



# Abiotic versus biotic controls on soil nitrogen cycling in drylands along a 3200 km transect

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**Abstract.** Nitrogen (N) cycling in drylands under changing climate is not well understood. Our understanding of N cycling over larger scales to date relies heavily on the measurement of bulk soil N, and the information about internal soil N transformations remains limited. The <sup>15</sup>N natural abundance ( $\delta^{15}\text{N}$ ) of ammonium and nitrate can serve as a proxy record for the N processes in soils. To better understand the patterns and mechanisms of N cycling in drylands, we collected soils along a 3200 km transect at about 100 km intervals in northern China, with mean annual precipitation (MAP) ranging from 36 to 436 mm. We analyzed N pools and  $\delta^{15}\text{N}$  of ammonium, dual isotopes (<sup>15</sup>N and <sup>18</sup>O) of nitrate, and the microbial gene abundance associated with soil N transformations. We found that N status and its driving factors were different above and below a MAP threshold of 100 mm. In the arid zone with MAP below 100 mm, soil inorganic N accumulated, with a large fraction being of atmospheric origin, and ammonia volatilization was strong in soils with high pH. In addition, the abundance of microbial genes associated with soil N transformations was low. In the semiarid zone with MAP above 100 mm, soil inorganic N concentrations were low and were controlled mainly by biological processes (e.g., plant uptake and denitrification). The preference for soil ammonium over nitrate by the dominant plant species may enhance the possibility of soil nitrate losses via denitrification. Overall, our study suggests that a shift from abiotic to bi-

otic controls on soil N biogeochemistry under global climate changes would greatly affect N losses, soil N availability, and other N transformation processes in these drylands in China.

## 1 Introduction

Drylands cover approximately 41 % of the Earth's land surface and play an essential role in providing ecosystem services and regulating carbon (C) and nitrogen (N) cycling (Hartley et al., 2007; Poulter et al., 2014; Reynolds et al., 2007). After water, N availability is the most important limiting factor for plant productivity and microbial processes in dryland ecosystems (Collins et al., 2008; Hooper and Johnson, 1999). Despite low soil N mineralization rates, N losses are postulated to be higher relative to N pools in dryland ecosystems compared with mesic ecosystems (Austin, 2011; Austin et al., 2004; Dijkstra et al., 2012). However, we still lack a full understanding of the constraints on N losses in drylands because multiple processes contribute to N losses, and the response of those processes to changing climate is highly variable (Nielsen and Ball, 2015). The precipitation regimes in drylands are predicted to change during the 21st century (IPCC, 2013), and more extreme climatic regimes will make dryland ecosystems more vulnerable to enhanced drought in some regions and intensive rain in others (Hunt-

ington, 2006; Knapp et al., 2008). Therefore, improving our understanding of N cycling and its controls would greatly enhance our ability to predict the responses of dryland ecosystems to global changes.

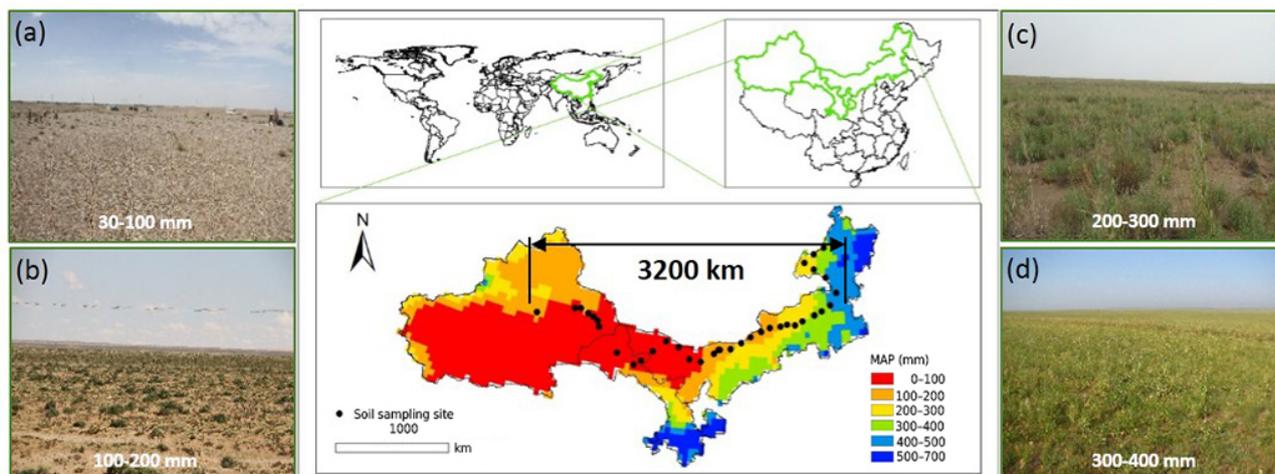
The natural abundance of  $^{15}\text{N}$  (expressed as  $\delta^{15}\text{N}$ ) provides critical information on N cycling and thus assists in understanding ecosystem N dynamics on large scales (Amundson et al., 2003; Austin and Vitousek, 1998; Houlton et al., 2006). The general pattern that foliar and soil  $\delta^{15}\text{N}$  increases as precipitation decreases has been observed at both the regional (Aranibar et al., 2004; Austin and Vitousek, 1998; Cheng et al., 2009; Peri et al., 2012) and global scales (Amundson et al., 2003; Craine et al., 2009; Handley et al., 1999), suggesting that N cycling is more open (i.e., greater input and output relative to internal cycling) in dryland ecosystems compared with mesic ecosystems. The underlying explanation is that in drylands N supply is higher than biotic demand, resulting in proportionally more N loss through leaching and gaseous N emission relative to the internal N pool (Austin and Vitousek, 1998). Given that the isotope fractionation during N loss is against the heavier isotope, soils and plant tissues become enriched in  $^{15}\text{N}$  with increasing N losses (Robinson, 2001). However, the effects of atmospheric deposition on N cycling are often ignored in N isotope studies, in which N isotopes derived from atmospheric deposition and biological N fixation are assumed to be uniform over large regional scales (Bai et al., 2012; Handley et al., 1999; Houlton and Bai, 2009). In addition, N losses in dryland ecosystems are likely dominated by gaseous losses (McCalley and Sparks, 2009; Peterjohn and Schlesinger, 1990). The natural abundance of  $^{15}\text{N}$  in soil total N is limited in its usefulness in interpreting the specific processes governing gaseous N losses. Therefore, it seems that the measurement of total N alone is not sufficient to reveal the responses of N cycling to changing precipitation because there are multiple processes that contribute to the  $\delta^{15}\text{N}$  variability in plant–soil systems.

Ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) isotopes can serve as a proxy record for N processes in soils because they directly respond to the in situ processes that control production and consumption of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . For example, comparing  $\delta^{15}\text{N}$  values of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and bulk soil N could reveal the relative importance of N transformation processes (such as between ammonification and nitrification) (Koba et al., 1998, 2010). Dual isotope analysis of  $\text{NO}_3^-$  ( $^{15}\text{N}$  and  $^{18}\text{O}$  of soil  $\text{NO}_3^-$ ) provides evidence for microbial denitrification in oceans (Sigman et al., 2009), forests (Fang et al., 2015; Houlton et al., 2006; Wexler et al., 2014), and groundwater (Minet et al., 2012). In addition, the  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$  has been used to partition microbially produced  $\text{NO}_3^-$  from atmospheric sources because microbial and atmospheric sources cover a different range of  $\delta^{18}\text{O}$  (Böhlke et al., 1997; Brookshire et al., 2012; Kendall et al., 2007). The positive correlations between N isotopes of available soil N ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,

and dissolved organic N) and plant leaves have been used to study the preferences of plant N uptake (Cheng et al., 2010; Houlton et al., 2007; Mayor et al., 2012; Takebayashi et al., 2010). With newly developed methods (Lachouani et al., 2010; Liu et al., 2014; Tu et al., 2016), the analysis of isotopic values in soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  has the potential to elucidate the N cycling characteristics and their controls; however, compared with the  $\delta^{15}\text{N}$  of bulk soil N, the  $\delta^{15}\text{N}$  of soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  has rarely been reported, especially in drylands.

Soil microbes constitute a major portion of the biota in terrestrial ecosystems and play key roles in regulating ecosystem functions and biogeochemical cycles (van Der Heijden et al., 2008). Linking soil microbial communities and N processes is critical for evaluating the response of N transformations to climate changes. However, despite the rapid development of high-throughput sequencing techniques in recent decades, there is still a great challenge for researchers to establish such linkages due to technical limitations, especially at large spatial scales (Zhou et al., 2011). Alternatively, a microarray-based metagenomics technology, GeoChip, has been developed for the analysis of microbial communities (He et al., 2007, 2010b; Tu et al., 2014). This technique can be used not only to analyze the functional diversity, composition, and structure of microbial communities but also to directly reveal the linkages between microbial communities and ecosystem functions (He et al., 2007). Functional gene microarray approaches have been used to examine the response of microbially mediated N processes under different environmental conditions. Denitrification genes from the soils in Antarctica, for example, are associated with increased soil temperatures, and  $\text{N}_2$ -fixation genes are associated with the presence of lichens (Yergeau et al., 2007). Research along an elevation gradient in the Tibetan grassland noted that some denitrification genes (*nirS* and *nosZ*) are more abundant at higher elevations, with nitrification as the major process of nitrous oxide ( $\text{N}_2\text{O}$ ) emission (Yang et al., 2013). The latest version, GeoChip 5.0S, contains probes covering more than 144 000 functional genes, which enables us to explore key microbially mediated biogeochemical processes more thoroughly than ever before (Cong et al., 2015; Wang et al., 2014).

In this study, we studied the effects of water availability on ecosystem-level N availability and cycling along a 3200 km transect in northern China. This natural gradient of precipitation provides an ideal system for identifying the response of soil N dynamics to water availability. In a previous study we reported a hump-shaped pattern of  $\delta^{15}\text{N}$  in bulk soil N along this precipitation gradient, with a threshold at an aridity index of 0.32 (mean annual precipitation of approximately 250 mm), demonstrating the respective soil microbial versus plant controls (Wang et al., 2014). Here, we further analyzed the concentrations and N isotopic compositions of soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (as well as oxygen (O) isotopes for  $\text{NO}_3^-$ ) and the abundance of microbial genes associated with soil N trans-



**Figure 1.** Vegetation type and sampling site distribution along the transect. Across the 3200 km precipitation gradient in northern China, four typical vegetation types are distributed from west to east, which are desert (a), desert steppe (b), typical steppe (c), and meadow steppe (d). The dominant plant genera change gradually from shrub (*Nitraria* spp., *Reaumuria* spp., and *Salsola* spp.) to perennial grasses (*Stipa* spp., *Leymus* spp., and *Cleistogenes* spp.). Soil types are predominantly arid, sandy, and brown loess rich in calcium from west to east of the transect. A total of 36 soil sampling sites were selected.

formation. The principal objectives of this study were to examine (1) the patterns of concentrations and  $\delta^{15}\text{N}$  values for soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , (2) the patterns of gene abundance associated with microbially regulated soil processes, and (3) the responses of soil N cycling to changes in water availability along the precipitation gradient in dryland ecosystems.

## 2 Materials and methods

### 2.1 Study areas

The research was carried out along a 3200 km transect across Gansu Province and Inner Mongolia in northern China, covering a longitude from 87.4 to 120.5° E and a latitude from 39.9 to 50.1° N (Fig. 1). The climate is predominantly arid and semiarid continental. From west to east along the transect, the mean annual precipitation (MAP) increases from 36 mm to 436 mm, the mean annual temperature (MAT) decreases from 9.9 to  $-1.8$  °C (Fig. S1 in the Supplement), and the aridity index (the ratio of precipitation to potential evapotranspiration) ranges from 0.04 to 0.60 (Fig. S1). Vegetation types distributed along the transect were mainly desert, desert steppe, typical steppe, and meadow steppe; the three dominant grass genera were *Stipa* spp., *Leymus* spp., and *Cleistogenes* spp., and the three shrub genera were *Nitraria* spp., *Reaumuria* spp., and *Salsola* spp. Soil types from west to east along the transect are predominantly arid, sandy, and calcium-rich brown loess.

### 2.2 Soil sampling and sample preparation

Soil sampling was conducted from July to August 2012, the peak of the plant growing season. This is the same transect as described in Wang et al. (2014), but with slightly different site coverage. We selected 36 sites at approximately 100 km intervals between adjacent sites due to limited time to extract soil with KCl solution on the same day after intensive sampling (Fig. 1), whereas 50 sites at approximately 50 km intervals were used for bulk soil N isotopes measurement in Wang et al. (2014). At each site, we set a 50 m  $\times$  50 m plot and five 1 m  $\times$  1 m subplots at the four corners and the center of the plot. In each subplot, 20 mineral soil samples were randomly collected using soil cores (2.5 cm diameter  $\times$  10 cm depth) and were then thoroughly mixed into one composite sample. The fresh soils were sieved (2 mm) to remove roots and rocks, homogenized by hand, and separated into three portions. The first portion was extracted in 2 M KCl (1 : 5 w/v) for 1 h on the same sampling day; the extracts were stored at 4 °C during the sampling trip. The second portion was placed in a sterile plastic bag and immediately stored at  $-40$  °C for later DNA extraction. The third portion was placed in a plastic bag and stored in a refrigerator at 4 °C for subsequent analyses.

### 2.3 Analyses of soil physicochemical properties and isotopes

Soil pH was measured using a pH meter and a soil-to-water ratio of 1 : 2.5. Soil N content and natural abundance of  $^{15}\text{N}$  were determined by an elemental analyzer connected to an isotope ratio mass spectrometer (IRMS) (Wang et al., 2014). The concentrations of soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in the KCl extracts

were analyzed using conventional colorimetric methods (Liu et al., 1996). Ammonium concentrations were determined using the indophenol blue method, and nitrate concentrations were determined using the sulfanilamide-NAD reaction following cadmium (Cd) reduction.

