

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

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20 **Key Points:**

- 21 • Soil N₂O efflux of the forest was significantly influenced by only the NP treatment in the
22 dry season.
- 23 • Soil *nirK* and *nosZ* denitrifiers played more dominant roles than soil nitrifiers (AOB,
24 AOA, NOB) in regulating N₂O emissions.
- 25 • Precipitation change was a more dominant factor than nitrogen deposition elevation in
26 influencing soil denitrifiers and N₂O emission.

27 **Abstract**

28 Nitrogen-rich tropical/subtropical forest soil acts as a terrestrial source of nitrous oxide (N₂O)
29 emissions, a greenhouse gas commonly affected by soil nitrogen availability and soil moisture.
30 However, in tropical and subtropical regions experiencing both elevated nitrogen deposition and
31 altered precipitation regimes, it is unclear whether nitrogen deposition and precipitation regimes
32 have interactive effects on forest soil N₂O emissions and what roles N₂O-associated
33 nitrifiers/denitrifiers play in these interactions. We conducted a two-year field study in a
34 subtropical evergreen broadleaf forest in southern China by applying four treatments: nitrogen
35 addition (N), seasonal precipitation distribution change (P), both nitrogen addition and seasonal
36 precipitation distribution change (NP) and a control (C). The Results showed that N₂O efflux
37 from the forest soil was significantly greater in the wet season than in the dry season, but was
38 promoted by the NP treatment only in the dry season. Soil moisture and pH decreased in the P
39 and N treatments, respectively. The abundances of the nitrifying gene *AOA-amoA* and
40 denitrifying gene *nosZ* in the wet season and the abundance of the denitrifying gene *nirK* in the
41 dry season differed significantly among the four treatments. A structural equation model showed
42 that precipitation change was more important than nitrogen addition in affecting soil properties
43 (e.g. moisture and pH) and N₂O-associated nitrifiers/denitrifiers, while soil *nirK*- and *nosZ*-
44 denitrifiers were the dominant functional microbes in regulating N₂O emissions. The results
45 support predictions of future nitrogen losses (N₂O) in subtropical forests in the context of
46 interactions between elevated nitrogen deposition and altered precipitation regimes.

47

48 **Plain Language Summary**

49 In this study, we examined the interactive effects of nitrogen (N) deposition increases and
50 precipitation regime changes on soil nitrous oxide (N₂O) emissions from a subtropical forest and
51 the underlying changes in soil functional microbial groups. Soil pH rather than soil available N
52 was significantly affected by simulated N deposition, while soil moisture was significantly
53 affected by simulated precipitation changes. Soil N₂O emissions were greater in the wet season
54 than in the dry season but were enhanced by the interaction of simulated N deposition increases
55 and precipitation changes only in the dry season. Moreover, the abundance of soil nitrifying and
56 denitrifying functional genes responded differently to the interaction of N deposition increases
57 and precipitation changes. Structural equation modelling results indicated that precipitation
58 change was more important than increased N deposition in affecting N₂O-associated nitrifiers
59 and denitrifiers, while soil *nirK*- and *nosZ*-type denitrifiers were the dominant functional
60 microbial groups in regulating N₂O emissions.

61

62 **1 Introduction**

63 As the largest nitrogen (N) pool in terrestrial ecosystems, soil acts not only as a carrier
64 for various forms of N (e.g. ammonium N, nitrate N, and organic N) for the growth of plants but
65 also as a source or a sink for main greenhouse gases such as nitrous oxide (N₂O) (Oertel et al.,
66 2016). However, soil N dynamics have been disrupted by a series of global/regional
67 environmental changes in recent decades, such as increases in N deposition and changes in
68 precipitation regimes (Dore, 2005; Kanakidou et al., 2016). N deposition and precipitation
69 changes have been demonstrated to induce changes in soil N availability and water availability,

70 respectively (Chen et al., 2017; Cheng et al., 2019), inducing changes in N₂O emissions by
71 influencing the community, abundance and activity of N₂O-associated nitrifying and denitrifying
72 functional microbes in soil (Avrahami et al., 2002; Levy-Booth et al., 2014). Moreover,
73 atmospheric N deposition is generally closely linked to precipitation because of coupling
74 between the nitrogen and water cycles, which is particularly pronounced in subtropical/tropical
75 regions where a significant wet-dry seasonality exists and wet N deposition depends strongly on
76 precipitation. For instance, southern China experiences not only a high amount of atmospheric N
77 deposition but also changes in precipitation patterns (Zhou et al., 2011; Yu et al., 2019). The
78 total natural N deposition rate of this region is over 35 kg N ha⁻¹ yr⁻¹ (Zhu et al., 2015), and a
79 transition has occurred from the traditional reduced N (NH_x) deposition dominance to nearly
80 equal NH_x and oxidized N (NO_y) in wet deposition, particularly in recent decades (Yu et al.,
81 2019). The annual precipitation in this region has changed little over the past 60 yr (1950-2009),
82 but incidences of heavy rain events have increased in the wet season and chances of drought
83 have increased in the dry season (Zhou et al., 2011). Due to these new trends and coupled
84 changes in N and water dynamics, it is necessary to predict the interactive effects of atmospheric
85 N deposition increases and changes in seasonal precipitation distribution on soil N₂O emissions
86 in this region. Notably, subtropical/tropical forests are commonly considered the largest natural
87 terrestrial sources of N₂O globally (Werner et al., 2007; Cheng et al., 2014). Therefore, the
88 seasonal wet-dry variations in forest N₂O emissions due to the typical subtropical monsoon
89 climate (Tang et al., 2006) may become more pronounced under the changing seasonal
90 distribution of precipitation, and more complex interactions might occur between N deposition
91 and precipitation changes. However, to date, few studies have been focused on solving this
92 problem, and the underlying mechanisms are still poorly understood.

93 N₂O is produced mainly via microbial nitrification and denitrification processes and is
94 reduced via microbial denitrification (Conrad, 1996; Levy-Booth et al., 2014). The N₂O-
95 associating steps of these two biological processes are catalysed by enzymes of different
96 microbial groups, and each enzyme is encoded by specific functional genes (Levy-Booth et al.,
97 2014). According to previous studies, soil nitrifiers and denitrifiers show different sensitivities to
98 changes in soil N availability (Avrahami et al., 2002), water availability (Szukics et al., 2010) or
99 other soil abiotic factors such as pH (Nicol et al., 2008; Giles et al., 2012), that regulate the final
100 emission rates of N₂O (Zhang et al., 2021; Chen et al., 2022). For instance, ammonia-oxidizing
101 archaea (AOA) are generally more abundant than ammonia-oxidizing bacteria (AOB) in many
102 acidified forest soils but are less responsive to N deposition than are AOB (Isobe et al., 2012;
103 Carey et al., 2016; Han et al., 2018). Moreover, the ammonia monooxygenase-encoding gene
104 *amoA* of AOA and the nitrous oxide reductase-encoding gene *nosZ* were reported to play roles in
105 regulating nitrification rates and N₂O emissions in response to seasonal precipitation changes
106 (Chen et al., 2017). Soil *nirK* (nitrite reductase encoding gene)-denitrifiers were demonstrated to
107 be sensitive to moisture changes, while AOA responded negatively, but AOB were more stable
108 than AOA in responding to soil moisture increases (Szukics et al., 2010; Wang et al., 2017).
109 AOA-*amoA* abundance was suggested to be more sensitive than AOB-*amoA* abundance to pH
110 changes in acidic soils (Cuhel et al., 2010), while *nosZ*-encoding N₂O reductase was shown to be
111 influenced by pH (Giles et al., 2012), further indicating that soil nitrifiers and denitrifiers play
112 different roles in N₂O emissions from acidified soils caused by N deposition (Liu et al., 2011).
113 As a consequence, (i) forest soil N₂O emissions are often enhanced by N deposition or N
114 addition because of the increase in N substrates (NH₄⁺-N, NO₃⁻-N) for nitrification or
115 denitrification (Huang et al., 2013; Bai et al., 2014); (ii) N₂O emissions are inhibited by

