



The combined influence of microbiome and soil environment contributes to the chemotype differentiation in *Atractylodes lancea*.

Hongyang Wang^{1,2,3#}, Zheng Peng^{1,2,3#}, Chengcai Zhang^{1,2,3#}, Chuanzhi Kang^{1,2},
Yan Zhang^{1,2,3}, Xiuzhi Guo^{1,2}, Yiheng Wang^{1,2}, Guang Yang^{1,2},
Zengxu Xiang⁴, Li Zhou^{1,2}, Zhixian Jing^{1,2}, Dahui Liu⁵
Sheng Wang^{1,2,3*}, Luqi Huang^{1,2*}, Lanping Guo^{1,2*}

¹State Key Laboratory for Quality Ensurance and Sustainable Use of Dao-di Herbs,
National Resource Center for Chinese Materia Medica,
China Academy of Chinese Medical Sciences, Beijing, China

²Key Laboratory of Biology and Cultivation of Herb Medicine,
Ministry of Agriculture and Rural Affairs, Beijing, China

³Dexing Research and Training Center of Chinese Medical Sciences, Dexing, China

⁴College of Horticulture, Nanjing Agricultural University, Nanjing, China

⁵Pharmacy Faculty, Hubei University of Chinese Medicine, Wuhan, China



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Introduction



Atractylodes lancea

- ❑ *Atractylodes lancea*, a perennial herb of the *Atractylodes* in the Compositae, is widely distributed in China.
- ❑ Volatile oils, especially hinesol, β -eudesmol, atractylon, and atractylodin, are key active ingredients of *A. lancea*, responsible for its medicinal effects including drying dampness, invigorating the spleen, and providing antibacterial and anti-inflammatory benefits.
- ❑ Previous studies have categorized *A. lancea* into three genotypes: the Maoshan-Dabie Mountains Group (MA), the North Yanshan Mountains Group (NA), and the Qinling-Taihang Mountains Group (SA), as well as two chemotypes: the Maoshan Cangzhu chemotype (MSA) and the Hubei Cangzhu chemotype (HBA).



Highlight

- ❑ The genetic differentiation of the MA and SA genotypes of *Atractylodes lancea* takes place along gradients of altitude and climate.
- ❑ *Atractylodes lancea* undergoes chemotype differentiation influenced by soil chemistry and microbiota, rather than being determined by its genotype.
- ❑ Rhizosphere and endophytic core microbes drive the chemotype differentiation of *Atractylodes lancea*.

Experimental design for Part One

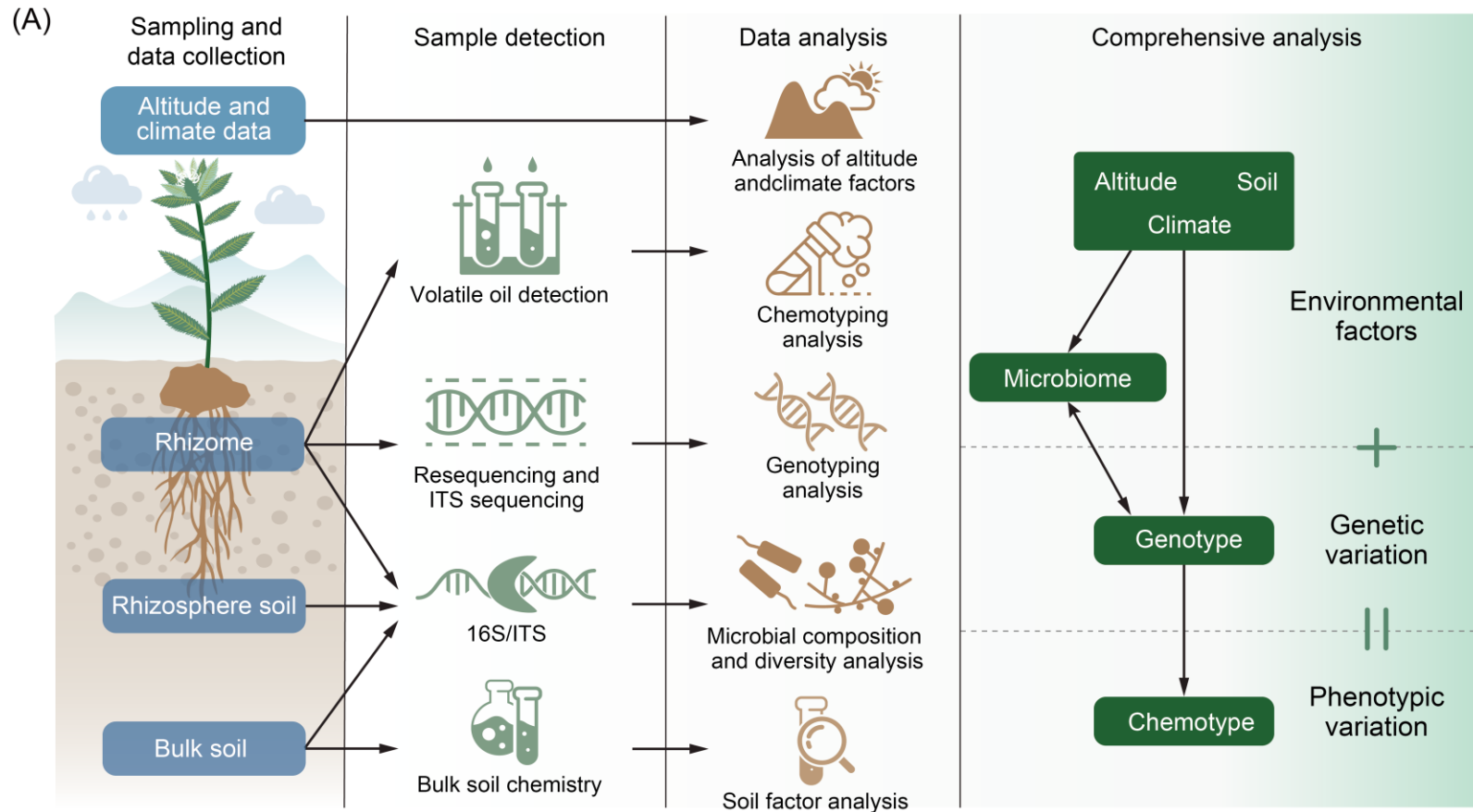


Figure 1A Flow diagram of sample collection and data analysis.

- ❑ A total of 187 wild and cultivated *A. lancea* rhizome were collected from 16 populations across five provinces in China. Among them, 109 were utilized for genome resequencing.
- ❑ The remaining 78 rhizomes of *A. lancea* were utilized for testing volatile oils, ITS sequences, and the 16S/ITS of endophytic microorganisms. The corresponding rhizosphere soil and bulk soil from these rhizomes were employed for microbial analysis and the determination of soil background nutrient content.
- ❑ Ultimately, a comprehensive analysis was carried out to examine the impact of these factors (altitude, climate, soil, and microorganisms) on the formation of genotypes and chemotypes in *A. lancea*.



