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Soil nitrogen transformation responses to seasonal precipitation changes are regulated by changes in functional microbial abundance in a subtropical forest

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Abstract

More dry-season droughts and wet-season storms have been predicted in subtropical areas. Since subtropical forest soils are significant sources of N_2O and NO_3^-, it is important to understand the features and determinants of N transformation responses to the predicted precipitation changes. A precipitation manipulation field experiment was conducted to reduce dry-season precipitation and increase wet-season precipitation, while keeping the annual precipitation unchanged in a subtropical forest. Net N mineralization, net nitrification, N_2O emission, nitrifying (bacterial and archaeal *amoA*) and denitrifying (*nirK*, *nirS* and *nosZ*) genes abundance, microbial biomass carbon (MBC) and soil physicochemical properties were monitored to characterize and explain soil N transformation responses. Dry-season precipitation reduction decreased net nitrification and N mineralization rates by 13 - 20%, while wet-season precipitation addition increased both rates by 50%. More than 20% of the total variation of net nitrification and N mineralization could be explained by microbial abundance and soil water content (SWC), but archaeal *amoA* abundance was the main factor. Increased net nitrification in wet season together with large precipitation events caused substantial NO_3^- losses via leaching. However, N_2O emission decreased moderately either in dry or wet seasons due to changes in *nosZ* gene abundance, MBC, net nitrification and SWC (decreased by 10 - 21%). We conclude that reducing dry-season precipitation and increasing wet-season precipitation affect N transformation mainly through altering functional microbial abundance and MBC, which are further determined by changes in DOC and NH_4^+

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44 availabilities. Such contrasting precipitation pattern will increase droughts and NO_3^-

45 leaching in subtropical forests.

46 **Key-words:** Denitrification, functional genes, nitrification, nitrogen cycle,

47 precipitation change, N_2O emission

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1 Introduction

Precipitation changes caused by global climate change are increasingly severe over the century (IPCC, 2007; Seager et al., 2007). Future projected precipitation patterns vary spatially and temporally, and the complexity and unpredictability of precipitation changes have exceeded other climate changes such as elevated CO_2 and temperature (Beier et al., 2012). Despite the frequency and intensity of precipitation events, seasonal precipitation changes are of increasing severity (Easterling et al., 2000). Recent study of 60 years precipitation data showed remarkable seasonal precipitation redistribution in a subtropical forest, with more frequent droughts in dry season and extremely rainfall events in wet season (Zhou et al., 2011). In contrast to annual precipitation amount, seasonal distribution may be more important in controlling the ecosystem functioning in subtropical forests, because of strong contrast between dry and wet seasons (Wang et al., 2009). Recent meta-analyses on precipitation manipulation experiments pointed out the lack of data in the warm and humid monsoon zones (Wu et al., 2011; Liu et al., 2016), and that more than 60% of all manipulative field experiments only focused on changes in precipitation amounts ((Beier *et al.*, 2012). The consequences of seasonal precipitation redistribution at ecosystem levels are still under investigation. Altogether, field experiments simulating seasonal precipitation changes in subtropical regions are urgently needed for better understanding of the ecosystem responses.

Since hydrological cycle is highly coupled with soil biogeochemical cycles, changes in precipitation can strongly affect the nutrient transformations, particularly nitrogen (N) cycling and balance, thus exerting a feedback on climate (Davidson et al., 2008; Wieder et al., 2011). For instance, Annual N_2O emission decreased by a rainfall exclusion in moist

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70 tropical forests, but recovered within the first year after the rainfall exclusion was stopped
71 (Davidson *et al*. (2008). Net N mineralization rate declined sharply in response to increased
72 rainfall, but increased during drought in grasslands (Jamieson et al., 1998). Opposite
73 response patterns were also obtained in temperate forests (Emmett et al., 2004; Chen et al.,
74 2011; Fuchslueger et al., 2014). Nevertheless, limited information was known about the
75 responses of N cycle to seasonal precipitation changes in subtropical forests which serve as
76 important sources of N_2O emission and inorganic N leaching (Fang et al., 2009; Isobe et al.,
77 2012).

78 Seasonal precipitation changes may disturb the natural seasonal dynamics of microbial
79 activities, soil moisture, temperature, plant nutrient uptake, carbon (C) and N availabilities,
80 and consequently the N transformations (Reichmann et al., 2013). Although the direct effects
81 of soil physicochemical properties and microbial communities on N transformations are well
82 documented, the predominant factors in determining N transformations under precipitation
83 changes are still debatable (Petersen et al., 2012; Auyeung et al., 2015).

84 Ammonium oxidation, the central and rate-limiting step in N cycle is driven by
85 ammonia-oxidizing archaea (*AOA*) and bacteria (*AOB*), which are marked by the *amoA*
86 functional gene (van der Heijden et al., 2008). The release and consumption of N_2O by
87 denitrification are mainly driven by nitrite-reducing bacteria marked by the *nirK* and *nirS*
88 genes and nitrous oxide-reducing bacteria marked by *nosZ* gene (Schimel and Bennett,
89 2004; Levy-Booth et al., 2014), respectively. Thus, changes in these microbial functions
90 can shed lights on the underlying mechanisms driving N transformation responses. The
91 abundance, composition and activity of these microbial functional groups largely depend

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on soil moisture, temperature, O_2 diffusion, C and N availabilities - all of these factors are strongly influenced by precipitation (Bell et al., 2014). For instance, reduced precipitation decreases soil moisture and increases aeration and O_2 diffusion, which stimulates the activity of nitrifiers (*AOA*/*AOB*) and nitrification, but constrain the activity of denitrifiers, and consequently the N_2O/N_2 emissions (Stark and Firestone, 1995; Zhalnina et al., 2012). However, both the denitrifiers and nitrifiers can be suppressed by decreased moisture and available C during drought (Bárta et al., 2010; Zhalnina et al., 2012). In addition, increased precipitation raises the NH_4^+:NO_3^- ratio as NO_3^- is easily leached (Reichmann et al., 2013), and consequently alter the predominant microbial groups (Nautiyal and Dion, 2008). The potential for mixotrophic growth and low substrate tolerance of nitrifying communities (Levy-Booth et al., 2014) suggests a broader ecological niche occupied by the nitrifying groups. Therefore, the nitrifying and denitrifying microorganisms may respond differently to seasonal precipitation changes, leading to non-synchronously changes in nitrification and denitrification, and consequently different changes in soil NO_3^-, NH_4^+ and N_2O pools. Nonetheless, the extent to which microorganisms control N transformations remains unclear because soil physicochemical properties can also affect N pools through erosion, leaching, plant uptake and physiological changes in microbial activity, regardless of microbial composition or abundance (Cregger et al., 2014; Auyeung et al., 2015). As a result, the effects of soil physicochemical properties and microbial communities on N transformation rates are difficult to differentiate, which make it difficult to uncover the underlying drivers.

