Effects of climate warming and elevated CO₂ on autotrophic nitrification and nitrifiers in dryland ecosystems

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ABSTRACT

Global climate change is predicted to enhance atmospheric temperature and CO₂, with important consequences on biogeochemical nitrogen cycling in dryland ecosystems, which are highly vulnerable and characterized by extremely low nutrient availability. Belowground nitrification processes, predominantly mediated by ammonia-oxidizing archaea (AOA) and bacteria (AOB), are central to plant nitrogen availability and soil N₂O emissions, but their responses to future climatic scenarios in drylands remain largely unknown. Here we investigated the impact of factorial combinations of elevated CO₂ (+200 ppm) and elevated temperature (+3 °C) on dynamics of ammonia oxidizers and nitrification in three dryland soils planted with *Eucalyptus tereticornis*. Soil properties (including total carbon, H₂O%, and nitrate) and potential nitrification rates were strongly impacted by elevated temperature after nine months, accompanied by significantly higher AOA abundance in two soils and a gradual decrease in AOB abundance under elevated temperature. DNA-stable isotope probing showed increased assimilation of ¹³CO₂ by AOA, but not AOB, under warming, indicating that AOA were actively growing and utilizing the ¹³CO₂ substrates, which was coupled with significantly higher net nitrogen mineralization and nitrification rates. High-throughput microarray analysis revealed temperature selections of particular AOA assemblages and a significant reduction in diversity and co-occurrence of the metabolically active AOA phylotypes. Although these responses were soil specific, structural equation modelling by compiling all the data together showed that warming had significant direct and indirect impacts on soil nitrification which were driven by changes in AOA community structure, but no obvious effects of elevated CO₂ could be identified. Our findings suggest that warming has stronger effects than elevated CO₂ on autotrophic nitrification, and AOA are more responsive to elevated temperature than AOB in the tested dryland ecosystems.

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1. Introduction

The global averaged land surface temperature has experienced a successive warming of 0.85 °C over the period from 1880 to 2012,

and is projected to exceed 3.7 °C by the end of the 21st century (IPCC, 2013). In the meantime, the atmospheric CO₂ concentrations have increased by 40% since the pre-industrial times to current levels of >400 ppm, primarily from fossil fuel combustion and land-use changes (IPCC, 2013). These global climate changes are expected to have direct consequences on aboveground plant communities (Ghannoum et al., 2010), or indirectly influence the net primary production (NPP) by enhancing the nutrient competition between plants and microorganisms (Rütting et al., 2010) and altering the microbial turnover rates of soil nutrients (Karhu et al., 2014). The elevated CO₂ could enhance the NPP of Earth's

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ecosystems to increase carbon and nitrogen sequestration in soil organic matter and plant biomass, which in the long term may drive progressive nitrogen limitation of natural settings (Luo et al., 2004). Such climatic impacts were found to vary across soils with different nutrient conditions (Karhu et al., 2014), and might be particularly relevant within dryland ecosystems, which are highly vulnerable and characterized by extremely low availability of nutrients (Delgado-Baquerizo et al., 2014).

Drylands (including arid, semi-arid, and dry sub-humid ecosystems) constitute the largest terrestrial biome on the planet, comprising 41% of the Earth's land surface (Schimel, 2010), and provide essential ecosystem services (e.g., biofuel, biodiversity, food, and fibre) to over 38% of the global population (Reynolds et al., 2007). As a consequence of climate change, the total area of drylands is predicted to expand by 10% at the end of this century (Feng and Fu, 2013), causing a significant drop in primary productivity (Fraser et al., 2011). Microbial communities have been considered as principal drivers in nutrient cycling processes (Singh et al., 2010), and their competition with plants for limited nutrients will ultimately determine the sustainability of NPP in drylands. A number of Free-air CO₂ enrichment (FACE) experiments have been carried out to assess effects of climate change on dryland ecosystem functioning and nutrient cycling (Del Galdo et al., 2006; Jin and Evans, 2007; Steven et al., 2013), which revealed complex responses of soil microbial communities to the projected climate change. Despite the global distribution of drylands and their important ecosystem services, considerable uncertainty remains about how future climatic scenarios will affect the microbe-mediated nutrient cycling in these ecosystems.

Nitrogen, as an essential nutrient element, is recognized as the most limiting factor affecting the NPP and organic matter decomposition in natural dryland ecosystems (Delgado-Baquerizo et al., 2014). Among all the nitrogen cycling processes, ammonia oxidation is considered to be the rate-limiting step for nitrification (Kowalchuk and Stephen, 2001), exerting significant control over the balance between relatively immobile ammonium and more mobile nitrite and nitrate, and thus is crucial for plant nitrogen availability, groundwater nitrate leaching, and greenhouse gas N₂O production (Singh et al., 2010; Hu et al., 2015a). Ammonia oxidation is catalyzed by two functional microbial guilds: ammonia-oxidizing archaea (AOA) affiliated within the novel *Thaumarchaeota* phylum (Brochier-Armanet et al., 2008), and ammonia-oxidizing bacteria (AOB) belonging to the β - or γ -proteobacteria (Purkhold et al., 2000). The slow-growing ammonia oxidizers obtain their sole energy source through oxidizing ammonia, and therefore ammonia substrate availability is considered to be the principal determinant shaping the differential growth, metabolic divergence, and ecological niches of AOA and AOB (He et al., 2012; Prosser and Nicol, 2012). Given the extremely low nutrient levels in drylands, competition for limited substrates among ammonia oxidizers and plants would be intense in these ecosystems (Hu et al., 2015b).

There is increasing evidence suggesting the cellular, genomic, and physiological differences between AOA and AOB, for example: (1) AOA had a significantly high affinity for ammonia than AOB (Martens-Habbena et al., 2009), and were reported to prefer the ammonia-poor and acidic environments (He et al., 2012; Zhang et al., 2012), by contrast, AOB tended to play a more important role in nitrification in the nitrogen-rich and alkaline environments (Verhamme et al., 2011; Xia et al., 2011); (2) AOA's pathway of assimilating inorganic carbon via a hydroxypropionate/hydroxybutyrate cycle is far more energy efficient than AOB, and requires one third less energy than AOB via the costly Calvin–Benson cycle (Könneke et al., 2014). AOA's high efficiency of metabolism perfectly suits the lifestyle of AOA to thrive in oligotrophic

environments (Könneke et al., 2014); and (3) the size of AOA cells are generally 10-fold smaller than that of AOB, and the increased surface-to-volume could offer AOA a better adaptation under nutrient-poor conditions (Martens-Habbena and Stahl, 2011). Therefore, the contrasting substrate affinity and divergent nitrification pathways between AOA and AOB might lead to differential responses to above- and below-ground resource partitioning induced by climate change, and will eventually modulate the fate of nitrogen resources for plant.

