Supplementary Information for

The Effect of the Conversion from Natural Broadleaved Forests into Chinese Fir (*Cunninghamia Lanceolata*) Plantations on Soil Microbial Communities and Nitrogen Functional Genes

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2. Materials and Methods

2.1 Site description and soil collection

The natural broadleaved forest was dominated by tree species *Schima superba*, *Lithocarpus glaber*, *Hevea brasiliensis*, *Tsuga chinensis*, *Zelkova serrata*, and *Castanea henryi*; shrub species *Adinandra millettii*, *Rhododendron simsii*, *Vaccinium vitis-idaea*, *Symplocos sumuntia*, *Diplospora dubia*, *Piper austrosinense*, *Eurya japonica*, *Ficus hirta*, and *Itea chinensis*; and herbaceous species *Adiantum capillus-veneris*, *Alpinia pumila*, *Odontosoria chinensis*, *Alsophila spinulosa*, *Cibotium barometz*, *Dryopteris crassirhizoma*, *Ophiopogon bodinieri*, *Adiantum flabellulatum*, *Indocalamus tessellatus*, *Ophiopogon japonicus*, and *Cymbidium goeringii*.

The mixed-species plantation and Chinese fir plantation were developed from natural broadleaved forests 30 years ago. The mixed-species plantation was dominated by the tree species *Cunninghamia lanceolate* and *Schima superba*; shrub species *Litsea cubeba*, *Adinandra millettii*, *Maesa japonica*, *Dalbergia dyeriana*, *Eurya japonica*, *Ficus hispida*, *Ficus hirta*, *Piper austrosinense*, *Smilax glabra*, and *Rhododendron simsii*; and herbaceous species *Adiantum capillus-veneris*, *Odontosoria chinensis*, *Miscanthus floridulus*, *Alpinia pumila*, *Cymbidium goeringii*, *Lophatherum gracile*, *Alsophila spinulosa*, *Cibotium barometz*, *Dryopteris crassirhizoma*, *Ophiopogon bodinieri*, *Pteris semipinnata*, *Adiantum flabellulatum*, and *Begonia grandis*.

The Chinese fir plantation was dominated by tress species *Cunninghamia lanceolate*; shrub species *Litsea cubeba*, *Adinandra millettii*, *Dalbergia dyeriana*, *Rhododendron simsii*, and *Eurya japonica*; and herbaceous species *Adiantum capillus-veneris*, *Miscanthus floridulus*, *Lophatherum gracile*, *Ophiopogon bodinieri*, *Pteris semipinnata*, *Adiantum flabellulatum*, *Alsophila spinulosa*, and *Dicranopteris dichotoma*.

Table S1. Basic status of natural broadleaved forest (N), mixed-species forest (M), and Chinese fir (*Cunninghamia lanceolata*) plantation (P) (mean ± Standard deviation).

