

Dear Dr. Michael 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 reviewers for his/her constructive comments and suggestions to our manuscript. The kind corrections on writing encourage us to improve our current and future work. In the revised version of this manuscript, 1) we rethought our data carefully and fixed many confusing sentences that the reviewer had pointed out; 2) the discussion section has been rewritten according to the reviewers’ and your comments. The relevant references have been cited accordingly; and 3) we have also made further efforts to improve the English writing by asking a native speaker to proof our revised manuscript before the resubmission.

Please find our line-by-line response to the review comments below. In addition, we have highlighted the changes in the marked-up manuscript with yellow background.

Thank you again for handling and editing our manuscript!

Respectively,

Yunting Fang, on behalf of all co-authors

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## Response to reviewer #1

### General comments

Overall, this manuscript provides an insightful data set that I believe will be of interest to anyone interested in aridland biogeochemistry. The large geographic scale and compound-specific isotopic analysis are especially valuable and the conclusions reached seem valid. Generally, the discussion of the mechanisms driving the observed patterns is thorough. There are some issues that need addressing, primarily in the discussion where several important processes have not been raised (mainly NO production), and there are several mechanisms that do not make sense (perhaps partly as a result of unclear English). This section would benefit from revision. Generally, the figures are clear and informative. Editing for English language is necessary prior to publication.

Reply: Thank you very much for your appreciation of our study. The comments from the reviewer are really helpful. In the revised version of the manuscript, we have rewritten part of the discussion section as suggested by the reviewer. We have also improved general writing by asking a native speaker (Ben Eisenkop from Binghamton University) to carefully edit our revised manuscript before the submission.

### Specific comments:

1) Methods: Is it just a coincidence that there is a gap in sampling sites around 100 mm MAP?

Reply: You are correct that it is just a coincidence that a gap of MAP seems appear around 100 mm. Our sampling sites were well-distributed at the distance of about 100 km between two adjacent sites. The threshold happened between site #15 (MAP = 102 mm) and site #16 (MAP = 142 mm). Please see Figure 1 in the main text. The ‘unintended gap’ serves nicely for us to break the entire gradient into 1) arid zone and 2) semiarid zone for data synthesis and discussion.

2) Discussion: What about loss of NO during nitrification? NO can be the dominant trace gas emitted from arid soils, and would explain loss of ammonium without subsequent appearance of nitrate. For process see Firestone & Davidson (1989) For arid land NO production see Homyak et al (2016, PNAS) and Soper et al (2016, Global Biogeochemical Cycles). This process belongs on Figure 8! A discussion of the isotopic consequences of this process should also be included.

Reply: Thank you for your suggestion. We agree with you that NO emission during N transformation is really important in arid soils. In the revised manuscript, we have added details about this NO losses in the section of ‘The losses of nitrate and ammonium’. Please see line 250-252 and line 260-265.

In Figure 8, we tried to compare and contrast the major soil N pools and N transformations (ammonification, nitrification, denitrification, among others) between mainly abiotic driven arid zone soils and biotic dominated semi-arid

zone soils. Specific losses of N, including NO, N<sub>2</sub>O, and N<sub>2</sub>, are not shown; instead, were discussed in the text.

3)The foliar <sup>15</sup>N reflecting <sup>15</sup>N of NH<sub>4</sub><sup>+</sup> in the soil- this could also reflect shifting plant physiology across the significant precipitation gradient, rather than just plant source preference for ammonium. Many aspects of plant internal N cycling likely shift as a function of water availability and would influence foliar <sup>15</sup>N. This should at least be acknowledged as an alternative explanation. Also, ammonium shows a larger range of isotopic values along the transect than nitrate, making it less likely that plant <sup>15</sup>N would correlate with nitrate <sup>15</sup>N anyway.

Reply: Thank you for your suggestion. In the revised manuscript, we have added plant physiology as an alternative reason for the changing pattern of foliar δ<sup>15</sup>N. Please see line 277-278.

Seen from the data of foliar <sup>15</sup>N and inorganic <sup>15</sup>N, foliar <sup>15</sup>N range (-2.1~8.7‰) was basically smaller than that of ammonium (-9~16‰) and nitrate (1.6~13.3‰). Significant <sup>15</sup>N range for soil NO<sub>3</sub><sup>-</sup> did exist but did not correlate to the plant <sup>15</sup>N.

4) Deposition- I think you need to be clear about the difference between wet versus dry deposition (with different isotopic signatures) and how you might expect them to change along the transect. Are there any measures of deposition anywhere on the transect you could mention?

Reply: Good point. In our companion work along this transect (Wang et al. 2014), we reported that rates of bulk N deposition (wet + dry) were increasing from west to east along this transect; data were estimated from a published paper (Lelieveld and Dentener 2000).

We expect that dry deposition could be higher than wet deposition in the arid zone soils, and that in the semiarid zone soils the contribution of wet deposition will increase significantly. In previous studies, higher δ<sup>15</sup>N values in dry deposition than in wet deposition had been reported for nitrate (e.g., by 1 to 3 permil in the northeastern US) (Garten 1996, Elliott et al. 2009) and ammonium (up to 33 permil) (Heaton et al. 1997). We have incorporated those points in the revised manuscript, and please see line 331-339.

Line 225- nitrate could also be removed from the soil by biological uptake. This also has potential to be a fractionating process (although evidence for fractionation by directly by plants under field conditions is limited, mycorrhizal fractionation is likely). You posit later that plant uptake of nitrate is low, but this may not necessarily be the case.

Reply: Thank you. We have incorporated uptake of NO<sub>3</sub><sup>-</sup> via plant and microbes in the revision. Please see line 222-223.

This study area is highly N-limited according to previous N manipulation experiment. Plant would take in both <sup>15</sup>N and <sup>14</sup>N in N limited areas (Craine et al. 2015). So, the fractionation effect during the plant N uptake could be low. Please see our statement in line 278-279.

Line 235- increasing compared to what? This is important.

Reply: The sentence has been modified as 'In addition, our preliminary study of a  $^{15}\text{N}$ -labeled  $\text{NO}_3^-$  incubation experiment showed that potential  $\text{N}_2$  losses via denitrification were also increasing with increasing precipitation in the semiarid soils (Liu and Fang, unpublished data)'. Please see line 236-238.

Line 244- There are several potential mechanisms for chemodenitrification (see again Homyak 2016).

Reply: Thank you. We have further discussed chemodenitrification based on the references provided. Please see line 260-265.

Line 255- Soper et al (2016, Global Biogeochemical Cycles) did find increased  $\text{NH}_3$  flux with wetting in an arid system.

Reply: Soper et al. (2016) reported increased  $\text{NH}_3$  flux 24 hours after a 15 mm artificial rain in soils with  $\text{pH} \sim 7.1$ , likely due to the stimulation of  $\text{NH}_3$  production (ammonification) followed by  $\text{NH}_3$  volatilization. However, the dominant post-rain N loss was still NO loss in that study, likely due to the enhanced nitrification, as this reviewer had emphasized. In our transect within the semi-arid zone spanning a precipitation gradient from 140 mm to 436 mm, and pH decrease from 8.6 to 6.7, plus associated vegetation change and plant  $\text{NH}_4^+/\text{NO}_3^-$  uptake, we believe the dominant drivers on soil  $\text{NH}_4^+$  consumption are 1) plant uptake, and 2) nitrification. Nevertheless, the study from Soper et al. (2016) is interesting for our understanding of dryland N loss and has been cited in our revision. Thank you! Please see line 270.

Line 256- "First, plant uptake will be enhanced when it is coupled with the microbe-regulating N cycling"- I'm not sure what this means.

Reply: Sorry for the confusing. The stimulation of pulse rainfall events to microbes and plant N uptake is different, with a lower stimulation threshold for microbes in extremely dry areas (Dijkstra et al. 2012). Below the MAP threshold, soil microbes may be activated by small rainfall events compared with plants, producing a pulse of high N availability to plants. But if there is an asynchrony in N-cycling via water limitation on plant N uptake, the mineralized N is subject to nitrification and denitrification losses. Above the MAP threshold, these two processes are probably coupled (i.e., microbial mineralized N immediately used by plant), resulting in higher N retention efficiency (Wang et al., 2014).

We have rewritten the entire paragraph to discuss both plant N uptake and nitrification on the consumption of soil  $\text{NH}_4^+$  and its isotopic signal. Please see the whole paragraph in line 270-284.

Line 268- This doesn't make sense. I don't know of any evidence showing preference for enriched substrates- I would expect it to be exactly the reverse in fact.

Reply: Deleted.

Line 324- This paragraph should be rewritten for clarity- right now it's just listing off a bunch of processes and it's confusing. What are the processes that would explain more  $\text{NH}_4^+$ , with higher enrichment, at low precipitation? Increasing volatilization with precip explains the concentration gradient, but would induce the opposite isotopic pattern (though it depends really on how much volatilization occurs as a fraction of the standing pool). A greater proportion of atmospheric deposition versus mineralization at low precip might explain the higher  $^{15}\text{N}$ .

If you invoke fixation by BSCs at low precip, this would also tend to decrease, rather than increase, the  $^{15}\text{N}$  at those sites. This also needs to be clarified- are there BSCs on the transect? However it looks from the gene abundance data like N fix genes increase along the transect. Rethink this paragraph.

Reply: We have rethought the whole paragraph and rewritten it to address the respective contribution of aerosol deposition and BSC to  $\text{NH}_4^+$  accumulation in the arid zone soils. Please see our revised discussion in line 329-345.

Line 336- Does fractionation during mineralization actually increase with mineralization rate though? I don't recall any evidence for this. Also I think invoking heterotrophic nitrification, when as far as I know there isn't a lot of evidence this is an important process in the field, is a stretch. Maybe remove.

Reply: Though the isotope effect of N mineralization is most often said to be low or negligible, it might be higher than we expected. Our lab recently reported that  $\delta^{15}\text{N}$  values of soil  $\text{NH}_4^+$  were lower than that of bulk soil N by 6-8 permil in two forest soils collected in northern China (Zhang et al. 2015). As had also suggested by review #2, the isotope effect of N mineralization can be as high as 20 permil if one looks at enzymes level and their isotope effects that are most likely involved in deamination of organic N forms in cells (Werner and Schmidt 2002). We are not sure about the changes of fractionation effect during mineralization along the precipitation gradient, but fractionation effect could exist. We are not stating that fractionation itself increased with increasing mineralization rate. Please see our modifications in line 348-354.

Clearly nitrification occurs in our study area. Ammonium was  $^{15}\text{N}$  depleted relative to bulk soil, indicating that there might be only a small fraction of soil ammonium was oxidized by autotrophic nitrifies. Heterotrophic nitrification therefore could be one of the reasons for the source of soil nitrate, and contributes to pool size of soil nitrate, but not to the  $^{15}\text{N}$  signal of soil ammonium. Both the fractionation effect over mineralization and the occurrence of heterotrophic nitrification were the alternative reason for  $^{15}\text{N}$  depleted soil ammonium in the semiarid zone soils. Please see our explanation in line 324-328.

Line 359- "Increasing ammonification with increasing MAP both reduced  $\text{NH}_3$  volatilization" Why would more

mineralization reduce volatilization? Unless you mean that volatilization decreases with precip? Again, I'm not sure that this is necessarily true. pH probably the main driver.

Reply: Sorry for the confusing. This sentence has been modified as 'Increasing N mineralization with increasing MAP, accompanied with reduced NH<sub>3</sub> volatilization associated with lower pH produce soil NH<sub>4</sub><sup>+</sup> pool with lighter N isotopes'. Please see line 372-373.

Line 360- why does more mineralization mean more plant uptake? Plant uptake is likely more a function of water availability. These things likely co-occur, but it's not causal. Perhaps misinterpretation of wording- re-write.

Reply: We have rewritten this sentence as 'Ammonification (N mineralization) supplies NH<sub>4</sub><sup>+</sup> for both plant uptake and favour soil nitrification'. Please see line 373-374.

Figure 8- I think it needs to be clear that the size of arrows indicates qualitative interpretation of these fluxes rather than actual measurement- the presence of pool sizes on the boxes makes this especially necessary. And again, NO loss is likely much more important than anammox and should appear here.

Reply: Thank you for your suggestion. We have added 'qualitative interpretation' in the figure legend. This figure was going to illustrate relative importance of various N processes. So, the specific N loss was not shown in the figure. We recognized that NO loss was very important in the N losses in these drylands, however, it is part of the nitrification process (as well as part of the denitrification process according to the 'Leaking Pipe Hypothesis'). Similarly, we did not show N<sub>2</sub>O/N<sub>2</sub> loss separately from denitrification. Rather, NO loss would be discussed in specific section (e.g. soil nitrate losses) in the discussion (please see line 250-252 and 260-265).