Part 1 Results: Genotypic Variation in *A. lancea*

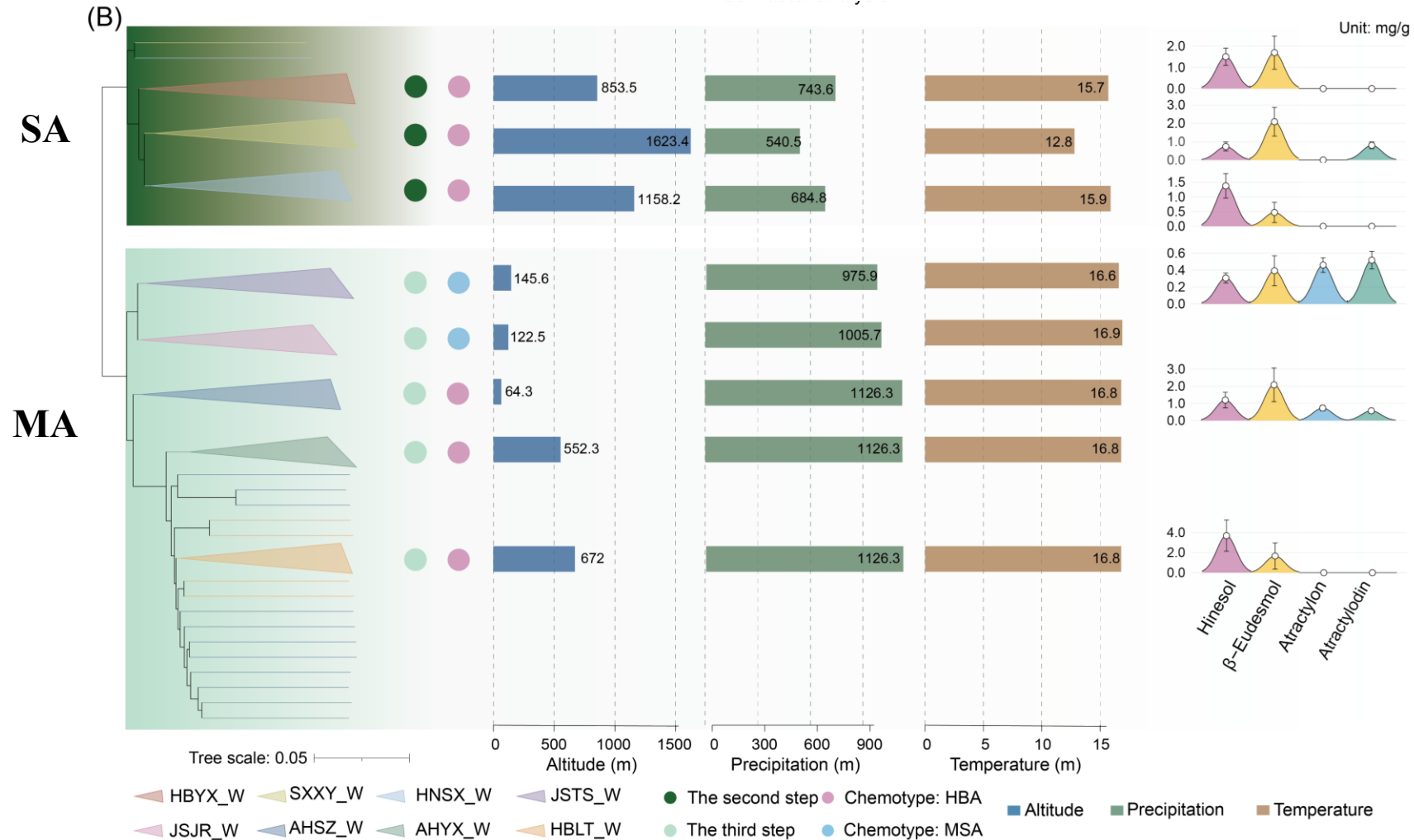


Figure 1B Phylogenetic clustering of genotypes in *A. lancea*

- ❑ *A. lancea* from five distinct producing areas can be classified into two genotypes: MA and SA.
- ❑ The SA genotype is predominantly distributed in high-altitude regions (China's second-tier terrain), while the MA genotype is solely found in low-altitude areas (China's third-tier terrain).



