

Supporting Information for

N₂O emission from a subtropical forest is dominantly regulated by soil denitrifiers under exogenous N enrichment and precipitation season distribution change

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Introduction

This supporting information includes Figures S1-S3 and Tables S1-S2. Figure S1 provides detailed information of the simulated N deposition and precipitation change site in the Heshan Heshan National Field Research Station of Forest Ecosystem, Chinese Academy of Sciences, including site location, plot distribution and experimental treatment design. Figure S2 provides the conceptual model used for the Structural Equation Modeling analysis in the present study. Figure S3 provides the terminal dynamics of soil nitrifying and denitrifying functional genes during two-year treatments. Table S1 provides the detailed primer information of the five studied functional genes. Table S2 provides the reaction program of qPCR for each gene.

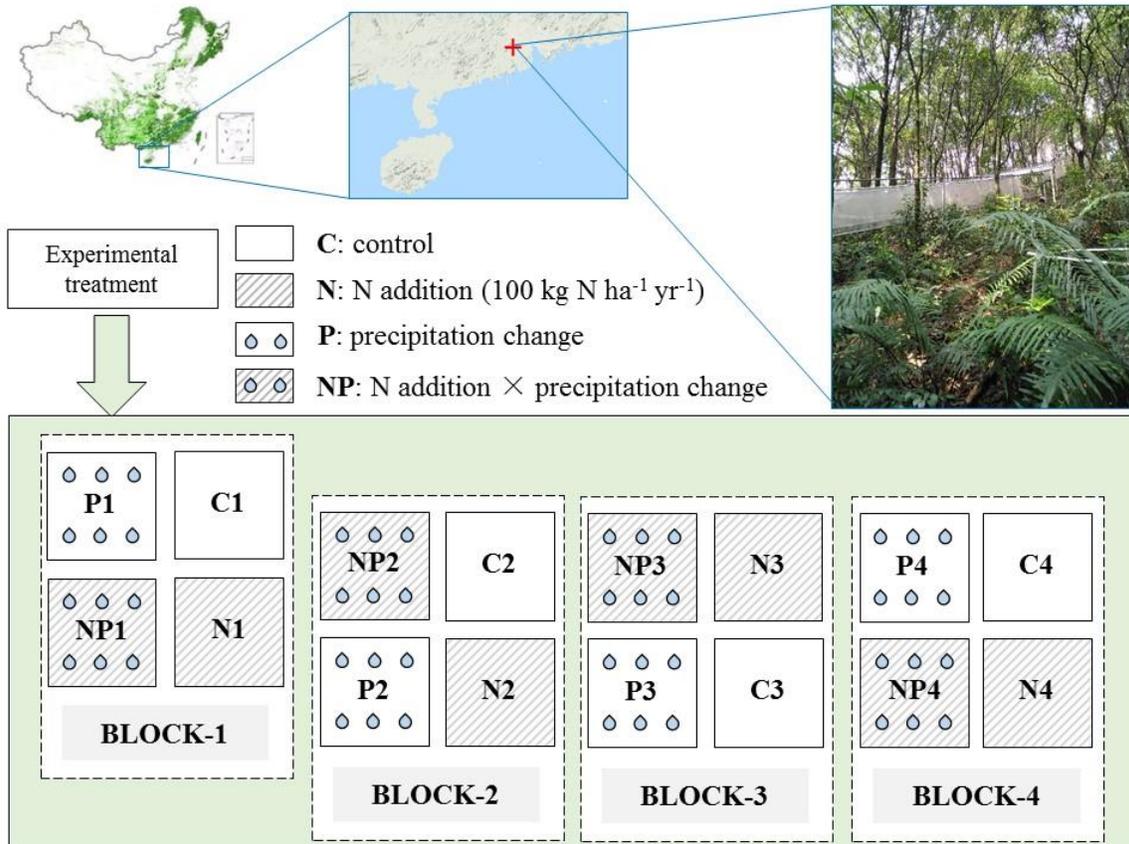


Figure S1. The location information and the experiment design of the study site.

