

Responses to the comments made by Reviewer

Dear Reviewer:

Thank you very much for helping us to handle the manuscript entitled “Response patterns of moss to atmospheric nitrogen deposition and nitrogen saturation in an urban-agro-forest transition” (egusphere-2023-2718). I am writing a response to the reviewer’s comments. The detailed revisions are highlighted in yellow in the manuscript, and the responses to the comments are listed as follows:

1. In the title part, response pattern, nitrogen saturation, and urban-agro-forest transition is hard to be understood.

A: First, moss responses to atmospheric N deposition are influenced by factors such as moss sampling time, frequency, and various N deposition forms. Therefore, this study explored these factors that influence moss response, aiming to understand the pattern of moss response to nitrogen deposition and to refine moss monitoring of atmospheric nitrogen deposition. Second, nitrogen saturation represents a phenomenon where mosses become less responsive to further nitrogen deposition, and the expected increases in moss nitrogen content may not occur. In fact, in such scenarios, moss nitrogen content might even decrease due to growth limitations and physiological disruptions. Finally, “urban-agro-forest transition” refers to the transition zone in this study area, which transitions from urban to agricultural areas and then to forests.

2. In the introduction part, what is the N saturation state of mosses? I think in the present study, the data can not support the N saturation state of mosses.

A: As mentioned in the introduction part, N-saturation is defined as the level of pollution below which there are no significant harmful environmental effects (*UBA, 2005*). The absorption of N deposition by mosses is limited because N deposition modulates mosses to take up N by altering their physiological indicators (*Liu et al., 2017; Shi et al., 2017*). Thus, there exists a N saturation state for mosses, in which the

rate of increase in moss N content with atmospheric N deposition slows down and may instead show a decreasing trend.

As detailed in “*section 4.3 Relationships between various N forms and the N-saturation state*”, the N-saturation state was discussed. In this study, we found that the increase in moss total N content with increasing atmospheric total N deposition was much faster at low levels than at high levels of N deposition. This phenomenon signifies that there is a point at which the response of mosses to N deposition becomes saturated. This also indicates that the moss response to N deposition is indeed influenced by N saturation.

Later, we constructed response models using the data from this study, which indicated that the moss N content exhibited a relatively subdued reaction to TN deposition increases exceeding approximately $4.0 \text{ kg N hm}^{-2} \text{ mon}^{-1}$. This observation suggested that the mosses were approaching the N-saturation state.

Although the data from this study didn't provide an accurate value for the N saturation state of mosses, the phenomenon of N saturation states constraining the response of mosses to N deposition was observed in this study and approximate values for the saturation value of N deposition could be suggested.

3. In the method part, it is better to give the land use types to show the transition of urbans-agro-forest in the fig. 1.

A: Thank you very much for your comments. We have taken your suggestion into account and redrawn Fig. 1. on page 6, Line 115. In the new version, the land use types are presented. At the same time, we have added the source of the land use data: "*The land-use data (2016) used here were provided by the Center of Land Acquisition and Consolidation in Sichuan Province.*" to the description of the figure on page 6, Line 121-122.

4. Lines 121-123, why add water to the collector? This will affect quantify precipitation. Besides, the rain samples were collected at one-month interval, which will cause nitrogen loss and the variation of nitrogen forms as cause by microbial activities.

A: Thank you very much for your comments. First, we added water when placing the collectors to collect partial dry deposition through the wet surface method. Second, the volume of the device obtained by measurement, however, was only used to support calculations when accurate precipitation could not be obtained. The actual precipitation data used in this study were provided by the Chongzhou Meteorological Bureau, Sichuan Province, China. Therefore, adding water to the collectors should not affect the quantity of precipitation.

Third, during the preliminary experiments, we also observed the issue raised by the reviewer regarding nitrogen loss. Subsequently, we increased the maximum capacity of our collectors to minimize N loss due to sample overflow. Finally, during the summer, we added 1 mL of 2 mol/L copper sulfate solution to the collectors to prevent the growth of bacteria and algae. However, due to the lower temperatures in winter, we disregard microbial activity. Additionally, we used a stainless-steel net to minimize disturbance from birds and contamination from crop stubble, aiming to reduce microbial proliferation as much as possible. We added the sentences “*During the summer, 1 mL of 2 mol/L copper sulfate solution was added to the collectors to prevent the growth of bacteria and algae.*” on page 6, Line 131-132. Certainly, we will also refine our experiments to address the variation in N forms caused by microbial activities.

5. Line 133, this study only measured bulk N deposition, will dry N deposition affect moss N content?

A: Conventional monitoring methods for atmospheric N deposition generally include dry deposition, wet deposition, and bulk deposition. The available measurements in China thus far have focused primarily focus on wet or bulk deposition. Although dry

deposition is important, it is more challenging and often cost-prohibitive to measure. Among these methods, the bulk deposition monitoring method utilizes standardized glass deposition collectors to collect bulk deposition, collects liquid from the collectors at regular intervals, and then determines the concentration of N compounds to estimate atmospheric N deposition. This method partially compensates for the drawbacks of dry deposition and is widely applied.

