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 ^{15}N natural abundance ($\delta^{15}N$) 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) from 36 mm to 436 mm. We analysed N pools and $\delta^{15}N$ of ammonium, dual isotopes (^{15}N and ^{18}O) of nitrate, and the microbial gene abundance associated with soil N transformations. We found that N status and their 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. Ammonia volatilization was strong in high pH soils. 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 controlled mainly by biological processes (e.g., plant uptake and denitrification). The uptake 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 the shift from abiotic to biotic 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.

Key words: soil inorganic N; ^{15}N natural abundance; soil microorganisms; functional genes; spatial patterns

1 Introduction

Drylands cover approximately 41% of the Earth's land surface and play an essential role in providing ecosystem services as well as 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 to 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 to 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 (Huntington, 2006; Knapp et al., 2008). Therefore, improving our understanding of N cycling and its controls would greatly enhance our ability of predicting the responses of dryland ecosystems to global changes.

The ^{15}N natural abundance (expressed as $\delta^{15}N$) can provide critical information on N cycling and thus assist in understanding ecosystem N dynamics over large scales (Amundson et al., 2003; Austin and Vitousek, 1998; Houlton et al.,

2006). The general pattern that foliar and soil $\delta^{15}N$ increases as precipitation decreases has been found at both 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 in dryland ecosystems than in mesic ecosystems. The underlying explanation is when N supply is higher relative to biotic demand, more N is lost through leaching and gaseous N emissions (Austin and Vitousek, 1998), during which isotope fractionation is against the heavier isotope so that plant tissue and soil become enriched in ^{15}N (Robinson, 2001). However, the controls of atmospheric deposition on N cycling are often ignored in N isotope studies, in which N isotopes 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). Besides, N losses in dryland ecosystems are likely dominated by gaseous losses instead of hydrological losses (McCalley and Sparks, 2009; Peterjohn and Schlesinger, 1990). The ^{15}N natural abundance of total N are limited in interpreting the specific processes governing those 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 $\delta^{15}N$ variability in plant-soil systems.

Isotopes in ammonium (NH_4^+) and nitrate (NO_3^-) can serve as a proxy record for the N processes in soils because they directly respond to the *in situ* processes that controlling NH_4^+ and NO_3^- production and consumption. For example, comparing $\delta^{15}N$ values of NH_4^+, NO_3^-, and bulk soil N could reveal the relative importance of N transformation processes (such as between ammonification and nitrification) (Koba et al., 2010; Koba et al., 1998). The dual isotope analysis of NO_3^- (^{15}N and ^{18}O of soil 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, $\delta^{18}O$ of NO_3^- has also been used to partition microbial produced NO_3^- from atmospheric sources because they cover a different range of $\delta^{18}O$ (Böhlke et al., 1997; Brookshire et al., 2012; Kendall et al., 2007). The positive correlations between N isotopes of soil available N (NH_4^+, NO_3^-, and dissolved organic N) and plant leaves have been used to study the preferences for plant N uptake (Cheng et al., 2010; Houlton et al., 2007; Mayor et al., 2012; Takebayashi et al., 2010). With methods newly developed (Lachouani et al., 2010; Liu et al., 2014; Tu et al., 2016), the analysis of isotopic values in soil NH_4^+ and NO_3^- has the potential to elucidate the N cycling characteristics and their controls; however, in comparison to that of bulk soil N, the $\delta^{15}N$ of both soil NH_4^+ and 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 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; He et al., 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 in different environmental conditions. Denitrification genes from the soils in Antarctic, for example, were found to be linked to higher soil temperatures, and N_2-fixation genes were linked to the presence of lichens (Yergeau et al., 2007). Research along an elevation gradient pointed out that some of denitrification genes (*nir*S and *nos*Z) were more abundant at higher elevations, with nitrification as the major process of N_2O emission in the Tibetan grassland (Yang et al., 2013). The latest version, GeoChip 5.0S, contains probes covering over 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 $\delta^{15}N$ for bulk soil N along this precipitation gradient, with a threshold at aridity index of 0.32 (mean annual precipitation of about 250 mm), showing the respective *soil microbial vs. plant* controls (Wang et al., 2014). Here, we further analysed the concentrations, N isotopic composition of soil NH_4^+ and NO_3^- (for NO_3^-, also oxygen (O) isotopes) as well as the microbial gene abundances associated with soil N transformations. The principal objectives of this study were to examine: (1) the patterns of concentrations and $\delta^{15}N$ for soil NH_4^+ and NO_3^-; (2) the patterns of gene abundance associated with microbially regulated soil processes; (3) and how soil N cycling responds 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 transect across Gansu province and Inner Mongolia in northern China, covering a longitude from 87.4ºE to 120.5ºE and a latitude from 39.9ºN to 50.1ºN (Fig. 1).The climate is predominantly arid and semi-arid continental. From west to east of the transect, the mean annual precipitation (MAP) increased from 36 mm to 436 mm and mean annual temperature (MAT) decreased from 9.9ºC to -1.8ºC (Fig. S1), with aridity index (the ratio of precipitation to potential evapotranspiration) 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 were predominantly arid, sandy, and calcium-rich brown loess.

2.2 Soil sampling and sample preparation

Soil sampling was conducted from July to August in 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 about 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) while 50 sites at about 50 km intervals were used for bulk soil N isotopes measurement in Wang et al. (2014). In each site,

we set a 50 m × 50 m plot, and then five 1 m × 1 m subplots at the four corners and the centre of the plot. In each subplot, twenty random mineral soil samples were collected using soil cores (2.5 cm diameter × 10 cm depth) and 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 parts. The first part was extracted in 2 M KCl (1:5 w/v) for 1 h on the same sampling day; the extracts were stored at -4 ºC during the sampling trip. The second part was placed in a sterile plastic bag and immediately stored at -40 ºC for later DNA extraction. The third part was placed in a plastic bag and stored in a refrigerator at -4 ºC for subsequent analyses.

2.3 Analyses of soil physicochemical properties and isotopes

Soil pH was measured using a pH meter with a soil to water ratio of 1:2.5. Soil N content and ^{15}N natural abundance were determined by an elemental analyser connected to an Isotope Ratio Mass Spectrometer (IRMS) (Wang et al., 2014). The concentrations of soil NH_4^+ and NO_3^- in KCl extracts were analysed using conventional colorimetric methods (Liu et al., 1996). Ammonium concentrations were determined by the indophenol blue method, andnitrate by sulfanilamide-NAD reactionfollowing cadmium (Cd) reduction.