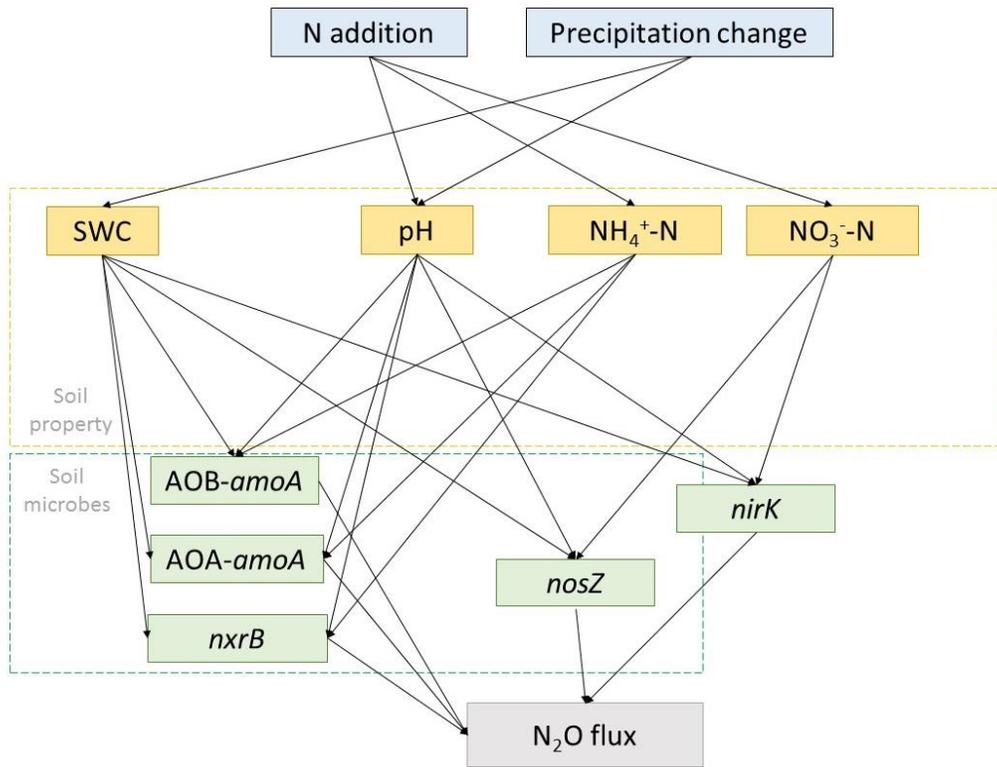


Figure S2. Structural equation model simulating the relationship among soil property, functional gene abundance and N₂O efflux. Arrows in different colors indicate the different relationships in the model: blue arrows indicate the relationships between soil water content (SWC) and functional genes, red arrows indicate the relationships between pH and functional genes, while black, middle-yellow green and dark-yellow colored arrows indicate the relationships between soil N indicators (ammonium N, nitrate N, microbial biomass N and total N), between ammonium/nitrate N and functional genes, between functional genes and N₂O effluxes, and between soil N status and N₂O effluxes, respectively.

