Response to Håkan Pleijel

The referee comments are in black, while the author comments are in **bold print** and blue.

The authors of this paper have analyzed how meteorological/climate variables as well as leaf traits affect the uptake rate of mercury (Hg) of leaves/needles in a range of tree species over a substantial part of Europe. The data base is large, which is an important asset in this type of analysis, formed by observations made using the ICP Forest level II plots and in addition data from a dense sampling network in Austria. Important results include the relationship of foliar Hg uptake rate with leaf nitrogen concentration and leaf mass per area (LMA) as well as links with soil moisture, water vapour pressure deficit (VPD) and other meteorological factors, suggesting a close link of Hg uptake rate with stomatal conductance and physiological activity. In general, this is a well-written and valuable piece of work which should be published. However, improvements are possible, and a number of mostly relatively minor changes and amendments should be considered.

Thank you for the constructive feedback and your comments.

Specific comments:

Line 41: "simulated start of the growing season" – some more details about how the simulation was made should be included.

We agree that the description of the model came off too shortly in the main manuscript. We will therefore add: "To evaluate the beginning of the growing season for deciduous trees, we applied a threshold based growing season model (de Beurs and Henebry, 2010) using the leaf area index (LAI) product by Copernicus Global Land Service based on PROBA-V satellite imagery at a resolution of 300 m and 10 days (Dierckx et al., 2014; Fuster et al., 2020). This growing season model follows an approach by (Bórnez et al., 2020) and defines the beginning of the growing season as the point in time, at which the LAI exceeds the 30% percentile threshold of the amplitude between minimum LAI early in the year and maximum LAI at peak season. For technical details of the model, modelling procedure and quality assurance please refer to supplementary information, Sect. S2.2."

Line 98: consider removing "balanced", it does not become clear what this refers to in this context, and it is unnecessary to include this word.

We will delete "balanced".

Lines 112-118. Information about which needle age classes were harvested should be added as well as which needle age classes were used in different analyses.

We describe the number of needles samples of different age classes in the last part of this Sect. 2.1: "Coniferous samples consist of needles of different age classes: most of the needle samples (n = 1958) flushed in the sampling season (current season; y_0), 600 samples are one-year old (y_1), 121 samples are two-year old (y_2), 125 are three-year old (y_3), 22 samples are four-year old (y_4), 60 samples are five-year old (y_5) and 3 samples are six-year old (y_6) needles." To this, we will add the sentence: "All

analysis of this study concerning tree species, foliage structure, nutrient contents and meteorological and site-specific parameters (Sect. 3.1 – 3.6) are based on Hg values of current-season (y_0) foliage."

Line 117: "typically"? Please be more specific.

Yes, "typically" is an unnecessary filler word here, so we will delete it.

Line 173: typo, "factors" should be "factor".

revised as suggested

Line 204 "leaf unfolding" seems not to be the appropriate expression here!? Rather: "emergence of the new flush of needles"? A similar comment applies to the text under 3.1 of the supplementary information. Here it is stated (line 5 and line 22 on page 3) that it is the beginning of the "growing season" that is determined from the PEP725 database. But isn´t it again the emergence of the new flush of needles which is treated here? The growing season of the older needles and thus "the beginning of the growing season of coniferous tree species" (line 5) – with stomatal gas exchange - starts much earlier.

We agree, that a more specific description of the beginning of the growing season of coniferous trees is necessary to avoid confusion, as there is no single point in time, at which the growing season of coniferous trees starts, which depends on the needle age class. We based the main parts of the analysis of this study on values in current-season needles, which indeed makes the emergence of the new flush of needles the starting point for the analysis. We will change the respective sentences: "While dates of harvest were available for all samples, we determined the start of the growing season <u>of current-season foliage</u> by combining available data sources with start-of-season modelling. These data sources comprise in-situ phenological observations, which were available for 15% of samples, and observations of the <u>emergence of the new flush of needles</u> of coniferous tree species from the Pan European Phenological database PEP725."

In the Supplement we will correct accordingly: "We matched observations on the beginning of the growing season <u>of current-season needles</u> from the Pan European Phenological database PEP725." Later in the same Section we will explicitly describe the mentioned BBCH codes: "We used data for the beginning of the season (needle age class: y_0) of BBCH codes 10, 11, 13, 31, 60, 61 and 223. These BBCH codes correspond to the following growth stages: first leaves separated (BBCH 10); first true leaf, leaf pair or whorl unfolded, first leaves unfolded (BBCH 11); 3 true leaves, leaf pairs or whorls unfolded (BBCH 13); leaf unfolding (>=50%) (BBCH 223); rosette 10% of final length (BBCH 31); first flowers open (BBCH 60); beginning of flowering (BBCH 61)."