The analyses of isotope compositions of NH_4^+ and NO_3^-, including $\delta^{15}N$ of NH_4^+, $\delta^{15}N$ of NO_3^-, and $\delta^{18}O$ of NO_3^- ($\delta = [(R_{sample}/ R_{standard}) -1] \times 1000$, where R denotes the ratio of heavy isotope to light isotope for N or O, in units of per mil, ‰), were based on the isotopic analysis of nitrous oxides (N_2O). Specifically, NH_4^+ in the extract was oxidized to NO_2^- by alkaline hypobromite (BrO^-), and then reduced to N_2O by hydroxylamine (NH_2OH) (Liu et al., 2014). Nitrate was firstly reduced to NO_2^- by Cd power, and then to N_2O by sodium azide (NaN_3) in an acetic acid buffer (McIlvin and Altabet, 2005; Tu et al., 2016). In order to correct machine drift and blank over the isotopic analyses, the international standards of NH_4^+ (IAEA N1, USGS 25, and USGS 26) and NO_3^- (IAEA N3, USGS 32, USGS 34, and USGS 35) were treated in identical analytical procedures as the samples to obtain the calibration curve between the measured and their expected isotope values. The isotopic signatures of produced N_2O were determined by an IsoPrime 100 continuous flow isotope ratio mass spectrometer connected to a Trace Gas (TG) pre-concentrator (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, 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 gene abundance of N fixation, ammonification, and anaerobic ammonia oxidation (anammox) were included in this paper. Briefly, microbial genomic DNA was extracted from 0.5 g soil using the MoBioPowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) and purified by agarose gel electrophoresis followed by phenol-chloroform-butanol extraction. DNA quality was assessed by the ratios of A260/280 and A260/230 using NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE), and final soil

DNA concentrations was quantified by PicoGreen using a FLUOstar Optima (BMG Labtech, Jena, Germany). 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 brief, the purified DNA samples (0.6 μg) was used for hybridization, which was labelled with the fluorescent dye Cy 3; subsequently, the labelled DNA was resuspended and hybridized at 67 ºC 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 photomultiplier tube gain of 75%, respectively. The images data were extracted by Agilent Feature Extraction program. The microarray raw data were further processed for subsequent analysis using an in-house pipeline that was built on the platform at the Institute for Environmental Genomics, University of Oklahoma (He et al., 2010a; He et al., 2007).

2.5 Statistical analyses

All analyses were conducted by using the SPSS 18.0 (SPSS, Chicago, IL) for Windows. Pearson correlation analysis was conducted to examine the linear relationships among different variables. Independent-Samples T-test was 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 NO_3^- and NH_4^+ concentrations

We found significant inorganic N accumulation in the investigated soil layer (0-10 cm) in sites with MAP less than 100 mm (Fig. 2b and c). Furthermore, the abundance of microbial genes associated with soil N transformations was significantly lower than those in sites with MAP of more than 100 mm (Fig.4). Together with the vegetation distribution along the transect (Fig.1), these results pointed out that soil N status and its controls could be different above and below a MAP threshold of 100 mm. Thereafter, we refer the area with MAP from 36 mm to 102 mm (15 sites) and from 142 mm to 436 mm (21 sites) as arid zone and semiarid zone in this study, respectively.

Soil NO_3^- and NH_4^+ concentrations in the arid zone were significantly higher than those in the semiarid zone ($P < 0.001$; Fig. 2b and c). In the arid zone, NO_3^- concentrations were highly variable and up to 1400 mg N kg^{-1}, with a mean of 87 mg N kg^{-1}. Ammonium concentrations varied from 2.0 to 9.9 mg N kg^{-1}, with a mean of 4.3 mg N kg^{-1}. In the semiarid zone, NO_3^- and NH_4^+ concentrations were low and in most samples less than 5 mg N kg^{-1}. Soil NH_4^+concentrations showed a quadratic relationship with increasing MAP in the semiarid zone, but NO_3^- concentrations remained low and did not change with increasing MAP. As expected, soil total N was significantly higher in the semiarid zone (on average 0.1%) than in the arid zone (on average 0.02%) and increased dramatically in the semiarid zone with increasing precipitation (Fig. 2a). Our results suggest a higher inorganic N availability in the arid zone than in the semiarid zone despite a smaller total N pool therein, which supports the idea that N availability is relatively higher in dry areas than in less dry areas.

3.2 The ^{15}N natural abundance of soil NO_3^- and NH_4^+

The $\delta^{15}N$ values of NO_3^- were significantly higher in the semiarid zone (0.5 to 19.2‰) than in the arid zone (-1.2 to 23.4‰; $P < 0.01$; Fig. 2f), with the mean of 8.4‰ and 6.3‰, respectively. With increasing MAP, the $\delta^{15}N$ value of NO_3^- increased in the arid zone but decreased in the semiarid zone, suggesting different controlling factors in the areas with different water availability. Different from that of soil NO_3^-, the $\delta^{15}N$ value of NH_4^+ was significantly higher in the arid zone (-1.2 to 20.2‰) than in the semiarid zone (-13.9 to 12.6‰; $P < 0.01$; Fig. 2e), with the mean of 9.2‰ and -0.3‰, respectively. The $\delta^{15}N$ of NH_4^+ was negatively correlated with MAP in the semiarid zone, but was stable as precipitation increased in the arid zone (Fig. 2e).

The N isotopic signature of NH_4^+ and NO_3^- reflects not only isotopic fractionation during N transformation processes, but also the N isotopic signature of their main sources (i.e., bulk soil N and NH_4^+, respectively). Therefore, we also calculated the relative ^{15}N enrichment of soil NH_4^+ (the difference between $\delta^{15}N$ of NH_4^+ and bulk soil N) and NO_3^- (the difference between $\delta^{15}N$ of NO_3^- and NH_4^+) to examine the isotopic imprint of N transformations on soil NH_4^+ and NO_3^-. The relative ^{15}N enrichment of soil NH_4^+ in the arid zone was mostly positive, while it was negative in the semiarid zone (Fig. 3a). There was a negative correlation between MAP and the relative ^{15}N enrichment of soil NH_4^+ across both the arid and semiarid zones (Fig. 3a). According to the Rayleigh model, sinks are always ^{15}N-depleted relative to their sources (Robinson, 2001). The positive values for the ^{15}N-enrichment of NH_4^+ support that net NH_4^+ losses occurred mainly in the arid zone, while the negative values imply that net NH_4^+ gain (e.g., via microbial N mineralization, biological N fixation and/or N deposition) might increase in the semiarid zone, and subsequently reduced the relative ^{15}N-enrichment of soil NH_4^+. In a similar way, we found that the relative ^{15}N-enrichment of NO_3^- were mostly negative in the arid zone and positive in the semiarid zone (Fig. 3b). A positive correlation was observed between MAP and the ^{15}N-enrichment of soil NO_3^- in both the arid and semiarid zones (Fig. 3b). Accordingly, these results suggest that NO_3^- losses along this dryland transect occurred when water becomes more available, and progressively enriched residual soil NO_3^- in ^{15}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 in all sites. In arid zone soils, gene abundances of all N cycling groups were found to be extremely low (Fig.4), indicating limited microbial potentials in the very dry environment. There was a sharp increase (by 8 to 9 fold) in the gene abundance from the arid zone to the semiarid zone (Fig. 4), even though soils were still mostly dry at the time of sampling (see soil moisture in Fig. S2). Gene abundances in the semiarid zone were 1-2 orders of magnitude higher than those in the arid zone. In addition, 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 control of water availability on soil microbial N processes.

