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Interactive comment on "Relationships between leaf δ^{15}N and leaf metallic nutrients" *by* Chongjuan Chen et al.

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1. Comment: Chen and coauthors investigated the relationships between leaf δ15N and metallic nutrients across a large number of sites from northeast to southwest China. They found leaf δ15N was positively correlated with leaf K, Ca, Mg and Zn but negatively correlated with leaf Fe, and these correlations were not affected by vegetation and soil type. However, the relationship between δ15N and Mn was dependent on vegetation and soil type. This is an interesting study which examined the relationships between leaf δ15N and leaf metallic nutrients for the first time. I have no major concerns with the content of the manuscript. However, the paper needs a strong hand in English editing. The language is often not precise/exact, sometimes appears to be ordinary and has grammatical errors. I strongly suggest to critically check the grammars, read and polish the manuscript using exact language. I listed some examples below but more need to be revised. I would recommend for publishing if this and the following comments could be addressed. Answer: Thanks for your comments. We have corrected the grammatical errors according to your comments, and we will send our manuscript to professional company for polishing.

2. Specific comments: (1) Line 15: "Calcium" should be "calcium". Answer: Corrected. (2) Line 25: meteallic →metallic. Answer: Corrected. (3) Line 29: Should be attention. Attention is an uncountable noun. Answer: Very thanks. Corrected. (4) Line 29-32: Need to be rephased. Delete "deemed as". This sentence is too long. Split to two sentences. Answer: Very thanks. Corrected as follows, Nitrogen (N) cycling has received considerable attention, because N is the key element in regulating productivity of terrestrial ecosystems (Fay et al., 2015; Wieder et al., 2015) and many nitrogenous compounds generating from N cycling are associated with major environment issues (Bourgeois et al., 2018; Desmit et al., 2018). (5) Line 34: Change to "Revealing the potential factors that influence leaf δ15N and investigating the relationships between them could help improve our current understanding of N cycling". Answer: Corrected. (6) Line 37: Should be "Much attention has". Answer: Corrected. (7) Line 43: Change "have exposed" to "demonstrated". Answer: Corrected. (8) Line 46-47: To our knowledge, no report exists for the relationships... Answer: Corrected. (9) Line 55: "plays a vital". Answer: Corrected. (10) Line 52-66: This paragraph is poorly written and needs to be revised. Answer: Very thanks. Corrected as follows, K is the activator of many enzymes, it promotes photosynthesis and has important influences on nitrate and ammonium utilization in plants (Coskun et al., 2017; Zhang et al., 2010). Ca is involved in and plays a vital role in nitrate signaling network (Krouk et al., 2017; Liu et al., 2017). Mg is an essential component of chlorophyll and associated closely with nitrate reduction process in plants (Bose et al., 2011). Fe participates in many physiological processes in plants, such as nitrogen assimilation, photosynthesis, respiration, DNA synthesis, dinitrogenase synthesis (Balk and Pilon, 2011; Shokrollahi et al., 2018). Mn and Zn are the important components of enzymes, which are included

in carbohydrate metabolism, N metabolism and RNA synthetase (Mukhopadhyay and Sharma, 1991; Henriques et al., 2012). Accordingly, these metallic elements are essential for plant N metabolism processes. Because N metabolism processes, including acquire different N forms, nitrate and ammonium assimilation and allocation, could cause isotopic fractionation (Evans, 2001; Tcherkez and Hodges, 2008; Liu et al., 2014), we hypothesized that leaf δ15N should be related to the contents of these metallic nutrients. Thus, in order to confirm this hypothesis, we sampled more than 600 plant samples from mainland China and analyzed leaf δ15N and contents of leaf K, Ca, Mg, Fe, Mn and Zn. (11) Line 80-88: Where is the description for soil sampling? Answer: We are very sorry for that. We have changed “Plant and soil sampling” into “Plant sampling”, and considering that plant metallic nutrients and δ15N should be the main content, we have added the description for soil sampling and measurements in the Supplementary Information. The description of soil sampling and measurements were as follows, Surface mineral soils (0 – 5 cm) were sampled after removing the litter layer. At each location, three squares (0.5 m $\times$ 0.5 m) within a 200 m2 area were set to collect the mineral soils. Samples were sieved through a 2 mm sieve to remove stones and plant residues in soil. Soil samples were air-dried and divided into three parts. First part of samples was used to determine soil δ15N and N content. Similar to the measurement of δ15N and N content in leaves, the dried soil sample was ground into fine powder, and ïĄď15N and N contents of soils were also determined by a DeltaPlus XP mass spectrometer coupled with an automated elemental analyzer in a continuous flow mode. The second part of soil sample was used to determined soil texture (clay, silt and sand content) and pH. Soil texture was analyzed using a particle size analyzer (Malvern Masterizer 2000, UK) after removing the calcium carbonates and organic matter. Soil pH was analyzed by a pH electrode in soil-water suspension, with a soil/water ratio was 0.4 (10 g soil and 25 mL deionized water without carbon dioxide). The third part of soil sample was treated to determine the soil organic carbon (SOC) content, soil sample was immersed in 1 mol/L HCl for 24 h and was stirred four times to remove carbonate, then, the soil sample was washed to neutrality

using distilled water and oven-dried at 50 âĎČ. In addition, at each location, another three soil samples were collected using a ring to measure soil bulk density. Soil bulk density was determined after oven-drying at 105 $\pm$ 2 âĎČ to a constant weight, and it was the dry weight divided by the certain volume of the ring knife. (12) Line 164: should be “positively correlated to leaf K...”. Answer: Corrected. (13) Line 161-175: Check the use of “while” and “whereas”. Both of them are conjunction and should connect two sentences. Answer: Thanks very much. We have checked them. (14) Line 224: “Whereas”$\rightarrow$ “Nevertheless”. Answer: Corrected. (15) Line 255: “but also served as...”. Answer: Corrected. (16) Line 261: “leaf and roots, and then...”. Answer: Corrected. (17) Line 277-278: Are there any correlations among metallic nutrients? The different relationships observed for Zn, Mn, Fe are very interesting and deserve further discussion. Answer: Thanks. As showed in Table S2, Almost all elements are related to each other. The relationships between leaf δ15N and leaf Fe, Mn, and Zn did need further discussion. According to your comments, we add some discussions about this part (as follows), but it is still not enough and need further exploration. Fe, Mn and Zn participate in N assimilation processes, even Fe and Zn are the key components of enzymes in nitrogen metabolism (Fischer et al., 2005; Henriques et al., 2012; Ventura et al., 2013). The reason why the relationship between δ15N and Fe is opposite to the relationship between δ15N and Zn might be related to the different roles of the two elements in plant N assimilation. Iron-sulfur protein is the electron donor for nitrate reductase, and Fe supply has important influences on nitrate reductase activity and then on NO3- utilization (Pandey, 2000). Zn is the component of glutamic dehydrogenase and might mediate the NH4+ utilization (Kitagishi and Obata, 1986). We do not know the underlying mechanisms as yet. Mn can promote the synthesis of chlorophyll and assimilation of nitrate (Dučič and Polle, 2005), we cannot also explain the non-significant relationship between leaf Mn and δ15N. Line 290-292: ...leaf metallic nutrients almost did not change..., which suggested ... Answer: Thanks for your detailed comments. We have corrected them.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2019-328/bg-2019-328-AC2-supplement.pdf

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