116 increased soil moisture because of the increase in soil anoxic conditions and the reduction of
117 N₂O to N₂ (Szukics et al., 2010; Cheng et al., 2014); and (iii) N₂O emissions, as a response to
118 soil N or water condition changes, are a result of changes in soil nitrifiers/denitrifiers and
119 corresponding nitrification/denitrification processes (Szukics et al., 2010). Notably, although
120 nitrite-oxidizing bacteria (NOB) only recently have been identified, the participation of NOB in
121 nitrification determines whether the fixed N (nitrite) remains in ecosystems or is lost to the
122 atmosphere (Daims et al., 2016; Daims and Wagner, 2018). Thus, quantifying the abundance of
123 the nitrite oxidoreductase-encoding gene *nxB* (Pester et al., 2014) in soil will facilitate an
124 understanding of the role of NOB in nitrification and nitrification-based N₂O production. Overall,
125 soil nitrogen and moisture changes, arising from N deposition increases and seasonal
126 precipitation distribution changes, respectively, may induce more complex interactive effects on
127 soil nitrifiers/denitrifiers and thereby forest soil N₂O emissions (Wang et al., 2017), but these
128 effects are still poorly understood, particularly in forest ecosystems.

129 The key objectives of this study were to reveal the responses of N₂O emissions to the
130 interaction between N deposition increases and precipitation changes and the underlying
131 microbial regulatory mechanisms in a subtropical broadleaf forest in southern China. The effects
132 of increases in N deposition were determined monthly by N addition. The effects of seasonal
133 precipitation distribution changes were simulated by artificially excluding precipitation in the dry
134 season and increasing precipitation in the wet season. We hypothesized that (1) N₂O emission is
135 stimulated by N addition, (2) N₂O emission is stimulated by precipitation reduction in the dry
136 season but is inhibited by precipitation increase in the wet season, (3) N₂O emission is
137 synergistically stimulated by the N addition-precipitation change interaction in the dry season but
138 is slightly inhibited by that in the wet season, and 4) the roles of soil nitrifiers/denitrifiers in N₂O
139 emissions are different among experimental treatments and between wet and dry seasons. The
140 results provide comprehensive insights for understanding forest soil N losses under future
141 environmental changes.

142 **2 Materials and Methods**

143 **2.1 Study site**

144 The field experimental site is located at the Heshan National Field Research Station of
145 Forest Ecosystem, Chinese Academy of Sciences (112°54' E , 22°41' N), Heshan city,
146 Guangdong Province of China (Fig. S1). The forest where the study site is located is a
147 subtropical broadleaf mixed forest. The dominant tree species of the forest are *Schima superba*
148 and *Michelia macclurei*. Dominant species of the shrub layer are *Psychotria asiatica*, *Melicope*
149 *pteleifolia* and *Ilex asprella*. The herb layer is dominated by *Lophatherum gracile*, *Blechnum*
150 *orientale* and *Adiantum flabellulatum*. The region where the forest belongs to has a typical
151 subtropical climate, with the whole year being divided into a wet season (from April to
152 September) and a dry season (from October to March). The average annual precipitation is 1700
153 mm. The average annual atmospheric temperature is 21.7 °C. The highest monthly average
154 temperature is 29.2 °C (July) while the lowest monthly average temperature is 12.6 °C (January)
155 (Wang et al., 2009). The forest soil belongs to typical laterite (or Oxisols in the USDA soil
156 taxonomy), developed from sandstone and has a high leaching potential (Chen et al., 2017). The
157 top layer (0-10 cm) of the forest soil has a pH value of 3.9, ammonium N of 1.4 mg kg⁻¹, nitrate
158 N of 2.3 mg kg⁻¹, total N of 0.2% and total phosphorous of 0.02%. The total inorganic N

159 deposition amount of this zone is $47.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, with the ratio of ammonium and nitrate
160 deposition rates being nearly 1.0 (Huang et al., 2015).

161 2.2 Experiment design

162 The field experiment simulating N deposition elevation and precipitation regime
163 alteration started in October 2018. Four experimental treatments were set at the site: N addition
164 (N), seasonal precipitation distribution change (P), interaction of N addition and precipitation
165 change (NP) and control (C). The N addition level is $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, which is doubled based
166 on the value of the natural inorganic N deposition (Huang et al., 2015), to simulate the N
167 deposition elevation. The precipitation change treatment was set as drier in the dry season and
168 wetter in the wet season, but the total annual precipitation did not change. The dry-season
169 precipitation reduction and the wet-season precipitation increase was done by excluding
170 throughfall and adding water in the two distinct seasons, respectively.

171 Four replicated plots, each $12 \text{ m} \times 12 \text{ m}$, are set up for each experimental treatment. A 3
172 m wide buffer strip is set to separate adjacent plots (Fig. S1). For each plot of the N and NP
173 treatments, starting in October 2018 and at the beginning of each month, 342.86 g ammonium
174 nitrate (NH_4NO_3) is dissolved in 20 L water and sprayed evenly under canopy (equals to an
175 increase of 1.7 mm precipitation in each year). As a control, each plot of the C and P treatments
176 receive the same volume of water to avoid the effect of extra water addition. For the P and NP
177 treatments, 11 or 12 pieces of transparent plastic film (length: 12 m, width: 0.5-1 m, total width:
178 8 m) are fixed on the top of each plot. The supporting system of the plastic film consists of 16
179 vertical galvanized steel tubes ($\varphi = 10 \text{ cm}$, 3 m length and $\sim 0.6 \text{ m}$ depth in soil) and 8 horizontal
180 stainless steel frames (12 m length). The films cover 67% of the total area of each plot when they
181 are fully expanded. Several water sprayers with a water pipe are fixed on top of each frame, to
182 guarantee an even water addition in the wet season. The throughfall exclusion occurs from mid-
183 September to mid-April (dry season) of each year by fully expanding the plastic film. After that,
184 all films are hanged beside the frame for each plot totally accepting natural precipitation. During
185 this period, a same volume water that equals to the excluded throughfall in the previous dry
186 season is added to the plot for several times (each time 50-55 mm). The added water is from a
187 pond near the site after filtration. The pH and other properties of the pond water are near to
188 natural precipitation (Chen et al., 2017).

189 During the first year of precipitation change (September^{16th} 2018 - September^{15th} 2019),
190 the natural precipitation was 528.0 mm in the dry season and 1691.5 mm in the wet season. The
191 throughfall that excluded in the dry season was 302.0 mm. Water that equaled to the amount of
192 the excluded throughfall was added 6 times (June^{16th} 2019, July^{8th} 2019, July^{19th} 2019, August^{10th}
193 2019, August^{24th} 2019 and September^{8th} 2019, each time 50.3 mm) in the wet season. During the
194 second year of precipitation change (September^{16th} 2019 - September^{15th} 2020), the natural
195 precipitation was 332.1 mm in the dry season and 1222.5 mm in the wet season, while the
196 excluded throughfall in the dry season of this year was 185.0 mm. Water that equaled to the
197 amount of the excluded throughfall was added 3 times (June^{12th} 2020, July^{8th} 2020 and August^{10th}
198 2020, each time 61.55 mm) in the wet season.

199 2.3 Gas sampling and analysis

200 The N_2O emission flux of the forest soil was measured using the closed chamber method
201 (Hutchinson and Mosier, 1981). The closed chamber consists of a PVC-made main body ($\varphi = 25$

202 cm, $H = 35$ cm) and a PVC-made base ($\varphi_{\text{out-ring}} = 33$ cm, $H_{\text{out-ring}} = 11$ cm; $\varphi_{\text{inner-ring}} = 25$ cm, $H_{\text{inner-}}$
 203 $\text{ring} = 8$ cm). A little fan is fixed inside the main body and linked to an external battery, to mix the
 204 gas of the chamber evenly when collecting gases. Two bases were randomly installed in each
 205 plot in 2012 and placed permanently. The corresponding main body of the closed chamber was
 206 placed nearby each base.