Lines 275-278 and other places: there is a strong focus on current year needles in the study, although not completely. The authors should explain why this is the case. Most conifers retain their needles ~4-10 years and Hg accumulation will continue over several years. In the years after the first, evergreen conifer needles will have a longer period of physiological activity and uptake of Hg, starting earlier in spring and ending later in autumn, compared to broadleaved trees. Thus, even if the uptake rate of Hg per day (mostly used in the paper) is smaller for conifers, this will partly be offset by the longer duration over the year of gas exchange in needles older than current. This is significant when analyzing biogeochemical fluxes on a (multi)annual scale. To focus only on the uptake rate per day obscures the importance of the variability in the duration of Hg uptake over the year in different types of trees.

We analyzed the relationship of physiological/climatic parameters with foliage Hg uptake rates, which correspond to Hg accumulation on a leaf/needle basis and do not agree with foliage Hg uptake fluxes (units of Hg per time period over unit ground area). Most needle samples of the dataset were harvested in late fall/early winter, while most broadleaves were sampled during peak season. Normalization for the number of days between spring and sampling day therefore ensures comparability between foliage Hg concentrations of the 23 tree species within the dataset, given that all samples represent current-season foliage. As you rightly point out, daily Hg uptake rates of needles 1 year and older might differ systematically from daily Hg uptake rates of all current-season foliage due to an early start of the growing season and a potential decrease in uptake rates with decreasing physiological activity of older needles. Comparability is the reason for analyzing exclusively current-season needles, with the exception of results presented in Fig. 7, which focuses on Hg concentrations of different needle age classes. We believe it is important to disentangle any confusion arising from the different reference units of Hg concentrations, uptake rates and fluxes. Thus, we suggest to amend Sect. 2.4: "We therefore calculated foliar Hg uptake rates (in ng Hg $g^{-1}_{d.w.}$ d^{-1}) of current-season samples by normalizing foliar Hg concentrations to their respective life period in days from the beginning of the growing season (emergence of new foliage) to date of harvest. These resulting foliar Hg uptake rates represent the average daily net Hg accumulation per gram dry weight on a leaf basis and should not be confused with foliar Hg fluxes on a whole-tree basis. Needles of age 1 year or older were excluded from calculating daily foliage Hg uptake fluxes, since Hg uptake might slow down in physiologically less active older needles (Wohlgemuth et al., 2020) and it is unclear, to which extend Hg uptake occurs in older needles in winter and in early spring before the emergence of new foliage."

To our knowledge, there is no study describing Hg uptake at the transition of winter to early spring before the emergence of current-season needles. It is an interesting idea to investigate the relevance of the early-spring uptake of needles $y_1 – y_n$ for the overall foliar Hg uptake flux.

Lines 287-288: as the authors state, the otherwise very informative paper Zhou et al (2021) does not distinguish between different needle age classes, which makes it hard to compare with needle age specific Hg concentration data. Some other reference should be used to support the statement.

We encountered difficulties to find studies, which compare Hg concentrations in broadleaves and <u>current-season</u> coniferous needles normalized for respective sample life periods, during which foliage was physiologically active. The paper Zhou et al. 2021 has the advantage, that their findings are based on a large dataset. However, given the lack of reporting on needles age classes in Zhou et al. 2021, we will add: "Similarly, Navrátil et al., 2016 reported higher foliar Hg concentrations in deciduous beech leaves (36.3 ng Hg g^{-1}) than in coniferous current-season spruce needles (14.1 ng Hg g^{-1}) of two adjacent forest plots sampled during peak season (August). Higher Hg concentrations in deciduous leaves (median: 28 ng Hg g^{-1} from 341 remote sites) than in composite multi-age coniferous needles (median: 15 ng Hg g^{-1} from 535 remote sites) were also reported in a global literature compilation (Zhou et al., 2021)."