4 Discussion

4.1 Losses of soil NO_3^- and NH_4^+

We observed different patterns of N cycling above and below a MAP threshold of 100 mm in this 3200 km transect. In the semiarid zone, the increased precipitation seems to lead to increased losses of soil NO_3^-, but not NH_4^+ (Fig. 3). Soil NO_3^- can be removed from the ecosystem via denitrification, leaching, and also plant and microbial uptake. The close correlation between measured dual isotopes ($\delta^{15}N$ and $\delta^{18}O$) of soil 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 O and N in NO_3^- (Granger et al., 2008). This kind of fractionation results in concurrent increases in the $\delta^{18}O$ and $\delta^{15}N$ values of the remaining NO_3^- with a ratio of 0.5 to 1 (Kendall et al., 2007). In the present study, the $\delta^{18}O$ values of soil NO_3^- were significantly correlated with the $\delta^{15}N$ values of soil 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 NO_3^- across five Hawaiian tropical forests (Houlton et al., 2006), indicating the occurrence of denitrification driven N losses when water becomes relatively available. Denitrification is regulated by proximal factors that immediately affect denitrifying communities, such as NO_3^- concentration and O_2 concentration (Saggar et al., 2013). Enhanced nitrification as a result of water addition and increased mineralization would yield a greater amount of NO_3^- for potential denitrification. Increased soil respiration in hot spots and/or hot moments caused by pulse precipitations consumes O_2, consequently favouring denitrification (Abed et al., 2013). In the semiarid zone, we observed that ^{15}N-enrichment of soil NO_3^- increased with increasing precipitation (Fig. 3b), suggesting that denitrification may become more favourable with increasing precipitation. In addition, our preliminary study of a ^{15}N-labeled NO_3^- incubation experiment showed that potential N_2 losses via denitrification were also increasing with increasing precipitation in the semiarid soils (Liu and Fang, unpublished data). Because gaseous N losses occur during both nitrification (more on this later) and denitrification, the coupled nitrification and denitrification could maintain soil NO_3^- concentration low while enriching its ^{15}N signal. These results support the idea that gaseous N losses is increasing as precipitation increases in dryland ecosystems (Wang et al., 2014).

In the arid zone, the $\delta^{15}N$ and ^{15}N-enrichment of soil NO_3^- also increased with increasing precipitation (Fig. 2f and 3b), indicating that denitrification may also take place. However, in these arid soils the microbial gene abundances were much lower (Fig.4), suggesting lower 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 attribute to soil NO_3^- losses in the arid zone. Chemodenitrification is an abiotic process, in which the reduction of NO_3^- or NO_2^- to NO and N_2O is coupled to the oxidation of reduced metals (e.g. Fe (II)) and humic substances (Medinets et al., 2015; Zhu-Barker et al., 2015). Ample soil NO_3^- was present in some arid zone soils (Fig. 2c), meanwhile our companion work also observed higher available Fe in arid zone soils (Luo et al., 2016). In a recent

publication, Homyak et al. (2016) reported both initial abiotic NO pulses after soil rewetting and biologically driven NO emissions later, pointed to multiple controls on NO emissions and N biogeochemistry in dryland ecosystems.

Different from the $\delta^{15}N$ of soil NO_3^-, the $\delta^{15}N$ values of soil NH_4^+ and their relative ^{15}N enrichment were higher in the arid zone than those in the semiarid zone (Fig. 2e and 3a), suggesting losses of NH_4^+ in the drier sites. We suggest that NH_3 volatilization should play a significant role in NH_4^+ losses, because soil pH was higher in the arid zone (from 7.3 to 9.7; Fig. 6a). The isotopic effect of NH_3 volatilization had been reported to be 40-60‰ (Robinson, 2001), resulting in ^{15}N-enriched soil NH_4^+. The significant negative correlation between the $\delta^{15}N$ values of NH_4^+ and soil pH in this study (Fig. 6b) supported our interpretation. Besides, 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 and result in higher potential nitrification in dry soils (Sullivan et al., 2012). In the process of nitrification, NO losses occur via a "hole-in-the-pipe" mechanism (Firestone and Davidson, 1989). In addition, nitrite (NO_2^-), produced from nitrification, can be reduced rapidly to NO via chemodenitrification. The reaction of chemodenitrification forms NO via nitrous acid (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 NO_2^- upon the recovery of nitrifiers from drought-induced stress (Homyak et al., 2016).

In the semiarid zone, soil NH_4^+ became gradually depleted in ^{15}N relative to the bulk soil N (Fig. 3a), suggesting the input of NH_4^+ (e.g., soil ammonification, N deposition), while simultaneously NH_4^+ was also consumed. NH_3 volatilization should be low due to relatively lower pH compared to those in the arid zone soils (Fig. 6a). Previous studies have found that water addition did not stimulate NH_3 volatilization (Yahdjian and Sala, 2010), although a recent study observed the opposite result in a semiarid subtropical savanna (Soper et al., 2016). With increasingly available water, biological N consumption by plants and microbes would dominate soil NH_4^+ sinks in the semiarid zone. The increased aboveground biomass with increasing MAP suggests a higher net plant N accumulation along this precipitation gradient (Wang et al., 2014). Since soil NH_4^+ concentration was higher relative to soil NO_3^- in the semiarid zone ($P < 0.001$), the dominant plant species might adapt to use soil NH_4^+ over NO_3^-. This is in accordance with the observed relationship of the $\delta^{15}N$ values of plant leaves (non-N fixing species) with soil NH_4^+ (R^2=0.40; Fig. 7a), but not with soil NO_3^- (Fig. 7b). When we plot this correlation for each plant species, three dominant species (*Stipa* spp., *Cleistogenes* spp., and *Reaumuria* spp.) were significantly correlated with soil NH_4^+. Besides, plant internal N cycling likely shifts as a function of water availability and would influence foliar $\delta^{15}N$ and such relationship, but the extent is hard to estimate at this stage. Plant N uptake may also exert fractionation effect on their N sources, but it might be negligible in the N limited areas (Craine et al., 2015). This may in part explain no strong ^{15}N-enrichment of soil NH_4^+ with increasing precipitation. The nitrification consumption of NH_4^+ 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}N enrichment of soil NO_3^-, without significantly altering NO_3^- concentration. On the other hand, enhanced plant uptake (of both soil NH_4^+ and 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 NH_4^+ and NO_2^- with N_2 as the end product (Thamdrup and Dalsgaard, 2002). Previous studies have found equal consumption of both soil NH_4^+ and NO_3^- through anammox in N-loaded and water-logged areas (Yang et al., 2014; Zhu et al., 2013). However, the only two anammox studies in drylands so far failed to confirm its importance (Abed et al., 2013; Strauss et al., 2012). Thus, although anammox possesses a fractionation effect of 23-29‰ (Brunner et al., 2013), it is difficult to tell its significance in our study transect at the present time.

Other abiotic processes have also been reported contributing 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 affect ^{15}N abundance of soil N. Other non-fractionation processes might also influence N cycle in dryland ecosystems, such as aeolian deposition and water erosion (Austin, 2011; Hartley et al., 2007). The importance of all these processes remains for further research.

