

## Responses to the comments made by Reviewer#2

### 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:

**Lines 28-29: I am not clear how to compare this? Please revise this sentence.**

**Moss N can be used to indicate total N deposition. Or Moss N is suggested to be used to indicate total N deposition instead of specific N species.**

**A:** Thank you very much for your comments. We modified the sentence “*In addition, the moss N content could better indicate total N deposition than the deposition of specific N species.*” to “*In addition, the moss N content serves as a more reliable indicator of total N deposition compared to the deposition of specific N species.*” on page 2, Line 28-30.

**Line 101: The reason why choosing this urban-agro-forest transition zone should be introduced.**

**A:** The reasons why we chose this urban-agro-forest transition zone are as follows: First, these regions comprise agricultural, urban, rural, and forest areas, which are typically formed during urbanization and are heavily influenced by human activities. Second, nitrogen deposition patterns and sources are more complex in these areas than in natural ecosystems. Finally, moss monitoring methods for nitrogen deposition are limited in such regions, and there is still a need for sufficient knowledge under high nitrogen deposition conditions. Those reasons were stated as “*The urban-agro-forest transition regions include agricultural, urban, rural and forest areas, which are commonly formed in the process of urbanization and are deeply influenced by human beings. The patterns and sources of N deposition are more complex here than in natural ecosystems. However, the method for moss monitoring N*”

*deposition is limited here, and sufficient knowledge is still needed in such high N deposition conditions.” on page 4, Line 91-95.*

**1. Different sampling points for QQ, CY, YT, HY, and JGM. For example, 4 sampling points at site QQ but only 2 points at JGM. Why?**

**A:** The number of moss sampling points selected around different atmospheric N deposition sampling sites varied. This variation is due to differences in field conditions at each site, requiring us to conduct on-site surveys to identify locations suitable for moss collection. All mosses were collected from natural rocks without canopies or overhanging vegetation to avoid the effect of throughfall N compounds. The sampling sites are more than 300 m away from the main roads and at least 100 m away from other roads or houses, free of the direct impact of stagnant water and surface water splashes, traffic, and other artificial pollution sources (human and animal excrement, fertilization, and stamping). Therefore, it cannot be guaranteed that the number of moss collection points around each deposition site will be consistent.

**Lines 121-122: Did you record the volume of water in collectors.**

**A:** During the period of water sample collection, we did not continuously monitor the volume of water in the collectors. Instead, we only measured the volume of water in the collectors monthly when collecting water samples for laboratory analysis. These data were then combined with rainfall data provided by the meteorological station to calculate atmospheric nitrogen deposition flux more accurately.

**Section 2.2: How did you sample the moss? What weight or area? Did you consider the period of moss growing?**

**A:** Details about moss sampling and analysis are described in Section 2.3. We have modified the sentences “*In this study, 2-5 subsample sites were selected for moss collection within 1 km of the N deposition sampling site (Fig. 1), with at least three replicates of mosses collected from each subsample site. Later, those replicates representing the same deposition sampling site were combined into a representative one.*” to “*In this study, 2-5 subsample sites were selected for moss collection within 1*

*km of the N deposition sampling site (Fig. 1). Within a 50-meter range (a square of 50×50 m), 5 to 10 samples were collected to combine into a representative one for each subsample site.” on page 7, Line 157-160.*

Besides, each subsample was of similar weight and distributed homogeneously and as separated as possible within the area, avoiding the collection of concentrated mops within the areas.