In order to investigate responses of N transformations to seasonal precipitation changes

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and the main controlling factors, a precipitation manipulation experiment was conducted in a subtropical forest in southern China, where the precipitation is projected to increase in wet seasons and decrease in dry seasons (Zhou et al., 2011). We simulated similar seasonal precipitation redistribution by reducing precipitation in dry seasons and increasing frequency of large precipitation events in wet seasons over two years. Changes in soil physiochemical properties, net N transformation rates, nitrifying (bacterial and archaeal *amoA*) and denitrifying (*nirK*, *nirS* and *nosZ*) genes abundance were analyzed and implicated in a hypothetical path model, aiming to test the effects of soil physicochemical properties and microbial abundance on N transformation rates (Fig. 1). The path coefficients and model fitness were analyzed by structure equation model (SEM). We hypothesized that (1) decreasing precipitation in the dry season will reduce N transformation rates via decreasing SWC, C and N availabilities and microbial abundance, but (2) precipitation addition during wet season will have little impact on N transformation due to the originally sufficient SWC and substrate supply; (3) The responses of N transformation rates to precipitation change will be more influenced by functional microorganisms than by other biotic and abiotic variables; (4) microbial abundance is directly influenced by soil physicochemical properties, but denitrifiers will be more easily affected than nitrifiers, because the nitrifiers has the potential for mixotrophic growth and low N and C substrate tolerance.

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2 Material and methods

2.1 Site description

The study site is located at the Heshan National Field Research Station of Forest Ecosystem, Chinese Academy of Sciences (112°54′E, 22°41′N), Heshan City, Guangdong province, southern China. This area has a pronounced wet season (April to September) receiving 80% of the annual rainfall, and a dry season (October to March) with only 20% of the annual rainfall. The soil is typical laterite (or Oxisols based on the USDA soil taxonomy), developed from sandstone, and is easily leached. This study was conducted in a 35-year old evergreen broadleaved mixed species (*EBMS*) forest dominated by *Schima superba* and *Michelia macclurei*. The forest consists about 30 woody species, with average tree height of 8 m, average diameter at breast eight (DBH) of 9.5 cm, stem density of 1430 trees ha^{-1}, and basal area of 11.6 m^2 ha^{-1}.

2.2 Experimental design

A replicated manipulative experiment of precipitation reduction in dry season and precipitation addition in wet season was employed for two years from October 2012 to September 2014. Eight 12 m × 12 m experimental plots were randomly assigned to 4 replicates of each of the 2 treatment types: the seasonal precipitation change manipulation (hereafter precip-change) and the trenched control (hereafter control). Distance between the adjacent plots was at least 2 m. Around the perimeter of each of the 8 plots, a 60-80 cm deep trench was excavated and 1 m height PVC segregation

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board was imbedded to reduce the potential for lateral movement of soil water from the surrounding areas into the plots. The precipitation reduction and addition was realized by throughfall exclusion and water addition facilities, respectively. Throughfall exclusion and water addition facilities were established in the 4 precip-change plots, but not in the control. The facilities included supporting structures, rainout shelters and water addition subsystems (Fig. S1). Within each of the 4 precip-change plots, 16 galvanized steel pipes (2.5-3 m length × 10 cm diameter) were vertically fixed in concrete bases which were imbedded in soil for 60 cm depth, and were welded together with 8 horizontal stainless steel frames (12 m length) at the top. Rainout sheets were fixed in two stainless steel frames and hanged on the supporting system with steel hook rivets. There were about 8-12 rainout sheets (with the width of 50-100 cm) within each precip-change plot depending on the density of tree stems. The rainout sheets were made from polyethylene plastic with > 90% light transmission and installed at approximately 1.5 m height above the soil surface. The total area of all the rainout sheets was 67% of the plot area (i.e., 144 m^2). The sheets were opened to exclude throughfall during dry season (October 1st to March 31) but folded without throughfall exclusion during wet season (April 1st to September 30th). Therefore, we reduced about 67% of the full incoming throughfall in the dry season. The intercepted rainfall was routed into an iron gutter placed at the lower slope of the plots, and then drained outside the plot with PVC pipes.

The water added into precip-change plots in the wet season was pumped from a pond (about 800 m away from the experimental plots) and transported with PVC

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pipes to the rubber sacs fixed on the supporting system, and then sprinkled out via 25 sprinklers distributed evenly in each plot. The pH was similar in the throughfall (6.42) and pond water (6.19), and no differences of the nutrient (e.g. nitrogen and organic carbon) contents between the pond water and throughfall were detected. The amount of water added into a precip-change plot during the wet season was calculated as a product of the above-canopy dry-season rainfall, the throughfall ratio, and the throughfall exclusion ratio (i.e. 0.67). The above-canopy rainfall was obtained from a standard meteorological station (Davis, Vaisala, Finland) about 80 m away from the experimental site. The throughfall ratio was 0.86 obtained from 8 rain gauges (TB4MM, Techno Solutions, Beijing, China) installed about 80 cm above soil surface in the 8 plots. As a result, the intensity of the dry season rainfall events was reduced and the frequency of large rainfall events in wet season was increased, while the annually total quantity of the throughfall was not changed. More specificly, the throughfall excluded was 220 mm in the 2013 dry season (Oct 1st 2012 to Mar 31st 2013) and the same amount water was added back into each PC plot with 4 large events (55 mm day^{-1}) in June through September 2013 (i.e., each event in one month) to mimic the projected occurrence of more large rainfall events in wet season in the region (Zhou et al., 2011). The throughfall exclusion was 170 mm in the 2014 dry season (Oct 1st 2013 to March 31st 2014) and the same amount water was added back into each precip-change plot with 3 large events (57 mm day^{-1}) in June through August 2014 (Fig. 2).