To date, there has been a large body of studies addressing how climate changes influence aboveground plant communities (Ghannoum et al., 2010) and soil physiochemical dynamics (Barnard et al., 2005; Rütting et al., 2010; Phillips et al., 2011) across various humid tropical, temperate and polar regions (Schimel, 2010; Lamb et al., 2011; Van Groenigen et al., 2011; Brown et al., 2012). Although climate impacts on belowground processes and microbial communities have been frequently reported in recent years (He et al., 2010; Dijkstra et al., 2012; Rousk et al., 2012; Zhou et al., 2012; Frey et al., 2013), relatively less effort was devoted to decipher the responses of nitrogen cycling microorganisms (Pereira et al., 2011, 2013), particularly ammonia oxidizers, to climate changes in dryland ecosystems. Among multiple environmental and climatic factors, temperature is recognized as one of the most important drivers influencing nitrification (Tournu et al., 2008) and shaping the large-scale distribution patterns of AOB in soils (Fierer et al., 2009). By conducting a meta-analysis of publicly-deposited AOA *amoA* gene sequences across a wide spectrum of habitat types, the distribution patterns of AOA was also found to be strongly driven by temperature on a global scale (Cao et al., 2013). Although no significant effects of CO₂ on the abundance of nitrifiers and denitrifiers were observed in laboratory microcosms (Zhang et al., 2010) and the FACE experiment of agricultural soils (Pereira et al., 2011, 2013), soil ammonia oxidizers could respond significantly to changes in root exudates and plant residues induced by elevated CO₂ in grassland ecosystems (Horz et al., 2004). Few studies have investigated the interactive effects of elevated temperature and elevated CO₂ on ammonia oxidizers, and we still lack a mechanistic understanding of how climate changes interact to mediate the autotrophic nitrification in dryland ecosystems, adding major uncertainties in projecting the future nitrogen cycles.

The main objective of this study was to examine the effects of elevated temperature and CO₂ on the abundance, community composition, and metabolic activity of ammonia oxidizers in dryland ecosystems during early stages of *Eucalyptus tereticornis* establishment. Intact soil monoliths taken from three sites differing in basic characteristics were subjected to factorial combinations of temperature and CO₂ treatment for nine months in climate controlled growth chambers (Fig. S1). The final harvested soils from the controlled growth chamber experiment were immediately used for a short-term ¹³CO₂-DNA-stable isotope probing (SIP) microcosm, which allowed deciphering detailed community structures of the metabolically active nitrifiers by a high-throughput functional gene microarray (Abell et al., 2012). We tested the following hypotheses: (1) elevated temperature will directly increase the nitrification rates by stimulating the activity of nitrifiers, or indirectly affect the nitrification activity by modifying mineralization, and plant and soil properties, (2) elevated CO₂ can only indirectly affect the nitrification rates by impacting the plant biomass, but such effects might be trivial in a short-term experiment of nine months; and (3) AOA and AOB will respond differently to climate changes, due to their significant divergence in substrate affinity, energy use efficiency, and genetic features.

2. Materials and methods

2.1. Sampling sites and collection of soil monoliths

Soil samples were collected from three distinct natural sites with Eucalyptus trees as the main vegetation types, that is, Rosemarys (R) and Camerons (C) about 1 km from each other developed on New England Granite series at University of New England's Newholme Farm, Armidale (30°25'S, 151°39'E), and Driftway (D) (33°36'S, 150°44'E) developed on Clarendon formation at the University of Western Sydney, Richmond, NSW, Australia. The average annual rainfall at the Rosemarys and Camerons sites is 790 mm, with the mean annual temperature at 21.1 °C. The average annual rainfall at Driftway is 801 mm, and the mean annual temperature is 17 °C. The soils are classified as Yellow-Chromosol at Rosemarys and Camerons, and as Clarendon Sand at Driftway according to the Australian Soil Classification system. The detailed information about the soil properties (including soil pH, H₂O₂, total nitrogen, total carbon, C:N ratios, and mineral nitrogen contents) is listed in Table S1. Soil types are predominantly sandy and arid, with precipitation/evapotranspiration ratios around 0.5 and 0.6 for Rosemarys/Camerons and Driftway sites, respectively, and thus classified as dry sub-humid ecosystems under the UNEP classification (Millennium Ecosystem Assessment, 2005). In the April 2011 field campaign, 72 intact soil monoliths were taken from the three field sites (24 monoliths for each site) by driving the PVC pipes (25 cm diameter × 45 cm height) into the ground (40 cm deep) and digging out. Monoliths were sealed at one end with caps containing drainage holes, and shipped to laboratory for the climate-controlled growth chamber experiment.

2.2. Climate-controlled growth chamber experiment

The growth chamber experiment was established with a full factorial design including four treatments: ambient CO₂ (400 ppm) plus ambient temperature (aCaT), elevated CO₂ (600 ppm) plus ambient temperature (eCaT), ambient CO₂ (400 ppm) plus elevated temperature (+3 °C) (aCeT), and elevated CO₂ (600 ppm) plus elevated temperature (+3 °C) (eCeT). Each treatment had six replicate monoliths, and each monolith was planted with one 3-month-old *E. tereticornis* seedling. Climate-controlled growth chambers were used to precisely manipulate light, temperature, humidity, and day–length cycles. The monthly day and night temperature was changed every month to simulate the realistic variations of climatic conditions based on 60 years' average data from the Meteorological Bureau Station (Table S2). A careful watering regime was implemented to ensure soil moisture levels in experimental pots fell within annual range of *in situ* field conditions. Soil monoliths were rotated between chambers every 4–6 weeks to avoid any potential chamber effects. Soil cores (2.5 cm in diameter, 15 cm in depth) were collected at the beginning of the experiment (T0), and 3, 7, and 9 months thereafter (T3, T7, and T9) from the monoliths, sieved through a 2.0 mm mesh, mixed thoroughly, and stored at –80 °C for DNA extraction and at 4 °C before physicochemical analyses. At the final harvest (T9), the above-ground plant properties including plant height and biomass were also recorded (Table S1).

2.3. DNA extraction and physicochemical analysis

The genomic DNA was extracted from 0.25 g of soil subsamples from four randomly-chosen replicates out of the six monoliths per treatment using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions, with slight modifications that the initial cell-lysis step

was achieved by using a FastPrep bead beating system (Bio-101, Vista, CA, USA) at a speed of 5.5 m s^{–1} for 30 s. The concentration and quality of extracted DNA were assessed using the NanoDrop® ND-2000c UV–Vis spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Soil ammonium and nitrate were extracted from 5 g of fresh soils with 25 ml of 2 M KCl by shaking at 170 rpm for 60 min, and the filtered solution was determined on a SEAL AQ2 Analyzer (SEAL Analytical Inc., Mequon, WI, USA). Soil moisture content was determined by oven-drying the samples at 105 °C overnight. Soil pH was assessed using a fresh soil to water ratio of 2.5 using a Delta pH-meter (Mettler-Toledo Instruments Co., Columbus, OH, USA). Total carbon and total nitrogen were measured on a LECO macro-CN analyzer (LECO, St. Joseph, MI, USA).

