Title: 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 in a subtropical forest to reduce dry-season precipitation and increase wet-season precipitation, ~~while keeping the~~ with 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*) gene~~s~~ abundance, microbial biomass carbon (MBC), extractable organic carbon (EOC), NO_3^-, NH_4^+ and soil water content (SWC) ~~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~~. Notably, archaeal *amoA* abundance showed the ~~high~~strongest correlation ~~coefficients (≥ 0.35)~~ with net N transformation rates (r≥ 0.35), suggesting the critical role of archaeal *amoA* abundance in determining N transformations. Increased net nitrification in the wet season, together with large precipitation events, caused substantial NO_3^- losses via leaching. However, N_2O emission decreased moderately ~~either~~ in both dry and~~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 soil N transformations ~~mainly~~ through altering functional microbial abundance and MBC, which are ~~mainly~~further affected by changes in ~~extractable organic carbon (EOC) and NH_4^+ which are further determined by changes in DEOC~~ and NH_4^+ availabilities. ~~Such contrasting precipitation pattern will increase droughts and NO_3^- leaching in subtropical forests.~~

Key-words: Denitrification, functional genes, nitrification, nitrogen cycle, precipitation change, N_2O emission

1 Introduction

Precipitation changes caused by global climate change are predicted to be increasingly severe over the coming 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~~ global changes such as elevated CO_2 and temperature (Beier et al., 2012). ~~Despite~~ In addition to the frequency and intensity of precipitation events, seasonal precipitation changes are of increasing severity in some regions of the world (Easterling et al., 2000). For example, an analysis ~~Recent study~~ of 60 years of precipitation data showed remarkable seasonal precipitation redistribution in ~~a~~ subtropical ~~forest~~China, with more frequent droughts in dry season and extreme~~ly~~ rainfall events in wet season (Zhou et al., 2011). In contrast to changes in total annual precipitation ~~amount~~, ~~seasonal~~ redistribution of seasonal precipitation may be more important in controlling ~~the~~ ecosystem function~~ing~~ in subtropical forests due to~~, because of~~ strong contrasts 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 annual precipitation amounts (Beier *et al*., 2012). The consequences of seasonal precipitation redistribution at ecosystem levels are still under investigation. ~~Altogether, f~~Field experiments simulating seasonal precipitation changes in subtropical regions are urgently needed for better understanding of the ecosystem responses.

~~Since the hydrological cycle is highly tightly coupled with soil biogeochemical N cycles,~~

changes Changes in precipitation can strongly affect the nutrient transformations, particularly soil nitrogen (N) cycling and balance, thus exerting a feedback on climate (Davidson et al., 2008; Wieder et al., 2011). For instance, Annual annual N_2O emission was decreased by a rainfall exclusion experiment in the moist tropical forest, but recovered within the first year after the rainfall exclusion was stopped Davidson *et al*. (2008). In grasslands, the Nnet N mineralization rate declined sharply in response to increased rainfall, but increased during drought in grasslands (Jamieson et al., 1998). Contrasting responses of N transformation have also been obtained in temperate forestsOpposite responses in N transformation patterns were also obtained in temperate forests (Emmett et al., 2004; Chen et al., 2011; Fuchslueger et al., 2014). NeverthelessHowever, limited information was knownexists about the responses of N cycle to seasonal precipitation changes in subtropical forests, which serve as important sources of N_2O emission and inorganic N leaching (Fang et al., 2009; Isobe et al., 2012). Seasonal precipitation changes may affect N transformations by disturbing the natural seasonal dynamics of microbial activities, soil moisture, temperature, plant nutrient uptake, and carbon (C) and N availabilities , and consequently the N transformations (Reichmann et al., 2013). Although the direct effects of soil physicochemical properties and microbial communities on N transformations are well documented, the predominant factors in determining N transformations under precipitation changes are still debatable (Petersen et al., 2012; Auyeung et al., 2015).

Ammonium oxidation, the central and rate-limiting step in N cycling,e is driven by ammonia-oxidizing archaea (*AOA*) and bacteria (*AOB*), which are marked by the *amoA* functional gene (van der Heijden et al., 2008). The release and consumption of N_2O by

denitrification are mainly driven by nitrite-reducing bacteria ~~marked by~~carrying the *nirK* and *nirS* genes and nitrous oxide-reducing bacteria ~~marked by~~carrying the *nosZ* gene (Schimel and Bennett, 2004; Levy-Booth et al., 2014)~~, respectively~~. Thus, changes in these functional microorganisms ~~microbial functions~~ can shed light~~s~~ on the underlying mechanisms ~~in~~ ~~driving~~of N transformation responses. The abundance, composition and activity of these microbial functional groups largely depend on soil moisture, temperature, O_2 diffusion, and C and N availabilities - all of these factors are strongly influenced by precipitation (Bell et al., 2014). For instance, previous research has~~es~~ shown~~ed~~ that reduced precipitation ~~decreases~~ decrease~~ds~~ soil moisture and increase~~ds~~~~s~~ aeration and O_2 diffusion, which ~~stimulates~~ stimulate~~ds~~ the activity of nitrifiers (*AOA*/*AOB*) and nitrification (Stark and Firestone, 1995; Zhalnina et al., 2012). In contrast, reduced precipitation could constrain the activity of denitrifiers, and consequently reduced the N_2O/N_2 emissions (Stark and Firestone, 1995; Zhalnina et al., 2012). ~~). 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 reduce the N_2O/N_2 emissions (Stark and Firestone, 1995; Zhalnina et al., 2012).~~ ~~However, b~~Both ~~the~~ denitrifiers and nitrifiers ~~can~~ might 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).~~ In addition, increased precipitation may raise~~d~~ the NH_4^+:NO_3^- ratio, as NO_3^-~~NO3~~ is easily leached (Reichmann et al., 2013). ~~Moreover, h~~High NH_4^+:NO_3^- ratios can consequently alter the

predominant microbial groups (Nautiyal and Dion, 2008). The potential for mixotrophic growth and starvation tolerance~~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-synchronous~~ly~~ changes in nitrification and denitrification, and consequently different changes in soil NO_3^-, NH_4^+ contents and N_2O emission ~~pools~~. ~~Nonetheless~~However, 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~~microorganisms, 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 makes it difficult to uncover the underlying drivers.