207 Starting in October 2018, gas samples were collected monthly at the middle of each
 208 month. The gas collection time of each sampling day is limited in 9:00-11:00 am, since the gas
 209 emission rate during this time period was proven to approximately equal to the average rate the
 210 whole day (Kessavalou et al., 1998; Tang et al., 2006). Before sample collection, the bottom of
 211 the chamber body is placed between the two rings of the base, while the gap between the inner
 212 and outer rings of the base is filled with water for creating a sealing environment. The fans of the
 213 chamber are also opened to mix the gas inside. Gas samples are taken from the sampling pot on
 214 top of the chamber at 0 min, 10 min, 20 min and 30 min using a plastic syringe (100 ml, Pingan,
 215 China). The sample of each time point is immediately transferred to a pre-evacuated air bag (0.2
 216 L, Shanghaieler LTD, ELCR, Shanghai, China) for next-step measurement. During the time
 217 period of gas collection, we recorded the atmospheric pressure and temperature using an aneroid
 218 barometer (Type DYM3, Fengyang, Tianjin, China), as well as soil moisture and temperature
 219 using a TDR soil water measurement system (Type TRIME-PICO, Aozuo, Beijing, China). The
 220 atmospheric temperature and precipitation are recorded by Heshan National Field Research
 221 Station of Forest Ecosystem, Chinese Academy of Sciences.

222 The gas samples were analyzed using the electron capture detector (ECD) of a gas
 223 chromatography (Type 7890A, Agilent, Santa Clara, USA) within one week of collection. The
 224 N_2O emission flux is calculated as follows:

$$225 \quad F = \rho \times \frac{V}{A} \times \frac{P}{P_0} \times \frac{T_0}{T} \times \frac{dC_1}{dt} \quad (1)$$

226 where, ρ : the N_2O density in the standard condition (g L^{-1}); V : the gas volume in the closed
 227 chamber (m^3); A : the chamber coverage area (m^2); P : the atmosphere pressure (Pa) of the
 228 sampling site; P_0 : the standard atmosphere pressure in the standard condition (Pa); T_0 : the
 229 absolute temperature in the standard condition; T : the absolute temperature of the sampling time;
 230 dC_1/dt : the liner slope of gas concentration changes within time (ppb h^{-1}). The N_2O flux is
 231 expressed in the unit of $\mu\text{g N m}^{-2} \text{h}^{-1}$.

232 2.4 Soil sampling and analysis

233 Starting in October 2018, soil samples were collected monthly at the middle of each
 234 month (on the same day of the gas collection). Six soil cores were randomly selected in each plot.
 235 After litter removal, the surface soil (0-20cm) of each soil core were collected using a corer ($\varphi =$
 236 4 cm). The surface soil that collected from all the six cores were then fully mixed into a
 237 composite sample. Therefore, a total of 16 soil samples were collected in each month. After
 238 taken back and sieved through a 2-mm mesh, each soil sample was divided into three subsamples
 239 and stored in different conditions for future laboratory analyses.

240 The first subsample was naturally dried, then directly used or used after more carefully
 241 sieved for the determination of soil pH and total nitrogen (total N). Soil pH was determined
 242 using a portable pH detector (F-71G, LAQUA, HORIBA, Japan) by mixing soil and water in a

243 ratio of 1:2.5 (m: v). Soil total N was determined by the Alpha-Naphthol Blue-spectrophotometer
244 method after sulfuric acid heating.

245 The second subsample was stored at 4 °C for the determination of soil water content
246 (SWC), ammonium nitrogen (NH₄⁺-N), nitrate nitrogen (NO₃⁻-N) and microbial biomass
247 nitrogen (MBN). SWC was determined using the oven-drying method. The ammonium N and
248 nitrate N were determined by using the flow injector (Type QC8000, Lachat, USA) after the
249 fresh soil was extracted by a 2 M KCl solution at a ratio of 1:5 (m: v). MBN were determined
250 using the chloroform-fumigation-extraction method (Vance et al., 1987).

251 The third subsample was stored at -80 °C for quantification of functional genes. Soil total
252 DNA was extracted from 0.25 g soil using a PowerSoil[®] DNA Isolation kit (MoBio, Anbiosci
253 Tech Ltd, USA) by following the manufacture's protocol. The extracted DNA was first tested
254 using a Nanodrophotometer (Thermo NanoDrop[™] One, Thermo Scientific, USA) and agarose
255 gel electrophoresis and then stored at -20 °C for further analyses.

256 2.6 Quantification of nitrifying and denitrifying functional genes

257 Five enzyme encoding functional genes of soil nitrifiers and denitrifiers were chosen in
258 this study, including 1) the *amoA* gene of ammonia-oxidizing bacteria (AOB-*amoA*), 2) the
259 *amoA* gene of ammonia-oxidizing archaea (AOA-*amoA*), 3) the *nxB* gene of nitrite-oxidizing
260 bacteria (NOB), 4) the nitrite reductase gene *nirK* and 5) the nitrous oxide reductase gene *nosZ*.
261 The primer information of each functional gene is listed in Table S1.

262 The abundance of functional genes was quantified by absolute real-time polymerase
263 chain reactions (PCR). The PCR reaction was carried out in a 384-well microplate (Labtite,
264 Greystone Biosciences, USA) with a LightCycler[®] 480II real-time fluorescent quantitative PCR
265 system (Roche, Switzerland). SYBR green was used as the detection system. Moreover, the
266 volume of the reaction mixture was 20 µl, including 10.4 µl SYBR Green Premix Ex Taq[™] II
267 (TaKaRa, Japan), 0.4 µl of 10 µM forward primer (0.2 µM in the final reaction system), 0.4 µl of
268 10 µM reverse primer (0.2 µM in the final reaction system), 2.0 µl DNA template and 6.8 µl
269 double distilled water. The standard plasmid of each functional gene, i.e. the plasmid that
270 contains the fragment of AOB-*amoA*, AOA-*amoA*, *nxB*, *nirK* or *nosZ*, was prepared from the
271 extracted DNA samples using same primers as above. The standard plasmid was then gradually
272 diluted into a serial plasmid solutions (10²-10¹⁰ copies µl⁻¹) to obtain the standard curve of the
273 target gene. The PCR reaction programs are listed in Table S2. Three technical replicates of all
274 the DNA samples and standard curves were designed when performing the qPCR amplifications.
275 The PCR amplification efficiency and the R² for the functional genes was in a range of 80.8-
276 118.5% and 0.900-9.994, respectively.

277 2.7 Statistical analysis

278 Comparisons were conducted mainly among the four experimental treatments (C, N, P,
279 NP) and between the wet and dry seasons. During the two-year investigation, the average soil
280 properties, functional gene abundances and N₂O effluxes of the dry season and the wet season
281 were calculated. Two-way ANOVA analyses followed by least significant difference (LSD)
282 testes were used to examine the difference of soil properties, functional gene abundances and
283 N₂O effluxes among the four treatments and between the two seasons. All data were assessed for
284 normality (Kolmogorov-Smirnov test) and quality of variances (Levène test) before analyses.
285 Data that did not fit normality or quality of variances were logarithmically transformed for

