Response to Associate Editor's comments

Manuscript #: bg-2017-3

Associate Editor: Dr. Denise M. Akob

Comment #1: Thank you for submitting your Research Article titled "Soil nitrogen transformation responses to seasonal precipitation changes are regulated by changes in functional microbial abundance in a subtropical forest" to Biogeosciences. The 3 referees provided thoughtful, constructive comments on your paper, and in your responses and revisions you have adequately addressed all major and minor issues. However, I have a few minor revisions (listed below) that should be addressed before acceptance. Please upload a revised version with these and the reviewers' comments addressed.

Response: Thank you very much for considering our revisions adequate and for providing valuable suggestions to further improve our manuscript. We have carefully studied each of your comments and incorporated them into this revision. The point-by-point responses to your comments are listed below and marked in the revised manuscript. We hope that you would find these revisions satisfactory.

Comment #2:

L. 219: write out "PC"

L218- elsewhere: write out the full month name, don't abbreviate.

Response: Done as suggested. Please see lines 198, 199, 202.

Comment #3: L. 259: do you mean microliters and not milliliters for the qPCR volume?

Response: Thanks for detecting this. It should be microliters, the unit has been corrected. Please see line 238.

Comment #4: L. 262: was this PCR- or molecular water?

Response: It was RNase free Ultra-Pure water, which could be used in PCR protocol. The term "double-distilled" has been revised. Please see line 241.

Comment #5: L. 265: please provide a reference for the standard construction and/or provide more details. E.g., what sequences were used in the plasmids?

Response: The standards were constructed using the method described in Henry et al. (2006) and Isobe et al. (2011). Briefly, to generate the standard curves, the target gene fragment of the soil clone obtained in this study were used. For example, we obtained the archaeal *amoA* PCR product with the same primers used in real-time PCR (i.e., CrenamoA 23f/CrenamoA 616r) and the extracted soil DNA as template. The archaeal *amoA* PCR product was cloned into the pMD20-T vector (TaKaRa, Dalian Division), and the cloning fragments were transformed into *Escherichia coli* JM109 strains. The recombinant *Escherichia coli* JM109 strains carrying the archaeal *amoA* recombinant

plasmids were inoculated into LB broth with ampicillin and incubated at 37°C overnight. The plasmid DNA was then extracted using the Hipure Plasmid Mini Kit (Magen, Guangzhou, China) and quantified on a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., USA). The presence of archaeal *amoA* inserts was verified by PCR with the same primers (i.e., CrenamoA 23f/CrenamoA 616r) and gel electrophoresis. The copy numbers of the standard archaeal *amoA* gene numbers was expressed as the DNA copy numbers of the extracted plasmid DNA carrying archaeal *amoA* gene, which was calculated from the plasmid DNA size, concentration, and average base pair molecular weight. Standard curve was then generated from a tenfold serial dilution (10^3-10^8 copies per µl) of the plasmid DNA. The references and more sentences have been added to describe the standard construction as suggested. Please see lines 242-255.

Henry S, Bru D, Stres B, Hallet S, Philippot L (2006) Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. Applied and Environmental Microbiology, 72, 5181-5189, doi:10.1128/AEM.00231-06.

Isobe, K., Koba, K., Suwa, Y., Ikutani, J., Fang, Y.T., Yoh, M., Mo, J.M., Otsuka, S., and Senoo, K.: High abundance of ammonia-oxidizing archaea in acidified subtropical forest soils in southern China after long-term N deposition, Fems Microbiol. Ecol., 80, 193-203, doi:10.1111/j.1574-6941.2011.01294.x, 2012.

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

Author: Jie Chen [1,2,3 #], Guoliang Xiao [1,2 #], Yakov Kuzyakov [3,4], G. Darrel Jenerette[5],Ying Ma [1,2], Wei Liu [1], Zhengfeng Wang [1], Weijun Shen [1 *]

[1] Center for Ecological and Environmental Sciences, South China Botanical Garden, Chinese Academy of Sciences, 723 Xinke Rd. Tianhe District, Guangzhou 510650, PR China

[2] University of Chinese Academy of Sciences, 19A Yuquan Road, Shijingshan District, Beijing 100049, PR China

[3] Department of Soil Science of Temperate Ecosystems, University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany

[4] Department of Agricultural Soil Science, University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany

[5] Department of Botany and Plant Sciences, Center for Conservation Biology, University of California Riverside, Riverside, CA92521, USA

[#] These authors contributed equally to this work.