In order to investigate responses of N transformations to seasonal precipitation changes and the main controlling factors,~~-~~ a precipitation manipulation experiment was conducted in a subtropical forest in southern China, where the precipitation is ~~projected~~ predicted to increase in wet seasons and decrease in dry seasons (Zhou et al., 2011). We simulated ~~the similar~~this seasonal precipitation ~~redistribution~~ pattern for two years~~, by reducing precipitation in dry seasons and increasing the frequency of large precipitation events in wet seasons over two years~~. Changes in soil physicochemical properties, net N transformation rates, and nitrifying (bacterial and archaeal *amoA*) and denitrifying (*nirK*, *nirS* and *nosZ*) gene~~s~~ abundance were analyzed and integrated~~implicated~~ in a hypothetical path model

which assumed that the precipitation-induced changes in soil physicochemical properties and microbial abundance ~~on~~ could alter N transformation rates (Fig. 1). The path coefficients and model fitness were analyzed by a structure equation model (SEM) (Petersen et al., 2012; Delgado-Baquerizo et al., 2014). We hypothesized that (1) decreasing precipitation in the dry season will reduce N transformation rates ~~via~~ by decreasing SWC, C and N availabilities, and microbial abundance, but (2) precipitation addition during the wet season will have little impact on N transformation due to the originally sufficient SWC and substrate supply; (3) ~~The~~ the responses of N transformation rates to the precipitation change will be associated with ~~the responses by of~~changes in functional ~~microorganism~~ ~~microbial~~gene ~~s~~abundance ~~than by other biotic and abiotic variables~~, because N transformation processes are primarily catalyzed by specific enzymes coded by functional genes ~~within~~; (4) microbial abundance is directly influenced by soil physicochemical properties, but denitrifiers will be more ~~easily~~ strongly affected than nitrifiers, because the nitrifiers ha~~s~~ves the potential for mixotrophic growth and are tolerant of low N and C ~~substrate tolerance~~substrate ~~supply~~availabilities.

2 Materials 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 (Wang et al., 2009). 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 vegetation inventory was conducted in the studyied forest by recording species name, diameter at breast height (DBH), tree height and density prior to the experiment. Generally, The 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. Prior to the experiment, the stand characteristics between the precip-change and control plots were compared, and no significant differences were detected. Generally, the four precip-change plots have average tree height and DBH of 10.2 ± 5.0 m and 10.7 ± 6.3, respectively, with average crown width of 46 ± 11 m^2 and total number of 64 tree individuals. The average tree height, DBH, crown width and total tree number in the four control plots are 7.7 ± 3.5 m, 9.5 ± 5.2 cm, 49 ± 13 m^2 and 68, respectively. Around the perimeter of each of the 8 plots, a 60-80 cm deep trench was excavated and 1 m height PVC segregation 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

196 sheets was 67% of the plot area (i.e., 144 m^2). The sheets were opened to exclude
197 throughfall during dry season (October 1st to March 31) but folded without throughfall
198 exclusion during wet season (April 1st to September 30th). Therefore, we reduced about
199 67% of the full incoming throughfall in the dry season. The intercepted rainfall was
200 routed into an iron gutter placed at the lower slope of the plots, and then drained outside
201 the plot with PVC pipes.
202 The water added into precip-change plots in the wet season was pumped from a pond
203 (about 800 m away from the experimental plots) and transported with PVC pipes to the
204 rubber sacs fixed on the supporting system, and then sprinkled out via 25 sprinklers
205 distributed evenly in each plot. The pH was similar in the throughfall (6.42) and pond
206 water (6.19)~~, and no differences of~~ but the nutrient (e.g. nitrogen and organic carbon)
207 contents was higher in throughfall than in ~~between~~ the pond water (Zhao et al., 2017),
208 which assures that we did not enrich nutrients while adding water ~~and throughfall were~~
209 ~~detected~~. The amount of water added into a precip-change plot during the wet season
210 was calculated as a product of the above-canopy dry-season rainfall, the throughfall
211 ratio, and the throughfall exclusion ratio (i.e. 0.67). The above-canopy rainfall was
212 obtained from a standard meteorological station (Davis, Vaisala, Finland) about 80 m
213 away from the experimental site. The throughfall ratio was 0.86 obtained from 8 rain
214 gauges (TB4MM, Techno Solutions, Beijing, China) installed about 80 cm above soil
215 surface in the 8 plots. As a result, the intensity of the dry season rainfall events was
216 reduced and the frequency of large rainfall events in wet season was increased, while
217 the annually total quantity of the throughfall was not changed. More specifically, 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. 2a).

2.3 Soil sampling and analyses

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

Soil physicochemical properties were measured using the methods as described by Liu *et al*. (1996). Briefly, soil water content (SWC) was obtained by drying fresh soils in an oven at 105 °C for 24 h. Total nitrogen (TN) and total phosphorus (TP) were determined using the H_2SO_4 digestion-indophenol blue colorimetry and H_2SO_4

digestion-Mo-Sb colorimetry methods, respectively. NH_4^+ and NO_3^- contents were determined from the 2 M KCl extraction liquid by using the indophenol blue colorimetry and copperized cadmium reduction methods, respectively.

Soil extractable~~dissolved~~ organic carbon (~~DOC~~EOC) and microbial biomass carbon (MBC) were measured immediately after the soil sampling using the fumigation extraction method described as Vance, Brookes and Jenkinson (1987). In detail, a pair of fresh soil subsamples (10 g) was placed into two glass breakers. One was fumigated in a vacuum dryer with alcohol-free chloroform and NaOH solution for 24 h in dark, and the other one was placed in dark for 24 h without fumigation. The two subsamples were extracted with 0.5 M K_2SO_4 after fumigation, and the ~~DOC~~ EOC concentration was determined using a total organic C analysis instrument (TOC-VCSH, Shimadzu, Japan). The difference of ~~DOC~~ EOC 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 cores (0-10 cm) were randomly sampled within each plot at the beginning of January, March, May, August and October from May 2012 to September 2014. One core of each pair was sieved through a 2-mm sieve after removing litter and stones, and stored at 4 °C for the initial pre-incubation measurements of SWC, NO_3^- and NH_4^+. The other core was incubated for one month in a PVC pipe that was open on both sides and was oriented vertically with an ion exchange resin bag placed at the bottom to collect inorganic N leached from the core. Soil cores and resin bags in the PVC pipes were collected after the one-month incubation, and the soil was sieved and stored at 4 °C for the final post-incubation measurements of SWC, NO_3^- and NH_4^+. The net N mineralization rate was calculated as the final NO_3^- and NH_4^+ content minus the initial NO_3^- and NH_4^+ content, and the net nitrification rate was calculated as the final NO_3^- content minus the initial NO_3^- content (Reichmann et al., 2013). Concentrations of NO_3^- and NH_4^+ extracted from the resin were considered as the leaching rates of NO_3^- and NH_4^+ per

month.