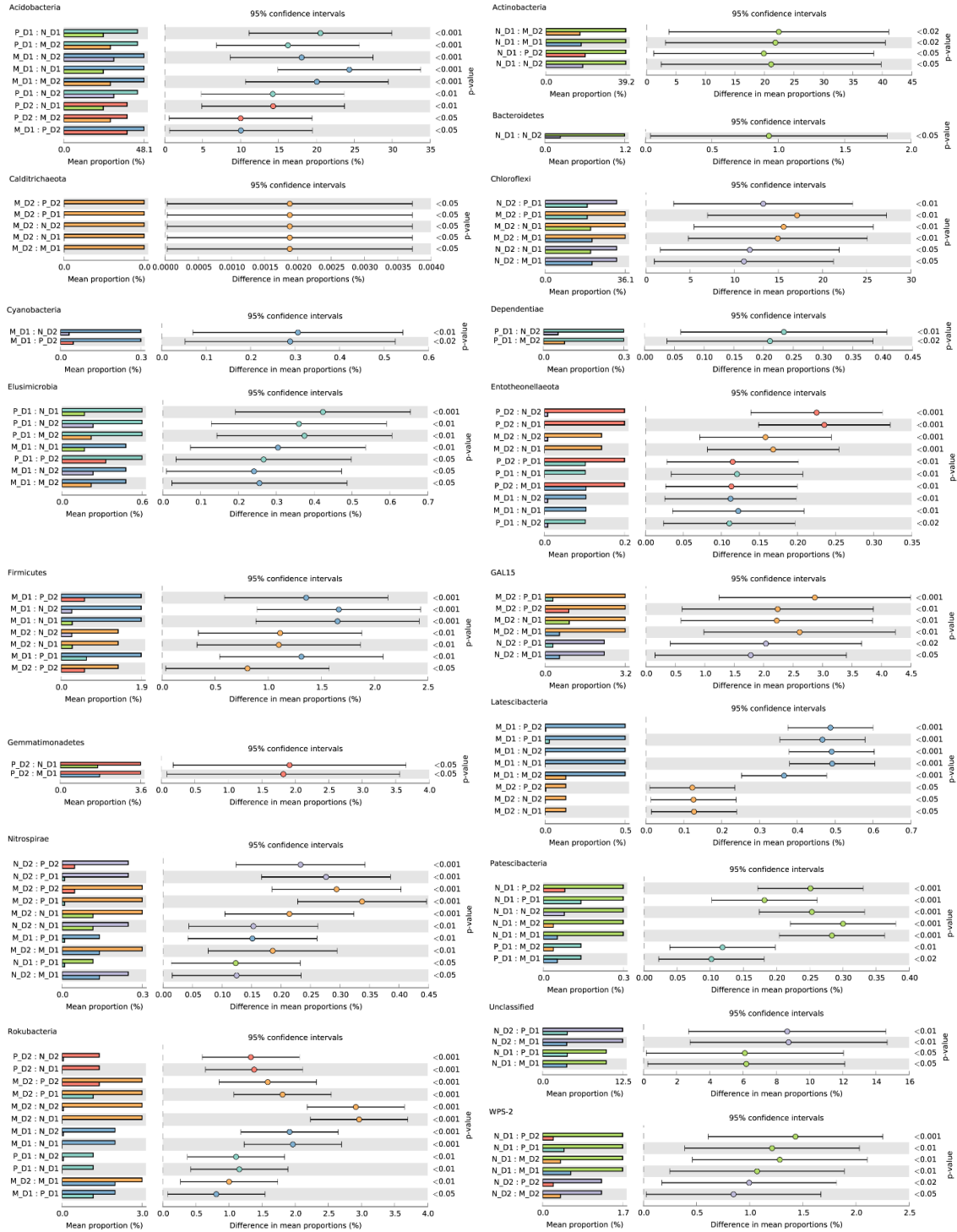| Stand	Age (a)	Slope aspect	Slope (°)	Mean tree height (m)	Mean DBH (cm)	Stand density (plant ha^{-1})	Land use history
N	≥ 30	Southwest	28	18.38 ± 1.66	18.60 ± 1.05	2210	N
M	30	Southwest	29	21.02 ± 1.28	16.23 ± 0.87	2505	N
P	30	Southwest	29	22.56 ± 0.93	14.98 ± 0.93	2500	N

DBH: diameter at breast height.

Table S2. Primers and PCR amplification conditions used in qPCR analysis of nitrogen cycle functional genes.