Technical corrections:

The manuscript contains many examples of awkward or technically incorrect English that can obscure meaning and requires editing by a native English speaker before publication. E.g. in the abstract –'our understanding of' might replace 'understanding about' and 'nitrogen cycling in drylands' rather than 'nitrogen cycling of drylands'. Also 'the patterns and mechanisms of water availability on soil N cycling' doesn't make technical sense. 'Driving' rather than 'driven'. 'Above and below' rather than 'on the two sides of'. 'Preference for' rather than 'preference of', etc.

Reply: Thank you very much for your comments and correction. In the revised version of the manuscript, we have made further efforts to improve writing. The reviewer #2 have also given a lot of suggestions on how to improve the writing.

References

Dijkstra, F. A., D. J. Augustine, P. Brewer, and J. C. von Fischer. 2012. Nitrogen cycling and water pulses in semiarid

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- Garten, C. T. 1996. Stable nitrogen isotope ratios in wet and dry nitrate deposition collected with an artificial tree. *Tellus B* **48**:60-64.
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## **Response to reviewer #2**

Review *Biogeosciences* Discussion BG-2016-226

The paper "Abiotic versus biotic controls on soil nitrogen cycling in drylands along a 3200 km transect" provides a great dataset on soil N cycling across a precipitation gradient in dryland ecosystems in China, based on the natural <sup>15</sup>N (18O) abundances of bulk soils and ammonium and nitrate, and on the abundances of marker genes involved in N cycling. These novel data allow deep and unprecedented insights into the controls of inorganic N cycling of these ecosystems, and clear trends emerge in abiotic versus biotic controls. The paper therefore addresses relevant questions within the scope of *Biogeosciences*. Methods and assumptions are valid, and the results definitely sufficient to support the interpretations and implications raised by the authors. The description of Materials and methods and calculations are sufficiently complete. The authors cited relevant work and demonstrate their novel contribution to the field. The title is concise and reflects the content of work, and the abstract concise and complete in summarizing the main points of this study. The presentation/manuscript is well structured and clear, but the language should be edited by a native speaker. The number and quality of references is fair and appropriate, and supplementary material is of high quality and appropriate. Beyond that I have the following comments (according to the lines in the manuscript, the language

corrections are by far not complete :)

Reply: Thank you very much for the high regard on our work. In the revised version of the manuscript, we have made many efforts to improve the writing.

L36: should read “driving” not driven;

Reply: Changed as suggested. See line 35.

L39: delete significantly;

Reply: Changed as suggested.

L41: rewrite “the uptake preference for soil...”.

Reply: Changed as suggested. See line 40.

L42: soil nitrate loss could also occur by hydrological pathways (leaching) during heavy rain storms.

Reply: Yes, that is true. Leaching is an alternative pathway by which soil nitrate could be lost. However, given the arid nature of our study sites (annual precipitation from 36 mm to 436 mm) it is less likely the significant pathway of N losses. Therefore, we did not mention it in the abstract section.

L42: rewrite “our study suggests that the shift from abiotic...”;

Reply: Changed as suggested. See line 41.

L51: rewrite “factor” not factors;

Reply: Changed as suggested. See line 49.

L54: rewrite “still lack a full understanding of the...”

Reply: Changed as suggested. See line 52.

L61: rewrite “over large scales”;

Reply: Changed as suggested. See line 59.

L67: change “are” to “become”

Reply: Changed as suggested. See line 65.



L71: change “water-driven” to “hydrological losses by leaching”

Reply: Changed as suggested. See line 68-69.

L73: change to “..alone is not..”

Reply: Changed as suggested. See line 71.

L74: change to “processes that contribute”

Reply: Changed as suggested. See line 72.

L77: what is the meaning of “integrate over their characteristics”? Please be more concise.

Reply: This sentence has been modified as ‘Isotopes in ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) can serve as a proxy record for the N processes in soils because they directly respond to in situ processes and reflect the processes controlling  $\text{NH}_4^+$  and  $\text{NO}_3^-$  production and consumption’. Please see line 73-74.

L79: rewrite “..provided evidence for: : :”

Reply: Changed as suggested. See line 77.

L81: “they cover a different range”.

Reply: Changed as suggested. See line 79.

L83/84: rewrite “..to study the preferences for plant N uptake”

Reply: Changed as suggested. See line 81-82.

L105: change to “gradient”.

Reply: Changed as suggested. See line 102.

L109: “gene abundances”.

Reply: Changed as suggested. See line 106.

L111/112: “with microbially regulated soil processes; and 3) how does soil N cycling: : :”.

Reply: Changed as suggested. See line 108-109.

L116: “the climate is: : :”.

Reply: Changed as suggested. See line 113.

L118: define the aridity index here.

Reply: It has been modified as ‘Aridity index (the ratio of precipitation to potential evapotranspiration) increased from 0.04 to 0.60’. See line 115-116.

L120: “: : :the three : : :”

Reply: Changed as suggested. See line 118.

L124: how do the authors decide which is the peak of soil N transformations? Is that peak vegetation season? Or the short season where the majority of rainfall occurs? Please be more specific here.

Reply: Our soil sampling was conducted from July to August in 2012. The most of the rainfall was occurred at this period of time along the transect. The sentence has been revised as ‘Soil sampling was conducted from July to August in 2012, the peak of plant growing season’. Please see line 121.

L131: correct “into” to “in”, twice.

Reply: Changed as suggested. See line 129 and 130.

L134: “using a pH meter”.

Reply: Changed as suggested. See line 133.

L141: “based on the isotopic analysis of nitrous oxide”.

Reply: Changed as suggested. See line 140.

L142/143: change “into” to “to”, three times.

Reply: Changed as suggested. See line 141 and 142.

L146: rewrite “samples”.

Reply: Changed as suggested. See line 145.

L148:change “to a Trace: : :”.

Reply: Changed as suggested. See line 147.

168: change to “Pearson correlation analysis”.

Reply: Changed as suggested. See line 167.

174: it should be “at” not “in” sites.

Reply: Changed as suggested. See line 173.

L175: “genes”.

Reply: Changed as suggested. See line 174.

L177: rewrite “that the soil N status and its controls could be different: : :”.

Reply: Changed as suggested. See line 176.

L185: “was significantly higher: : :”. By the way if I get the numbers correct in the arid zone bulksoil N (soil total N) would be 200 mg N/kg, with nitrate 87 mgN/kg and ammonium 4 mg N/kg, i.e. inorganic N would on average contribute 46% to soil total N, and only 54% on average is bound as organic N in humus?

Reply: (1) Please see line 184. (2) Yes, in some sites of the arid zone with extremely limited precipitation, soil N mainly is in inorganic form, and it is mostly driven by inorganic N accumulation by atmospheric deposition (as indicated by the <sup>18</sup>O isotopes of soil nitrate in Figure 5a), not by the formation and mineralization of organic matter (and organic N). This is a key point of our result, and has also been observed in the desert soils of northern China (Qin et al. 2012) and northern Chile (Michalski et al. 2004). Please see our discussion in the whole paragraph from line 297 to 319 and line 331-340.

188: “supports”.

Reply: Changed as suggested. See line 187.

203: “<sup>15</sup>N depleted relative to their sources”.

Reply: Changed as suggested. See line 202.

205: please specify what you mean with “via microbial and plant regulation”. <sup>15</sup>N depletion of soil ammonium or less <sup>15</sup>N enrichment can arise from microbial N mineralization (if this process exerts significant N isotope fractionation) or

biological N fixation (causing inputs of N with  $\delta^{15}\text{N}$  around 0 to -2 permil). Maybe also atmospheric ammonium/ammonia deposition.

Reply: Thank you. The sentence has been modified as ‘The positive values for the  $^{15}\text{N}$  enrichment of soil  $\text{NH}_4^+$  support that net  $\text{NH}_4^+$  losses occurred mainly in the arid zone, while the negative values imply that net  $\text{NH}_4^+$  gain (e.g. via microbial mineralization, biological N fixation and/or N deposition) might increase in the semiarid zone, and subsequently reduced the relative  $^{15}\text{N}$  enrichment of soil  $\text{NH}_4^+$ .’ Please see line 203-205.

In the later discussion in the manuscript, we also discussed that higher  $^{15}\text{N}$  of deposited ammonium may explain the  $^{15}\text{N}$ -enriched soil ammonium in the arid zone. Our preliminary study found that  $\delta^{15}\text{N}$  values of aerosol ammonium 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‰. The similar results have been found in Japan (Kawashima and Kurahashi 2011);  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$  in SPM (suspended particulate matter) ranged from 1.3‰ to 38.5‰, with the average of 11.6‰. These higher  $\delta^{15}\text{N}$  of ammonium in dry deposition may result from the exchange of atmospheric ammonia gas and aerosol ammonium (Heaton et al. 1997). Please see line 331-339.

207: rewrite “A positive correlation was : :”

Reply: Changed as suggested. See line 207.

212: “genes”

Reply: Changed as suggested. See line 211.

213: “rewrite “was measured at all sites”

Reply: Changed as suggested. See line 212.

214: “were found to be...”

Reply: Changed as suggested. See line 212.

215 “in the gene abundance of all detected N cycling groups”

Reply: The sentence has been modified as ‘There was a sharp increase (by 8 to 9 fold) in the gene abundance from the arid zone to the semiarid zone’. Please see line 213-214.

217: “dry at the time: :”. “gene abundances in the semiarid zone were: : :”

Reply: Changed as suggested. See line 215.

218: “gene abundances of the five: : :”

Reply: Changed as suggested. See line 216.

219: “potential control of water availability on soil microbial N processes”.

Reply: Changed as suggested. See line 217-218.

223: “water availability drives different patterns” is not meaningful. Please rephrase.

Reply: The sentence has been modified as ‘We observed different patterns of N cycling above and below a MAP threshold of 100 mm in this 3200 km transect’. Please see line 221.

223: “at both sides of about MAP = 100 mm” is really not the best phrasing, maybe rather “above and below a MAP threshold of 100 mm”.

Reply: Thank you. We changed the sentence as suggested. Please see line 221.

224: “seems to lead to losses of N: : :”.

Reply: Changed as suggested. See line 222.

226: “we found direct evidence”.

Reply: The sentence has been deleted.

226/227: of course denitrification is a kinetic process. So what? Simply say that denitrification exerts isotope fractionation against the isotopically heavier compounds, ranging between 5 and 25permil: : :”

Reply: Thank you. This sentence has be modified as ‘Microbial denitrification exerts large fractionation against the isotopically heavier compounds, ranging between 5 and 25‰ for O and N in NO<sub>3</sub><sup>-</sup>’. Please see line 225-226.

232/233: please specify this sentence on availability of N and O<sub>2</sub> supply –to me the meaning is not clear.

Reply: Thank you. This sentence has been modified as ‘Denitrification is regulated by proximal factors that immediately affect denitrifying communities, such as NO<sub>3</sub><sup>-</sup> concentration and O<sub>2</sub> concentration’. Please see line 230-232.

235: “in addition, a preliminary study: : . an increasingN<sub>2</sub> loss via: : :”

Reply: Thank you. For the first suggestion, we would like to keep the expression of ‘our preliminary study’. For the

second one, we have modified the expression as ‘potential N<sub>2</sub> losses via denitrification’. Please see line 237.

240: “in some sites, : : : pointing to losses of soil : : :”.

Reply: Deleted.

241: “after heavy precipitation events”.

Reply: Changed as suggested. See line 246.

239-245: the main pattern for soil nitrate at the arid sites is <sup>15</sup>N depletion of nitrate relative to ammonium. Only a few sites had more positive <sup>15</sup>N values in nitrate compared to ammonium. The explanation by enhanced denitrification during heavy rain or chemodenitrification is therefore only secondary. The main pattern has to be explained – why is soil nitrate <sup>15</sup>N depleted relative to ammonium. My best guess is its production through nitrification which causes ammonium to become <sup>15</sup>N enriched and nitrate <sup>15</sup>N depleted (this is also an alternative explanation for the <sup>15</sup>N enrichment of ammonium at many arid sites). I also would not expect large amounts of reduced iron (FeII) to be present at arid sites. Only in some places denitrification may also play a role, where nitrate was <sup>15</sup>N enriched relative to ammonium. Another input of nitrate is atmospheric deposition, but its isotopic composition for that region is most probably unknown (Fig 5(a) indicates that atmospheric nitrate lies between 0 and 5 permil).

