



# Arbuscular mycorrhizal fungal interactions bridge the support of root-associated microbiota for slope multifunctionality in an erosion-prone ecosystem

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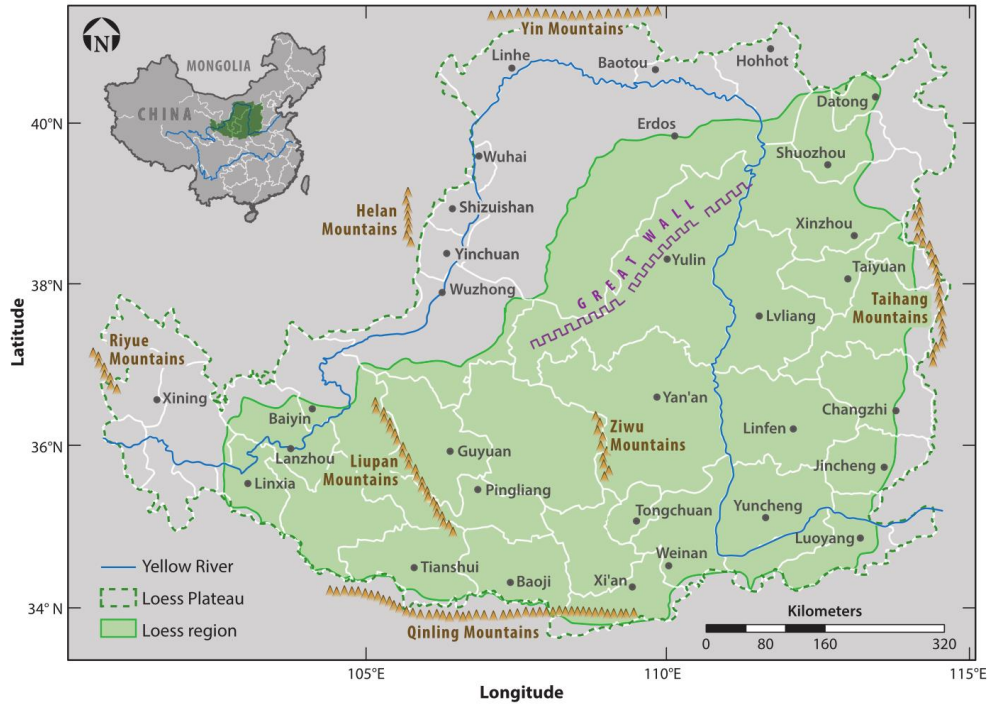
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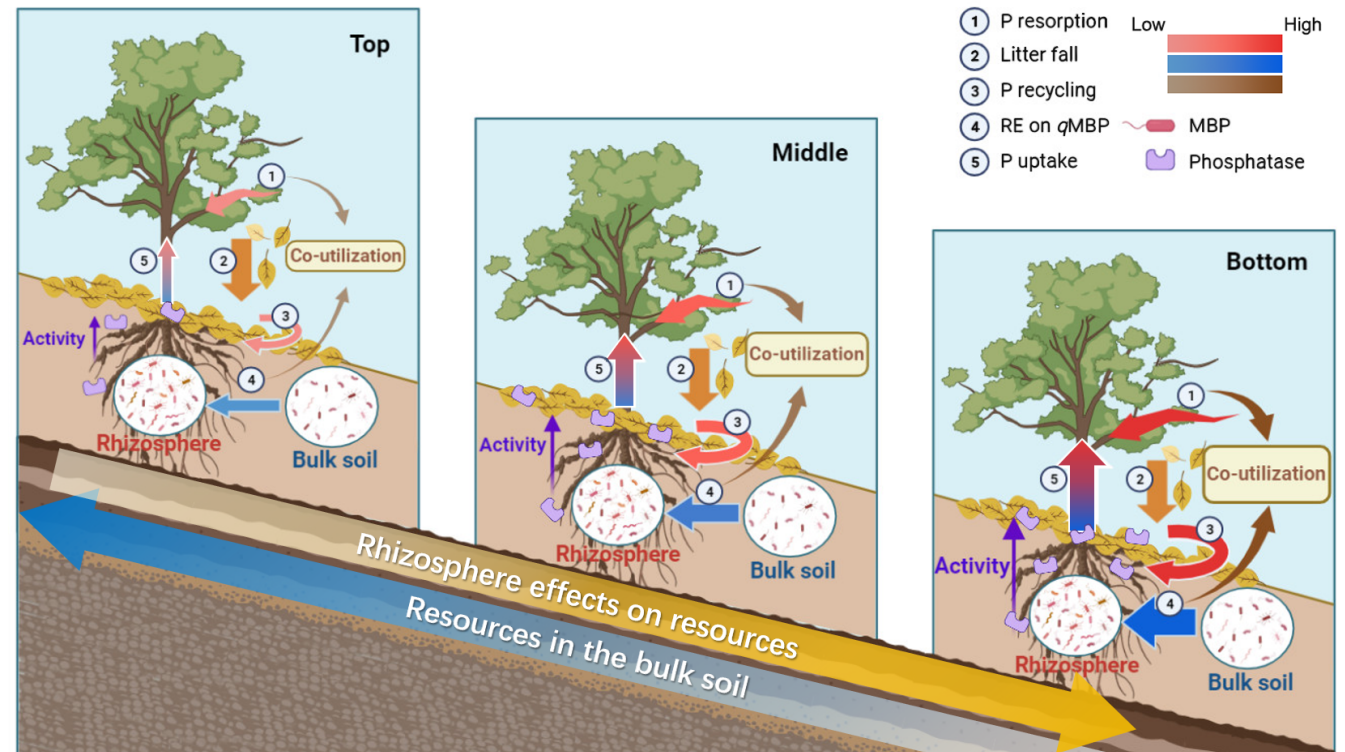
# Introduction



