



Effect of the Carbon Nanosol on the Tobacco Growth by Modulating the Root-associated Microbiome

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2023/10/17

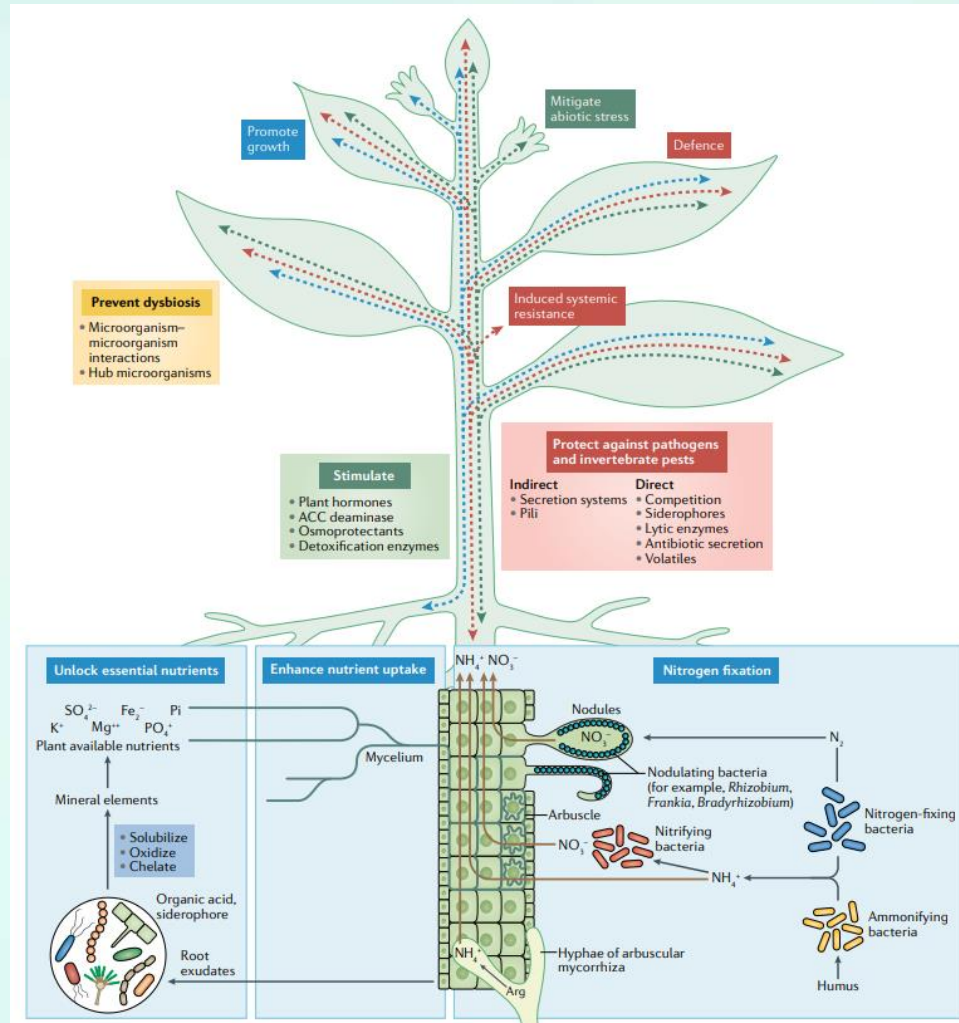
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- Introduction
- Experiment design
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Root-associated Microbiome: Critical Component for Plant Growth and Health



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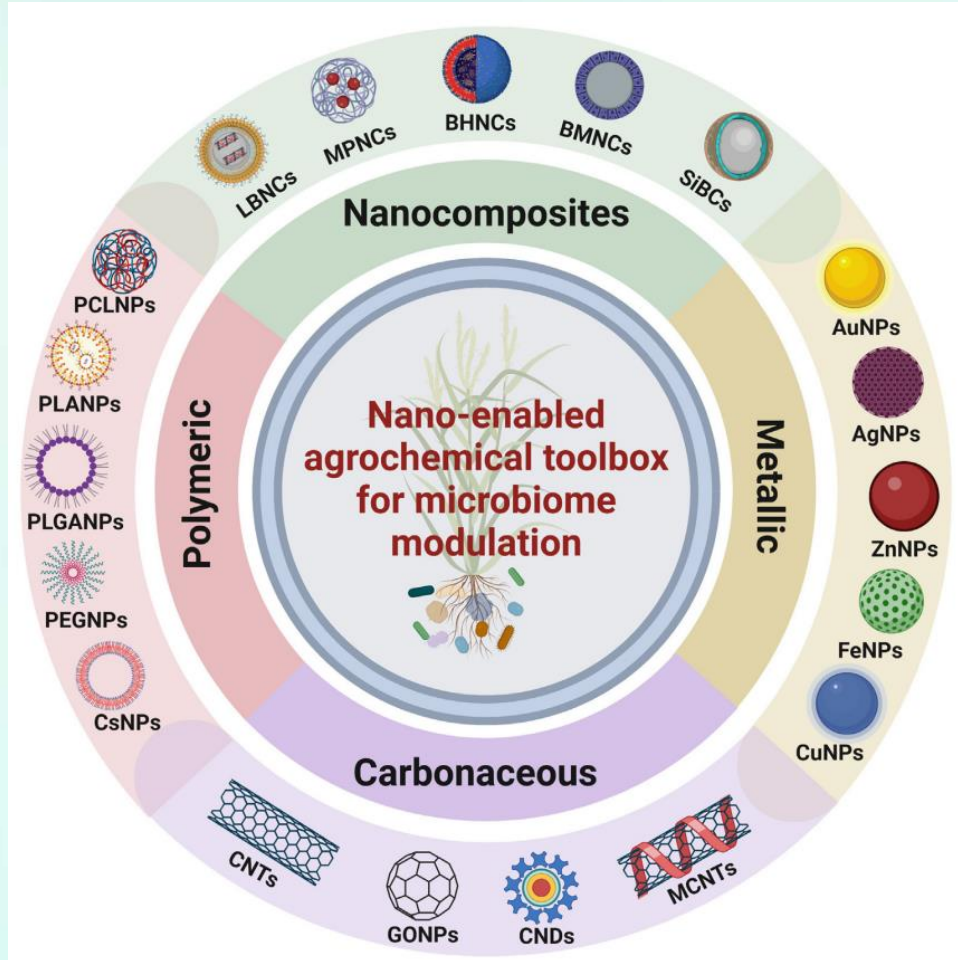