Soil nNitrous oxide (N_2O) effluxes from soils were measured twice per month, from October 2012 to September 2014, using static chamber and gas chromatography techniques. The static chambers were made from white PVC materials and consisted of a removable cover box (26 cm diameter ×35 cm height) and a base (33 cm diameter × 11 cm height). The removable cover box with diameter of 26 cm and height of 35 cm, was an open-bottom PVC pipe, equipped with a 12 V fan on the internal top wall to make turbulence sufficiently during gas sampling. The base of the static chamber was nested together by an inside (25 cm diameter × 11 cm height) and an outside (33 cm diameter × 8 cm height) PVC pipes, with a water groove left between the two pipes for sealing during gas samples collection. The bottom of the base was inserted into soil depth of 5 cm. cut sharply to facilitate soil insertion. Two months before gas sampling, four static chambers were deployed randomly at each plot to minimize effects of installation disturbance. The N_2O samples were collected between 09:00 and 11:00 a.m. local time. Prior to gas sampling, the cover box was placed on the collar filled with water in the groove, and the fan was turned on simultaneously. The static chamber was closed for 30 minutes, and gas samples (80 ml) were taken using 100 ml plastic syringes at the initial closed time as well as every 10 minute thereafter during the closed period. When collecting gas samples, the soft rubber hose connected with static chamber was cleaned thoroughly by pumping plastic syringe for three times, then 80 ml gas sample inside the chamber was collected and transferred into a 500 ml polyethylene-aluminum coated gas sampling bag. At the same time, values of

atmospheric pressures and air temperatures inside static chambers were measured for three times. ~~After gas sampling, cover boxes were removed to reduce disturbance to experimental plots as much as possible.~~ N_2O concentrations were analyzed in the laboratory by gas chromatography (Agilent 7890A, Agilent Technologies, USA) equipped with an electron capture detector set at 300 °C and a stainless porapak-Q column set at 70 °C within 24 hours following gas sampling. ~~N_2 was used as carrier gas at the flow rate of 30 ml min^{-1}. The N_2O concentration of standard gas for system calibration was 332 ppbV.~~ The N_2O flux was calculated by changes of N_2O concentrations inside static chamber during periods of gas sampling, with the equation as follows:

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

~~Where~~ 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 analyses

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).

~~The objective of s~~Structural equation modeling (SEM) is often used to detect complex relationships between one or more dependent or independent variables by using a series of statistical methods. The complex relationships among the target variables are expressed as paths in a hypothetical model, and finally tested by a series of statistical methods, such as univariate and multivariate regressions, ANOVA and factor analysis (Bagozzi and Yi, 2012). In this study, we used SEM ~~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 conceptual model (Fig. 1), and the SEM was performed with AMOS 21.0 (SPSS Inc., Chicago, IL, USA). How the effects of soil physicochemical properties and microbial abundance determine the responses of N transformation rates were evaluated. In order to explicitly illustrate the pathways of soil physicochemical properties and microbial abundance involved in each N transformation process, three individual models were constructed corresponding to the conceptual model to explain the responses of (a) net nitrification, (b) net N

mineralization and (c) N_2O emission rates. The hypothetical relationships among variables in the models are constructed based on the results of correlation analyses (Fig. S2). We used three models since it would ~~may~~ be easier to discover the controlling factors than using one complex model ~~which~~ that implicates all the measured processes (Delgado-Baquerizo et al., 2014). In these models, the precip-change treatments are categorical exogenous variables with two levels: 0 representing control and 1 representing seasonal precipitation changes (Delgado-Baquerizo et al., 2014). Abundance of both *nirK* and *nirS* genes were evidenced correlated with nitrification or N mineralization rates (Levy-Booth et al., 2014). Therefore, *nirK* and *nirS* abundance were added as one (*nirK*+*nirS*) endogenous factors in model. Net nitrification rate was included in model (b) as an endogenous factor because it may influence N_2O emission through altering the production of NO_3^- as the substrate for N_2O production. Prior to the SEM analyses, normal distribution of all the involved variables were examined, and genes abundance were log-transformed. Goodness of model fits was evaluated by chi-square test ($p > 0.05$), comparative fit index (CFI > 0.95), and root square mean errors of approximation (RMSEA < 0.05) (Hu and Bentler, 1998; Schermelleh-Engel et al., 2003). Pathways without significant effects were not shown ($p > 0.05$) in the final models.

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~~EOC, 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 ($p < 0.01$, Table S3 and Fig. 2d). Similarly, NO_3^- concentration decreased by 35 % and 24 % in 2013 and 2014, respectively ($p < 0.01$, Table S3 and Fig. 2j). Opposite patterns were observed for NH_4^+ concentration, which increased with the precipitation reduction (Fig. 2l). In the wet seasons with precipitation addition, SWC, NO_3^- concentration, ~~DOC~~ EOC and MBC remained lower in the precip-change plots than in the control plots in both years (Table S3 and Fig. 2d, f, h and j). After the experiment, soil pH in the precip-change plots was 3.82 ± 0.02 in dry seasons and 3.78 ± 0.07 in wet seasons~~, and~~. I~~i~~n the control plots, it was 4.06 ± 0.05 in dry and 3.86 ± 0.1 in wet seasons. It has no significant changes when compared with the pH values before experiment, with 4.01 ± 0.04 and 4.05 ± 0.08 in dry and wet seasons of the precip-change plots, and 4.23 ± 0.01 and 4.11± 0.07 in dry and wet seasons of the control plots.

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 ($p < 0.1$, Table S4 and Fig. 3b and d). The NO_3^- leaching also declined with precipitation reduction, especially in 2014 with a marked decrease by 22 % ($p < 0.001$, Table S4 and Fig. 3e and f). 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. 3b, d and f). Throughout the two years, moderate decreases were detected in N_2O emission either during dry-season precipitation reduction (35%) or during wet-season precipitation addition (15%) (Table S4 and Fig. 3j).

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 detect~~ion~~ limitation caused by low soil pH (4.08 ± 0.05) (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. 4a and b). The abundance of three denitrifying genes (*nirK*, *nirS* and *nosZ*) increased with precipitation reduction by 30-80% in the 2013 dry season ($p < 0.05$, Table S5 and Fig. 4d, f and h). 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 (Table S5 and Fig. 4c, d, e, f, g and h).

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 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. 5a and b). 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. 5c).