[*]Corresponding author: Dr. Weijun Shen

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, with annual precipitation unchanged. Net N mineralization, net nitrification, N_2O emission, nitrifying (bacterial and archaeal *amoA*) and denitrifying (*nirK*, *nirS* and *nosZ*) gene abundance, microbial biomass carbon (MBC), extractable organic carbon (EOC), NO_3^-, NH_4^+ and soil water content (SWC) 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 SWC. Notably, archaeal *amoA* abundance showed the strongest correlation with net N transformation rates ($r \geq 0.35$), 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 in both dry and 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

through altering functional microbial abundance and MBC, which are further affected by changes in EOC and NH_4^+ availabilities.

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 global changes such as elevated CO_2 and temperature (Beier et al., 2012). 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 of 60 years of precipitation data showed remarkable seasonal precipitation redistribution in subtropical China, with more frequent droughts in dry season and extreme rainfall events in wet season (Zhou et al., 2011). In contrast to changes in total annual precipitation, redistribution of seasonal precipitation may be more important in controlling ecosystem function in subtropical forests due to 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. Field experiments simulating seasonal precipitation changes in subtropical regions are urgently needed for better understanding of the ecosystem responses.

Changes in precipitation can strongly affect soil 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 was decreased by a rainfall exclusion experiment in the moist tropical forest, but recovered within the first year after rainfall exclusion was stopped Davidson *et al*. (2008). In grasslands, the net N mineralization rate declined sharply in response to increased rainfall, but increased during drought (Jamieson et al., 1998). Contrasting responses of N transformation have also been obtained in temperate forests (Emmett et al., 2004; Chen et al., 2011; Fuchslueger et al., 2014). However, limited information exists 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 seasonal dynamics of microbial activities, soil moisture, temperature, plant nutrient uptake, and carbon (C) and N availabilities (Reichmann et al., 2013). Although the direct effects of soil physicochemical properties and microbial communities on N transformations are well documented, the dominant 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, 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 carrying the *nirK* and *nirS* genes and nitrous oxide-reducing bacteria carrying the *nosZ* gene (Schimel and Bennett, 2004; Levy-Booth et al., 2014). Thus, changes in these functional microorganisms can shed light on the underlying mechanisms 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 shown that reduced precipitation decreases soil moisture and increases aeration and O_2 diffusion, which stimulates 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). Both denitrifiers and nitrifiers might be suppressed by decreased moisture and available C during drought (Bárta et al., 2010; Zhalnina et al., 2012). In addition, increased precipitation may raise the NH_4^+:NO_3^- ratio, as NO_3^- is easily leached (Reichmann et al., 2013). 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 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 changes in nitrification and denitrification, and consequently different changes in soil NO_3^-, NH_4^+ contents and N_2O emission. 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 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 predicted to increase in wet seasons and decrease in dry seasons (Zhou et al., 2011). We simulated this seasonal precipitation pattern for two years. Changes in soil physicochemical properties, net N transformation rates, and nitrifying (bacterial and archaeal *amoA*) and denitrifying (*nirK*, *nirS* and *nosZ*) gene abundance were analyzed and integrated in a hypothetical path model which assumed that the precipitation-induced changes in soil physicochemical properties and microbial abundance 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 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 responses of N transformation rates to the precipitation change will be associated with changes in functional gene abundance, because N transformation processes are primarily catalyzed by specific enzymes coded by functional genes; (4) microbial abundance is directly influenced by soil physicochemical properties, but denitrifiers will be more strongly affected than nitrifiers, because the nitrifiers have the potential for mixotrophic growth and are tolerant of low N and C substrate 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 study forest by recording species name, diameter at breast height (DBH), tree height and density prior to the experiment. Generally, 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 $\pm$ 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 $\pm$ 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

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 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) but the nutrient (e.g. nitrogen and organic carbon) contents was higher in throughfall than in the pond water (Zhao et al., 2017), which assures that we did not enrich nutrients while adding water. 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 specifically, the throughfall excluded was 220 mm in the 2013 dry

season (October 1st 2012 to March 31st 2013) and the same amount water was added back into each ~~PC~~precip-change 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 (October 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

