Dear Dr. Weintraub:

Please find enclosed the revision of our manuscript entitled "Abiotic versus biotic controls on soil nitrogen cycling in drylands along a 3200 km transect" (Manuscript # bg-2016-226).

We would like to extend our grateful thanks to all reviewers and you for the constructive comments and suggestions to our manuscript. In the revised version of this manuscript, 1) we fixed many confusing sentences that the reviewer had pointed out; 2) the discussion section has been rewritten according to the reviewers'. The relevant references have been cited accordingly; and 3) we have sought professional editorial service (Springer Nature Author Services) for a thorough language editing. All changes are highlighted in the submitted manuscript with yellow background. We hope these will satisfy your request and we are looking forward to the expedited publication of this manuscript on Biogeosciences.

Thank you again for handling and editing our manuscript!

Reviewer 1

There are still a number of language errors, and the paper would benefit from an additional close proofread prior to publication, but is otherwise sound

Reply: Thank you for your suggestion. We have found a language company to improve the English writing.

Reviewer 2

There's not a lot to say here. It's an excellent study that seems to have benefited from excellent reviews and editorial management. I mainly point out typos below with a few other minor comments. Also I am often critical of figures but these ones are beautifully done. Overall A+ for all involved in the process, especially study authors.

Reply: Thank you very much for your appreciation of our study. Reviewer 2 really gave us a lot of useful and constructive suggestions, from grammar to logic. The kindly provided suggestions on writing encourage us to improve our current and future work. We really appreciated!

L74. typo: controlling = control

Reply: Accepted.

L79 microbially produced

Reply: Done.

L95 Antarctica

Reply: Done.

L 112: 3200 km

Reply: Done.

L 176. recommend change "pointed out" to "suggest" or "indicate." If using suggest, "could be" can be removed.

Reply: Thank you, we changed 'pointed out' to 'indicated'. Please see line 175.

L 177. Rewriting suggestion: Thereafter, we refer to the areas with MAP from 36 mm to 102 mm (15 sites) and from 142 mm to 436 mm (21 sites) as the arid zone and the semiarid zone, respectively.
Reply: Accepted.

L 182. missing a space after NH4+
Reply: Done.

L 285. cool finding. I might mention this in the abstract. I think part of this is that anammox is so energetically inefficient it will occur really slowly and so might not have a huge impact on fluxes.
Reply: Thanks. But we choose not to include this in the abstract as we focus on nitrification and denitrification induced N losses.

L 295. last sentence here doesn't add to paper. make more specific about what should be done or delete.
Reply: We agreed with you and deleted the sentence.

L 312. yes very good analysis, very interesting. I've seen in mountain systems with rich soils that have lots of organic matter the opposite--almost all NO3 of biological origin except for a big flush of atmospheric N from snowmelt. This contrast in a dryland makes a lot of sense.
Reply: Thanks! We hope this paper on dryland N biogeochemistry may stimulate more discussion on the biotic vs. abiotic controls on N cycling across the globe.

L 319. Another point I might make here is that lack of water means that the highly mobile NO3- ions don't leach into streams and groundwater as much as in more mesic areas.
Reply: Thanks! We changed the sentence to '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 transformation (Fig. 4), that combined with the lack of leaching, would facilitate the preservation of NO_3^-'. Please see line 316-318.

L 342. Sentence not grammatical. If I understand the point of the sentence, I might rewrite the second phrase to say something like "..., the expected d15N value for NH4+ derived from fixation."
Reply: Thanks! We changed the sentence to 'We found that with decreasing precipitation, the $\delta^{15}N$ of bulk soil N decreased to close to zero, which is the expected $\delta^{15}N$ value for NH_4^+ derived from biological N fixation'. Please see line 340-341.

L 344. change "research" to "study"
Reply: Done.

L 367. Effects
Reply: Done.

L 369. Compared
Reply: Done.

L 373. Pools

Reply: Done.

L. 373. "Ammonification (N mineralization) BOTH supplies NH4 + for plant uptake and favourS soil nitrification"
Reply: Accepted.

L 374. enrich THE remaining
Reply: Done.

L 377. I am in favor of deleting the final throwaway line about effects of climate change. A stronger concluding sentence would summarize the key findings of the study, which show that readily interpretable broad-scale patterns of N cycling can be seen across a wide gradient of arid systems.
Reply: After considering the reviewer's recommendation, we choose to keep the global change link but modify the sentence to ‘The precipitation regulation of the abiotic vs. biotic controls on N cycling and N losses suggest that global climate changes would have a great impact on these dryland ecosystems.’ Please see line 373-375.

Reviewer 3
Dear Mike,

I have reviewed the manuscript titled: “Abiotic versus biotic controls on soil nitrogen cycling in drylands along a 3200 km transect.” Overall, I think the manuscript provides useful data and information about processes controlling N cycling—it advances understanding of N cycling. I am confident it will be of use to the scientific community exploring how aridity impacts ecosystem N dynamics.
Reply: Thank you very much for your appreciation of our study.

I think it would be useful for a native English speaker to do one more round of editing. I think the data and scope of the paper are great and this begs for the additional editing work to increase overall clarity.
Reply: we have sought professional editorial service (Springer Nature Author Services) for a thorough language editing. All changes are highlighted in the submitted manuscript with yellow background.

I have a few concerns regarding interpretation of results and highlight below issues that I think require attention before publishing.

L62. I suggest reminding the audience what the authors mean by “open” N cycling.
Reply: Thank you! The sentence changed to ‘suggesting that N cycling is more open (i.e., more input and output relative to internal cycling) in dryland ecosystems compared with mesic ecosystems’. Please see line 61-62.

L63-65. Good example of a sentence that can use editing
Reply: We have changed the sentence to two separate ones. ‘The underlying explanation for openness is when N supply is higher relative to biotic demand, more N is lost through leaching and gaseous N emissions (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}N with increasing N losses (Robinson, 2001)’.

Please see line 62-65 in the modified manuscript'.

L68. I suggest clarifying this sentence as there are drylands in which hydrological N losses are larger than gaseous losses (at least on an annual basis and especially during a wet year).
Reply: We agreed with you and deleted 'instead of hydrological losses'.

L74. "That" can be deleted
Reply: Yes, you are right. To keep a simple sentence, we changed 'controlling' to 'control'.

L130. Perhaps clarify these were frozen. Refrigerator, at least to me, means +4 C.
Reply: It should be +4 C. Please see our corrections in line 129 and 131.

L167. Please specify that SPSS is software.
Reply: Done. Please see line 166.