Nature reviews microbiology, 2020, 18, 607–621

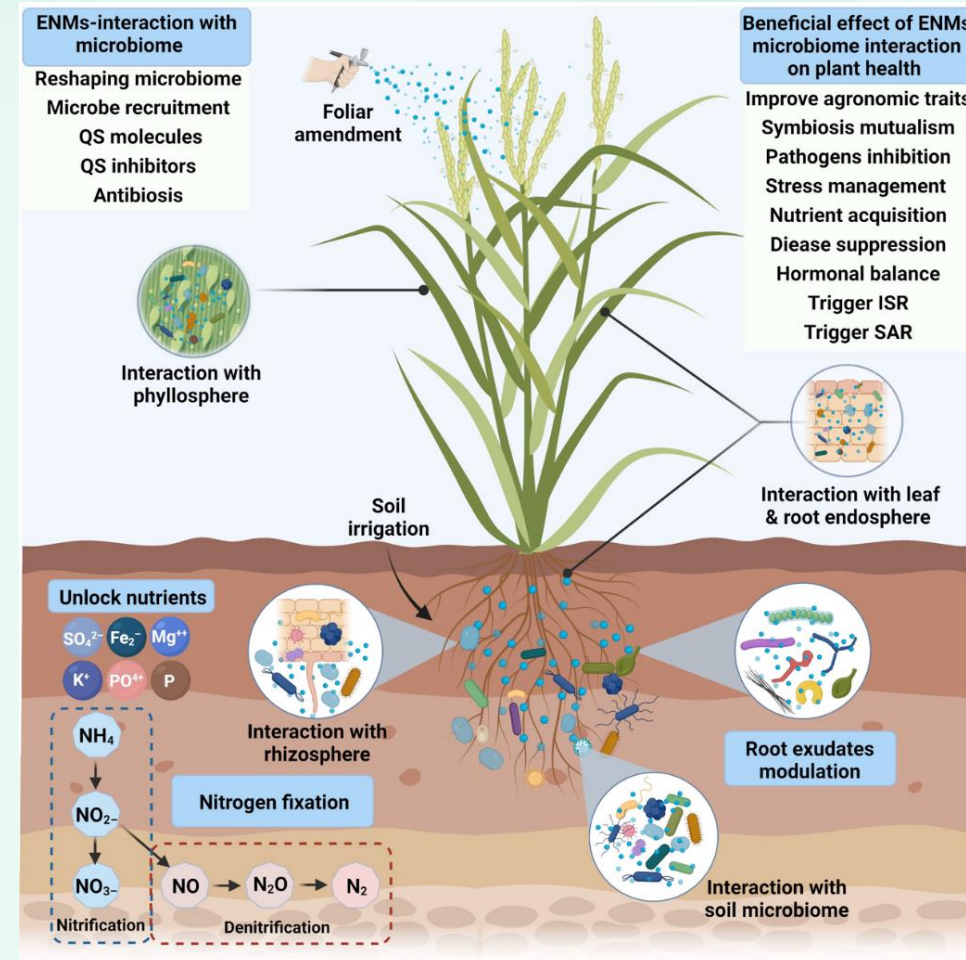
Beneficial effects of the plant-associated microbiome

- growth promotion
- nutrient acquisition
- chemical antagonism
- nutrient cycling
- pathogen resistance
- environmental stress resilience

Engineered Nanomaterials (ENMs) as Critical Tools for Microbiome Modulation



Various Engineered Nanomaterials

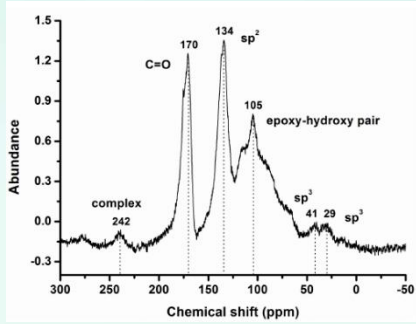


Trends in Plant Science, 2023

Harnessing Carbon Nanosol for Plant Growth Enhancement



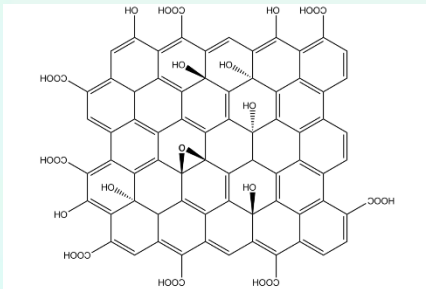
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- Definition:

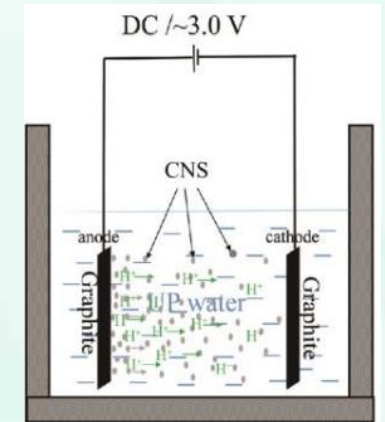
Carbon nanosol (CNS) as a carbon-based nanomaterial, synthesized through a electrolysis of graphite in water.

Synthesis of Carbon Nanosols via Pulse Electrodeposition Method Using Graphite (Chen et.al, 2020)



- Characteristics:

CNS surfaces are rich in abundant functional groups, rendering them remarkably stable, well-dispersed, and biocompatible.



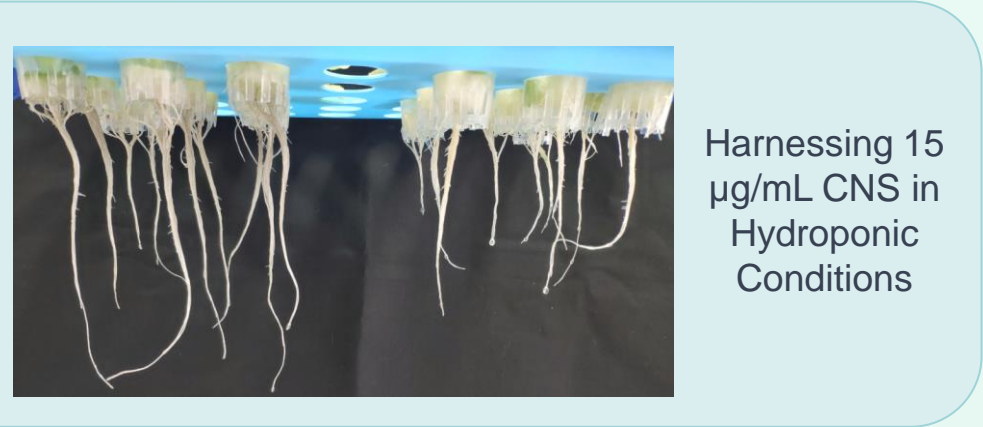
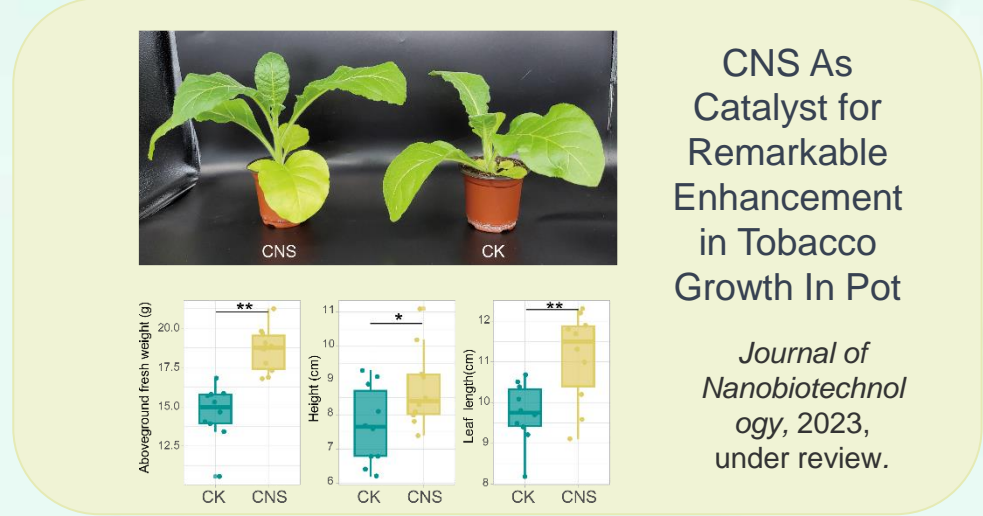
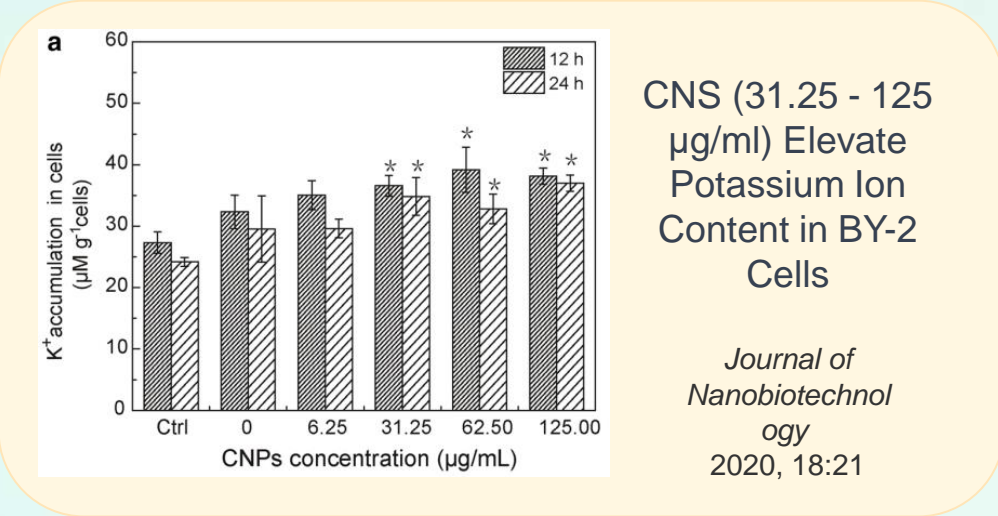
Journal of Nanobiotechnology 2020, 18:21