219 determined from the 2 M KCl extraction liquid by using the indophenol blue
220 colorimetry and copperized cadmium reduction methods, respectively.
221 Soil extractable organic carbon (EOC) and microbial biomass carbon (MBC) were
222 measured immediately after the soil sampling using the fumigation extraction method
223 described as Vance, Brookes and Jenkinson (1987). In detail, a pair of fresh soil
224 subsamples (10 g) was placed into two glass breakers. One was fumigated in a
225 vacuum dryer with alcohol-free chloroform and NaOH solution for 24 h in dark, and
226 the other one was placed in dark for 24 h without fumigation. The two subsamples were
227 extracted with 0.5 M K_2SO_4 after fumigation, and the EOC concentration was
228 determined using a total organic C analysis instrument (TOC-VCSH, Shimadzu, Japan).
229 The difference of EOC concentration between the fumigated and un-fumigated was
230 multiplied by 0.45 to calculate MBC content.
231 Soil total DNA was extracted from 0.3 g fresh soil using the HiPure Soil DNA Mini
232 Kit (Magen, Guangzhou, China), quantified with a NanoDrop 2000 spectrophotometer
233 (Thermo Fisher Scientific Inc., USA) and stored at -20 °C for further analyses. The
234 abundance of bacterial and archaeal ammonia-monooxygenase gene (*amoA*), nitrite
235 reductase genes (*nirK* and *nirS*) and nitrous oxide reductase gene (*nosZ*) were
236 quantified by using absolute Real-time PCR on an ABI 7500 thermocycler system with
237 primers and thermal profiles presented in the supplementary material (Table S1). The
238 Real-time PCR reactions was performed on 96-well plates (Axygen, USA), with 20 µl
239 ~~ml~~ volume in each well including 12.5 µl SYBR Premix Ex Taq (TaKaRa
240 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 RNase free Ultra-Pure water~~double-distilled water~~. The standards were constructed using the method described ~~by~~in Henry et al. (2006) and Isobe et al. (2011). Briefly, the target functional gene PCR products were obtained with the same primers used in real-time PCR and the extracted soil DNA as template. The PCR products were cloned using the pMD20-T vector (TaKaRa, Dalian Division), and then transformed into *Escherichia coli* JM109 strains. The recombinant *Escherichia coli* JM109 strains carrying the target functional gene recombinant plasmids were inoculated into LB broth with ampicillin and incubated at 37°C overnight. The plasmid DNA was then extracted using the Hipure Plasmid Mini Kit (Magen, Guangzhou, China) and quantified on a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., USA). The DNA copy numbers of the extracted plasmid DNA carrying the target functional gene was calculated from the plasmid DNA size, concentration, and average base pair molecular weight, which could stand for the copy numbers of the standard functional gene. Finally, the standard curve was generated from a tenfold serial dilution (10^3-10^8 copies per µl) of the plasmid DNA.~~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 nitrous oxide (N_2O) effluxes s 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 bottom of the base was inserted into soil depth of 5 cm. 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. 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. At the same time, values of atmospheric pressures and air temperatures inside static chambers were measured for three times. 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. 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 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).

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 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 be easier to

discover the controlling factors than using one complex model 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, 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, 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. In 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 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

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

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

409 # 4 Discussion

410 ## 4.1 Drivers of N transformation processes

411 Consistent with our hypotheses, seasonal precipitation redistribution induced

significant changes in net N mineralization and nitrification rates 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), 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 significant losses from the soil NO_3^- pool. During the two-year experiment, SWC was always lower in precip-change plots than in control plots, despite 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). There were no significant differences in these stand characteristics, but the bigger trees in precip-change plots might have greater transpiration rates and therefore caused more soil water loss in the summer wet season (Gao et al., 2017). Another reason might be the large amount of precipitation added (55 mm per event). Large precipitation events may result in flood-irrigation that can 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 precipitation in the dry season would suppress N transformation, and precipitation addition during the wet season would have little impact on N transformation processes because the soils are water-saturated

and substrate sufficient. In agreement with the first hypothesis, net nitrification and N mineralization rates decreased sharply with the reduction of throughfall in the dry season (Fig. 3a, b, c and d). However, contrary to the second hypothesis, nitrification and N mineralization rates increased markedly with precipitation supplementation in the wet seasons (Fig. 3 a, b, c and d). These results can be explained by the interactions between microbial abundance, soil moisture and substrate availability (Fig. 5a, b and S3). Specifically, soil EOC of the dry season was less in the precip-change plots than in the control plots (Fig. 2e and f), probably attributable to reduced C input due to lower root production and exudation after drying (Kuzyakov and Domanski, 2000; Borken and Matzner, 2009). The reduced supply of soil C substrate (i.e., EOC) could have restricted the growth of soil microorganisms (e.g. MBC and *AOA*), resulting in decreased net nitrification and mineralization rates (Fig. 5a and b). Although increased NH_4^+ concentrations with reduced precipitation could provide more N substrate for nitrifiers, the negative effects of decreased SWC and EOC may have outweighed the positive effects of increased NH_4^+. 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 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 response to decreased SWC, MBC (Fig. 2d, h) and archaeal *amoA* gene abundance (Fig. 4a) with precipitation addition might be such a case (also see Fig. S2). A 10%

456 lower SWC in the precip-change plots in natural humid wet season might create better
457 redox conditions for microbial nitrification, as excessive soil moisture could reduce soil
458 oxygen concentration. According to Borken & Matzner (2009), the increases of soil
459 microbial activity by rewetting usually occurred due to an increased pulse in organic
460 substrate availability as well as reconstituting mineralization of SOM . Substantial
461 decreases in MBC and archaeal *amoA* gene abundance in our study indicated that some
462 microorganisms may die from starvation or competition caused by limited substrate
463 concentrations, and consequently release MBC and microbial biomass nitrogen (MBN).
464 These available substrates released by dead microorganisms could be reused by the
465 surviving microorganisms, which could support the increased energy demand of
466 accelerated microbial processes (Borken and Matzner, 2009).

