlation plotted against the number of transcripts with nonzero expression ($\times 10^3$); points are labelled Y1-Y4.

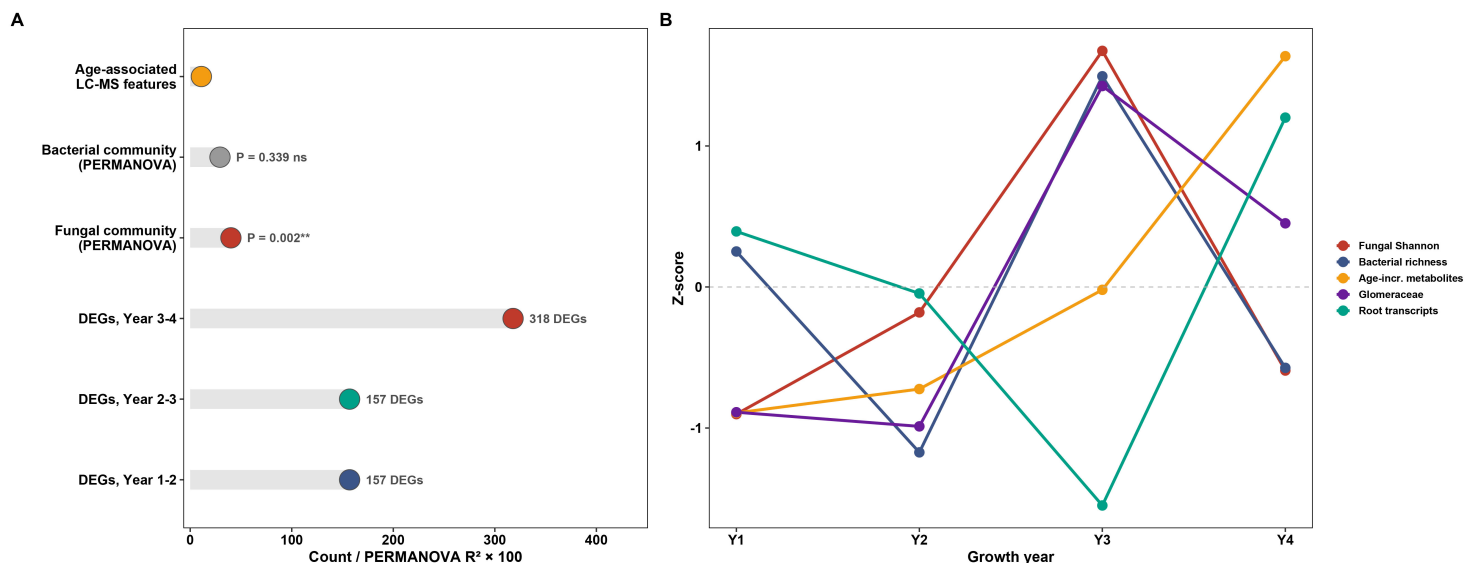


Figure 6

Cross-layer evidence across growth years. (A) Descriptive summary of age-associated LC-MS feature counts, adjacent-year DEG counts, and bacterial and fungal community effect sizes. PERMANOVA R^2 values were multiplied by 100 for display, whereas LC-MS features and DEGs are shown as counts; P values identify the community-level tests. Counts and effect sizes retain their original statistical meanings, and the common scale provides a compact visual comparison. (B) Within-metric z-score profiles for fungal Shannon diversity, bacterial richness, age-increasing metabolite features, Glomeraceae relative abundance, and expressed root-transcript count across years 1-4.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile1.docx](#)