Line 338: should be "Leaf Mass per Area".

revised as suggested

Line 345: should be "Figure 4" (not 44).

thank you, corrected

Figure 4: This figure contains very interesting information, especially the relationship between daily Hg uptake rate and foliar N concentration (representing physiological activity). Such a relationship could become very useful for large-scale modelling of Hg fluxes if supported also by further data. However, it is confusing that the daily Hg uptake rates, especially of the conifers, in Figure 4 are substantially higher than those reported for the same species in Table 1 based on a larger number of observations. Also, the N concentration values differ between Figure 4 and Table 1 for the same species. The authors should discuss these discrepancies, their causes and implications.

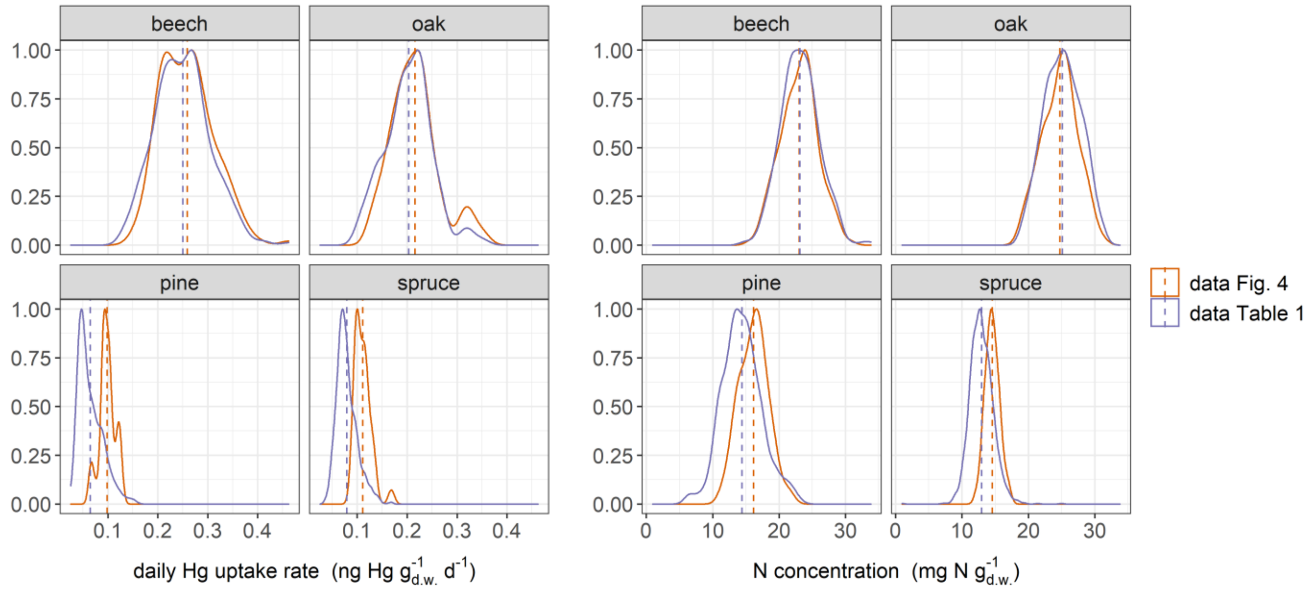
For an overview, we assembled all values in a table, highlighting pine and spruce. Average values of beech, oak, hornbeam and Douglas fir fall within the respective range of ± one sd. to each other.

	relatively larger dataset **(Table 1)**			relatively smaller sub-dataset **(Fig. 4)**		
Species group	daily Hg uptake (ng Hg $g^{-1}_{d.w.}$ d^{-1}) (mean ± sd.)	foliar N conc. (mg N g^{-1}d.w.) (mean ± sd.)	n	daily Hg uptake (ng Hg $g^{-1}_{d.w.}$ d^{-1}) (mean ± sd.)	foliar N conc. (mg N g^{-1}d.w.) (mean ± sd.)	n
beech	0.25 ± 0.05	23.1 ± 2.9	312	0.26 ± 0.05	23.0 ± 2.8	164
oak	0.20 ± 0.05	25.1 ± 2.8	252	0.22 ± 0.05	24.7 ± 2.7	106
hornbeam	0.19 ± 0.03	19.4 ± 2.1	10	0.19 ± 0.04	18.9 ± 1.4	9
Douglas fir	0.13 ± 0.02	17.0 ± 3.5	26	0.12 ± 6e-5	19.0 ± 2.2	2
pine	0.06 ± 0.02	14.4 ± 3.0	355	0.10 ± 0.02	16.1 ± 2.1	35
spruce	0.08 ± 0.02	12.9 ± 1.7	1509	0.11 ± 0.02	14.5 ± 1.0	33