Fu et al., 2017, *Annu. Rev. Earth Planet. Sci.*

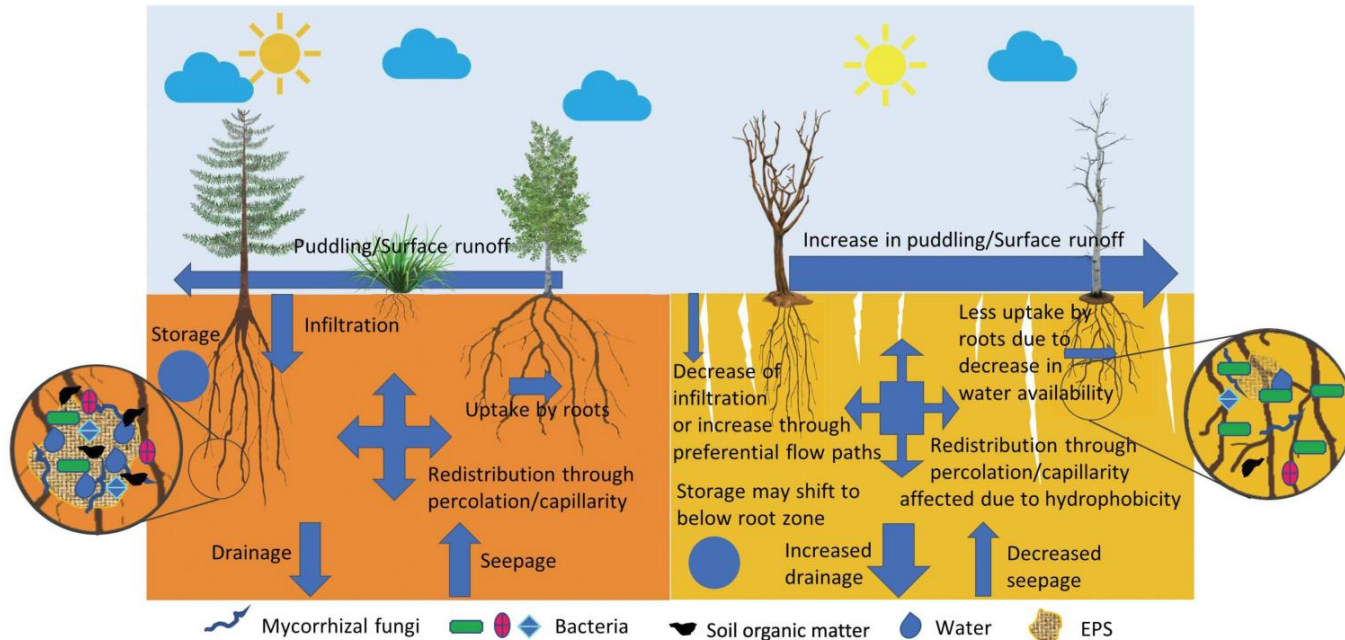
Eroded **slopes** are the basic landscape unit of the Loess Plateau. Slope position can strongly affect biological phosphorus utilization and even **the biodiversity–ecosystem functioning relationship**.

The Loess Plateau is a traditionally **erosion-induced degraded region**, characterized by soil erosion and nutrient immobilization, resulting in generally low soil **phosphorus** availability.

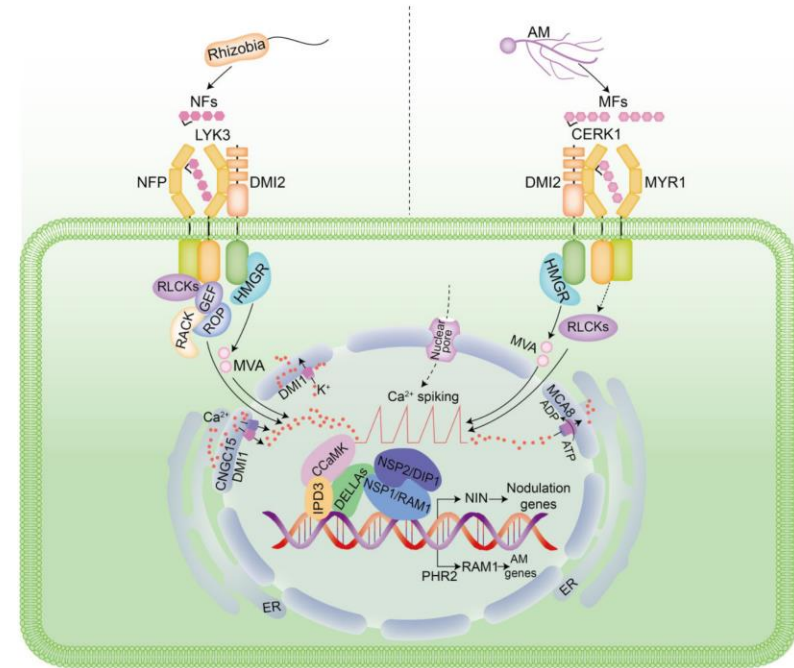


Qiu et al., 2023, *Catena*

# Introduction



Coban et al., 2022, *Science*



Wang et al., 2022, *Plant Cell*

- Soil microbiota, especially the **root-associated microbiota**, plays a pivotal role in restoring degraded ecosystems by executing an array of essential functions, such as nutrient cycling, organic matter decomposition, and primary production.
- Rhizobia and arbuscular mycorrhizal (AM) fungi share a signaling pathway and mutually promote colonization in the rhizosphere, implying their co-evolution that may largely contribute to phosphorus-limited ecosystems being restored with **woody legumes**, such as *Robinia pseudoacacia*.