The analyses of the isotope compositions of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , including  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$ ,  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$ , and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  ( $\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where  $R$  denotes the ratio of the heavy isotope to the light isotope for N or O in units per mil, ‰), were based on the isotopic analysis of  $\text{N}_2\text{O}$ . Specifically,  $\text{NH}_4^+$  in the extract was oxidized to  $\text{NO}_2^-$  by alkaline hypobromite ( $\text{BrO}^-$ ) and then reduced to  $\text{N}_2\text{O}$  by hydroxylamine ( $\text{NH}_2\text{OH}$ ) (Liu et al., 2014). Nitrate was firstly reduced to  $\text{NO}_2^-$  by Cd powder and then to  $\text{N}_2\text{O}$  by sodium azide ( $\text{NaN}_3$ ) in an acetic acid buffer (McIlvin and Altabet, 2005; Tu et al., 2016). To correct for machine drift and to blank over the isotopic analyses, international standards of  $\text{NH}_4^+$  (IAEA N1, USGS 25, and USGS 26) and  $\text{NO}_3^-$  (IAEA N3, USGS 32, USGS 34, and USGS 35) were treated in identical analytical procedures as the samples to obtain a calibration curve between the measured and expected isotope values. The isotopic signatures of the produced  $\text{N}_2\text{O}$  were determined by an IsoPrime 100 continuous flow isotope ratio mass spectrometer connected to a trace gas (TG) preconcentrator (Liu et al., 2014). The analytical precision for isotopic analyses was better than 0.3 ‰ ( $n = 5$ ).

## 2.4 DNA extraction and GeoChip analysis

For soil DNA extraction, purification, and quantification and the analysis of functional structure of soil microbial communities, we adopted the same approaches as described previously (Wang et al., 2014). In addition to the abundance of nitrification and denitrification genes reported in Wang et al. (2014), the abundance of N fixation, ammonification, and anaerobic ammonia oxidation (anammox) genes was included in this paper. Briefly, microbial genomic DNA was extracted from 0.5 g soil using the MO BIO Power-Soil DNA isolation kit (MO BIO Laboratories, Carlsbad, CA, USA) and purified by agarose gel electrophoresis followed by phenol–chloroform–butanol extraction. DNA quality was assessed by the A260/280 and A260/230 ratios using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA), and final soil DNA concentrations were quantified with PicoGreen using a FLU-Ostar Optima (BMG Labtech, Jena, Germany). The GeoChip 5.0S, manufactured by Agilent (Agilent Technologies Inc., Santa Clara, CA), was used for analyzing DNA samples. The experiments were conducted as described previously (Wang et al., 2014). In short, the purified DNA samples (0.6  $\mu\text{g}$ ) were used for hybridization and were labeled with the fluorescent dye Cy 3. Subsequently, the labeled DNA was re-suspended and hybridized at 67 °C in an Agilent hybridization oven for 24 h. After washing and drying, the slides were scanned by a NimbleGen MS200 scanner (Roche, Madison,

WI, USA) at 633 nm using a laser power of 100 % and a photomultiplier tube gain of 75 %. The image data were extracted using the Agilent Feature Extraction program (Agilent Technologies, Santa Clara, CA, USA). The raw microarray data were further processed for subsequent analysis using an in-house pipeline that was built on a platform at the Institute for Environmental Genomics, University of Oklahoma (He et al., 2007, 2010a).

## 2.5 Statistical analyses

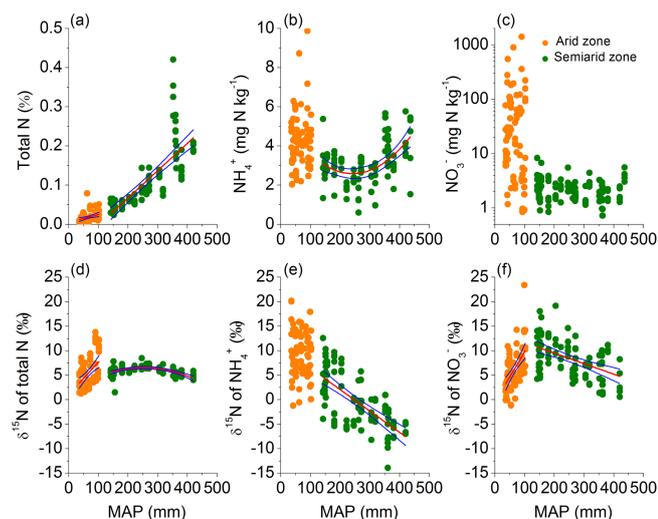
All analyses were conducted using the software package SPSS 18.0 (SPSS, Chicago, IL) for Windows. Pearson correlation analysis was conducted to examine the linear relationships between different variables. Independent-sample  $T$  tests were performed to examine the differences in the investigated variables between arid-zone soils and semiarid-zone soils. Statistically significant differences were set at a  $P$  value of 0.05 unless otherwise stated.

## 3 Results

### 3.1 Soil $\text{NO}_3^-$ and $\text{NH}_4^+$ concentrations

We found significant inorganic N accumulation in the investigated soil layer (0–10 cm) at sites with a MAP less than 100 mm ( $P < 0.01$ ; Fig. 2b and c). Furthermore, the abundance of microbial genes associated with soil N transformations was significantly reduced compared with that at sites with a MAP greater than 100 mm (see below). Together with the vegetation distribution along the transect (Fig. 1), these results indicated that soil N status and its controls could be different above and below a MAP threshold of 100 mm. Therefore, we hereafter refer to the areas with MAP from 36 to 102 mm (15 sites) and from 142 to 436 mm (21 sites) as the arid zone and semiarid zone, respectively.

In the arid zone, soil  $\text{NO}_3^-$  concentrations were highly variable and reached up to 1400  $\text{mg N kg}^{-1}$ , with a mean of 87  $\text{mg N kg}^{-1}$ . Ammonium concentrations varied from 2.0 to 9.9  $\text{mg N kg}^{-1}$ , with a mean of 4.3  $\text{mg N kg}^{-1}$ . In the semiarid zone,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations were low – less than 5  $\text{mg N kg}^{-1}$  in most samples. Soil  $\text{NH}_4^+$  concentrations exhibited a quadratic relationship, with increasing MAP in the semiarid zone, but  $\text{NO}_3^-$  concentrations remained low and did not change with increasing MAP. As expected, bulk soil N was significantly greater in the semiarid zone (on average 0.1 %) compared with the arid zone (on average 0.02 %) and increased dramatically in the semiarid zone with increasing precipitation (Fig. 2a). Our results suggest increased inorganic N availability in the arid zone compared with the semiarid zone despite a smaller total N pool, which supports the idea that N availability is greater in dry areas compared with less-dry areas.