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198 ## 2.3 Soil sampling and analysis

199 Soil samples were collected at the beginning and end of January, March, May, August
200 and October from May 2012 to September 2014 for physicochemical properties, and
201 from January 2013 to September 2014 for microbial functional genes analyses. Soil
202 samples were collected from 0 to 10 cm depth with an auger (Φ35 mm), sieved
203 through a 2 mm mesh to remove litter and stones. One composite soil sample,
204 consisting of six subsamples randomly collected within each plot, was used for the
205 physicochemical (stored at 4 °C) and microbial (stored at -20 °C) analyses. All
206 samples were analyzed within two weeks.

207 Soil physicochemical properties were measured using the methods as described by
208 Liu *et al*. (1996). Briefly, soil water content (SWC) was obtained by drying fresh soils
209 in an oven at 105 °C for 24 h. Total nitrogen (TN) and total phosphorus (TP) were
210 determined using the H_2SO_4 digestion-indophenol blue colorimetry and H_2SO_4
211 digestion-Mo-Sb colorimetry methods, respectively. NH_4^+ and NO_3^- contents were
212 determined from the 2 M KCl extraction liquid by using the indophenol blue
213 colorimetry and copperized cadmium reduction methods, respectively.

214 Soil dissolved organic carbon (DOC) and microbial biomass carbon (MBC) were
215 measured immediately after the soil sampling using the fumigation extraction method
216 described as Vance, Brookes & Jenkinson (1987). In detail, a pair of fresh soil
217 subsamples (10 g) was placed into two glass breakers. One was fumigated in a
218 vacuum dryer with alcohol-free chloroform and NaOH solution for 24 h in dark, and
219 the other one was placed in dark for 24 h without fumigation. The two subsamples

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were extracted with 0.5 M K_2SO_4 after fumigation, and the DOC concentration was determined using a total organic C analysis instrument (TOC-VCSH, Shimadzu, Japan). The difference of DOC concentration between the fumigated and un-fumigated was multiplied by 0.45 to calculate MBC content.

Soil total DNA was extracted from 0.3 g fresh soil using the HiPure Soil DNA Mini Kit (Magen, Guangzhou, China), quantified with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., USA) and stored at -20 °C for further analyses. The abundance of bacterial and archaeal ammonia-monooxygenase gene (*amoA*), nitrite reductase genes (*nirK* and *nirS*) and nitrous oxide reductase gene (*nosZ*) were quantified by using absolute Real-time PCR on an ABI 7500 thermocycler system with primers and thermal profiles presented in the supplementary material (Table S1). The Real-time PCR reactions was performed on 96-well plates (Axygen, USA), with 20 ml volume in each well including 12.5 µl SYBR Premix Ex Taq (TaKaRa Biotechnology, Japan), 1 µl of each primer (10 mmol L^{-1}), 2 µl of DNA template (10 ng), 1 µl Dimethyl sulfoxide and 4.5 µl double-distilled water. Standard curve was generated from a tenfold serial dilution (10^3-10^8 copies per µl) plasmid extracted from clones containing the target genes fragment for the calculation of functional genes abundance in each sample.

2.4 Measurement of N transformation rates

Net N mineralization and nitrification rates were measured through the *in situ* field soil incubation using the resin-core method (Reichmann et al., 2013). Six paired soil

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241 cores (0-10 cm) were randomly sampled within each plot at the beginning of January,
242 March, May, August and October from May 2012 to September 2014. One core of
243 each pair was sieved through a 2-mm sieve after removing litter and stones, and
244 stored at 4 °C for the initial pre-incubation measurements of SWC, NO_3^- and NH_4^+.
245 The other core was incubated for one month in a PVC pipe that was open on both
246 sides and was oriented vertically with an ion exchange resin bag placed at the bottom
247 to collect inorganic N leached from the core. Soil cores and resin bags in the PVC
248 pipes were collected after the one-month incubation, and the soil was sieved and
249 stored at 4 °C for the final post-incubation measurements of SWC, NO_3^- and NH_4^+.
250 The net N mineralization rate was calculated as the final NO_3^- and NH_4^+ content
251 minus the initial NO_3^- and NH_4^+ content, and the net nitrification rate was calculated
252 as the final NO_3^- content minus the initial NO_3^- content (Reichmann et al., 2013).
253 Concentrations of NO_3^- and NH_4^+ extracted from the resin were considered as the
254 leaching rates of NO_3^- and NH_4^+ per month.
255 Nitrous oxide (N_2O) fluxes from soils were measured twice per month, from
256 October 2012 to September 2014, using static chamber and gas chromatography
257 techniques. The static chambers were made from white PVC materials and consisted
258 of a removable cover box and a base. The removable cover box with diameter of 26
259 cm and height of 35 cm, was an open-bottom PVC pipe, equipped with a 12 V fan on
260 the internal top wall to make turbulence sufficiently during gas sampling. The base of
261 the static chamber was nested together by an inside (25 cm diameter × 11 cm height)
262 and an outside (33 cm diameter × 8 cm height) PVC pipes, with a water groove left

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263 between the two pipes for sealing during gas samples collection. The bottom of the
264 base was cut sharply to facilitate soil insertion. Two months before gas sampling, four
265 static chambers were deployed randomly at each plot to minimize effects of
266 installation disturbance.

267 The N_2O samples were collected between 09:00 and 11:00 a.m. local time. Prior to
268 gas sampling, the cover box was placed on the collar filled with water in the groove,
269 and the fan was turned on simultaneously. The static chamber was closed for 30
270 minutes, and gas samples were taken using 100 ml plastic syringes at the initial closed
271 time as well as every 10 minute thereafter during the closed period. When collecting
272 gas samples, the soft rubber hose connected with static chamber was cleaned
273 thoroughly by pumping plastic syringe for three times, then 80 ml gas sample inside
274 the chamber was collected and transferred into a 500 ml polyethylene-aluminum
275 coated gas sampling bag. At the same time, values of atmospheric pressures and air
276 temperatures inside static chambers were measured for three times. After gas
277 sampling, cover boxes were removed to reduce disturbance to experimental plots as
278 much as possible. N_2O concentrations were analyzed in the laboratory by gas
279 chromatography (Agilent 7890A, Agilent Technologies, USA) equipped with an
280 electron capture detector set at 300 °C and a stainless porapak-Q column set at 70 °C
281 within 24 hours following gas sampling. N_2 was used as carrier gas at the flow rate of
282 30 ml min^{-1}. The N_2O concentration of standard gas for system calibration was 332
283 ppbV. The N_2O flux was calculated by changes of N_2O concentrations inside static
284 chamber during periods of gas sampling, with the equation as follows:

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$$\mathbf{F} = \boldsymbol{\rho} \times \frac{V}{A} \times \frac{P}{P\mathbf{0}} \times \frac{T\mathbf{0}}{T} \times \frac{dC}{d_t}$$

Where F stands for the flux of N_2O (mg m^{-2} hr^{-1}), ρ stands for the density of N_2O under standard condition (g L^{-1}), V stands for the effective volume of chamber (m^3), A stands for the area of soil covered by chamber (m^2), P and T stand for the atmospheric pressures (Pa) and absolute air temperature inside chamber (K) when gas sampling, P_0 and T_0 stand for the atmospheric pressures (Pa) and the absolute temperature (K) under standard condition, and $\frac{dC}{dt}$ stands for changes of N_2O concentrations in the chamber during gas sampling.

2.5 Statistical analysis

Two-way repeated-measures analysis of variance (ANOVA) with sampling time as the repeated factor was used to examine the effects of precip-change and sampling time on all measured parameters. Pillai's trace from multivariate test was used for within-subjects test when the assumption of multisample sphericity was not met. Independent samples *t* tests were used to detect the difference of each variable between precip-change and control at each sampling time. All the parameters were explored for normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levène test) prior to the analyses, and log-transformed If necessary. All statistical analyses described above were performed using SPSS v.16.0 (SPSS Inc., Chicago, IL, USA).

Structural Equation Modeling (SEM) were performed with AMOS 21.0 (SPSS Inc., Chicago, IL, USA) to test the hypothetical causal relationships among soil physicochemical properties, microbial abundance and N transformation rates in the

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306 conceptual model (Fig. 1). How the effects of soil physicochemical properties and
307 microbial abundance determine the responses of N transformation rates were
308 evaluated. In order to explicitly illustrate the pathways of soil physicochemical
309 properties and microbial abundance involved in each N transformation process, three
310 individual models were constructed corresponding to the conceptual model to explain
311 the responses of (a) net nitrification, (b) net N mineralization and (c) N_2O emission
312 rates. Three models may be easier to discover the controlling factors than one
313 complex model which implicates all the measured processes. In these models, the
314 precip-change treatments are categorical exogenous variables with two levels: 0
315 representing control and 1 representing seasonal precipitation changes (Delgado-
316 Baquerizo et al., 2014). Abundance of both *nirK* and *nirS* genes were evidenced
317 correlated with nitrification or N mineralization rates (Levy-Booth et al., 2014).
318 Therefore, *nirK* and *nirS* abundance were added as one (*nirK*+*nirS*) endogenous
319 factors in model. Net nitrification rate was included in model (b) as an endogenous
320 factor because it may influence N_2O emission through altering the production of NO_3^-
321 as the substrate for N_2O production. Prior to the SEM analyses, normal distribution of
322 all the involved variables were examined, and genes abundance were log-transformed.
323 Goodness of model fits was evaluated by chi-square test ($p > 0.05$), comparative fit
324 index (CFI > 0.95), and root square mean errors of approximation (RMSEA < 0.05)
325 (Hu and Bentler, 1998; Schermelleh-Engel et al., 2003). Pathways without significant
326 effects were not shown ($p > 0.05$) in the final models.

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3 Results

3.1 Responses of soil physicochemical properties, N transformation rates and microbial abundance to precipitation changes

Before the precipitation manipulation from May to September in 2012, average net N transformation (i.e. N nitrification, mineralization and leaching) rates, N (NO_3^-, NH_4^+, TN) and organic C (MBC, DOC, TOC) contents as well as soil temperature were similar among all plots (Table S2). In the two dry seasons with precipitation reduction, SWC decreased by 16 % in 2013 and by 21 % in 2014 ($p < 0.01$, Fig. 2, Table S3). Similarly, NO_3^- concentration decreased by 35 % and 24 % in 2013 and 2014, respectively ($p < 0.01$, Fig. 2, Table S3). Opposite patterns were observed for NH_4^+ concentration, which increased with the precipitation reduction (Fig. 2). In the wet seasons with precipitation addition, SWC, NO_3^- concentration, DOC and MBC remained lower in the precip-change plots than in the control plots in both years (Fig. 2, Table S3).

Precipitation reduction strongly decreased the average dry-season net nitrification rate by 13 % in 2013 and by 20 % in 2014, and decreased net N mineralization rate by 16 % in 2013 and by 18 % in 2014 ($p < 0.1$, Fig. 3, Table S4). The NO_3^- leaching also declined with precipitation reduction, especially in 2014 with a marked decrease by 22 % ($p < 0.001$, Fig. 3, Table S4). Contrastingly, the rates of three N transformation processes increased by 50% with precipitation addition in the 2013 wet season whereas changed little in the 2014 wet season (Fig. 3). Throughout the two years, moderate decreases were detected in N_2O emission either during dry-season precipitation

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reduction (35%) or during wet-season precipitation addition (15%) (Fig. 3, Table S4).

No amplification of bacterial *amoA* gene was detected in soil neither from the precip-change plots nor from the control plots, which was mainly because soil *AOB* community abundance in the studied forest was under the detection limit (Isobe et al., 2012). The average seasonal archaeal *amoA* gene was $6.5\times10^6 \pm 1.9\times10^6$ copies g^{-1} dry soil, and varied significantly according seasonal precipitation changes. With precipitation reduction, the archaeal *amoA* gene abundance changed little in the 2013 dry season but decreased by 70% in the 2014 dry season (Fig. 4). The abundance of three denitrifying genes (*nirK*, *nirS* and *nosZ*) increased with precipitation reduction by 30-80% in the 2013 dry season ($p < 0.05$, Fig. 4, Table S5). In both seasons of 2014, neither dry-season precipitation reduction nor wet-season precipitation addition had significant impacts on the abundance of the three denitrifying genes (Fig. 4, Table S5).