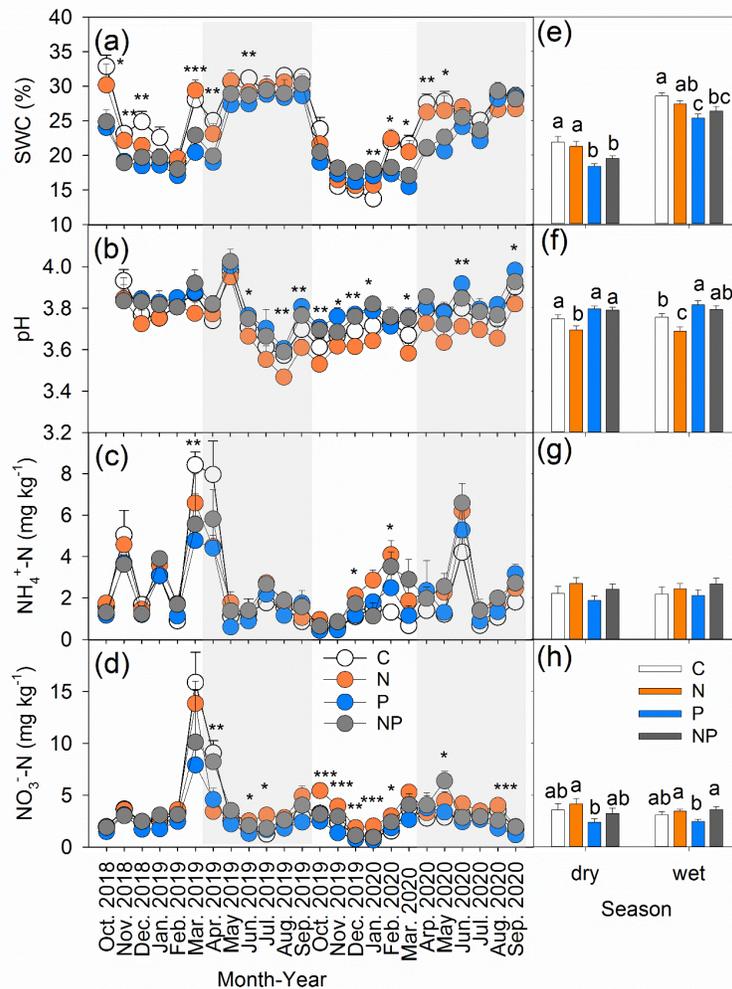
286 further analyses. The statistically significant difference was analyzed at $p < 0.05$ level. All
 287 statistical analyses were performed in SPSS 20.0 (SPSS Inc. Chicago, USA) and Sigmaplot 12.5
 288 (Systat Software Inc.).

289 A principal component analysis (PCA) was performed separately in each treatment, to
 290 test the relationship of the soil properties, functional gene abundances and N₂O effluxes. A
 291 structural equation model (SEM) was constructed to explore the effects of N addition and
 292 precipitation change on soil properties, nitrifying/denitrifying functional gene abundances and
 293 N₂O effluxes (Fig. S2). Briefly, the conceptual model includes variables of mainly three aspects:
 294 1) soil properties, including soil water content (SWC), soil pH, ammonium N (NH₄⁺-N) and
 295 nitrate N (NO₃⁻-N). 2) Abundances of nitrifying and denitrifying functional genes, including
 296 AOB-*amoA*, AOA-*amoA* and *nxrB* for nitrification, and *nirK* and *nosZ* for denitrification. (3)
 297 Soil N₂O effluxes. Moreover, different relationships were established among the soil, microbial
 298 and N₂O indicators based on previous studies. The conceptual model was aimed to test that under
 299 the N addition and precipitation change treatments, how changes in soil water and soil inorganic
 300 N conditions induce responses in soil nitrifiers and denitrifiers and further influencing the
 301 microbial regulations in N₂O emission. Based on the model running results, we got the standard
 302 regression coefficient and significant level of each relationship, and the squared multiple
 303 correlation (R²) of each variable in the model. The SEM analyses were conducted by AMOS
 304 21.0 (SPSS Inc., Chicago, IL, USA). The final model analysis results were illustrated and
 305 expressed schematically.

306 **3 Results**

307 **3.1 Soil properties**

308 During the two-year period, the soil water content (SWC) of the study site ranged from
 309 14% to 33%, with significantly greater values in the wet season than in the dry season ($p < 0.001$)
 310 (Fig. 1a, e). The soil pH ranged from 3.6 to 4.0, with a relatively low value in the first wet season
 311 of the experimental treatments (Fig. 1b). The soil ammonium N and nitrate N concentrations
 312 ranged from 0.4 mg kg⁻¹ to 8.4 mg kg⁻¹ and from 0.7 mg kg⁻¹ to 15.9 mg kg⁻¹, respectively, but
 313 fluctuated weakly with time, expect for an increase in March 2019 and June 2020 (Fig. 1c, d).
 314 Moreover, soil nitrate N was generally greater than ammonium N, indicating the potential for
 315 nitrification in the forest soil. According to two-way ANOVA, there were significant differences
 316 among the four treatments in terms of SWC, pH and nitrate N ($p < 0.05$). The SWC decreased in
 317 the experimental treatments and was greatest in the C treatment but lowest in the P treatment (p
 318 < 0.05) in both seasons (Fig. 1e). In contrast, soil pH was significantly lower in the N treatment
 319 ($p < 0.05$) but greater in the P and NP treatments in both seasons than in the C treatment, with
 320 the lowest value in the N treatment and the greatest value in the P treatment (Fig. 1f). Compared
 321 with the C treatment, the N treatment increased the soil ammonium N by 18.3% and 10.6% in the
 322 dry season and wet season, respectively ($p > 0.05$), while the N treatment increased the soil
 323 nitrate N by 13.9% and 10.6% in the dry season and wet season, respectively ($p > 0.05$). In
 324 contrast, the P treatment decreased the soil ammonium N by 14.5% and 2.7% in the dry season
 325 and wet season, respectively ($p > 0.05$) (Fig. 1g, h), while the P treatment decreased the soil
 326 nitrate N by 33.8% and 20.9% in the dry season and wet season, respectively ($p > 0.05$) (Fig. 1g,
 327 h). Except for the nitrate N content in the wet season, the contents of all four soil indicators in
 328 the NP treatment were between those in the N and P treatments, showing no significant
 329 difference from those in the C treatment ($p > 0.05$) (Fig. 1 e-h).



330

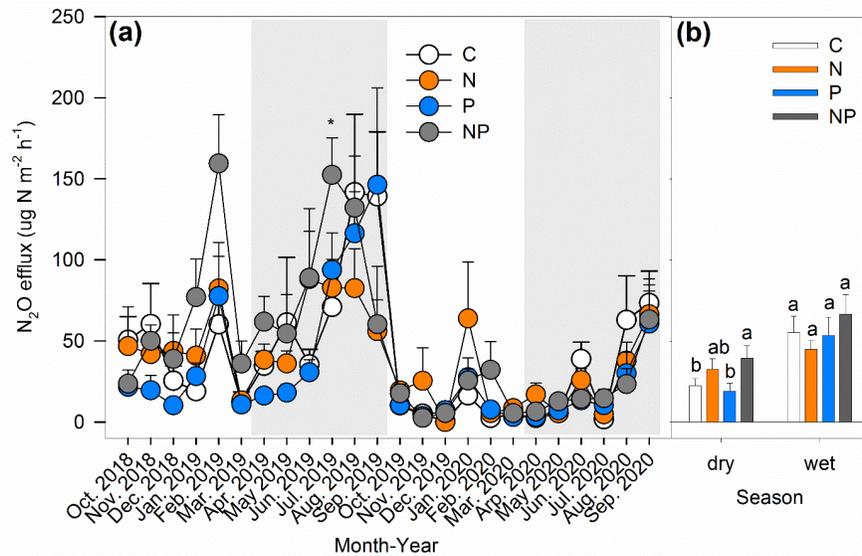
331 **Figure 1** Soil (0-20 cm) physiochemical property (average \pm standard error, $n = 4$) of each
 332 month (a-d) and of wet/dry season (e-h). C, N, P and NP indicate the treatment control, N
 333 addition, precipitation change, and interaction of N addition and precipitation change. Gray
 334 shades and white regions indicate wet seasons and dry seasons, respectively. Follows are the
 335 same. Asterisks in (a) - (d) indicate that the difference is significant ($p < 0.05$) among the four
 336 treatments (One-way ANOVA), while *, ** and *** indicate the difference is significant at $p <$
 337 0.05 , $p < 0.01$ and $p < 0.001$ level, respectively. Same letters in (e)-(h) indicate that the
 338 difference between any two treatments of a specific season is nonsignificant ($p > 0.05$) (One-way
 339 ANOVA). Follows are the same.