4.2 Sources of soil NO_3^- and NH_4^+

We observed much higher concentrations of soil NO_3^- in the arid zone (Fig. 2c), on average about 20 times higher than those in the semiarid zone. Nitrate can be formed via microbial nitrification, deposited from N-bearing gaseous (e.g., HNO_3) or dry aerosol NO_3^- (Kendall et al., 2007), plus as dissolved nitrate in rainwater or snow. If NO_3^- is formed by nitrification, NO_3^- will obtain its O atoms from soil O_2 and H_2O in a 1:2 ratio (Kendall et al., 2007). The $\delta^{18}O$ value of atmospheric O_2 is relatively stable (23.5‰; we assume that the isotope composition of O_2 in the atmosphere and soils are the same). The $\delta^{18}O$ value of nitrified NO_3^- will depend on the $\delta^{18}O$ value of the local water. The $\delta^{18}O$ 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), that would yield the corresponding $\delta^{18}O$ of nitrified NO_3^- from -5.3 to 11.3‰ (Fig. 5a). However, the $\delta^{18}O$ values of soil NO_3^- in the arid zone varied from 5.5 to 51.8‰ (Fig. 5a). This disparity between calculated and measured $\delta^{18}O$ values provides evidence for the minor importance of nitrification. The higher $\delta^{18}O$ values of soil NO_3^- we observed in the arid zone have rarely been reported for nitrified NO_3^-, according to previous studies (Kendall et al., 2007). For example, an *in situ* study conducted in the forest floor soils found that $\delta^{18}O$ values of nitrified NO_3^- changed from 3.1 to 10.1‰ (Spoelstra et al., 2007). By comparison, atmospheric origin NO_3^- normally has higher $\delta^{18}O$ values because of the chemical oxidation of NO_3^- precursor, NO_x (NO and NO_2) (Fang et al., 2011). Previous research found that $\delta^{18}O$ values of aerosol 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 NO_3^- in the surface soils of the arid zone were from the atmospheric deposition. Nitrate would be accumulated on the surface soil when experiencing prolonged droughts, as also reported in northern Chile and southern California sites (Böhlke et al., 1997), as well as in the Turpan-Hami area of northwestern China (Qin et al., 2012). A pronounced trend in Figure 5a (green arrow) toward higher $\delta^{18}O$ and lower $\delta^{15}N$ values is obvious for elevated NO_3^- concentrations, which might be the results of mixed NO_3^- from both soil nitrification and atmospheric deposition, as previously observed in groundwater of Saharan desert (Dietzel et al., 2014). In the arid zone, extreme dryness and high

alkalinity (with an average pH of 8.3) might limit microbial activities, as suggested by the low gene abundance involving N transformation (Fig. 4), thus facilitate the preservation of NO_3^-.

In the semiarid zone, the $\delta^{18}O$ values of soil NO_3^- were low (0.9-21.0‰), indicating much less atmospheric contribution. The deposited NO_3^- will experience postdepositional microbial processes, and the original signature of $\delta^{18}O$ will vanish after biological processes (Qin et al., 2012). With increasing MAP, nitrification would progressively provide more NO_3^- with lower $\delta^{18}O$ values. The calculated $\delta^{18}O$ of NO_3^- from nitrification were from 2.5 to 6.5‰ based on the $\delta^{18}O$ of soil H_2O (-8 to -2‰; Shenyang site) (Liu et al., 2010). Both autotrophic and heterotrophic nitrification could generate soil NO_3^-. Heterotrophic nitrification is the process that oxidize organic N to NO_3^-, bypassing NH_4^+. If this process was important, it would provide an additional explanation for the lack of ^{15}N-enrichment in soil NH_4^+ (Fig. 3a). The importance of heterotrophic nitrification have been recognized recently in grasslands (Müller et al., 2014; Müller et al., 2004) and forests (Zhang et al., 2014).

There was NH_4^+ accumulation in the arid zone soils and the accumulated NH_4^+ was characterized with higher ^{15}N-enrichment (Fig. 2b, e). Ammonium has been shown to be the dominant species in bulk N deposition in China (Liu et al., 2013). Dry deposition is generally the dominant form of deposition in arid climates (Elliott et al., 2009). It has been reported that the $\delta^{15}N$ values of NH_4^+ and 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}N-enrichment. Our preliminary study also showed that $\delta^{15}N$ values of aerosol NH_4^+ in one arid site (Dunhuang in Gansu Province, MAP = 46 mm) in northwestern China ranged from 0.35 to 36.9‰ with the average of 16.1‰ (Liu and Fang, unpublished data). Similar results have been found in a site of Japan (Kawashima and Kurahashi, 2011), where the $\delta^{15}N$ of NH_4^+ in suspended particulate matter ranged from 1.3 to 38.5‰ with the average of 11.6‰. It remains unclear why the $\delta^{15}N$ of NH_4^+ in dry deposition is so positive, but it may result from the isotope exchange of atmospheric ammonia gas and aerosol NH_4^+, which causes aerosol NH_4^+ enriched in ^{15}N (with an isotope 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 soil NH_4^+ pool and soil organic N, and the contribution may be proportionally more important with decreasing precipitation. We find that the $\delta^{15}N$ of bulk soil N decreased to close to zero with decreasing precipitation, notice that biological N fixation provided NH_4^+ with the $\delta^{15}N$ value around zero. BSCs were observed during soil sampling in the arid zone. A previous research has 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 with MAP from 100 mm to 200 mm, soil NH_4^+ concentrations were lower than those in the arid zone, which may be caused by a tight coupling between microbial production of NH_4^+ and plant consumption (Nielsen and Ball, 2015). Soil NH_4^+ was depleted in ^{15}N relative to bulk soil N and their differences in $\delta^{15}N$ increased with increasing MAP (Fig. 3a), likely due to gradually enhanced N mineralization in less dry soils. The isotope effect of N mineralization might be also higher than commonly expected. Our laboratory recently reported that ^{15}N fractionation during mineralization

was up to 6-8‰ in two forest soils in northern China (Zhang et al., 2015). The fractionation during mineralization can be even as high as 20‰ if one looks at the enzyme level (Werner and Schmidt 2002). With increasing water availability in semiarid zone (MAP > 200 mm), N turnover linking biological uptake (plant and microbes) of N and return could further enhance and progressively fuel soil ammonification, which in turn result in lower $\delta^{15}N$ in soil NH_4^+. In addition, there is also a possibility of dissimilatory nitrate reduction to ammonium (DNRA), although we did not measure this process in our study. DNRA is even less sensitive to oxygen level than denitrification and therefore may occur in aerobic soils (Müller et al., 2004), contributing to the availability of soil NH_4^+.

5 Summary

To the best of our knowledge, our study reported, for the first time, the pattern of $\delta^{15}N$ in soil inorganic N (NH_4^+ and NO_3^-) across a precipitation gradient from very arid land to semiarid grassland. Together with the analysis of soil N concentration, soil properties like pH and soil moisture, and functional gene abundance, the compound-specific $\delta^{15}N$ analyses presented here demonstrate a clear shifting contribution of *abiotic vs. biotic* (microbes and plants) controls on N cycling along this 3200 km dryland transect in China.