3.2 Paths determining N transformation rates and functional microbial abundance

Although the annual precipitation amount was kept constant, the redistribution of seasonal precipitation imposed an overall negative impact on SWC and NO_3^- concentration (Fig. 5). SWC affected net nitrification and N mineralization through a direct negative path and N_2O emission through a direct positive path (Fig. 5). Net N mineralization, nitrification and N_2O emission rates were also affected by the functional genes abundance and MBC paths. Since bacterial *amoA* gene was not detected, we only

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use the archaeal *amoA* abundance as the dominant nitrifying microbial abundance in the SEM analyses. Specifically, the archaeal *amoA* gene abundance and MBC had direct positive impacts on net N mineralization and nitrification rates, whereas the *nosZ* gene abundance had a direct negative impact on N_2O emission (Fig. 5). As a result, 21% and 22% of the net N mineralization and nitrification variability are explained, respectively (see the r^2 in Fig. 5). Among the direct influential factors, archaeal *amoA* abundance showed the strongest correlations either with net N mineralization or with net nitrification rates. Soil N_2O emission was mostly affected by positive effects of net nitrification rate and SWC, followed by negative effects of *nosZ* abundance and MBC, and as much as 42% of the total variation could be explained (see the r^2 in Fig. 5).

Precip-change-induced changes in SWC had no direct impacts on functional genes abundance. Instead, the functional genes abundance was indirectly affected by the precip-change-induced alterations in NO_3^-, NH_4^+ concentrations and DOC (Fig. 5). Specifically, NO_3^- and NH_4^+ had direct positive effects on archaeal *amoA* abundance whereas DOC had a direct negative effect on *nirK*+*nirS* abundance. Both NH_4^+ and DOC concentration had direct positive impacts on the *nosZ* abundance (Fig. 5). Changes in MBC were directly positively influenced by SWC and DOC.

4 Discussion

4.1 Drivers of N transformation processes

Consistent with our hypotheses, seasonal precipitation redistribution induced significant changes in net N mineralization and nitrification rates through altering SWC,

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391 MBC and archaeal *amoA gene* abundance. N_2O emission decreased moderately either
392 in precipitation reduction or addition, which indicated that soil N loss by N_2O emission
393 in subtropical forests would be alleviated by the predicted seasonal precipitation
394 changes. In contrast, increased NO_3^- leaching during precipitation addition in wet
395 season led to a significant loss of soil NO_3^- pool. During the two years' experiment,
396 SWC was always lower in precip-change plots than in control plots, despite of the
397 precipitation addition in the wet seasons (Fig. 2). One reason is the higher transpiration
398 loss resulting from bigger trees in the precip-change plots than in the control plots. The
399 average tree height and DBH were respectively 10.2 ± 5.0 m and 10.7 ± 6.3 cm in the
400 four precip-change plots with the total number of 64 tree individuals, compared to 7.7
401 ± 3.5 m and 9.5 ± 5.2 cm in the four the control plots having the total number of 68 tree
402 individuals.

403 Initially, we hypothesized that decreased precipitaiton in the dry season would
404 suppress N transformation, and precipitation addition during wet season would have
405 little impact on the N transformation processes because the soils are water saturated and
406 substrate sufficient. Agreeing with the first hypothesis, the net nitrification and N
407 mineralization rates decreased sharply with the reduction of throughfall in dry season
408 (Fig. 3). However, disagreeing with the second hypothesis, the nitrification and N
409 mineralization rates increased markedly during precipitation supplement in the wet
410 seasons (Fig. 3). These results were caused by the interactions among microbial
411 abundance, soil moisture and substrate availability (Fig. 5 and S3). Specifically, DOC
412 of the dry season was less available in the precip-change plots than in the control plots

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(Fig. 2), probably due to reduced C input by less root production and exudation after drying (Kuzyakov and Domanski, 2000; Borken and Matzner, 2009). The reduced soil C substrate (or DOC) could suppress the growth of soil microorganisms (e.g. MBC and *AOA*), and therefore resulted in decreased net nitrification and mineralization rates (Fig. 3 and 5). Although increased NH_4^+ concentration during precipitation reduction could provide more N substrate for nitrifier, the negative effects of decreased SWC and DOC may have overwhelmed the positive effects of increased NH_4^+ on microbial nitrification process in dry season. Instead, the accumulated NH_4^+ after dry season precipitation reduction might have a positive legacy effect on soil microbial activity in wet season, leading to increased N transformations. In addition, SWC also directly affected N transformations by physiological changes in microbial activity, regardless of microbial abundance and composition (Auyeung et al., 2015). The increased N transformation rates in respond to decreased SWC, MBC and archaeal *amoA* gene abundance during precipitation addition might be one of such cases. A 10% decrease of SWC in the natural humid wet season might create a better redox conditions for microbial nitrification, as excessive soil moisture could reduce soil oxygen concentration. According to Borken & Matzner (2009), the increases of soil microbial activity by rewetting usually occurred with an increased pulse in reconstituting mineralization of SOM as well as an increase of organic substrate availability. This study revealed substantial decrease in MBC and archaeal *amoA* gene abundance, which indicate that microorganisms may reduce microbial abundance and release the MBC and MBN from dead or non-active microorganisms to support the increased energy demand caused by increased microbial

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activity and accelerated microbial processes (Borken and Matzner, 2009).

We also hypothesized that the N transformation processes are predominantly influenced by functional microbial abundance. As expected, net N mineralization and nitrification rates showed stronger relationships with archaeal *amoA* abundance than that with MBC and other soil properties (Fig. 5). However, MBC and denitrifying gene abundance had similar effects on N_2O emission, and only *nosZ* gene abundance exerted a pronounced effect on N_2O emission (Fig. 5), which may through reducing the N_2O consumption (Henderson et al. 2010; Levy-Booth et al. 2014). No significant correlation between N_2O emission and *nirK* + *nirS* gene abundance was detected, which was inconsistent with previous researches (Levy-Booth et al., 2014). The N_2O emission related denitrification can be also performed by nitrifiers and fungi in soils with high aeration and limited substrate availability (Levy-Booth et al. 2014). The experimental seasonal precipitation strongly decreased SWC and DOC content (Fig. 1), which could lead to higher aeration while lower substrate availability, and consequently predominant roles of nitrifier and fungi denitrification in controlling N_2O emission. In addition, SWC and nitrification rate also directly affected N_2O emission via altering substrate availability and consequent microbial activity, despite of high microbial abundance (Fig. 5). Overall, net nitrification and N mineralization were mainly regulated by *AOA* abundance, while the controlling factors of N_2O emission were complex.