Reply: Thank you. We agree with you that <sup>15</sup>N depletion for soil nitrate relative to soil ammonium in arid region is in part due to soil nitrification, which exerts a strong isotope fractionation against <sup>15</sup>N. Weak denitrification in arid region may have also contributed to low <sup>15</sup>N values in soil nitrate. However, we think that main source of soil nitrate in arid region is atmospheric deposition, as indicated by <sup>18</sup>O of nitrate in soil nitrate and atmospheric deposition, instead of nitrification, since in those areas, microbial activity may be quite weak even for nitrification. We have further discussed those issues in the whole paragraph, which are from line 242 to 252, and from line 297 to 319.

243: “chemodenitrification is an abiotic process..”

Reply: Changed as suggested. See line 247.

244: change “preserved” to “present”

Reply: Changed as suggested. See line 249.

247: “suggesting losses of: : :”

Reply: Changed as suggested. See line 254.

248: what is the meaning of “ammonia volatilization can be strong for the ammonium loss”???

Reply: Sorry for the confusing. The sentence has been modified as ‘we suggest that NH<sub>3</sub> volatilization should play a significant role in NH<sub>4</sub><sup>+</sup> losses, because soil pH was higher in the arid zone (from 7.3 to 9.7; Fig. 6a)’. Please see line 254-256.

249: “The isotope effect of: : :”

Reply: Changed as suggested. See line 256.

250 “significant negative: : :”

Reply: Changed as suggested. See line 257.

250/251: the alternate explanation is that nitrification can also cause <sup>15</sup>N enrichment of ammonium, and <sup>15</sup>N depleted nitrate in many arid soils actually point to a significant role of this process, aside of ammonia volatilization.

Reply: Thank you. We agree with you that <sup>15</sup>N depletion for soil nitrate relative to soil ammonium in arid region is in part due to soil nitrification, which exerts a strong isotope fractionation against <sup>15</sup>N. However, we think that main source of soil nitrate in arid region is atmospheric deposition, as indicated by <sup>18</sup>O of nitrate in soil nitrate and atmospheric deposition, instead of nitrification, since in those areas, microbial activity may be quite weak even for nitrification. We have further discussed those issue in the whole paragraph from line 297 to 319.

252/253: what does “suggesting the net ammonium gain” mean? Please rephrase.

Reply: The sentence has been fixed as ‘In the semiarid zone, soil NH<sub>4</sub><sup>+</sup> became gradually depleted in <sup>15</sup>N relative to the bulk soil N (Fig. 3a), suggesting the input of NH<sub>4</sub><sup>+</sup> (e.g., soil ammonification, N deposition, etc.), while simultaneously NH<sub>4</sub><sup>+</sup> was also consumed’. Please see line 266-267.

252: soil ammonium “became” gradually <sup>15</sup>N depleted relative to: : :

Reply: The sentence has been modified as ‘NH<sub>3</sub> volatilization should be low due to relatively lower pH compared to those in the arid zone soils’. Please see line 267-268.

252-270: the main pattern of soil ammonium is that it becomes <sup>15</sup>N depleted with higher MAP in semiarid sites. This CANNOT be explained with consumption processes such as plant uptake and nitrification, as in both cases (plants and nitrifiers) exert an isotope effect meaning that plants or nitrate become <sup>15</sup>N depleted and soil ammonium <sup>15</sup>N enriched.

An inverse isotope effect has never been shown for any biological process involved in the (production) consumption of ammonium. Lines 268-270 therefore are wrong because microbes will not prefer  $^{15}\text{N}$  enriched ammonium during immobilization. The whole paragraph therefore is misleading and has to be rewritten. The explanation can therefore only come from  $^{15}\text{N}$  depleted N inputs (biological N fixation, 0 to -2 permil; atmospheric ammonium/ammonia deposition, isotope range for the region unknown?) or its production through mineralization of organic N. Though the isotope effect of N mineralization is most often said to be low or negligible, it might be high if one looks at enzymes and their isotope effects that are most likely involved in deamination of organic N forms in cells (they can be as high as 20 permil). Please consult the respective N isotope reviews such as Werner and Schmidt *Phytochemistry* 61 (2002)465–484.

Reply: Thank you for your points. First, we agree with you that both the processes of plant N uptake and nitrification exert isotope effect. However, they may be different in different area. 1) The area in this study is highly N-limited according to previous N manipulation experiment. Plants would take in both  $^{15}\text{N}$  and  $^{14}\text{N}$  in N-limited areas (Craine et al. 2015). So, the fractionation effect during plant N uptake could be low. 2) Nitrification includes two types, i.e. autotrophic nitrification and heterotrophic nitrification. To our knowledge, only autotrophic nitrification leaves  $^{15}\text{N}$  footprint on the soil ammonium. If the oxidized ammonium by autotrophic nitrification only accounted for a small proportion of total ammonium pool, then this nitrification would not influence  $^{15}\text{N}$  of ammonium. We have incorporated those explanations in the manuscript, and please see line 271-284.

Indeed, the isotope effect of N mineralization is most often said to be low or negligible. However, it might be higher than we expected. Our lab recently reported that  $\delta^{15}\text{N}$  values of soil  $\text{NH}_4^+$  were lower than that of bulk soil N by 6-8 permil in two forest soils in northern China (Zhang et al. 2015). As had pointed here, they can be as high as 20 permil if one looks at enzymes and their isotope effects that are most likely involved in deamination of organic N forms in cells (Werner and Schmidt 2002). Thus large  $^{15}\text{N}$  depletion in ammonium (by above 10 permil) compared to soil organic matter observed in the semi-arid regions of our study also supports the idea that N mineralization may exert a larger isotope effect. We have corrected that paragraph regarding this issue, and please see line 346-357.

259: “prefer soil ammonium over nitrate”.

Reply: The expression has changed as ‘the dominant plant species might adapt to use soil  $\text{NH}_4^+$  over  $\text{NO}_3^-$  as nutrient’. Please see line 273-274.

263: “demonstrates the ammonium preference of plants”.

Reply: Deleted.

265: sentence is meaningless – “soil nitrification have been observed to be enhanced with more water widely: : :”?



Reply: Deleted.

271: “we detected anammox genes in these dryland ecosystems”.

Reply: The sentence has been modified as ‘we detected high anammox gene abundances in these dryland ecosystems’.  
Please see line 285.

275: “water-logged”.

Reply: Changed as suggested. See line 287.

275/276: “studies of anammox process rates so far failed to : :”.

Reply: The sentence has been modified as ‘However, the only two anammox studies in drylands so far failed to confirm its importance’. Please see line 288.

280: “responsible for gaseous losses: : :”.

Reply: Changed as suggested. See line 292.

282: “aeolian”.

Reply: Changed as suggested. See line 294.

285: “observed the highest: : :”.

Reply: The sentence has been modified as ‘we observed much higher concentrations of soil  $\text{NO}_3^-$  in the arid zone’. Please see line 297.

287: besides small deposition as dissolved nitrate in rainwater or snow.

Reply: It has been incorporated into the main text. Please see line 299.

288: “since the  $\delta^{18}\text{O}$ : : :”.

Reply: Please see the correction in line 300.

289: “depends on the  $\delta^{18}\text{O}$ : : :”.

Reply: Please see the correction in line 302.

290: “from the areas closest to: : :”.

Reply: Accepted. See line 302-303.

291: “ranged from: : : to: : :”.

Reply: Accepted. See line 303.

294: I don't understand the reasoning behind this sentence, why is atm. O<sub>2</sub> and its d18O important. It is not directly expressed in the d18O of NO<sub>3</sub><sup>-</sup> formed in the atmosphere because this is more<sup>18</sup>O enriched. So...?

Reply: Thank you. The sentence has been fixed as ‘The higher  $\delta^{18}\text{O}$  values of soil NO<sub>3</sub><sup>-</sup> we observed in the arid zone have rarely been reported for nitrified NO<sub>3</sub><sup>-</sup>, according to previous studies’. Please see line 306-307.

285-311: as said before there is also evidence for nitrification in the data set, as in many arid soils nitrate is <sup>15</sup>N depleted relative to ammonium, which indicates nitrification also to contribute to soil nitrate accumulation, aside of atmospheric deposition. There are several typos in this paragraph.

Reply: Accepted. The processes of soil nitrification has been incorporated into our revised manuscript. Please also see line 258-262. In addition, we have made great efforts to improve the writing.

316-318: what does this coincidence of KIE denitrification and d18O of nitrate mean? This is totally dis-connected. Delete.

Reply: Done.

319-323: the gradual <sup>15</sup>N depletion of ammonium in itself, but also relative to soil total N indicates that mineralization is the main input process of soil ammonium, and that N mineralization causes <sup>15</sup>N fractionation. Obviously nitrification also occurs, but as long as only a small fraction (like 10-20%) of soil ammonium is oxidized by autotrophic nitrifies ammonium would still be <sup>15</sup>N depleted relative to bulk soil. Heterotrophic nitrification is another explanation, as stated by the authors.

Reply: We agree with you. Please see line 320-328.

337: why do the authors believe that soil ammonification was stimulated with higher MAP? Where is the evidence for that? Only the ammonium concentrations?

Reply: Besides of increasing ammonium concentration, we also observed that ammonium <sup>15</sup>N was more depleted relative to bulk soil N with higher MAP. Please see further explanation in line 350-354.

347: was the precipitation range really large?

Reply: The precipitation range was between 36 mm and 436 mm in this study, and may not large enough. This paper focuses on the N cycling in drylands with changing water availability, and especially focus on the available N. From this point of view, the sentence has been modified as ‘To the best of our knowledge, our study reported for the first time the pattern of  $\delta^{15}\text{N}$  in soil inorganic N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) across a precipitation gradient from very arid land to semiarid grassland’. Please also see corrections in line 359-360.

355: what is phytochemical nitrate loss?

Reply: Deleted.

360: what is “provided lighter N isotope for soil ammonium? And as this sentence states “increasing ammonification reduced ammonia volatilization”. How should that happen?

Reply: Sorry for the confusing. This sentence has been modified as ‘Increasing N mineralization with increasing MAP, accompanied with reduced  $\text{NH}_3$  volatilization associated with lower pH produce soil  $\text{NH}_4^+$  pool with lighter N isotopes’. Please see line 372-374.

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

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## 28 **Abstract**

29 Nitrogen (N) cycling in drylands under changing climate is not well understood. Our understanding of N cycling over larger  
30 scales to date relies heavily on the measurement of bulk soil N, and the information about internal soil N transformations  
31 remains limited. The  $^{15}\text{N}$  natural abundance ( $\delta^{15}\text{N}$ ) of ammonium and nitrate can serve as a proxy record for the N processes  
32 in soils. To better understand the patterns and mechanisms of N cycling in drylands, we collected soils along a 3200 km  
33 transect at about 100 km intervals in northern China, with mean annual precipitation (MAP) from 36 mm to 436 mm. We  
34 analysed N pools and  $\delta^{15}\text{N}$  of ammonium, dual isotopes ( $^{15}\text{N}$  and  $^{18}\text{O}$ ) of nitrate, and the microbial gene abundance  
35 associated with soil N transformations. We found that N status and their **driving factors** were different above and below a  
36 MAP threshold of 100 mm. In the arid zone with MAP below 100 mm, soil inorganic N accumulated, with a large fraction  
37 being of atmospheric origin. Ammonia volatilization was strong in high pH soils. The abundance of microbial genes  
38 associated with soil N transformations was low. In the semiarid zone with MAP above 100 mm, soil inorganic N  
39 concentrations were low and controlled mainly by biological processes (e.g., plant uptake and denitrification). The uptake  
40 **preference for soil ammonium** over nitrate by the dominant plant species may enhance the possibility of soil nitrate losses  
41 *via* denitrification. Overall, **our study suggests that the shift from abiotic to biotic controls** on soil N biogeochemistry under  
42 global climate changes would greatly affect N losses, soil N availability, and other N transformation processes in these  
43 drylands in China.

