- 1 **Note:** Comments by the Editor and Reviewers are presented in red, while the first (i.e. initial response)
- 2 and second responses (i.e. lines of changes that have been made in the manuscript) of the authors are
- 3 given in black and blue, respectively.

4 By the Editor

5 Thanks for submitting your work to Biogeosciences. This manuscript was read and commented on by two

6 reviewers as well as a non-assigned reader; in general all provide thoughtful, interesting comments and

7 useful suggestions. I have read and reviewed this feedback, the authors' responses, and of course the

8 manuscript itself.

9 In general I agree with the reviewers that this is fundamentally a strong, interesting manuscript, and your 10 responses are thoughtful and adequate. I do also, however, share some of their concerns about the 11 experiments treatments and how they're described. In particular, R2's concern about heat stress being 12 confounded with drought should be carefully considered, and the potential problem (or not) of different 13 light levels comprehensively discussed. The comments about notation and R package descriptions are well 14 taken although optional from my point of view; the critical thing is that things are clearly documented. On that note, the availability of your data and analytical code needs to be clearly specified, ideally with a link 15 16 to a permanent repository.

17 In summary, this is an interesting manuscript that needs moderate to major revisions before further18 consideration.

19	Dear Editor,
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21	Thank you for your suggestions and comments.
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23	We have improved the description of the treatments used in our sapling experiments (L. 161 -
24	179), adapted our terminology of the treatment effects (L. 171 – 179; L. 178; L. 491; L. 580; L.
25	585; L. 588: L. 620; L. 662 and L 691 – 693) and improved the discussion about the treatments (L.
26	589 - 594). We also added data on the volumetric soil water content measured at the flux tower
27	in Brasschaat (see L. 204; fig. 2; L. 219 – 231 and L. 244 -252) and commented on this data (L.
28	477 – 479 and L. 612 - 618). Significant effort has been made to clarify and discuss the (potential)
29	effect of the polycarbonate roof in L. 125 – 126; L. 184 – 189 and L. 650 – 659), although
30	knowing the exact effect would require a different experimental set-up. All data and code has
31	been made available on Zenodo. We referred to the changes (i.e. lines) we made in the
32	manuscript underneath each appropriate comment of the reviewers.
33	
34	Kind regards,

36 By Fabrizio D'Aprile

Certainly, water stress and/or related soil parameters cannot explain all the time variability in foliage 37 38 coloration. However, it can contribute and therefore depict reaction of vegetation at both the small parcel 39 and landscape level. I tested it in two works (please see the attached publications). This does not mean to 40 contrast the authors' work but providing them with some more information that might be useful. And, I 41 would also like to attract the attention on the fact that crown transparency and coloration/withering, 42 which have frequently been used as parameters related to water stress due to both direct and indirect 43 causes, may not necessarily be indicative of the level of the humidity content of the tree and/or health 44 status. 45 Dear Mr. Aprile, 46 Thank you for your comments and articles. We will consider them for revision and future work. 47 48 Certainly, further studies over a more extensive geographical range could aid to further unravel 49 the effects of various water stress and/or soil parameters on the leaf coloration dynamics. Note 50 that we added data on the volumetric soil water content measured at the flux tower in 51 Brasschaat (see L. 204; fig. 2; L. 219 – 231 and L. 244 -252) and commented on this data (L. 477 –

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479 and L. 612 - 618).

54 Kind regards, 55

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57 By Anonymous Referee #1

- 58 Mariën and co-authors presented an experimental study in which they assessed the effect of drought 59 stress on leaf senescence for 3 tree species in Belgium forests (mature trees) over 2017-2019 and from 60 manipulative experiments with saplings. Results did not show any effect of drought stress on the timing 61 of leaf senescence. However, the authors observed an effect of drought on the chlorophyll content and 62 the canopy greenness of trees. Overall, this study is well written. The experimental design is sound, and 63 limits in the protocol and analysis are clearly highlighted and discussed. Results support the conclusions 64 of the manuscript. I don't have major comments on the manuscript, only a few suggestions that might 65 strengthen the analysis:
- 1) The authors used piecewise logistic regression to estimate the timing of leaf. Some studies suggested
 that a simple threshold approach leads to better results and maybe a more robust comparison of
 phenological events. Did the authors tried to compare the timing of senescence using a threshold
 approach? The absence of observed effect might come from the definition of leaf senescence.
- 2) Drought stress is defined here as the rainfall deficit. Instead of a meteorological drought index, did the
 authors tried other physiological drought indices? For example, the ratio of actual over potential
 evapotranspiration (Stocker et al. 2018) that might be more representative of the stress than the rainfall
 deficit.
- 3) Some studies suggested that the timing in leaf unfolding impacts senescence (Fu et al. 2014). Was this
 effect observed on site? Did the authors include other effects than precipitation, temperature and
 drought stress in their model? It might be interesting to discuss this point in the discussion section.
- 4) I suggest the authors to highlight the effect of drought stress on CCI and greenness in the abstract. As
 they discussed (L. 464), the effect on greenness is probably an important source of confusion in the
 literature and I think it is an important message of this paper. I hope the authors will find these comments
 useful for improving their manuscript. Best regards,
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Dear Anonymous Referee 1,