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~~ EOC (Fig. 5). Specifically, NO_3^- and NH_4^+ had direct positive effects on archaeal *amoA* abundance whereas ~~DOC~~ EOC had a direct negative effect on *nirK*+*nirS* abundance. Both NH_4^+ and ~~DOC~~ EOC concentration had direct positive impacts on the *nosZ* abundance (Fig. 5c). Changes in MBC were directly positively influenced by SWC and ~~DOC~~EOC.

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~~ ~~altered~~ by altering SWC, MBC and archaeal *amoA* gene abundance. N_2O emission was decreased by both precipitation enhancement (wet season) and precipitation reduction (dry season)~~decreased moderately either both in precipitation reduction and~~or ~~addition treatments~~, which indicated that soil N loss by N_2O emission in subtropical forests would be alleviated by the predicted seasonal precipitation changes. In contrast, increased NO_3^- leaching during precipitation addition in the wet seasons led to ~~a~~ significant losses ~~of~~ from the soil NO_3^- pool. During the ~~two~~ two-years~~'~~ experiment, SWC was always lower in precip-change plots than in control plots, despite ~~of~~ the precipitation addition in the wet seasons (Fig. 2c and d). One reason is the higher transpiration loss resulting from relatively bigger trees in the precip-change plots (tree height: 10.2 ± 5.0 m, DBH: 10.7 ± 6.3 cm) than that in the control plots (tree height: 7.7 ± 3.5 m, DBH: 9.5 ± 5.2 cm). ~~The average tree height and DBH were respectively 10.2 ± 5.0 m and 10.7 ± 6.3 cm in the four precip-change plots with the total number of 64 tree individuals, compared to 7.7 ± 3.5 m and 9.5 ± 5.2 cm in the four the control plots having the total number of 68 tree individuals.~~There were no significant differences in these stand characteristics, but the bigger trees in ~~the~~ precip-change plots ~~may~~might have ~~had~~ greater transpiration rates and therefore caused more soil water loss in the summer wet season (Gao et al., 2017). ~~–~~Another ~~possible~~ reason might be the large ~~size~~amount of precipitation ~~events~~added (55 mm per event). Large ~~sized~~ precipitation

events may result in flood-irrigation~~, which~~ that can ~~could~~ break the soil pores or reduce pore number, leading to soil structural decline (Barber et al., 2001). These changes in soil structure may affect soil water content, as soil water retention capacity is related to pore ~~-~~size and pore ~~-~~distribution (Loll and Moldrup, 2000).

Initially, we hypothesized that decreased precipita~~i~~tion in the dry season would suppress N transformation, and precipitation addition during the wet season would have little impact on ~~the~~ N transformation processes because the soils are ~~water~~ water-saturated and substrate sufficient. ~~Agreeing~~ In agreement with the first hypothesis, ~~the~~ net nitrification and N mineralization rates decreased sharply with the reduction of throughfall in the dry season (Fig. 3a, b, c and d). However, ~~disagreeing~~ contrary to~~with~~ the second hypothesis, ~~the~~ nitrification and N mineralization rates increased markedly ~~during~~ with precipitation supplementation ~~in~~ in the wet seasons (Fig. 3 a, b, c and d). These results ~~were caused~~ can be explained by the interactions ~~among~~ between microbial abundance, soil moisture and substrate availability (Fig. 5a, b and S3). Specifically, ~~DOC~~ soil EOC of the dry season was less ~~available~~ in the precip-change plots than in the control plots (Fig. 2e and f), probably ~~due to~~attributable to reduced C input ~~by less~~due to lower root production and exudation after drying (Kuzyakov and Domanski, 2000; Borken and Matzner, 2009). The reduced supply of soil C substrate (~~or DOC~~i.e., EOC) could have ~~suppress~~ restricted the growth of soil microorganisms (e.g. MBC and *AOA*), ~~and therefore~~ resulting~~ed~~ in decreased net nitrification and mineralization rates (Fig. 5a and b). Although increased NH_4^+ concentrations ~~during~~ with reduced precipitation ~~reduction~~ could provide more N

substrate for nitrifiers, the negative effects of decreased SWC and ~~DOC~~ EOC may have ~~overwhelmed~~ outweighed the positive effects of increased NH_4^+ ~~on microbial nitrification processes in the dry season~~. Instead, the accumulated NH_4^+ after dry season precipitation reduction might have had a positive legacy effect on soil microbial activity in the wet season, leading to increased N transformations. In addition, SWC differences are also known to directly affect N transformations by~~SWC differences also directly affected N transformations by~~ stimulating physiological changes in microbial activity, regardless of microbial abundance and composition (Auyeung et al., 2015). The increased N transformation rates (Fig. 3b, d) in ~~responding~~ response to decreased SWC, MBC (Fig. 2d, h) and archaeal *amoA* gene abundance (Fig. 4a) ~~during~~ with precipitation addition might be ~~one of~~ such a case~~s~~ (~~Fig. 2d, h, Fig. 3b, d, Fig. 4a and~~ also see Fig. S2). A 10% ~~decrease of~~lower SWC in the precip-change plots in 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~~ due to an increased pulse in organic substrate availability as well as 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~~ Substantial decreases in MBC and archaeal *amoA* gene abundance in our study indicated that some microorganisms may die from starvation or competition caused by limited substrate concentrations, and consequently ~~reduce microbial abundance and~~ release ~~the~~ MBC and microbial biomass nitrogen (MBN). These available substrates

released by dead microorganisms could be reused by the survivinged microorganisms, and which couldconsequently from dead or non-active microorganisms to support the increased energy demand caused byof increased microbial activity and accelerated microbial processes (Borken and Matzner, 2009).

We also hypothesized that the N transformation processes are predominantly influenced byassociated with functional microbial abundance. As expected, net N mineralization and nitrification rates showed stronger relationships with archaeal *amoA* abundance than that with MBC and or other soil properties (Fig. 5a and b). However, MBC and denitrifying gene abundance had similar effects on N_2O emission., Our results also showed thatand only *nosZ* gene abundance exerted a pronounced effect on N_2O emission (Fig. 5c), probably viaby, 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 researchesin contrast to previous studies (Levy-Booth et al., 2014; Gao et al., 2016). The N_2O emission emission-related denitrification can be also be 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 EOC content (Fig. 1), which could leading to higher aeration while lowering substrate availability., These changes in soil physicochemical properties could result in and consequentlythe predominantenhance the roles of nitrifier and fungi denitrification in controlling N_2O emission. In addition, SWC and nitrification rate also directly affected N_2O emission