On this issue of whether the period of moss growth is considered, we provide the following explanation. We collected mosses every month. However, we did not attempt to destroy the moss roots as much as possible during the moss collection process and only used the green part. Afterwards, we considered the period of moss growth when we found that significant positive correlations between the moss N content and TN-N, NH<sub>4</sub><sup>+</sup>-N, and NO<sub>3</sub><sup>-</sup>-N deposition in winter (January and February), autumn (October and November) and summer (July and August), but these correlations were absent during winter and spring. This phenomenon is relevant to the growing season of moss. The detailed explanations are provided in **section 4.1**, “*The covariation between the moss N content and atmospheric N deposition depends on the season. For example, significant positive correlations were found between the moss N content and TN-N, NH<sub>4</sub><sup>+</sup>-N, and NO<sub>3</sub><sup>-</sup>-N deposition in winter (January and February), summer (July and August) and autumn (October and November) (Fig. 3, P < 0.05), but these correlations were absent during spring. This phenomenon is relevant to the growing season of mosses. As mentioned in several studies, the growth of mosses generally occurs from March to May and from October to December (Thöni et al., 2011; Yurukova et al., 2009). Since mosses undergo a period of nutrient accumulation during growth (Faus-Kessler et al., 2001), they can better monitor atmospheric N deposition after growth (Boquete et al., 2011; Thöni et al., 2011). Thus, the optimal sampling seasons are winter (January and February), summer (July and August) and autumn (October and November) within this area. Moss growth status and regional N deposition level influence the moss response patterns, subsequently influencing the design of effective sampling strategies.” on page 15-16, Line 319-331.*

**Lines 194-196: Details for the certified reference material and laboratory standards are required.**

**A:** Based on your comments, we added the following sentences “*The certified reference materials used in the experiment all conformed to national standards. The standard solutions of  $\text{NH}_4^+-\text{N}$ ,  $\text{NO}_3--\text{N}$  and  $\text{TN}$  complied with GSB 04-2832-2011, GSB 04-1772-2004 and GSB 04-2837-2011 (b). These certified reference materials were stored and utilized correctly.*” on page 9, Line 208-211.

**2 Subplot f is the study areas. What do you mean the study areas? Average of subplots a-e? You can put this one into SI or change it into average N deposition from study sites.**

**A:** Subplot f is the study area, which is the average of five sites, subplots a-e. The detailed description of Subplot f “*Additionally, the averages of atmospheric N deposition and moss N content across the five sites are shown in Fig. 2f, providing an overview of the temporal variations in the study area. It was found that the variation in the N content in moss highly matched the monthly fluctuation patterns of N deposition (all N species) in the study area.*” This information was added to better illustrate the meaning of Fig. 2f, on page 10, Line 228-231.

**Line 231: change “R” to “r”, usually we use lowercase letter to show correlation coefficient.**

**A:** We changed “R” to “r” on page 12, Line 245 and on page 16, Line 342.

**Table 1. I am not clear about the sampling frequencies. In the Section 2.2, the author only mentioned a one-month interval of sampling.**

**A:** Thank you very much for your comments. Our sampling interval, as mentioned in **Section 2.2**, is indeed one month. The correlation analysis was subsequently divided into two steps.

First, by analyzing the relationship between the moss N content of the current month and atmospheric N deposition under different accumulation time scales (1, 3, 6, 9, and

12 months). This approach enabled identification of the appropriate sampling frequency for continuous monitoring of N deposition, revealing that the moss N content in a given month exhibited responsiveness to the cumulative N deposition of preceding months. For example, to analyze the correlation between moss N content in October 2018 and N deposition under the sampling frequency of three months, the value of moss N content should be given as a value in October 2018, while the N deposition should be the sum of August, September and October 2018.

After analyzing the maximum cumulative N deposition time scale that mosses can respond to within six months, we can refine the moss monitoring method for N deposition, controlling the frequency of moss collection to less than six months per time. This methodology is described in detail in **Section 2.4**, *“The correlation between the moss total N content and various atmospheric N deposition under different accumulation time scales (1, 3, 6, 9, and 12 months) was analyzed. This approach enabled the study to discern the appropriate sampling frequency for continuous monitoring of N deposition, revealing that the moss N content in a given month was sensitive to the cumulative N deposition in the preceding months. For example, to analyze the correlation between the moss N content in October 2018 and N deposition under the sampling frequency of three months, the value of moss N content should be given as a value in October 2018, while the N deposition should be the sum of August, September and October 2018.”*, on page 8, Line 175-182.