44

45 **Key words:** soil inorganic N;  $^{15}\text{N}$  natural abundance; soil microorganisms; functional genes; spatial patterns

## 46 **1 Introduction**

47 Drylands cover approximately 41% of the Earth's land surface and play an essential role in providing ecosystem services as  
48 well as regulating carbon (C) and nitrogen (N) cycling (Hartley et al., 2007; Poulter et al., 2014; Reynolds et al., 2007). After  
49 water, N availability is the most important limiting **factor** to plant productivity and microbial processes in dryland  
50 ecosystems (Collins et al., 2008; Hooper and Johnson, 1999). Despite low soil N mineralization rates, N losses are postulated  
51 to be higher relative to N pools in dryland ecosystems compared to mesic ecosystems (Austin, 2011; Austin et al., 2004;  
52 Dijkstra et al., 2012). However, **we still lack a full understanding of** the constraints on N losses in drylands, because multiple  
53 processes contribute to N losses and the response of those processes to changing climate is highly variable (Nielsen and Ball,  
54 2015). The precipitation regimes in drylands are predicted to change during the 21st Century (IPCC, 2013), and more  
55 extreme climatic regimes will make dryland ecosystems more vulnerable to enhanced drought in some regions and intensive  
56 rain in others (Huntington, 2006; Knapp et al., 2008). Therefore, improving our understanding of N cycling and its controls  
57 would greatly enhance our ability of predicting the responses of dryland ecosystems to global changes.

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

60 2006). The general pattern that foliar and soil  $\delta^{15}\text{N}$  increases as precipitation decreases has been found at both regional  
61 (Aranibar et al., 2004; Austin and Vitousek, 1998; Cheng et al., 2009; Peri et al., 2012) and global scales (Amundson et al.,  
62 2003; Craine et al., 2009; Handley et al., 1999), suggesting that N cycling is more open in dryland ecosystems than in mesic  
63 ecosystems. The underlying explanation is when N supply is higher relative to biotic demand, more N is lost through  
64 leaching and gaseous N emissions (Austin and Vitousek, 1998), during which isotope fractionation is against the heavier  
65 isotope so that plant tissue and soil **become** enriched in  $^{15}\text{N}$  (Robinson, 2001). However, the controls of atmospheric  
66 deposition on N cycling are often ignored in N isotope studies, in which N isotopes from atmospheric deposition and  
67 biological N fixation are assumed to be uniform over large regional scales (Bai et al., 2012; Handley et al., 1999; Houlton  
68 and Bai, 2009). Besides, N losses in dryland ecosystems are likely dominated by gaseous losses instead of **hydrological**  
69 **losses** (McCalley and Sparks, 2009; Peterjohn and Schlesinger, 1990). The  $^{15}\text{N}$  natural abundance of total N are limited in  
70 interpreting the specific processes governing those gaseous N losses. Therefore, it seems that the measurement of total N  
71 **alone is not** sufficient to reveal the responses of N cycling to changing precipitation, because there are multiple processes  
72 **that contribute to** the  $\delta^{15}\text{N}$  variability in plant-soil systems.

73 **Isotopes in ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) can serve as a proxy record for the N processes in soils because they**  
74 **directly respond to the *in situ* processes that controlling  $\text{NH}_4^+$  and  $\text{NO}_3^-$  production and consumption.** For example,  
75 comparing  $\delta^{15}\text{N}$  values of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and bulk soil N could reveal the relative importance of N transformation processes  
76 (such as between ammonification and nitrification) (Koba et al., 2010; Koba et al., 1998). The dual isotope analysis of  $\text{NO}_3^-$   
77 ( $^{15}\text{N}$  and  $^{18}\text{O}$  of soil  $\text{NO}_3^-$ ) **provides evidence for** microbial denitrification in oceans (Sigman et al., 2009), forests (Fang et al.,  
78 2015; Houlton et al., 2006; Wexler et al., 2014) and groundwater (Minet et al., 2012). In addition,  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  has also  
79 been used to partition microbial produced  $\text{NO}_3^-$  from atmospheric sources because **they cover a different range of**  $\delta^{18}\text{O}$   
80 (Böhlke et al., 1997; Brookshire et al., 2012; Kendall et al., 2007). The positive correlations between N isotopes of soil  
81 available N ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and dissolved organic N) and plant leaves have been used to **study the preferences for plant N**  
82 **uptake** (Cheng et al., 2010; Houlton et al., 2007; Mayor et al., 2012; Takebayashi et al., 2010). With methods newly  
83 developed (Lachouani et al., 2010; Liu et al., 2014; Tu et al., 2016), the analysis of isotopic values in soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  has  
84 the potential to elucidate the N cycling characteristics and their controls; however, in comparison to that of bulk soil N, the  
85  $\delta^{15}\text{N}$  of both soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  has rarely been reported, especially in drylands.

86 Soil microbes constitute a major portion of the biota in terrestrial ecosystems and play key roles in regulating ecosystem  
87 functions and biogeochemical cycles (Van Der Heijden et al., 2008). Linking soil microbial communities and N processes is  
88 critical for evaluating the response of N transformations to climate changes. However, despite rapid development of high-  
89 throughput sequencing techniques in recent decades, there is still a great challenge for researchers to establish such linkages  
90 due to technical limitations, especially at large spatial scales (Zhou et al., 2011). Alternatively, a microarray-based  
91 metagenomics technology, Geochip, has been developed for the analysis of microbial communities (He et al., 2007; He et al.,  
92 2010b; Tu et al., 2014). This technique can be used not only to analyze the functional diversity, composition and structure of  
93 microbial communities, but also to directly reveal the linkages between microbial communities and ecosystem functions (He

94 et al., 2007). Functional gene microarray approaches have been used to examine the response of microbially mediated N  
95 processes in different environmental conditions. Denitrification genes from the soils in Antarctic, for example, were found to  
96 be linked to higher soil temperatures, and N<sub>2</sub>-fixation genes were linked to the presence of lichens (Yergeau et al., 2007).  
97 Research along an elevation gradient pointed out that some of denitrification genes (*nirS* and *nosZ*) were more abundant at  
98 higher elevations, with nitrification as the major process of N<sub>2</sub>O emission in the Tibetan grassland (Yang et al., 2013). The  
99 latest version, GeoChip 5.0S, contains probes covering over 144,000 functional genes, which enables us to explore key  
100 microbially mediated biogeochemical processes more thoroughly than ever before (Cong et al., 2015; Wang et al., 2014).

101 In this study, we studied the effects of water availability on ecosystem-level N availability and cycling along a 3200 km  
102 transect in northern China. This natural **gradient** of precipitation provides an ideal system for identifying the response of soil  
103 N dynamics to water availability. In a previous study we reported a hump-shaped pattern of  $\delta^{15}\text{N}$  for bulk soil N along this  
104 precipitation gradient, with a threshold at aridity index of 0.32 (mean annual precipitation of about 250 mm), showing the  
105 respective *soil microbial vs. plant* controls (Wang et al., 2014). Here, we further analysed the concentrations, N isotopic  
106 composition of soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> (for NO<sub>3</sub><sup>-</sup>, also oxygen (O) isotopes) as well as the microbial **gene abundances** associated  
107 with soil N transformations. The principal objectives of this study were to examine: (1) the patterns of concentrations and  
108  $\delta^{15}\text{N}$  for soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>; (2) the patterns of gene abundance associated **with microbially regulated soil processes; and (3)**  
109 **how soil N cycling responds to changes** in water availability along the precipitation gradient in dryland ecosystems.

## 110 **2 Materials and methods**

### 111 **2.1 Study areas**

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

### 120 **2.2 Soil sampling and sample preparation**

121 **Soil sampling was conducted from July to August in 2012, the peak of the plant growing season.** This is the same transect as  
122 described in Wang et al. (2014), but with slightly different site coverage; we selected 36 sites at about 100 km intervals  
123 between adjacent sites due to limited time to extract soil with KCl solution on the same day after intensive sampling (Fig. 1)  
124 while 50 sites at about 50 km intervals were used for bulk soil N isotopes measurement in Wang et al. (2014). In each site,



125 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,  
126 twenty random mineral soil samples were collected using soil cores (2.5 cm diameter × 10 cm depth) and thoroughly mixed  
127 into one composite sample. The fresh soils were sieved (2 mm) to remove roots and rocks, homogenized by hand and  
128 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  
129 were stored at -4 °C during the sampling trip. The second part was placed in a sterile plastic bag and immediately stored at -  
130 40 °C for later DNA extraction. The third part was placed in a plastic bag and stored in a refrigerator at -4 °C for subsequent  
131 analyses.

### 132 2.3 Analyses of soil physicochemical properties and isotopes

133 Soil pH was measured using a pH meter with a soil to water ratio of 1:2.5. Soil N content and <sup>15</sup>N natural abundance were  
134 determined by an elemental analyser connected to an Isotope Ratio Mass Spectrometer (IRMS) (Wang et al., 2014). The  
135 concentrations of soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> in KCl extracts were analysed using conventional colorimetric methods (Liu et al.,  
136 1996). Ammonium concentrations were determined by the indophenol blue method, and nitrate by sulfanilamide-NAD  
137 reaction following cadmium (Cd) reduction.

138 The analyses of isotope compositions of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>, including δ<sup>15</sup>N of NH<sub>4</sub><sup>+</sup>, δ<sup>15</sup>N of NO<sub>3</sub><sup>-</sup>, and δ<sup>18</sup>O of NO<sub>3</sub><sup>-</sup> (δ =  
139 [(R<sub>sample</sub>/ R<sub>standard</sub>) - 1] × 1000, where R denotes the ratio of heavy isotope to light isotope for N or O, in units of per mil, ‰),  
140 were based on the isotopic analysis of nitrous oxides (N<sub>2</sub>O). Specifically, NH<sub>4</sub><sup>+</sup> in the extract was oxidized to NO<sub>2</sub><sup>-</sup> by  
141 alkaline hypobromite (BrO<sup>-</sup>), and then reduced to N<sub>2</sub>O by hydroxylamine (NH<sub>2</sub>OH) (Liu et al., 2014). Nitrate was firstly  
142 reduced to NO<sub>2</sub><sup>-</sup> by Cd powder, and then to N<sub>2</sub>O by sodium azide (NaN<sub>3</sub>) in an acetic acid buffer (McIlvin and Altabet, 2005;  
143 Tu et al., 2016). In order to correct machine drift and blank over the isotopic analyses, the international standards of NH<sub>4</sub><sup>+</sup>  
144 (IAEA N1, USGS 25, and USGS 26) and NO<sub>3</sub><sup>-</sup> (IAEA N3, USGS 32, USGS 34, and USGS 35) were treated in identical  
145 analytical procedures as the samples to obtain the calibration curve between the measured and their expected isotope values.  
146 The isotopic signatures of produced N<sub>2</sub>O were determined by an IsoPrime 100 continuous flow isotope ratio mass  
147 spectrometer connected to a Trace Gas (TG) pre-concentrator (Liu et al., 2014). The analytical precision for isotopic  
148 analyses was better than 0.3‰ (n = 5).

### 149 2.4 DNA extraction and GeoChip analysis

150 For soil DNA extraction, purification, quantification, and the analysis of functional structure of soil microbial communities,  
151 we adopted the same approaches as described previously (Wang et al., 2014). In addition to the abundance of nitrification  
152 and denitrification genes reported in Wang et al. (2014), the gene abundance of N fixation, ammonification, and anaerobic  
153 ammonia oxidation (anammox) were included in this paper. Briefly, microbial genomic DNA was extracted from 0.5 g soil  
154 using the MoBioPowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) and purified by agarose gel  
155 electrophoresis followed by phenol-chloroform-butanol extraction. DNA quality was assessed by the ratios of A260/280 and  
156 A260/230 using NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE), and final soil

157 DNA concentrations was quantified by PicoGreen using a FLUOstar Optima (BMG Labtech, Jena, Germany). GeoChip 5.0S,  
158 manufactured by Agilent (Agilent Technologies Inc., Santa Clara, CA), was used for analyzing DNA samples. The  
159 experiments were conducted as described previously (Wang et al., 2014). In brief, the purified DNA samples (0.6  $\mu$ g) was  
160 used for hybridization, which was labelled with the fluorescent dye Cy 3; subsequently, the labelled DNA was resuspended  
161 and hybridized at 67 °C in an Agilent hybridization oven for 24 h. After washing and drying, the slides were scanned by a  
162 NimbleGen MS200 scanner (Roche, Madison, WI, USA) at 633 nm using a laser power of 100% and photomultiplier tube  
163 gain of 75%, respectively. The images data were extracted by Agilent Feature Extraction program. The microarray raw data  
164 were further processed for subsequent analysis using an in-house pipeline that was built on the platform at the Institute for  
165 Environmental Genomics, University of Oklahoma (He et al., 2010a; He et al., 2007).

## 166 2.5 Statistical analyses

167 All analyses were conducted by using the SPSS 18.0 (SPSS, Chicago, IL) for Windows. Pearson correlation analysis was  
168 conducted to examine the linear relationships among different variables. Independent-Samples T-test was performed to  
169 examine the differences in the investigated variables between arid zone soils and semiarid zone soils. Statistically significant  
170 differences were set at a *P*-value of 0.05 unless otherwise stated.