2.4. Potential nitrification rates (PNR) measurement

PNR was measured using the chlorate inhibition soil-slurry method as previously described (Kurola et al., 2005) with modifications, which represent the soil ammonia oxidation activity incubated with adequate substrate within 24 h. Adjustment of soil slurry pH to 7.1 was thought to limit activity of acidophilic ammonia oxidizing strains (Prosser and Nicol, 2012), hence in this study, PNR was assessed at the natural pH for each soil without adjustment of slurry pH. In brief, for each sample, three subsamples (5 g of fresh soil) were incubated in 50 ml falcon tubes containing 20 ml of 1 mM (NH₄)₂SO₄. Potassium chlorate was added to the tubes at a final concentration of 10 mg ml^{–1} to inhibit nitrite oxidation. The suspension was incubated in the dark at 25 °C for 24 h, and nitrite was extracted with 5 ml of 2 M KCl and determined using a spectrophotometer at a wavelength of 540 nm with N-(1-naphthyl) ethylenediamine dihydrochloride (Hu et al., 2015b). PNR was calculated as the linear accumulation in concentrations of nitrite between time 0 and after 24 h during the incubation.

2.5. Quantitative PCR (qPCR) analysis of *amoA* genes

Abundance of AOA and AOB *amoA* genes in controlled growth chambers was determined using the primer sets CrenamoA23f/CrenamoA616r (Tourna et al., 2008) and *amoA*-1F/*amoA*-2R (Rotthauwe et al., 1997), respectively. The 25 µl reaction mixture contained 12.5 µl of SensiMix SYBR No-ROX reagent (Bioline, Sydney, Australia), 0.6 µl of each primer (20 µM), 1 µl of bovine serum albumin (20 mM), and 2 µl of 5-fold diluted template DNA (1–10 ng). All qPCR analysis was carried out on a Corbett Rotor-Gene 6000 cycler (Qiagen, Doncaster, VIC, Australia) using the thermal-cycling conditions as follows: 5 min initial denaturation at 95 °C, 40 cycles of 30 s at 95 °C, 30 s at 53 °C for AOA and 55 °C for AOB, 1 min at 72 °C, followed by a plate read at 83 °C. Standard curves were generated using ten-fold serial dilutions of plasmids containing correct inserts of AOA or AOB *amoA* genes. Melting curve analyses were conducted following each assay to evaluate the specificity of PCR products, and the amplification efficiencies for all assays ranged between 87% and 99%.

2.6. Community structure analysis by terminal restriction fragment length polymorphism (T-RFLP) and diagnostic *amoA*-based microarray

The community structure of AOA and AOB at the T9 sampling point was characterized by T-RFLP analysis of the *amoA* genes using the fluorescently-labelled primers FAM-CrenamoA23f/CrenamoA616r and VIC-*amoA*-1F/*amoA*-2R, respectively. Amplification conditions, PCR product purification, digestion, and data processing were performed as described previously (Hu et al., 2015b). The total genomic DNA extracted from a randomly chosen

subset of samples (three replicates for each treatment) from the growth chamber experiment at the T9 sampling point was further characterized by the high-throughput microarray to target a broad variety of ammonia oxidizers. PCR amplification, *in vitro* transcription, fragmentation, hybridization, and scanning followed the detailed protocol as described previously (Abell et al., 2012).

2.7. DNA-SIP, net nitrogen mineralization and nitrification rates

Soils collected from the final harvest (the T9 sampling point) were immediately incubated in a SIP microcosm to identify the active autotrophic ammonia oxidizers (Fig. S1). Two sets of treatments were established in triplicate microcosms: 5% (v/v) $^{12}\text{CO}_2$ or 5% $^{13}\text{CO}_2$ (99 atoms%, Sigma–Aldrich Co., St Louis, MO, USA) was injected to the headspace (Zhang et al., 2012). Sealed 160-ml serum bottles were incubated in their original controlled growth chambers, and destructively sampled after 30 days of incubation. The light, temperature, humidity, and day–length cycles were maintained, which were identical to that used at the last month of the growth chamber experiment. Soil moisture content was kept constant (as that determined at the T9 sampling point) by weekly replacing the water loss of soils. Net nitrogen mineralization rates and net nitrification rates were estimated via the changes in concentrations of ammonium and nitrate during the incubation (Gleeson et al., 2010).

Isopycnic density-gradient centrifugation was performed for triplicate SIP microcosms in 1.8-ml seal tubes in a S120VT vertical rotor (Hitachi, Japan), subjected to ultracentrifugation at 140,000 g_{av} (70,000 rpm) for 36 h at 20 °C. The CsCl gradient was fractionated into 18 equal fractions, and genomic DNA was retrieved from both light and heavy fractions as described in Supplementary Methods, and used for qPCR and microarray analysis. We compared qPCR and T-RFLP results from the T9 of the growth chamber experiment and the day 30 of the DNA-SIP incubation (Figs. S2 and S3), and found no significant changes in abundance and community structure of AOA or AOB, indicating that 30-day DNA-SIP incubation did not cause a disturbance.

2.8. Network analysis and visualization

The co-occurrence patterns between the metabolically active AOA phylotypes (retrieved from heavy fractions of the SIP incubation using microarray probes) were explored in network analysis using the CoNet Cytoscape plug-in (Soffer et al., 2014). Briefly, for all the pairwise interactions, correlation scores were calculated using Pearson correlation, Spearman correlation, mutual information, Bray–Curtis dissimilarity, and Kullback–Leibler dissimilarity. All AOA phylotypes below a minimum occurrence of 3 across the three soils were discarded to avoid introduction of spurious correlations. The ReBoot procedure with 100 permutations was conducted to control the potential false-positive correlations, and the resultant distribution was further refined with 1000 bootstraps. The *P* values for correlations were combined from the five correlation measures using the *Simes* method, and only correlations found to be significant by at least two correlation methods were included (Soffer et al., 2014). Network topology was visualized and explored using the Fruchterman Reingold algorithm on the open-source interactive platform Gephi (Bastian et al., 2009). Only correlations with a ρ -value above 0.6, and a significance level below 0.05 were displayed.

2.9. Statistical analyses

The *amoA* gene copy numbers were log-transformed prior to statistical analysis to meet normality assumptions. A three-way

analysis of variance (ANOVA) with temperature and CO_2 as between-subjects factors and sampling time as within-subjects variable was used to determine the effects of eC, eT, sampling time, and their interactions, on soil properties, PNR, and molecular measures. A two-way ANOVA was performed to analyze the effects of eC, eT, and their interactions on rates of net mineralization and nitrification. Multiple comparisons of group means between treatments were performed with Student–Newman–Keuls test after one-way ANOVA on soil properties, PNR, net mineralization and nitrification rates, and the *amoA* gene copies in SPSS 20 (IBM Co., Armonk, NY, USA). $P < 0.05$ was considered to be statistically significant. Non-metric multidimensional scaling (NMDS) was used to visualize the Bray–Curtis dissimilarity matrices based on the hybridization intensity of AOA probes. The significance of sample discrimination between different treatments was tested via the two-way permutational multivariate analysis of variance (PerMANOVA) by using the *adonis* function in the *vegan* package of the R platform with 999 permutations. The heat map illustrating the microarray results of AOA was generated using the *gplots* package in R.