467 We also hypothesized that N transformation processes are associated with functional
468 microbial abundance. As expected, net N mineralization and nitrification rates showed
469 stronger relationships with archaeal *amoA* abundance than with MBC or other soil
470 properties (Fig. 5a and b). However, MBC and denitrifying gene abundance had similar
471 effects on N_2O emission. Our results also showed that only *nosZ* gene abundance
472 exerted a pronounced effect on N_2O emission (Fig. 5c), probably by reducing N_2O
473 consumption (Henderson et al., 2010; Levy-Booth et al., 2014). No significant
474 correlation between N_2O emission and *nirK* + *nirS* gene abundance was detected, in
475 contrast to previous studies (Levy-Booth et al., 2014; Gao et al., 2016). The N_2O
476 emission-related denitrification can also be performed by nitrifiers and fungi in soils
477 with high aeration and limited substrate availability (Levy-Booth et al., 2014). The

experimental seasonal precipitation strongly decreased SWC and EOC content (Fig. 1), leading to higher aeration while lowering substrate availability. These changes in soil physicochemical properties could enhance the role of nitrifier and fungi denitrification in controlling N_2O emission. In addition, SWC and nitrification rate also directly affected N_2O emission by altering substrate availability and consequently microbial activity, despite 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, a large proportion of the variation remained unexplained (Fig. 5). This unexplained variation 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, gene abundance based on DNA may not fully reflect gene expression.

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 abundance was positively affected by EOC and NH_4^+ concentration, suggesting substrate constraints for these two functional microbial groups. This disagreed with previous studies that reported that the *AOA* community had greater potential for mixotrophic growth and better low-substrate tolerance than its counterpart *AOB* (Erguder et al., 2009; Shen et al., 2012). However, these previous results were mainly

499 due to greater competitiveness of *AOA* than *AOB*, as these studies mainly focused on
500 the comparison of effects of substrate availability on *AOA* and *AOB* communities. Both
501 *nosZ* and *amoA* gene abundance increased with EOC and NH_4^+ concentration (Fig. 5),
502 which indicated that the *AOA* community could be constrained by C and N substrates
503 when competing with other microbes that have different functions. Otherwise, the
504 existing *AOA* species that have the potential for mixotrophic growth and starvation
505 tolerance would not dominate in the studied subtropical forest, as the soil is rich in
506 SOM (Zhou et al., 2006; Chen et al., 2015). Therefore, the *AOA* community in the
507 studied soil could be strongly influenced by changes in soil C and N availability.
508 The abundance of *nirK* and *nirS* genes was positively controlled by soil NH_4^+
509 concentration and negatively controlled by EOC content (Fig. 5). This confirmed that
510 higher NH_4^+ content could favor more abundant microorganisms containing *nirK* or
511 *nirS* genes (Yi et al., 2015), because higher NH_4^+ concentration could supply sufficient
512 NO_3^- as the direct substrate or provide optimum pH values for growth of the
513 denitrifying microorganisms. The negative effect of EOC on *nirK* and *nirS* gene
514 abundance was inconsistent with previous reports that denitrifiers are primarily
515 heterotrophic (Bárta et al., 2010). One reason is that high EOC concentrations can
516 constrain the growth of microorganisms carrying *nirK* and *nirS* genes through effects
517 on other factors, such as pH and C:N ratio (Henderson et al., 2010; Levy-Booth et al.,
518 2014). Generally, the abundance of both nitrifying and denitrifying genes changed with
519 precipitation redistribution, and the direction and magnitude of the changes depended
520 mainly on soil N and C substrate availabilities.

5 Conclusion

Soil net nitrification and N mineralization rates responded significantly to seasonal precipitation redistribution. 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-year experiment decreased moderately, and as much as 42% of the total variation in N_2O emission was attributed to the combined effects of SWC, nitrification rate, MBC and *nosZ* gene abundance. The accumulation of NH_4^+ due to dry-season precipitation reduction may stimulate nitrification in the wet season, and consequently accelerate N loss by NO_3^- leaching. Therefore, the predicted long-term seasonal precipitation changes in subtropical forests may result in profound changes to different N pools and fluxes, including reduced N_2O emission and enhanced NO_3^- leaching. These, in turn, could exert a feedback to climate and environmental changes. Meanwhile, changes in functional microbial abundance induced by soil EOC and NH_4^+ substrate availabilities will determine the extent and direction of soil N transformation changes.