## 171 3 Results

### 172 3.1 Soil NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations

173 We found significant inorganic N accumulation in the investigated soil layer (0-10 cm) at sites with MAP less than 100 mm  
174 (Fig. 2b and c). Furthermore, the abundance of microbial genes associated with soil N transformations was significantly  
175 lower than those in sites with MAP of more than 100 mm (Fig.4). Together with the vegetation distribution along the  
176 transect (Fig.1), these results pointed out that soil N status and its controls could be different above and below a MAP  
177 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  
178 mm (21 sites) as arid zone and semiarid zone in this study, respectively.

179 Soil NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations in the arid zone were significantly higher than those in the semiarid zone (*P* < 0.001;  
180 Fig. 2b and c). In the arid zone, NO<sub>3</sub><sup>-</sup> concentrations were highly variable and up to 1400 mg N kg<sup>-1</sup>, with a mean of 87 mg  
181 N kg<sup>-1</sup>. Ammonium concentrations varied from 2.0 to 9.9 mg N kg<sup>-1</sup>, with a mean of 4.3 mg N kg<sup>-1</sup>. In the semiarid zone,  
182 NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations were low and in most samples less than 5 mg N kg<sup>-1</sup>. Soil NH<sub>4</sub><sup>+</sup> concentrations showed a  
183 quadratic relationship with increasing MAP in the semiarid zone, but NO<sub>3</sub><sup>-</sup> concentrations remained low and did not change  
184 with increasing MAP. As expected, soil total N was significantly higher in the semiarid zone (on average 0.1%) than in the  
185 arid zone (on average 0.02%) and increased dramatically in the semiarid zone with increasing precipitation (Fig. 2a). Our  
186 results suggest a higher inorganic N availability in the arid zone than in the semiarid zone despite a smaller total N pool  
187 therein, which supports the idea that N availability is relatively higher in dry areas than in less dry areas.

### 188 3.2 The $^{15}\text{N}$ natural abundance of soil $\text{NO}_3^-$ and $\text{NH}_4^+$

189 The  $\delta^{15}\text{N}$  values of  $\text{NO}_3^-$  were significantly higher in the semiarid zone (0.5 to 19.2‰) than in the arid zone (-1.2 to  
190 23.4‰;  $P < 0.01$ ; Fig. 2f), with the mean of 8.4‰ and 6.3‰, respectively. With increasing MAP, the  $\delta^{15}\text{N}$  value of  $\text{NO}_3^-$   
191 increased in the arid zone but decreased in the semiarid zone, suggesting different controlling factors in the areas with  
192 different water availability. Different from that of soil  $\text{NO}_3^-$ , the  $\delta^{15}\text{N}$  value of  $\text{NH}_4^+$  was significantly higher in the arid zone  
193 (-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‰,  
194 respectively. The  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$  was negatively correlated with MAP in the semiarid zone, but was stable as precipitation  
195 increased in the arid zone (Fig. 2e).

196 The N isotopic signature of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  reflects not only isotopic fractionation during N transformation processes,  
197 but also the N isotopic signature of their main sources (i.e., bulk soil N and  $\text{NH}_4^+$ , respectively). Therefore, we also  
198 calculated the relative  $^{15}\text{N}$  enrichment of soil  $\text{NH}_4^+$  (the difference between  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$  and bulk soil N) and  $\text{NO}_3^-$  (the  
199 difference between  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) to examine the isotopic imprint of N transformations on soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$ .  
200 The relative  $^{15}\text{N}$  enrichment of soil  $\text{NH}_4^+$  in the arid zone was mostly positive, while it was negative in the semiarid zone  
201 (Fig. 3a). There was a negative correlation between MAP and the relative  $^{15}\text{N}$  enrichment of soil  $\text{NH}_4^+$  across both the arid  
202 and semiarid zones (Fig. 3a). According to the Rayleigh model, sinks are always  $^{15}\text{N}$ -depleted relative to their sources  
203 (Robinson, 2001). The positive values for the  $^{15}\text{N}$ -enrichment of  $\text{NH}_4^+$  support that net  $\text{NH}_4^+$  losses occurred mainly in the  
204 arid zone, while the negative values imply that net  $\text{NH}_4^+$  gain (e.g., via microbial N mineralization, biological N fixation  
205 and/or N deposition) might increase in the semiarid zone, and subsequently reduced the relative  $^{15}\text{N}$ -enrichment of soil  $\text{NH}_4^+$ .  
206 In a similar way, we found that the relative  $^{15}\text{N}$ -enrichment of  $\text{NO}_3^-$  were mostly negative in the arid zone and positive in the  
207 semiarid zone (Fig. 3b). A positive correlation was observed between MAP and the  $^{15}\text{N}$ -enrichment of soil  $\text{NO}_3^-$  in both the  
208 arid and semiarid zones (Fig. 3b). Accordingly, these results suggest that  $\text{NO}_3^-$  losses along this dryland transect occurred  
209 when water becomes more available, and progressively enriched residual soil  $\text{NO}_3^-$  in  $^{15}\text{N}$ .

### 210 3.3 The abundance of microbial functional genes

211 The abundances of microbial genes of five main N cycling groups (N fixation, ammonification, nitrification, denitrification,  
212 and anammox) were measured at all sites. In arid zone soils, gene abundances of all N cycling groups were found to be  
213 extremely low (Fig.4), indicating limited microbial potentials in the very dry environment. There was a sharp increase (by 8  
214 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  
215 the time of sampling (see soil moisture in Fig. S2). Gene abundances in the semiarid zone were 1-2 orders of magnitude  
216 higher than those in the arid zone. In addition, microbial gene abundances of the five main N cycling groups all increased  
217 with increasing precipitation in both the arid and semiarid zones (Fig. 4), suggesting a potential control of water availability  
218 on soil microbial N processes.

## 219 4 Discussion

### 220 4.1 Losses of soil NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>

221 We observed different patterns of N cycling above and below a MAP threshold of 100 mm in this 3200 km transect. In the  
222 semiarid zone, the increased precipitation seems to lead to increased losses of soil NO<sub>3</sub><sup>-</sup>, but not NH<sub>4</sub><sup>+</sup> (Fig. 3). Soil NO<sub>3</sub><sup>-</sup> can  
223 be removed from the ecosystem via denitrification, leaching, and also plant and microbial uptake. The close correlation  
224 between measured dual isotopes ( $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ ) of soil NO<sub>3</sub><sup>-</sup> suggests the occurrence of denitrification in the semiarid zone.  
225 Microbial denitrification exerts large fractionation against the isotopically heavier compounds, ranging between 5 and 25%  
226 for O and N in NO<sub>3</sub><sup>-</sup> (Granger et al., 2008). This kind of fractionation results in concurrent increases in the  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$   
227 values of the remaining NO<sub>3</sub><sup>-</sup> with a ratio of 0.5 to 1 (Kendall et al., 2007). In the present study, the  $\delta^{18}\text{O}$  values of soil NO<sub>3</sub><sup>-</sup>  
228 were significantly correlated with the  $\delta^{15}\text{N}$  values of soil NO<sub>3</sub><sup>-</sup> in the semiarid zone, with a slope of 0.7 (Fig.5b). This slope  
229 is very similar to the slope of 0.8 observed in soil NO<sub>3</sub><sup>-</sup> across five Hawaiian tropical forests (Houlton et al., 2006),  
230 indicating the occurrence of denitrification driven N losses when water becomes relatively available. Denitrification is  
231 regulated by proximal factors that immediately affect denitrifying communities, such as NO<sub>3</sub><sup>-</sup> concentration and O<sub>2</sub>  
232 concentration (Saggar et al., 2013). Enhanced nitrification as a result of water addition and increased mineralization would  
233 yield a greater amount of NO<sub>3</sub><sup>-</sup> for potential denitrification. Increased soil respiration in hot spots and/or hot moments caused  
234 by pulse precipitations consumes O<sub>2</sub>, consequently favouring denitrification (Abed et al., 2013). In the semiarid zone, we  
235 observed that <sup>15</sup>N-enrichment of soil NO<sub>3</sub><sup>-</sup> increased with increasing precipitation (Fig. 3b), suggesting that denitrification  
236 may become more favourable with increasing precipitation. In addition, our preliminary study of a <sup>15</sup>N-labeled NO<sub>3</sub><sup>-</sup>  
237 incubation experiment showed that potential N<sub>2</sub> losses via denitrification were also increasing with increasing precipitation  
238 in the semiarid soils (Liu and Fang, unpublished data). Because gaseous N losses occur during both nitrification (more on  
239 this later) and denitrification, the coupled nitrification and denitrification could maintain soil NO<sub>3</sub><sup>-</sup> concentration low while  
240 enriching its <sup>15</sup>N signal. These results support the idea that gaseous N losses is increasing as precipitation increases in  
241 dryland ecosystems (Wang et al., 2014).

242 In the arid zone, the  $\delta^{15}\text{N}$  and <sup>15</sup>N-enrichment of soil NO<sub>3</sub><sup>-</sup> also increased with increasing precipitation (Fig. 2f and 3b),  
243 indicating that denitrification may also take place. However, in these arid soils the microbial gene abundances were much  
244 lower (Fig.4), suggesting lower biological activities. It is therefore more likely that microbial denitrification is only a minor  
245 process in arid zone soils, and may only occur after a large rain event. Microbial denitrification has been observed in  
246 hotspots after heavy precipitation events in some desert soils (Abed et al., 2013; Zaady et al., 2013). Alternatively,  
247 chemodenitrification may attribute to soil NO<sub>3</sub><sup>-</sup> losses in the arid zone. Chemodenitrification is an abiotic process, in which  
248 the reduction of NO<sub>3</sub><sup>-</sup> or NO<sub>2</sub><sup>-</sup> to NO and N<sub>2</sub>O is coupled to the oxidation of reduced metals (e.g. Fe (II)) and humic  
249 substances (Medinets et al., 2015; Zhu-Barker et al., 2015). Ample soil NO<sub>3</sub><sup>-</sup> was present in some arid zone soils (Fig. 2c),  
250 meanwhile our companion work also observed higher available Fe in arid zone soils (Luo et al., 2016). In a recent

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

253 Different from the  $\delta^{15}\text{N}$  of soil  $\text{NO}_3^-$ , the  $\delta^{15}\text{N}$  values of soil  $\text{NH}_4^+$  and their relative  $^{15}\text{N}$  enrichment were higher in the  
254 arid zone than those in the semiarid zone (Fig. 2e and 3a), suggesting losses of  $\text{NH}_4^+$  in the drier sites. We suggest that  $\text{NH}_3$   
255 volatilization should play a significant role in  $\text{NH}_4^+$  losses, because soil pH was higher in the arid zone (from 7.3 to 9.7; Fig.  
256 6a). The isotopic effect of  $\text{NH}_3$  volatilization had been reported to be 40-60‰ (Robinson, 2001), resulting in  $^{15}\text{N}$ -enriched  
257 soil  $\text{NH}_4^+$ . The significant negative correlation between the  $\delta^{15}\text{N}$  values of  $\text{NH}_4^+$  and soil pH in this study (Fig. 6b) supported  
258 our interpretation. Besides, despite the low microbial gene abundance, nitrification may be able to occur in the arid zone  
259 soils. Although nitrifiers are sensitive to water availability, they can remain active in thin water films and result in higher  
260 potential nitrification in dry soils (Sullivan et al., 2012). In the process of nitrification, NO losses occur via a “hole-in-the-  
261 pipe” mechanism (Firestone and Davidson, 1989). In addition, nitrite ( $\text{NO}_2^-$ ), produced from nitrification, can be reduced  
262 rapidly to NO via chemodenitrification. The reaction of chemodenitrification forms NO via nitrous acid ( $\text{HNO}_2$  (aqueous  
263 phase), HONO (gas phase)) decomposition (Medinets et al., 2015). Alternatively, nitrifier denitrification can also serve as a  
264 mechanism for NO emission by the reduction of  $\text{NO}_2^-$  upon the recovery of nitrifiers from drought-induced stress (Homyak  
265 et al., 2016).