Considering that the study area includes both outdoor and backward facilities, we chose the bulk deposition monitoring method to monitor N deposition in the region. Bulk deposition, which encompasses wet deposition plus a fraction of dry deposition, has recently been incorporated into a 43-site monitoring network across China. On average, bulk and dry N deposition rates were equally significant (50% each) (*Xu et al., 2015*). Additionally, one study reported that trends in bulk deposition may provide a useful guide for increasing in total N deposition rates (*Song et al., 2017*). Therefore, we believe that measuring bulk N deposition is also meaningful.

Above all, the current experimental results cannot accurately determine whether N dry deposition affects moss N content or quantify its impact. We can only quantify the relationship between bulk N deposition and moss N content.

The following are the relevant references cited in this response:

- (1) Xu, W., Luo, X., Pan, Y., et al.: Quantifying atmospheric nitrogen deposition through a nationwide monitoring network across China. *Atmos. Chem. Phys.* 15, 12345-12360, <https://doi.org/10.5194/acp-15-12345-2015>, 2015.
- (2) Song, L., Kuang, F., Skiba, U., et al.: Bulk deposition of organic and inorganic nitrogen in southwest China from 2008 to 2013, *Environ. Pollut.*, 227, 157-166, <https://doi.org/10.1016/j.envpol.2017.04.031>, 2017.

6. Lines 154-155, moss usually were found in the places with trees. Is it difficult to find mosses living under this condition?

A: Thanks for your comments. Finding mosses that meet the conditions mentioned in the paper "from natural rocks without canopies or overhanging vegetation" is indeed challenging. During site selection, we conducted rigorous field investigations primarily to mitigate sources of N for mosses other than atmospheric nitrogen deposition. These sources include leaf litter deposition and soil nitrogen, which could influence the results.

7. In the results part, as showed in figure 4, the R² between N deposition and nitrogen content in moss is low, so how is the accuracy for using moss as the bio-monitor of atmospheric N deposition?

A: In our analysis, we considered the correlation between N deposition and moss N content to be accurate and valid when it met a significance level of $P < 0.05$. The reasons for the low R² in our analysis are as follows. First, our study covered a wide range. Five distinct sites were strategically chosen within the urban-agro-forest transition. These sites represented the four primary land-use types, each representing one of the four primary land-use types. Second, the time scale of our data spans one year, resulting in a large volume of data. Above all, these factors collectively contributed to the lower R² of the regression equation.

8. In the conclusion part, if the moss can only be sampled in autumn and summer, how can it be used for as bio-monitor of N deposition in a year?

A: We are very sorry for our negligence. With your reminder, we referred to Figure 3 and checked the results section "**3.2 Correlations between moss N content and N deposition**". We identified our oversight and made the following modifications.

(1) We modified "*the optimal sampling time is autumn (October and November) and summer (July and August)*" to "*the optimal sampling times are winter (January*

and February), autumn (October and November) and summer (July and August)” on page 2, Line 25-26.

- (2) We modified “*in autumn (October and November) and in summer (July and August)”* to “*winter (January and February), summer (July and August) and autumn (October and November)”* on page 15, Line 321-322.
- (3) We modified “*autumn (October and November) and summer (July and August) within this area”* to “*winter (January and February), summer (July and August) and autumn (October and November)”* on page 16, Line 328-329.
- (4) We modified “*Mosses should be sampled more frequently than every six months and during autumn (October and November) and summer (July and August) as a method of monitoring N deposition.”* to “*Mosses should be sampled more frequently than every six months and during winter (January and February), autumn (October and November) and summer (July and August) as a method of monitoring N deposition.”* on page 19, Line 435-437.
- (5) We modified “*Second, the optimal sampling periods are autumn and summer, the growing period, allowing for a more accurate estimation of atmospheric N deposition.”* to “*Second, the optimal sampling periods were winter, summer and autumn, allowing for a more accurate estimation of atmospheric N deposition.”* on page 20, Line 454-455.

Regarding this issue, we made descriptions as following:

First, sampling conducted during winter (January and February), summer (July and August), and autumn (October and November) was deemed the optimal sampling time. However, this does not render samples collected during other months meaningless; they can still offer qualitative or semi-quantitative insights for monitoring atmospheric N deposition.

Additionally, this study optimized several parameters of moss monitoring for nitrogen deposition. Among them, the optimal sampling time was based on the optimal sampling frequency, which represents the longest cumulative N deposition time scale

to which mosses collected in the current month can respond to. This study revealed that the optimal sampling frequency was within six months per time. When using a bio-monitor for nitrogen deposition, integrating the optimal sampling frequency with the optimal sampling time can not only enhance the accuracy of this method, but also can extend the monitoring time scale beyond just the optimal sampling period to encompass the entire year. We obtained the monthly nitrogen deposition flux by inputting the moss nitrogen content collected in each month into the regression equation (Fig. 4). Then, based on the optimal sampling frequency, we multiply it by the total number of months. By repeating this process, we can derive the annual deposition flux.