340

3.2 N_2O efflux

341 The N_2O efflux varied over the months and showed significant wet-dry seasonality, with
 342 an increasing trend in the wet season in comparison to the previous dry season ($p < 0.001$) (Fig.
 343 2). The average N_2O efflux of the C treatment was $22.3 \pm 4.5 \mu\text{g N m}^{-2} \text{ h}^{-1}$ in the dry season and
 344 $55.4 \pm 9.9 \mu\text{g N m}^{-2} \text{ h}^{-1}$ in the wet season (Fig. 2b). Compared with the C treatment, N_2O efflux
 345 in the N treatment increased nonsignificantly by 31.8% ($p > 0.05$) in the dry season but
 346 decreased nonsignificantly by 21.7% in the wet season ($p > 0.05$). Compared with the C
 347 treatment, the N_2O efflux in the P treatment decreased nonsignificantly by 14.7% and 3.3%,

348 respectively, in the dry season and wet season ($p > 0.05$). In contrast, the N₂O efflux in the NP
 349 treatment increased significantly (77.4%, $p < 0.05$) and nonsignificantly (20.4%, $p > 0.05$) in the
 350 dry season and wet season, respectively (Fig. 2b). Therefore, the soil N₂O efflux of the forest
 351 was significantly influenced by only the NP treatment in the dry season.

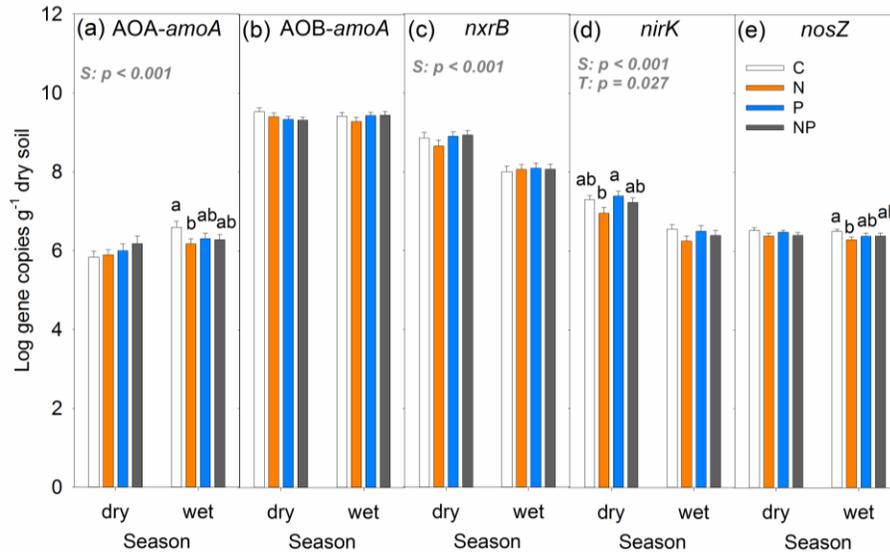


352

353 **Figure 2** Monthly dynamics of N₂O effluxes (a) and average N₂O effluxes during the wet season
 354 and the dry season (b). Significance levels are indicated by * at $p < 0.05$ level. Same letters in (b)
 355 indicate that the difference between different treatments is nonsignificant ($p < 0.05$) (One-way
 356 ANOVA).

357 3.3 Abundances of soil nitrifying and denitrifying functional genes

358 AOB-*amoA* was most abundant in the soil, followed by *nxrB*, *nirK*, *nosZ* and AOA-*amoA*
 359 (Fig. S3, Fig. 3). The AOB-*amoA* abundance and *nxrB* abundance accounted for approximately
 360 68.5% and 28.5%, respectively, while the abundances of AOA-*amoA*, *nirK* and *nosZ* accounted
 361 for approximately 3% of the total abundance of all five genes. Compared with the dry season soil,
 362 the wet season soil showed a significant increase in AOA-*amoA* abundance ($p < 0.001$),
 363 significant decreases in *nxrB* and *nirK* abundances ($p < 0.001$), and nonsignificant decreases in
 364 AOB-*amoA* and *nirK* abundances ($p > 0.05$) (Fig. 3a, c, d). Compared with those in the C
 365 treatment, the AOA-*amoA* and *nosZ* abundances in the N treatment soil decreased significantly
 366 in the wet season, and the *nirK* abundance in the N treatment soil decreased significantly in the
 367 dry season ($p < 0.05$) (Fig. 3a, d, e), indicating an inhibitory effect of N addition. Moreover, the
 368 soils in the N treatment had the lowest abundances of AOA-*amoA*, *nirK* and *nosZ*, followed by
 369 the soils in the NP and P treatments, but there were no significant differences among the C, P and
 370 NP treatments ($p > 0.05$) (Fig. 3a, d, e). According to two-way ANOVA, the *nirK* abundance
 371 significantly differed among the four treatments ($p < 0.05$) (Fig. 3d).

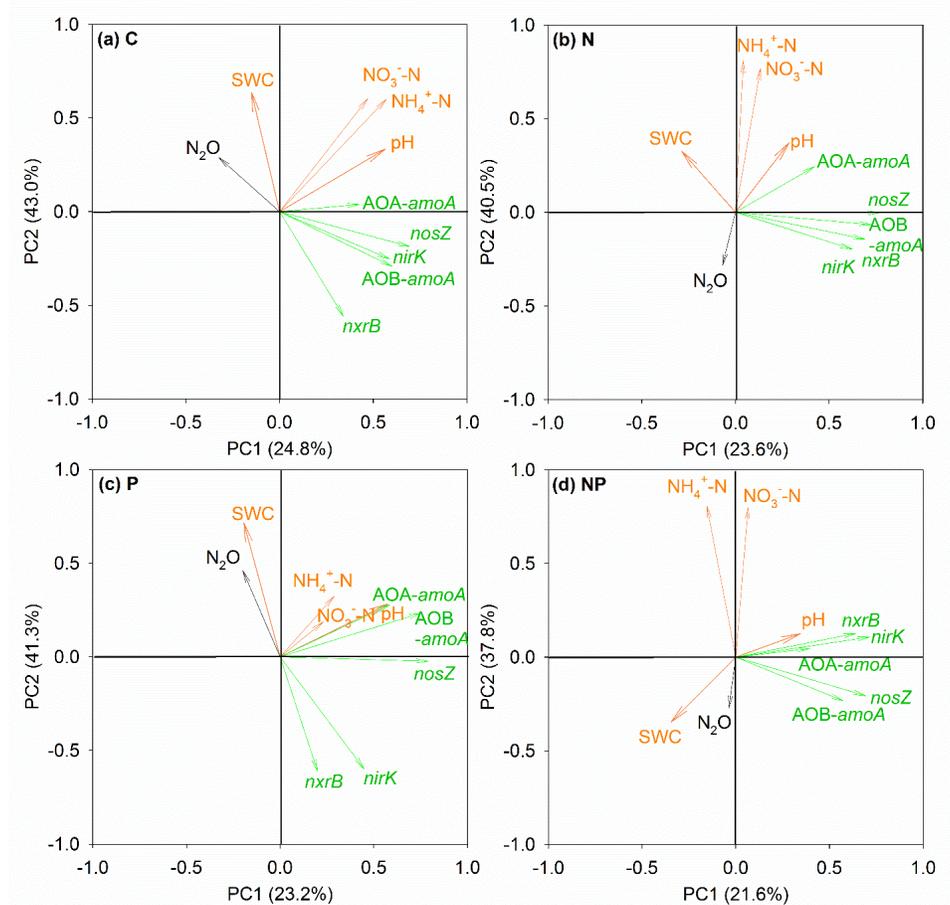


372

373 **Figure 3** Abundances (average \pm standard error) of nitrifying and denitrifying functional genes
 374 in the dry season and wet season. S and T indicate the significance of differences between
 375 seasons and among treatments, respectively, according to two-way ANOVA. Same letters
 376 indicate that the difference between any two treatments of a specific season is nonsignificant ($p >$
 377 0.05) (One-way ANOVA).