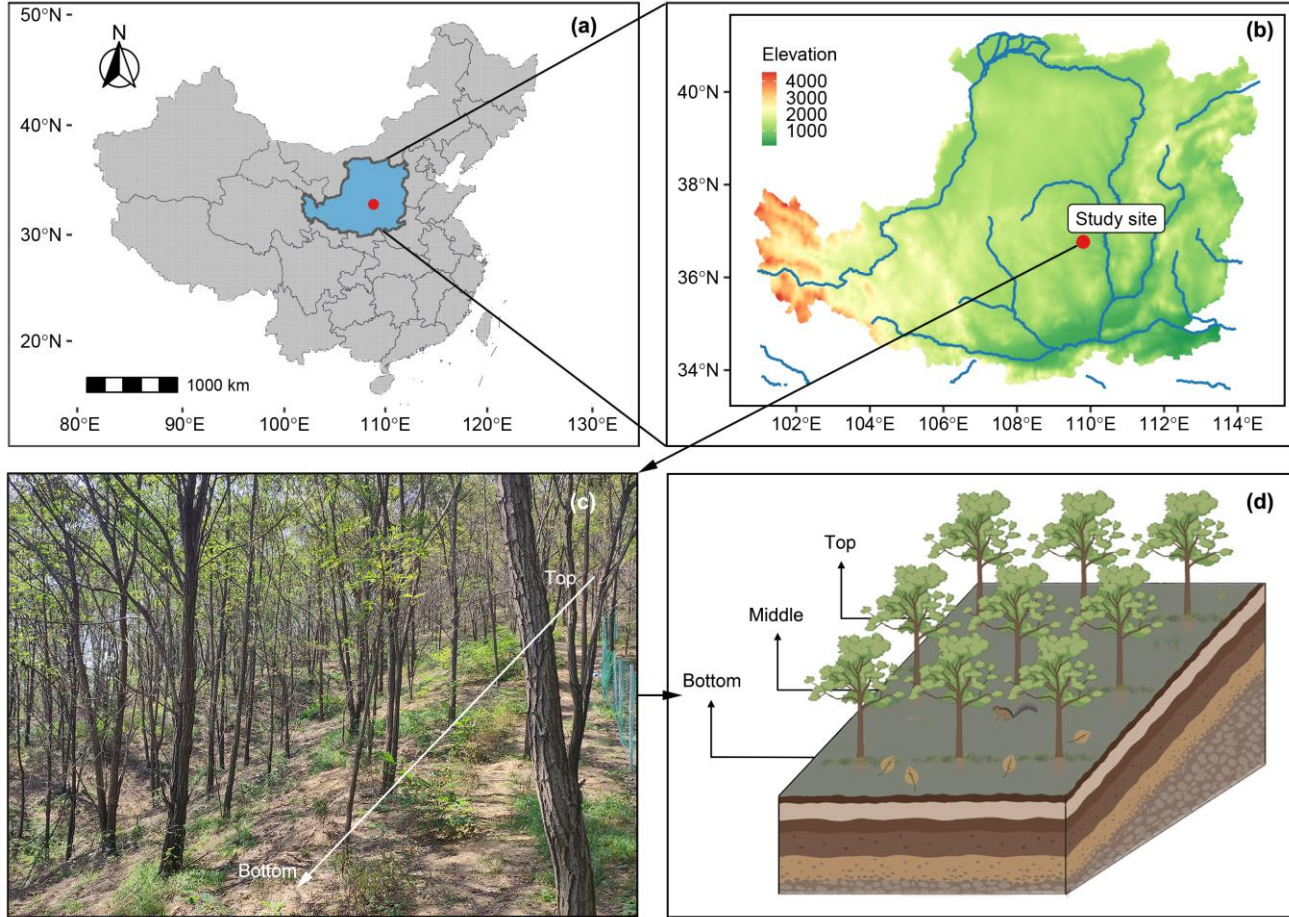
# Introduction

Unfortunately, existing knowledge about microbial diversity and functionality shifts along slopes is fragmented, with the **focus limited to** a single microbial kingdom or ecosystem function; Ecological networks serve as useful tools for comprehending complex biological interactions and can disentangle the roles of keystone microbial taxa in community assembly and ecosystem functioning. In eroded landscapes, dominant keystone taxa in network clusters have been identified as the engine to support multifunctionality and mitigate erosion impacts. However, microbial network complexity and interactions within and among fungal and bacterial kingdoms that are critical for maintaining multifunctionality during natural restoration processes, **are still unclear**.

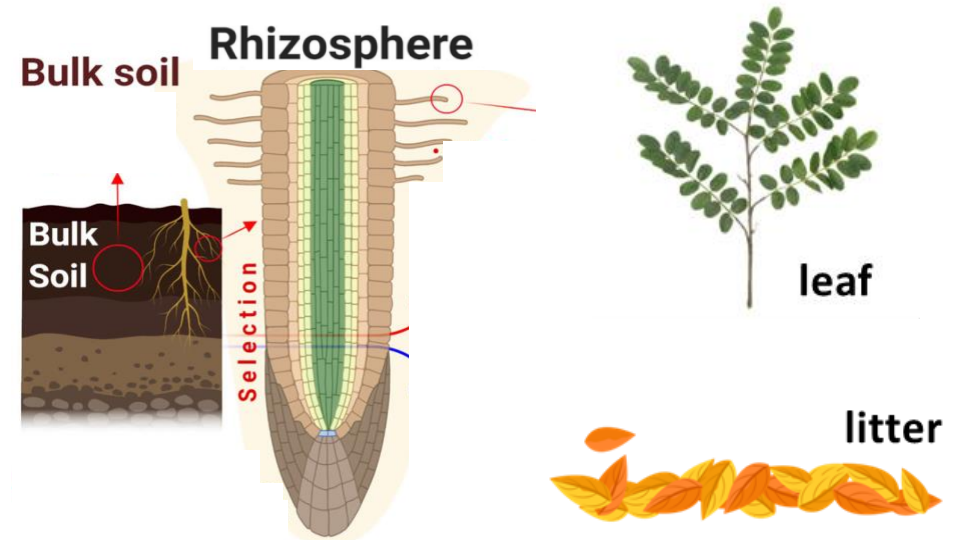
## Hypothesis:

- a) All facets of multifunctionality would be driven by and dependent on the diversity of root-associated microbiota, among which AM fungi were the foremost given their proficiency in alleviating P limitation for *R. pseudoacacia*;
- b) Cross-kingdom networks would be more connected than single-kingdom networks, with AM fungi serving as the central figure due to their fundamental role in niche partitioning and interactions with other microbial assemblies;
- c) Regardless of the resource distribution along the slope, AM fungal interactions would robustly support multifunctionality, particularly the mutualism with rhizobia that benefits *R. pseudoacacia* by supplying N and P synergistically.

# Methods



- *Robinia pseudoacacia*
- 60 m × 30 m, three positions were established at an interval of 20 m
- 6 replications, 18 plots (10 m × 10 m)