~~via~~ by altering substrate availability and consequently microbial activity, despite ~~of~~ high microbial abundance (Fig. 5c). Although functional microbial abundance showed the most significant correlations with N transformation rates~~,~~ and could explain more than 20% of their variation ~~in N transformation rates~~, a large proportion of the variation remained unexplain~~able~~ed (Fig. 5). The~~se~~is unexplained variation~~s~~ ~~are~~is mainly attributed to the changes in other functional microbial genes involved in the nitrogen cycle, such as *narG* and *napA* responsible for NO_3^- reduction, and *nifH* responsible for N fixation (Widmer et al., 1999; Tavares et al., 2006). Moreover, ~~the~~ gene abundance based on DNA may not fully reflect gene expression. ~~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 changes in substrate concentrations. SEM analysis showed that both *amoA* and *nosZ* gene~~s~~ abundance ~~were~~ was positively affected by ~~DOC~~ EOC and NH_4^+ concentration, suggesting substrate constraints for these two functional microbial groups. This disagreed with previous studies ~~reporting which~~that reported that the *AOA* community had ~~the~~ greater potential ~~of~~ for mixotrophic growth and better ~~low~~ low-substrate tolerance ~~when compared with~~than its counterpart *AOB* (Erguder et al., 2009; Shen et al., 2012). However, these previous results were mainly ~~caused by~~due to ~~a~~ ~~stronger~~greater competitiveness of *AOA* ~~than its counterpart~~than *AOB*, as these studies

mainly focused on the comparison of effects of substrate availability on *AOA* and *AOB* communities.~~relative effects of substrate availability on *AOA* and *AOB*.~~ Both *nosZ* and *amoA* gene~~s~~ abundance increased with ~~DOC~~ EOC and NH_4^+ concentration (Fig. 5), which indicated that the *AOA* community could be constrained by C and N substrates when competing with other microbes that have different functions. Otherwise, the existing *AOA* species that have the potential ~~ability~~ for~~of~~ mixotrophic growth and starvation tolerance~~low~~ low ~~substrate tolerance~~ ~~may~~ would not domina~~n~~te in the studied subtropical forest, as the soil is ~~originally~~ rich in SOM (Zhou et al., 2006; Chen et al., 2015). Therefore, the *AOA* community in the studied soil could be ~~easily~~ strongly influenced by changes in soil C and N availability.

The abundance of *nirK* and *nirS* genes was positively controlled by soil NH_4^+ concentration and negatively controlled by ~~DOC~~ EOC content (Fig. 5). This ~~further~~ confirmed that ~~more~~ higher NH_4^+ content could favor more abundant microorganisms containing *nirK* or *nirS* genes (Yi et al., 2015), because higher NH_4^+ concentration could supply sufficient NO_3^- as the direct substrate or provide optimum pH value~~s~~ for growth of the denitrifying microorganisms. The negative effect of ~~DOC~~ EOC on *nirK* and *nirS* gene abundance was inconsistent with ~~most of~~ previous reports that denitrifiers are primari~~l~~y heterotrophic (Bárta et al., 2010). One reason is ~~because~~ that high ~~DOC~~ EOC concentration~~s~~ can constrain the growth of microorganisms ~~containing~~ carrying *nirK* and *nirS* genes through ~~effecting~~ effects on other factors, such as pH and C:N ratio (Henderson et al., 2010; Levy-Booth et al., 2014). Generally, the abundance of both nitrifying and denitrifying gene~~s~~ ~~abundance~~ changed with precipitation

redistribution, and the direction and magnitude of the changes depended ~~mostly~~ mainly on soil N and C substrate availabilities.

5 Conclusion

~~To summarize, s~~Soil net nitrification and N mineralization rates responded significantly to seasonal precipitation redistribution~~, and~~. ~~Additionally, m~~More than 20% of the variation could be explained by the effects of microbial abundance, SWC, and 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~~ two-year~~s'~~ ~~seasonal precipitation redistribution~~experiment decreased moderately, and as much as 42% of the total variation in N_2O emission was attributed to the ~~total~~ combined effects of SWC, nitrification rate, MBC and *nosZ* gene abundance. The accumulation of~~ed~~ NH_4^+ due to dry-season precipitation reduction may stimulate nitrification ~~process~~ in the wet season, and consequently accelerate N loss ~~from~~ by NO_3^- leaching. Therefore, ~~long term of~~ the predicted long-term seasonal precipitation changes in subtropical forests may result in profound changes ~~in~~ to different N pools and fluxes~~ size~~, including ~~with~~ ~~less~~reduced N_2O emission ~~while~~ and ~~more~~ ~~more~~ enhanced NO_3^- leaching. These~~, which~~ in turn, could exert a feedback to climate and environmental changes. Meanwhile, changes in functional microbial abundance induced by soil ~~DOC~~ EOC and NH_4^+ substrate availabilities will ~~be the predominant driver in regulating~~determine the extent and direction of soil N transformation changes.

Author contribution

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

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595 Competing interests

596 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 extractable~~dissolved~~ organic carbon (~~DOC~~EOC) 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 precipitation change (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.

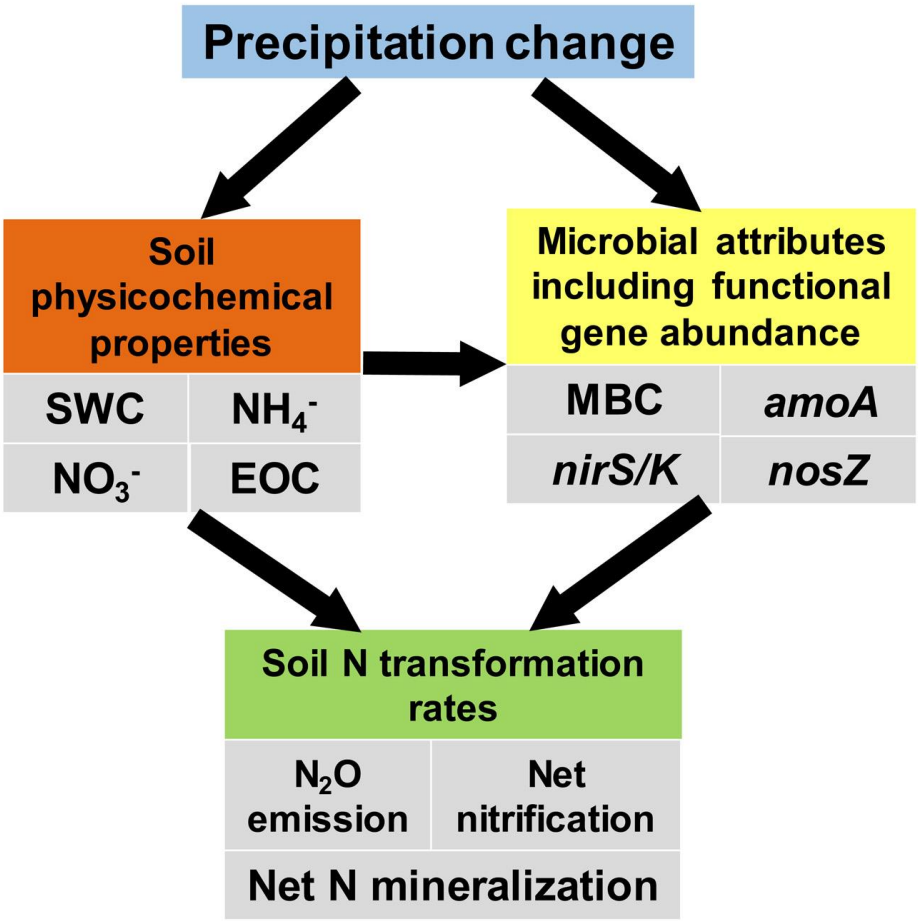
Fig. 3. Nitrogen transformation rates measured in control and precipitation change (precip-change) plots over the course of experiment. Points and bars with standard error (n = 4) show mean values at each sampling time and in dry (DS) and wet (WS) seasons. Grey shades indicate the periods of precipitation 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 precipitation change (precip-change) plots over the course of experiment. Points and bars with standard error (n = 4) show mean values at each