378 3.4 Relationships among soil properties, abundance of soil nitrifiers/nitrifiers and N₂O
 379 efflux

380 The PCA results showed different patterns among the four treatments (C, N, P, and NP).
 381 The first two PCs explained 67.8%, 64.1%, 64.5% and 59.4% of the total variability in the C, N,
 382 P, and NP soils, respectively (Fig. 4). Generally, the functional gene abundances of all four
 383 treatment soils had positive PC1 values, while the soil properties had positive PC2 values expect
 384 for SWC in the NP soil. Moreover, pH showed close relationships with the AOA-amoA
 385 abundance in the C, N and P soils and with the AOA-amoA, nxrB and nirK abundances in the NP
 386 soil. The N₂O efflux showed close relationships with SWC in the C, P and NP soils, but did not
 387 show close relationships with any soil property indicators in the N soil. In contrast, the N₂O
 388 efflux did not show a close relationship with the abundances of soil nitrifying and denitrifying
 389 functional genes in each treatment (Fig. 4).



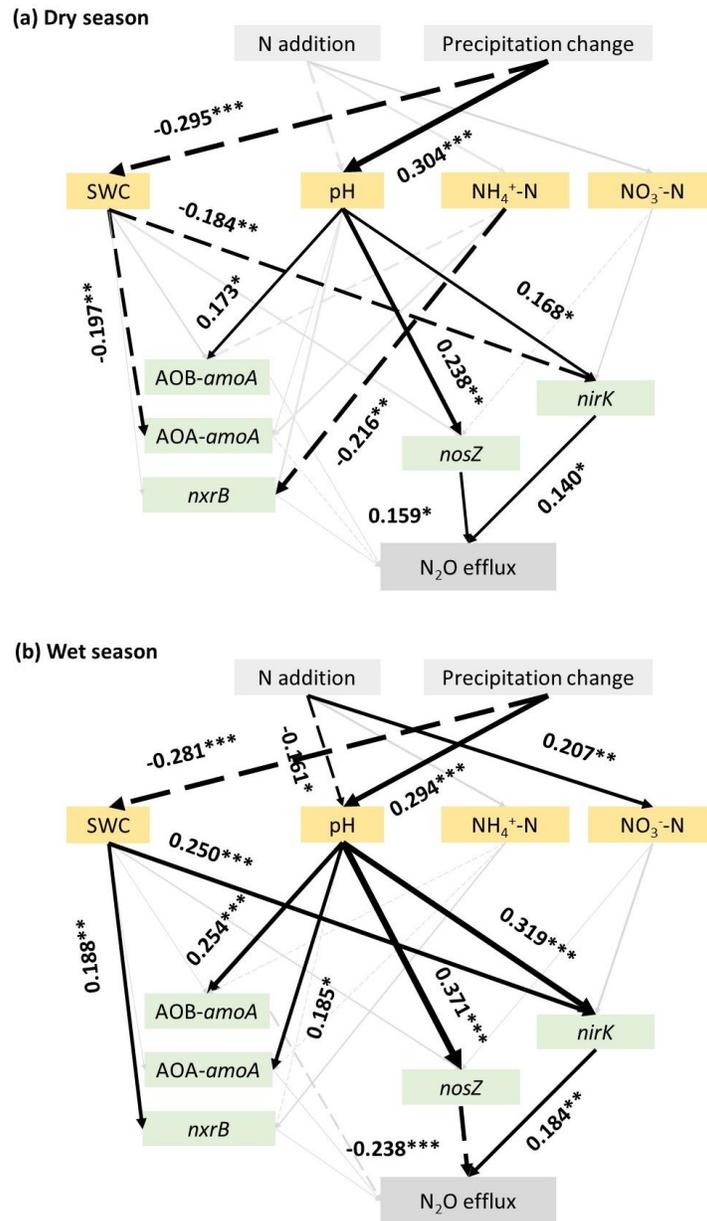
390

391 **Figure 4** Principal component analysis (PCA) illustrating the relationships among soil properties,
 392 functional gene abundances and N₂O effluxes. Arrows in orange, green and black indicate the
 393 factor loadings of soil property, nitrifying/denitrifying functional gene abundance and N₂O efflux,
 394 respectively, on the PC1/PC2 axes.

395 The SEM results showed that the effects of N addition and precipitation change on soil
 396 properties, nitrifying/denitrifying functional gene abundance and N₂O efflux differed between
 397 the wet and dry seasons. In the dry season, there were no significant relationships between N
 398 addition and soil properties (Fig. 5a). In contrast, precipitation change (precipitation decrease)
 399 was negatively related to SWC ($r = -0.295$, $p < 0.001$) but had a positive relationship with pH (r
 400 $= 0.304$, $p < 0.001$). SWC was negatively related to the AOA-*amoA* abundance ($r = -0.197$, $p =$
 401 0.007) and *nirK* abundance ($r = -0.184$, $p = 0.009$). In contrast, pH showed significant positive
 402 relationships with the abundances of AOB-*amoA* ($r = 0.173$, $p = 0.019$), *nosZ* ($r = 0.238$, $p =$
 403 0.001) and *nirK* ($r = 0.168$, $p = 0.02$). Both the *nirK* and *nosZ* abundances showed significant
 404 positive relationships with the N₂O efflux ($r = 0.140$, $p = 0.048$; $r = 0.159$, $p = 0.025$). Except for a
 405 negative relationship between soil ammonium N and *nxrB* abundance ($r = -0.126$, $p = 0.002$),
 406 there were no significant relationships between soil inorganic N and nitrifying/denitrifying
 407 functional gene abundances (Fig. 5a).

408 The soil in the wet season showed relationships similar to those in the dry season, that is,
 409 negative relationships between precipitation change (precipitation increase) and SWC ($r = -0.281$,

410 $p < 0.001$), between precipitation change and pH ($r = 0.294$, $p < 0.001$), between pH and the
 411 abundances of AOB-*amoA* ($r = 0.254$, $p < 0.001$), *nosZ* ($r = 0.371$, $p < 0.001$) and *nirK* ($r = 0.319$,
 412 $p < 0.001$), and between *nirK* abundance and N₂O efflux ($r = 0.184$, $p = 0.008$). In contrast, *nosZ*
 413 abundance showed a negative relationship with N₂O efflux ($r = -0.238$, $p < 0.001$). Moreover, N
 414 addition had a negative relationship with pH ($r = -0.161$, $p = 0.018$) but a positive relationship
 415 with soil nitrate N ($r = 0.207$, $p = 0.004$), while pH had a positive relationship with AOA-*amoA*
 416 abundance ($r = 0.185$, $p = 0.013$) (Fig. 5b).



417

418 **Figure 5** Structural equation model simulating the effects of N addition and precipitation change
 419 on soil property, functional gene abundance and N₂O efflux. Squares in light gray, yellow, light
 420 green and middle gray indicate the indicators of N addition/precipitation change, soil property,
 421 functional gene abundance and N₂O efflux, respectively. (1) Arrows in black indicate the
 422 relationship is significant ($p < 0.05$), while arrows in gray indicate the relationships is

423 nonsignificant ($p > 0.05$). (2) Solid black arrows indicate the relationship is positive, while
 424 dotted black arrows indicate the relationship is negative, according to the model results. (3)
 425 Numbers on adjacent to each arrow indicate the standard regression index of corresponding
 426 relationship, while numbers of nonsignificant relationships were not shown in this figure. *, **,
 427 and *** indicate that the relationship is significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ level,
 428 respectively. (4) The arrows are of different thickness, the higher the absolute value of the
 429 standard regression value, the thicker the arrow.