Target genes	Primers	Sequences (5′–3′)	Fragment length (pb)	Amplification condition	References
Bacterial *amoA*	amoAF	STAATGGTCTGGCTTAGACG	270	95°C–3min, 37 cycles, 95°C–30s, 53°C–30s, 72°C–45s, 72°C–10min, 10°C hold	[1]
	amoAR	GCGGCCATCCATCTGTATGT			
Archaeal *amoA*	amoA-1F	GGGGTTTCTACTGGTGGT	371	95°C–3min, 37 cycles, 95°C–30s, 55°C–30s, 72°C–45s, 72°C–10min, 10°C hold	[2]
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC			
nirS	cd3AF	GTSAACGTSAAGGARACSGG	353	95°C–3min, 37 cycles, 95°C–30s, 55°C–30s, 72°C–45s, 72°C–10min, 10°C hold	[3]
	R3cdR	GASTTCGGRTGSGTCTTGA			
nirK	F1aCu	ATCATGGTSCTGCCGCG	385	95°C–3min, 37 cycles, 95°C–20s, 63°C–30s, 72°C–30s, 72°C–10min, 10°C hold	[4]
	R3Cu	GCCTCGATCAGRTTGTGGTT			
norZ	nosZ-F	CGYTGTTCMTCGACAGCCAG	360	95°C–3min, 37 cycles, 95°C–5s, 58°C–30s, 72°C–30s, 72°C–10min, 10°C hold	[5]
	nosZ-1622R	CGSACCTTSTTGCCSTYGCG			

				95°C–3min, 37 cycles,	
	nifHF	AAAGGYGGWATCGGYAARTCCAC-		95°C–30s, 55°C–30s,	
nifH		CAC	390	72°C–45s, 72°C–	[6]
	nifHR	TTGTTSGCSGCRTACATSGCCATCAT		10min, 10°C hold	

(a) ------- *Bacteria* -------
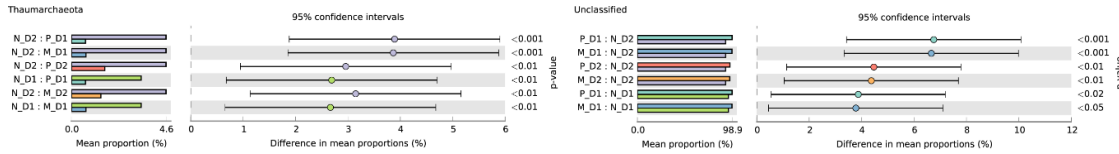
(b) ------- *Archaea* -------

Figure S1. Comparison of the relative abundance of bacteria (a) and archaea (b) at the phyla level in different soil depths (D1: 0–20 cm, D2: 20–50 cm) from natural broadleaved forests (N), mixed-species plantations (M), and Chinese fir (*Cunninghamia lanceolata*) plantations (P). The plot presents only the phyla with a statistical difference by the Tukey-Kramer test (*P* < 0.05, after Bonferroni correction).
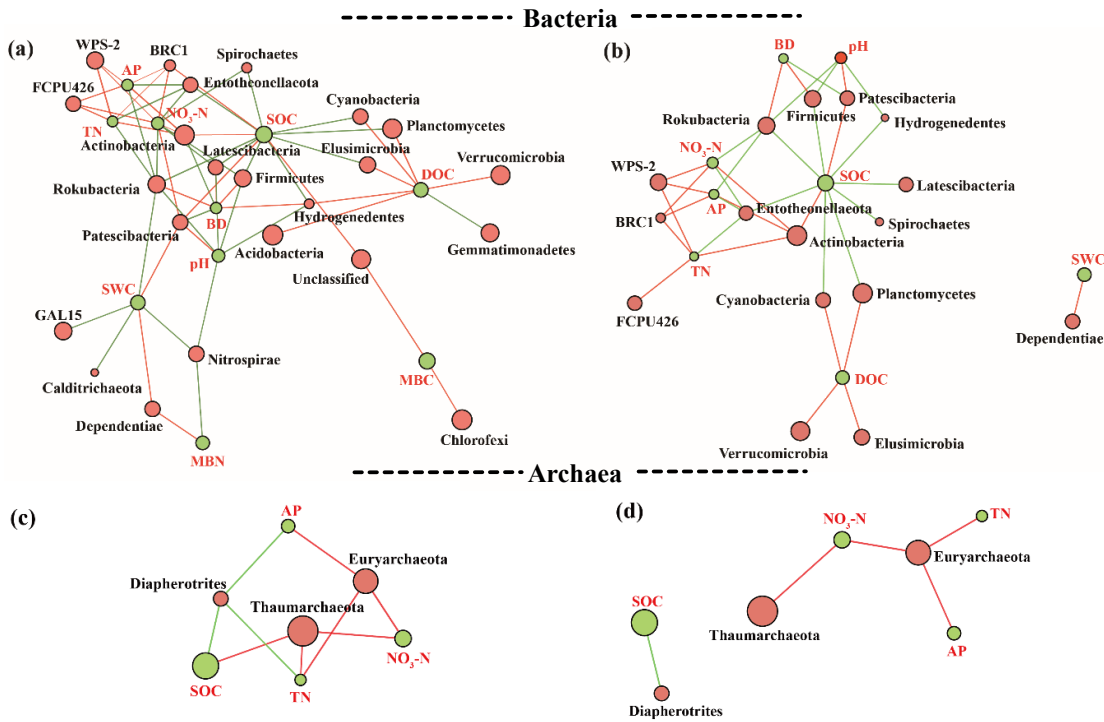