In the arid zone with extreme aridity (36 mm < MAP < 100 mm; Fig. 8a), plant cover is sparse and microbial activity is limited (Fig. 1 and 4). Nitrogen input, mostly in the form of atmospheric deposition, is largely accumulated, creating "enriched" inorganic N pools despite a much smaller pool of soil total N. The accumulation of inorganic N drives abiotic processes that lead to N losses with strong isotopic fractionation effect on the remaining soil N. Higher pH associated with lower MAP is likely a dominant driver of NH_3 volatilization, causing soil NH_4^+ enriched in ^{15}N. The very high yet variable NO_3^- accumulation in soil comparing to NH_4^+ suggests limited NO_3^- loss under extreme aridity.

In the semiarid zone (100 mm < MAP < 436 mm; Fig. 8b), controls on N cycling increasingly shift from abiotic to biotic factors. Microbial gene abundances associated with N cycling groups were much higher when water became more available (Fig. 3). Increasing N mineralization with increasing MAP, accompanied with reduced NH_3 volatilization due to lower pH, produce soil NH_4^+ pool with lighter N isotopes. Ammonification (N mineralization) supplies NH_4^+ for both plant uptake and favour soil nitrification. Both nitrification and denitrification could lead to N loss and isotopically enrich remaining soil N. Soil heterogeneity and pulse precipitation events could provide hotspots for these microbial processes, while increased plant cover and N uptake could reduce soil NH_4^+ and NO_3^- pools and minimize overall N losses. The abiotic vs. biotic controls on N cycling and N losses around a threshold of MAP 100 mm suggest global climate changes, in particular the change of precipitation pattern, would have great impact on these dryland ecosystems.

Author contribution

Y. Fang, D. Liu, W. Zhu, and X. Han designed the study; D. Liu, X. Wang, Y. Pan, C. Wang, D. Xi, Y. Wang, and X. Han performed the experiment; D. Liu, W. Zhu, Y. Fang, X. Wang, Y. Pan, C. Wang, D. Xi, E. Bai and Y. Wang analysed the data. D. Liu, W. Zhu, and Y. Fang wrote the manuscript; X. Wang, Y. Pan, C. Wang, E. Bai, and X. Han contributed to discussion of the results and manuscript preparation.

Acknowledgements

The work was financially supported by the National Key Research and Development Program of China (2016YFA0600802), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB15020200, XDB15010401 and XDA05100100), the National Natural Science Foundation of China (31370464, 31422009, and 41405144), Hundred Talents Program of Chinese Academy of Sciences (No.Y1SRC111J6), and State Key Laboratory of Forest and Soil Ecology (LFSE2015-19). We would like to thank Ying Tu, Haiyan Ren, Shasha Zhang, Feifei Zhu, and Xiaoming Fang for their assistance in field sampling and laboratory analysis, and Shaonan Huang for sharing the unpublished data. We thank all members of the sampling team from the Institute of Applied Ecology, Chinese Academy of Sciences for their assistance during field sampling. We would like to thank Ben Eisenkop for his assistance on the English editing. We also thank two anonymous reviewers for their helpful comments and many constructive suggestions.

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Figure captions

Figure 1. Vegetation types and sampling sites 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), and 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.

Figure 2. Nitrogen concentrations and isotopic composition of bulk soil N, NH_4^+, and NO_3^-. The significant ($P < 0.05$) trends are shown with a regression line (red) and 95% confidence intervals (blue). In each site, n = 5.

Figure 3. The relative ^{15}N enrichment of soil NH_4^+ and NO_3^-. The relative ^{15}N enrichment of soil NH_4^+ and NO_3^- were calculated as the difference between $\delta^{15}N$ of bulk soil N and NH_4^+, and between $\delta^{15}N$ of soil NH_4^+ and NO_3^-, respectively. The significant ($P < 0.05$) trend is shown with a regression line (red) and 95% confidence intervals (blue). In each site, n = 5.

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. Data are the site-averaged value; results of the abundance of nitrification and denitrification genes have been reported in a previous study (Wang et al., 2014). The significant ($P < 0.05$) trends are shown with a regression line (red) and 95% confidence intervals (blue).

Figure 5. Relationship between $\delta^{18}O$ and $\delta^{15}N$ of soil NO_3^-. The range of $\delta^{18}O$ and $\delta^{15}N$ from atmospheric NO_3^- was based on the limited isotope measurement of precipitation. Black points represent precipitation NO_3^- collected from an urban site in Beijing in the year of 2012, with data derived from Tu et al. (2016). Grey points represent precipitation NO_3^- collected from Qingyuan forest CERN (Chinese Ecosystem Research Network, CERN) in Northern China in the year of 2014 (Huang and Fang, unpublished data). The range of $\delta^{15}N$ and $\delta^{18}O$ produced by nitrified NO_3^- are positioned by using the $\delta^{15}N$ of soil NH_4^+ in this study (Fig. 2e), and the estimated $\delta^{18}O$ from soil nitrification based on the 1:2 ratio of soil O_2 and H_2O (see Text), respectively.

Figure 6. Soil pH and the relationship with $\delta^{15}N$ of soil NH_4^+. The different patterns of soil pH was 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 $\delta^{15}N$ of soil 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). In each site, n = 5.

Figure 7. Relationship between the $\delta^{15}N$ of foliage and $\delta^{15}N$ of soil NH_4^+ and NO_3^-. Data on foliar $\delta^{15}N$ (*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).

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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 (g N m^{-2}) of each soil N pool based on the bulk soil density of top 10 cm were present in the brackets.

640

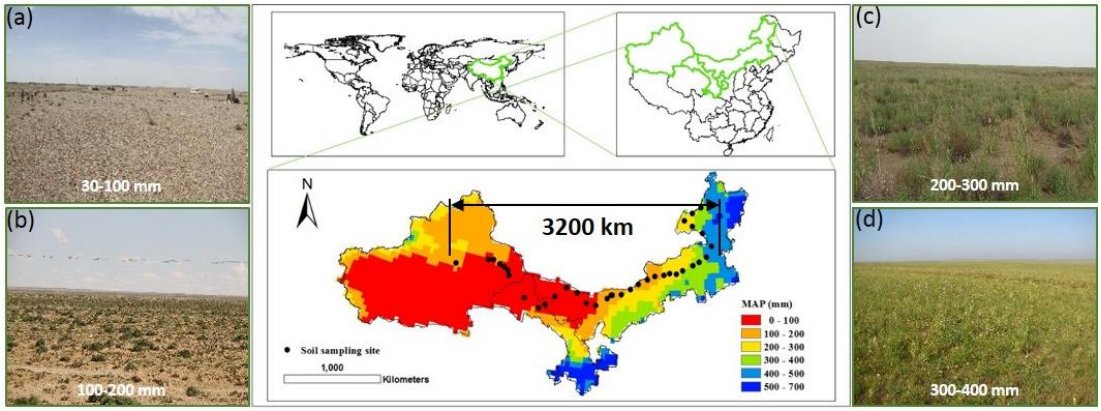

(a)
30-100 mm
(b)
100-200 mm
N
3200 km
Soil sampling site
1,000
Kilometers
MAP (mm)
0 - 100
100 - 200
200 - 300
300 - 400
400 - 500
500 - 700
(c)
200-300 mm
(d)
300-400 mm