Part 1 Results: Chemotypic Variation in *A. lancea*

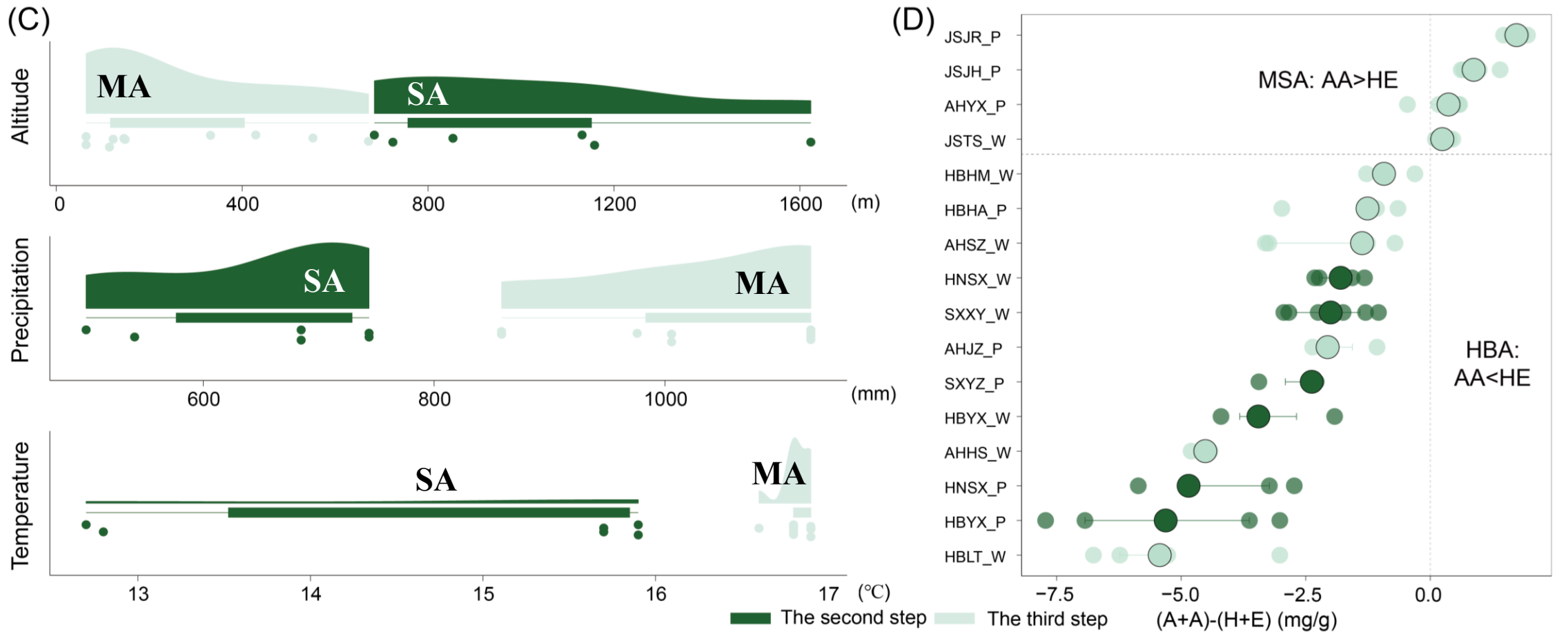


Figure 1C and D Geographical environment of distribution and chemotype variation of *A. lancea*

- ❑ The SA distribution region experiences lower levels of rainfall and temperature, whereas the MA distribution region is characterized by higher rainfall and temperature.
- ❑ The *A. lancea* from five distinct producing areas can be further classified into two chemotypes: MSA and HBA.



Part 1 Results: Microbiome Analysis

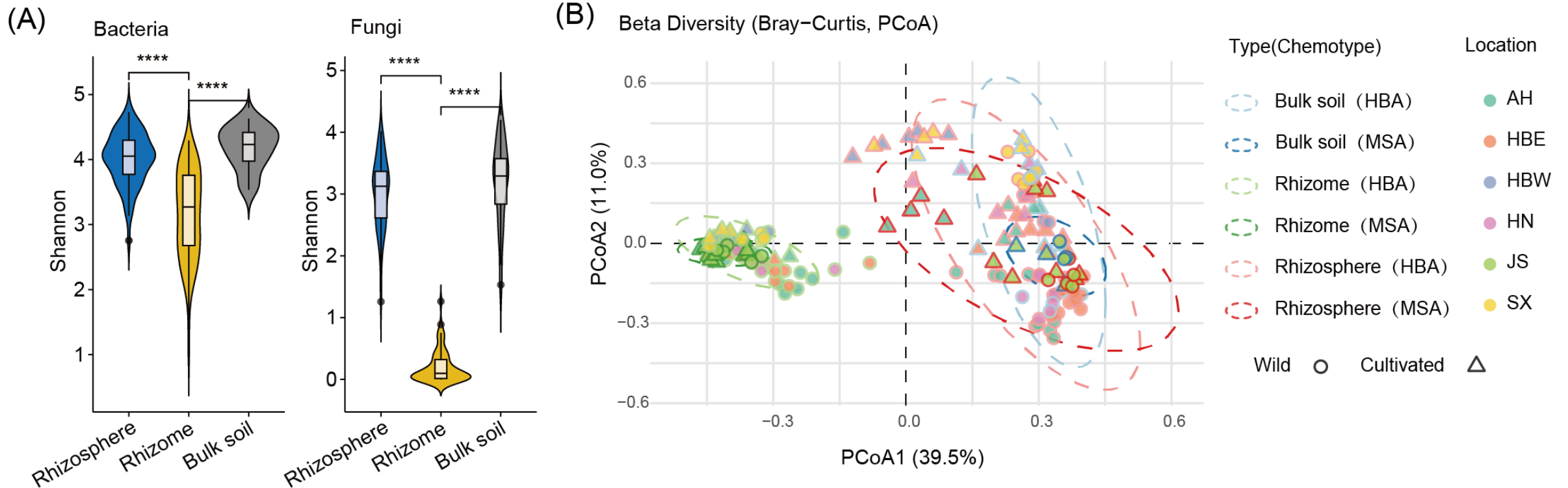


Figure 2A and B Analysis of alpha diversity and PCOA for rhizosphere and endophytic microorganisms of *A. lancea* from various producing areas

- Both the bacterial and fungal diversity in bulk soil and rhizosphere soil were significantly higher than endophytes, and the microbial community composition in the rhizosphere soil closely resembled that in the bulk soil.
- A unique microbial community has developed within the rhizome of *A. lancea*.