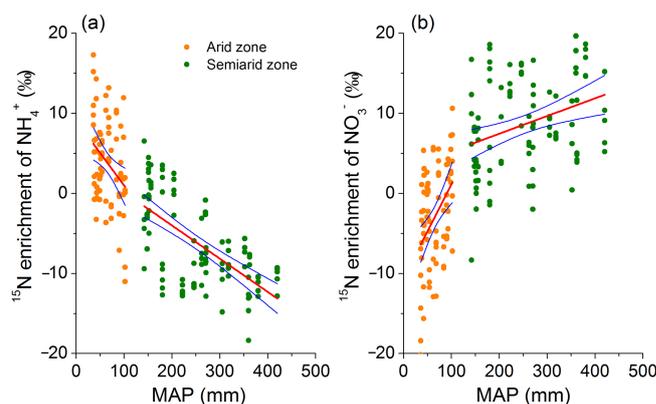


**Figure 2.** Nitrogen concentrations and isotopic composition of bulk soil N,  $\text{NH}_4^+$ , and  $\text{NO}_3^-$ . The significant ( $P < 0.05$ ) trends are shown with a regression line (red) and 95 % confidence intervals (blue). At each site  $n = 5$ .

### 3.2 The $^{15}\text{N}$ natural abundance of soil $\text{NO}_3^-$ and $\text{NH}_4^+$

The  $\delta^{15}\text{N}$  values for  $\text{NO}_3^-$  were significantly greater in the semiarid zone (0.5 to 19.2‰) compared with the arid zone (−1.2 to 23.4‰,  $P < 0.01$ ; Fig. 2f), with means of 8.4 and 6.3‰, respectively. With increasing MAP, the  $\delta^{15}\text{N}$  value for  $\text{NO}_3^-$  increased in the arid zone but decreased in the semiarid zone, suggesting different controlling factors in areas with different water availability. Unlike the  $\delta^{15}\text{N}$  for soil  $\text{NO}_3^-$ , the  $\delta^{15}\text{N}$  value for  $\text{NH}_4^+$  was significantly greater in the arid zone (−1.2 to 20.2‰) compared with the semiarid zone (−13.9 to 12.6‰,  $P < 0.01$ ; Fig. 2e), with means of 9.2 and −0.3‰, respectively. The  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$  was negatively correlated with the MAP in the semiarid zone but was stable as precipitation increased in the arid zone (Fig. 2e).

The N isotopic signature of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  reflects not only the isotopic fractionation during N transformation processes but also the N isotopic signature of their main sources (i.e., bulk soil N and  $\text{NH}_4^+$ , respectively). Therefore, we also calculated the relative  $^{15}\text{N}$  enrichment of soil  $\text{NH}_4^+$  (the difference between the  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$  and bulk soil N) and  $\text{NO}_3^-$  (the difference between the  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) to examine the isotopic imprint of N transformations on soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . The relative  $^{15}\text{N}$  enrichment of soil  $\text{NH}_4^+$  in the arid zone was mostly above zero, whereas its value was below zero in the semiarid zone (Fig. 3a). A negative correlation was observed between MAP and the relative  $^{15}\text{N}$  enrichment of soil  $\text{NH}_4^+$  across both the arid and semiarid zones (Fig. 3a). According to the Rayleigh model, sinks are always  $^{15}\text{N}$ -depleted relative to their sources (Robinson, 2001). The positive values for the relative  $^{15}\text{N}$  enrichment of  $\text{NH}_4^+$  support the notion that net  $\text{NH}_4^+$  losses occurred mainly in the

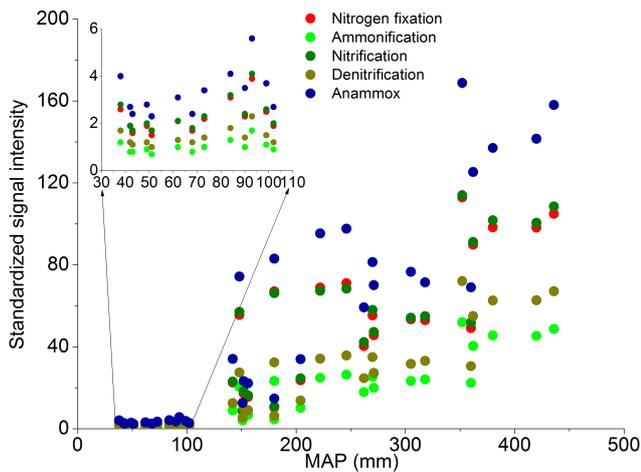


**Figure 3.** The relative  $^{15}\text{N}$  enrichment of soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . Data in the figures were calculated as the difference between  $\delta^{15}\text{N}$  of bulk soil N and  $\text{NH}_4^+$  and between  $\delta^{15}\text{N}$  of soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , respectively. The significant ( $P < 0.05$ ) trend is shown with a regression line (red) and 95 % confidence intervals (blue). At each site  $n = 5$ .

arid zone, whereas the negative values imply that net  $\text{NH}_4^+$  gain (e.g., via microbial N mineralization, biological N fixation, and/or N deposition) might increase in the semiarid zone, and subsequently reduce the relative  $^{15}\text{N}$  enrichment of soil  $\text{NH}_4^+$ . In a similar manner, we found that the relative  $^{15}\text{N}$  enrichment of  $\text{NO}_3^-$  was mostly below zero in the arid zone and above zero in the semiarid zone (Fig. 3b). A positive correlation was observed between the MAP and the relative  $^{15}\text{N}$  enrichment of soil  $\text{NO}_3^-$  in both the arid and semiarid zones (Fig. 3b). Accordingly, these results suggest that  $\text{NO}_3^-$  losses increase when water becomes more available, and the residual soil  $\text{NO}_3^-$  becomes progressively enriched in  $^{15}\text{N}$ .

### 3.3 The abundance of microbial functional genes

The abundances of microbial genes of five main N cycling groups (N fixation, ammonification, nitrification, denitrification, and anammox) were measured at all sites. In arid-zone soils, the abundances of all N cycling genes were extremely low (Fig. 4), indicating limited microbial potential in this very dry environment. A sharp increase (by eight- to nine-fold) in the gene abundance was noted from the arid zone to the semiarid zone (Fig. 4), even though the soils were still mostly dry at the time of sampling (see soil moisture in Fig. S2). The gene abundances in the semiarid zone were 1 to 2 orders of magnitude greater than those in the arid zone. In addition, the microbial gene abundances of the five main N cycling groups all increased with increasing precipitation in both the arid and semiarid zones (Fig. 4), suggesting a potential effect of water availability on soil microbial N processes.

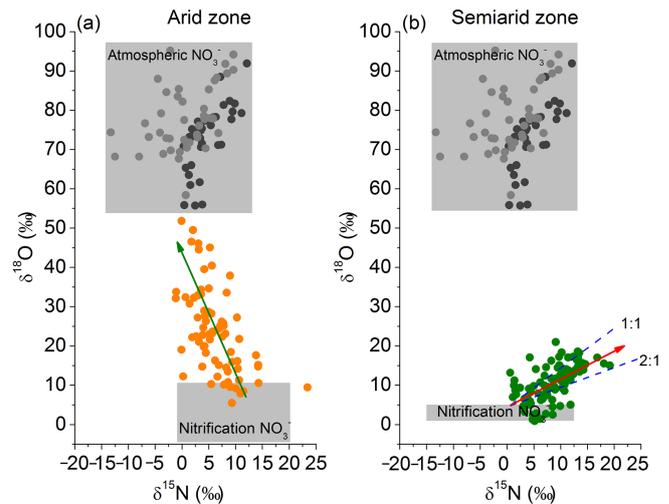


**Figure 4.** Changes in the abundance of microbial gene involved in N cycling. Signal intensity was standardized based on both the number of array probes and DNA quantity in a gram of dry soil. Each point is the site-averaged value; results of the abundance of nitrification and denitrification genes were reported in a previous study (Wang et al., 2014).