Figure 1

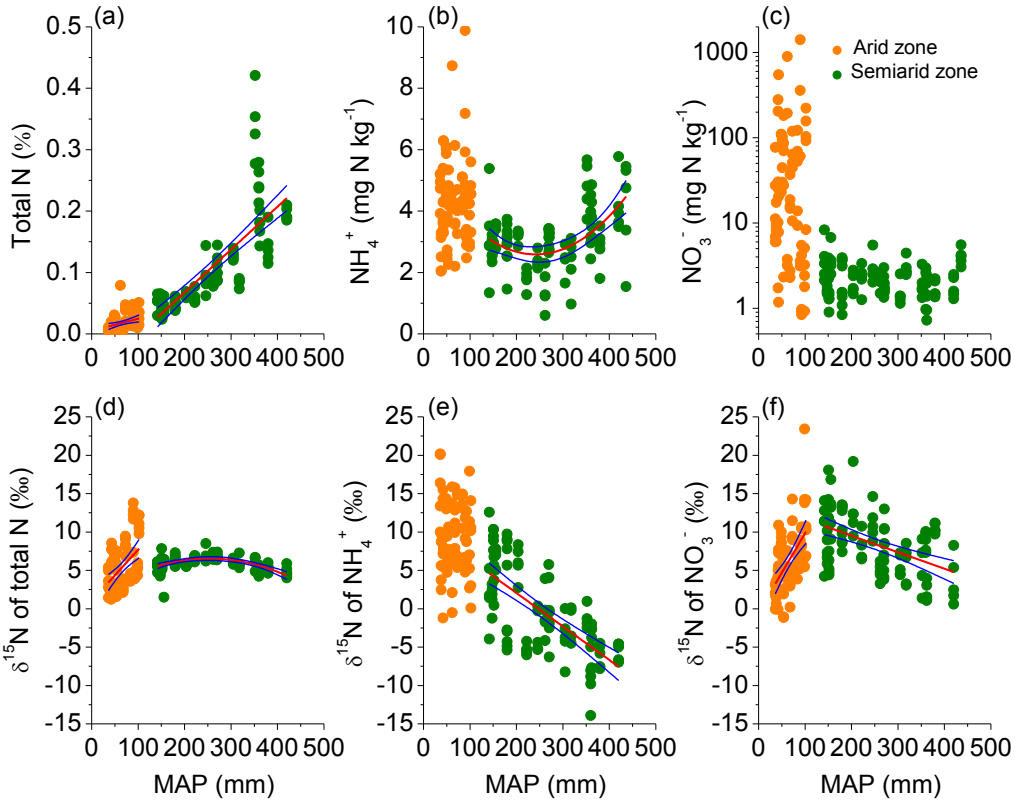

(a)
(b)
(c)
(d)
(e)
(f)
Total N (%)
NH4+ (mg N kg-1)
NO3- (mg N kg-1)
δ15N of total N (‰)
δ15N of NH4+ (‰)
δ15N of NO3- (‰)
MAP (mm)
Arid zone
Semiarid zone

Figure 2

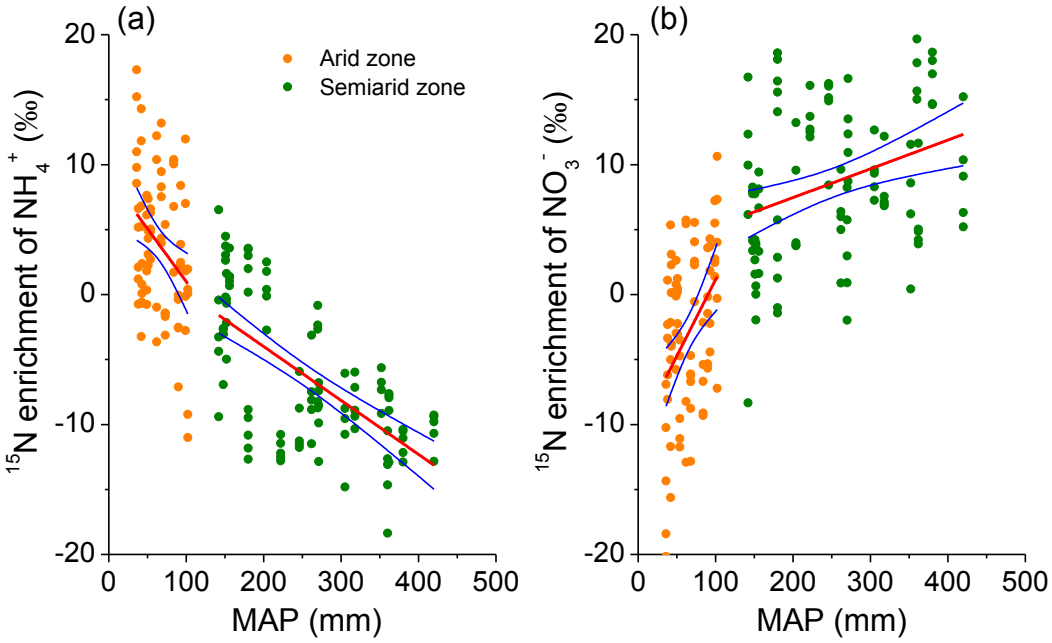

(a)
Arid zone
Semiarid zone
^{15}N enrichment of NH_4^+ (‰)
MAP (mm)
(b)
^{15}N enrichment of NO_3^- (‰)
MAP (mm)