Author contribution

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

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Competing interests

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 organic carbon (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.

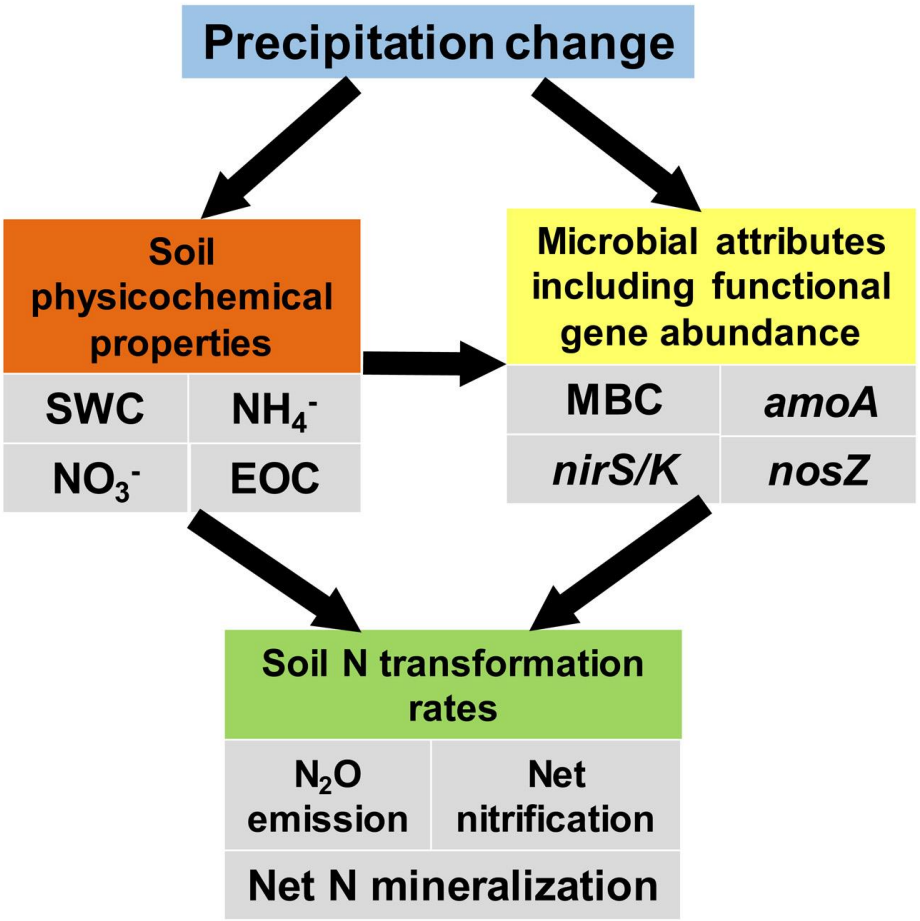
Fig. 3. Nitrogen transformation rates measured in control and precipitation change (precip-change) plots over the course of experiment. Points and bars with standard error (n = 4) show mean values at each sampling time and in dry (DS) and wet (WS) seasons. Grey shades indicate the periods of precipitation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