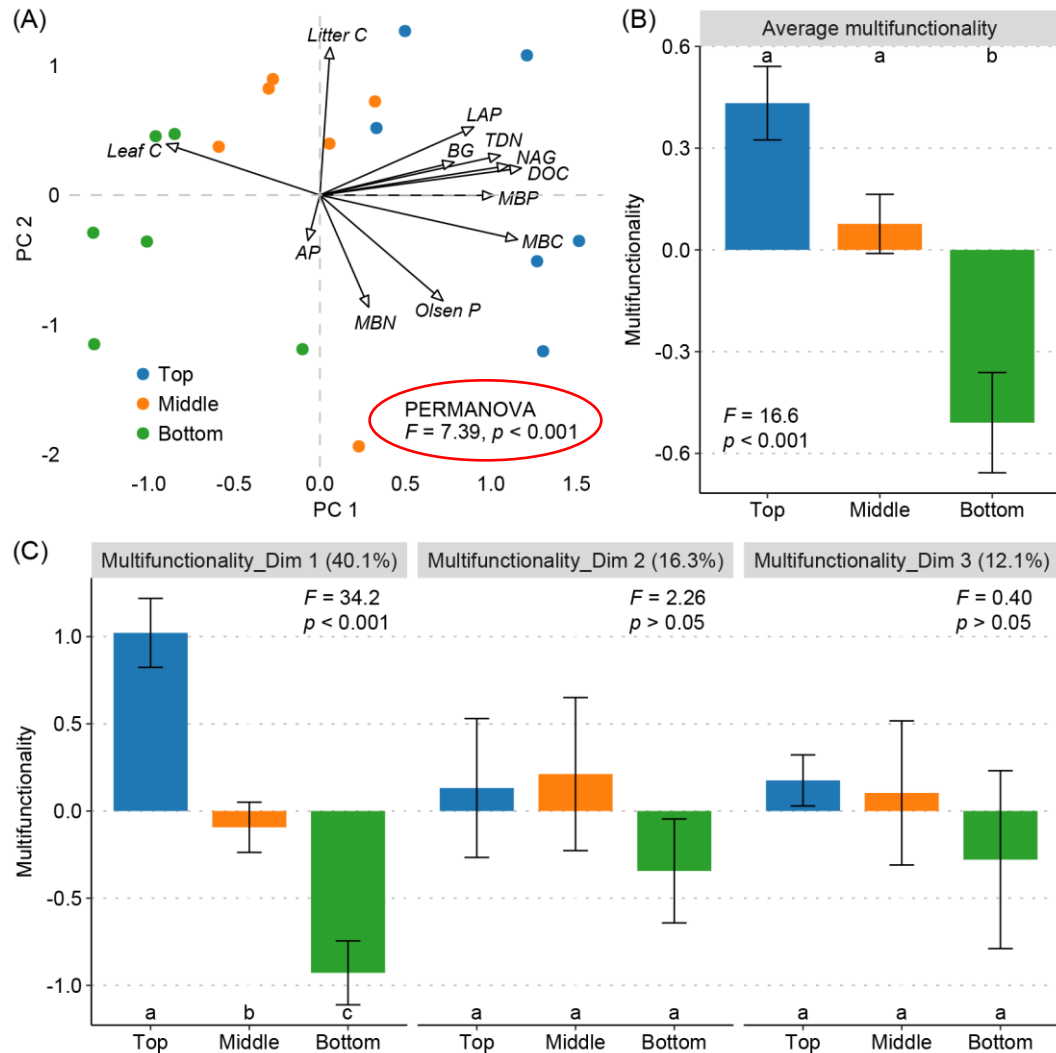


**Environmental variables:** Moisture; pH; Bulk density; SOC; Soil N:P; TP

**Root-associated microbiota:** AM fungi; Bacteria; Rhizobia

**Ecosystem functioning:** Microbially driven C pools (dissolved organic C and microbial biomass C); Nutrient cycling (total dissolved N, Olsen-P, microbial biomass N, microbial biomass P); Organic matter decomposition ( $\beta$ -glucosidase, N-acetylglucosaminidase, leucine aminopeptidase, and alkaline phosphatase); Plant production (leaf C content and litter C content)

# Results

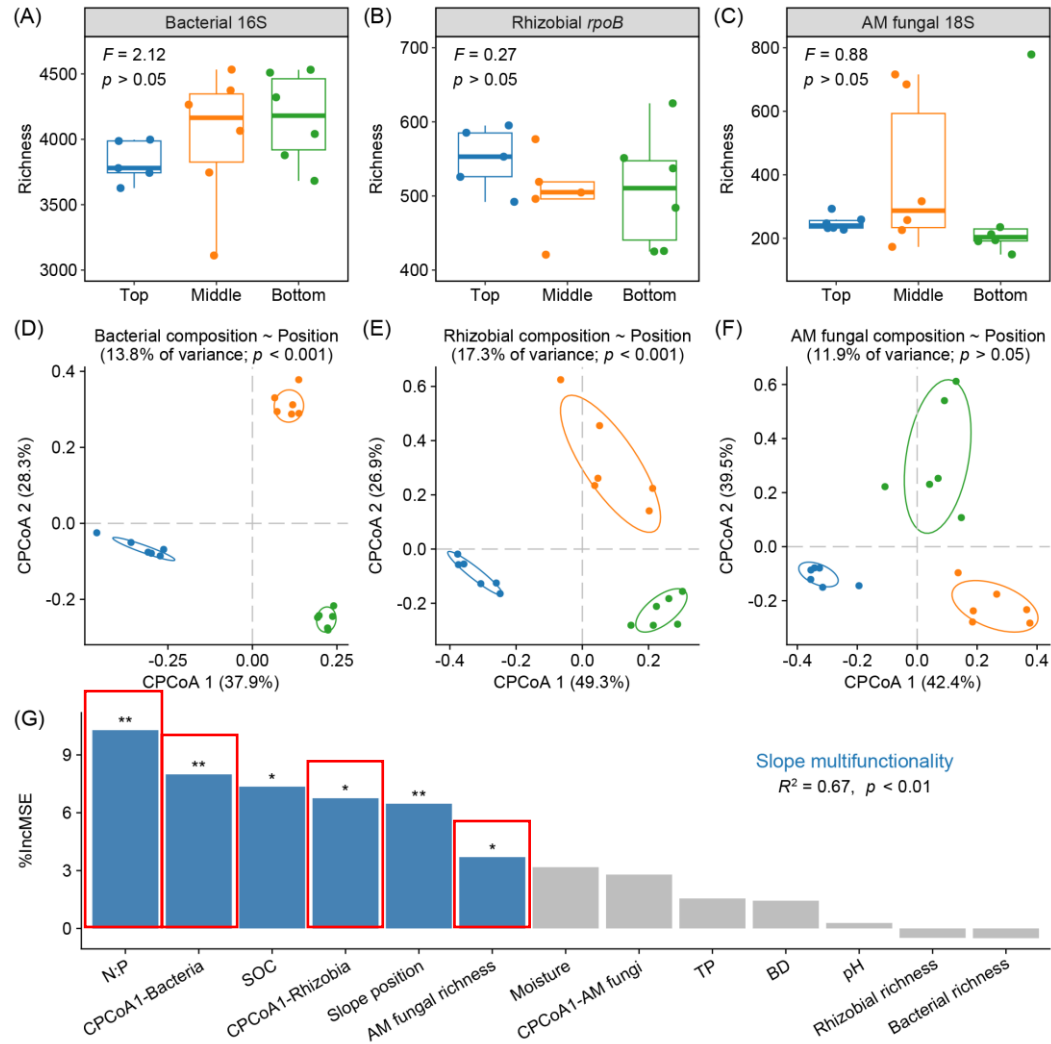


- Slope position **significantly** impacted multiple ecosystem functions and thereafter the average multifunctionality;
- Three dimensions of slope multifunctionality were identified using principal component analysis, jointly capturing **68.4%** of the spatial variation in ecosystem functions;
- The first dimension (PC1), which was dominated by **microbially driven C pools** and **organic matter decomposition**, exhibited a distribution similar to that of the average multifunctionality.