266 In the semiarid zone, soil  $\text{NH}_4^+$  became gradually depleted in  $^{15}\text{N}$  relative to the bulk soil N (Fig. 3a), suggesting the  
267 input of  $\text{NH}_4^+$  (e.g., soil ammonification, N deposition), while simultaneously  $\text{NH}_4^+$  was also consumed.  $\text{NH}_3$  volatilization  
268 should be low due to relatively lower pH compared to those in the arid zone soils (Fig. 6a). Previous studies have found that  
269 water addition did not stimulate  $\text{NH}_3$  volatilization (Yahdjian and Sala, 2010), although a recent study observed the opposite  
270 result in a semiarid subtropical savanna (Soper et al., 2016). With increasingly available water, biological N consumption by  
271 plants and microbes would dominate soil  $\text{NH}_4^+$  sinks in the semiarid zone. The increased aboveground biomass with  
272 increasing MAP suggests a higher net plant N accumulation along this precipitation gradient (Wang et al., 2014). Since soil  
273  $\text{NH}_4^+$  concentration was higher relative to soil  $\text{NO}_3^-$  in the semiarid zone ( $P < 0.001$ ), the dominant plant species might adapt  
274 to use soil  $\text{NH}_4^+$  over  $\text{NO}_3^-$ . This is in accordance with the observed relationship of the  $\delta^{15}\text{N}$  values of plant leaves (non-N  
275 fixing species) with soil  $\text{NH}_4^+$  ( $R^2=0.40$ ; Fig. 7a), but not with soil  $\text{NO}_3^-$  (Fig. 7b). When we plot this correlation for each  
276 plant species, three dominant species (*Stipa* spp., *Cleistogenes* spp., and *Reaumuria* spp.) were significantly correlated with  
277 soil  $\text{NH}_4^+$ . Besides, plant internal N cycling likely shifts as a function of water availability and would influence foliar  $\delta^{15}\text{N}$   
278 and such relationship, but the extent is hard to estimate at this stage. Plant N uptake may also exert fractionation effect on  
279 their N sources, but it might be negligible in the N limited areas (Craine et al., 2015). This may in part explain no strong  $^{15}\text{N}$ -  
280 enrichment of soil  $\text{NH}_4^+$  with increasing precipitation. The nitrification consumption of  $\text{NH}_4^+$  could also increase as indicated  
281 by the microbial gene abundance along the precipitation gradient (Fig. 4). The coupled nitrification and denitrification in the  
282 semiarid zone could lead to N loss and the  $^{15}\text{N}$  enrichment of soil  $\text{NO}_3^-$ , without significantly altering  $\text{NO}_3^-$  concentration.  
283 On the other hand, enhanced plant uptake (of both soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) would diminish soil inorganic N pools and greatly  
284 reduce gaseous N losses through either nitrification (Homyak et al., 2016) or denitrification.

285 Unexpectedly, we detected high anammox gene abundance in these dryland ecosystems (Fig. 4). Anammox is the  
286 microbial reaction between  $\text{NH}_4^+$  and  $\text{NO}_2^-$  with  $\text{N}_2$  as the end product (Thamdrup and Dalsgaard, 2002). Previous studies  
287 have found equal consumption of both soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  through anammox in N-loaded and water-logged areas (Yang et  
288 al., 2014; Zhu et al., 2013). However, the only two anammox studies in drylands so far failed to confirm its importance  
289 (Abed et al., 2013; Strauss et al., 2012). Thus, although anammox possesses a fractionation effect of 23-29‰ (Brunner et al.,  
290 2013), it is difficult to tell its significance in our study transect at the present time.

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

#### 296 4.2 Sources of soil $\text{NO}_3^-$ and $\text{NH}_4^+$

297 We observed much higher concentrations of soil  $\text{NO}_3^-$  in the arid zone (Fig. 2c), on average about 20 times higher than those  
298 in the semiarid zone. Nitrate can be formed via microbial nitrification, deposited from N-bearing gaseous (e.g.,  $\text{HNO}_3$ ) or dry  
299 aerosol  $\text{NO}_3^-$  (Kendall et al., 2007), plus as dissolved nitrate in rainwater or snow. If  $\text{NO}_3^-$  is formed by nitrification,  $\text{NO}_3^-$   
300 will obtain its O atoms from soil  $\text{O}_2$  and  $\text{H}_2\text{O}$  in a 1:2 ratio (Kendall et al., 2007). The  $\delta^{18}\text{O}$  value of atmospheric  $\text{O}_2$  is  
301 relatively stable (23.5‰; we assume that the isotope composition of  $\text{O}_2$  in the atmosphere and soils are the same). The  $\delta^{18}\text{O}$   
302 value of nitrified  $\text{NO}_3^-$  will depend on the  $\delta^{18}\text{O}$  value of the local water. The  $\delta^{18}\text{O}$  values of rainwater taken from the areas  
303 closest to the arid zone of our dryland transect (Lanzhou City and its surrounding areas) ranged from -19.1 to 5.2‰ (Chen et  
304 al., 2015), that would yield the corresponding  $\delta^{18}\text{O}$  of nitrified  $\text{NO}_3^-$  from -5.3 to 11.3‰ (Fig. 5a). However, the  $\delta^{18}\text{O}$  values  
305 of soil  $\text{NO}_3^-$  in the arid zone varied from 5.5 to 51.8‰ (Fig. 5a). This disparity between calculated and measured  $\delta^{18}\text{O}$  values  
306 provides evidence for the minor importance of nitrification. The higher  $\delta^{18}\text{O}$  values of soil  $\text{NO}_3^-$  we observed in the arid zone  
307 have rarely been reported for nitrified  $\text{NO}_3^-$ , according to previous studies (Kendall et al., 2007). For example, an *in situ*  
308 study conducted in the forest floor soils found that  $\delta^{18}\text{O}$  values of nitrified  $\text{NO}_3^-$  changed from 3.1 to 10.1‰ (Spoelstra et al.,  
309 2007). By comparison, atmospheric origin  $\text{NO}_3^-$  normally has higher  $\delta^{18}\text{O}$  values because of the chemical oxidation of  $\text{NO}_3^-$   
310 precursor,  $\text{NO}_x$  ( $\text{NO}$  and  $\text{NO}_2$ ) (Fang et al., 2011). Previous research found that  $\delta^{18}\text{O}$  values of aerosol  $\text{NO}_3^-$  ranged from 60  
311 to 111‰ in the Dry Valleys of Antarctica (Savarino et al., 2007). This combined information supports the hypothesis that a  
312 sizable fraction of  $\text{NO}_3^-$  in the surface soils of the arid zone were from the atmospheric deposition. Nitrate would be  
313 accumulated on the surface soil when experiencing prolonged droughts, as also reported in northern Chile and southern  
314 California sites (Böhlke et al., 1997), as well as in the Turpan-Hami area of northwestern China (Qin et al., 2012). A  
315 pronounced trend in Figure 5a (green arrow) toward higher  $\delta^{18}\text{O}$  and lower  $\delta^{15}\text{N}$  values is obvious for elevated  $\text{NO}_3^-$   
316 concentrations, which might be the results of mixed  $\text{NO}_3^-$  from both soil nitrification and atmospheric deposition, as  
317 previously observed in groundwater of Saharan desert (Dietzel et al., 2014). In the arid zone, extreme dryness and high

318 alkalinity (with an average pH of 8.3) might limit microbial activities, as suggested by the low gene abundance involving N  
319 transformation (Fig. 4), thus facilitate the preservation of  $\text{NO}_3^-$ .

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

329 There was  $\text{NH}_4^+$  accumulation in the arid zone soils and the accumulated  $\text{NH}_4^+$  was characterized with higher  $^{15}\text{N}$ -  
330 enrichment (Fig. 2b, e). Ammonium has been shown to be the dominant species in bulk N deposition in China (Liu et al.,  
331 2013). Dry deposition is generally the dominant form of deposition in arid climates (Elliott et al., 2009). It has been reported  
332 that the  $\delta^{15}\text{N}$  values of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in dry deposition were higher than those in wet deposition (Elliott et al., 2009; Garten,  
333 1996; Heaton et al., 1997) and might contribute to the observed  $^{15}\text{N}$ -enrichment. Our preliminary study also showed that  
334  $\delta^{15}\text{N}$  values of aerosol  $\text{NH}_4^+$  in one arid site (Dunhuang in Gansu Province, MAP = 46 mm) in northwestern China ranged  
335 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  
336 Japan (Kawashima and Kurahashi, 2011), where the  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$  in suspended particulate matter ranged from 1.3 to 38.5‰  
337 with the average of 11.6‰. It remains unclear why the  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$  in dry deposition is so positive, but it may result from  
338 the isotope exchange of atmospheric ammonia gas and aerosol  $\text{NH}_4^+$ , which causes aerosol  $\text{NH}_4^+$  enriched in  $^{15}\text{N}$  (with an  
339 isotope effect of 33‰, (Heaton et al., 1997)). In the drylands, biological N fixation is another important N input (Evans and  
340 Ehleringer, 1993). In this study, we speculated that biological N fixation by biological soil crusts (BSCs) could contribute to  
341 soil  $\text{NH}_4^+$  pool and soil organic N, and the contribution may be proportionally more important with decreasing precipitation.  
342 We find that the  $\delta^{15}\text{N}$  of bulk soil N decreased to close to zero with decreasing precipitation, notice that biological N fixation  
343 provided  $\text{NH}_4^+$  with the  $\delta^{15}\text{N}$  value around zero. BSCs were observed during soil sampling in the arid zone. A previous  
344 research has also reported the potential N-fixing activity and ecological importance of BSCs in soil stability and N  
345 availability in the grasslands of Inner Mongolia (Liu et al., 2009).

346 In the semiarid zone with MAP from 100 mm to 200 mm, soil  $\text{NH}_4^+$  concentrations were lower than those in the arid  
347 zone, which may be caused by a tight coupling between microbial production of  $\text{NH}_4^+$  and plant consumption (Nielsen and  
348 Ball, 2015). Soil  $\text{NH}_4^+$  was depleted in  $^{15}\text{N}$  relative to bulk soil N and their differences in  $\delta^{15}\text{N}$  increased with increasing  
349 MAP (Fig. 3a), likely due to gradually enhanced N mineralization in less dry soils. The isotope effect of N mineralization  
350 might be also higher than commonly expected. Our laboratory recently reported that  $^{15}\text{N}$  fractionation during mineralization

351 was up to 6-8‰ in two forest soils in northern China (Zhang et al., 2015). The fractionation during mineralization can be  
352 even as high as 20‰ if one looks at the enzyme level (Werner and Schmidt 2002). With increasing water availability in  
353 semiarid zone (MAP > 200 mm), N turnover linking biological uptake (plant and microbes) of N and return could further  
354 enhance and progressively fuel soil ammonification, which in turn result in lower  $\delta^{15}\text{N}$  in soil  $\text{NH}_4^+$ . In addition, there is also  
355 a possibility of dissimilatory nitrate reduction to ammonium (DNRA), although we did not measure this process in our study.  
356 DNRA is even less sensitive to oxygen level than denitrification and therefore may occur in aerobic soils (Müller et al.,  
357 2004), contributing to the availability of soil  $\text{NH}_4^+$ .

## 358 5 Summary

359 To the best of our knowledge, our study reported, for the first time, the pattern of  $\delta^{15}\text{N}$  in soil inorganic N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ )  
360 across a precipitation gradient from very arid land to semiarid grassland. Together with the analysis of soil N concentration,  
361 soil properties like pH and soil moisture, and functional gene abundance, the compound-specific  $\delta^{15}\text{N}$  analyses presented  
362 here demonstrate a clear shifting contribution of *abiotic vs. biotic* (microbes and plants) controls on N cycling along this  
363 3200 km dryland transect in China.

364 In the arid zone with extreme aridity (36 mm < MAP < 100 mm; Fig. 8a), plant cover is sparse and microbial activity is  
365 limited (Fig. 1 and 4). Nitrogen input, mostly in the form of atmospheric deposition, is largely accumulated, creating  
366 "enriched" inorganic N pools despite a much smaller pool of soil total N. The accumulation of inorganic N drives abiotic  
367 processes that lead to N losses with strong isotopic fractionation effect on the remaining soil N. Higher pH associated with  
368 lower MAP is likely a dominant driver of  $\text{NH}_3$  volatilization, causing soil  $\text{NH}_4^+$  enriched in  $^{15}\text{N}$ . The very high yet variable  
369  $\text{NO}_3^-$  accumulation in soil comparing to  $\text{NH}_4^+$  suggests limited  $\text{NO}_3^-$  loss under extreme aridity.