4.2 Determinants of nitrifying and denitrifying gene abundance

The responses of both nitrifying and denitrifying genes were mainly related to the

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456 changes in substrate concentrations. SEM analysis showed that both *amoA* and *nosZ*
457 genes abundance were positively affected by DOC and NH_4^+ concentration,
458 suggesting substrate constraints for these two functional microbial groups. This
459 disagreed with previous studies reporting that *AOA* community had the potential of
460 mixotrophic growth and low substrate tolerance when compared with its counterpart
461 *AOB* (Erguder et al., 2009; Shen et al., 2012). However, these results were mainly
462 caused by a stronger competitiveness of *AOA* than its counterpart *AOB*, as these
463 studies mainly focused on the relative effects of substrate availability on *AOA* and
464 *AOB*. Both *nosZ* and *amoA* genes abundance increased with DOC and NH_4^+
465 concentration (Fig. 5), which indicated *AOA* community could be constrained by C
466 and N substrates when competing with other microbes that have different functions.
467 Otherwise, the existing *AOA* species that have the potential ability of mixotrophic
468 growth and low substrate tolerance may not dominante in the studied subtropical
469 forest, as the soil is originally rich in SOM (Zhou et al., 2006; Chen et al., 2015).
470 Therefore, *AOA* community in the studied soil could be easily influenced by changes
471 in soil C and N availability.

472 The abundance of *nirK* and *nirS* genes was positively controlled by soil NH_4^+
473 concentration and negatively controlled by DOC content (Fig. 5). This further
474 confirmed that more NH_4^+ content could favor more abundant microorganisms
475 containing *nirK* or *nirS* genes (Yi et al., 2015), because higher NH_4^+ concentration
476 could supply sufficient NO_3^- as the direct substrate or optimum pH value for growth
477 of the denitrifying microorganisms. The negative effect of DOC on *nirK* and *nirS*

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gene abundance was inconsistent with most of previous reports that denitrifiers are primary heterotrophic (Bárta et al., 2010). One reason is because high DOC concentration can constrain the growth of microorganisms containing *nirK* and *nirS* genes through effecting other factors, such as pH and C:N ratio (Henderson et al., 2010; Levy-Booth et al., 2014). Generally, abundance of both nitrifying and denitrifying gene abundance changed with precipitation redistribution, and the direction and magnitude of the changes depended mostly on soil N and C substrate availabilities.

5 Conclusions

To summarize, soil net nitrification and N mineralization rates responded significantly to seasonal precipitation redistribution, and more than 20% of the variation could be explained by the effects of microbial abundance, SWC, soil C and N substrates. *AOA* community abundance was the main factor in regulating these two N transformation processes. N_2O emission during the two years' seasonal precipitation redistribution decreased moderately, and as much as 42% of the total variation in N_2O emission was attributed to the total effects of SWC, nitrification rate, MBC and *nosZ* gene abundance. The accumulated NH_4^+ due to precipitation reduction may stimulate nitrification process in wet season, and consequently accelerate N loss from NO_3^- leaching. Therefore, long term of the predicted seasonal precipitation changes in subtropical forests may result in profound changes in different N pool size, with less N_2O emission while more NO_3^- leaching, which in turn exert a feedback to climate and environmental changes. Meanwhile, changes in functional microbial abundance

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500 induced by soil DOC and NH_4^+ substrate availabilities will be the predominant driver
501 in regulating the extent and direction of soil N transformation changes.

502 Author contribution

503 Jie Chen and Guoliang Xiao carried out the experiment, analyzed the data and wrote
504 the draft manuscript. Weijun Shen conceived the study. All authors contributed to
505 manuscript writing and revision.

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512 Competing interests

513 The authors declare that they have no conflict of interest.

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Figure captions

Fig. 1. A conceptual model illustrating the effects of physiochemical properties and functional microorganisms on N transformation rates. Soil water content (SWC), ammonium (NH_4^+), nitrate (NO_3^-) and dissolved organic carbon (DOC) concentrations were included in the group of soil physiochemical property. Microbial biomass carbon (MBC), nitrifying (*amoA*) and denitrifying (*nirK*, *nirS* and *nosZ*) gene abundance were included in the microbial attributes group. The solid lines with arrows indicate the direction of the effect.

Fig. 2. Seasonal dynamics of precipitation and soil physiochemical properties in control and precip-change plots over the course of experiment. Points and bars with standard error ($n = 4$) show mean values at each sampling time and in dry (DS) and wet (WS) seasons. Grey shades indicate the periods of precipitation reduction. The significance levels are presented as: $*p < 0.05$.

Fig. 3. Nitrogen transformation rates measured in control and precip-change plots over the course of experiment. Points and bars with standard error ($n = 4$) show mean values at each sampling time and in dry (DS) and wet (WS) seasons. Grey shades indicate the periods of precipitation reduction. The significance levels are presented as: $*p < 0.05$.

Fig. 4. Copy numbers of archaeal *amoA*, *nirK*, *nirS* and *nosZ* gene per gram dry soil measured in control and precip-change plots over the course of experiment. Points and bars with standard error ($n = 4$) show mean values at each sampling time and in

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688 dry (DS) and wet (WS) seasons. Grey shades indicate the periods of precipitation
689 reduction. The significance levels are presented as: $*p < 0.05$.

690 **Fig. 5.** Path diagrams demonstrating the effects of soil physicochemical properties
691 and functional genes abundance on net nitrification, N mineralization and N_2O efflux
692 rates in response to precipitation change during two years. Numbers adjacent to
693 arrows are path coefficients, which indicate the relationships between the two
694 variables on both sides of the arrows. Solid and dash lines represent positive and
695 negative paths, respectively. The r^2 above or below each response variable in the
696 model denotes the proportion of variance which could be explained. Size of the lines
697 indicate significant levels of path coefficients.