Finally, we constructed a structural equation model (SEM) to evaluate how climate changes influence plant properties (including plant height, leaf number, leaf area, leaf dry weight, leaf carbon, leaf nitrogen, leaf C/N, and plant biomass at the T9 sampling point; Table S1), soil properties (including $\text{NH}_4^+ - \text{N}$, $\text{NO}_3^- - \text{N}$, soil pH, water content, total carbon, and total nitrogen at the T9 sampling point; Table S1), AOA community abundance (at the T9 sampling point) and structure (including only the $^{13}\text{CO}_2$ -labelled active AOA phylotypes detected by the microarray), net nitrification rates and net nitrogen mineralization rates. Prior to the SEM analysis, the pairwise correlations among these variables were examined by the Mantel test using the *Ecodist* package in R. The matrix of *R* values derived from Mantel test was imported into AMOS 21.0 (SPSS Inc., Chicago, IL, USA) for construction of SEM. The *a priori* and theoretical assumptions made to establish the structural equation model were (1) eT could directly increase the nitrification rates by stimulating the enzymatic activity of nitrifiers; (2) eT could also indirectly affect nitrification by enhancing nitrogen mineralization rates, by altering the abundance and community structure of AOA, or by impacting the plant biomass, which in turn increased nitrogen mineralization rates and subsequently affected AOA community and nitrification; (3) eC can only indirectly affect the nitrification rates by impacting the plant growth, which consequently affected soil properties, mineralization, AOA community and nitrification. The data matrix was fitted to the model using the maximum-likelihood estimation method. The overall goodness-of-fit of model fits was indicated by a non-significant chi-square test ($P > 0.05$), high goodness-of-fit index (>0.90), low Akaike information criteria (AIC), and low root square mean errors of approximation (RMSEA < 0.05).

3. Results

3.1. Differentiations in soil properties, PNR and abundances of AOA and AOB in climate-controlled growth chambers

Soil pH remained largely unchanged during the course of growth chamber experiment (Tables S1 and S3). Soil moisture contents, total nitrogen, total carbon, $\text{NH}_4^+ - \text{N}$, and $\text{NO}_3^- - \text{N}$ were significantly affected by eC, eT, or eC $\times$ eT interactions (Table S3), but these effects were not consistent across the three soils. PNR tended to increase with time and temperature in all the three soils (Fig. S4), and the effect of time $\times$ eT was significant in the R and C soils (Table 1). The AOA *amoA* gene copies, ranging from 6.4×10^7 – 1.3×10^8 copies g^{-1} soil in the R soil to 2.7 – 7.2×10^7 and

Table 1

Summary of two-way ANOVA results (repeated measures) indicating the effects of eC and eT on potential nitrification rates (PNR), abundances of AOA and AOB *amoA* genes ($n = 4$), net nitrogen mineralization rates (NNMR), and net nitrification rates (NNR) ($n = 6$).

	PNR	AOA	AOB	NNMR	NNR
<i>Rosemarys</i>					
eC	0.505	0.370	8.811	3.358	2.156
eT	3.910	15.88	11.12	16.15	15.37
eC $\times$ eT	1.551	0.047	0.187	0.521	0.041
Time	4.340	8.479	0.508	NA	NA
Time $\times$ eC	7.947	0.472	0.008	NA	NA
Time $\times$ eT	35.25	0.816	6.391	NA	NA
Time $\times$ eC $\times$ eT	0.349	1.000	0.209	NA	NA
<i>Cameron's</i>					
eC	3.702	6.591	0.166	0.586	0.286
eT	3.616	20.15	7.604	1.372	3.128
eC $\times$ eT	0.060	1.751	0.432	0.409	0.097
Time	3.376	8.541	1.966	NA	NA
Time $\times$ eC	10.05	0.285	5.464	NA	NA
Time $\times$ eT	15.21	4.068	7.051	NA	NA
Time $\times$ eC $\times$ eT	0.147	0.278	0.026	NA	NA
<i>Driftway</i>					
eC	3.984	2.581	1.852	0.186	2.208
eT	3.944	5.803	2.101	9.732	18.15
eC $\times$ eT	0.997	1.772	4.299	2.106	1.387
Time	5.697	6.560	2.466	NA	NA
Time $\times$ eC	0.368	0.898	2.664	NA	NA
Time $\times$ eT	1.466	0.611	4.273	NA	NA
Time $\times$ eC $\times$ eT	1.867	0.160	0.156	NA	NA

Values showed in this table are the *F*-value exported from the two-way ANOVA. Bold values indicate a significant difference at $P < 0.05$, and NA indicates that data are not available.

6.6×10^7 – 1.3×10^8 in the C and D soils, respectively, were more than one order of magnitude higher than AOB *amoA* gene (Fig. 1). Significantly positive effects of eT were observed on AOA abundance in the R and C soils (Table 1), with increasing AOA *amoA* gene copies observed in the aCeT and eCeT treatments (Fig. 1), but no significant impact of climate change on AOA abundance was found in the D soil. By contrast, AOB abundance tended to decrease with time from above 5.37×10^6 copies g^{-1} soil to below 3.49×10^6 copies g^{-1} soil in the C and D soils, and a significant impact of eT on AOB abundance was found only in the R soil (Table 1).

3.2. Community profiling of ammonia oxidizers by T-RFLP

The T-RFLP analysis of AOA *amoA* gene by the *RsaI* enzyme yielded 10 distinct terminal restriction fragments (TRFs) across all the treatments at the T9 sampling point, of which TRF-187, -195, and -276 were the most dominant phylotypes and differentially responded to eT in the R and C soils (Fig. 2a). The D soil harboured a highly divergent AOA community, with the relative abundances of all the dominant phylotypes (TRF-187, -195, -293, -336, and -388) obviously responsive to climate change. Notably, TRF-195, being an important fragment in the aCaT and eCaT treatments, was totally absent from the warming treatments in the D soil. By contrast, digestion of AOB *amoA* gene by the *MspI* enzyme resulted in 11 distinct TRFs, dominated by TRF-150 throughout the different treatments and soils (Fig. 2b). AOB community structures seemed to be highly resistant to climate change, and no apparent differences could be observed between different treatments.