Part 1 Results: Microbiome Analysis

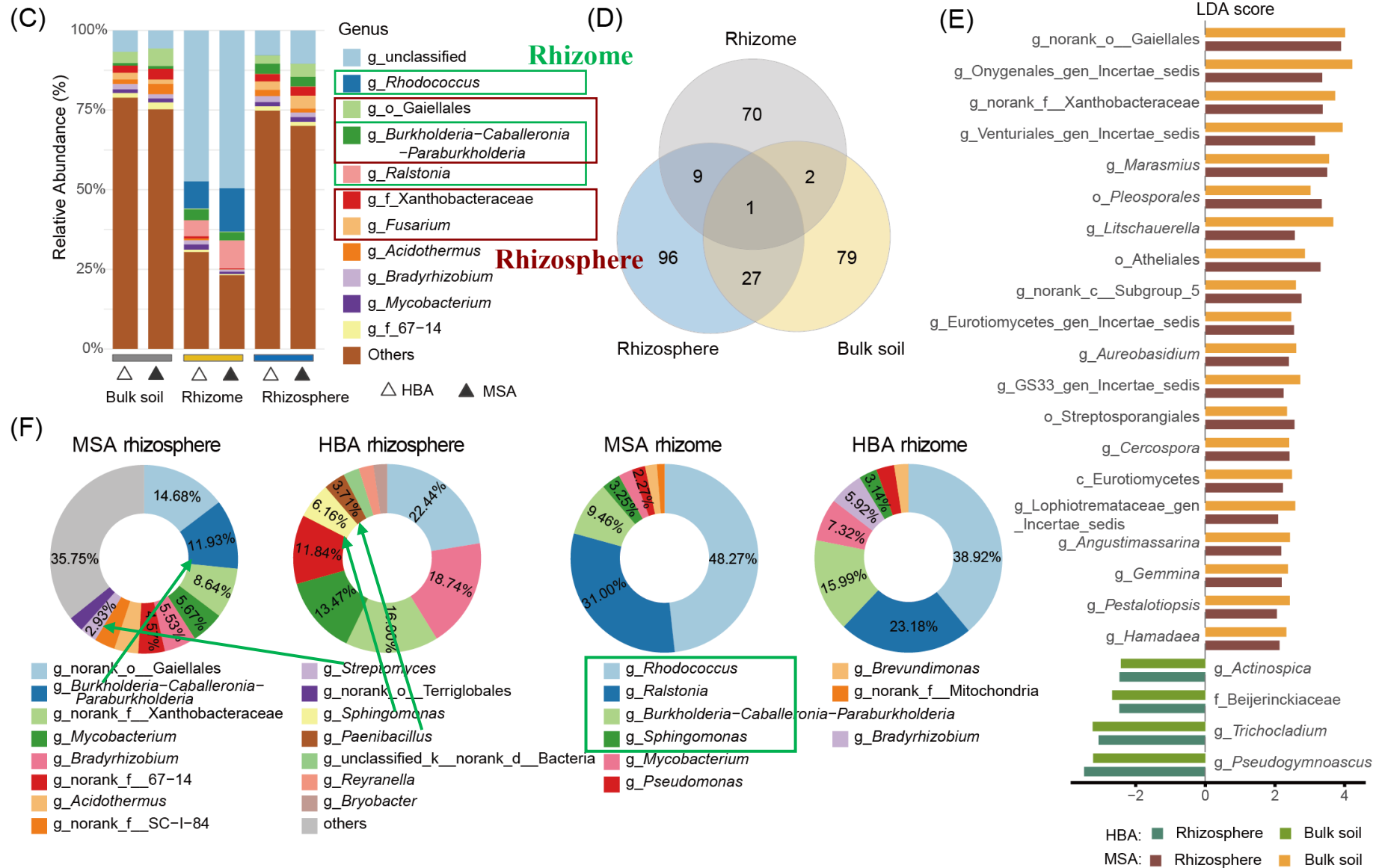


Figure 2C-F Microbial composition in the rhizosphere and as endophytes of *A. lancea* from different origins



Part 1 Results: Microbiome Analysis

(G) Top 15 Significant EC

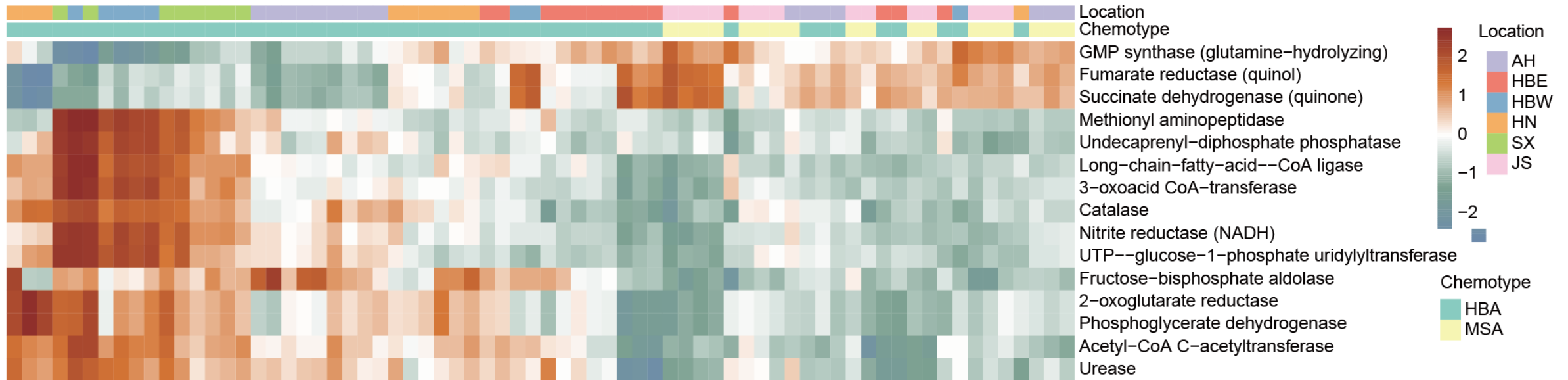


Figure 2G Heatmap of the top 15 significantly enriched Enzyme Commission (EC) functional categories

- Functional prediction analysis showed that GMP synthase, fumarate reductase, and succinate dehydrogenase exhibited higher abundances in the MSA chemotype, whereas Methionyl aminopeptidase and other related enzymes displayed relatively higher abundances in the HBA chemotype, highlighting the functional disparities between the HBA and MSA chemotypes in the microbiomes of *A. lancea*.



