

## ***Interactive comment on “Abiotic versus biotic controls on soil nitrogen cycling in drylands along a 3200 km transect” by Dongwei Liu et al.***

**Dongwei Liu et al.**

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Dear editor,

It is really our honor to have so many constructive comments and suggestions on our manuscript from the reviewer #2. The kind corrections on writing encourage us to improve our current and future work. In the revised version of this manuscript, we will fix many sentences according to the suggestions, and improve the English writing by asking a native speaker to correct our revised manuscript before resubmission. Please find our specific revisions to each comments below.

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

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China, based on the natural  $^{15}\text{N}$  ( $^{18}\text{O}$ ) 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 high regard on our work. In the revised version of the manuscript, we will make many efforts to improve the writing.

L36: should read “driving” not driven;

Reply: Will be changed as suggested.

L39: delete significantly;

Reply: Will be changed as suggested.

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

Reply: Will be changed as suggested.

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

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Reply: Yes, that is true. Leaching is also 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: Will be changed as suggested.

L51: rewrite “factor” not factors;

Reply: Will be changed as suggested.

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

Reply: Will be changed as suggested.

L61: rewrite “over large scales”;

Reply: Will be changed as suggested.

L67: change “are” to “become”

Reply: Will be changed as suggested.

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

Reply: Will be changed as suggested.

73: change to “.. alone is not..”

Reply: Will be changed as suggested.

74: change to “processes that contribute”

Reply: Will be changed as suggested.

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

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Reply: This sentence will be modified as “Isotopes in ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) can serve as a proxy record for the N processes in soils because they directly respond to in situ processes and integrate their N cycling characteristics across temporal and spatial scales”.

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

Reply: Will be changed as suggested.

81: “they cover a different range”.

Reply: Will be changed as suggested.

83/84: rewrite “..to study the preferences for plant N uptake”

Reply: Will be changed as suggested.

105: change to “gradient”.

Reply: Will be changed as suggested.

109: “gene abundances”.

Reply: Will be changed as suggested.

111/112: “with microbially regulated soil processes; and 3) how does soil N cycling: : :”.

Reply: Will be changed as suggested.

116: “the climate is: : :”.

Reply: Will be changed as suggested.

118: define the aridity index here.

Reply: It will be changed as suggested. ‘Aridity index (the ratio of precipitation to potential evapotranspiration) increased from 0.04 to 0.60’.

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120: “: : :the three : : :”

Reply: Will be changed as suggested .

124: 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 will be revised as ‘Soil sampling was conducted from July to August in 2012, around the peak of plant growing season’.

131: correct“into” to “in”, twice.

Reply: Will be changed as suggested.

134: “using a pH meter”.

Reply: Will be changed as suggested.

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

Reply: Will be changed as suggested.

142/143: change “into” to “to”, three times.

Reply: Will be changed as suggested.

146: rewrite “samples”.

Reply: Will be changed as suggested.

148:change “to a Trace: : :”.

Reply: Will be changed as suggested.

168: change to “Pearson correlation analysis”

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Reply: Will be changed as suggested.

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

Reply: Will be changed as suggested.

175: “genes”.

Reply: Will be changed as suggested.

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

Reply: Will be changed as suggested.

185: “was significantly higher: : :”. By the way if I get the numbers correct in the arid zone bulk soil 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: 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  $^{18}\text{O}$  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 also has been observed in the desert soils of northern China (Qin et al. 2012) and northern Chile (Michalski et al. 2004). We will make that clear in our discussion and contrast that to N cycling pattern in the semiarid zone.

188: “supports”.

Reply: Will be changed as suggested.

203: “ $^{15}\text{N}$  depleted relative to their sources”.

Reply: Will be changed as suggested.

205: please specify what you mean with “via microbial and plant regulation”.  $^{15}\text{N}$  depletion of soil ammonium or less  $^{15}\text{N}$  enrichment can arise from microbial N min-

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eralization (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 will be 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  $\text{NH}_4^+$ .' In the later discussion in the manuscript, we will also discuss 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 resulted from the exchange of atmospheric ammonia gas and aerosol ammonium (Heaton et al. 1997).

207: rewrite "A positive correlation was : :"

Reply: Will be changed as suggested.

212: "genes"

Reply: Will be changed as suggested.

213: "rewrite "was measured at all sites"

Reply: Will be changed as suggested.

214: "were found to be . . ."

Reply: Will be changed as suggested.

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

Reply: Will be changed as suggested.

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

Reply: Will be changed as suggested.

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

Reply: Will be changed as suggested .

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

Reply: Will be changed as suggested.

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

Reply: The sentence will be modified as ‘We observed different patterns of N cycling above and below a MAP threshold of 100 mm in this transect’.

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. It will be changed as suggested.

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

Reply: Will be changed as suggested.

226: “we found direct evidence”.

Reply: Will be changed as suggested.