L175. Figure 4 is out of order because figure 3 has not been mentioned yet.
Reply: Yes, reviewer 2 are technically correct. However, the aim of this paragraph (line 172-177) is to tell the audience why we have the arid and semiarid zone in this study. So, the expression here is just a preface or introduction. Because we would like to keep the current structure in the results section, from soil N concentration, ^{15}N characteristics to the microbial gene abundance, we changed 'fig. 4' to 'see below'. Please see our corrections in line 174.

L179. This sentence repeats the same information in L173
Reply: The sentence in line 179 was deleted.

L200. It would be useful to edit the sentence so that "positive" is not perhaps interpreted as slope.
Reply: The sentence has been modified as 'The relative ^{15}N enrichment of soil NH_4^+ in the arid zone was mostly above zero, while they were below zero in the semiarid zone (Fig. 3a)'. Please see line 197-199.

We also change Line 204 to 'In a similar way, we found that the relative ^{15}N-enrichment of NO_3^- were mostly below zero in the arid zone and above zero in the semiarid zone'. Please see line 204-205.

L208. But the 15N-NO3- could have also been enriched through other processes not involving NO3- losses. For example, during the transformation of NH4+ to NO3-, NO2- could have produced NO, enriching the leftover N oxidized to NO3-.
Reply: True. We will have extensive discussion on that later. For now, we simplified that sentence to 'Accordingly, these results suggest that NO_3^- losses may increase when water becomes more available, and progressively enriched residual soil NO_3^- in ^{15}N'.

L224. But this could have also been produced by NO loss enriching NO2- during nitrification. Can the authors separate between these two processes and be sure of denitrification alone?

Reply: True. Both nitrification and denitrification would promote NO and N_2O loss and also enrich the NO_2^- and NO_3^-. However, the dual isotopic analysis data on $\delta^{18}O$ and $\delta^{15}N$ provided direct evidence for denitrification and NO_3^- loss in the semiarid zone.

We cannot rule out the nitrification contribution based on NO_3^- concentration and its ^{15}N enrichment. Later, in line 235-237, we addressed this possibility. 'Because gaseous N losses occur during both nitrification (see below) and denitrification, the coupled nitrification and denitrification could maintain low soil NO_3^- concentration while enriching the ^{15}N signal'. Further discussion on NO loss is provided in the next two paragraphs.

L232. This is a rough transition because the authors had been discussing denitrification. Nitrification appears suddenly and abruptly.
Reply: Thanks! Rewrite to 'Nitrate can be provided by enhanced microbial processes, including nitrification, when water becomes more available'. Please see line 229-230.

L247-249. To the best of my knowledge, the abiotic reduction of NO3- to NO2- (i.e., the "ferrous wheel hypothesis") has not been confirmed within the context of "natural" soils—if it has, please provide references. Therefore, I suggest minimizing speculation and concentrating on NO2- rather than NO3-; NO2- can react abiotically to produce NO (Heil, J., Vereecken, H., Bruggemann, N., 2016. A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil. European Journal of Soil Science 67, 23-39). Perhaps a mechanism for this NO3- reduction may involve coupled biotic-abiotic processes. I think linking these two papers together might work very well in this manuscript:
Roco, C.A., Bergaust, L.L., Shapleigh, J.P., Yavitt, J.B., 2016. Reduction of nitrate to nitrite by microbes under oxic conditions. Soil Biology & Biochemistry 100, 1-8.
Homyak, P.M., Kamiyama, M., Sickman, J.O., Schimel, J.P., 2016. Acidity and organic matter promote abiotic nitric oxide production in drying soils. Global Change Biology 10.1111/gcb.13507.

By linking these papers it may be possible to explain the increasing δ15N-NO3- in the arid zone as precipitation increases. In extremely arid regions the NO3- signature is dominated by atmospheric deposition but as soils become wetter, the biological signal begins to enrich δ15N-NO3-.

Reply: No, we did not intend to apply the "ferrous wheel hypothesis", which was proposed by Davidson et al. to address the 'rapid' fixation of labeled NO_3^- into soil organic matter (the reduction of NO_3^- by Fe (II) to form NO_2^-, then chemically react with soil organic matter).

We have now modified writing to focus more on NO_2^-. 'Chemodenitrification is an abiotic process, in which the reduction of 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)'. Please see line 244-246.

The Heil et al. review provided several possible coupled biotic-abiotic processes that could affect fates of soil inorganic N and their isotopic signals, which has been cited in this revision, thanks! Please see line 246-248. The Roco et al. paper provided an important mechanism explaining NO_3^- reduction to NO_2^-, which is also cited in the revision. Please see line 249-251.

We have already cited a related Homyak et al. paper. The mechanisms involved in N loss in arid ecosystems can be extremely diverse and complicated and their significances on N budget under field conditions are often poorly known (Heil et al.). Our paper presented a large scale spatial pattern (over a 3200 km transect in China) of inorganic N concentration and related $^{15}NH_4^+$ and $^{15}NO_3^-$ enrichment. The exact mechanisms beyond the patterns are hard to pinpoint and we do not intend to over speculate in the discussion.

L 267. Please clarify: "while simultaneously NH4+ was also consumed." Why would a gradual depletion in 15N-NH4+ suggest consumption? This is opposite to our understanding.
Reply: Deleted.

L 266-284. I have problems understanding how the decrease in 15N-NH4+ as precipitation increases can be explained by plant consumption. I agree that if N is limiting then isotope discrimination would be low (i.e., both 14 and 15 N would be taken up, say at the same rate). However, to cause a decrease in δ15N-NH4+ as precipitation increases you must consume more 15N than 14N or inject more 14N into the system. That mineralization caused fractionation sounds more plausible to me, but this is not well stated and it is confusing. It is critical to the paper to address this well. The shape of figure 3a is controlled by both 15N-NH4+ and bulk soil 15N. I suggest the authors systematically how 15N-NH4+ changes relative to the bulk soil. Doing so would help clarify against figure 2e.
Reply: The focus of discussion here is on NH_4^+ consumptions. So, we removed the first sentence to avoid confusing.
We agree with the reviewer's assessment that enhanced N mineralization contributed to the 'dilution' of heavy isotope N. We have talked about this viewpoint in line 344-355.

L300. It would be useful to remind the reader that it is one O from air and two O's from water. The ratio somewhat implies it but it is not clear.
Reply: The sentence has been modified as 'If NO_3^- is formed by nitrification, NO_3^- will contain one O atom from soil O_2 and two O atoms from H_2O'.