3.3. Community profiling of ammonia oxidizers by microarray

The high-resolution *amoA*-based functional gene microarray was further employed to elucidate the detailed phylogenetic changes in AOA and AOB communities during the controlled

growth chamber experiment at the T9 sampling point. Due to the low abundance of AOB in all the examined soils (Fig. 1), obtaining sufficient PCR products for AOB as required by microarray was not possible, therefore only the fingerprinting profiles of AOA were demonstrated (Fig. 2c). Consistent with the T-RFLP results, the R and C soils harboured contrasting AOA populations from the D soil, in which the sequences related to the *Nitrosotalea* and *Nitrososphaera* clusters were found to be dominated by only six major probes (AamoA-101, -100, -104, -72, -73, and -69). Intriguingly, remarkably stronger hybridization signals for probes AamoA-72 and -73 targeting *Nitrososphaera* were observed in the aCeT treatment of the R and D soils, relative to the aCaT treatment, while probes AamoA-64, -65, -48, -18, and -12 targeting *Nitrososphaera* showed weaker signals in the aCeT and eCeT treatments of the R soil, indicating differential adaptation of AOA phylotypes to climate changes. The NMDS analysis based on the Bray–Curtis dissimilarity matrices (Fig. 3) revealed a clear separation of the aCeT treatment from other treatments in the R and D soils, whereas the eCeT treatment harboured the most distinct AOA community in the C soil, but the significant effects of eT could only be observed in the D soil as revealed by the two-way PerMANOVA analysis (Table S4).

3.4. Net nitrogen mineralization (NNMR) and net nitrification rates (NNR) during the DNA-SIP incubation

To provide more direct evidence for the metabolic activity of ammonia oxidizers in response to climate treatments, soil samples collected from the final harvest of growth chamber experiment were immediately used in a 30-day DNA-SIP microcosm incubation. NNMR was calculated as the changes in concentrations of ammonium and nitrate during the DNA-SIP incubation, whereas NNR as the change in nitrate concentrations (Gleeson et al., 2010). Active mineralization and nitrification were observed for all the three soils, and no strong differences were found between the $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ microcosms, indicating that $^{13}\text{CO}_2$ did not result in biases against ammonia oxidizers. Therefore, the data from the $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ microcosms for each treatment were combined together for analysis (Fig. 4). NNMR ranged from -0.034 to 0.219 mg N kg^{-1} soil per day in the R soil, to -0.010 to 0.118 and -0.062 to 0.192 mg N kg^{-1} soil per day in the C and D soils, respectively. Significantly positive effects of eT on NNMR were observed in both R and D soils (Table 1; Fig. 4). The patterns of NNR are quite similar to that of NNMR, with values varying from 0.096 to 0.316 $\text{mg NO}_3^- \text{N kg}^{-1}$ soil per day in the R soil, to 0.120 – 0.226 and -0.002 to 0.250 $\text{mg NO}_3^- \text{N kg}^{-1}$ soil per day in the C and D soils, respectively. There was a significantly positive effect of eT on NNR in the R and D soils (Table 1), but not significant effect of warming was found in the C soil. No significant impacts of eC or interactive effects of eC $\times$ eT could be found on NNMR or NNR in any of the examined soils.

3.5. ^{13}C -labelled functionally active ammonia oxidizers

To reveal which groups of ammonia oxidizers could be actively growing by assimilating CO_2 , isopycnic gradient ultracentrifugation was performed with genomic DNA isolated from each treatment to differentiate between the ^{12}C -DNA (inactive) and ^{13}C -enriched-DNA (active). AOA abundance in the $^{12}\text{CO}_2$ microcosms for all the three soils peaked only in the light fractions around a buoyant density of 1.69 g ml^{-1} (Fig. 5). After 30-day incubation in the presence of $^{13}\text{CO}_2$, the peak of AOA communities clearly shifted towards the heavy fractions around 1.71 – 1.73 g ml^{-1} , indicating assimilation of $^{13}\text{CO}_2$ into the genomic DNA of AOA. In treatments with eT, AOA were labelled to significantly higher extents compared with aT, except the eCeT treatment of the C soil, in which only a

Fig. 1. Changes in abundances of AOA and AOB *amoA* genes across different treatments at four sampling time points during the controlled growth chamber experiment. Data plotted are mean values and standard errors ($n = 4$) from replicate monoliths. The asterisk indicates significant difference between different treatments ($P < 0.05$).

minor proportion of AOA was observed in the active region. By contrast, AOB communities in both $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ microcosms reached the maximal abundance in the light SIP regions around a buoyant density of 1.70 g ml^{-1} (Fig. 5), suggesting that a majority of AOB remained inactive during the incubation. The active AOB populations comprised a minor percentage of the total AOB communities in the C and D soils, and the labelling extent in the warming treatments tended to be slightly lower than that under aT.

Genomic DNA retrieved from the light and heavy SIP regions were subjected to T-RFLP analysis of AOA by using the *RsaI* and *HpyCH4V* enzymes, which produced contrasting fingerprinting patterns between the 'active' and 'inactive' assemblages for nearly all the treatments (Fig. 6). The dominant active AOA phylotypes (TRF-187, -293 and -388 digested by *RsaI*; and TRF-74 and -160 digested by *HpyCH4V*) were observed to evidently respond to eT. High-throughput microarray was also performed to characterize the metabolically active AOA populations retrieved from the heavy SIP regions (Fig. 2c). Generally, the hybridization patterns of the active AOA members represented a subset of the total AOA community, in particular, the diversity of active AOA (measured by the frequency of positive probes) significantly decreased in the warming treatments in the R and C soils, but not in the D soil (Fig. 2d). Diverse probes targeting the *Nitrosotalea* and

Nitrososphaera clusters produced strong hybridization signals in all the soils. Intriguingly, probes AamoA-101, -100 and -104 targeting *Nitrosotalea* were increasingly positive in the aCeT treatment of the R soil, and probes AamoA-72 and -73 targeting *Nitrososphaera* were increasingly hybridized in the aCeT treatment of the R and D soils. By contrast, probes AamoA-64, -65, -46, -47, -45, -48, -19, -23, -18, -3, and -12 targeting *Nitrososphaera* were very weak or totally absent from the warming treatments of both R and C soils. The intensity of probes targeting *Nitrosopumilus* decreased to be undetectable in the aCeT and eCeT treatments of the C soil. We failed to get sufficient PCR products of AOB *amoA* genes for microarray analysis, due to the low AOB abundance in the heavy SIP fractions (Fig. 5). The NMDS analysis based on the Bray–Curtis dissimilarity matrices indicated that the active AOA community was highly divergent from the total AOA community for all the three soils (Fig. 3). Significant effects of eT on the active AOA communities could be found in the R and C soils, and no effects of eC could be observed (Table S4).