Part 2 Results: Functional Verification of Indigenous Microbe

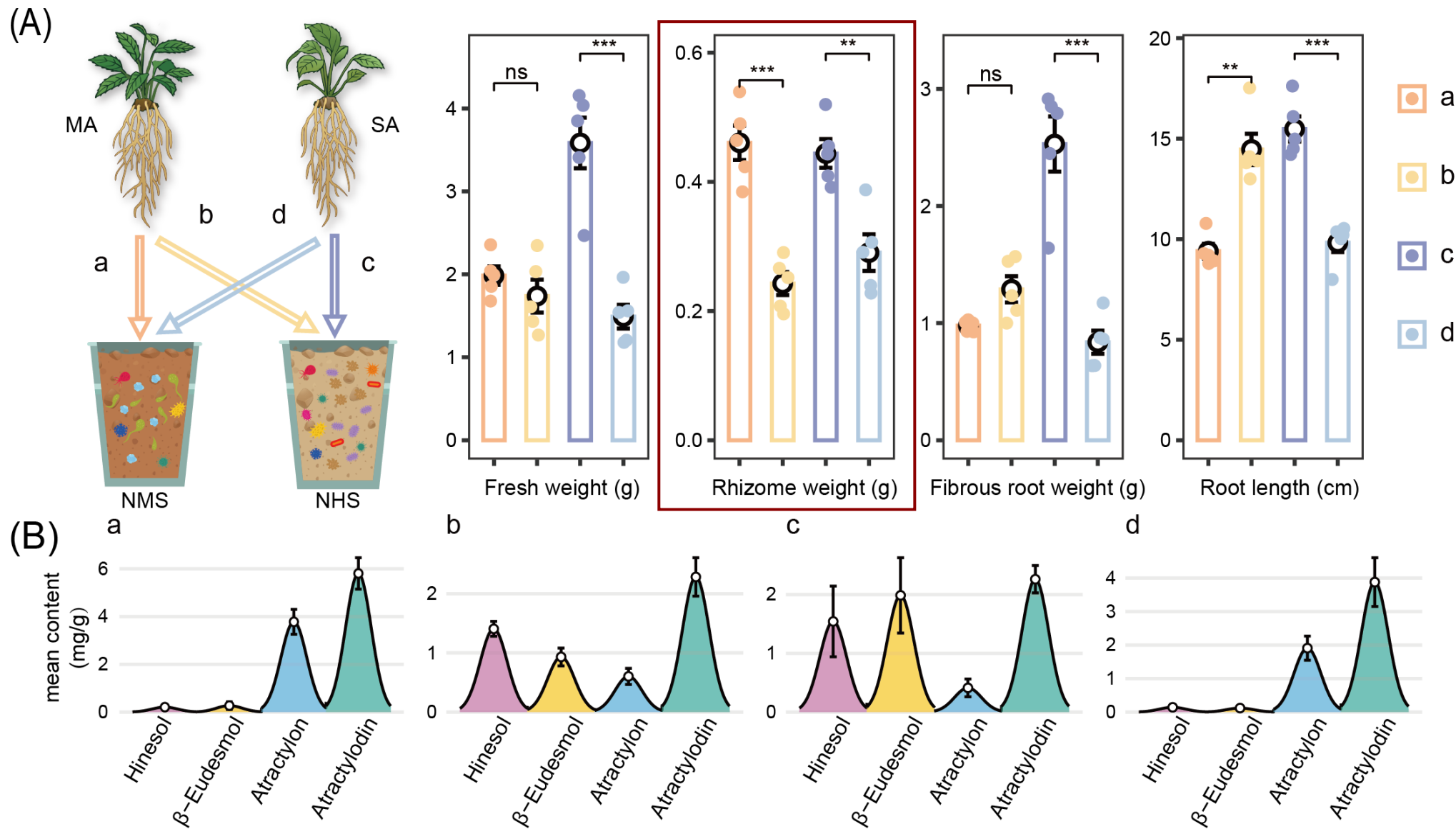


Figure 3A and B The biomass and volatile oil content of various genotypes of *A. lancea* planted in untreated soil sourced from the production areas of different chemotypes of *A. lancea*



Part 2 Results: Functional Verification of Indigenous Microbe

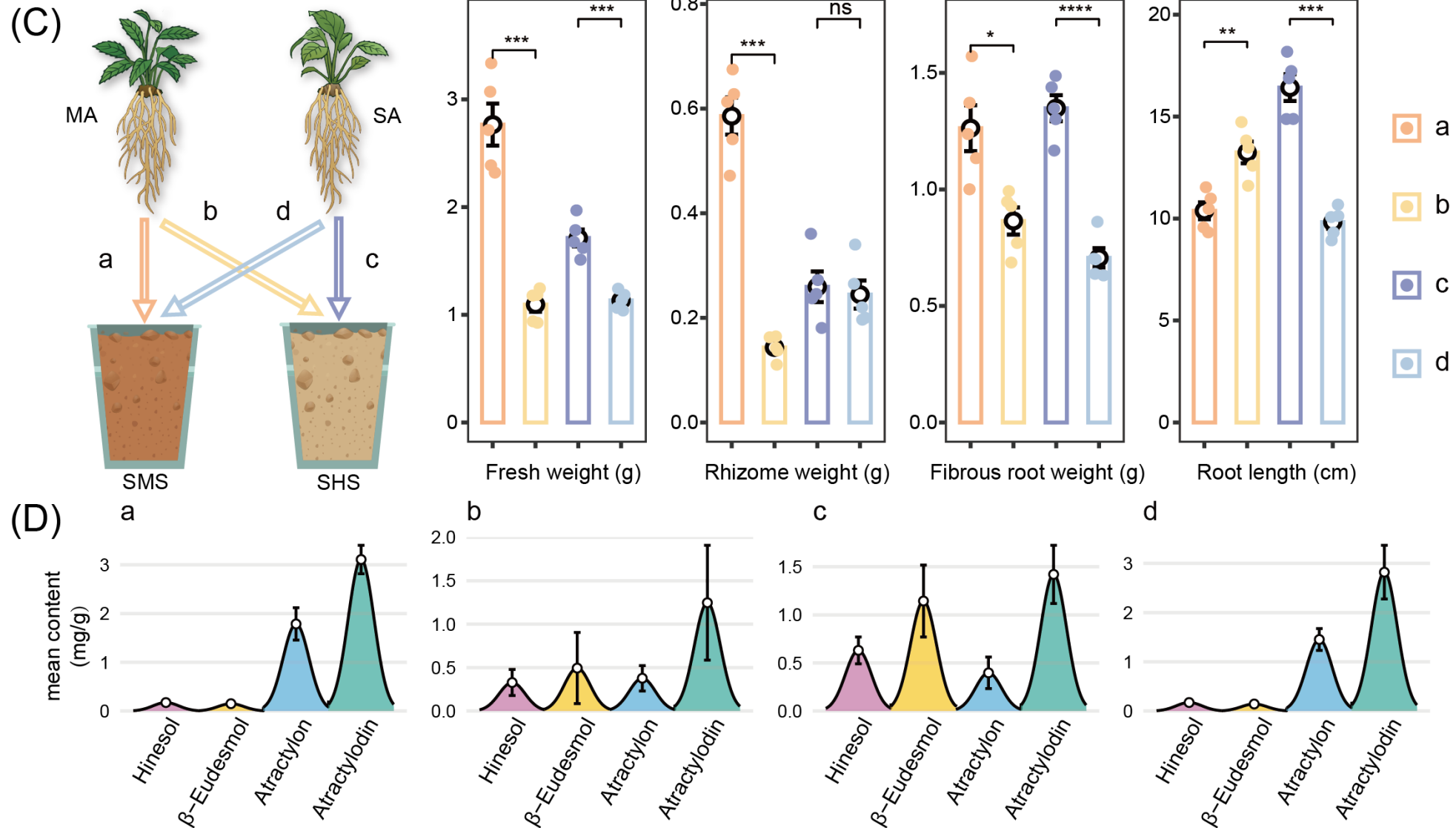


Figure 3C and D Biomass and volatile oil content of various genotypes of *A. lancea* cultivated in sterilized soil sourced from regions producing different chemotypes of *A. lancea*



Part 2 Results: Functional Verification of Indigenous Microbe

(E)