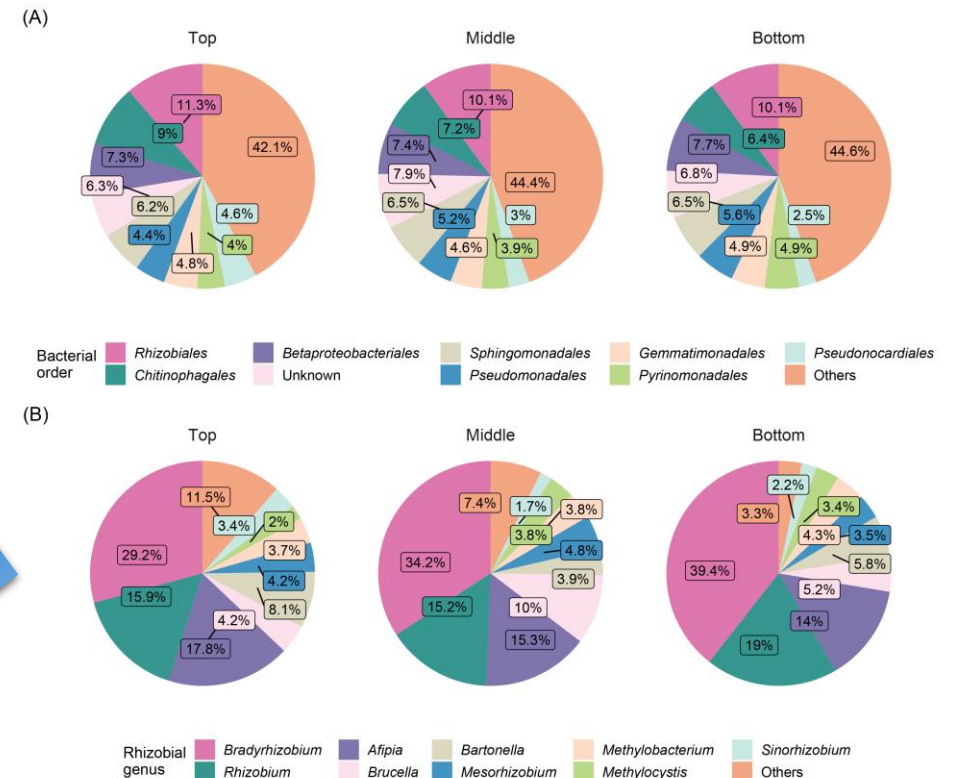
**Table S1** Distribution of ecosystem functions related to four important services on the slope using linear mixed-effects models.

Service	Function	Top	Middle	Bottom	P
Microbially driven C pools	DOC (mg kg <sup>-1</sup> )	103±4.51 <sup>a</sup>	89.4±5.23 <sup>ab</sup>	76.4±3.40 <sup>b</sup>	**
	MBC (mg kg <sup>-1</sup> )	164±16.8 <sup>a</sup>	127±13.28 <sup>b</sup>	84.2±6.65 <sup>c</sup>	***
	TDN (mg kg <sup>-1</sup> )	29.4±1.84 <sup>a</sup>	28.3±1.67 <sup>a</sup>	21.4±1.76 <sup>b</sup>	*
Nutrient cycling	Olsen-P (mg kg <sup>-1</sup> )	0.38±0.10 <sup>a</sup>	0.35±0.14 <sup>a</sup>	0.16±0.03 <sup>a</sup>	n.s.
	MBN (mg kg <sup>-1</sup> )	14.3±1.31 <sup>a</sup>	13.3±1.68 <sup>a</sup>	12.9±2.06 <sup>a</sup>	n.s.
	MBP (mg kg <sup>-1</sup> )	6.02±0.93 <sup>a</sup>	5.67±0.41 <sup>a</sup>	2.91±0.30 <sup>b</sup>	**
Organic matter decomposition	BG (nmol g <sup>-1</sup> h <sup>-1</sup> )	15.9±1.23 <sup>a</sup>	11.3±1.23 <sup>a</sup>	11.3±2.30 <sup>a</sup>	n.s.
	NAG (nmol g <sup>-1</sup> h <sup>-1</sup> )	8.70±0.30 <sup>a</sup>	1.93±0.51 <sup>b</sup>	1.80±0.40 <sup>b</sup>	***
	LAP (nmol g <sup>-1</sup> h <sup>-1</sup> )	40.7±2.16 <sup>a</sup>	33.2±1.63 <sup>b</sup>	27.7±1.21 <sup>b</sup>	**
Plant production	AP (nmol g <sup>-1</sup> h <sup>-1</sup> )	73.2±5.94 <sup>a</sup>	74.4±5.21 <sup>a</sup>	82.9±4.86 <sup>a</sup>	n.s.
	Leaf C (g kg <sup>-1</sup> )	544±6.80 <sup>b</sup>	582±8.24 <sup>a</sup>	573±4.87 <sup>a</sup>	**
	Litter C (g kg <sup>-1</sup> )	509 ±6.73 <sup>a</sup>	518±7.70 <sup>a</sup>	497±6.66 <sup>a</sup>	n.s.