L315-316. Since figure 5a does not show elevated NO3- concentrations, I think it would be useful to clarify that the concentrations are high because these measurements were made in arid regions. Is this right?
Reply: Yes, you are right. The sentence has been modified as 'As shown in Figure 5a, a pronounced trend (green arrow) toward higher $\delta^{18}O$ and lower $\delta^{15}N$ values is obvious for elevated NO_3^- concentrations found in the arid zone soils, which might be the result of mixed NO_3^- from both soil nitrification and atmospheric deposition'. Please see line 313-315.

L325. For the definition of heterotrophic nitrification to work better, it is best to modify this sentence so that the definition is located in the middle of the sentence—as a reminder—rather than as a definition. By starting the sentence with the definition it implies it is a new term not well known by readers of the journal.

Reply: Rewrite as 'Heterotrophic nitrification, a process that oxidizes organic N to NO_3^-, bypasses NH_4^+'. Please see line 324.

L340-345. The argument about biological soil crusts (BSC) needs to be better developed. Supposedly BSCs contribute to NH4+ inputs as precipitation decreases. However, figure 4 shows the complete opposite: that BSC gene abundance increases in wetter regions and are less important as precipitation decreases. Please elaborate. And this becomes a problem because the authors use the gene abundance data to suggest that nitrification is not likely to dominate N cycling in arid regions. So for nitrification the data are true but somehow for BSCs we are asked to not believe it.

Also, I am confused as to what is meant by "notice that biological N fixation provided NH4+ with the δ15N value around zero." Is the reader supposed to be looking at data showing rates of N fixation in relation to δ15N-NH4+? If so, I don't see those data in the manuscript.
Reply: We believe that reviewer is right here; we should not overplay the significance of BSCs, which we did not directly measured. We have also fixed that confusing sentence.

Changed to: '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. We found with decreasing precipitation, the $\delta^{15}N$ of bulk soil N decreased to close to zero, the expected $\delta^{15}N$ value for NH_4^+ derived from biological N fixation. 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)'. Please see line 340-343.

L359. I think the authors have a nice study and great dataset such that they do not need to justify its importance by saying they were first to make this measurement—that is not too useful. What is useful is what we have learned from it.
Reply: Thank you. Agree and changed to 'Our study reported 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'.

L366. Perhaps say "15N-enriched" instead.
Reply: Accepted.

L600. Figure 1 legend: there is an extra "and"
Reply: We deleted second "and", and started another sentence, 'The dominant plant genera change gradually from shrub ... '.

L609. Figure 3 legend: the first two sentences are identical and can be condensed.
Reply: The first sentence is the figure title, and the second is the explanation for the data calculation. Now, we changed the second one, which started as 'Data in the figures were calculated as…'.

Figure 8. I suggest adding N losses via NO during the transformation of NH4+ to NO3- in both the arid and semiarid zone. The legend of figure 8 requires this information be present. If the authors wish to omit these processes from figure 8, they

must rewrite the legend to be specific as to why they are excluded. The authors are showing several processes removing N from the system (NH3 volatilization, annamox, denitrification) and by that logic NO losses should be identified during the transformation of NH4+ to NO3- . Or at least acknowledge that nitrification can produce losses

Reply: The purpose of Fig. 8 is to contrast abiotic processes (deposition input, volatilization) against biological processes (e.g., nitrification and denitrification), and their different contributions in the arid and semiarid conditions. The loss of NO and N_2O is part of the nitrification and denitrification processes. The losses of N trace gases also often involve coupled biotic-abiotic interactions, as had been extensively discussed in the paper. Consequently, we decide not to include them in the figure but do change figure legend to remind readers the significance of these pathways. Please see our modification in line 636-637.

A suggestion to increase overall clarity: I suggest ending paragraphs with a synthetic "take-home" message summarizing and concluding the paragraph. Ending paragraphs with citations, reinforcing information already known, does not help highlight how this paper is advancing our knowledge.

Reply: We appreciated your useful suggestion! We will go over the entire manuscript, in particular the Discussion section, to see whether such changes are necessary and would improve the overall reading of the paper. Some changes have already been made according to the suggestion of reviewer #2. Thanks!

Respectively,

D. Liu, W. Zhu, and Y. Fang, on behalf of all co-authors.

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 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 (Huntington, 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 ^{15}N natural abundance of (expressed as $\delta^{15}N$) provides 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 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., more input and output relative to internal cycling) in dryland ecosystems compared with mesic ecosystems. The underlying explanation for openness is when the N supply is higher relative to biotic demand, more N is lost through leaching and gaseous N emissions (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}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}N in total N is 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.

Ammonium (NH_4^+) and nitrate (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 NH_4^+ and NO_3^-. 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). 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, the $\delta^{18}O$ in NO_3^- has been used to partition microbially produced NO_3^- from atmospheric sources because microbial and atmospheric sources 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 available soil 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 newly developed methods (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, compared with the $\delta^{15}N$ 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 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; 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 Antarctica, for example, are associated with increased soil temperatures, and N_2-fixation genes are associated with the presence of lichens (Yergeau et al., 2007). Research along an elevation gradient noted that some denitrification genes (*nir*S and *nos*Z) are more abundant at higher elevations, with nitrification as the major process of nitrous oxides (N_2O) emission in the Tibetan grassland (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 $\delta^{15}N$ 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 vs. plant* controls (Wang et al., 2014). Here, we further analyzed the concentrations and N isotopic compositions of soil NH_4^+ and NO_3^- (as well as oxygen (O) isotopes for NO_3^-) and the abundance of microbial genes associated with soil N transformation. The principal objectives of this study were to examine (1) the patterns of concentrations and $\delta^{15}N$ values for soil NH_4^+ and NO_3^-; (2) the patterns of gene abundance associated with microbially regulated soil processes; (3) and 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 87.4ºE 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 along the transect, the mean annual precipitation (MAP) increased from 36 mm to 436 mm, the mean annual temperature (MAT) decreased from 9.9 ºC to -1.8 ºC (Fig. S1), and the 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 2012, the peak of the plant growing season. This location 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). In each site, we set a 50 m × 50 m plot and five 1 m × 1 m subplots at the four corners and the center of the plot. In each subplot, twenty random mineral soil samples were collected using soil cores (2.5 cm diameter × 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 ºC during the sampling trip. The second portion was placed in a sterile plastic bag and immediately stored at -40 ºC for later DNA extraction. The third portion 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 natural abundance of ^{15}N 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 the KCl extracts were analysed using conventional colorimetric methods (Liu et al., 1996). Ammonium concentrations were determined using the indophenol blue method, and nitrate by sulfanilamide-NAD reactionfollowing cadmium (Cd) reduction.