## 4 Discussion

### 4.1 Losses of soil $\text{NO}_3^-$ and $\text{NH}_4^+$

We observed different patterns of N cycling above and below a MAP threshold of 100 mm along this 3200 km transect. In the semiarid zone, the increased precipitation seemed to lead to increased losses of soil  $\text{NO}_3^-$  but not  $\text{NH}_4^+$  (Fig. 3). Soil  $\text{NO}_3^-$  can be removed from the ecosystem via denitrification, leaching, and plant and microbial uptake. The close correlation between the measured dual isotopes ( $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ ) of soil  $\text{NO}_3^-$  suggests the occurrence of denitrification in the semiarid zone. Microbial denitrification exerts large fractionation against the isotopically heavier compounds, ranging between 5 and 25‰ for both O and N in  $\text{NO}_3^-$  (Granger et al., 2008). This type of fractionation results in concurrent increases in the  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  values of the remaining  $\text{NO}_3^-$ , with a ratio of 0.5 to 1 (Kendall et al., 2007). In the present study, the  $\delta^{18}\text{O}$  values of soil  $\text{NO}_3^-$  were significantly correlated with the  $\delta^{15}\text{N}$  values of soil  $\text{NO}_3^-$  in the semiarid zone, with a slope of 0.7 (Fig. 5b). This slope is very similar to the slope of 0.8 observed in soil  $\text{NO}_3^-$  across five Hawaiian tropical forests (Houlton et al., 2006), indicating the occurrence of denitrification-driven  $\text{NO}_3^-$  losses. Denitrification is regulated by proximal factors, such as  $\text{NO}_3^-$  concentration and  $\text{O}_2$  concentration, that immediately affect denitrifying communities (Saggar et al., 2013). Nitrate can be supplied through enhanced microbial processes, including nitrification, when water becomes more available. Increased soil respiration in hot spots and/or hot moments caused by pulse precipitation consumes  $\text{O}_2$ , consequently favoring denitrification (Abed et al., 2013). In the semiarid zone, we observed that the relative



**Figure 5.** Relationship between  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  of soil  $\text{NO}_3^-$ . The range of  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  from atmospheric  $\text{NO}_3^-$  was based on the limited isotope measurement of precipitation. Black points represent precipitation  $\text{NO}_3^-$  collected from an urban site in Beijing in the year 2012, with data derived from Tu et al. (2016). Grey points represent precipitation  $\text{NO}_3^-$  collected from Qingyuan forest CERN (Chinese Ecosystem Research Network, CERN) in northern China in the year 2014 (Huang and Fang, unpublished data). The ranges of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  produced by nitrified  $\text{NO}_3^-$  are positioned by using the  $\delta^{15}\text{N}$  of soil  $\text{NH}_4^+$  in this study (Fig. 2e) and the estimated  $\delta^{18}\text{O}$  from soil nitrification based on the 1 : 2 ratio of soil  $\text{O}_2$  and  $\text{H}_2\text{O}$  (see Text), respectively.

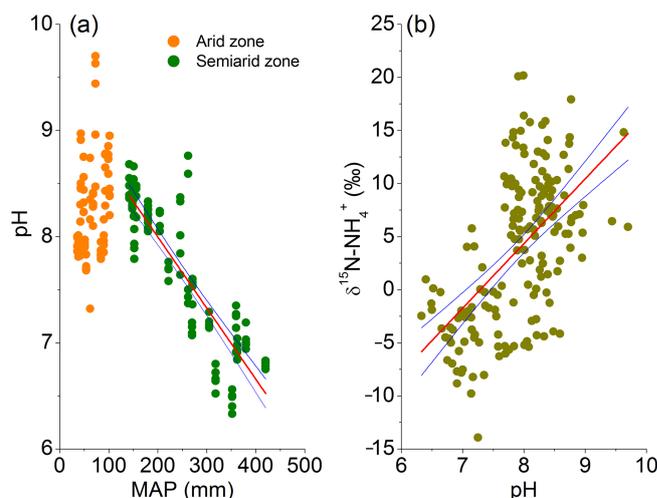
$^{15}\text{N}$  enrichment of soil  $\text{NO}_3^-$  increased with increasing precipitation (Fig. 3b), suggesting that denitrification may become more favorable with increasing precipitation. In addition, in our preliminary study, a  $^{15}\text{N}$ -labeled  $\text{NO}_3^-$  incubation experiment revealed that potential  $\text{N}_2$  losses via denitrification also increased with increasing precipitation in the semiarid soils (Liu and Fang, unpublished data). Because gaseous N losses occur during both nitrification (see below) and denitrification, the coupled nitrification and denitrification could maintain low soil  $\text{NO}_3^-$  concentrations while enriching the  $^{15}\text{N}$  signal of soil  $\text{NO}_3^-$ .

In the arid zone, the  $\delta^{15}\text{N}$  and the relative  $^{15}\text{N}$  enrichment of soil  $\text{NO}_3^-$  both increased with increasing precipitation (Figs. 2f and 3b), indicating that denitrification may also occur. However, in these arid soils, microbial gene abundances were considerably lower (Fig. 4), suggesting limited biological activities. It is therefore more likely that microbial denitrification is only a minor process in arid-zone soils and may only occur after a large rain event. Microbial denitrification has been observed in hotspots after heavy precipitation events in some desert soils (Abed et al., 2013; Zaady et al., 2013). Alternatively, chemodenitrification may cause soil  $\text{NO}_3^-$  losses in the arid zone. Chemodenitrification is an abiotic process in which the reduction of  $\text{NO}_2^-$  to  $\text{NO}$  and  $\text{N}_2\text{O}$

is coupled to the oxidation of reduced metals (e.g., Fe (II)) and humic substances (Medinets et al., 2015; Zhu-Barker et al., 2015). In a recent review, Heil et al. (2016) discussed several abiotic reactions involving  $\text{NO}_2^-$ , including the self-decomposition of  $\text{NO}_2^-$ , reactions of  $\text{NO}_2^-$  with reduced metals, nitrosation of soil organic matter (SOM) by  $\text{NO}_2^-$ , and the reaction between  $\text{NO}_2^-$  and  $\text{NH}_2\text{OH}$ . In this study, ample soil  $\text{NO}_3^-$  was present in some arid-zone soils (Fig. 2c). In addition, our companion work also observed higher available Fe in arid-zone soils (Luo et al., 2016). Roco et al. (2016) demonstrated that the first step of denitrification, the dissimilatory reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$ , was much more common under aerobic conditions than commonly realized, could occur in diverse bacteria groups, and has multiple types of physiological controls. Homyak et al. (2016) reported both initial abiotic NO pulses after soil rewetting and subsequent biologically driven NO emissions, suggesting multiple biotic and abiotic controls on NO emissions and N losses in dryland ecosystems.