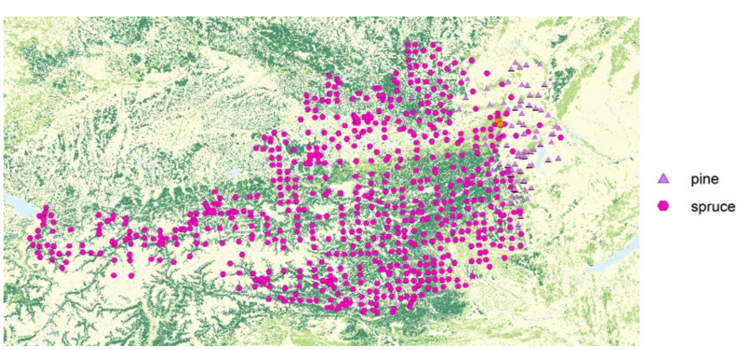
Median values of daily Hg uptake rates and N concentrations of pine and spruce coincide with respective average values.

	relatively larger dataset **(Table 1)**		relatively smaller sub-dataset **(Fig. 4)**	
	daily Hg uptake (ng Hg $g^{-1}_{d.w.}$ d^{-1}) median (min – max)	foliar N conc. (mg N g^{-1}d.w.) median (min – max)	daily Hg uptake (ng Hg $g^{-1}_{d.w.}$ d^{-1}) median (min – max)	foliar N conc. (mg N g^{-1}d.w.) median (min – max)
pine	0.06 (0.03 – 0.15)	14.2 (6.2 – 23.3)	0.10 (0.06 – 0.13)	16.2 (11.7 – 21.4)
spruce	0.08 (0.03 – 0.17)	12.8 (1.0 – 25.0)	0.11 (0.09 – 0.17)	14.5 (12.3 – 17.0)

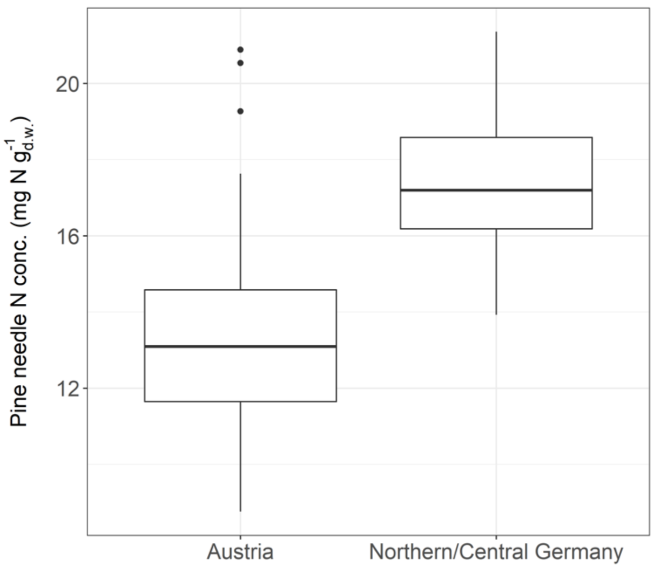
Density plots reveal that by tendency, the sub-dataset of Fig. 4 is indeed skewed towards higher values of daily Hg uptake rates and N concentrations for pine and spruce compared to the larger dataset of Table 1. This is not the case for beech and oak.



Relatively higher/lower average daily foliar Hg uptake rates of pine and spruce are associated with respective higher/lower average N concentrations between the two datasets (compare density plots above). We suggest that this concurrence is consistent with the relationship between Hg and N contents described in Sect. 3.3 and Sect. 3.4. The Hg/N sub-datasets (data Fig. 4) of pine and spruce consist of 10% and 2% of data from the main dataset (data Table 1) respectively, so the shift in average Hg/N values could be coincidental. It is noticeable, however, that the main dataset of Table 1 contains a high proportion of Hg and N measurements from the Austrian Bio-Indicator Grid, while the sub-dataset is exclusively composed of values from ICP Forests Level II Plots. The average daily Hg uptake rate between all sites of the Austrian Bio-Indicator Grid is 0.05 ± 0.01 ng Hg g^{-1}d.w. d^{-1} (mean ± sd; n = 162 sites of 2015 and 2017) for pine and 0.08 ± 0.02 ng Hg g^{-1}d.w. d^{-1} (mean ± sd; n = 1274 sites of 2015 and 2017) for spruce. The average daily Hg uptake rate between all ICP Forests plots is 0.07 ± 0.02 ng Hg g^{-1}d.w. d^{-1} (mean ± sd; n = 80 plots of 2015 and 2017) for pine and 0.09 ± 0.02 ng Hg g^{-1}d.w. d^{-1} (mean ± sd; n = 84 plots of 2015 and 2017) for spruce. Most of the pine sampling sites are located in the eastern part of Austria (see Fig. 1 and map below).