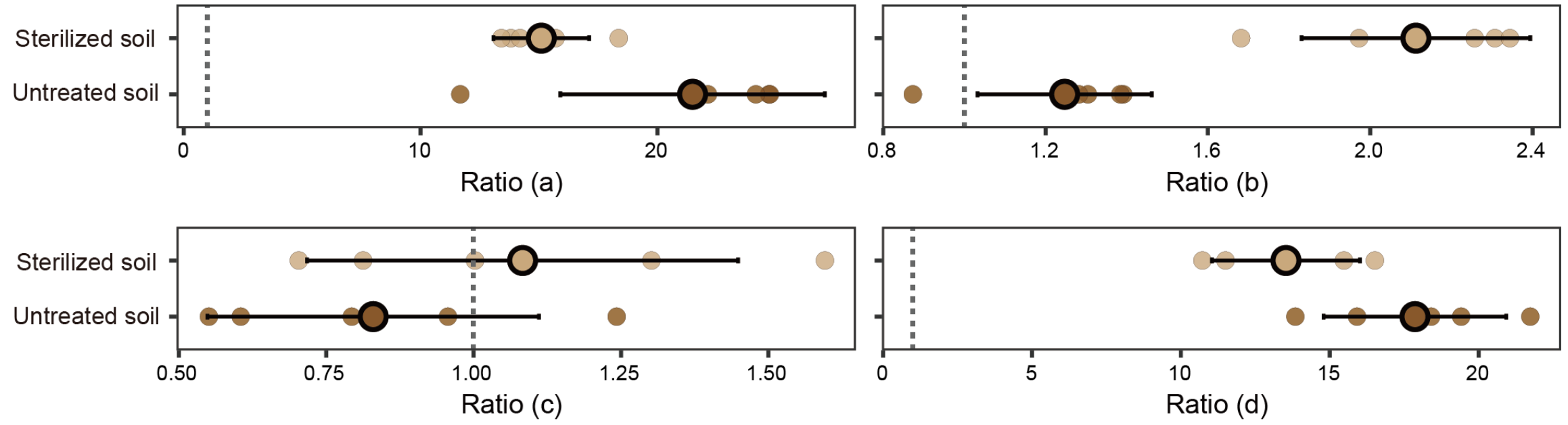
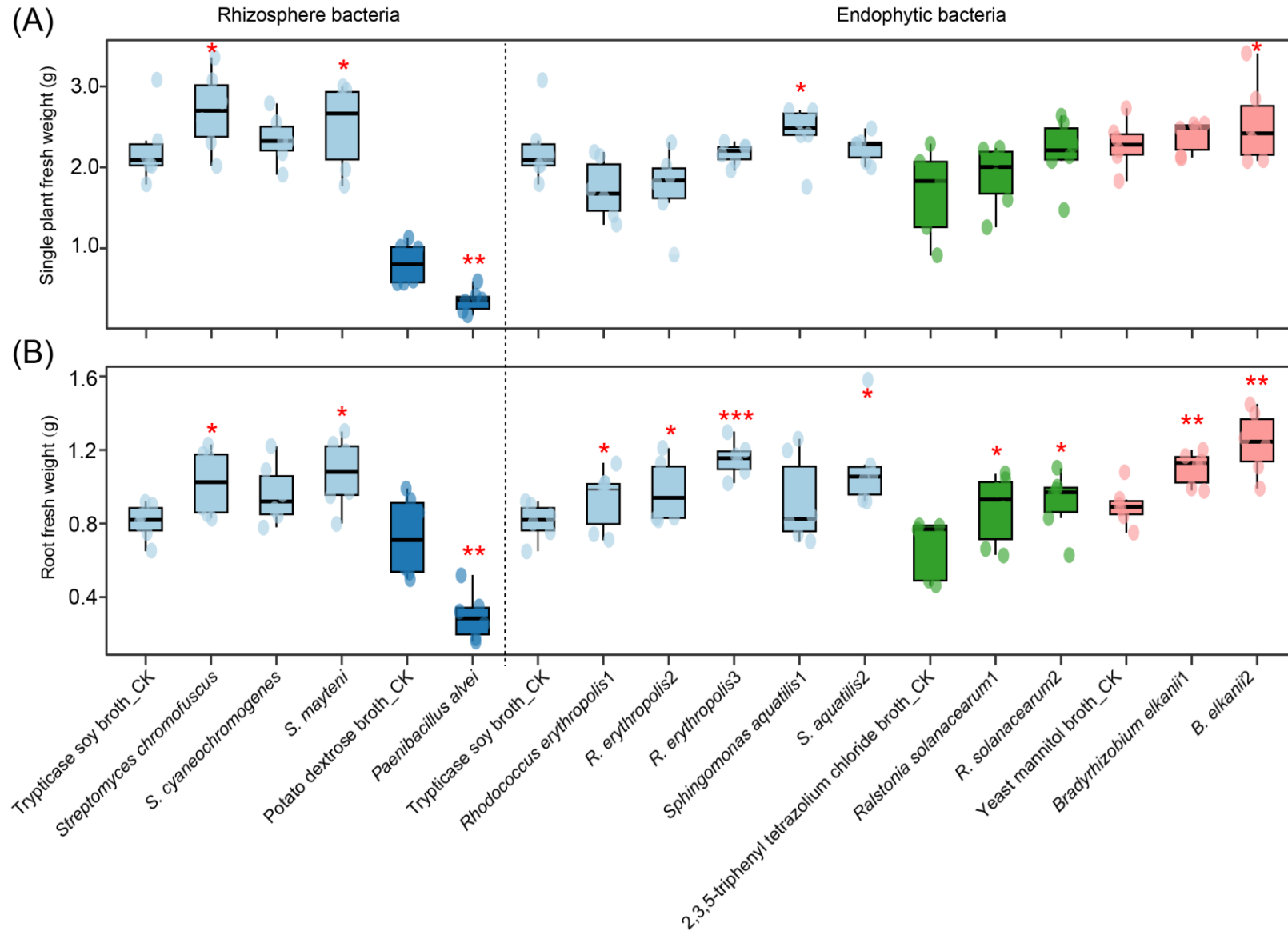


Figure 3E Ratios of (atractylon + atractylodin) / (hinesol + β -eudesmol) across sterilized (top) and untreated (bottom) soils

□ The impact of sterilized soil on the composition of volatile oil in *A. lancea* is less pronounced than that of unsterilized soil.