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

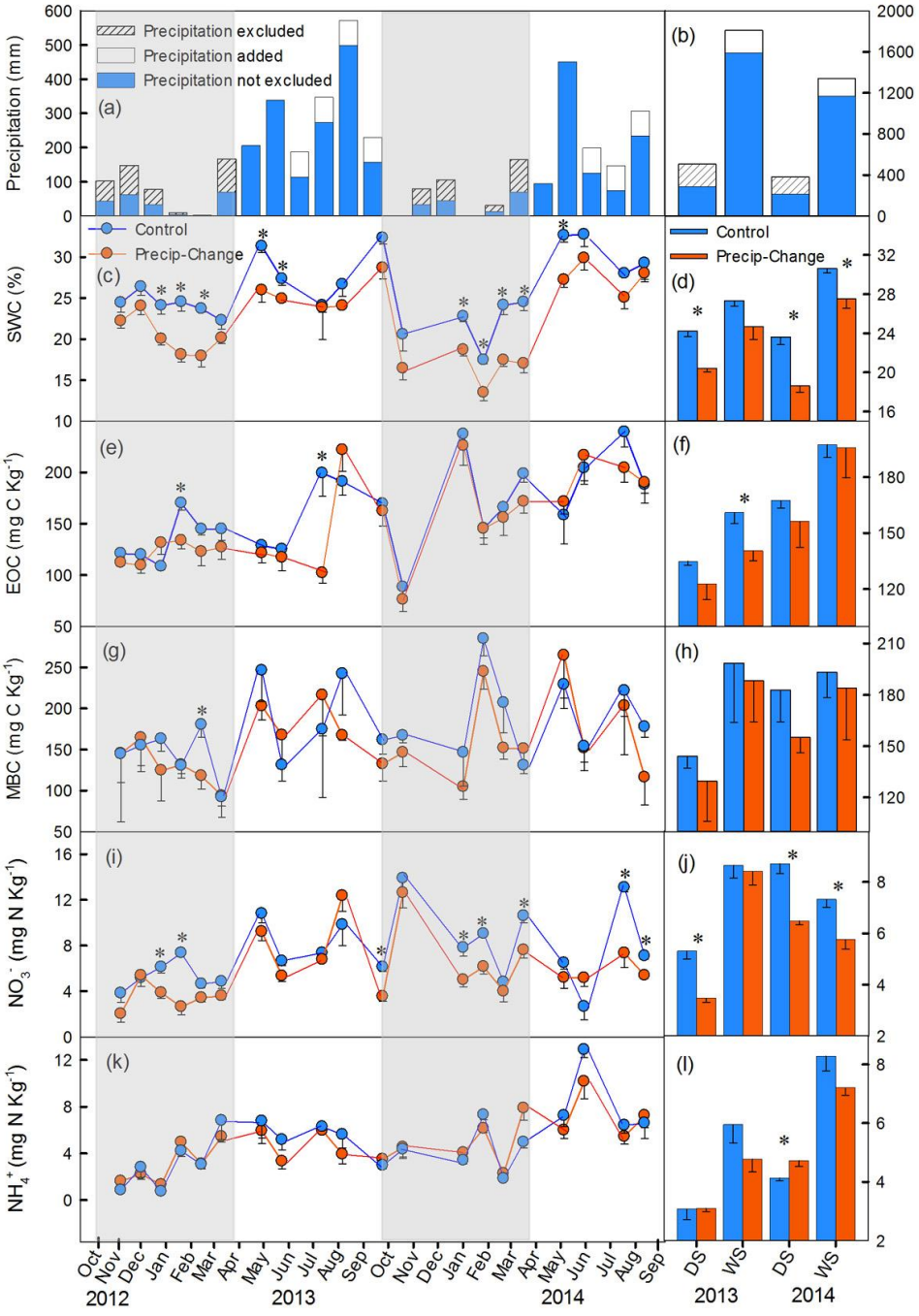
754 **Fig. 5.** Path diagrams demonstrating the effects of soil physicochemical properties and
755 functional genes abundance on net nitrification, N mineralization and N_2O efflux rates
756 in response to precipitation change (precip-change) during two years. Numbers
757 adjacent to arrows are path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