3.6. Co-occurrence patterns between active AOA phylotypes

Network analysis was conducted to determine whether eC and eT have significant impacts on the structural connections among

Fig. 2. Terminal restriction fragment length polymorphism fingerprints of the AOA (a, digested using the *RsaI* enzyme) and AOB (b, digested using the *MspI* enzyme) *amoA* gene fragments across different treatments at the T9 sampling point of the growth chamber experiment. Heat maps showing the *amoA*-based microarray results of AOA from total genomic DNA extracted from the T9 sampling point of the growth chamber (GR-T9) experiment and from ^{13}C -labelled DNA in the heavy SIP fractions at the day 30 of the DNA-SIP microcosm incubation (^{13}C -D30) (c), and the right panel (d) indicates the diversity of AOA represented by the averaged frequencies of positive microarray signals for each treatment, based on the microarray results as shown in the heat maps. Results of microarray were first normalized to the positive control probes, and then to the reference values determined individually for each probe. Only those probes with normalized signal intensity higher than 0.02 in at least one of the triplicate samples were displayed. Black arrows below the heat maps indicate the probes which are of particular interest in this study.

the co-occurring AOA phylotypes. The results showed apparent differences in network topology properties (determined by the number of nodes and edges, average degrees, modularity, clustering coefficient, and average path length) across different treatments (Fig. 7; Table 2). More frequent correlations could be found between AOA phylotypes in the eCaT (37 nodes and 479 edges) and aCaT treatment (33 nodes and 379 edges), compared to aCeT (24 nodes and 190 edges) and eCeT (30 nodes and 280 edges). Interestingly, the nodes representing the AamoA-72 and -73 phylotypes (affiliated within *Nitrososphaera*) had the highest degree among all nodes in the network derived from the aCaT treatment (Table S5), however, they were highly responsive to the changing climatic scenarios, with significantly less correlations with other phylotypes observed in other three treatments. By contrast, the phylotypes AamoA-100, -101, and -104 (affiliated within *Nitrosotalea*) had mostly negative correlations (mutual exclusion) with other phylotypes under aCaT (Fig. 7), and such correlations became much more frequent in climate treatments, as represented by the increased sizes of their nodes in networks of other three treatments.

3.7. Relationships among climate change, plant, soil, AOA community, and rates of net mineralization and nitrification

SEM was constructed to explore the direct and indirect effects of eC and eT on AOA communities and nitrification (Fig. 8). Our data showed that eT significantly increased plant biomass but eC had no effect. No significant effects of eC on soil properties or mineralization could be observed ($P > 0.05$). By contrast, eT had significantly positive influences on AOA community structure ($\lambda = 0.133$; $P < 0.001$) and nitrification ($\lambda = 0.144$; $P < 0.001$). Strongly positive effects of soil characteristics were also detected on mineralization ($\lambda = 0.091$; $P = 0.021$), nitrification ($\lambda = 0.236$; $P < 0.001$), and AOA abundance ($\lambda = 0.236$; $P < 0.001$). We also found that mineralization was the most important driver of nitrification ($\lambda = 0.412$; $P < 0.001$), and plant biomass had significantly positive effects on AOA abundance ($\lambda = 0.209$; $P < 0.001$) but not on AOA community structure. Overall, eT could indirectly influence AOA abundance by modifying plant biomass, and directly enhance soil nitrification or indirectly contribute to increased nitrification through the changes

Fig. 3. Non-metric multidimensional scaling (NMDS) ordinations derived from the Bray–Curtis dissimilarity matrices showing differences in AOA community compositions between different treatments at the T9 sampling time point and the day 30 of the DNA-SIP incubation, based on the microarray data. The stress values for all NMDS plots are lower than 0.11 indicating that the differences are well-represented by the two-dimensional ordinations.

in community structure of the active AOA populations. In addition, soil properties as represented by nutrient factors (*e.g.*, total carbon, total nitrogen, ammonium, and nitrate), soil pH and water contents (which can affect availability of nutrients), could also play an important role in impacting AOA abundance, mineralization, and nitrification.

4. Discussion

4.1. Elevated temperature has more important roles than elevated CO₂ in impacting ammonia oxidation within dryland soils

The nitrogen cycling within dryland ecosystems account for a major part of the global nitrogen cycle, and the nitrogen turnover and balance are increasingly influenced by climate changes (Delgado-Baquerizo et al., 2014). Results from previous studies on the effects of climate change on nitrogen cycling are mainly focused on soil enzymatic activities, nitrogen dynamics, and aboveground plant properties (Ghannoum et al., 2010; Rütting et al., 2010; Van Groenigen et al., 2011; Dijkstra et al., 2012), with less understanding of the belowground microbial communities (He et al., 2010; Pereira et al., 2011, 2013; Rousk et al., 2012; Zhou et al., 2012). Meanwhile, emerging biogeochemical process-based models are attempting to directly link microbial physiology with soil nutrient cycling (Frey et al., 2013), but they are facing difficulties of integrating and parameterizing less-understood microbial dynamics into modelling effort. In this study, by simulating eT (+3 °C) and eC (+200 ppm) in climate-controlled growth chambers for nine months, nitrogen cycling processes (including potential/net nitrification and net nitrogen mineralization rates), soil properties, plant biomass, and abundance, community composition, and metabolic activity of ammonia oxidizers in three dryland soils were comprehensively assessed. Our results suggested a much more

dominant role of eT than eC in impacting autotrophic nitrification within the tested dryland soils. Although these climate effects are not always consistent and might be soil specific, the SEM analysis collectively suggested that warming effects could be directly exerted on nitrification possibly by enhancing the enzymatic activity, and indirectly on nitrification by shifting the community structure of the active AOA phylotypes, or by changing the substrate availability for nitrification through modifying nitrogen mineralization activity (Fig. 8). However, in contrast with our hypothesis, changes in plant biomass induced by eT did not have direct effects on soil properties, mineralization or nitrification (Fig. 8), this might be due to the short duration of the growth chamber experiment in which the decomposition of plant residues would take more time to apparently affect the substrates available for nitrification.

The significant effects of eT on autotrophic nitrification and ammonia oxidizers might be explained by three possible mechanisms. Firstly, nitrification is a microbially-mediated process predominantly controlled by ammonia oxidizers and their temperature-sensitive enzyme activity, which could directly respond to warming through thermal adaptation (genetic changes) and acclimation (physiological responses) (Karhu et al., 2014). Higher temperature could provide beneficial conditions through directly increasing cell maintenance and survival and concomitantly decreasing the lag time for cell growth (Avrahami and Bohannan, 2007). As evidenced in this study, enhanced nitrification rates are closely related to increase in AOA abundance, shifts in AOA community composition, and stimulation of AOA metabolic activity, which might be due to direct responses to climate warming. Furthermore, it is also possible that these soils contain AOA phylotypes pre-adapted to relatively higher temperature (De la Torre et al., 2008; Hatzenpichler et al., 2008), which stay less active or dormant (Lennon and Jones, 2011) at lower temperature with low resource availability, but are potentially capable of being

Fig. 4. Changes in net nitrogen mineralization rates (NNMR) and net nitrification rates (NNR) across different treatments in the DNA-SIP microcosms during 30 days' incubation. The data from the $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ treatments were pooled, because no significant differences in the NNMR and NNR were found between the two treatments. Error bars represent standard errors of replicate samples ($n = 6$). Different letters above the bars indicate a significant difference ($P < 0.05$).