Figure S2. Pearson's rank correlation coefficients and statistical significance between phyla of bacteria and archaea to soil physiochemical properties. Left figures (a and c) show the difference at 0.05 level, and right figures (b and d) show the difference at 0.01 level. The size of red nodes in the graph represents abundance of species. The red line represents a positive correlation, and the green line represents a negative correlation. The thickness of the line indicates the size of the correlation coefficient. The thicker the line, the higher the correlation, vice versa. BD: bulk density; SWC: soil water content; NO_3^-–N: nitrate nitrogen; NH_4^+–N: ammonium nitrogen; TN: total nitrogen; SOC: soil organic carbon; DOC: dissolved organic carbon; MBC: microbial

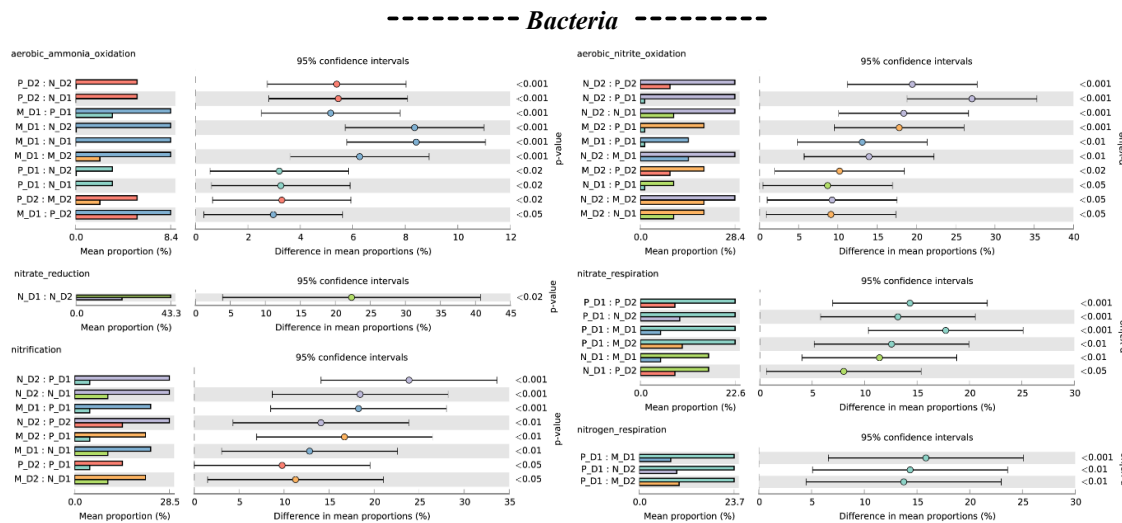biomass carbon; MBN: microbial biomass nitrogen; AP: available phosphorus.

Figure S3. Comparison of the relative abundance of nitrogen functional bacterial communities in different soil depth (D1: 0–20 cm, D2: 20–50 cm) from natural broadleaved forests (N), mixed-species plantations (M), and Chinese fir (*Cunninghamia lanceolata*) plantations (P). The plot presents only the predicted functions with a statistical difference by the Tukey-Kramer test ($P < 0.05$, after Bonferroni correction).

References

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