Figure 3

650

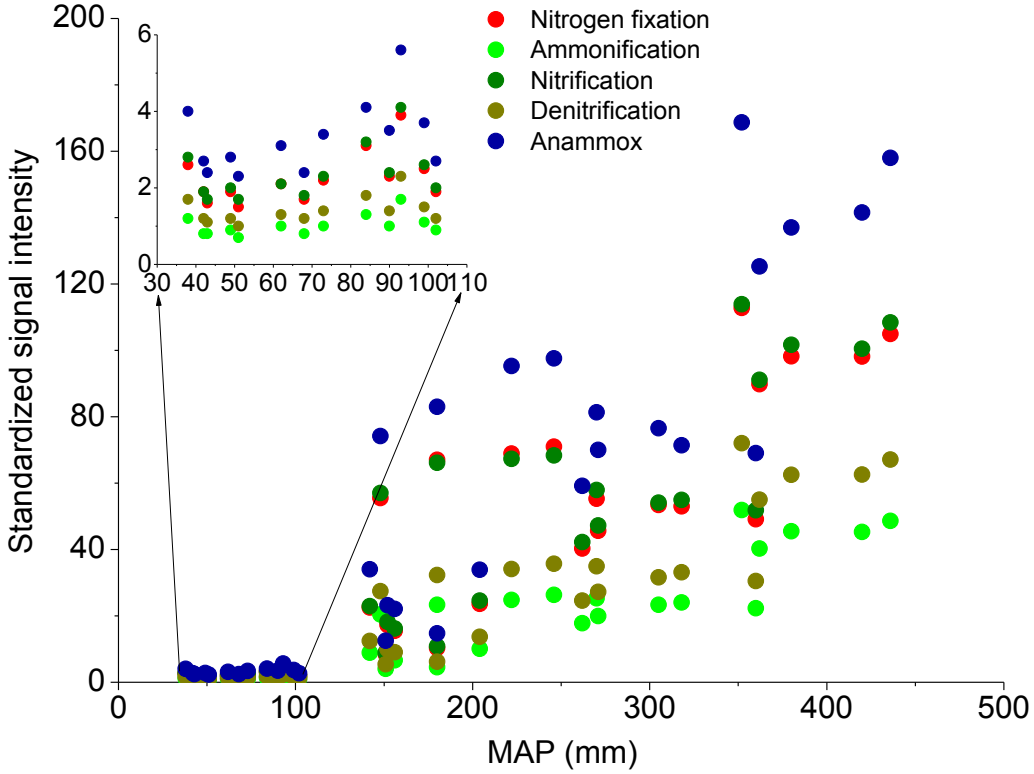
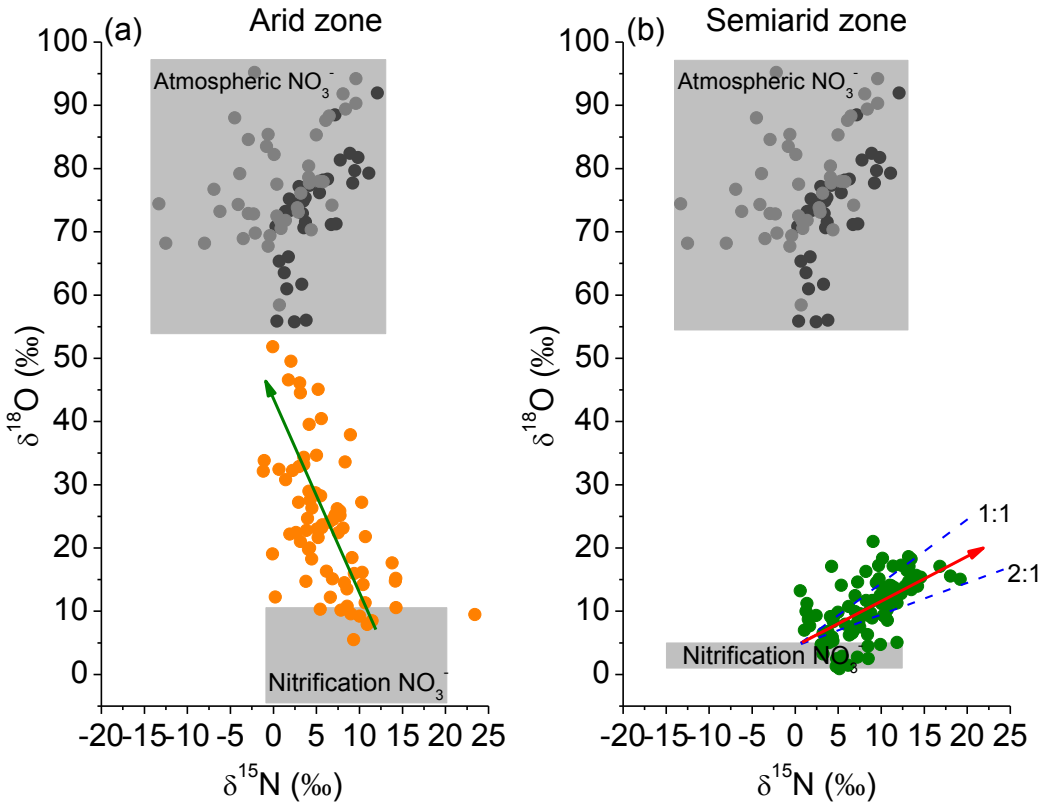

Nitrogen fixation
Ammonification
Nitrification
Denitrification
Anammox
Standardized signal intensity
MAP (mm)
0
40
80
120
160
200
0
100
200
300
400
500
0
2
4
6
30
40
50
60
70
80
90
100
110

Figure 4


(a)
Arid zone
Atmospheric NO_3^-
Nitrification NO_3^-
$\delta^{18}O$ (‰)
$\delta^{15}N$ (‰)
(b)
Semiarid zone
Atmospheric NO_3^-
Nitrification NO_3^-
1:1
2:1
$\delta^{18}O$ (‰)
$\delta^{15}N$ (‰)