The analyses of the 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 the heavy isotope to the light isotope for N or O, in units per mil, ‰), were based on the isotopic analysis of 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). To correct for machine drift and blank over the isotopic analyses, 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 a calibration curve between the measured and expected isotope values. The isotopic signatures of the 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 greater 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 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 A260/280 and A260/230 ratios using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE), and final soil DNA concentrations were quantified with PicoGreen using a FLUOstar 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 brief, the purified DNA samples (0.6 μg) were used for hybridization, and were 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 a photomultiplier tube gain of 75%, respectively. 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., 2010a; He et al., 2007).

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-samples 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 NO_3^- and NH_4^+ concentrations

We found significant inorganic N accumulation in the investigated soil layer (0-10 cm) in sites with a MAP less than 100 mm ($P < 0.01$; Figs. 2b and c). Furthermore, the abundance of microbial genes associated with soil N transformations was significantly reduced compared with that in sites with a MAP of 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 mm to 102 mm (15 sites) and from 142 mm to 436 mm (21 sites) as arid zone and semiarid zone, respectively.

In the arid zone, NO_3^- concentrations were highly variable and reached 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 -less than 5 mg N kg^{-1} in most samples. Soil NH_4^+ concentrations exhibited 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, 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 relatively greater in dry areas compared with less dry areas.

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

The $\delta^{15}N$ values for 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 $\delta^{15}N$ value for 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 $\delta^{15}N$ for soil NO_3^-, the $\delta^{15}N$ value for 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 $\delta^{15}N$ of 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 NH_4^+ and 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 NH_4^+, respectively). Therefore, we also calculated the relative ^{15}N enrichment of soil NH_4^+ (the difference between the $\delta^{15}N$ of NH_4^+ and bulk soil N) and NO_3^- (the difference between the $\delta^{15}N$ 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 above zero, whereas its value was below zero in the semiarid zone (Fig. 3a). A negative correlation was noted 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 the notion that net NH_4^+ losses occurred mainly in the arid zone, whereas 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 reduce the relative ^{15}N-enrichment of soil NH_4^+. In a similar manner, we found that the relative ^{15}N-enrichment of 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 ^{15}N-enrichment of soil NO_3^- in both the arid and semiarid zone (Fig. 3b). Accordingly, these results suggest that NO_3^- losses increase when water becomes more available, and the residual soil NO_3^- progressively becomes enriched 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 at all sites. In arid zone soils, the abundances of all N cycling groups genes were extremely low (Fig .4), indicating limited microbial potentials in very dry environment. A sharp increase (by 8- to 9-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.

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 plant and microbial uptake. The close correlation between the 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 both O and N in NO_3^- (Granger et al., 2008). This type 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 NO_3^- losses. Denitrification is regulated by proximal factors, such as NO_3^- concentration and O_2 concentration that immediately affect denitrifying communities (Saggar et al., 2013). Nitrate can be provided by 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 O_2, consequently favoring 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 favorable with increasing precipitation. In addition, in our preliminary study, a ^{15}N-labeled NO_3^- incubation experiment revealed that potential 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 NO_3^- concentration while enriching the ^{15}N signal. These results support the idea that gaseous N losses increase 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 (Figs. 2f and 3b), indicating that denitrification may also occur. However, in these arid soils, microbial gene abundances were considerably reduced (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 cause soil NO_3^- losses in the arid zone. Chemodenitrification is an abiotic process in which the reduction of 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). In a recent review, Heil et al. (2016) discussed several abiotic reactions involving NO_2^-, including the self-decomposition of NO_2^-, reactions of NO_2^- with reduced metal cations, nitrosation of soil organic matter (SOM) by NO_2^-, and the reaction between NO_2^- and NH_2OH. Ample soil 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 NO_3^- to NO_2^-, might be much more common under aerobic conditions than commonly realized, occurring in diverse bacteria groups and having 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 biogeochemistry in dryland ecosystems.

In contrast to the $\delta^{15}N$ of soil NO_3^-, the $\delta^{15}N$ values of soil NH_4^+ and their relative ^{15}N enrichment were increased in the arid zone compared with the semiarid zone (Figs. 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 to 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. 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 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 (Heil et al., 2016; Homyak et al., 2016).

In the semiarid zone, 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 NH_3 volatilization (Yahdjian and Sala, 2010), however, a recent study observed the opposite result in a semiarid subtropical savanna (Soper et al., 2016). The increasing available water would also stimulate biological N consumption by plants and microbes. The increased aboveground biomass with increasing MAP suggests an increased net plant N accumulation along this precipitation gradient (Wang et al., 2014). Given that the soil NH_4^+ concentration was greater than that of the 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 notion is in accordance with the observed relationship between the $\delta^{15}N$ values of plant leaves (non-N fixing species) and soil NH_4^+ (R^2=0.40; Fig. 7a), but not soil NO_3^- (Fig. 7b). When we plotted this correlation for each plant species, three dominant species (*Stipa* spp., *Cleistogenes* spp., and *Reaumuria* spp.) were significantly correlated with soil NH_4^+. In addition, internal plant N cycling likely shifts as a function of water availability and influences foliar $\delta^{15}N$, but the extent of this relationship is difficult to estimate at this stage. Plant N 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 the lack of strong ^{15}N enrichment of soil NH_4^+ with increasing precipitation. The consumption of NH_4^+ in 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}N enrichment of soil NO_3^-, without significantly altering the 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^-, and N_2 is 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 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 transect 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 affect ^{15}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 NO_3^- and NH_4^+

We observed much higher concentrations of soil NO_3^- in the arid zone (Fig. 2c); on average, they were approximately 20-fold 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 one O atom from soil O_2 and two O atoms from H_2O (Kendall et al., 2007). The $\delta^{18}O$ value of atmospheric O_2 is relatively stable (23.5‰; we assume that the isotopic composition of O_2 in the atmosphere and soils are the same). The $\delta^{18}O$ value of nitrified NO_3^- depends 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), yielding corresponding $\delta^{18}O$ values of nitrified NO_3^- ranging 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 the calculated and measured $\delta^{18}O$ values provides evidence for the minor importance of nitrification. According to previous studies, the higher $\delta^{18}O$ values of soil NO_3^- we observed in the arid zone have rarely been reported for nitrified NO_3^- (Kendall et al., 2007). For example, an *in situ* study conducted on the forest floor soils found that the $\delta^{18}O$ 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 is from atmospheric deposition. Nitrate accumulates on the surface soil when experiencing prolonged droughts, as also 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 Figure 5a, a pronounced trend (green arrow) toward higher $\delta^{18}O$ and lower $\delta^{15}N$ values is obvious for elevated NO_3^- concentrations found in the arid zone soils, which might be the result of mixed NO_3^- from both soil nitrification and atmospheric deposition. A similar results was observed in groundwater of the Saharan Desert (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), that combined with the lack of leaching, would facilitate the preservation of soil NO_3^-.