430 **4 Discussion**

431 4.1 Effects of N addition and seasonal changes in the distribution of precipitation on N₂O 432 emissions

433 (1) Unlike the first hypothesis and previous studies in subtropical forests showing that N
 434 addition promoted N₂O emissions (Zhang et al., 2008; Nie et al., 2019), in this study, forest soil
 435 N₂O effluxes were not significantly stimulated in the dry season but inhibited in the wet season
 436 by N addition (Fig. 2). However, this is in line with the findings of a study showing the response
 437 of N₂O emissions to a gradient of N addition in a subtropical forest of southern China (Han et al.,
 438 2019). Moreover, soil ammonium N and nitrate N were nonsignificantly stimulated by N
 439 addition in either season (Fig. 1g, h). Therefore, we speculated that in the dry season, the
 440 increased N₂O emissions under N addition were a result of increased substrate (NH₄⁺-N, NO₃⁻-N)
 441 availability for nitrification and denitrification, while the decreased N₂O effluxes in the wet
 442 season were influenced by soil factors (e.g., soil moisture) other than soil inorganic N
 443 availability. (2) The precipitation change treatment resulted in relatively lower soil N₂O effluxes
 444 and significantly lower soil moisture in both seasons (Fig. 1 a, e; Fig. 2). This finding did not
 445 support the second hypothesis. It was unexpected that in the wet season, soil moisture in the
 446 surface layer (0–20 cm) was inhibited by increased precipitation. Possible reasons for this
 447 finding might be that there was high natural rainfall in the wet season, which induced high
 448 moisture levels in the forest soil. The water holding capacity of the surface soil might have been
 449 exceeded under simulated precipitation, and the extra water was lost via leaching to deeper soil
 450 layers (20–40 cm and 40–60 cm). This was supported by the findings that the soil water content
 451 in the soil in the P treatment was higher than that in the C treatment (data not shown). Thus, the
 452 inhibition of N₂O emissions in the wet season may have occurred due to the decreased soil
 453 nitrifier and denitrifier abundances under the precipitation change treatment (Fig. 3a, c, d). (3)
 454 The N₂O effluxes were significantly stimulated by the NP treatment in the dry season but were
 455 nonsignificantly affected in the wet season (Fig. 2b). This is partly consistent with the third
 456 hypothesis, suggesting that the added N may stimulate soil N usage and N loss, while
 457 precipitation changes may cause less changes in N₂O production and gaseous emissions. To
 458 confirm this speculation, a field ¹⁵N labelling method for tracing the transformation and fate of
 459 added N (Gurmesa et al., 2016) must be employed in future studies.

460 4.2 Effects of N addition and seasonal precipitation distribution changes on the 461 abundances of soil nitrifiers and denitrifiers

462 The AOB-*amoA* gene outnumbered the AOA-*amoA* gene in the studied forest soil (Fig.
 463 3), which contrasts with previous findings showing that AOA are generally more dominant than
 464 AOB in acidic forest soils (Isobe et al., 2012) but the finding is in line with the study of Petersen
 465 (2012) in a black spruce forest. Moreover, the *nxrB* gene was less abundant than AOB-*amoA* but

466 much more abundant than AOA-*amoA* (Fig. 3a-c), implying a greater potential for N₂O
467 production than NO₃⁻ production during autotrophic nitrification. The *nirK* gene was more
468 abundant than the *nosZ* gene (Fig. 3d-e), showing the potential for more N₂O production than
469 N₂O reduction during denitrification. Notably, the *nirK* gene was less abundant than the AOB-
470 *amoA* gene, which contrasts with findings of Yin et al. (2023) in four highly acidic soils.
471 Compared with the dry season soil, the wet season soil showed significantly greater AOA-*amoA*
472 abundance, but the AOB-*amoA* abundance did not change markedly, while the trends in AOA-
473 *amoA* with season were similar with those of SWC (Fig. 1e), showing that AOA may be more
474 sensitive than AOB to soil moisture changes. However, this finding is inconsistent with the
475 results of Wang et al. (2017), who reported that unlike AOA, AOB responded positively to soil
476 moisture increases. In contrast, the *nxrB* abundance decreased significantly in the wet season,
477 implying that higher soil moisture may not benefit NOB community or further facilitate the
478 production of N₂O. This might be one reason why significantly greater N₂O effluxes were
479 observed in the wet season than in the dry season (Fig. 2b). The *nirK* abundance decreased
480 significantly but the *nosZ* abundance varied less from the dry to wet season, showing that higher
481 soil moisture may limit the production of N₂O via the denitrification pathway.

482 Our results showed that the three experimental treatments (N, P, and NP) did not induce
483 significant changes in the abundance of AOB-*amoA* or *nxrB* in either season (Fig. 3b, c),
484 indicating that the soil AOB and NOB communities were not sensitive to either N addition or
485 precipitation increase/decrease. This is reflected in the generally similar PCA patterns of
486 functional genes in the soils of the four treatments (Fig. 4). Moreover, this finding is inconsistent
487 with previous findings showing that AOB abundance increased by both N supply and soil
488 moisture increase (Wang et al., 2017). Possible reasons for this difference might be that AOB
489 were dominant in the studied forest soil and were adapted to the soil conditions (Petersen et al.,
490 2012) induced by long-term high N input via N deposition and to the high precipitation in the
491 subtropical region. Thus, short-term (2 yr) exogenous N input or increasing/decreasing seasonal
492 precipitation did not change the AOB community much. On the other hand, the *nxrB* abundance
493 decreased significantly from the dry to wet season but did not change significantly with
494 increasing precipitation, implying that the soil moisture change induced by natural wet-dry
495 seasonal variation was more influential than that induced by the precipitation change treatment,
496 although the soil moisture content decreased significantly under this treatment (Fig. 1e).
497 However, more evidence is required to confirm this speculation. AOA-*amoA* was
498 nonsignificantly stimulated in the dry season but significantly inhibited in the wet season by N
499 addition (Fig. 3a), implying that increased NH₄⁺ supply (Fig. 1g) may stimulate AOA in the dry
500 season, but this was not the dominant factor influencing the AOA community in the wet season.
501 The abundance of soil denitrifiers, especially *nosZ* denitrifiers, was inhibited by N addition (Fig.
502 3d, e), which conflicts with the observation that the NO₃⁻ content increased but is consistent with
503 the decreased pH of the soil (Fig. 1h, e), implying that pH may play more an essential role than
504 substrate (NO₃⁻) availability in soil denitrifier groups (Bárta et al., 2010). Although not
505 significant, the *nirK* and *nosZ* abundances were generally reduced by the three experimental
506 treatments in both seasons (Fig. 3d, e), showing that soil denitrifiers were sensitive to soil N and
507 water changes to some extent.

508 4.3 Effects of soil abiotic and biotic factors on N₂O emissions

509 Soil N₂O efflux has been proven to be closely linked to many soil abiotic factors such as
510 moisture (Zhang et al., 2008; Cheng et al., 2014), pH (Cuhel et al., 2010) and N availability

511 (Huang et al., 2014; Zhang et al., 2014). This is partly reflected in the PCA results of the four
 512 treated soils in present study (Fig. 4). Other than the soils of the N addition treatment (N), the
 513 soils in the treatments (C, P and NP), especially those in the P treatment, exhibited close
 514 relationships between N₂O efflux and soil moisture (Fig. 4a, c, d). This implies that soil moisture
 515 was more important in influencing forest soil N₂O emissions than pH and inorganic N.
 516 According to the PCA, no close relationships existed between the abundances of
 517 nitrifiers/denitrifiers and N₂O effluxes in the soils of all four treatments (Fig. 4), implying that
 518 the final emission of N₂O was not determined by single pathway of nitrification or denitrification.
 519 On the other hand, all four treated soils showed close relationships between pH and AOA-*amoA*
 520 abundance, again demonstrating the high sensitivity of the AOA community in the acidic forest
 521 soil (Isobe et al., 2012). Moreover, the close relationship between pH and the abundance of
 522 AOA-*amoA*, *nxrB* and *nirK* in the NP soil further increase the impact of the interaction of N
 523 addition and precipitation changes on the microbial community.

524 4.4 Role of soil functional microbes in the production and emission of N₂O under 525 elevated N deposition and seasonal precipitation distribution changes

526 We conducted pathway analyses by combining indicators of soil properties,
 527 nitrifying/denitrifying functional microbes and N₂O emissions (Fig. 5). Nitrogen addition had
 528 negative and positive impacts on soil pH and nitrate N, respectively, but only in the wet season
 529 (Fig. 5b). This implies that the increase in precipitation and increase in soil moisture during the
 530 wet season may accelerate soil acidification and autotrophic nitrification, which is reflected in
 531 the relatively high AOA-*amoA* abundance during this season (Fig. 4a). This was also supported
 532 by the results of Nie et al. (2019), who reported that in a tropical forest, the rate of net
 533 nitrification was greater in the wet season than dry season under N addition. However, to
 534 confirm this speculation, the nitrification rate of the forest soil further must be quantified.