- Potential for Enhancing Crop Growth:

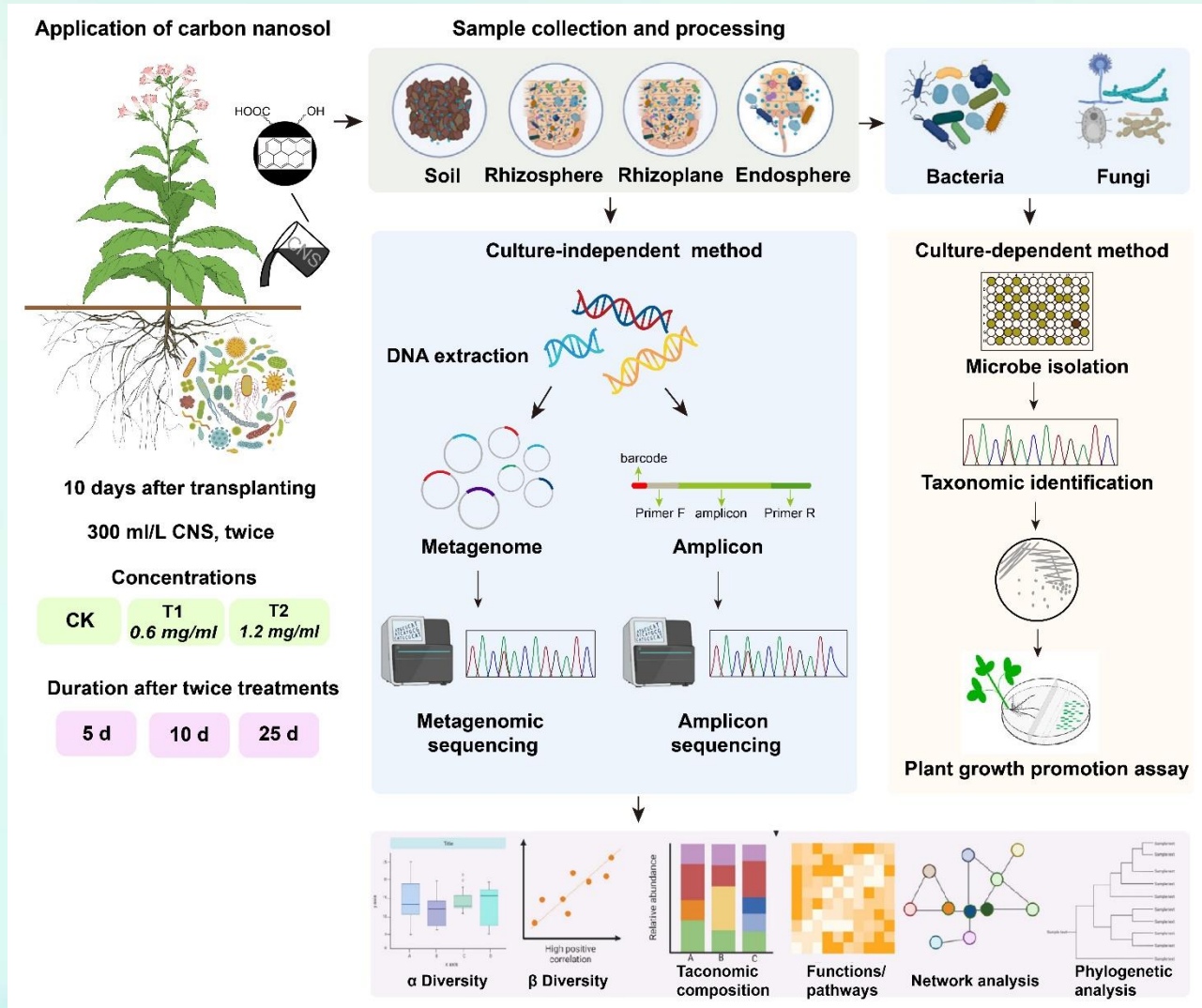
Carbon nanomaterials exhibit promising potential in regulating nutrient absorption, stimulating plant growth, and enhancing biomass accumulation in crops.

This underscores their promising application prospects.