# Results

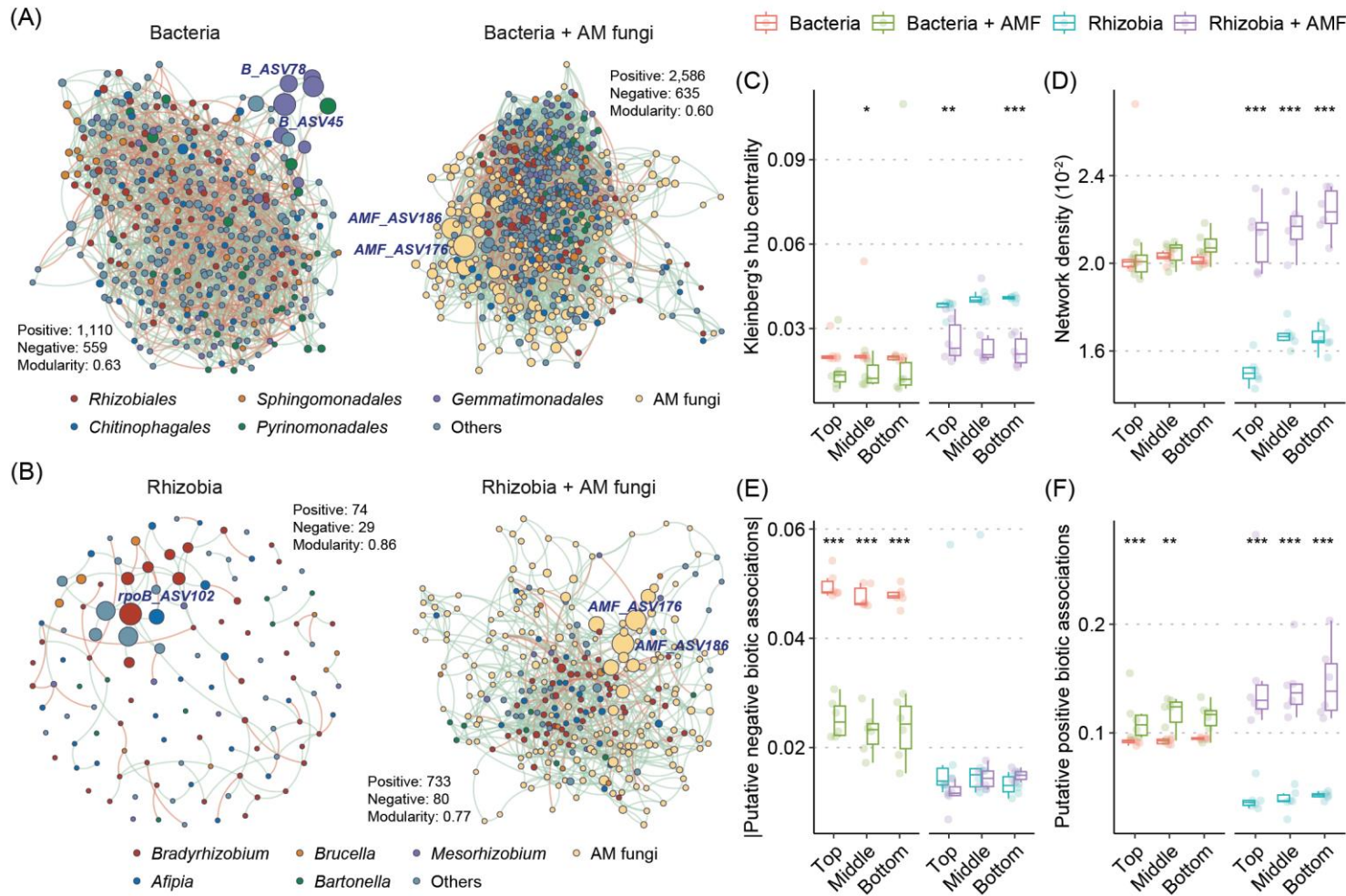


- AM fungal richness and the composition of bacterial and rhizobial communities collectively drove slope multifunctionality.
- Soil N:P that decreased from the top to the bottom position, emerged as a crucial abiotic factor influencing slope multifunctionality.



- The dominant bacterial order and rhizobial genus were *Rhizobiales* and *Bradyrhizobium*, respectively.

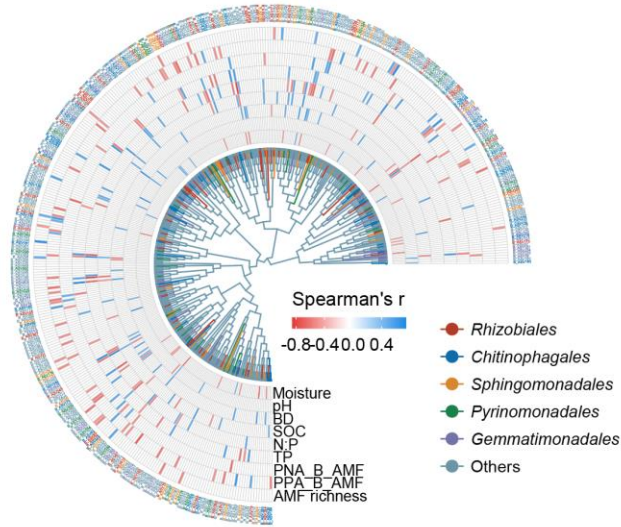
# Results



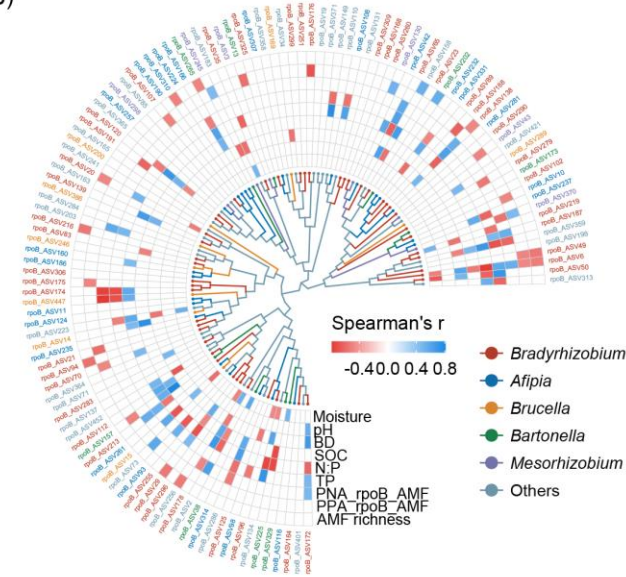
- The networks became **more interconnected** and exhibited reduced modularity when AM fungal interactions were incorporated.
- Two AM fungal ASVs were also recognized as keystone species in the cross-kingdom networks, assuming the topological roles of **network connectors**.
- The strength of positive associations was **much higher** than that of negative associations, aligning with the ratio of positive and negative edges.

# Results

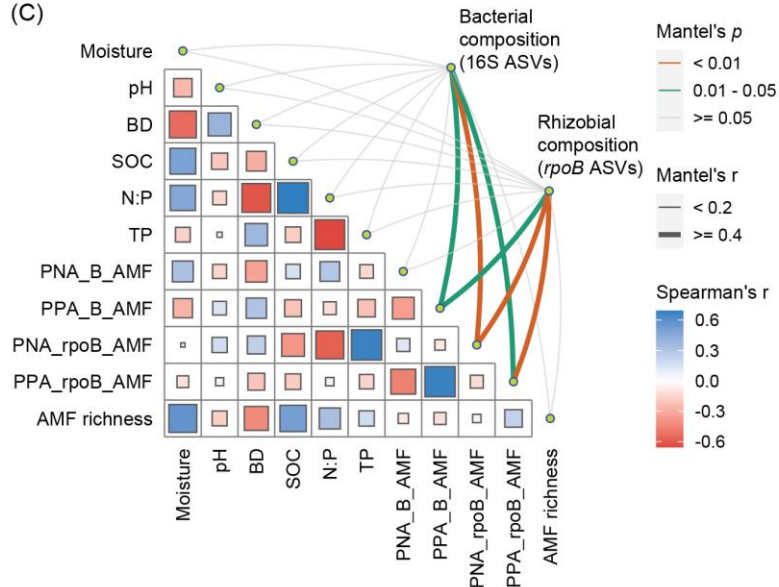
(A)



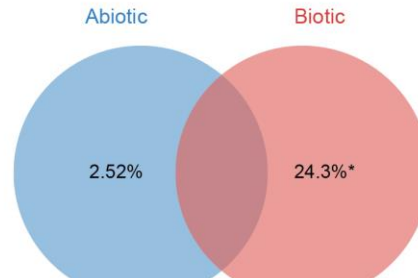
(B)