In the semiarid zone, the $\delta^{18}O$ values of soil NO_3^- were low (0.9-21.0‰), indicating reduced atmospheric contribution. The deposited NO_3^- will experience postdepositional microbial processes, and the original signature of $\delta^{18}O$ will vanish after biological processes occur (Qin et al., 2012). With increasing MAP, nitrification would progressively provide more NO_3^- with lower $\delta^{18}O$ values. The calculated $\delta^{18}O$ values of NO_3^- from nitrification ranged 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, a process that oxidizes organic N to NO_3^-, bypasses NH_4^+. If this process is 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 has been recognized recently in grasslands (Müller et al., 2014; Müller et al., 2004) and forests (Zhang et al., 2014).

Ammonium accumulation was noted in the arid zone soils and the accumulated NH_4^+ was characterized by increased ^{15}N enrichment (Figs. 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 in arid climates (Elliott et al., 2009). The $\delta^{15}N$ values of NH_4^+ and NO_3^- in dry deposition were increased compared with 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 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 $\delta^{15}N$ of NH_4^+ in suspended particulate matter ranged from 1.3 to 38.5‰ with an average of 11.6‰. It remains unclear why the $\delta^{15}N$ 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 creates 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 the soil NH_4^+ pool and soil organic N. We found that with decreasing precipitation, the $\delta^{15}N$ of bulk soil N decreased to close to zero, which is the expected $\delta^{15}N$ value for NH_4^+ derived from biological N fixation. BSCs were 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 NH_4^+ was depleted in ^{15}N relative to bulk soil N, and their differences in $\delta^{15}N$ increased with increasing MAP, suggesting the input of NH_4^+ (e.g., soil ammonification, N deposition). The increasing precipitation was closely correlated to the microbial gene abundance associated with N transformations (Fig. 3). The $\delta^{15}N$ of bulk soil N was quite stable in the semiarid zone, about 5‰ (Fig. 2d). An increase in N mineralization as precipitation increases would bring in more $^{14}NH_4^+$ and progressively lower the $\delta^{15}N$ of soil NH_4^+ (Fig. 2e). The isotope effect of N mineralization might also be higher than commonly expected. Our laboratory recently reported that ^{15}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 increasing water availability in the semiarid zone (MAP > 200 mm), N turnover linking the biological uptake (plant and microbes) and return of N could further enhance soil ammonification, which results in lower $\delta^{15}N$ in soil NH_4^+. In addition, there is also a possibility of dissimilatory nitrate 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 NH_4^+.

5 Summary

Our study reported 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, such as soil pH and moisture, and functional gene abundance, the compound-specific $\delta^{15}N$ analyses presented here demonstrate a clearly 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, characterized by extreme aridity (36 mm < MAP < 100 mm; Fig. 8a), plant cover is sparse, and microbial activity is limited (Figs. 1 and 4). Nitrogen input, mostly in the form of atmospheric deposition, largely accumulates, creating "^{15}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 NH_3 volatilization, causing soil NH_4^+ enriched in ^{15}N. The very high yet variable accumulation of NO_3^- in soil compared with 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 considerably greater when water became more available (Fig. 3). Increasing N mineralization with increasing MAP was accompanied by reduced NH_3 volatilization due to lower pH, producing soil NH_4^+ pools with lighter N isotopes. Ammonification (N mineralization) both supplies NH_4^+ for plant uptake and favors soil nitrification. Both nitrification and denitrification could lead to N loss and isotopically enrich the remaining soil N. Soil heterogeneity and pulse precipitation events could provide hotspots for these microbial processes, whereas increased plant cover and N uptake could reduce the soil NH_4^+ and NO_3^- pools and minimize overall N losses. The precipitation regulation of the abiotic vs. biotic controls on N cycling and N losses suggest that global climate changes would have a 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.

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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). 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^-. Data in the figures 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).

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. Notice during both nitrification and denitrification, N trace gases NO and N_2O can be produced and escape the system ('hole-in-the-pipe' model, not shown in the figure), affecting both NH_4^+ and NO_3^- concentrations and their $\delta^{15}N$ values.

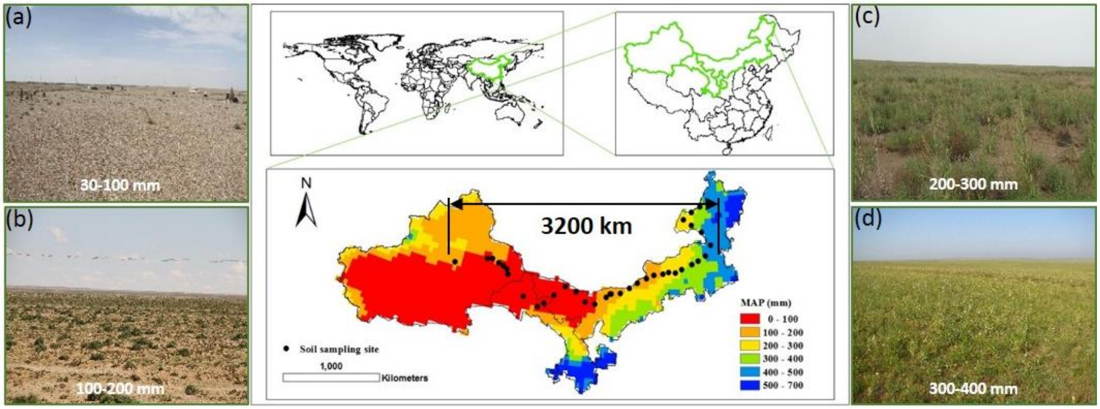

(a)
30-100 mm
(b)
100-200 mm
N
3200 km
Soil sampling site
1,000
Kilometers
MAP (mm)
0 - 100
100 - 200
200 - 300
300 - 400
400 - 500
500 - 700
(c)
200-300 mm
(d)
300-400 mm