These Austrian pine sites are characterized by sandy and nutrient-poor soils and pine needles with lower N content compared to e.g. pine samples in Central Germany (see figure below). We therefore assume that the difference of daily needle Hg uptake rates and N concentrations between the datasets presented in Table 1 and Fig. 4 is a result of the geographic shift in nutrient conditions among the sampling sites included in both datasets.



We will present all values included in Fig. 4 in a separate table in the Supplement to make these values available. We will also add the density plot to the Supplement to visualize the differences in mean Hg and N between main dataset and sub-dataset.

Figure 5 (this applies also to figures in the supplementary): While the relationship for pine has a reasonable distribution of data, permitting the authors to derive a quite clear linear relationship, this is not the case for spruce. For the latter species there are two clusters of data separated by a large empty space with respect to the x-variable, which contains no data. Linear regression is not to recommend for such a data set. An option would be to compare and test the difference between the two clusters with a t-test.

Yes, linear regression for spruce values is not ideal here. A two sided t-test on the two clusters revealed, that the average daily foliar Hg uptake rate is significantly ($p = 0.008$) different between the two clusters at a water VPD threshold value of 3 kPa. Respective t-tests at all other VPD threshold values revealed non-significant ($p > 0.05$) differences in average daily Hg uptake rates between the two clusters. For reasons of consistency and comparability with the other datasets, we would prefer to keep presenting results of a linear regression for spruce values in the Supplement, but will amend Sect. 3.5: "Average daily needle Hg uptake rates of spruce needles were clustered between two groups of forest plots with high and low daytime proportions of VPD > threshold (Fig. S9) relative to each other. T-tests revealed a significant ($p = 0.008$) difference in average daily spruce needle Hg uptake rates between the two clusters for a VPD threshold value of 3 kPa and non-significant ($p > 0.05$) differences for all other VPD threshold values."

Line 371: It is not fully explained why the specific water VPD thresholds were used for different species. From where were these thresholds taken?

The reason for working with water VPD thresholds is, that we were primarily interested in the length of time period of high evaporative demand (time periods of VPD > threshold) within the growing season, during which foliar Hg(0) uptake should be reduced according to theory of stomatal closure. The values of 1.2 kPa, 1.6 kPa, 2 kPa and 3 kPa represent incremental test steps within a VPD range, in which trees show a species-specific stomatal response. We explain this in Sect. 2.5: "We chose these four VPD thresholds because they were reported in literature to incrementally induce leaf stomatal closure of temperate forest trees, ranging from initial stomatal closure (around 0.8 kPa – 1 kPa (Körner, 2013)) to maximum stomatal closure (at around 3 kPa – 3.2 kPa (CLRTAP, 2017))." To make it clear, that these threshold values were chosen as test values we will add: "We chose these four VPD thresholds as test values because they were reported…"

Line 389: replace "had" by "has".

revised as suggested

Lines 393-394: "… the high degree of stomatal closure under drought stress of spruce". I do not understand this statement in relation to the data presented. The average daily Hg uptake rate, suggested by authors to be a possible proxy for stomatal conductance, does not differ very much between high and low VPD (Figure 5b), indicating a small response in stomatal conductance by high water VPD in spruce!?

Both spruce and pine respond to rising water VPD by reducing their stomatal conductance (Zweifel et al., 2009). Regarding daily foliar Hg uptake rates, we detected a significant linear regression coefficient versus a time proportion above a threshold of VPD > 3 kPa for spruce and a threshold of 1.2 kPa for pine (compare threshold values of Fig. 5a and 5b). From literature we derive, that spruce is a bit slower to respond to rising VPD as compared to pine, but that spruce stomata are almost completely closed at high VPD like 3 kPa (Zweifel et al., 2009). We will refine the description of findings from literature: "Among isohydric species, pine has been discovered to reduce tree conductance and stomatal aperture during the onset of dry conditions earlier and at a greater rate than spruce (Lagergren and Lindroth, 2002; Zweifel et al., 2009). Spruce was observed to keep stomata almost completely closed under drought stress, i.e. high VPD and/or soil water deficit (Zweifel et al., 2009)."