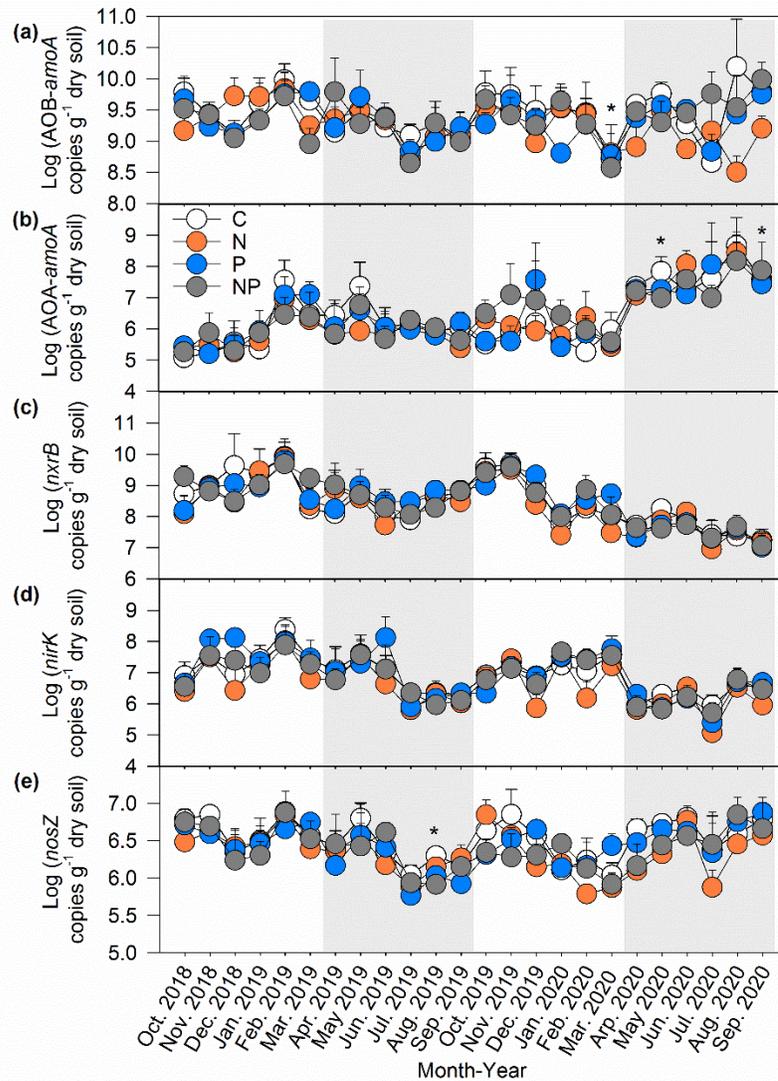


Figure S3. Monthly dynamics of the abundance of soil microbial nitrifying and denitrifying functional genes. * indicate the difference is significant ($p < 0.05$) among the four treatments according to One-way ANOVA. Gray shades and white regions indicate wet seasons and dry seasons, respectively.

Table S1. Primer information of each functional gene in this study.

Target gene	Primer set	Amplicon Length (bp)	Reference
AOB- <i>amoA</i>	Bac- <i>amoA</i> -1F (GGGGTTTCTACTGGTGGT)	491	(Rasche et al., 2011)
	Bac- <i>amoA</i> -2R (CCCCTCKGSAAAGCCTTCTTC)		
AOA- <i>amoA</i>	CrenamoA23F (ATGGTCTGGCTWAGACG)	624	(Könneke et al., 2005)
	CrenamoA616R (GCCATCCATCTGTATGTCCA)		
<i>nxB</i>	<i>nxB</i> 169F (TACATGTGGTGGAAACA)	485	(Pester et al., 2014)
	<i>nxB</i> 638R (CGGTTCTGGTCRATCA)		
<i>nirK</i>	<i>nirK</i> F560-589 (GGGCATGAACGGCGCGCTCATGGTGCTGCC)	376	(Levy-Booth and Winder, 2010)
	<i>nirK</i> R906-935 (CGGGTTGGCGAACTTGCCGGTGGTCCAGAC)		
<i>nosZ</i>	<i>nosZ</i> -F (CGCTGTTTCNTCGACAGYCAG)	380	(Rich et al., 2003)
	<i>nosZ</i> -R (ATGTGCAKNGCRTGGCAGAA)		

Table S2. PCR reaction programs of the functional genes.

Target gene	Denaturation temperature and time	Annealing temperature and time	Elongation temperature and time	Number of cycles
AOB- <i>amoA</i>	95 °C, 10s	55 °C, 15s	72 °C, 30s	40
AOA- <i>amoA</i>	95 °C, 10s	55 °C, 15s	72 °C, 30s	40
<i>nxB</i>	95 °C, 10s	52 °C, 15s	72 °C, 30s	40
<i>nirK</i>	95 °C, 10s	60 °C, 15s	72 °C, 15s	40
<i>nosZ</i>	95 °C, 10s	56 °C, 30s	72 °C, 45s	40