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Thank you for your review and suggestions. We will respond here to your comments:

85 1) The Referee asks whether we considered alternatives (e.g. simple thresholds in canopy 86 coloration percentage) to the piecewise linear regressions to determine the timing of the onset 87 of leaf senescence. We are aware that different methodologies (e.g. from simple thresholds to 88 complex network-based approaches) can, and are, used to estimate the timing of leaf 89 senescence. In fact, we compared the results obtained using piecewise linear regressions and 90 50% canopy coloration / leaf fall thresholds (i.e. assuming that the onset of leaf senescence 91 can be approximated with the timing when 50% of the canopy lost the green color) in previous 92 work, and showed that the methods provide different results, with 50% thresholds giving 93 results that are consistently later (Mariën et al., 2019). We agree that comparing different 94 methods might nevertheless yield advantages, as the timing of leaf senescence is inherently a 95 problem of deriving a trend in complex ecological data (i.e. data that is, for example, 96 hierarchical and non-linear). Exactly for deriving this trend, and as extra regression method to 97 compare to the piecewise linear regressions, we used the generalized additive mixed models

and plotted the resulting factor-smooth interaction smoothers with 95% simultaneous confidence intervals.

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2) The Referee asks whether we considered different physiological drought indices (e.g. the ratio 102 of actual over potential evapotranspiration as in Stocker et al. (2018)). We agree that other 103 indices would be useful. However, calculating the index proposed in Stocker et al. (2018) would 104 not be feasible in a short term. An additional difficulty is that these calculations would require 105 a hydrological model and are strongly dependent on local soil characteristics. Furthermore, 106 most local meteorological stations do not provide evaporation data. Finally, note that long-107 term values of the rainfall deficit, as reported in Fig. 3, are rather exceptional. Therefore, the 108 drought stress index that is reported here should be sufficiently representative for our 109 purposes. Note that we actually do not use the drought index in our calculations or models but 110 only use it to describe the meteorological conditions within the three year study period. 111 We also added data on the volumetric soil water content measured at the flux tower in Brasschaat (see L. 204; fig. 2; L. 219 – 231 and L. 244 -252) and commented on this data (L. 112 113 477 – 479 and L. 612 - 618), which can give an additional indication of the water deficit in the 114 study area during the considered period.

116 The Referee asks whether we observed an effect of the timing of the leaf unfolding on the 117 senescence timing, and whether we considered including other variables into our model. The 118 age of leaves might indeed affect the timing of the onset of senescence, especially in species 119 with an indeterministic growth pattern (e.g. birch). Therefore, we will test the correlation 120 between leaf unfolding and senescence timing (some preliminary results are available in the supplementary file 'TEST_BB_OLS_markdown'). However, our dataset will be limited to 121 122 mature trees in 2018 and 2019, as spring data for 2017 are not available and leaf unfolding 123 for the trees in the manipulative experiment was affected by establishment effects. However, 124 note that we did not follow the exact same leaves from bud burst to senescence. In addition, 125 it is hard to disentangle whether the different timing of the bud burst affects the timing of leaf senescence, or whether the opposite is the case (Marchand et al 2020). Our models simply 126 127 included "treatment", "leaf position", "day of year" and "individual tree" for the manipulative 128 experiment, and "year", "species", "leaf position", "day of year" and "individual_tree" for 129 mature trees. So, they did not include meteorological variables. A significant upgrade in the 130 modeling work is the application of GAMM or GAMLSS models, where correlations between 131 e.g. seasonal chlorophyll data and meteorological data, are accounted for. The amount of work in applying these models to senescence trends is significant and we are working on this 132 133 in a next manuscript. The following line was added at L. 630 – 633. "Although Fu et al. (2014) 134 suggested a correlation between the bud burst and the onset of autumn leaf senescence, we 135 have found no relationships for 2018 and 2019 in birch and beech, and a positive relationship 136 in oak (every delay of one day in the bud burst corresponded to a delay of ± two days in the 137 onset of autumn leaf senescence)". More details about this are added in a specific file 138 uploaded in Zenodo.