Figure 7: Although there is a general trend for higher Hg concentrations in older needles in Figure 7, also seen in other studies, the pattern in the figure is not completely clear. This may partly be caused by the heterogeneity of the data, with a strongly varying number of observations for the different needle age classes. It may also be the case that the larger number of needle age classes included in the figure compared to most other studies, indicate a levelling off in the rate of annual increase in Hg concentration of the oldest needles. The authors should discuss the data presented in Figure 7 in further depth including the possible consequences of the heterogeneity of the data. The quite strong levelling off in annual Hg concentration increase for y4-y6 in Figure 7 is not in complete agreement with the statement on lines 432-436.

We will discuss in more detail: "In agreement with previous studies (Ollerova et al., 2010; Hutnik et al., 2014; Navrátil et al., 2019; Wohlgemuth et al., 2020; Pleijel et al., 2021), we found a trend of Hg concentrations in differently aged needles with older needles exhibiting higher Hg concentrations (Fig. 7), demonstrating that Hg accumulation continues in older needles. Annual Hg net accumulation seems to slow down in older spruce needles of age classes $y_3 - y_6$ in contrast to needles of age classes $y_0 - y_2$ (Fig. 7), albeit ranges of average Hg concentrations ± standard deviation overlap among older and younger spruce needles, which might be the result of relatively lower sample numbers of older needles compared to younger needles (e.g. 3 samples for y_6 vs. 301 samples for y_1). A decline in foliar Hg uptake by older needles could be caused by lower physiological activity, cuticular wax degradation or an increase in Hg re-emission with needle age (Wohlgemuth et al., 2020)."

Lines 450-453 on stomatal conductance modelling: the phrase "model in a multiplicative way" is not appropriate and hard to understand for people not strongly involved in stomatal conductance modelling. Also, there exist other types (e.g., photosynthesis based) stomatal conductance models than multiplicative which could be considered. It would be appropriate to use a more recent reference than Emberson et al (2000), since a lot of development has taken place in stomatal conductance modelling over the last two decades. The authors may consider Emberson et al (2018) Ozone effects on crops and consideration in crop models. *European Journal of Agronomy* 100, 19–34, although it is on crops.

Thank you, we will delete "in a multiplicative way" and add the suggested reference.

Line 480-481: The suggestion by the authors to assume that foliar Hg can represent the stomatal conductance (integrated over longer time scales) is interesting and thought-provoking. This statement must be based on the assumption that the atmospheric concentration of Hg is essentially constant, from year to year and from place to place. To what extent is this assumption valid? This should be discussed.

Based on comments by reviewer Frank Wania, we will add a discussion on GEM to Sect. 3.2 and will comment on the topic throughout the manuscript. Generally, the horizontal variability of GEM during the season is relatively low. We evaluated a GEM concentration of 1.39 ± 0.09 ng m^{-3} (mean ± sd) based on average GEM concentrations at 6 EMEP stations in Central Europe between May – Sept. In principle, however, we agree that atmospheric GEM should be taken into account and revised the text in the manuscript accordingly: "We therefore suggest, that foliar Hg measurements bear the potential to serve as a proxy for stomatal conductance, providing a time-integrated measure for stomatal aperture when taking into account the spatial and temporal variation in atmospheric Hg(0)."

In the Conclusion part it would be appropriate to discuss the relationship between the net accumulation in leaves/needles over time periods of weeks to years, which is the focus in the paper, and the dynamic bi-directional fluxes of Hg to/from vegetation observed in many studies using highly time resolved measurements.

We are not sure, if a discussion about short-scale temporal (uptake/re-emission) fluxes in the Conclusion will advance the main message of this manuscript as the focus of this study is on long-term foliar net Hg accumulation rates. However, we will add an explanatory sentence to Sect. 2.4:

“Please also note, that daily foliar Hg uptake rates in this study represent average values over the growing season. The actual daily foliar Hg uptake on a given day might differ from the average value depending on the time period within the growing season (e.g. early season vs. peak season) (Laacouri et al., 2013).”

Essentially, this is a very interesting and valuable paper.

Thank you very much for this kudo.