370 In the semiarid zone (100 mm < MAP < 436 mm; Fig. 8b), controls on N cycling increasingly shift from abiotic to  
371 biotic factors. Microbial gene abundances associated with N cycling groups were much higher when water became more  
372 available (Fig. 3). Increasing N mineralization with increasing MAP, accompanied with reduced  $\text{NH}_3$  volatilization due to  
373 lower pH, produce soil  $\text{NH}_4^+$  pool with lighter N isotopes. Ammonification (N mineralization) supplies  $\text{NH}_4^+$  for both plant  
374 uptake and favour soil nitrification. Both nitrification and denitrification could lead to N loss and isotopically enrich  
375 remaining soil N. Soil heterogeneity and pulse precipitation events could provide hotspots for these microbial processes,  
376 while increased plant cover and N uptake could reduce soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  pools and minimize overall N losses. The abiotic  
377 vs. biotic controls on N cycling and N losses around a threshold of MAP 100 mm suggest global climate changes, in  
378 particular the change of precipitation pattern, would have great impact on these dryland ecosystems.



379 **Author contribution**

380 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  
381 performed the experiment; D. Liu, W. Zhu, Y. Fang, X. Wang, Y. Pan, C. Wang, D. Xi, E. Bai and Y. Wang analysed the  
382 data. D. Liu, W. Zhu, and Y. Fang wrote the manuscript; X. Wang, Y. Pan, C. Wang, E. Bai, and X. Han contributed to  
383 discussion of the results and manuscript preparation.

384 **Acknowledgements**

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

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597

598 **Figure captions**

599

600 **Figure 1.** Vegetation types and sampling sites distribution along the transect. Across the 3200 km precipitation gradient in  
601 northern China, four typical vegetation types are distributed from west to east, which are desert (a), desert steppe (b), typical  
602 steppe (c), and meadow steppe (d), and the dominant plant genera change gradually from shrub (*Nitraria* spp., *Reaumuria*  
603 spp., and *Salsola* spp.) to perennial grasses (*Stipa* spp., *Leymus* spp., and *Cleistogenes* spp.). Soil types are predominantly  
604 arid, sandy, and brown loess rich in calcium from west to east of the transect. A total of 36 soil sampling sites were selected.  
605

606 **Figure 2.** Nitrogen concentrations and isotopic composition of bulk soil N,  $\text{NH}_4^+$ , and  $\text{NO}_3^-$ . The significant ( $P < 0.05$ )  
607 trends are shown with a regression line (red) and 95% confidence intervals (blue). In each site,  $n = 5$ .  
608

609 **Figure 3.** The relative  $^{15}\text{N}$  enrichment of soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . The relative  $^{15}\text{N}$  enrichment of soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were  
610 calculated as the difference between  $\delta^{15}\text{N}$  of bulk soil N and  $\text{NH}_4^+$ , and between  $\delta^{15}\text{N}$  of soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , respectively.  
611 The significant ( $P < 0.05$ ) trend is shown with a regression line (red) and 95% confidence intervals (blue). In each site,  $n = 5$ .  
612

613 **Figure 4.** Changes in the abundance of microbial gene involved in N cycling. Signal intensity was standardized based on  
614 both the number of array probes and DNA quantity in a gram of dry soil. Data are the site-averaged value; results of the  
615 abundance of nitrification and denitrification genes have been reported in a previous study (Wang et al., 2014). The  
616 significant ( $P < 0.05$ ) trends are shown with a regression line (red) and 95% confidence intervals (blue).  
617

618 **Figure 5.** Relationship between  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  of soil  $\text{NO}_3^-$ . The range of  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  from atmospheric  $\text{NO}_3^-$  was based  
619 on the limited isotope measurement of precipitation. Black points represent precipitation  $\text{NO}_3^-$  collected from an urban site  
620 in Beijing in the year of 2012, with data derived from Tu et al. (2016). Grey points represent precipitation  $\text{NO}_3^-$  collected  
621 from Qingyuan forest CERN (Chinese Ecosystem Research Network, CERN) in Northern China in the year of 2014 (Huang  
622 and Fang, unpublished data). The range of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  produced by nitrified  $\text{NO}_3^-$  are positioned by using the  $\delta^{15}\text{N}$  of soil  
623  $\text{NH}_4^+$  in this study (Fig. 2e), and the estimated  $\delta^{18}\text{O}$  from soil nitrification based on the 1:2 ratio of soil  $\text{O}_2$  and  $\text{H}_2\text{O}$  (see  
624 Text), respectively.  
625

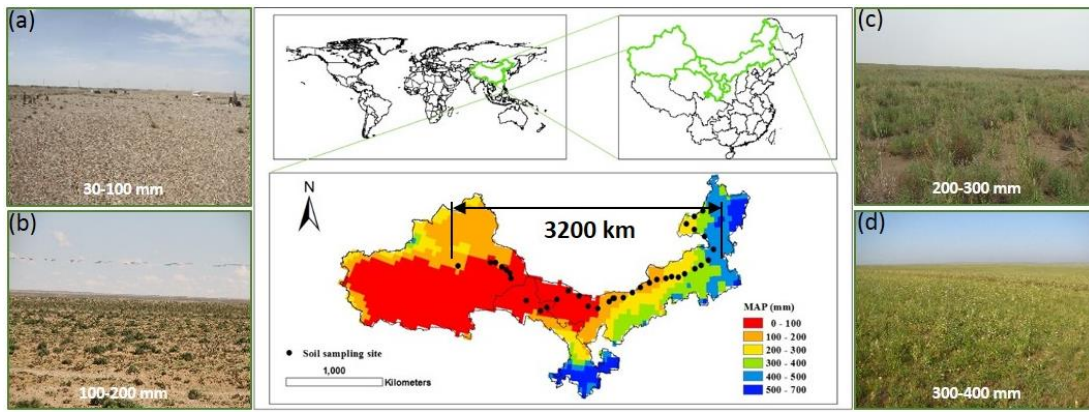
626 **Figure 6.** Soil pH and the relationship with  $\delta^{15}\text{N}$  of soil  $\text{NH}_4^+$ . The different patterns of soil pH was observed above and  
627 below the threshold at MAP of about 100 mm; data were derived from Wang et al. (2014). There was a positive correlation  
628 between  $\delta^{15}\text{N}$  of soil  $\text{NH}_4^+$  and pH across the transect. The significant ( $P < 0.05$ ) trend is shown with a regression line (red)  
629 and 95% confidence intervals (blue). In each site,  $n = 5$ .  
630

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

635

636 **Figure 8.** A framework of N biogeochemical cycling in dryland ecosystems in northern China. Width of arrows and size of  
637 boxes indicate the relative importance (**qualitative interpretation**) of soil N processes and pools between the arid zone (a) and  
638 semiarid zone (b). The mean pool sizes ( $\text{g N m}^{-2}$ ) of each soil N pool based on the bulk soil density of top 10 cm were  
639 present in the brackets.

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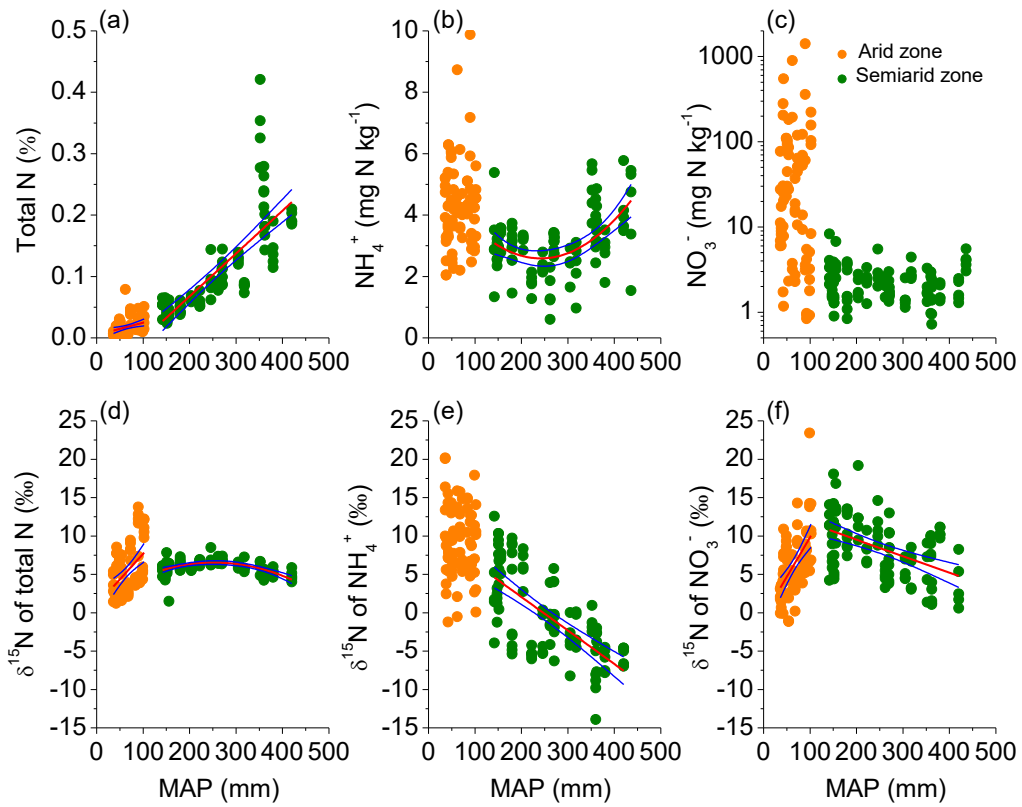


641

642 **Figure 1**

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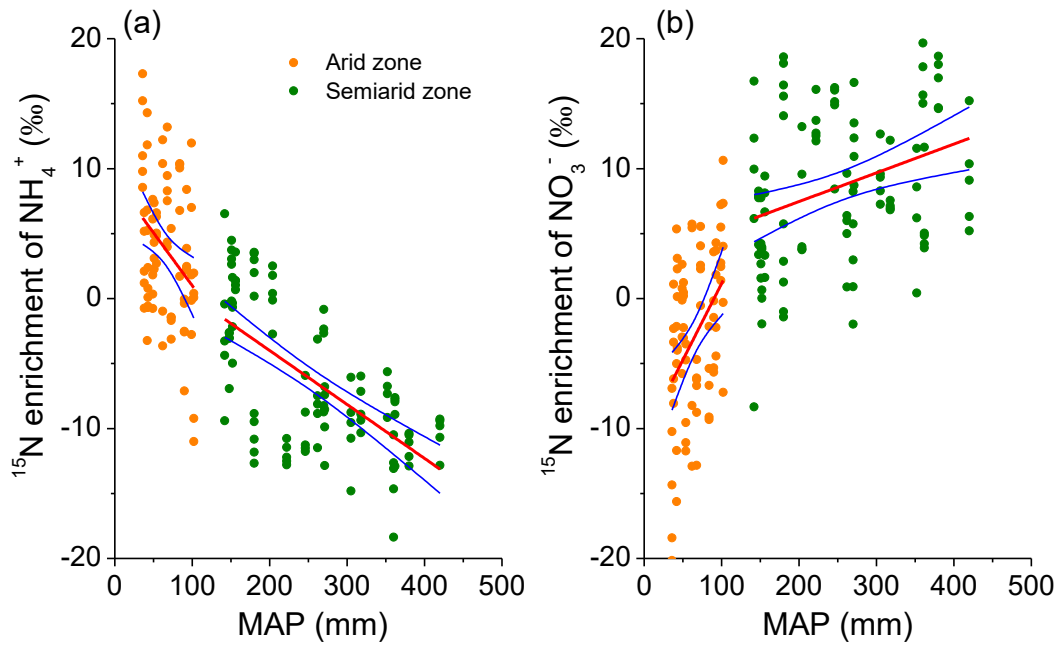