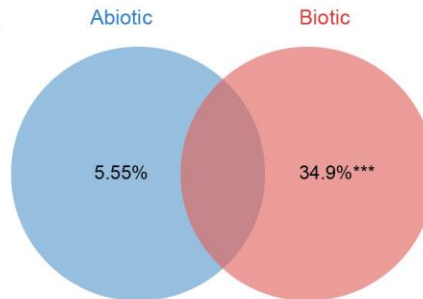
(C)



(D)



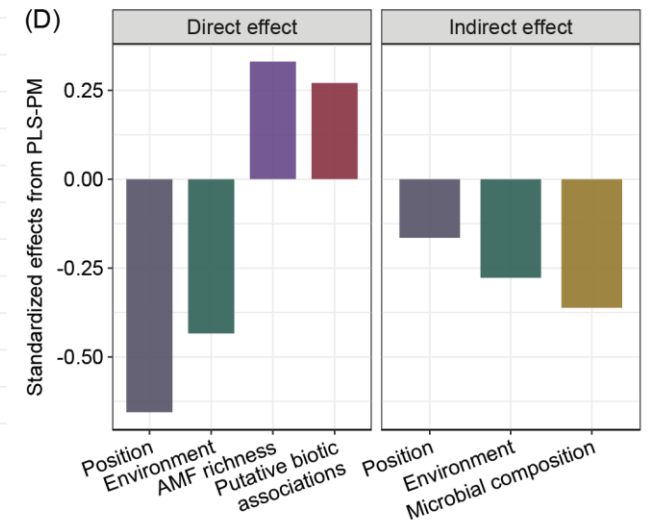
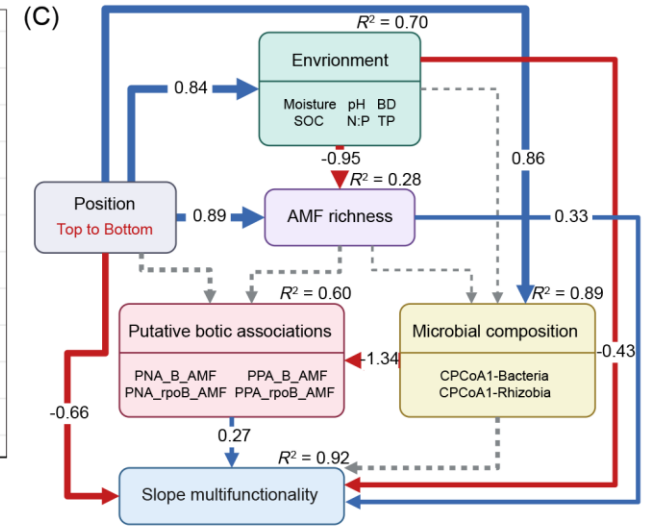
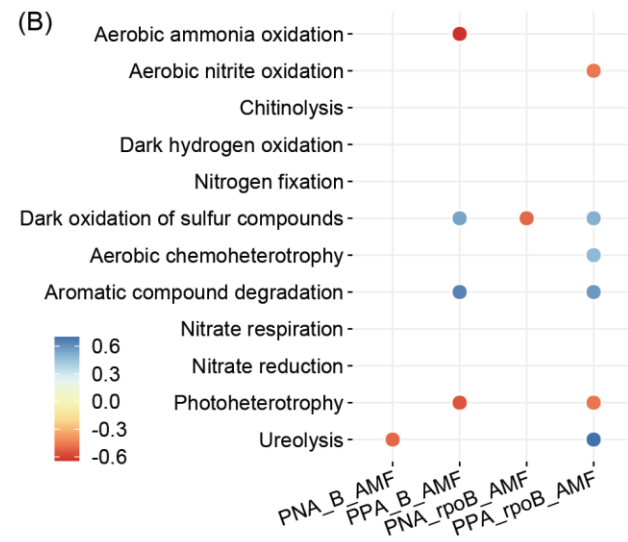
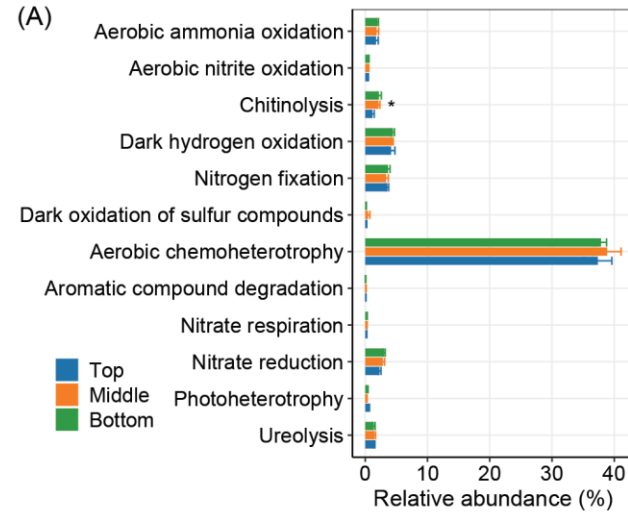
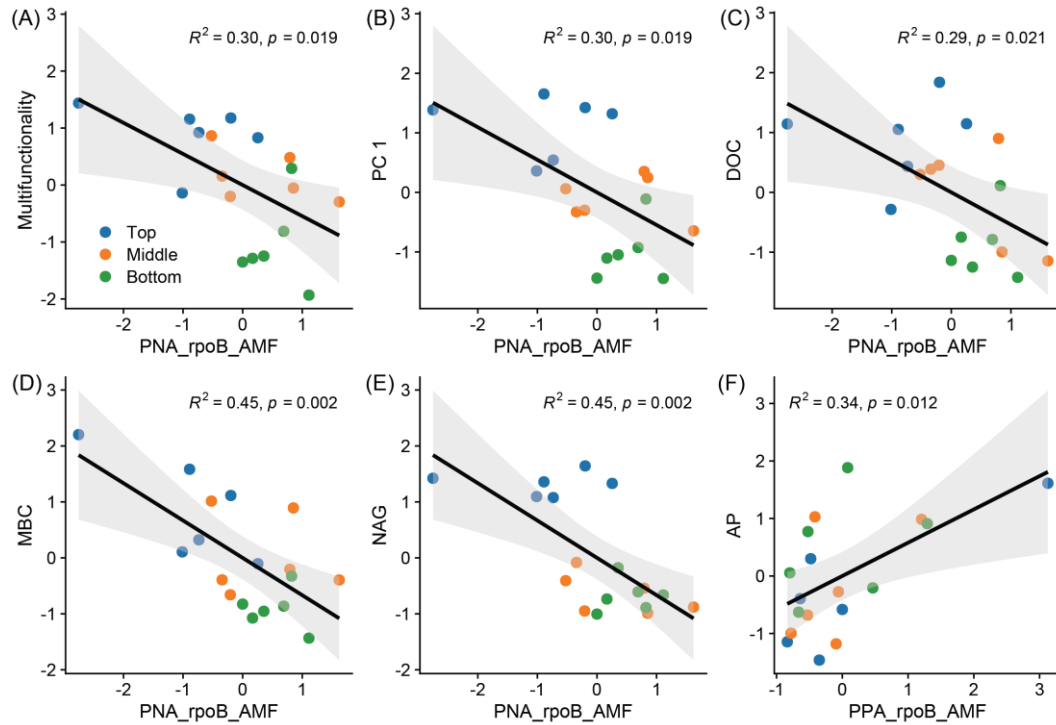
(E)



➤ Biotic factors contributed **much more** than abiotic factors to the explained variations in bacterial and rhizobial compositions, especially those **biotic associations between rhizobia and AM fungi**.