Figure 1

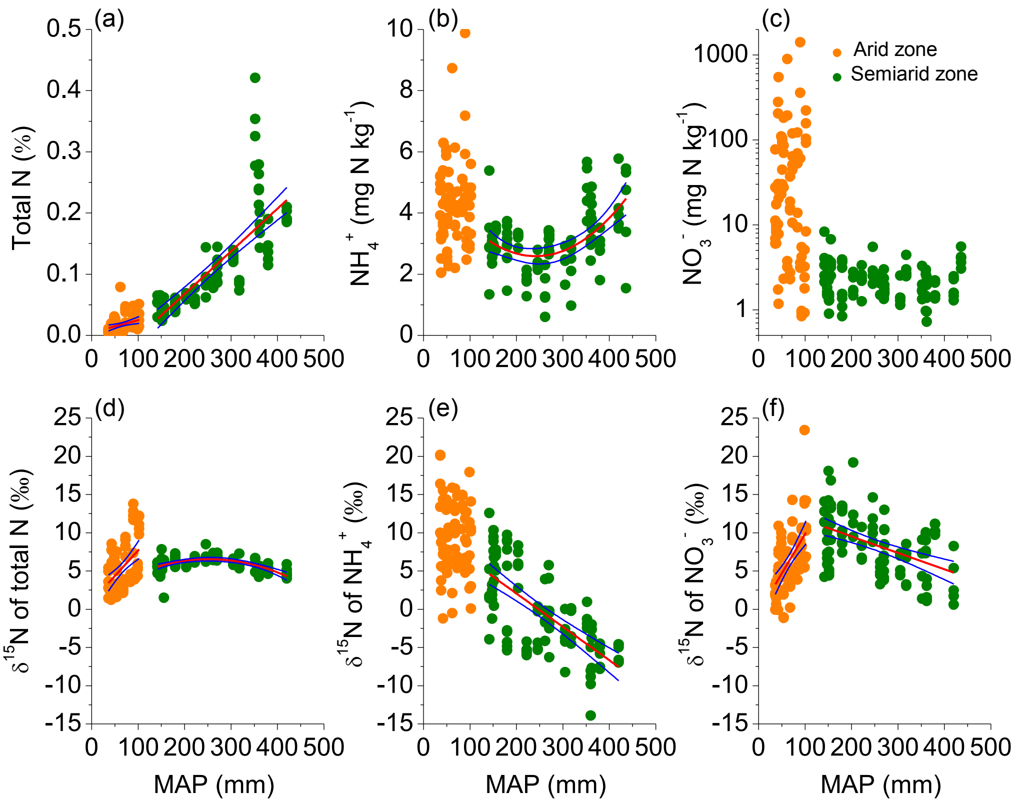

(a)
(b)
(c)
(d)
(e)
(f)
Arid zone
Semiarid zone
Total N (%)
NH4+ (mg N kg-1)
NO3- (mg N kg-1)
δ15N of total N (‰)
δ15N of NH4+ (‰)
δ15N of NO3- (‰)
MAP (mm)
MAP (mm)
MAP (mm)

Figure 2

645

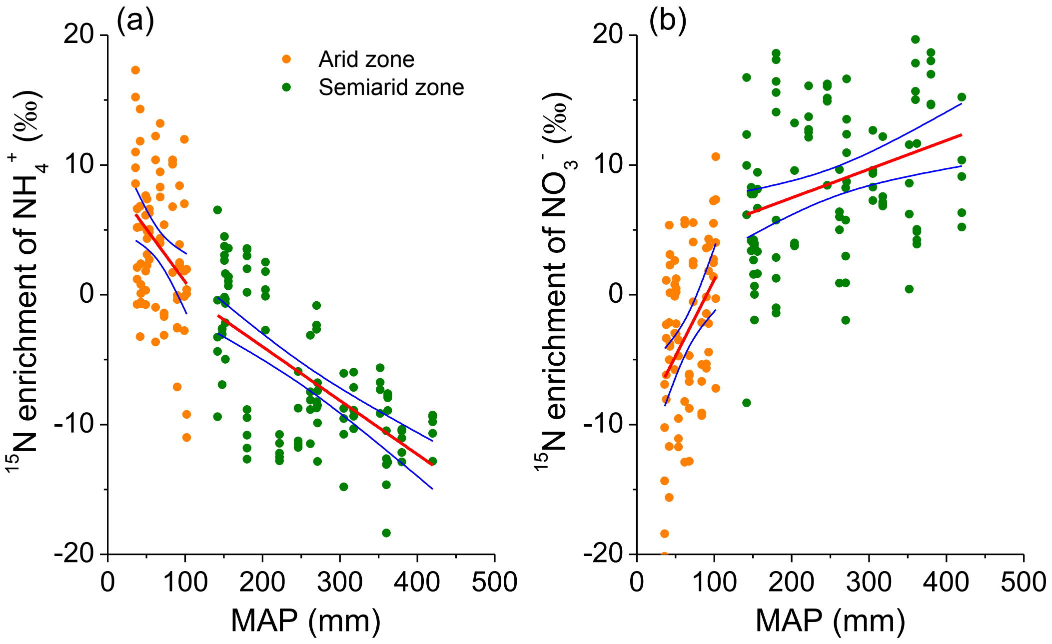
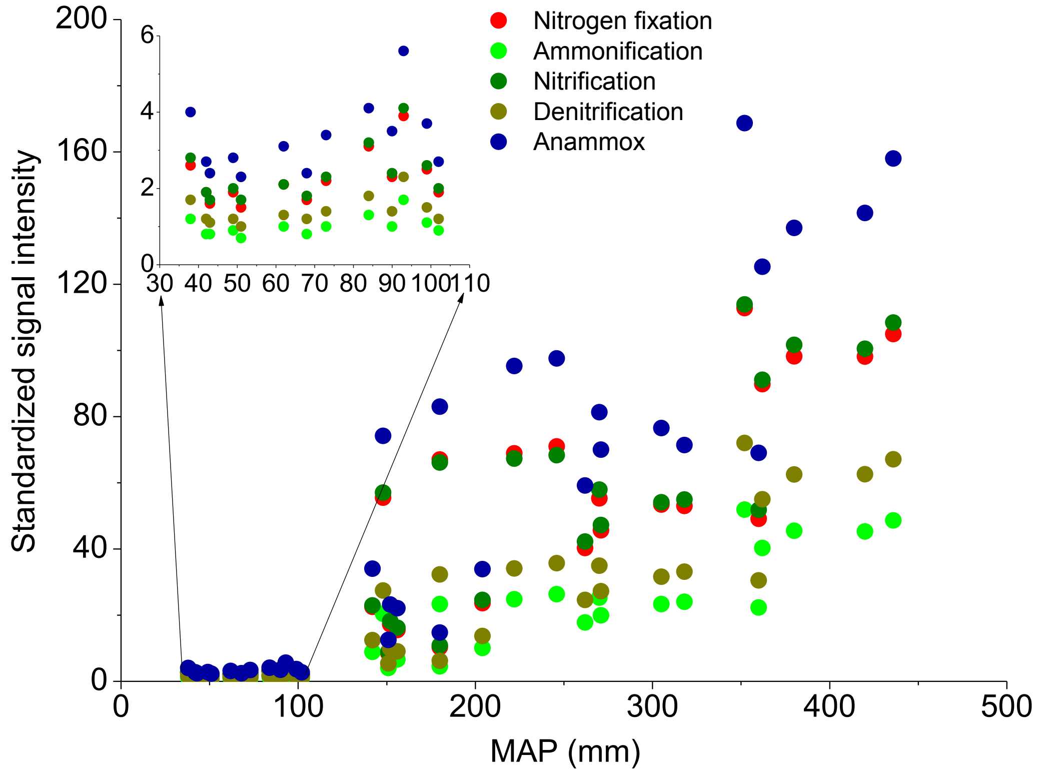
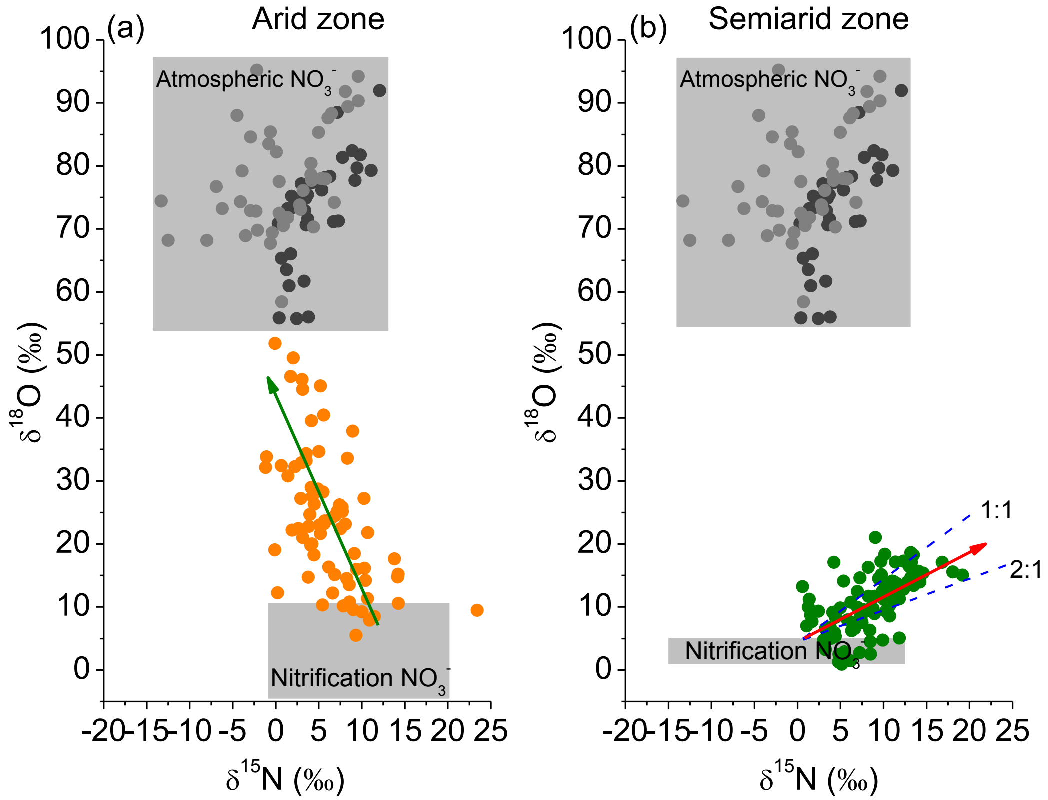