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

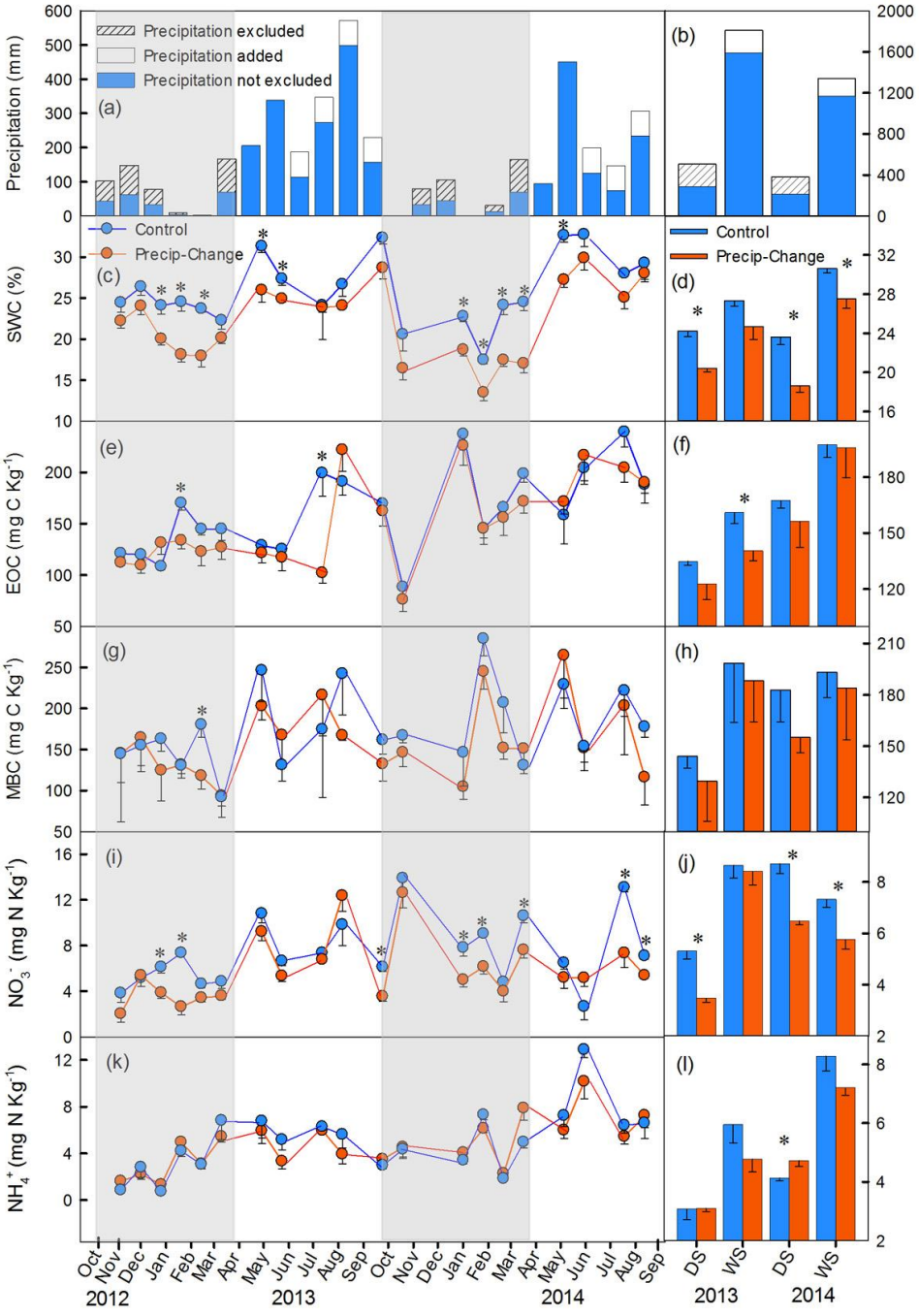
798 **Fig. 5.** Path diagrams demonstrating the effects of soil physicochemical properties and
799 functional genes abundance on net nitrification, N mineralization and N_2O efflux rates
800 in response to precipitation change (precip-change) during two years. Numbers
801 adjacent to arrows are path coefficients, which indicate the relationships between the
802 two variables on both sides of the arrows. Solid and dash lines represent positive and
803 negative paths, respectively. The r^2 above or below each response variable in the model
804 denotes the proportion of variance which could be explained. Size of the lines indicate
805 significant levels of path coefficients.