resuscitated and contribute to nitrification at higher and optimal temperature (Fig. 5) when resource limitation is relieved by warming-stimulated nitrogen mineralization. Based on the microarray results of the AOA communities in the DNA-SIP incubation, the phylotypes (AamoA-100, -101, and -104 affiliated with *Nitrosotalea*; AamoA-72 and -73 affiliated with *Nitrososphaera*) in the R soil were increasingly labelled by $^{13}\text{CO}_2$ (Fig. 2c), suggesting their high ability of adaptation to high temperature. Secondly, the functional genes, microbial enzyme activities and microbes involved in organic matter degradation have been reported to be greatly stimulated (Zhou et al., 2012; Nie et al., 2013) under eT, resulting in increased capacity to mineralize organic matter and enhanced nitrogen availability (Frey et al., 2013). The diffusion rates of ammonia substrate into microbial cells were also presumed to increase with increased temperature in soil environment (Avrahami and Bohannan, 2007). In addition, soil warming could accelerate intracellular enzyme kinetics and increase microbial cell turnover, which may enter the soil organic matter pool (Hagerty et al., 2014). As a result, we observed an increasing trend of net nitrogen mineralization rates under eT (Fig. 4), which might provide a pulse of high-level substrates for ammonia oxidizers. Thirdly, it was reported that climate warming could significantly alter the amount of root exudation (Uselman et al., 2000; Yin et al., 2013),

leading to increased availability of nitrogen and carbon sources. Higher temperature could also stimulate the decomposition processes of leaf materials and rhizodeposition by increasing the capacity and rates of microorganisms (Singh et al., 2010). Although plant litters and root exudation were not examined in this study, we did find a significantly positive impact of eT on plant biomass (Fig. 8), which could lead to above-belowground resource partitioning and eventually affect the growth of ammonia oxidizers.

Beyond the significant impacts of climate warming, eC was also reported to significantly influence the abundance and community structure of ammonia oxidizers (Horz et al., 2004). It is believed that the influences of eC on microbial communities are generally indirect via the higher plant residue inputs (Singh et al., 2010), and eC has only modest effects on the CO_2 concentrations within the soil matrix (Drigo et al., 2008). Previous findings from FACE and open top chamber studies found that eC has small or no inhibitive effects on gross/net nitrogen mineralization (Gill et al., 2002; De Graaff et al., 2006), nitrifying and denitrifying enzyme activity is decreased by 18–20% (Barnard et al., 2005) and gross nitrogen immobilization and microbial nitrogen content was stimulated by 22% and 5.8%, respectively, under eC (De Graaff et al., 2006). Although effects of eC were not significant in this study, the SEM analysis found that eC has marginally negative effects on

Fig. 5. Quantitative distribution of the relative abundance of AOA and AOB *amoA* genes retrieved from CsCl gradients from the $^{13}\text{CO}_2$ or $^{12}\text{CO}_2$ treatments during the DNA-SIP microcosms after 30 days' incubation. Eighteen fractions of genomic DNA extracted from each 1.8-ml centrifuge tube covered a buoyant density gradient from 1.64 to 1.75 g ml^{-1} . The plotted values are the proportion of AOA *amoA* gene copies in each fraction to the total abundance across the entire gradient. For easy interpretation of results, the standard error bars from the triplicate microcosms were removed. Because the $^{12}\text{CO}_2$ microcosms from all the treatments had good reproducibility, only the data from the aCaT treatment were plotted.

mineralization, plant and soil properties (Fig. 8). In addition, the negative impacts of warming on network complexity was compromised by eC as observed in the co-occurrence patterns that the aCaT and aCeT treatments harboured 379 and 190 edges, respectively, which were increased to 479 and 280 edges in the eCaT and eCeT treatments, respectively (Fig. 7 and Table 2). The possible antagonistic interaction between eC and eT on nitrification observed in this study suggested that the strong impacts of eT on nitrogen availability might be slightly relieved by eC in dryland soils. It should be noted that the duration of the growth chamber experiment is only nine months in this study, a longer term might be needed to observe a significant effect of eC on nitrification and nitrifiers.

4.2. Increasing advantages of AOA over AOB under climate warming

Recent studies have attempted to unravel the differential responses of AOA and AOB to temperature alternations, which suggested that temperature selection effects are much stronger on the abundance and transcriptional activity of AOA phylotypes (Tournau et al., 2008), and shifts in AOB community toward warming are not significant in microcosm incubations of agricultural soils (Tournau et al., 2008) and open-top chamber experiments of Antarctic soils (Yergeau et al., 2012). Among these studies, AOA were assumed to prefer the relatively higher temperatures compared with their counterpart AOB. For example, in arid and semiarid soils, AOA abundance was remarkably higher in the summer than in the

Fig. 6. Comparisons of T-RFLP fingerprinting of the AOA *amoA* gene TRFs (digested using the *RsaI* or *HpyCH4V* enzymes) from both light (L) and heavy (H) SIP fractions across different treatments. Error bars represent standard errors ($n = 3$).

winter, while a reverse trend was observed for AOB abundance (Sher et al., 2013). In agricultural and grassland soils, non-thermophilic AOA phylotypes responded positively to increasing temperature (Tournai et al., 2008), paralleled with increased PNR, but AOB abundance showed an opposite trend (Avrahami and Bohannan, 2007). However, these studies did not differentiate between the functionally active and inactive microorganisms or the relative contribution of AOA and AOB to nitrification. In this study, by constructing $^{13}\text{CO}_2$ -DNA-SIP microcosms, we provide direct evidence that climate warming is more likely to favour the metabolic activity of AOA, which are the dominant nitrifiers in dryland ecosystems and can be actively growing, replicating, and incorporating $^{13}\text{CO}_2$ substrates. The increasing $^{13}\text{CO}_2$ assimilation by AOA is well related to enhanced potential/net nitrification rates in the R and D soils (with a similar trend observed in the C soil), indicating a more important contribution of AOA to nitrification and CO_2 fixation in drylands under warming. However, only a minor fraction of AOB, representing less than 20% of the total AOB community, was labelled by $^{13}\text{CO}_2$, and the labelling extent was even reduced by eT in the C soil, implying that most of AOB stayed less active or dormant in nutrient-poor drylands. It should be noted that DNA-SIP cannot detect nitrification carried out by heterotrophic or non-growing ammonia oxidizers (Pratscher et al., 2011), even though these microorganisms might also play important roles in nitrification.

The dominance of AOA over AOB in drylands under eT could be attributed to their intrinsic physiological adaptation to warming or

significant divergence in substrate affinity. Firstly, substantial evidence has suggested that enriched and pure AOA strains generally have high temperature optima than their counterparts AOB. For instance, the first soil AOA strain *Nitrososphaera viennensis* shows optimal growth at 37 °C (Tournai et al., 2011), growth of the moderate thermophilic Candidatus *Nitrososphaera gargensis* is favoured under 46 °C (Hatzenpichler et al., 2008), and the thermophilic AOA *Nitrosocaldus yellowstonii* could grow up to 74 °C (De la Torre et al., 2008). A maximum growth rate of the marine AOA strain *Nitrosopumilus maritimus* SCM1 was observed at 32 °C, but no growth could be detected at 10 °C (Qin et al., 2014). By contrast, to date no AOB strains have been isolated or detected in environments with constant temperatures above 40 °C (Jiang and Bakken, 1999; Hatzenpichler, 2012). Our SEM analysis also suggested that eT could directly influence AOA community structures, which then resulted in a higher nitrification rate (Fig. 8). Secondly, eT could indirectly lead to niche differentiation of AOA and AOB via altering the intensity of resource competition. In nutrient-poor dryland soils, AOA and AOB have to compete with other ammonium-assimilating organisms, such as heterotrophic nitrifiers and plants, and it has been suggested that AOB are inferior competitors for limited substrates in soils (He et al., 2012). Kinetic studies established that AOA ammonia monooxygenase possesses remarkably higher affinity for substrates than most other known microorganisms (Martens-Habbena et al., 2009), and high density of ammonium/ammonia transporters within AOA cells could facilitate substrate uptake (Urakawa et al., 2011), which might