The Growth-Boosting Potential of CNS



Experiment design



Understanding CNS managing the microbiome

↑

Sample collection, processing and analysis

Reproducible lab-based studies with culture collection

↓

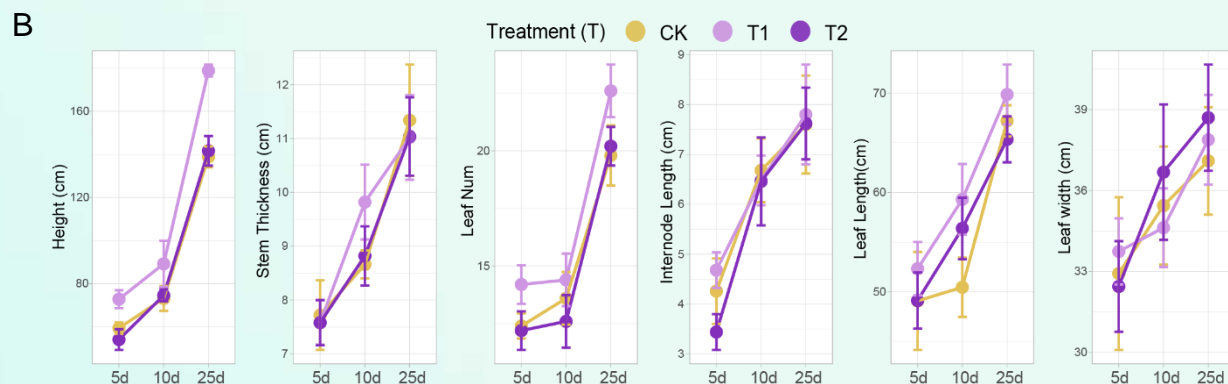
Microbial intervention in agriculture

1、 Effects of CNS on Tobacco Flowering and Growth Dynamics



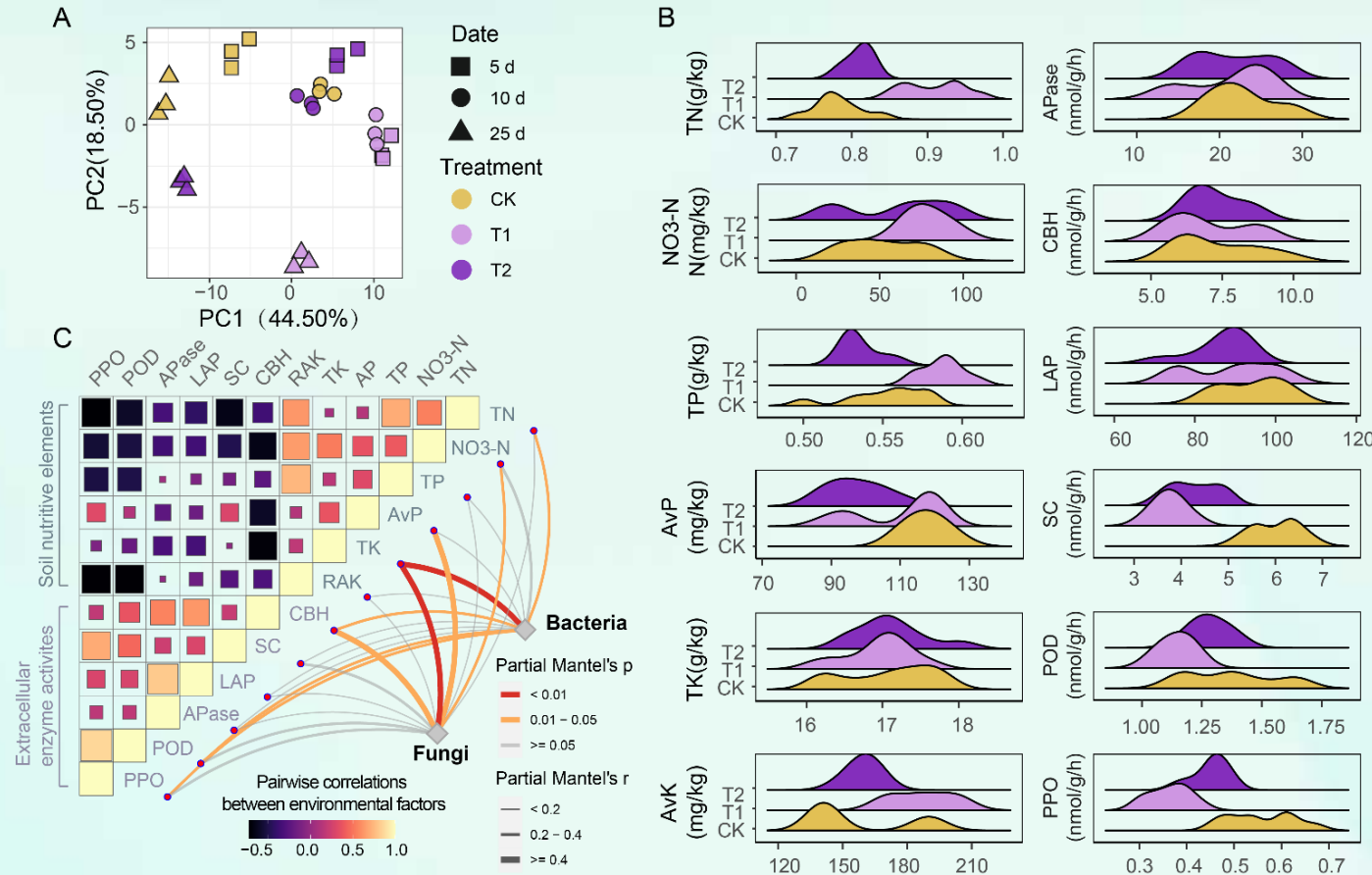
Growth Phenomenon: T1 (600 mg/L) CNS application leads to significant growth enhancement, with T1 exhibiting substantial height increase ($28.82\% \pm 8.54\%$)

Flowering Dynamics: CNS-treated plants exhibit diverse flowering patterns, with T1 promoting twofold increase in flowering, while T2 shows absence of flowering.



Flowering and Growth Nexus: Dose-dependent impact of CNS on tobacco plants observed.

2、 Impact of CNS on Soil Nutrients and Enzyme Activity



Environmental Effects: The effects of CNS on soil nutrient availability and enzyme activity were concentration-dependent.

Soil Nutrients:

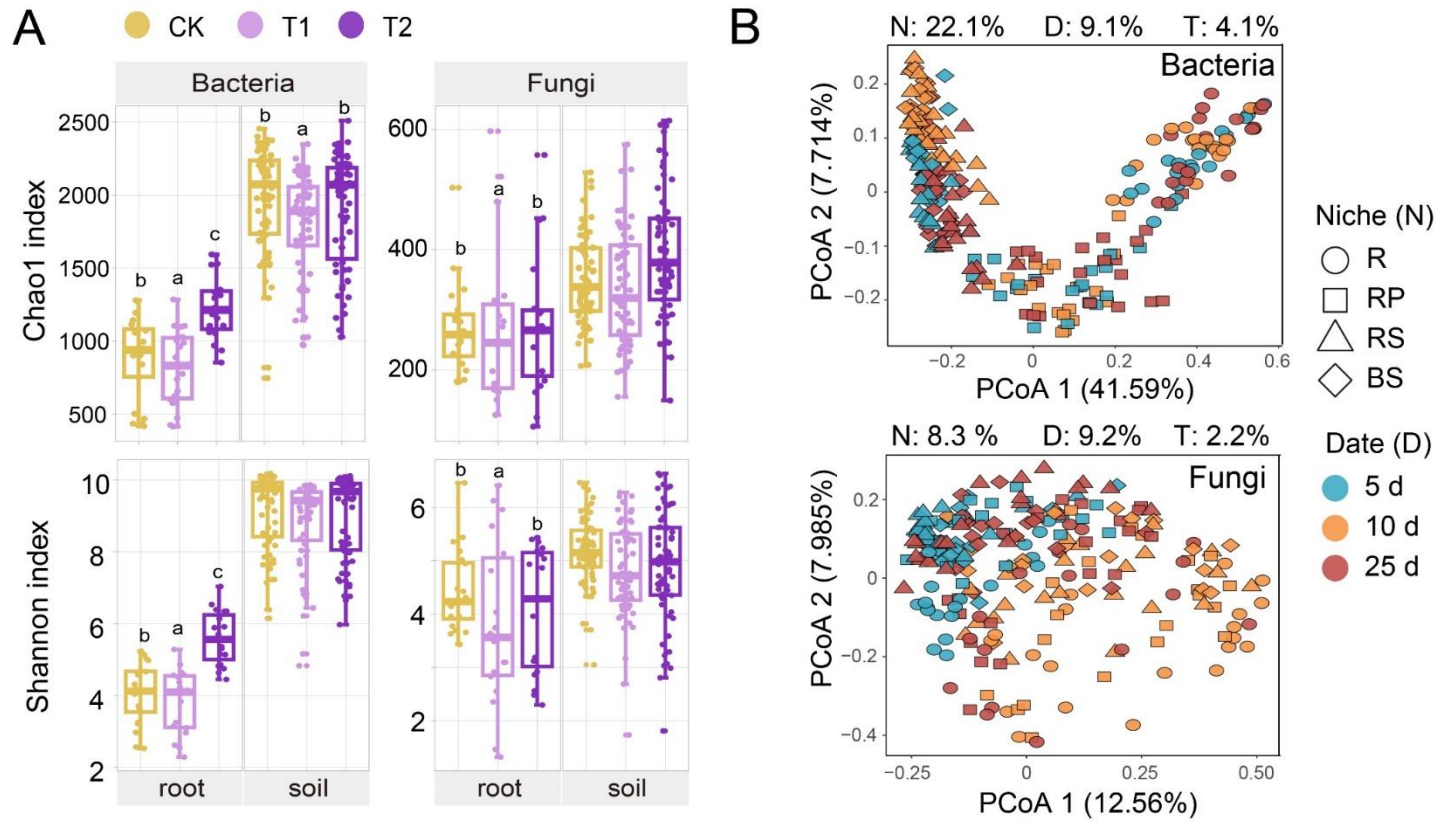
CNS improves nutrient content

Enzyme Activity Assessment: CNS decrease several enzyme activities, implying possible extracellular enzyme inhibition.