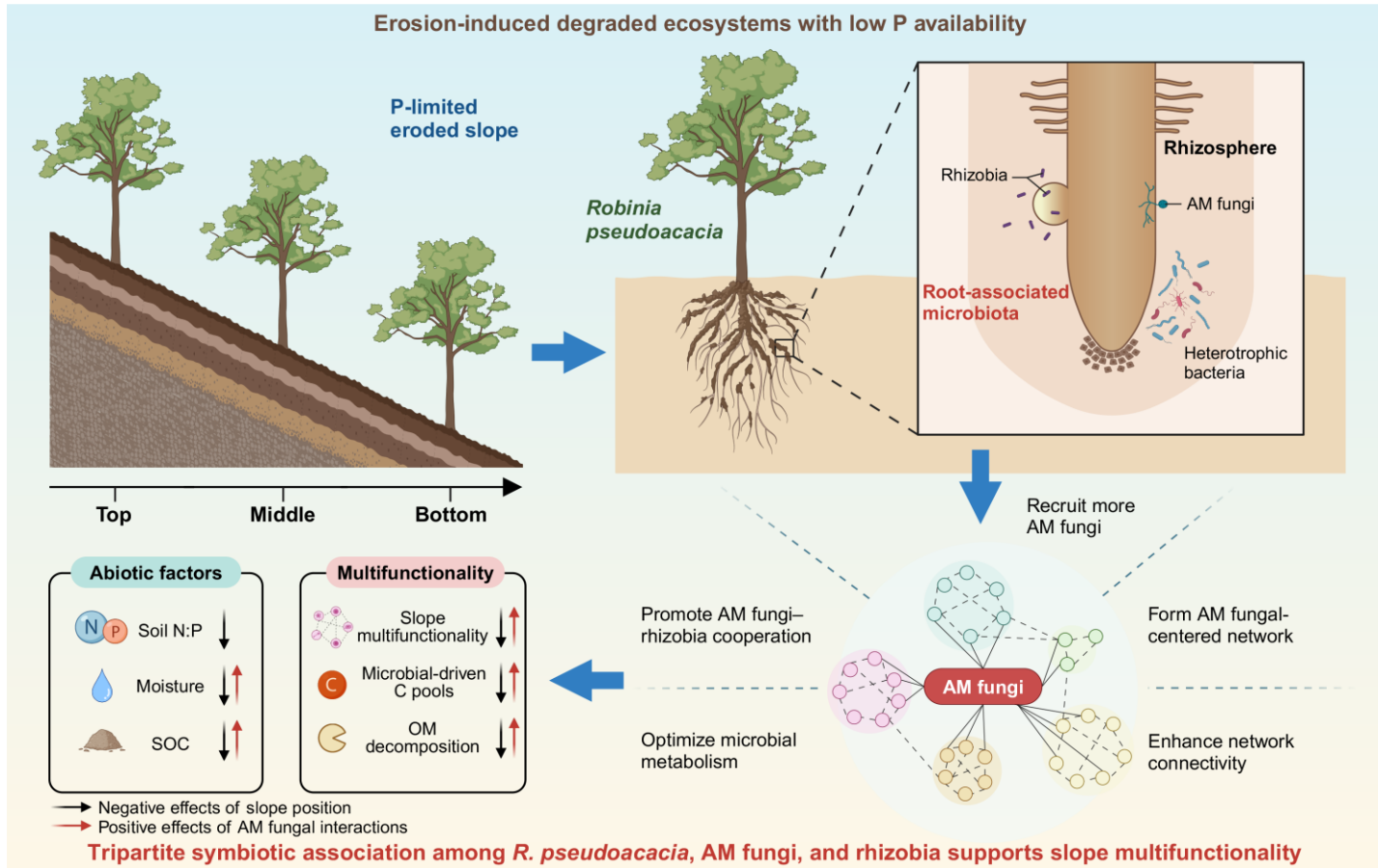
➤ AM fungi also **facilitated** the **assembly** and **phylogeny** of bacterial and rhizobial communities.

# Results



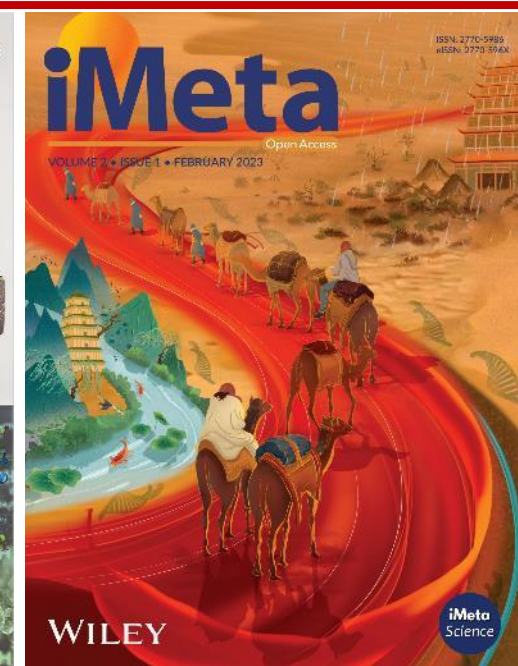
- The reduced negative rhizobia–AM fungi association (i.e., **less competition**) significantly supports slope multifunctionality, especially **microbially driven C pools** and **lignin decomposition**;
- Their **stronger positive association** releases greater **phosphorus mineralization potential**.

# Summary



- Explicit variations in slope multifunctionality were across different slope positions, associated with shifts in **limiting resources**, including soil P and moisture.
- In response to resource limitations, *R. pseudoacacia* strategically recruits AM fungi, forming an **AM fungal-centered network** in the rhizosphere.
- The *R. pseudoacacia*–AM fungi–rhizobia **tripartite association** robustly supports slope multifunctionality via enhanced decomposition of recalcitrant compounds, improved P mineralization potential, and optimized microbial metabolism.

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