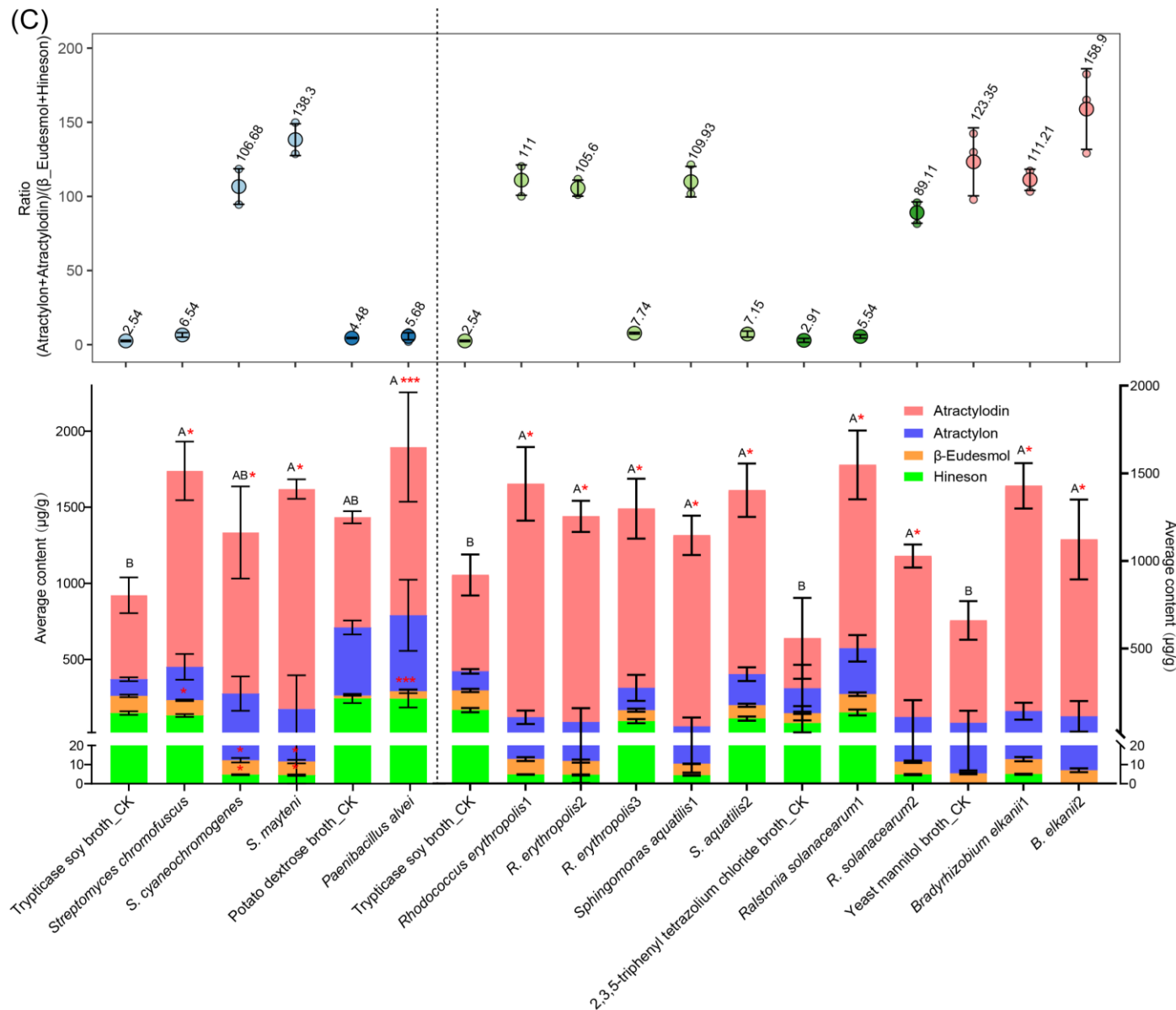
Part 3 Results: Functional Verification of core Microbe



Figures 4A and B Effects of rhizosphere and endophytic specific core bacteria on the biomass of *A. lancea*



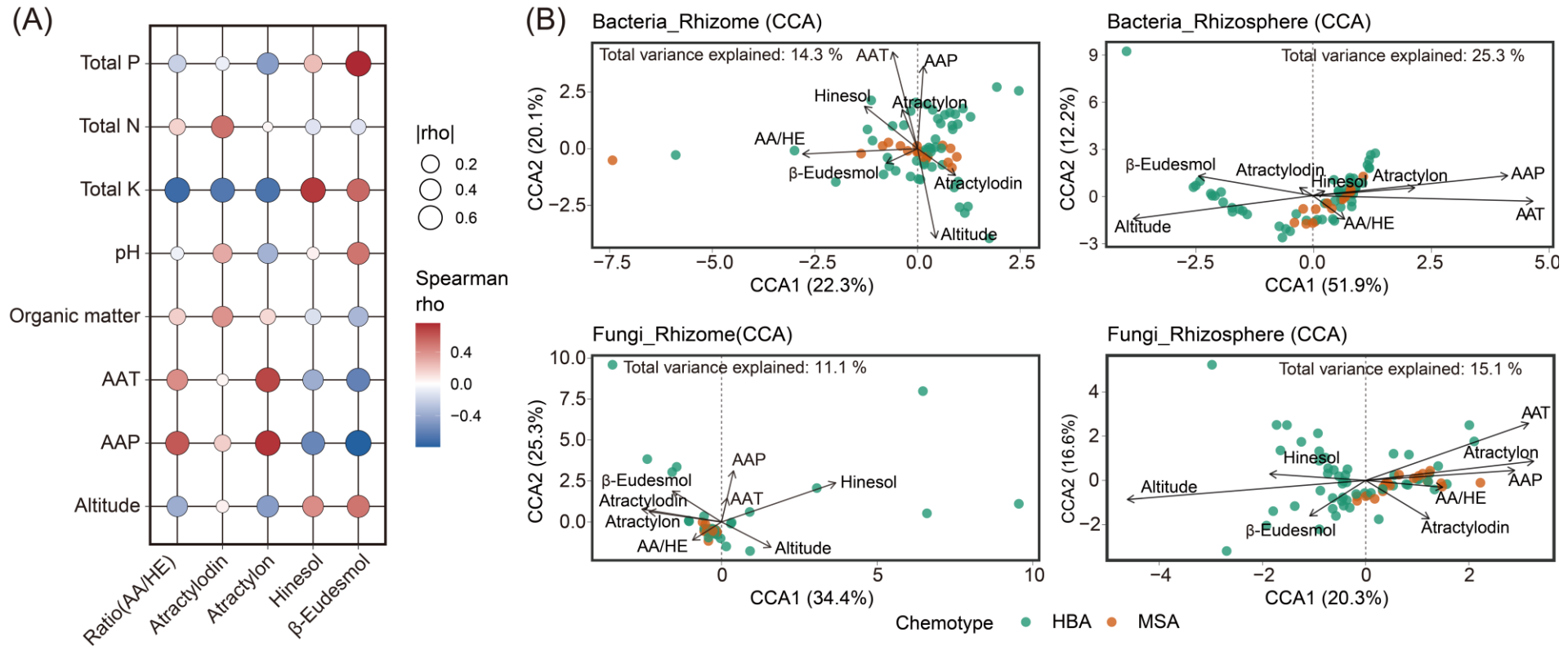
Part 3 Results: Functional Verification of core Microbe



Figures 4A and B Influence of rhizosphere and endophytic specific core bacteria on the biomass of *A. lancea*



Part 4: Comprehensive Analysis



Figures 5A and B The impact of environmental factors, including altitude, climate, and soil, on the four primary volatile oils and microbial composition of *A. lancea*.

- ❑ High levels of potassium and phosphorus suppress the accumulation of atractylone and atractylodin, while promoting the accumulation of hinesol and β -eudesmol.
- ❑ High temperatures and heavy rainfall facilitate the accumulation of atractylone and atractylin, while suppressing the accumulation of hinesol and β -eudesmol.
- ❑ High temperatures and heavy rainfall primarily strengthen the relationship between rhizosphere bacteria/fungi and atractylone, whereas altitude intensifies the relationship between hinesol or β -eudesmol and microbial communities.