(a)
(b)
Arid zone
Semiarid zone
^{15}N enrichment of NH_4^+ (‰)
^{15}N enrichment of NO_3^- (‰)
20
10
0
-10
-20
0
100
200
300
400
500
MAP (mm)

Figure 3



Standardized signal intensity
MAP (mm)
Nitrogen fixation
Ammonification
Nitrification
Denitrification
Anammox
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

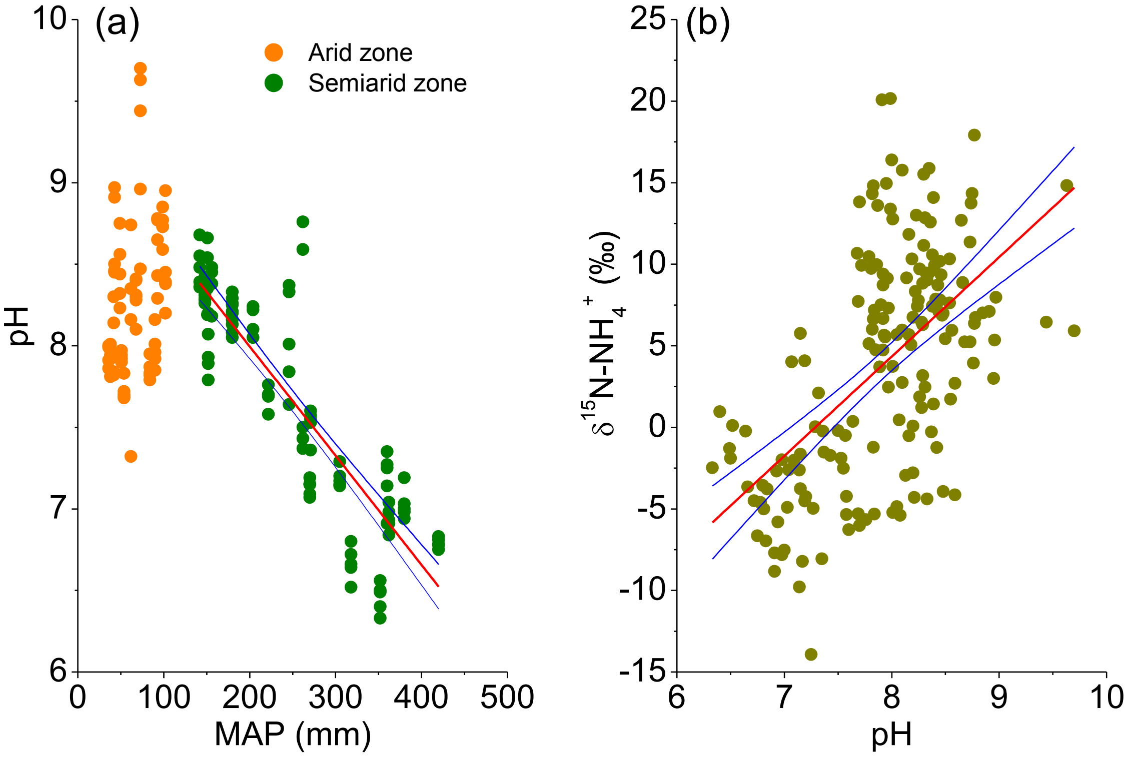
650 **Figure 4**



(a)
Arid zone
Atmospheric NO3-
Nitrification NO3-
δ18O (‰)
δ15N (‰)
(b)
Semiarid zone
Atmospheric NO3-
Nitrification NO3-
1:1
2:1
δ18O (‰)
δ15N (‰)