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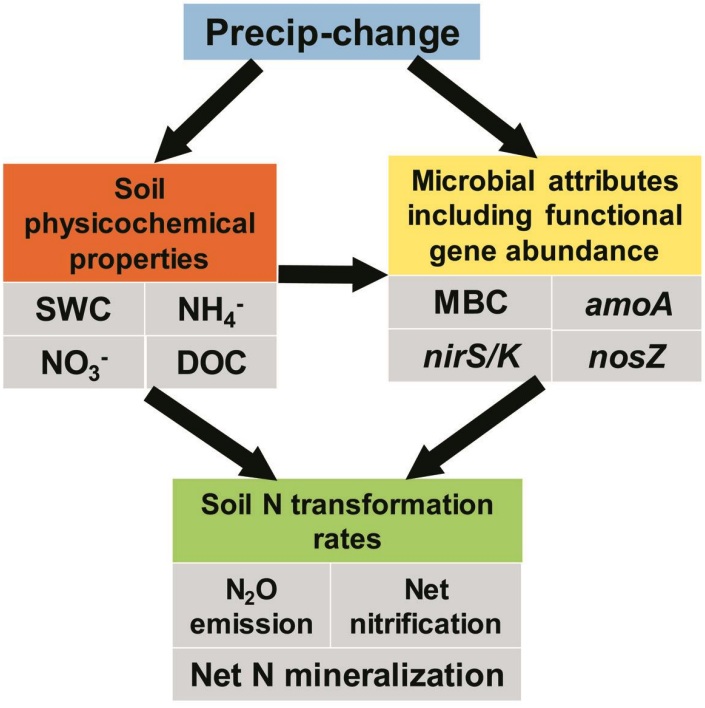
698 **Fig. 1**



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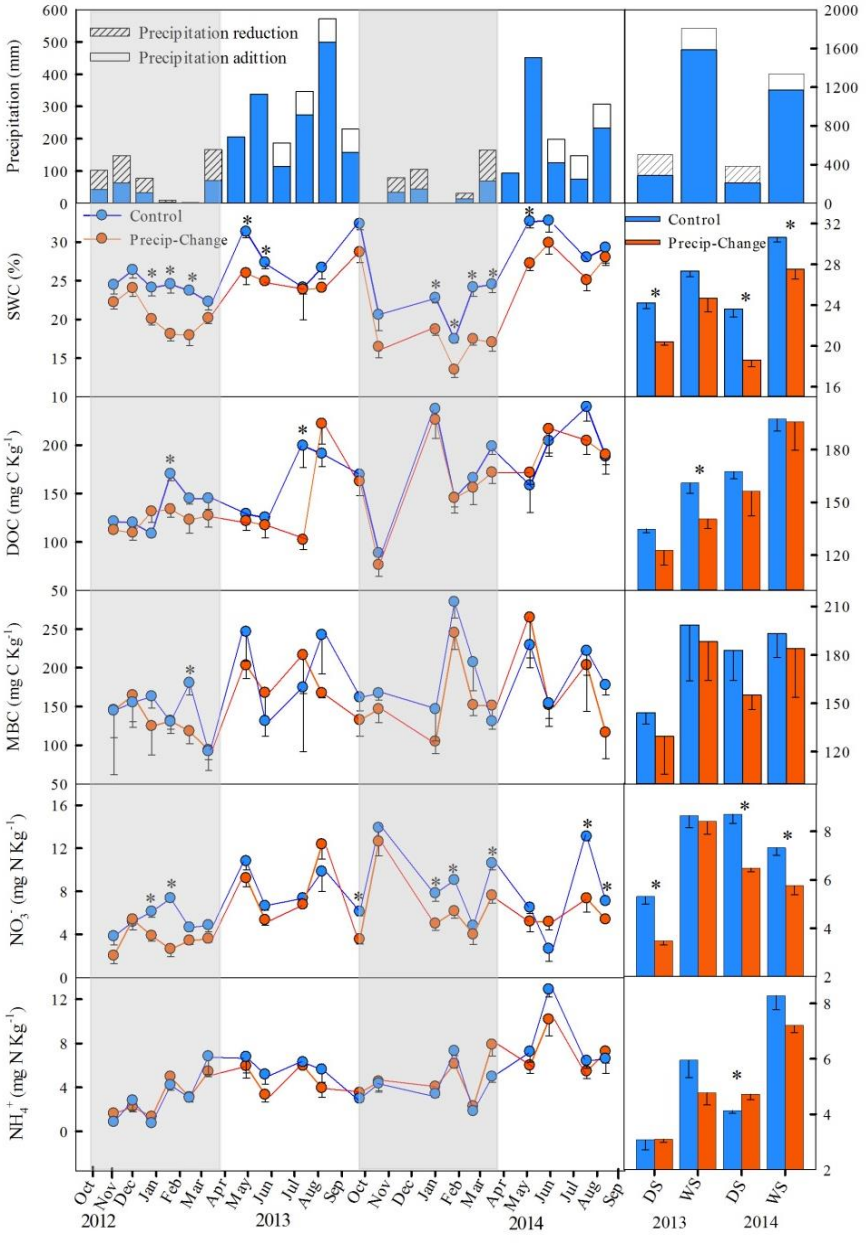
700 **Fig. 2**



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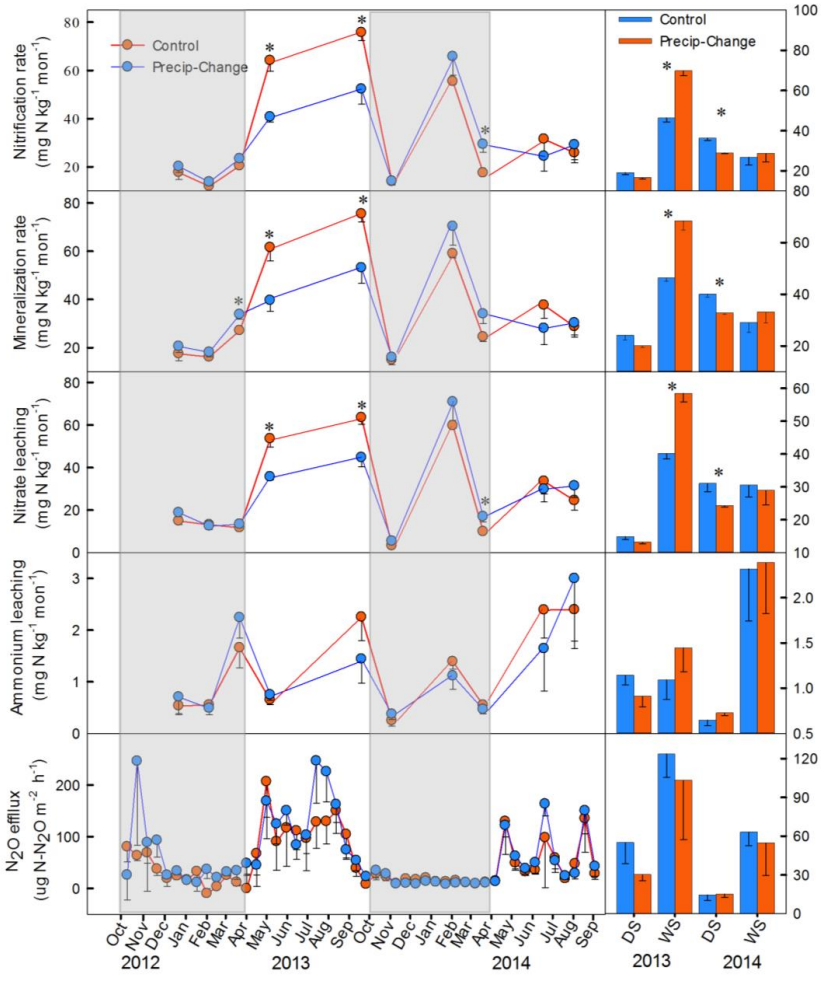
702 **Fig. 3**



