

Supplementary Material

Supporting Information Includes:

Number of pages: 8

Number of Tables: 4

Number of Figures: 3

Title: Promoted phosphorus transformation by increasing soil microbial diversity and network complexity- A case of long-term mixed-species plantations of *Eucalyptus* with N-fixing tree species

Jiyin Li ^{a, 1}, Yeming You ^{a, b}, Wen Zhang ^c, Yi Wang ^d, Yuying Liang ^a, Haimei Huang ^a, Hailun Ma ^a, Qinxia He ^a, Angang Ming ^{b, e}, Xueman Huang ^{a, b, *}

^a Guangxi Key Laboratory of Forest Ecology and Conservation, Guangxi Colleges and Universities Key Laboratory for Cultivation and Utilization of Subtropical Forest Plantation, College of Forestry, Guangxi University, Nanning, Guangxi 530004, China

^b Guangxi Youyiguan Forest Ecosystem National Observation and Research Station, Youyiguan Forest Ecosystem Observation and Research Station of Guangxi, Pingxiang 532600, Guangxi, China

^c Jiangxi Academy of Agricultural Sciences, Nanchang, Jiangxi 330000, China

^d Institute of Resources and Environment, Key Laboratory of National Forestry and Grassland Administration/Beijing for Bamboo & Rattan Science and Technology, International Centre for Bamboo and Rattan, Beijing, 100102, China

^e Experimental Centre of Tropical Forestry, Chinese Academy of Forestry, Pingxiang 532600, China

* Corresponding author. Tel.: +86 13667881541; E-mail address: huangxm168168@163.com (X. Huang).

¹ These authors contributed equally to this work.

Table S1 Main characteristics in PPs and MPs.

Stand type	Altitude (m)	Gradient (°)	Age (a)	SD (trees hm ⁻²)	DBH (cm)	TH (m)
PPs	224	24	17	595±28	20.11±0.27	23.88±0.38
MPs	227	21	17	610±12	19.61±0.50	23.16±0.47
<i>Eucalyptus</i> <i>urophylla</i>	—	—	—	310±17	22.26±0.28	25.83±0.40
<i>Acacia</i> <i>mangium</i>	—	—	—	300±18	16.13±1.20	19.62±0.65

PPs: pure plantations; MPs: mixed plantations; SD: stand density; D.B.H.: diameter at breast height; TH: tree height.

Table S2. Details of the various soil extracellular enzymes and associated substrates.

Enzyme Type	Enzyme	International Classification Number	Abbreviation	Substrate
N-acquiring enzyme	β -1,4-N- acetylglucosaminidase	EC 3.2.1.30	NAG	4-MUB-N-acetyl- β -D- glucosaminide (200 μ M)
	Leucine aminopeptidase	EC 3.4.11.1	LAP	L-Leucine-7-amino-4- methylcoumarin (200 μ M)
P-acquiring enzyme	Acid phosphatase	EC 3.1.3.2	ACP	4-MUB-phosphate (200 μ M)

EC: Enzyme Commission number describing enzymatic function in increasing level of detail (the first number distinguishes 1-oxidoreductases, 2-transferases, 3- hydrolases, 4-lyases, 5-isomerases, and 6-ligases)

Table S3 Quantitative real-time PCR primers for nitrogen and phosphorus cycling function genes.

Functional gene type	Target gene	Primer	Sequence (5' - 3')
Nitrogen cycle	<i>nifH</i>	Pol-F	TGCGAYCCSAARGCBGACTC
		Pol-R	ATSGCCATCATYTCRCCGGA
	<i>AOB-amoA</i>	amoA-1F	GGGGTTTCTACTGGTGGT
		amoA-2R	CCCCTCKGSAAAGCCTTCTTC
	<i>narG</i>	narG-f	TAYGTSGGGCAGGARAAACTG
		narG-r	CGTAGAAGAAGCTGGTGCTGT
	<i>nirK</i>	nirk876	ATYGGCGGVCAYGCGCA
		nirk1040	GCCTCGATCAGRTRTGGTT
	<i>nirS</i>	Nirs-Cd3aF	GTSAACGTSAAGGARACSGG
		Nirs-R3cdR	GASTTCGGRTGSGTCTTGA
	<i>nosZ</i>	nosZ2F	CGCRACGGCAASAAGGTSMSST
		nosZ2R	CAKRTGCAKSGCRTGGCAGAA
	<i>phoC</i>	phoc-A-F1	CGGCTCCTATCCGTCCGG
		phoc-A-R1	CAACATCGCTTTGCCAGTG
Phosphorus cycle	<i>phoD</i>	ALPS-F730	CAGTGGGACGACCACGAGGT
		ALPS-R1101	GAGGCCGATCGGCATGTCTG
	<i>BPP</i>	bpp-F	GACGCAGCCGAYGAYCCNGCNITNTGG
		bpp-R	CAGGSCGCANRTCIACRTTRTT
	<i>pqqC</i>	Fw	AACCGCTTCTACTACCAG
		Rv	GCGAACAGCTCGGTCAG