806 **Fig. 1**


Precipitation change
Soil physicochemical properties
SWC
NH_4^-
NO_3^-
EOC
Microbial attributes including functional gene abundance
MBC
amoA
nirS/K
nosZ
Soil N transformation rates
N_2O emission
Net nitrification
Net N mineralization

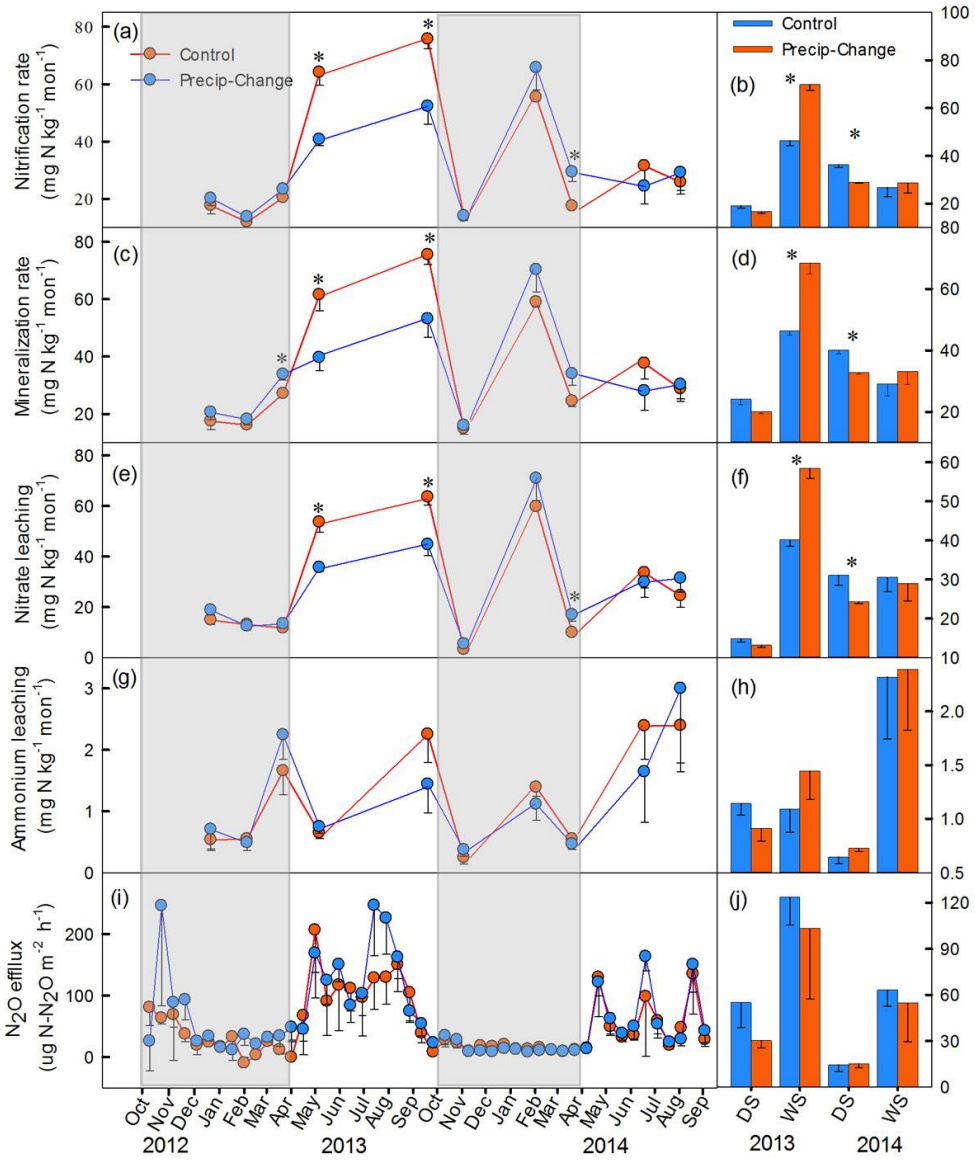
807

808 **Fig. 2**

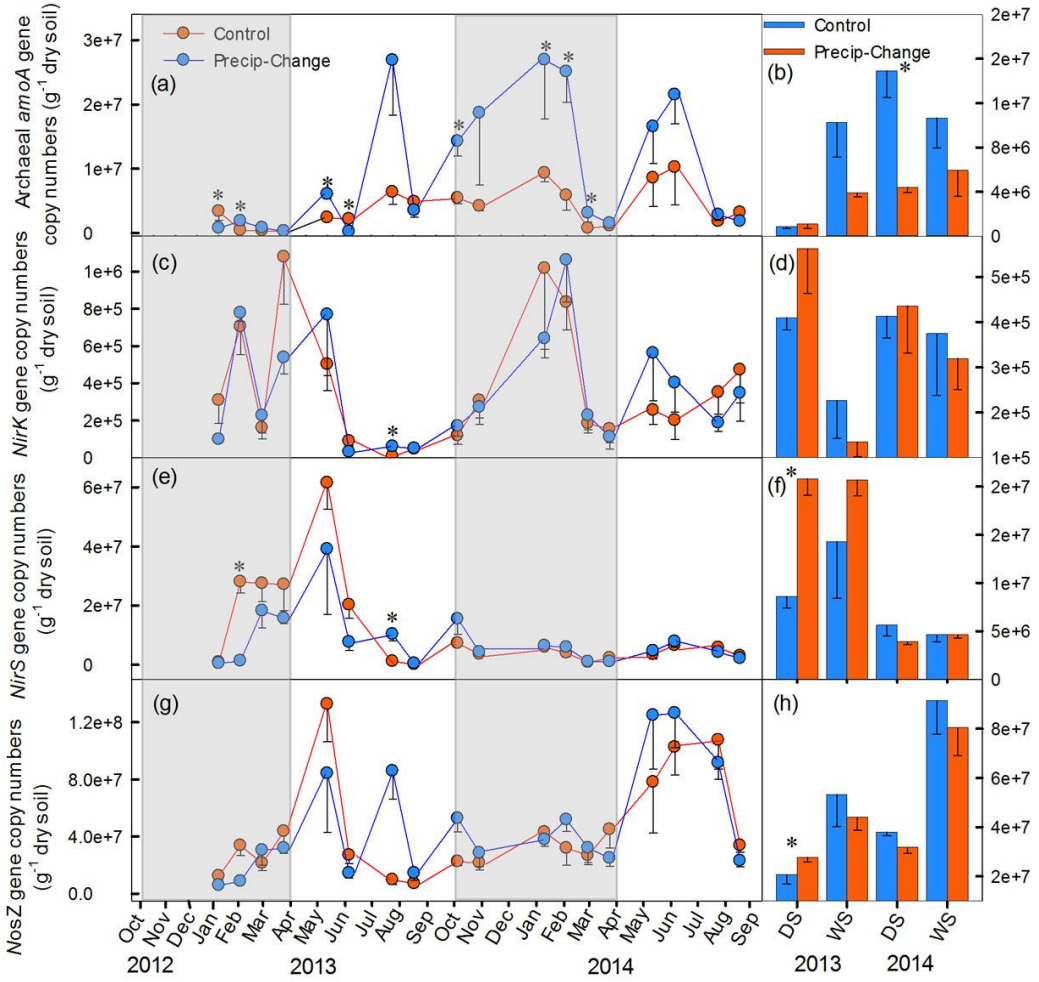

Precipitation excluded
Precipitation added
Precipitation not excluded
Precipitation (mm)
(a)
(b)
Control
Precip-Change
SWC (%)
(c)
(d)
EOC (mg C Kg-1)
(e)
(f)
MBC (mg C Kg-1)
(g)
(h)
NO3- (mg N Kg-1)
(i)
(j)
NH4+ (mg N Kg-1)
(k)
(l)
Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep
2012 2013 2014
DS WS DS WS
2013 2014