In contrast to the  $\delta^{15}\text{N}$  of soil  $\text{NO}_3^-$ , the  $\delta^{15}\text{N}$  values for soil  $\text{NH}_4^+$  and their relative  $^{15}\text{N}$  enrichment were greater in the arid zone compared with the semiarid zone (Figs. 2e and 3a), suggesting losses of  $\text{NH}_4^+$  at the drier sites. We suggest that  $\text{NH}_3$  volatilization should play a significant role in  $\text{NH}_4^+$  losses because soil pH was higher in the arid zone (from 7.3 to 9.7; Fig. 6a). The isotopic effect of  $\text{NH}_3$  volatilization had been reported to be 40 to 60‰ (Robinson, 2001), resulting in  $^{15}\text{N}$ -enriched soil  $\text{NH}_4^+$ . The significant positive correlation between the  $\delta^{15}\text{N}$  values for  $\text{NH}_4^+$  and soil pH in this study (Fig. 6b) supported our interpretation. In addition, despite the low microbial gene abundance, nitrification may be able to occur in the arid-zone soils. Although nitrifiers are sensitive to water availability, they can remain active in thin water films, resulting in increased nitrification in dry soils (Sullivan et al., 2012). In the process of nitrification, NO losses occur via a “leaky pipe” mechanism (Firestone and Davidson, 1989). In addition, nitrite ( $\text{NO}_2^-$ ) produced during nitrification can be reduced rapidly to NO via chemodenitrification. The reaction of chemodenitrification forms NO via nitrous acid ( $\text{HNO}_2$  (aqueous phase), HONO (gas phase)) decomposition (Medinets et al., 2015). Alternatively, nitrifier denitrification can also serve as a mechanism for NO emission by the reduction of  $\text{NO}_2^-$  upon the recovery of nitrifiers from drought-induced stress (Heil et al., 2016; Homyak et al., 2016).

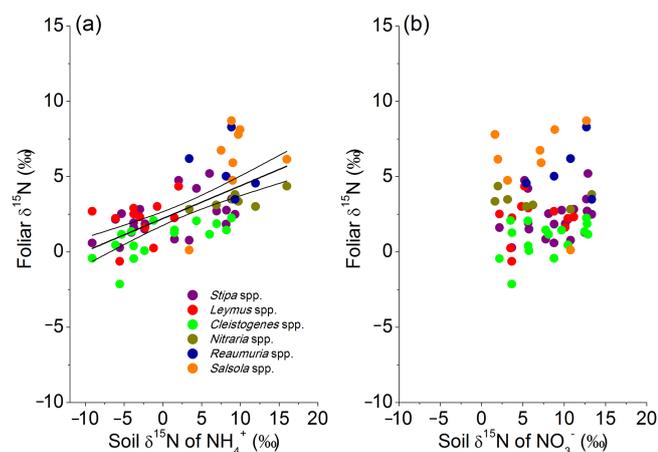
In the semiarid zone,  $\text{NH}_3$  volatilization should be low due to relatively lower pH compared with the arid-zone soils (Fig. 6a). Previous studies have found that water addition did not stimulate  $\text{NH}_3$  volatilization (Yahdjian and Sala, 2010); however, a recent study observed the opposite trend in a semiarid subtropical savanna (Soper et al., 2016). The increasing available water in the semiarid zone would also stimulate biological N consumption by plants and microbes. The increased aboveground biomass with increasing MAP



**Figure 6.** Soil pH and the relationship with  $\delta^{15}\text{N}$  of soil  $\text{NH}_4^+$ . The different patterns of soil pH were observed above and below the threshold at MAP of about 100 mm; data were derived from Wang et al. (2014). There was a positive correlation between  $\delta^{15}\text{N}$  of soil  $\text{NH}_4^+$  and pH across the transect. The significant ( $P < 0.05$ ) trend is shown with a regression line (red) and 95 % confidence intervals (blue). At each site  $n = 5$ .

suggests an increased net plant N accumulation along this precipitation gradient (Wang et al., 2014). Given that the soil  $\text{NH}_4^+$  concentration was greater than that of soil  $\text{NO}_3^-$  in the semiarid zone ( $P < 0.001$ ), the dominant plant species might adapt to prefer  $\text{NH}_4^+$  to  $\text{NO}_3^-$ . This notion is in accordance with the observed relationship between the  $\delta^{15}\text{N}$  values of plant leaves (non-N-fixing species) and soil  $\text{NH}_4^+$  ( $R^2 = 0.40$ ; Fig. 7a), but not soil  $\text{NO}_3^-$  (Fig. 7b). When we plotted this correlation for each plant species, three dominant species (*Stipa* spp., *Cleistogenes* spp., and *Reaumuria* spp.) all showed significant correlation between foliar  $\delta^{15}\text{N}$  and soil  $\text{NH}_4^+$ . Plant nitrogen uptake may also exert a fractionation effect on N sources, but it might be negligible in N-limited areas (Craine et al., 2015). This notion may in part explain a lack of strong  $^{15}\text{N}$  enrichment in soil  $\text{NH}_4^+$  with increasing precipitation. The consumption of  $\text{NH}_4^+$  during nitrification could also increase, as indicated by the microbial gene abundance along the precipitation gradient (Fig. 4). The coupled nitrification and denitrification in the semiarid zone could lead to N loss and the  $^{15}\text{N}$  enrichment of residual soil  $\text{NO}_3^-$ , without significantly changing the  $\text{NO}_3^-$  concentration. Conversely, enhanced plant uptake (of both soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) would diminish soil inorganic N pools and greatly reduce gaseous N losses through either nitrification (Homyak et al., 2016) or denitrification.

Unexpectedly, we detected high anammox gene abundance in these dryland ecosystems (Fig. 4). Anammox is the microbial reaction between  $\text{NH}_4^+$  and  $\text{NO}_2^-$ , and  $\text{N}_2$  is the end product (Thamdrup and Dalsgaard, 2002). Previous studies have found equal consumption of both soil  $\text{NH}_4^+$  and



**Figure 7.** Relationship between the  $\delta^{15}\text{N}$  of foliage and  $\delta^{15}\text{N}$  of soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . Data on foliar  $\delta^{15}\text{N}$  (*Stipa* spp., *Leymus* spp., *Cleistogenes* spp., *Reaumuria* spp., and *Salsola* spp.) were from the previous study of Wang et al. (2014). Almost all dominant plants were found in the area with MAP more than 100 mm (semiarid zone). Data are the site-averaged values. The significant ( $P < 0.05$ ) trend is shown with a regression line (thick) and 95 % confidence intervals (thin).