762 **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

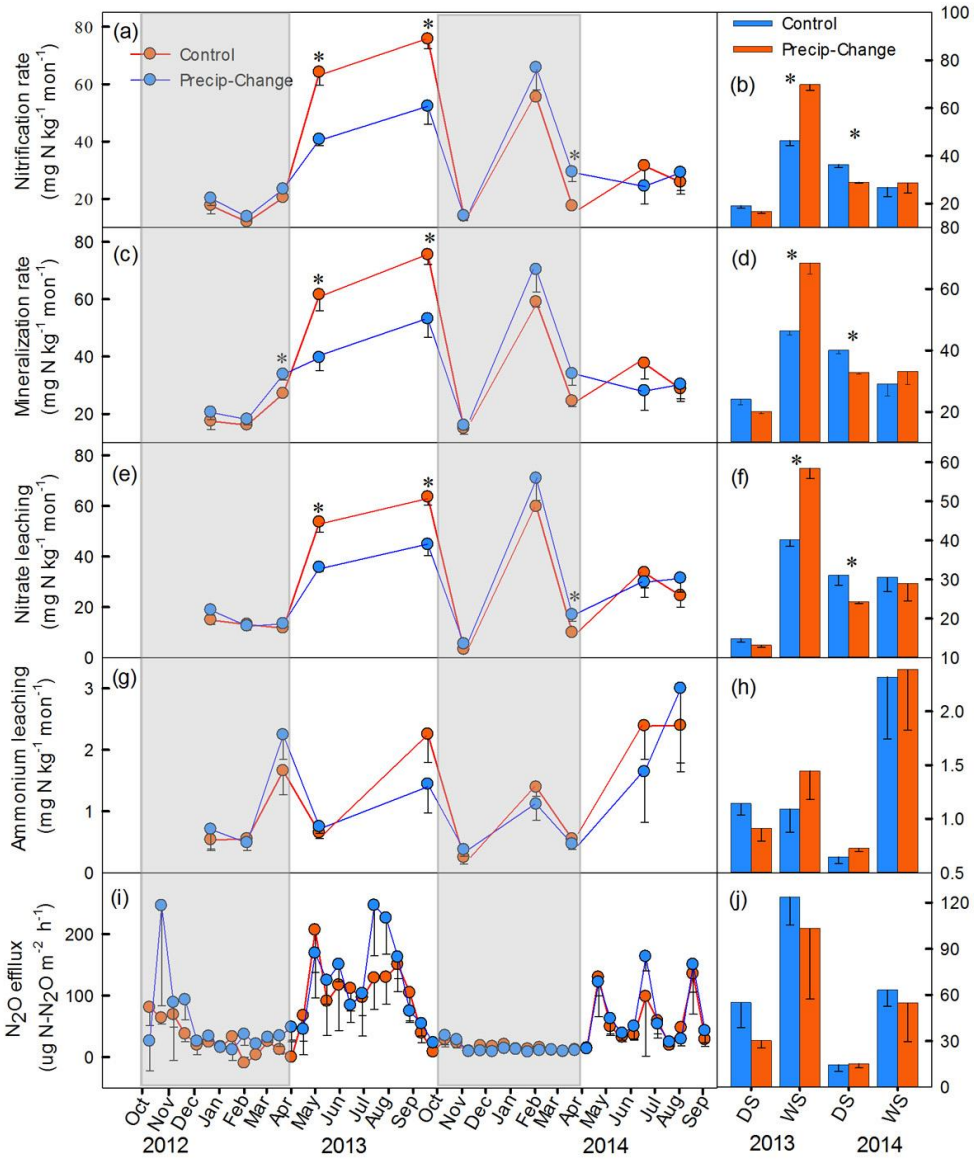
763

764 **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

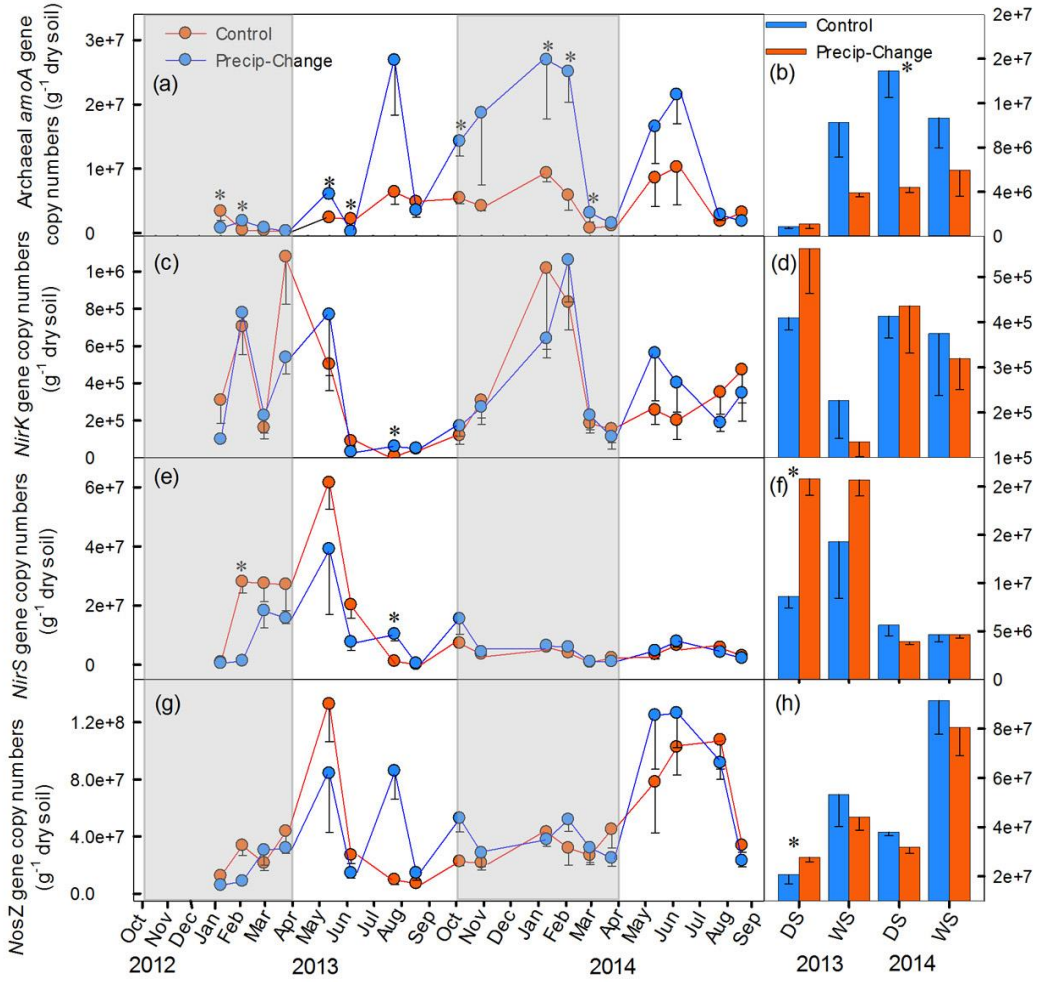
765

766 **Fig. 3**


(a)
Control
Precip-Change
Nitrification rate
(mg N kg-1 mon-1)
(b)
Control
Precip-Change
(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