Microbial Associations: Significant correlations link potassium content (TK), cellobiohydrolase (CBH), and microbial communities.

TN, total nitrogen. NO₃-N, nitrate nitrogen. TP, total phosphorus. AvK, Available potassium. TK, total potassium. AvK, Available potassium. CBH, β-D-cellobiosidase. BG, β-glucosidase. SC, Sucrase. LAP, leucine aminopeptidase. Apase, Phosphatase. POD, peroxidase. PPO, Polyphenoloxidase

3、 Effect of CNS on root-associated microbial community structure and diversity



Root Microbial Diversity Shifts: CNS treatment induces significant alterations in root endophytic microbial diversity.

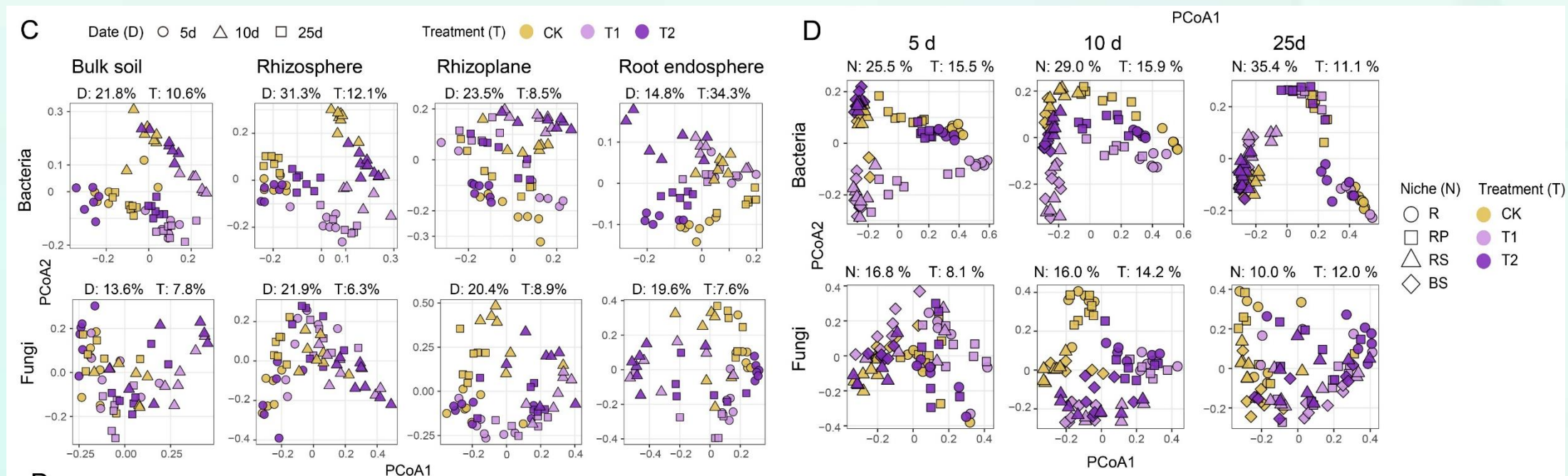
Ecological Niche Impact: PCoA results highlight significant differences between bacterial communities across distinct ecological niches, while developmental stages effectively differentiate fungal samples.

Bulk soil (BS)、Rhizosphere soil (RS)、Rhizoplane soil (RP) and Root endosphere (R)

3、 Effect of CNS on root-associated microbial community structure and diversity

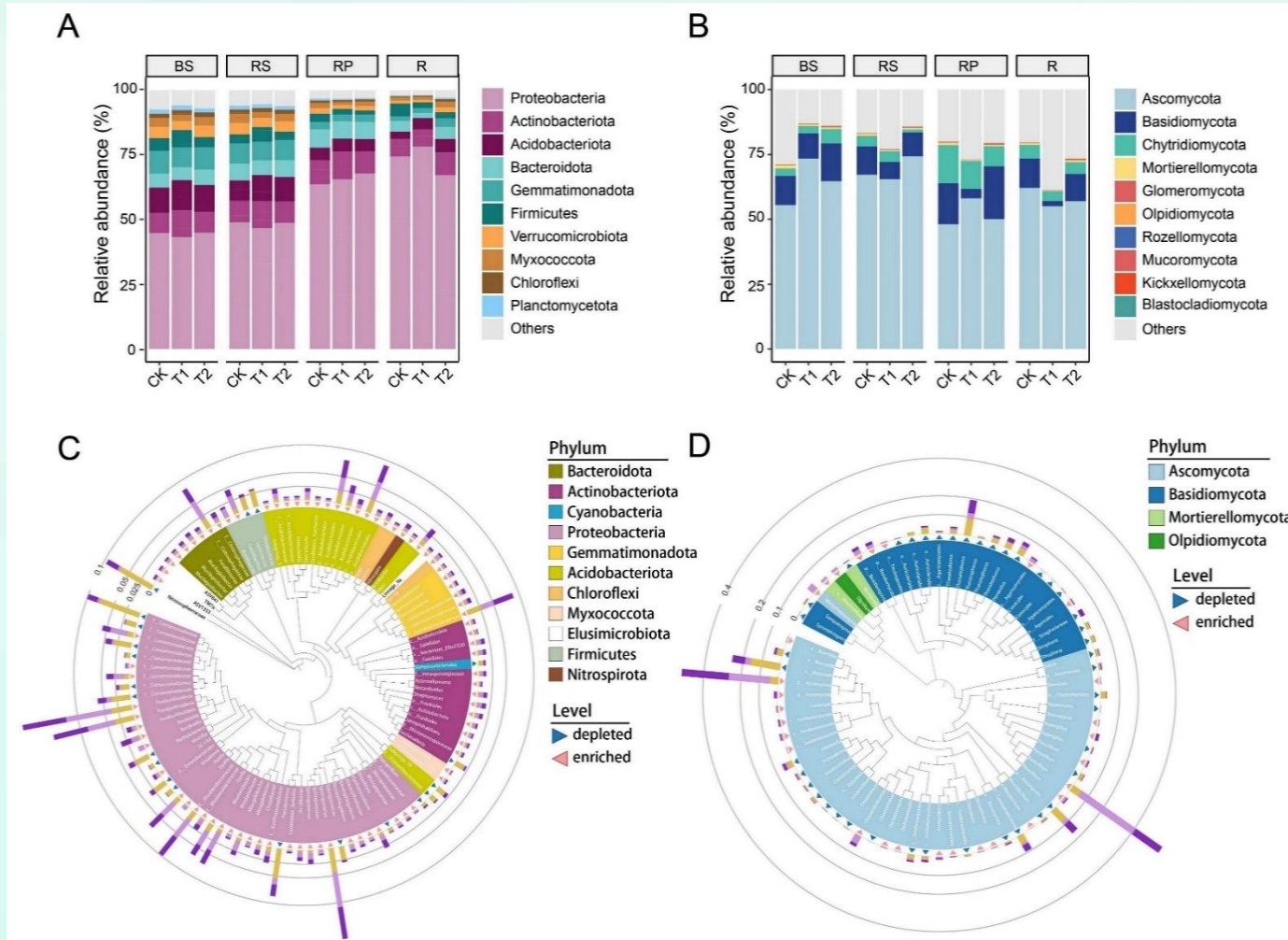
Ecological Niche Impact: CNS influences microbial communities across various ecological niches, notably root endophytic bacteria.

Enduring effects: The impact of CNS on community structure initially increases and then decreases, reaching its peak at 10 days.