$\text{NO}_3^-$  through anammox in N-loaded and waterlogged areas (Yang et al., 2014; Zhu et al., 2013). However, the only two studies of anammox in drylands to date failed to confirm its importance (Abed et al., 2013; Strauss et al., 2012). Thus, although anammox possesses a fractionation effect of 23 to 29 ‰ (Brunner et al., 2013), it is difficult to determine its significance in our study at the present time.

Other abiotic processes have also been reported to contribute to N losses in drylands. High soil surface temperature driven by solar radiation may be responsible for gaseous N losses in dryland ecosystems (Austin, 2011; McCalley and Sparks, 2009, 2008), and they may affect  $^{15}\text{N}$  abundance of soil N. Other non-fractionation processes, such as aeolian deposition and water erosion, might also influence N cycle in dryland ecosystems (Austin, 2011; Hartley et al., 2007).

#### 4.2 Sources of soil $\text{NO}_3^-$ and $\text{NH}_4^+$

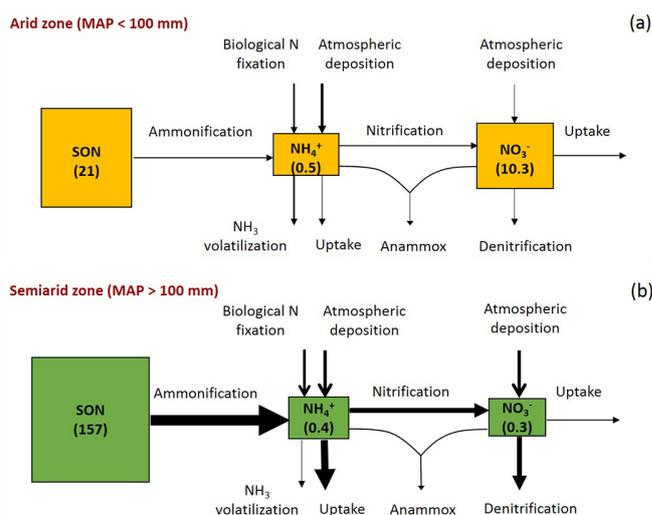
We observed much higher concentrations of soil  $\text{NO}_3^-$  in the arid zone (Fig. 2c); on average, they were approximately 20 times higher than those in the semiarid zone. Nitrate can be formed via microbial nitrification, deposited from N-bearing gaseous (e.g.,  $\text{HNO}_3$ ) or dry aerosol  $\text{NO}_3^-$  (Kendall et al., 2007) or as dissolved nitrate in rainwater or snow. If  $\text{NO}_3^-$  is formed by nitrification,  $\text{NO}_3^-$  obtains one O atom from soil  $\text{O}_2$  and two O atoms from  $\text{H}_2\text{O}$  (Kendall et al., 2007). The  $\delta^{18}\text{O}$  value of atmospheric  $\text{O}_2$  is relatively stable (23.5 ‰; we assume that the isotopic composition of  $\text{O}_2$  in the atmosphere and soils are the same). The  $\delta^{18}\text{O}$  value of nitrified  $\text{NO}_3^-$  depends on the  $\delta^{18}\text{O}$  value of the local water. The  $\delta^{18}\text{O}$  values of rainwater taken from the areas closest to the arid

zone of our dryland transect (Lanzhou city and its surrounding areas) ranged from  $-19.1$  to  $5.2$  ‰ (Chen et al., 2015), yielding corresponding  $\delta^{18}\text{O}$  values of nitrified  $\text{NO}_3^-$  ranging from  $-5.3$  to  $11.3$  ‰ (Fig. 5a). However, the  $\delta^{18}\text{O}$  values of soil  $\text{NO}_3^-$  in the arid zone varied from  $5.5$  to  $51.8$  ‰ (Fig. 5a). This disparity between the calculated and measured  $\delta^{18}\text{O}$  values provided evidence for the minor importance of nitrification. According to previous studies, the higher  $\delta^{18}\text{O}$  values of soil  $\text{NO}_3^-$  we observed in the arid zone have rarely been reported for nitrified  $\text{NO}_3^-$  (Kendall et al., 2007). For example, an in situ study conducted on forest floor soils found that the  $\delta^{18}\text{O}$  values of nitrified  $\text{NO}_3^-$  changed from  $3.1$  to  $10.1$  ‰ (Spoelstra et al., 2007). By comparison, atmospheric-origin  $\text{NO}_3^-$  normally has higher  $\delta^{18}\text{O}$  values because of the chemical oxidation of  $\text{NO}_3^-$  precursor  $\text{NO}_x$  ( $\text{NO}$  and  $\text{NO}_2$ ) (Fang et al., 2011). Previous research found that the  $\delta^{18}\text{O}$  values of aerosol  $\text{NO}_3^-$  ranged from  $60$  to  $111$  ‰ in the Dry Valleys of Antarctica (Savarino et al., 2007). This combined information supports the hypothesis that a sizable fraction of  $\text{NO}_3^-$  in the surface soils of the arid zone is from atmospheric deposition. Nitrate accumulates on the surface soil when experiencing prolonged droughts, which has also been reported in northern Chile, southern California (Böhlke et al., 1997), and the Turpan-Hami area of northwestern China (Qin et al., 2012). As shown in Fig. 5a, a pronounced trend (green arrow) toward higher  $\delta^{18}\text{O}$  and lower  $\delta^{15}\text{N}$  values is obvious for elevated  $\text{NO}_3^-$  concentrations in the arid-zone soils, which might be the result of mixed  $\text{NO}_3^-$  from both soil nitrification and atmospheric deposition. A similar result was observed in the groundwater of the Sahara (Dietzel et al., 2014). In the arid zone, extreme dryness and high alkalinity (an average pH of 8.3) might limit microbial activities, as suggested by the low gene abundance involving N transformations (Fig. 4), which combined with the lack of leaching, would facilitate the preservation of soil  $\text{NO}_3^-$ .

In the semiarid zone, the  $\delta^{18}\text{O}$  values of soil  $\text{NO}_3^-$  were low ( $0.9$ – $21.0$  ‰), indicating low atmospheric contribution. The deposited  $\text{NO}_3^-$  generally experiences postdepositional microbial processes, and the original signature of  $\delta^{18}\text{O}$  will vanish after biological processes occur (Qin et al., 2012). With increasing MAP, nitrification would progressively provide more  $\text{NO}_3^-$  with lower  $\delta^{18}\text{O}$  values. The calculated  $\delta^{18}\text{O}$  values of  $\text{NO}_3^-$  from nitrification ranged from  $2.5$  to  $6.5$  ‰ based on the  $\delta^{18}\text{O}$  of soil  $\text{H}_2\text{O}$  ( $-8$  to  $-2$  ‰; Shenyang site) (Liu et al., 2010). Both autotrophic and heterotrophic nitrification could generate soil  $\text{NO}_3^-$ . Heterotrophic nitrification, a process that oxidizes organic N to  $\text{NO}_3^-$ , bypasses  $\text{NH}_4^+$ . If this process were important, it would provide an additional explanation for the lack of  $^{15}\text{N}$  enrichment in soil  $\text{NH}_4^+$  (Fig. 3a). The importance of heterotrophic nitrification has been recently recognized in grasslands (Müller et al., 2004, 2014) and forests (Zhang et al., 2014).