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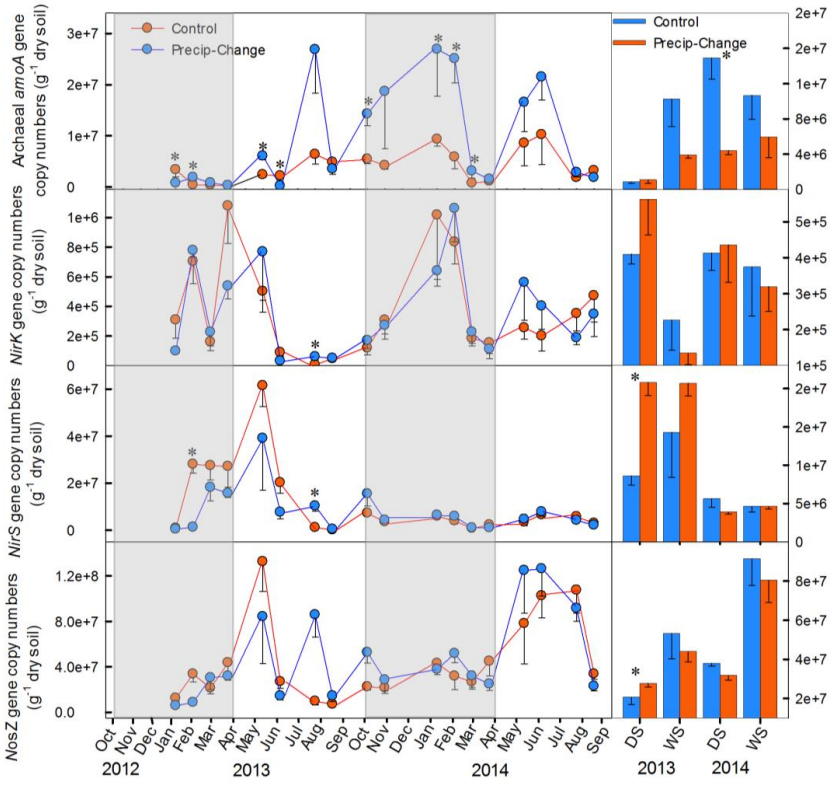
704 **Fig. 4**



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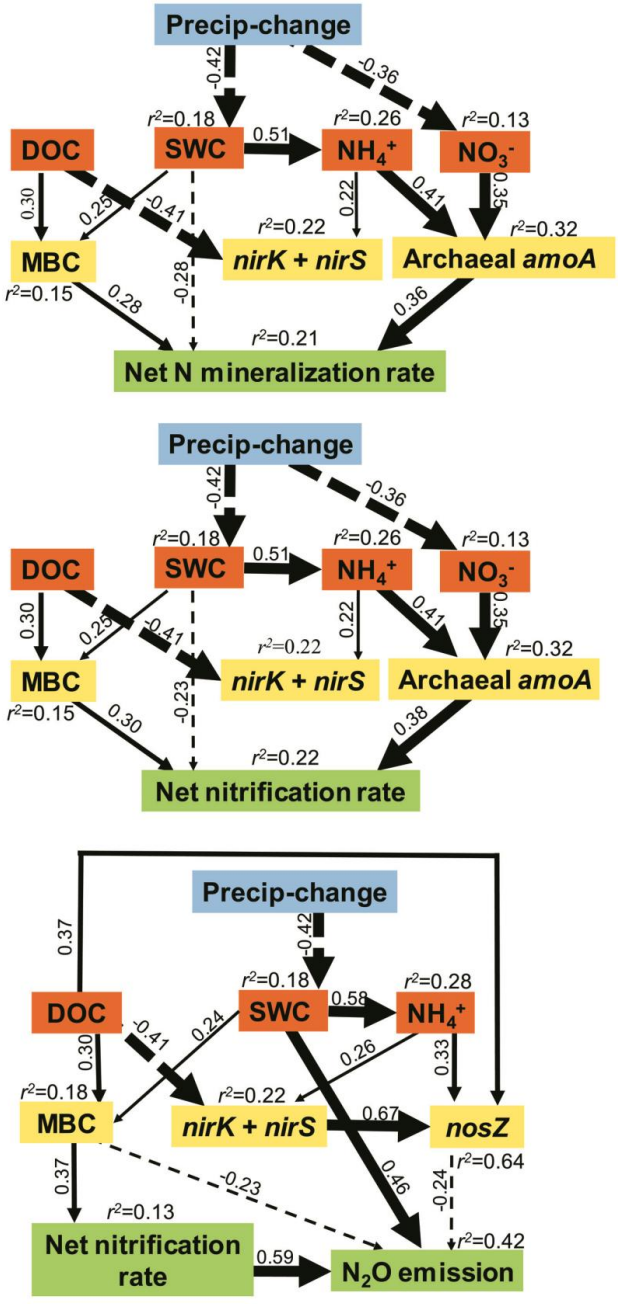
706 **Fig. 5**



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