Table S4 Statistical table of bacterial and fungi species in both 0–10 cm and 10–20 cm soil layers in PPs and MPs.

Microbial type	Soil layer (cm)	Stand type	Phylum	Class	Order	Family	Genus	OTU
Bacteria	0–10	PPs	20	50	112	155	229	1435
		MPs	21	62	131	187	283	1760
	10–20	PPs	20	47	108	155	224	1315
		MPs	20	58	126	179	268	1695
	Total	—	21	64	140	201	311	1869
Fungi	0–10	PPs	8	18	41	57	73	693
		MPs	8	21	45	73	93	723
	10–20	PPs	8	18	41	52	56	651
		MPs	8	19	43	64	87	654
	Total	—	8	24	62	104	157	1128

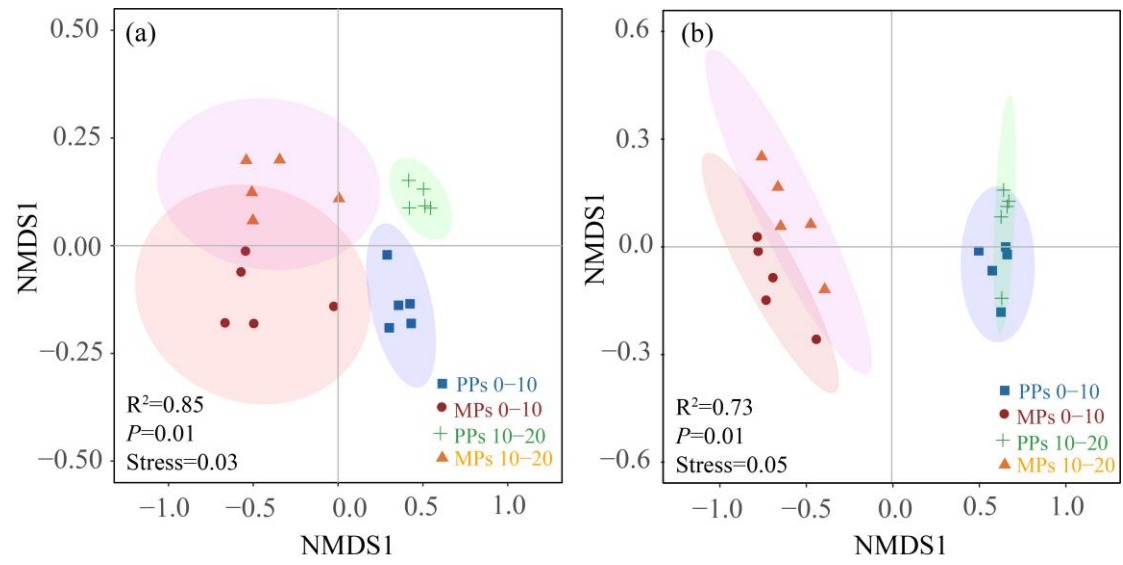


Fig. S1 Nonmetric multidimensional scaling analysis of (a) bacterial and (b) fungal, based on Bray-Curtis similarity in both 0–10 cm and 10–20 cm soil layers in PPs and MPs.