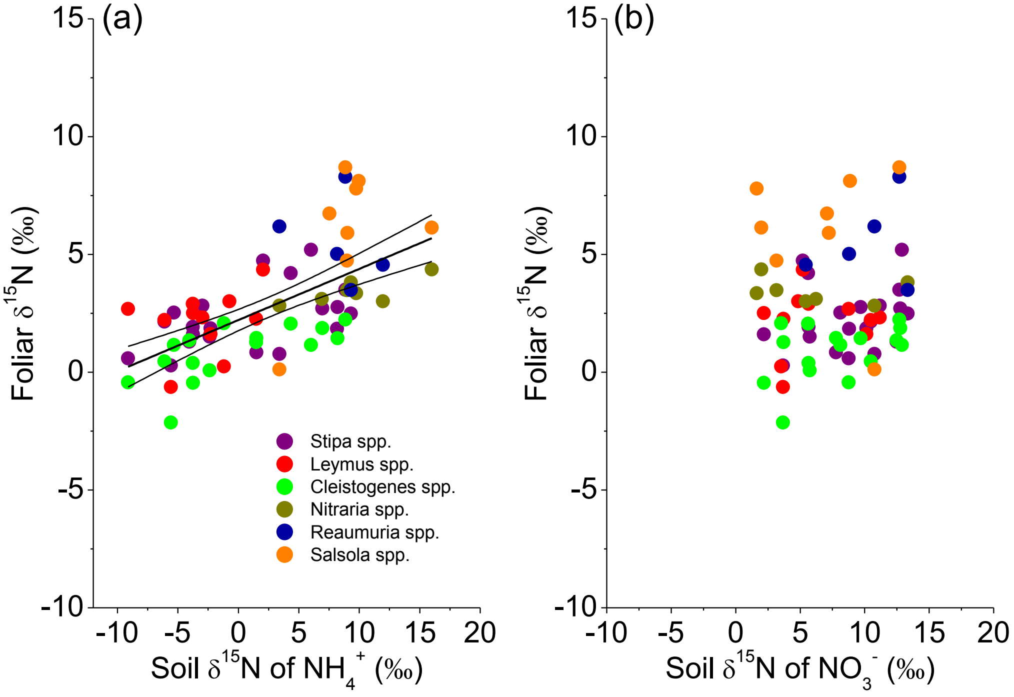
Figure 5

655



(a)
Arid zone
Semiarid zone
pH
MAP (mm)
10
9
8
7
6
0
100
200
300
400
500
(b)
$\delta^{15}N\text{-}NH_4^+$ (‰)
pH
25
20
15
10
5
0
-5
-10
-15
6
7
8
9
10

Figure 6



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

660

Figure 7

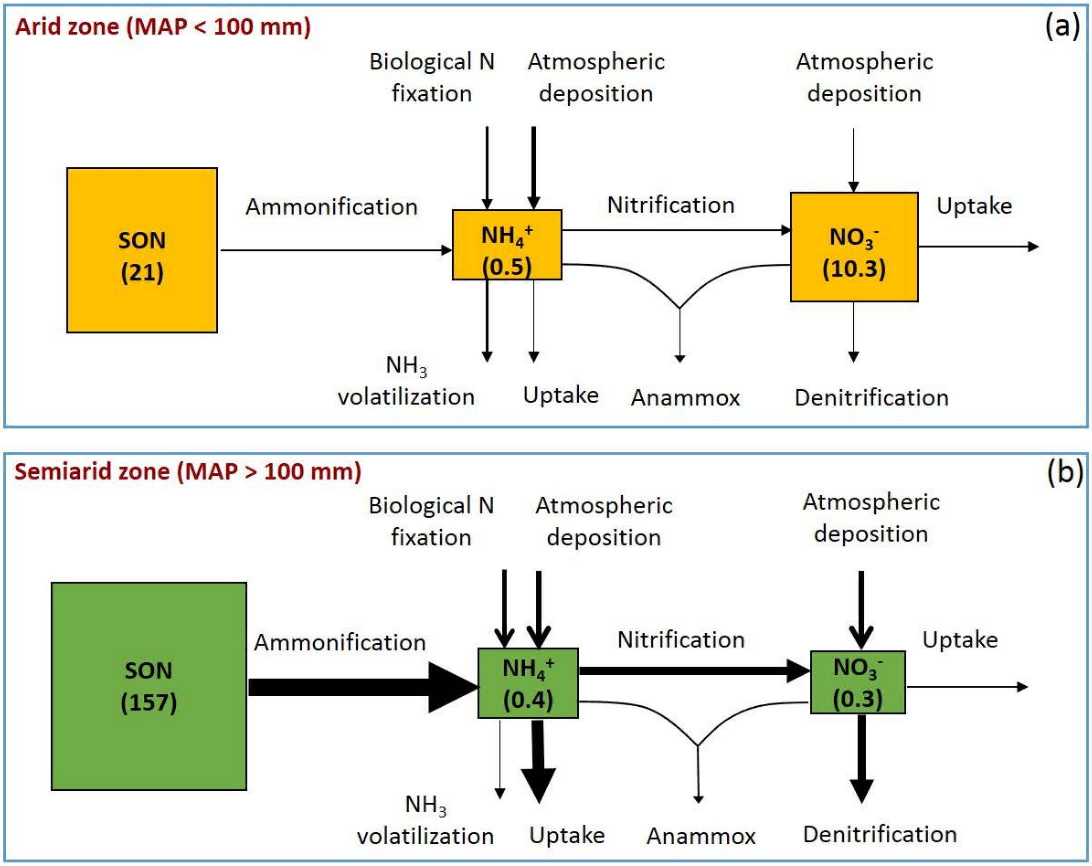

Arid zone (MAP < 100 mm)
(a)
Biological N fixation
Atmospheric deposition
Atmospheric deposition
SON (21)
Ammonification
NH_4^+ (0.5)
Nitrification
NO_3^- (10.3)
Uptake
NH_3 volatilization
Uptake
Anammox
Denitrification
Semiarid zone (MAP > 100 mm)
(b)
Biological N fixation
Atmospheric deposition
Atmospheric deposition
SON (157)
Ammonification
NH_4^+ (0.4)
Nitrification
NO_3^- (0.3)
Uptake
NH_3 volatilization
Uptake
Anammox
Denitrification

Figure 8