644

645 **Figure 2**

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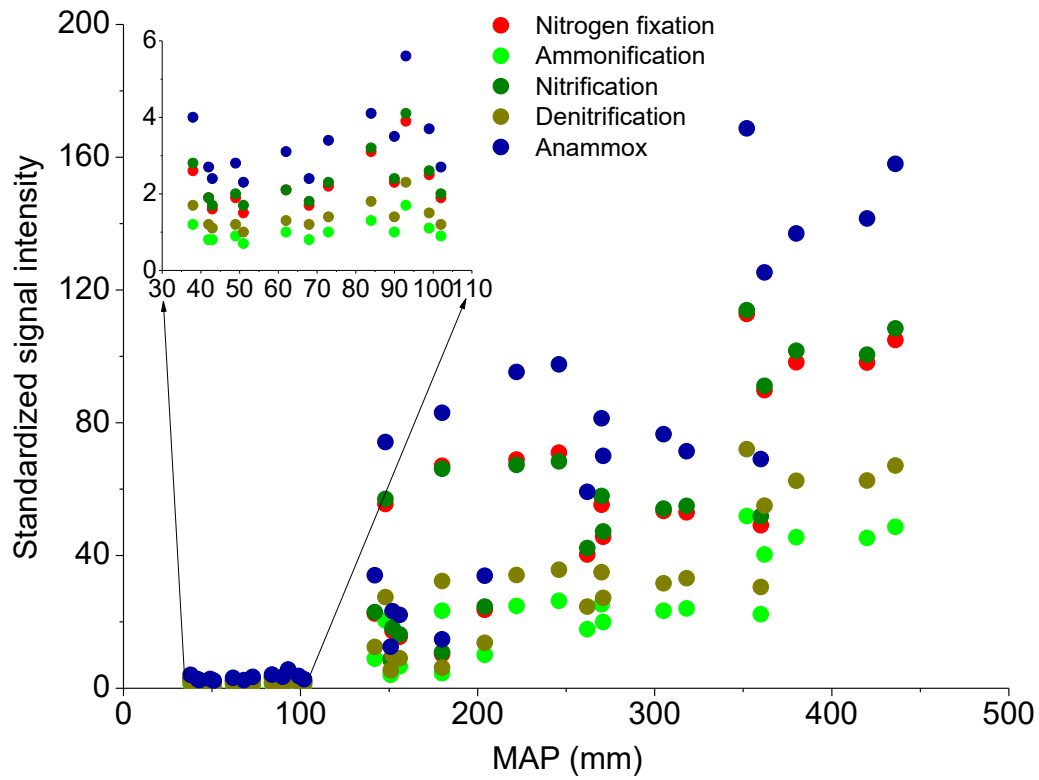
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649 **Figure 3**

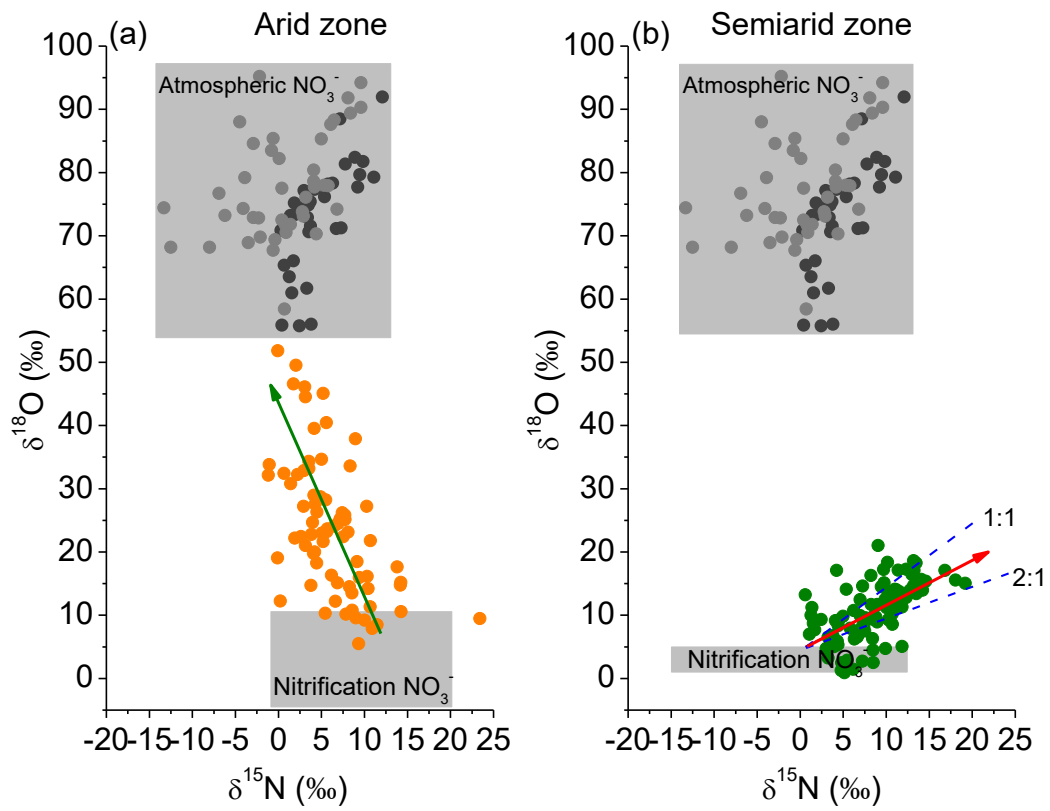
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652 **Figure 4**

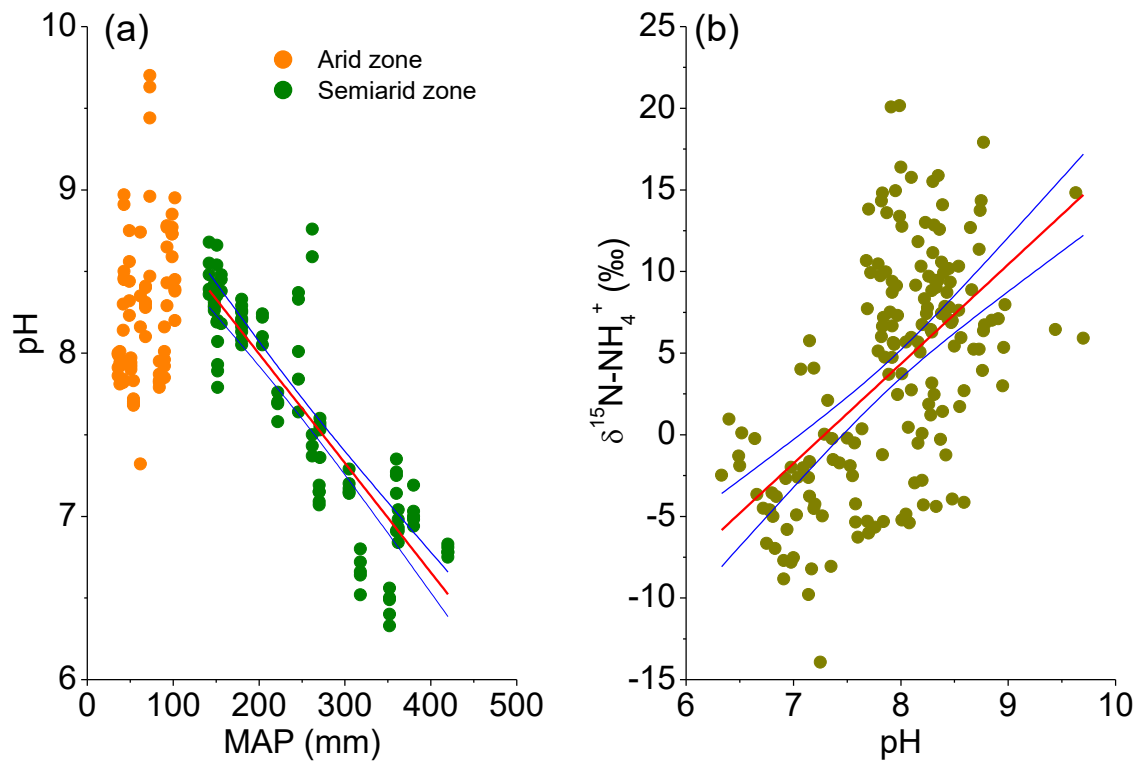
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655 **Figure 5**

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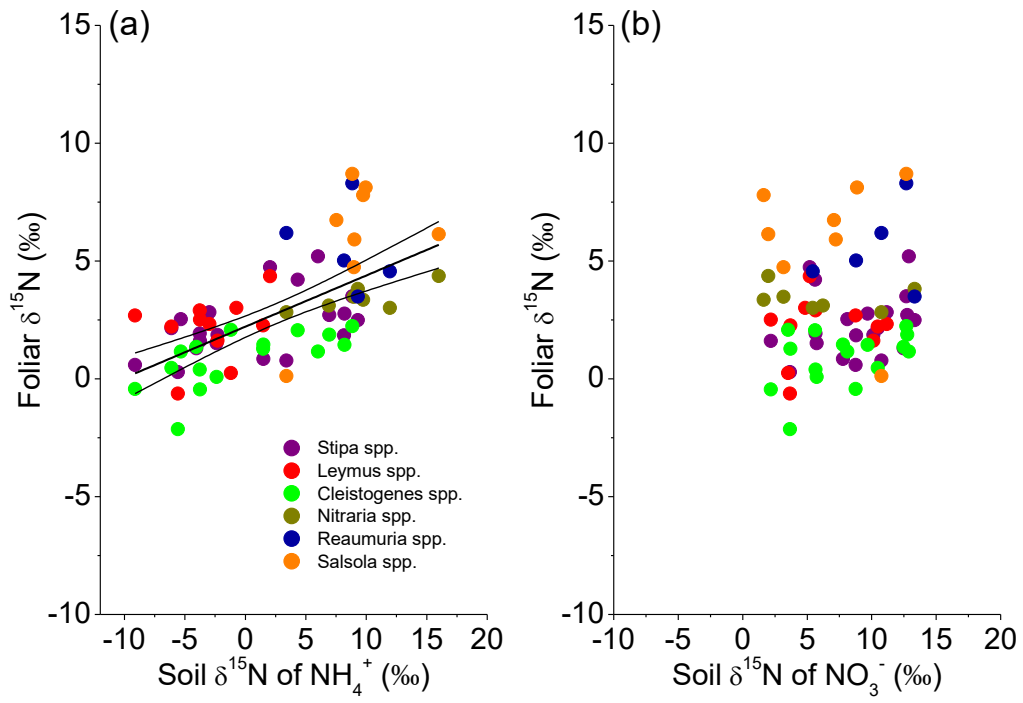


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659 **Figure 6**

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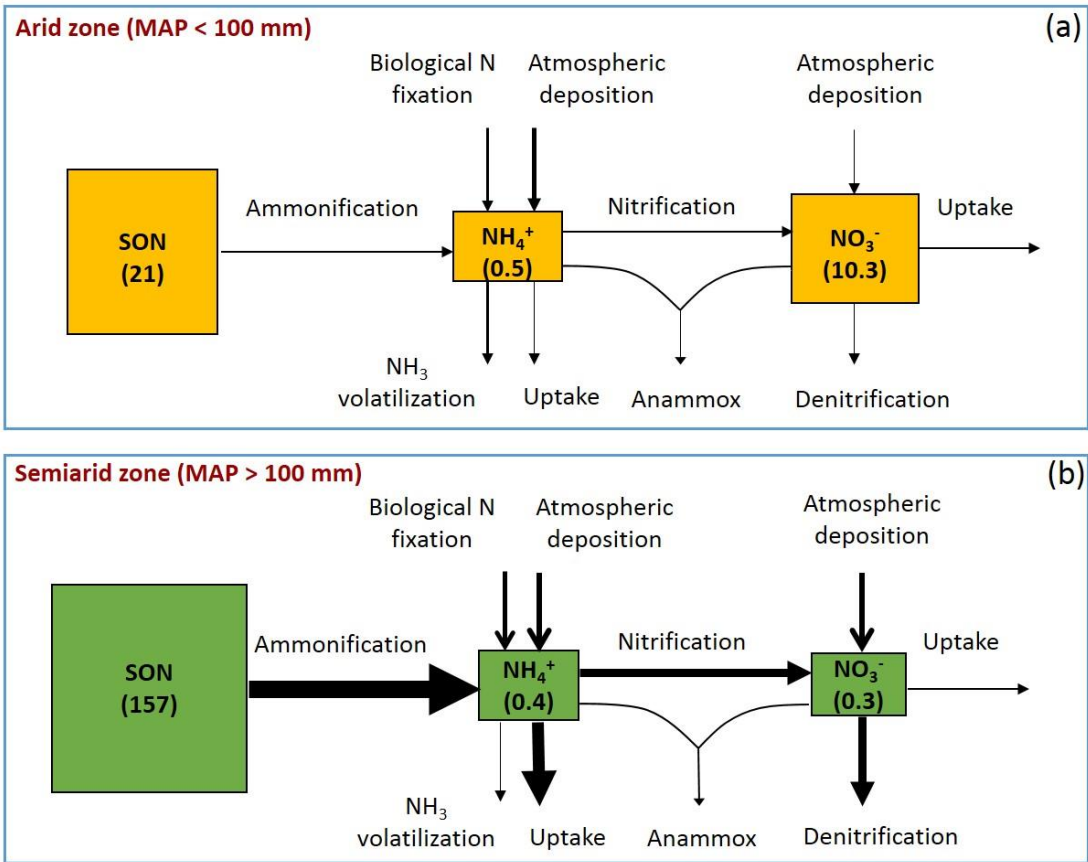
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662

663 **Figure 7**

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665

666 **Figure 8**

667

668