4、 CNS Effects on Root-associated Microbial Taxonomy Composition



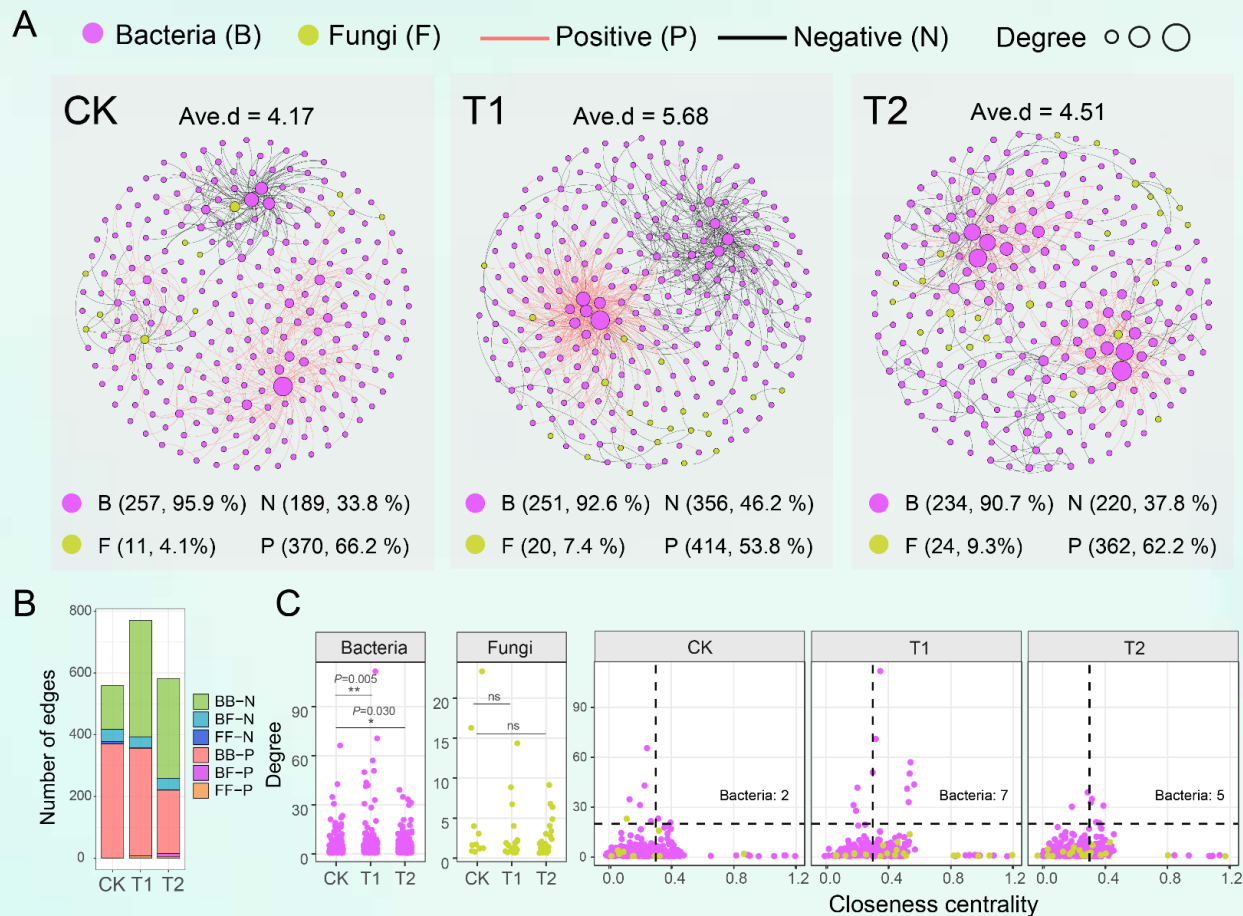
At Phylum Level: CNS treatment does not cause significant alterations in taxonomy composition.

Differential ASV: Identification of 1148 difference ASVs (923 bacteria, 225 fungi) following CNS treatment.

Enrichment of Beneficial Microbes: CNS treatment enhanced relative abundance of potential beneficial bacteria like *Burkholderia*, *Sphingomonas*, *Lactobacillus*, and beneficial fungi like *Penicillium*.

Bulk soil (BS)、 Rhizosphere soil (RS)、 Rhizoplane soil (RP) and Root endosphere (R)

5、 CNS Impact on Root-Associated Microbial Inter-Kingdom Co-occurrence Networks

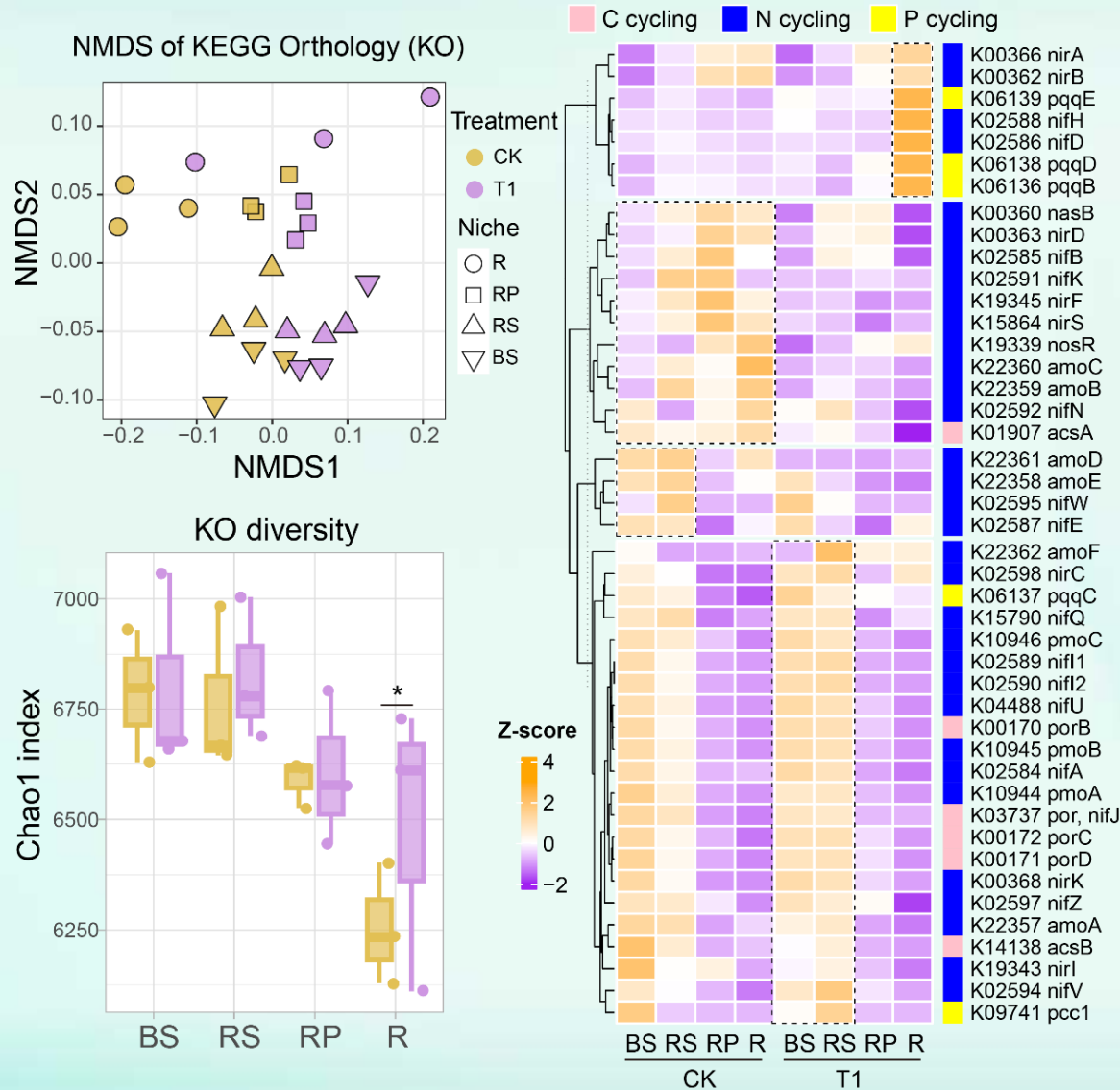


Network Complexity and Stability: CNS treatment enhanced complexity and stability of co-occurrence network.