655 **Figure 5**

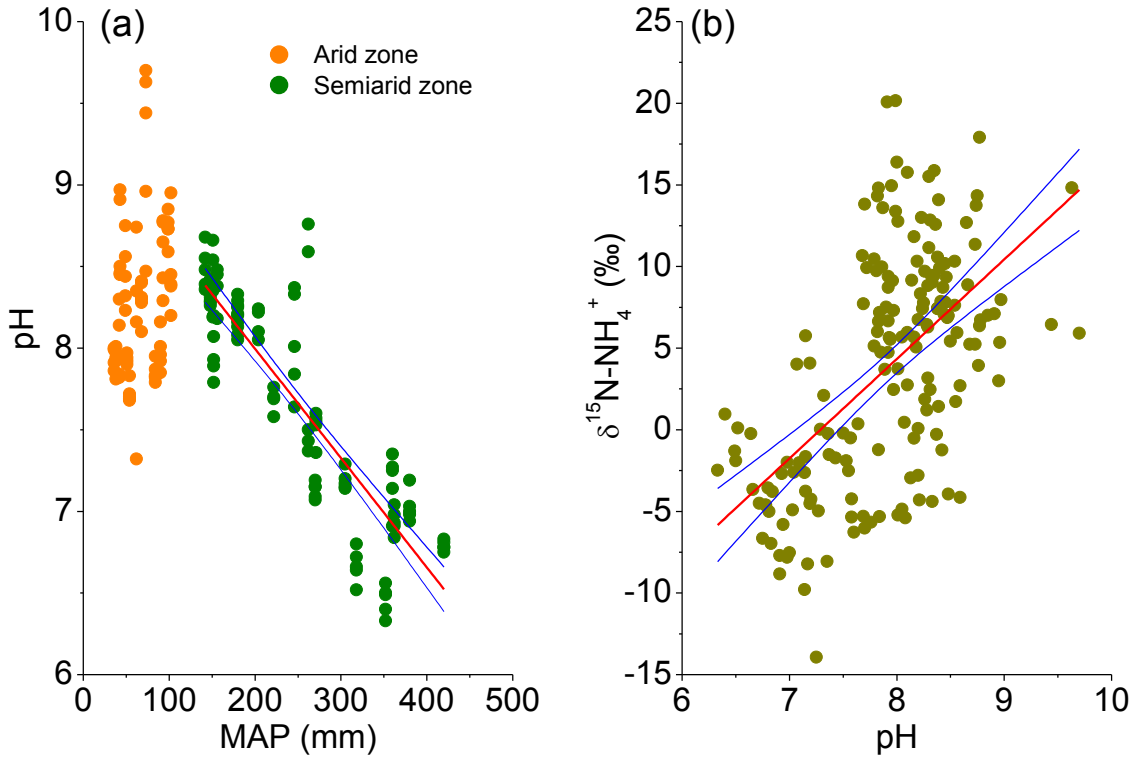


Figure 6

660

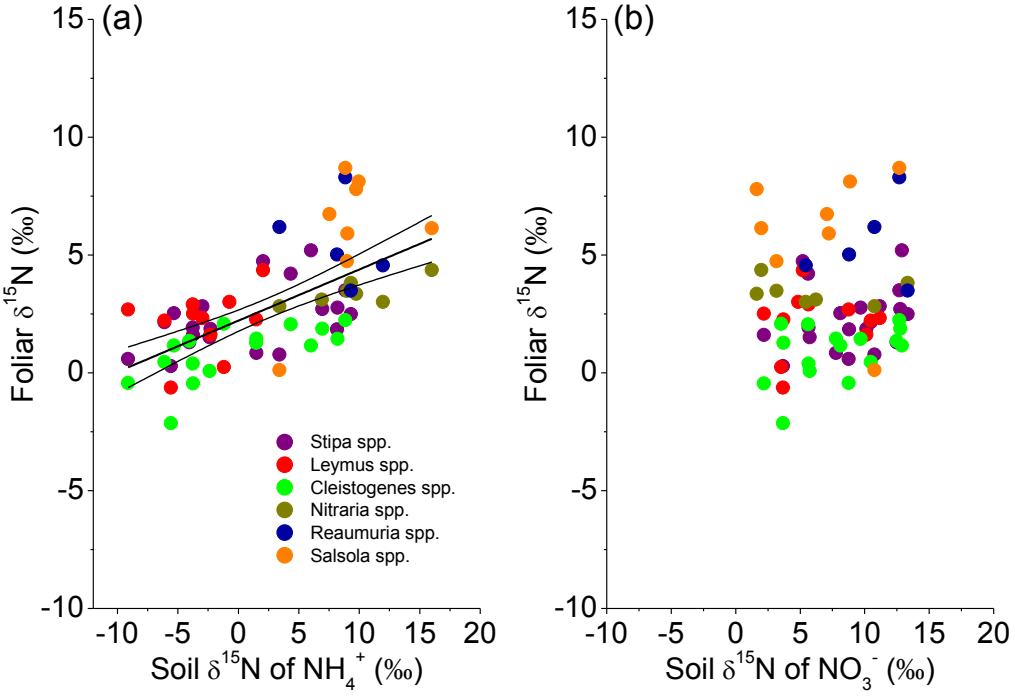

(a)
(b)
Foliar δ15N (‰)
Soil δ15N of NH4+ (‰)
Soil δ15N of NO3- (‰)
Stipa spp.
Leymus spp.
Cleistogenes spp.
Nitraria spp.
Reaumuria spp.
Salsola spp.

Figure 7

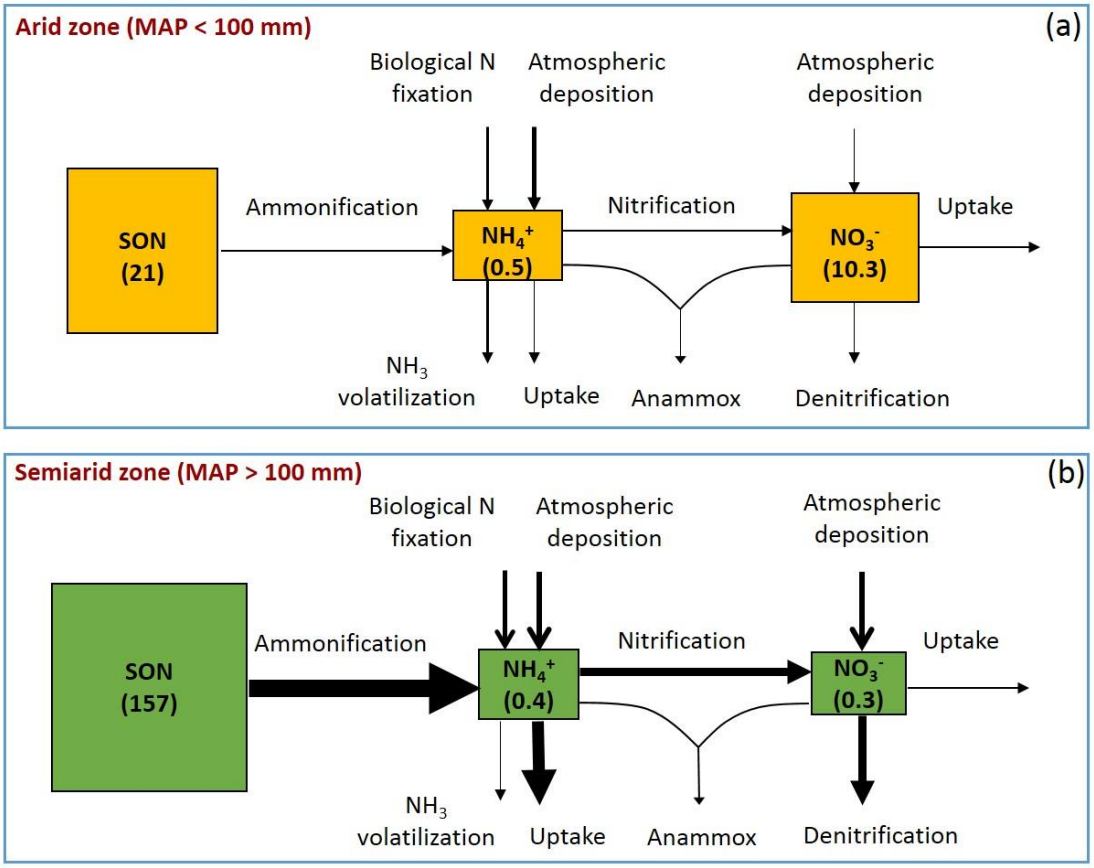

Arid zone (MAP < 100 mm)
(a)
Biological N fixation
Atmospheric deposition
Atmospheric deposition
SON (21)
Ammonification
NH4+ (0.5)
Nitrification
NO3- (10.3)
Uptake
NH3 volatilization
Uptake
Anammox
Denitrification
Semiarid zone (MAP > 100 mm)
(b)
Biological N fixation
Atmospheric deposition
Atmospheric deposition
SON (157)
Ammonification
NH4+ (0.4)
Nitrification
NO3- (0.3)
Uptake
NH3 volatilization
Uptake
Anammox
Denitrification

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Figure 8