535 The SEM showed that compared with N addition, precipitation change induced more
 536 significant impacts on soil nitrifier/denitrifier abundances and N₂O emissions in both seasons
 537 (Fig. 5). This reveals that microbial regulation of soil N₂O emissions is more dependent on soil
 538 water changes than on soil inorganic N availability changes, which is also supported by the PCA
 539 (Fig. 4). For dry season soil, a decrease in precipitation had negative impacts on SWC, and a
 540 change in SWC had negative impacts on the abundances of AOA-*amoA* and *nirK*, while a
 541 change in *nirK* abundance had positive impacts on N₂O efflux (Fig. 5a). This could have
 542 occurred because a decrease in precipitation in the dry season led to lower soil moisture levels,
 543 further inhibiting the growth of soil AOA and *nirK* denitrifiers. The change in AOA-*amoA*
 544 abundance did not significantly change N₂O production via the autotrophic nitrification pathway,
 545 but the decrease in *nirK* abundance contributed to a significant decrease in N₂O production via
 546 the denitrification pathway. This may be one of the reasons for the significantly lower N₂O
 547 effluxes in the soil in the dry season than in the wet season (Fig. 2b). On the other hand, we
 548 found that a decrease in precipitation had positive impacts on pH, and pH had positive impacts
 549 on the abundances of AOB-*amoA*, *nirK* and *nosZ*, while the abundances of the two denitrifying
 550 functional genes further had positive impacts on N₂O efflux (Fig. 5a). This might have occurred
 551 because the decrease in precipitation alleviated soil acidification (Fig. 1f), further facilitating the
 552 growth of soil AOB and *nirK/nosZ* denitrifiers, while pH had a more significant impact on *nosZ*
 553 denitrifiers than on *nirK* denitrifiers (Fig. 5a), which further facilitated N₂O reduction (N₂O →
 554 N₂) rather than N₂O production via the denitrification pathway. This may be another reason for
 555 the lower N₂O effluxes observed in the dry season (Fig. 2b). (2) The soil in the wet season

556 showed similar negative and positive impacts on the SWC and pH under precipitation increase,
557 respectively, as those in the dry season (Fig. 5). A possible reason for this might be that the
558 natural precipitation during the wet season kept the soil moisture level high (Fig. 1e), and the
559 water added during this season may have leached more to the deeper soil layer. SWC had
560 positive impacts on *nxrB* and *nirK* abundances, indicating that the growth of NOB and *nirK*
561 denitrifiers may be limited by high soil moisture levels and simulated precipitation in the wet
562 season. In the wet season, pH had positive impacts on *nirK* and *nosZ* abundances similar to that
563 in the dry season, but the *nirK* abundance had positive impacts, while the *nosZ* abundance had
564 negative impacts on N₂O efflux (Fig. 5b). Considering that *nirK* abundance decreased but *nosZ*
565 abundance changed little from the dry to wet season (Fig. 3d, e), we speculate that in the wet
566 season, the N₂O production of the denitrification pathway may be limited because of the
567 decreased *nirK* copy numbers in soil; however, the N₂O reduction was also limited, thus
568 alleviating N₂O consumption and further facilitating N₂O emission from the soil. This may be
569 one of the reasons for the high N₂O effluxes in the wet season (Fig. 2b).

570 Unlike soil moisture and pH, soil ammonium N and nitrate N did not significantly impact
571 the abundances of nitrifiers or denitrifiers in either season, even though nitrate N was positively
572 affected by N addition in the wet season (Fig. 1, Fig. 5). This indicates that soil inorganic N
573 availability was not a predominant factor regulating the abundance of soil nitrifiers and
574 denitrifiers, which is inconsistent with previous findings (Carey et al., 2016; Cheng et al., 2019).
575 However, this may have occurred because the studied forest soil was N-saturated after a high
576 level of long-term natural N deposition (Huang et al., 2015). On the other hand, no relationships
577 were detected between pH and *nxrB* abundance in either season, which is not in line with the
578 results of Li et al. (2019), who reported that *nxrB* abundance was significantly affected by pH.
579 One reason for this difference might be that the soil of the forest in this study is acidic, and the
580 variation in pH was relatively low (3.6-4.0) in comparison to the pH range gradient (4.8-7.0) in
581 the study by Li et al. (2019).

582 Generally, the SEM revealed that for the forest soils with interactions between N addition
583 and seasonal precipitation distribution changes, (1) changes in precipitation modified forest soil
584 physicochemical properties more than N addition and thus played more essential roles in
585 influencing soil nitrifier/denitrifier abundances and final N₂O emissions; (2) pH was most
586 sensitive to changes in precipitation change and N addition, followed by soil moisture, and
587 induced significant effects on soil nitrifiers and denitrifiers; (3) soil *nirK* and *nosZ* denitrifiers
588 played more dominant roles than soil nitrifiers (AOB, AOA, NOB) in regulating N₂O emissions;
589 and (4) N₂O emissions during the dry and wet seasons differed in terms of the potential to
590 regulate soil functional microbes due to the different responses of the microbes to seasonal
591 changes and the experimental treatments.

592 **5 Conclusions**

593 In this study, we tested whether N addition and changes in seasonal precipitation
594 distribution had individual and interactive effects on subtropical forest soil N₂O emissions and
595 investigated the underlying microbial mechanisms involved. We showed that soil N₂O emissions
596 were significantly greater in the wet season than in the dry season but were promoted by
597 interactions between N addition and precipitation change only during the dry season.
598 Precipitation change was more influential than N addition in affecting soil properties, especially
599 pH and moisture, while N addition and the interaction between N addition and precipitation

600 change did not induce significant changes in soil ammonium N and nitrate N contents. The
 601 structural equation model results showed that soil pH played a more essential role than soil
 602 moisture in regulating soil nitrifiers and denitrifiers and soil N₂O efflux. Soil nitrifiers, especially
 603 AOB-*amoA* and *nxrB*, were much more abundant than soil denitrifiers in the studied subtropical
 604 forest soil. However, under the N addition and precipitation change treatments, soil denitrifiers
 605 were more important in regulating N₂O emissions in both seasons, but there was a higher
 606 abundance of *nirK* than *nosZ* in the dry season soil, which contributed to N₂O production and
 607 reduction, but the decreased *nirK* abundance and the insubstantial change in *nosZ* abundance in
 608 the wet season soil limited the reduction of N₂O after production, contributing to more soil
 609 gaseous loss in the form of N₂O. In the future studies, soil nitrification rates and other N
 610 transformation rates should be determined to confirm the role of soil functional microbes in
 611 responding to soil N and water level changes and in regulating emissions of N₂O.

612

613 **Acknowledgements**

614 This work was financially supported by the National Natural Science Foundation of
 615 China (32101344, 31600384, 32071596, 31425005), the National Ten Thousand Talents
 616 Program, the Guangdong Province Baiqianwan Talents Program, Guangdong Basic and Applied
 617 Basic Research Foundation (Grant NO. 2021A1515011295) and the Open Project of Guangdong
 618 Provincial Key Laboratory of Plant Resources (2020B1212060027). We sincerely thank the
 619 Heshan National Field Research Station of Forest Ecosystem, Chinese Academy of Sciences, for
 620 the platform and meteorological data supports. We are also very grateful to Dr. Zhiming Guo,
 621 Zhipeng Chen and Shaoyun Lv for their assistance in the field investigation and laboratory
 622 analyses. There are no conflicts of interests for any authors.

623 **5 Data availability statement**

624 Data of N₂O efflux, soil property, soil nitrifying and denitrifying functional gene
 625 abundance are available in Bolin Centre Database <https://doi.org/10.17043/21ki6u-1> (Han et al.,
 626 2024).

627

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