Fig. 7. Schematic representation of the effects of eC and eT (a, aCaT; b, eCaT; c, aCeT; and d, eCeT) on the co-occurrence patterns of the metabolically active AOA phylotypes (recovered from the heavy fractions of the SIP incubations) based on dataset from the microarray analysis. The nodes represent the AOA lineages detected by different microarray probes, and the edges correspond to a significant correlation ($P < 0.05$) between nodes. The size of each node is proportional to the number of significant connections. Node colours denote different AOA lineages (*Nitrososphaera*, *Nitrosopumilus*, and *Nitrosotalea*). Red lines connecting nodes indicate significantly negative correlations (mutual exclusion), while green lines denote significantly positive patterns (co-occurrence).

selectively afford AOA greater ecological advantage in dryland and nutrient limited soils under warming. Thirdly, cellular, genomic, and physiological features of nearly all representative AOA cultures reflect an oligotrophic lifestyle, being well-adapted to grow with

low-level energy supply (Lehtovirta-Morley et al., 2011). AOA's pathway of assimilating inorganic carbon via a hydroxypropionate/hydroxybutyrate cycle is far more energy efficient than any other aerobic autotrophic pathways, and requires one third less energy

Table 2
Topology properties of the co-occurrence networks of AOA phylotypes across different treatments. All the data were exported from the Gephi platform.

	No. of nodes	No. of edges	Average degree ^a	Modularity ^b	Average clustering coefficient ^c	Average path length ^d
aCaT	33	379	11.485	0.364	0.408	1.27
eCaT	37	479	12.946	0.596	0.416	1.284
aCeT	24	190	7.917	0.537	0.41	1.236
eCeT	30	280	9.333	0.405	0.395	1.325

^a The degree of a node in a network is defined as the number of connections it has to other nodes.
^b Modularity is designed to measure the strength of division of a network into modules. Networks with high modularity have dense connections between nodes within modules but sparse correlations between nodes in different modules.
^c Average clustering coefficient represents the degree to which nodes in a network tend to cluster together.
^d Average path length is defined as the average number of steps along the shortest paths for all possible pairs of network nodes.

Fig. 8. The structural equation model showing the effects of eT and eC on plant and soil properties, abundance and community structure of AOA, and net nitrogen mineralization and nitrification rates (including data from all the three soils at the T9 sampling point). Plant properties are composed by plant height, leaf number, leaf area, leaf dry weight, leaf carbon, leaf nitrogen, leaf C/N, stem biomass, and branch biomass (Table S1). Soil properties are composed by $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, soil pH, water content, total carbon, and total nitrogen (Table S1). AOA community structures include only the $^{13}\text{CO}_2$ -labelled active AOA phylotypes detected by the microarray. Nitrification and mineralization include only net nitrification rates and net nitrogen mineralization rates, respectively. Bold and dashed arrows indicate positive and negative relationships, respectively; red and blue arrows denote significant and non-significant pathways, respectively. Numbers adjacent to arrows are path coefficients, and width of the arrows is proportional to the strength of path coefficients. R^2 values denote the proportion of variance explained for each variable. The hypothetical model fit our data well, as suggested by $\chi^2 = 14.5$, $P = 0.107$; GFI = 0.994; AIC = 68.47; and RMSEA = 0.031. Significance levels are as follows: ** $P < 0.05$ and *** $P < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

than AOB via the costly Calvin–Benson cycle (Könneke et al., 2014). AOA's high efficiency of metabolism by utilizing the energy obtained from ammonia oxidation for biomass formation perfectly suits the lifestyle of AOA to thrive in drylands with a constantly low-level energy supply (Könneke et al., 2014). Therefore, the high affinity for ammonia substrate and the most energy-efficient aerobic pathway might provide AOA an ecological advantage in dryland soils over their counterparts AOB in face of future climatic scenarios like warming.

High-resolution microarray analyses of AOA recovered from the active SIP regions revealed that the active AOA community was predominantly affiliated within *Nitrosotalea* and *Nitrososphaera*, with a minority belonging to *Nitrosopumilus* (Fig. 2c). Previous studies have recognized *Nitrososphaera* as the most abundant soil AOA lineage (Hu et al., 2013) with strong genetic capacity to use various ammonia sources and flexible carbon metabolism (Spang et al., 2012), and directly linked *Nitrososphaera* with nitrification activities in alkaline (Xia et al., 2011) and acidic agricultural soils (Zhang et al., 2012). Our findings provide new traits of *Nitrososphaera* that different phylotypes of this cluster occupy distinct ecological niches shaped by eT, and a small fraction of *Nitrososphaera* being tolerant of higher temperature (Fig. 2c) might be increasingly responsible for dryland nitrification under warming. Moreover, although the *Nitrosotalea* lineage was considered to be obligately acidophilic (Lehtovirta-Morley et al., 2011) and to be functionally active in acidic soils (Zhang et al., 2012), our study expanded the functional importance of *Nitrosotalea* to drylands (soil pH 5.29–6.35), which are, similar to acidic soils, characterized by low levels of ammonia substrates (Delgado-Baquerizo et al., 2014). The major phylotypes of *Nitrosotalea* have increasingly negative correlations with *Nitrososphaera* under warming (Fig. 7), implying that *Nitrosotalea* might strongly compete with *Nitrososphaera* if both groups occupy similar ecological niches in future dryland soils. In addition, the metabolic activity of the *Nitrosopumilus* lineage, being found in only one soil (Fig. 2c), could be inhibited by eT. Therefore, if atmospheric temperature is going to

continuously increase, *Nitrosopumilus* might significantly decline in dryland ecosystems with unknown long-term consequences.

5. Conclusions

In conclusion, by combining climate-controlled growth chamber experiment and DNA-SIP microcosms with high-throughput microarray techniques, we provide evidence that elevated temperature has stronger impacts than elevated CO_2 on autotrophic nitrification and ammonia oxidizers in the short term of nine months. Metabolic activity of AOA and AOB responded differently to elevated temperature, with increasing dominance of AOA in nitrification activity within dryland soils. Some effects of climate warming on nitrification and nitrifiers were variable and soil-specific. Nonetheless, our findings provide novel evidence for the responses of nitrogen dynamics in typical dryland and nutrient-poor ecosystems under projected warming, and future research should examine the long-term interactive impacts on nitrogen cycle under the field condition.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2015.09.008>.

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