Afterwards, based on the optimal sampling frequency, the correlations between moss N content and various species of N deposition were analyzed in each sampling months, which could obtain the optimal sampling time for moss response to atmospheric N deposition. This methodology is elaborated in detail in **Section 2.4**, *“Furthermore, correlations between the moss N content and various species of N deposition were analyzed in each sampling months, which could obtain the optimal sampling time for moss response to atmospheric N deposition. Note that the time scale of the moss N content was from October 2018 to September 2019, while the N deposition collection period was more than one year, from April 2018 to September*

2019, which could enhance the optimality of the sampling frequency for this study.”, on page 8, Line 183-188.

**2 and 3 are all correlations, but presented in different ways. Can you make one figure to show both correlations with similar ways?**

**A:** Thank you very much for your comments. Figures 2 and 3 represent different correlations and meanings.

Figure 2 is described in detail in “*Section 3.1 Monthly variation in N deposition and moss N content*” on pages 9-10. Fig. 2a-e shows the monthly N deposition flux and moss N content at the five sampling sites. Besides, the averages of atmospheric N deposition and moss N content across the five sites are shown in Fig. 2f, providing an overview of the temporal variations in the study area. In this section, the study did not actually calculate the correlation between them.

Figure 3 is described in detail in “*Section 3.2 Correlations between moss N content and N deposition*” on page 12-13, Line 253-264. Figure 3 was based on the optimal sampling frequency, which was calculated in Table 1. The correlations between the moss N content and the various N deposition species were analyzed in each sampling month to determine the optimal sampling time for determining the response of the moss to atmospheric N deposition. This methodology is elaborated in detail in *Section 2.4 Correlation between moss N content and atmospheric N deposition*.

Above all, we cannot make one figure to show both correlations in similar ways.

**In section 3.3. Both linear and logarithmic regressions were conducted. Here, only one regression is enough. Please selected a better regression with higher R2 Here, only TN follows a linear regression, whereas NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N follow a non-linear regression.**

**A:** In this study, both linear and logarithmic models were used to evaluate the response of moss N content to different forms of N deposition. By displaying both regressions, we aim to highlight the results of this section. The results showed that the logarithmic models had a high R<sup>2</sup> ( $P < 0.05$ ) for TN. However, for NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N, the linear models had high R<sup>2</sup> values ( $P < 0.05$ ). Based on the results, the relationships between the moss total N content and various N forms, and the N-saturation state, were discussed in *section 4.3*. Therefore, we consider that both regressions should be conducted simultaneously and are presented in Figure 4.

**In the section of Introduction, N deposition per year should be given.**

**A:** Thank you very much for your comments. Based on your comments, we supplemented “*Atmospheric N deposition has climbed by three-to-fivefold over the course of the 20th century (IPCC 2013). Global N deposition was estimated at 119 Tg N in 2010 (land, 60%; seas, 40%) (Liu et al., 2022).*” in the section of Introduction, on page 3, Line 46-49, to better illustrate the importance of atmospheric N deposition in the global N cycle and the need for its monitoring. Besides, the corresponding references were added to the reference lists on page 24, Line 562-563 and Line 571-574.

(1) IPCC. *Climate Change 2013-The Physical Science Basis*. New York: Cambridge University Press; 2013.

(2) Liu, L., Xu, W., Lu, X., Zhong, B., Guo, Y., Lu, X., Zhao, Y., He, W., Wang, S., Zhang, X., Liu, X., Vitousek, P.: *Exploring global changes in agricultural ammonia emissions and their contribution to nitrogen deposition since 1980. Proc. Natl. Acad. Sci., 119, <https://doi.org/10.1073/pnas.2121998119>, 2022.*