Differential Effects on Fungi and Bacteria: CNS augments positive interactions among fungi while attenuating similar interactions among bacterial species.

Central Role of Bacteria: Num of network hubs are increased after CNS treatment and exclusively composed of bacterial

6、 Exploring Functional Traits of Root-Associated Microbial Communities



Functional Pathway Alterations: Significant shifts observed in microbial community metabolic pathways following CNS treatment.

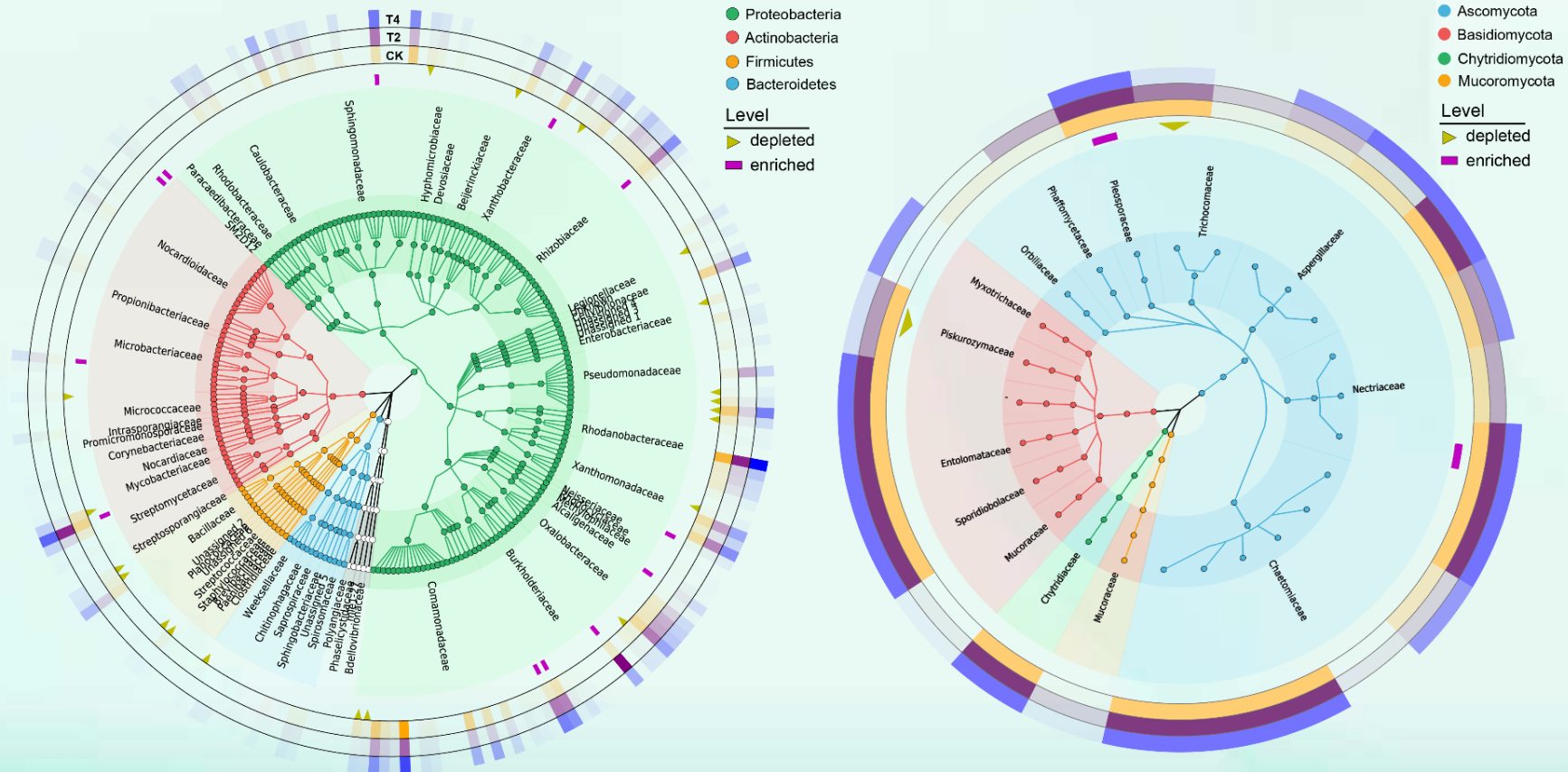
Enhanced Functional Diversity: CNS elevated functional diversity within endosphere after CNS treatment.

Enrichment of Key Functional Genes: Functional genes associated with P and N cycling are enriched within the root endophytic microbiome under CNS treatment.

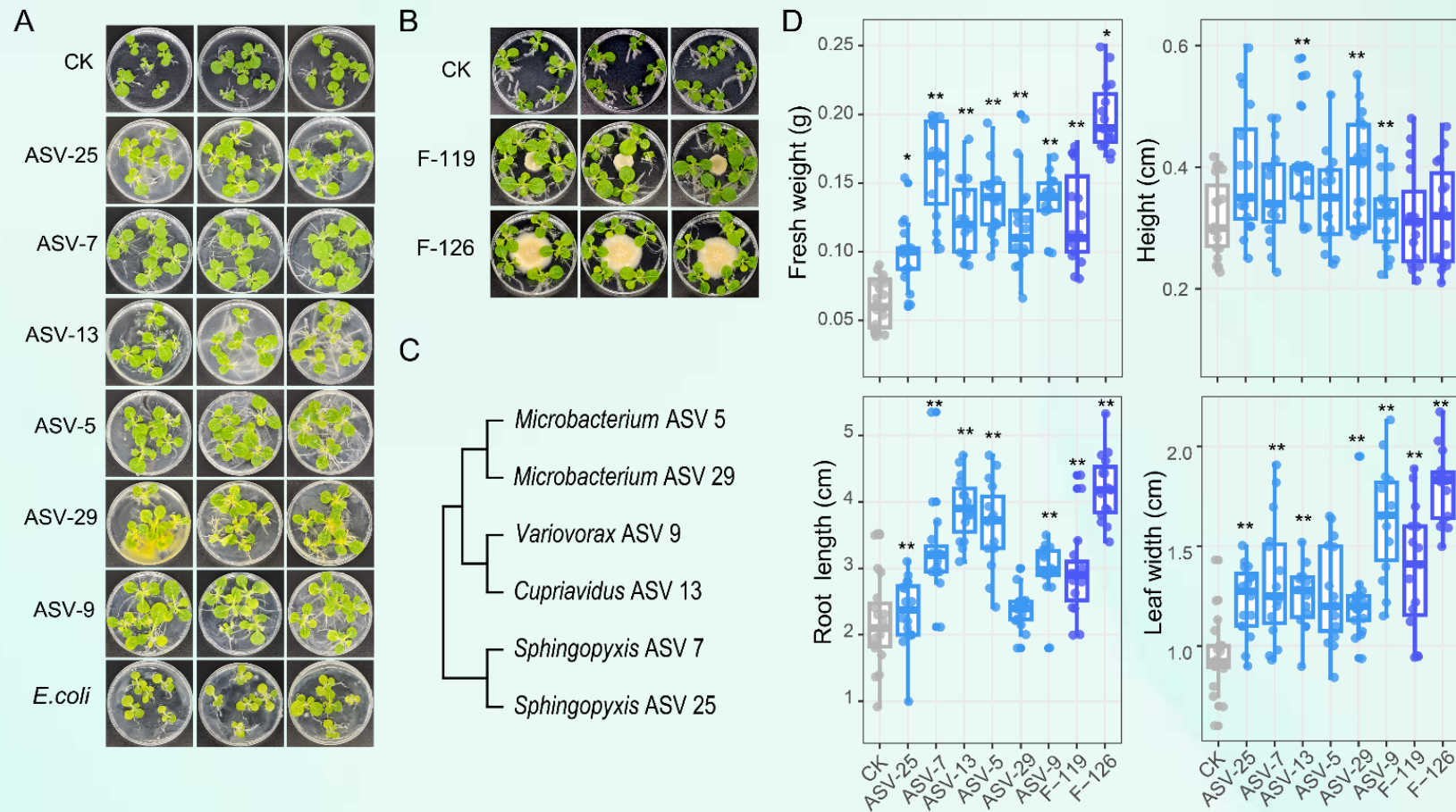
Bulk soil (BS), Rhizosphere soil (RS),
Rhizoplane soil (RP), Root endosphere (R)

7、 Isolation of culturable microorganisms

- **Culturable Microbes Isolated:** A total of 392 bacterial strains and 54 fungal strains were isolated, and identified through 16S rRNA and ITS RNA gene analysis.
- **Map to sequencing data:** Among these isolates, 12 bacterial strains and two fungal strains enriched after CNS treatment.