809

Fig. 3

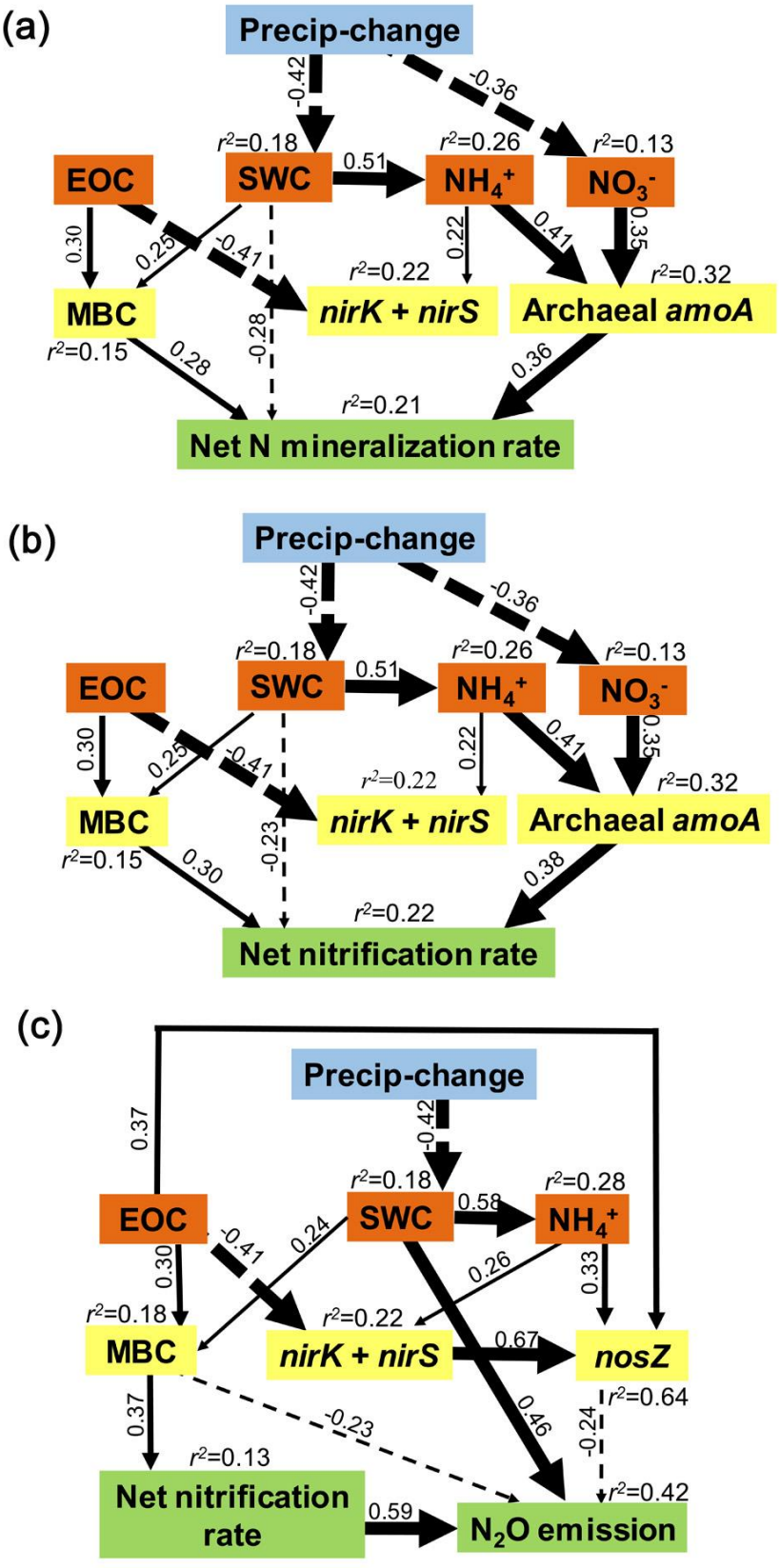

(a)
Control
Precip-Change
Nitrification rate
(mg N kg-1 mon-1)
(b)
(c)
Mineralization rate
(mg N kg-1 mon-1)
(d)
(e)
Nitrate leaching
(mg N kg-1 mon-1)
(f)
(g)
Ammonium leaching
(mg N kg-1 mon-1)
(h)
(i)
N2O efflux
(ug N-N2O m-2 h-1)
(j)
Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep
2012
2013
2014
DS WS DS WS
2013
2014

812 **Fig. 4**


Archaeal amoA gene copy numbers (g-1 dry soil)
NirK gene copy numbers (g-1 dry soil)
NirS gene copy numbers (g-1 dry soil)
NosZ gene copy numbers (g-1 dry soil)
Control
Precip-Change
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep
2012 2013 2014
DS WS DS WS
2013 2014

813

814 **Fig. 5**


(a)
Precip-change
-0.42
-0.36
r^2=0.18
r^2=0.26
r^2=0.13
EOC
SWC
0.51
NH_4^+
NO_3^-
0.30
0.25
-0.41
0.22
0.41
0.35
r^2=0.22
r^2=0.32
MBC
nirK + nirS
Archaeal amoA
r^2=0.15
0.28
-0.28
0.36
r^2=0.21
Net N mineralization rate
(b)
Precip-change
-0.42
-0.36
r^2=0.18
r^2=0.26
r^2=0.13
EOC
SWC
0.51
NH_4^+
NO_3^-
0.30
0.25
-0.41
0.22
0.41
0.35
r^2=0.22
r^2=0.32
MBC
nirK + nirS
Archaeal amoA
r^2=0.15
0.30
-0.23
0.38
r^2=0.22
Net nitrification rate
(c)
Precip-change
0.37
-0.42
r^2=0.18
r^2=0.28
EOC
SWC
0.58
NH_4^+
-0.41
0.24
0.30
0.26
0.33
r^2=0.18
r^2=0.22
MBC
nirK + nirS
0.67
nosZ
r^2=0.64
0.37
-0.23
0.46
-0.24
r^2=0.13
r^2=0.42
Net nitrification rate
0.59
N_2O emission

815