Part 4: Comprehensive Analysis

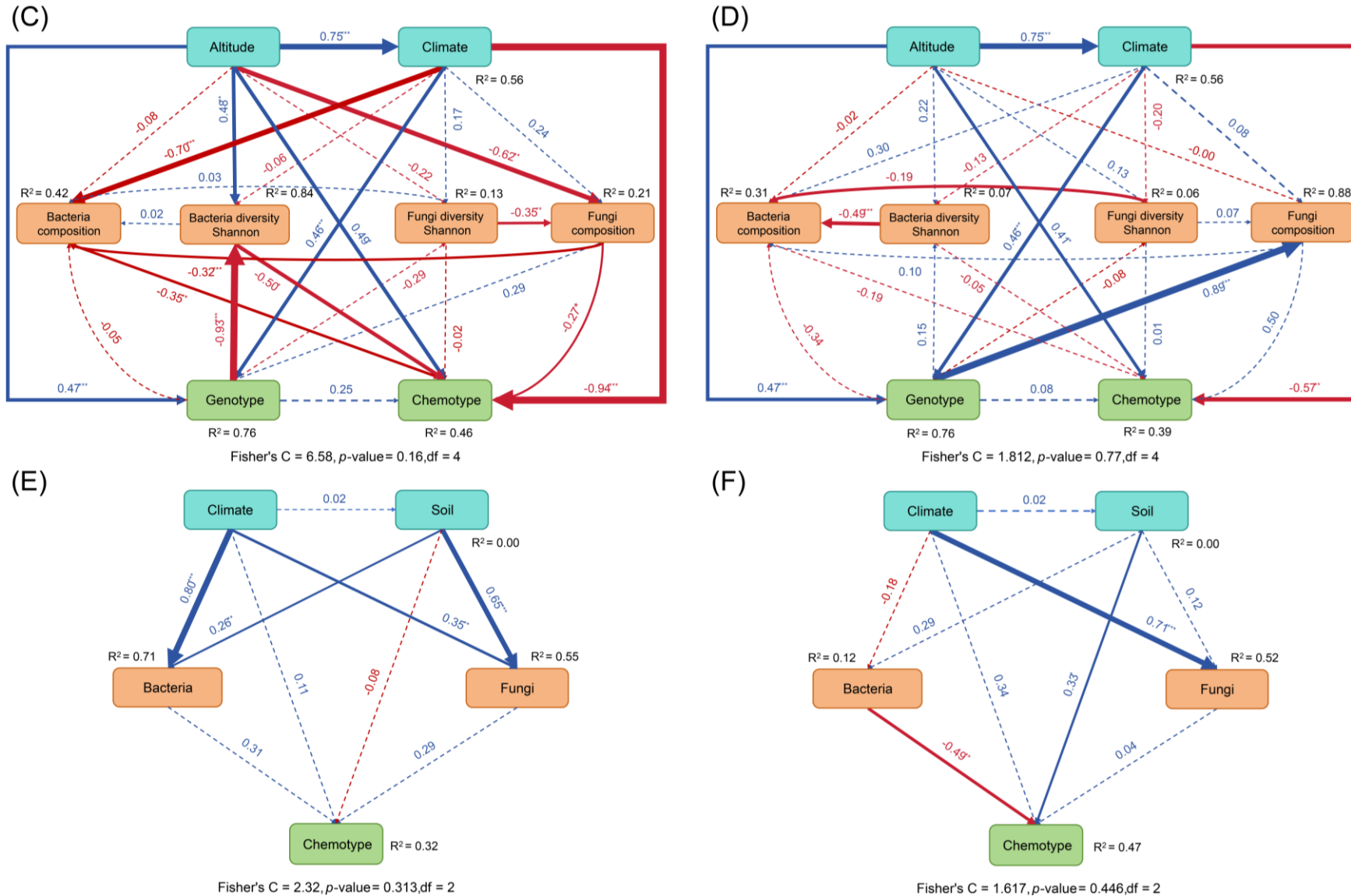


Figure 5C-F Structural equation model illustrating how environmental factors, including altitude, climate, soil, and microorganisms, drive the formation of genotypes and chemotypes in *A. lancea*.



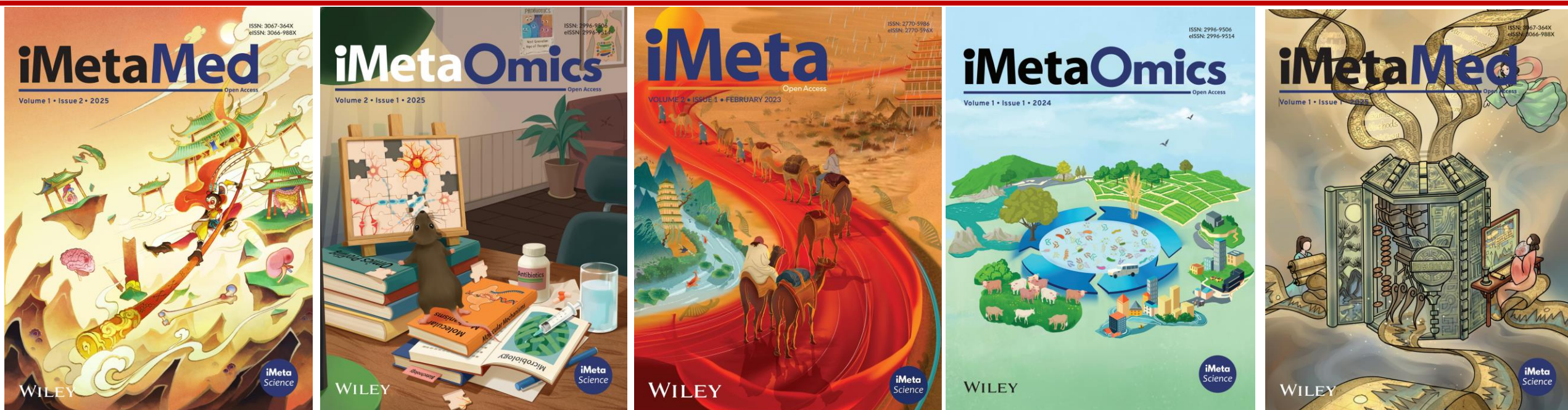
Summary

The study reveals that altitude and climate are the driving factors behind the genotypic differentiation of *A. lancea*, yet genotype alone does not determine chemotype. Instead, microbiome and soil environment are the key drivers of chemotype differentiation, with microorganisms exerting a particularly significant influence.

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