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 will be modified as ‘Microbial denitrification exerts

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strong isotopic isotopes fractionation against the isotopically heavier compounds, ranging between 5 and 25 ‰ for nitrate nitrogen and oxygen’.

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 will be modified as ‘Denitrification rate is regulated by proximal factors that immediately affect denitrifying communities, such as NO<sub>3</sub>– concentration and O<sub>2</sub> concentration’.

235: “in addition, a preliminary study: : ∴ an increasing N<sub>2</sub> loss via: : ∴”

Reply: Will be changed as suggested.

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

Reply: Will be changed as suggested.

241: “after heavy precipitation events”.

Reply: Will be changed as suggested.

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 δ<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 (Fe<sup>II</sup>) 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 ‰).

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Reply: Thank you. We agree with you that  $^{15}\text{N}$  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  $^{15}\text{N}$ . Weak denitrification in arid region may have also contributed to low  $^{15}\text{N}$  values in soil nitrate. However, we think that main source of soil nitrate in arid region is atmospheric deposition, as indicated by  $^{18}\text{O}$  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 will make this clear in the revised manuscript.

243: “chemodenitrification is an abiotic process..”

Reply: Will be changed as suggested.

244: change “preserved” to “present”

Reply: Will be changed as suggested.

247: “suggesting losses of : :”

Reply: Will be changed as suggested .

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

Reply: Sorry for the confusing. The sentence will be modified as ‘Because soil pH was higher in the arid zone (from 7.3 to 9.7; Fig. 6a),  $\text{NH}_3$  volatilization would play a significant role in  $\text{NH}_4^+$  losses’.

249: “The isotope effect of: : :”

Reply: Will be changed as suggested .

250 “significant negative: : :”

Reply: Will be changed as suggested .

250/251: the alternate explanation is that nitrification can also cause  $^{15}\text{N}$  enrichment

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of ammonium, and  $^{15}\text{N}$  depleted nitrate in many arid soils actually point to a significant role of this process, aside of ammonia volatilization.

Reply: Accepted.

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

Reply: The sentence will be fixed as ‘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 contribution of  $\text{NH}_4^+$  input processes, such as soil ammonification in this N limited areas.’

252: soil ammonium “became” gradually  $^{15}\text{N}$  depleted relative to: : :

Reply: Done.

252-270: the main pattern of soil ammonium is that it becomes  $^{15}\text{N}$  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  $^{15}\text{N}$  depleted and soil ammonium  $^{15}\text{N}$  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

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plant N uptake and nitrification exert isotope effect. However, they may be different in different area. 1) This study area 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 the 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. Please also see question to line 319-323. 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 as we thought before. We will correct that paragraph regarding this issue.

259: “prefer soil ammonium over nitrate”.

Reply: Will be changed as suggested.

263: “demonstrates the ammonium preference of plants”.

Reply: Will be changed as suggested.

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”.

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Reply: Will be changed as suggested.

275: “water-logged”.

Reply: Will be changed as suggested.

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

Reply: Will be changed as suggested.

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

Reply: Will be changed as suggested.

282: “aeolian”.

Reply: Will be changed as suggested.

285: “observed the highest: : :”.

Reply: Will be changed as suggested.

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

Reply: Accepted. It will be incorporated into the main text.

288: “since the d18O: : :”.

Reply: Will be changed as suggested.

289: “depends on the d18O: : :”.

Reply: Will be changed as suggested.

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

Reply: Will be changed as suggested.

291: “ranged from: : : to: : :”.

Reply: Will be changed as suggested.

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294: I don't understand the reasoning behind this sentence, why is atm. O<sub>2</sub> and its d<sup>18</sup>O important. It is not directly expressed in the d<sup>18</sup>O 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 will be fixed as 'In addition, 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'.

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 contribute to soil nitrate accumulation, aside of atmospheric deposition. There are several typos in this paragraph.

Reply: Accepted. The processes of soil nitrification will be incorporated into our revised manuscript. Please also see our response to line 239-245. In addition, we will make efforts to improve writing.

316-318: what does this coincidence of KIE denitrification and d<sup>18</sup>O 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 nitrifiers 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. See responses above. Thank you!

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

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Reply: Besides of increasing ammonium concentration, we also observed that ammonium  $^{15}\text{N}$  was more depleted relative to bulk soil N with higher MAP.

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 will be modified as 'To the best of our knowledge, our study for the first time showed the pattern of  $\delta^{15}\text{N}$  in soil inorganic N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) across a precipitation gradient in drylands'.

355: what is phytochemical nitrate loss?

Reply: It is a typo. It should be photochemical nitrate loss.

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 will be modified as 'With the increasing of precipitation, both the stimulated ammonification and reduced  $\text{NH}_3$  volatilization (with low pH) may contribute to  $^{15}\text{N}$  depleted soil ammonium pool'.

Respectively,

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

References

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