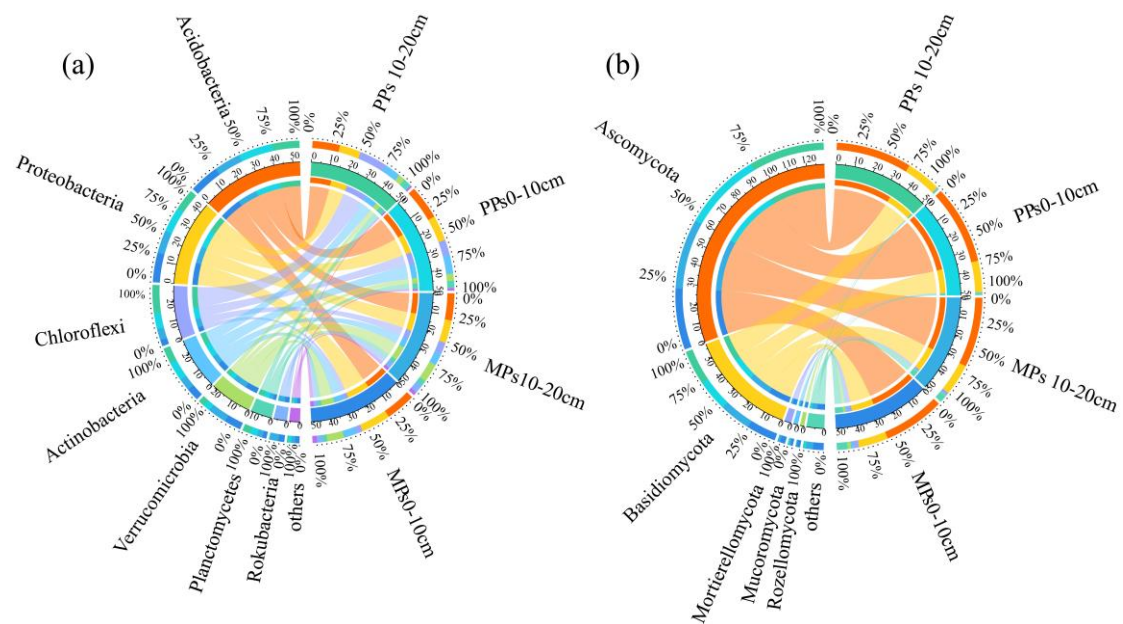


Fig. S2 Chord diagrams showing the bacterial (a) and fungal (b) community composition (at the relative abundance >1% phylum level). The outer circle scale represents the percentage information of relative abundance of OTU in the sample; The inner circle scale represents the absolute abundance information of OTU in the sample (unit: 1000).

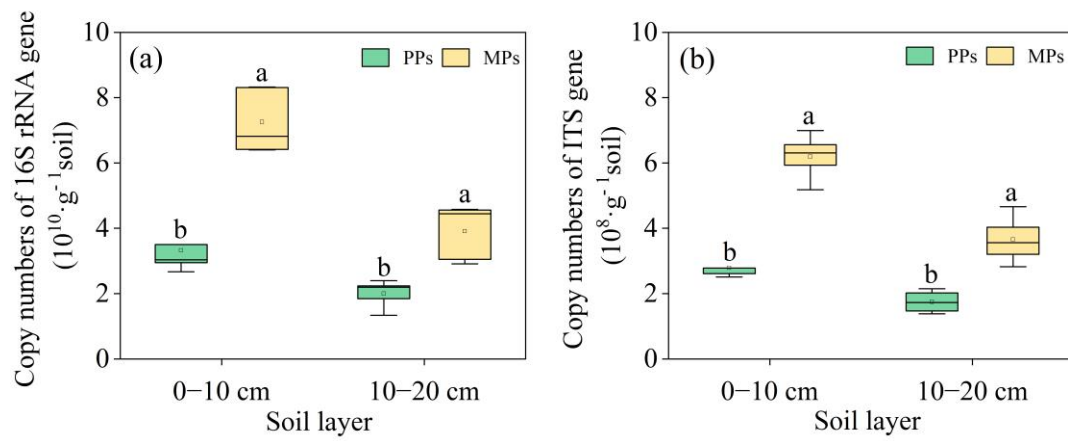


Fig. S3 Comparisons copy number of (a) 16SrRNA and (b) ITS in both 0–10 cm and 10–20 cm soil layers in PPs and MPs.