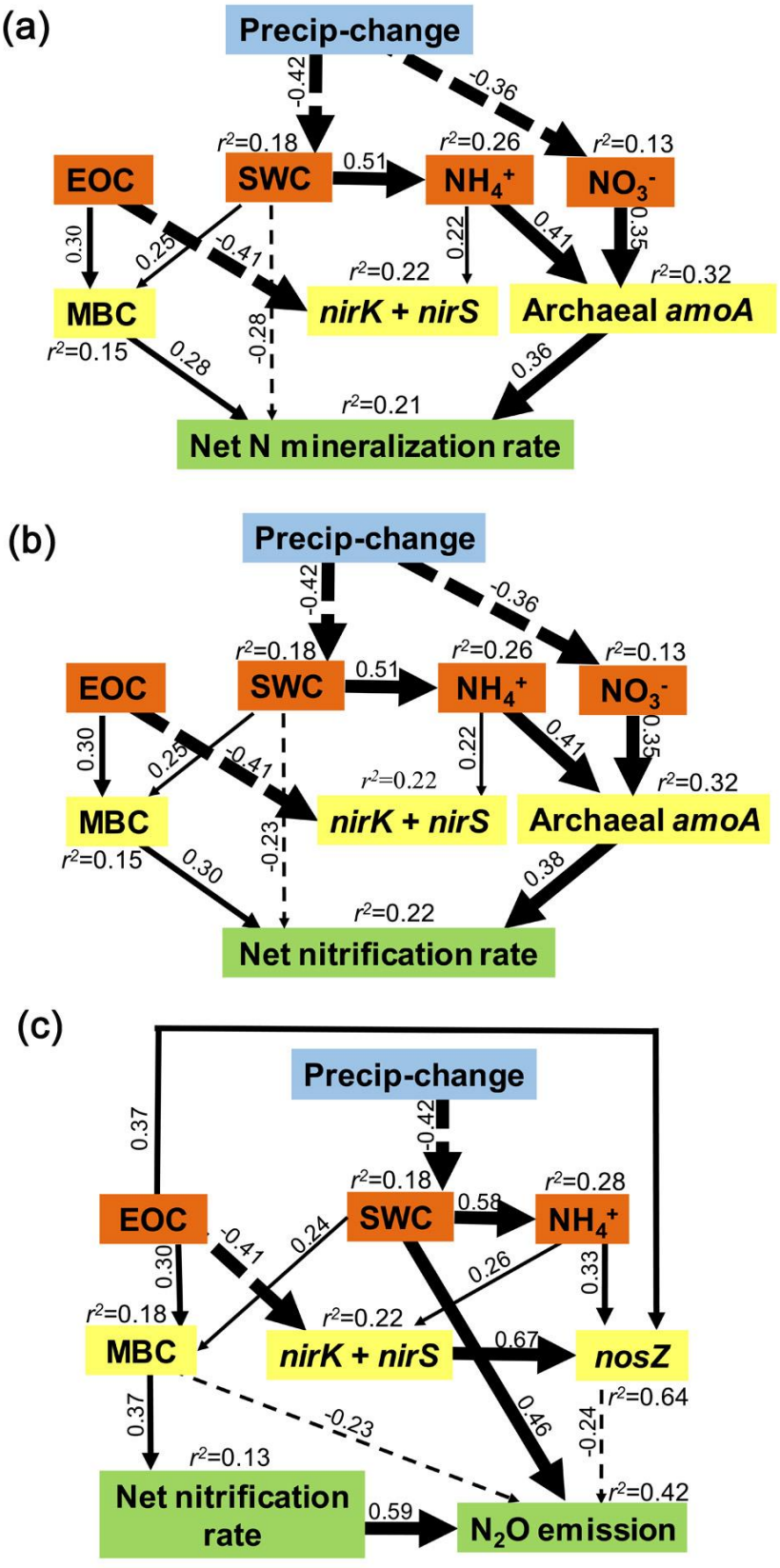
767

768 **Fig. 4**


Control
Precip-Change
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
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)
3e+7
2e+7
1e+7
0
1e+6
8e+5
6e+5
4e+5
2e+5
6e+7
4e+7
1.2e+8
8.0e+7
4.0e+7
0.0
2e+7
1e+7
8e+6
4e+6
5e+5
3e+5
1e+5
5e+6
8e+7
6e+7
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
2012
2013
2014
DS
WS

769

770 **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

771