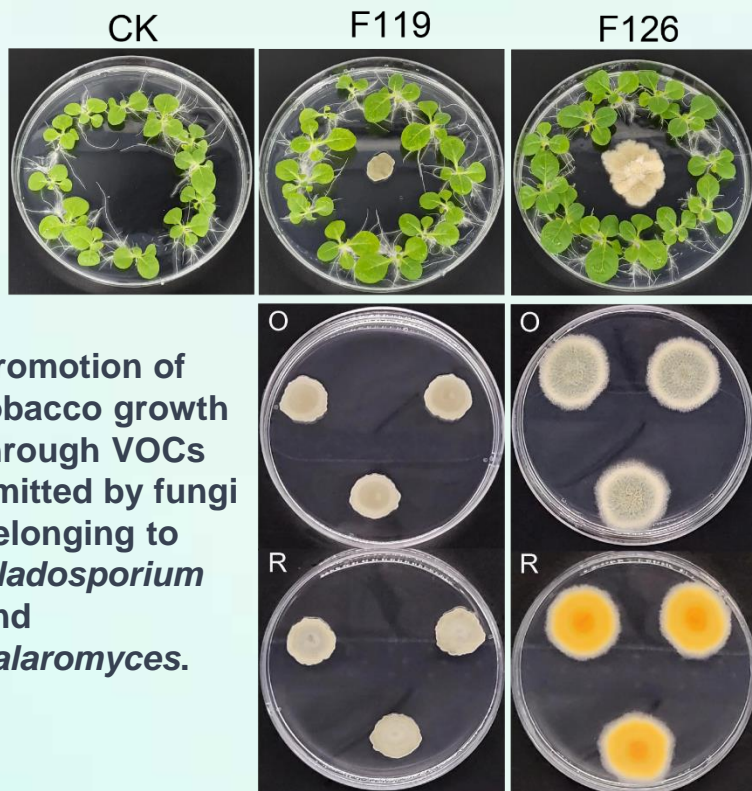


8、 Multiple Fungal and Bacterial Isolates Significantly Enhance Plant Growth

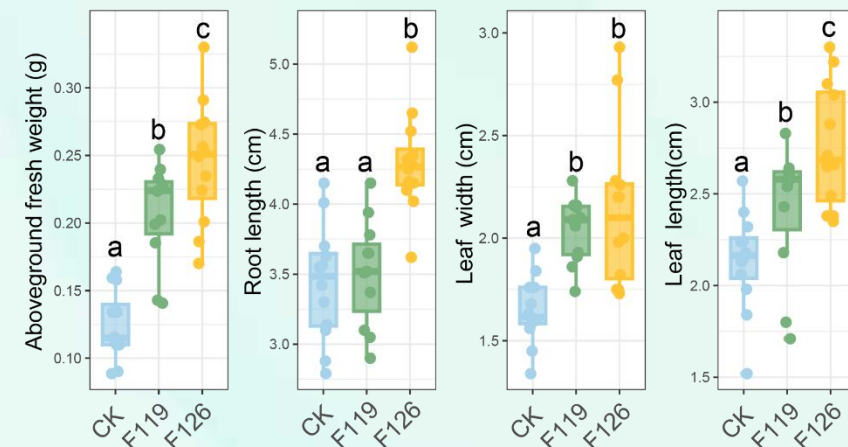
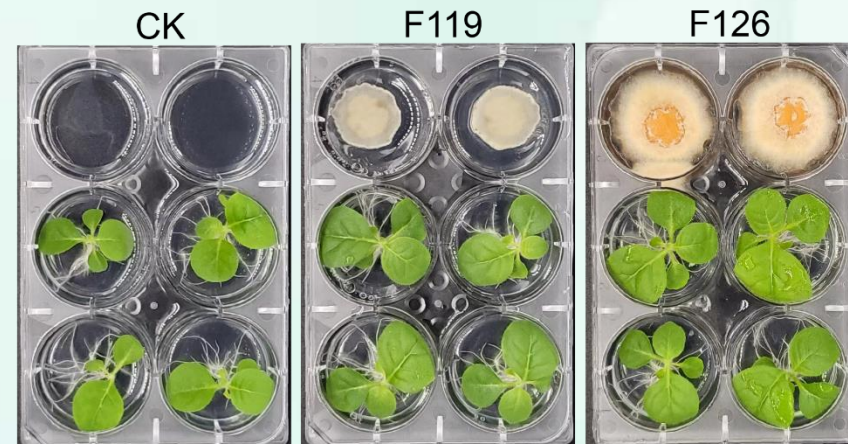
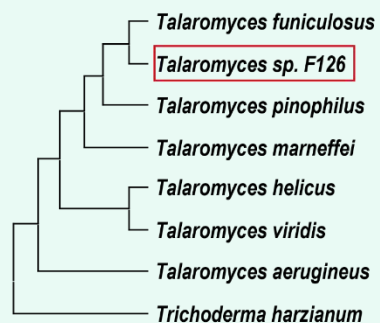
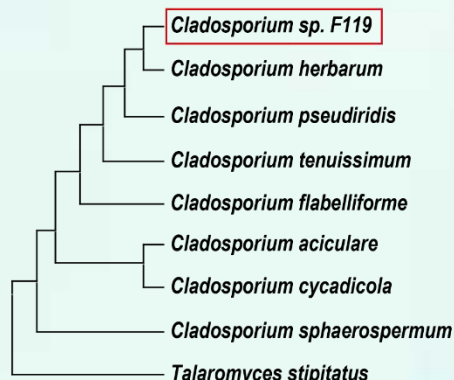


- 2 fungal and 6 bacterial strains enriched after CNS treatment exhibited growth-promoting effects in plate assays.

9、 Two Fungi might Promote Plant Growth via Volatile Organic Compounds (VOCs)



Promotion of tobacco growth through VOCs emitted by fungi belonging to *Cladosporium* and *Talaromyces*.



Conclusion

- **Impact on Plant Growth and Soil Dynamics:** CNS has demonstrated significant influence on tobacco growth, soil nutrient availability, and enzyme activities, with effects correlated to concentration.
- **Microbiota Diversity and Community Structure:** CNS concentrations have significant impacts on root endophytic microbial diversity, community structure, microbiota interactions and functional profile.
- **Beneficial Microbial Interactions:** Isolation of growth-promoting PGPR strains from CNS-enriched communities highlights the potential of CNS to enhance crop yield by targeting specific beneficial microbiomes.

Acknowledgement

- China Tobacco Gene Research Center, Zhengzhou Tobacco Research Institute of CNTC

Jiemeng Tao Peng Lu Taibo Liang Lijun Meng
Huan Su Jianfeng Zhang Peijian Cao Jingjing Jin

- China National Tobacco Corporation Henan Company



Thank you!