Ammonium accumulation was noted in the arid-zone soils and the accumulated  $\text{NH}_4^+$  was characterized by increased  $^{15}\text{N}$  enrichment (Fig. 2b, e). Ammonium is the dominant species in bulk N deposition in China (Liu et al., 2013). Dry deposition is generally the dominant form of deposition under an arid climate (Elliott et al., 2009). The  $\delta^{15}\text{N}$  values of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in dry deposition were higher than those in wet deposition (Elliott et al., 2009; Garten, 1996; Heaton et al., 1997) and might contribute to the observed  $^{15}\text{N}$  enrichment. Our preliminary study also showed that  $\delta^{15}\text{N}$  values of aerosol  $\text{NH}_4^+$  at one arid site (Dunhuang in Gansu Province, MAP = 46 mm) in northwestern China ranged from 0.35 to 36.9‰, with an average of 16.1‰ (Liu and Fang, unpublished data). Similar results were obtained at a site in Japan (Kawashima and Kurahashi, 2011), where the  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$  in suspended particulate matter ranged from 1.3 to 38.5‰, with an average of 11.6‰. It remains unclear why the  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$  in dry deposition is so positive, but it may result from the isotopic exchange of atmospheric ammonia gas and aerosol  $\text{NH}_4^+$ , which creates aerosol  $\text{NH}_4^+$  enriched in  $^{15}\text{N}$  (an isotopic effect of 33‰; Heaton et al., 1997). In the drylands, biological N fixation is another important N input (Evans and Ehleringer, 1993). In this study, we speculated that biological N fixation by biological soil crusts (BSCs) could contribute to the soil  $\text{NH}_4^+$  pool and soil organic N. We found that with decreasing precipitation, the  $\delta^{15}\text{N}$  of bulk soil N decreased to close to 0‰ (Fig. 2d), which is the expected  $\delta^{15}\text{N}$  value for  $\text{NH}_4^+$  derived from biological N fixation. BSCs were visually observed during soil sampling in the arid zone. A previous study also reported the potential N-fixing activity and ecological importance of BSCs in soil stability and N availability in the grasslands of Inner Mongolia (Liu et al., 2009).

In the semiarid zone, soil  $\text{NH}_4^+$  was depleted in  $^{15}\text{N}$  relative to bulk soil N, and the differences in  $\delta^{15}\text{N}$  increased with increasing MAP, which is likely due to gradually enhanced N mineralization (ammonification) in less-dry soils. The increase in precipitation was closely correlated to the microbial gene abundance associated with N transformations (Fig. 4). The  $\delta^{15}\text{N}$  of bulk soil N was quite stable in the semiarid zone, at approximately 5‰ (Fig. 2d). An increase in N mineralization as precipitation increases would bring in more  $^{14}\text{N}$  and progressively lower the  $\delta^{15}\text{N}$  of soil  $\text{NH}_4^+$  (Fig. 2e). The isotopic effect of N mineralization might also be higher than commonly expected. Our laboratory recently found that  $^{15}\text{N}$  fractionation during mineralization was up to 6 to 8‰ in two forest soils in northern China (Zhang et al., 2015). The fractionation during mineralization can even be as high as 20‰ at the enzyme level (Werner and Schmidt, 2002). With further increase in water availability in the semiarid zone, N turnover linking the biological uptake (plant and microbes) and return of N could further enhance soil ammonification, which would result in lower  $\delta^{15}\text{N}$  in soil  $\text{NH}_4^+$ . In addition, there is also a possibility of dissimilatory nitrate



**Figure 8.** A framework of N biogeochemical cycling in dryland ecosystems in northern China. Width of arrows and size of boxes indicate the relative importance (qualitative interpretation) of soil N processes and pools between the arid zone (a) and semiarid zone (b). The mean pool sizes ( $\text{g N m}^{-2}$ ) of each soil N pool based on the bulk soil density of the top 10 cm are in the brackets. It should be noted that during both nitrification and denitrification, N trace gases NO and  $\text{N}_2\text{O}$  can be produced and escape the system (leaky pipe model (Firestone and Davidson, 1989), not shown in the figure), affecting both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations and their  $\delta^{15}\text{N}$  values.

reduction to ammonium (DNRA); however, we did not measure this process in our study. DNRA is even less sensitive to oxygen levels than denitrification and may therefore occur in aerobic soils (Müller et al., 2004), contributing to the availability of soil  $\text{NH}_4^+$ .

## 5 Summary

Our study reported the pattern of  $\delta^{15}\text{N}$  in soil inorganic N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) across a precipitation gradient from very arid land to semiarid grassland. Together with the analyses of soil N concentration, soil pH and moisture, and functional gene abundance, the compound-specific  $\delta^{15}\text{N}$  analyses presented here demonstrate a clear shift of abiotic-versus-biotic (microbes and plants) controls on N cycling along this 3200 km dryland transect in China.

In the arid zone, characterized by extreme aridity ( $36 \text{ mm} < \text{MAP} < 100 \text{ mm}$ ; Fig. 8a), plant cover was sparse and microbial activity was limited (Figs. 1 and 4). Nitrogen input, mostly in the form of atmospheric deposition, largely accumulated, creating  $^{15}\text{N}$ -enriched inorganic N pools despite a much smaller pool of bulk soil N. The accumulation of inorganic N drives abiotic processes that lead to N losses, with strong isotopic fractionation effects on the remaining soil N. The higher pH associated with a lower MAP is likely

a dominant driver of  $\text{NH}_3$  volatilization, causing  $^{15}\text{N}$  enrichment in soil  $\text{NH}_4^+$ . The very high yet variable accumulation of  $\text{NO}_3^-$  in soil compared with  $\text{NH}_4^+$  suggests limited  $\text{NO}_3^-$  loss under extreme aridity.

In the semiarid zone ( $100\text{ mm} < \text{MAP} < 436\text{ mm}$ ; Fig. 8b), controls on N cycling increasingly shift from abiotic to biotic factors. Microbial gene abundances associated with N cycling groups were considerably greater when water became more available (Fig. 4). Increasing N mineralization with increasing MAP was accompanied by reduced  $\text{NH}_3$  volatilization due to lower pH, producing soil  $\text{NH}_4^+$  pools with lighter N isotopes. Ammonification (N mineralization) both supplies  $\text{NH}_4^+$  for plant uptake and favors soil nitrification. Both nitrification and denitrification could lead to N loss and isotopically enrich the remaining soil  $\text{NO}_3^-$ . Soil heterogeneity and pulse precipitation events could provide hotspots for these microbial processes, whereas increased plant cover and N uptake could reduce the soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  pools and minimize overall N losses. The regulation of abiotic-versus-biotic controls by precipitation on N cycling and N losses suggests that global climate changes would have a great impact on these dryland ecosystems.

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*Competing interests.* The authors declare that they have no conflict of interest.

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