1403) The referee suggests highlighting the effect of drought stress on CCI and greenness in the141abstract, as discussed on L. 464 ("For the mature trees, the different drought response of the142autumn pattern of chlorophyll (no effect) and the loss of canopy greenness (advanced and

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enhanced) is probably an important reason of confusion still present today in the literature on the relationship between drought and autumn senescence"). We thank the referee for this suggestion and will consider this in the revision. We highlighted the different effect of the drought on the CCI and canopy greenness in the abstract (L. 30 - 33) and text (L. 597 - 605).

- 146 147
- 148 Kind regards,
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150 By Anonymous Referee #2

The article analyzes the impact of drought on the onset of autumn senescence and the difference featured 151 152 by different temperate deciduous tree species. The authors used a manipulative experiment of tree beech 153 sapling and three years of data on beech, birch, and oak trees. The authors show that drought did not 154 affect the onset of senescence. Tree saplings showed high mortality with drought, and mature trees 155 showed higher leaf mortality. No significant differences across species were observed. The manuscript 156 deals with a significant subject, senescence, about which not much is yet known. Understanding the 157 senescence process, particularly in relation to drought, is fundamental to predict the phenological cycle 158 of temperate trees better.

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160 1) Regarding the greenhouse experiment, I have a methodological concern. From the data reported in Fig 161 1 seems that the "drought" treatment does not have a significant (should be tested statistically thought) 162 effect on soil moisture (Fig 3c). Instead, the effect was mainly an increase of VPD that is not drought but 163 an increase of the atmospheric evaporative demand. One of the factors linked to the earlier senescence 164 in the case of drought is abscisic acid accumulation (ABA). Long term ABA responses should be more 165 induced by soil moisture. Root perceives reducing soil moisture and upregulate ABA synthesis. ABA is a 166 factor controlling earlier senescence (and stomatal regulation). I am not aware of studies showing the 167 high VPD can trigger the same response in terms of upregulation of ABA. It could be that the lack of 168 response observed was simply due to the fact that the reduction of soil moisture was not enough to trigger 169 the physiological response inducing earlier senescence.

2) Also, in general, I would not call drought the treatment. Given the data shown in Figure 1, I think it is
more heat stress. Please provide more insights to understand whether the treatment can be indeed called
drought treatment. If not, I would suggest talking about heat stress and increased atmospheric aridity.
This would not diminish the paper. There is a lot of discussion on the different repose of plants to
decreasing soil moisture and/or increasing VPD, and here I think the authors are looking at increased VPD
and not necessarily at drought. This can also support the discussion of the differences between 2018
(more soil moisture stress) and 2019 (more heat and VDP stress) – see discussion at line 469-470.

3) The 20% reduction of incoming light should also be better addressed (Line 162-163): though unclear, it
seems that senescence is controlled by photoperiod. How does 20% - decrease in incoming radiation
affect the photoperiod? The authors should check this and evaluate if the reduction of light has an impact
on the results.

- 4) In the methods section 2.1.2, when the CCI is mentioned the first time, I expected a description of the
- sampling (that comes later). I think it would be beneficial to move section 2.2 above, where the CCI ismentioned the first time. Preferentially, put a reference in paragraph 2.1.2 to paragraph 2.2.

5) The meteo stations are 20 and 60 km from the sites. But there is no information about where these stations were located (in a city, in a forest, in a grassland, at which height). Even if the climate regimes can be similar at a distance of 20-60 km can we be sure that the microclimate is comparable? I suggest the authors carefully check all this information and provide a methods description that can prove the study's robustness.

6) The equation and symbols do not follow the scientific format. I suggest to rewrite them. Also many variables have names that are more for a programming language but not following the scientific notion. I suggest to follow the IUPAC standards, or at least try to go close to that format. Avoid using "Leaf_place" Also please define the variable the first time is used, and then stick with the symbol: one example is the "day of the year" that in the equation 2 (model 1) is Doy and in the text is "day of the year"

1947) If I am not wrong there is a mistake in Eq 1. First if rH should not be expressed in % as indicated but as195fraction (rH[%]/100) Here the result of VPD with the current equation > T <- 25 > rH <- 50 > e0 <-</td>195fraction (rH[%]/100) Here the result of VPD with the current equation > T <- 25 > rH <- 50 > e0 <-</td>

613.75*exp((17.502*T)/(240.97+T)) > e <- rH*e0 > VPD <- e0-e > VPD [1] -155829.6 Moreover, even if the
 rH is used in the correct unit, the VPD unit is wrong. The resulting VPD from this equation is in Pa and not

198 kPa as indicated at line 144. > T <- 25 > rH <- $50/100 > e0 <- 613.75^* exp((17.502*T)/(240.97+T)) > e <-$

199 rH*e0 > VPD <- e0-e > VPD [1] 1590.098 The VPD reported in the figures seems correct, therefore please

- 200 verify is there is a problem in the Equation.
- 8) There are few track changes and typos in the manuscript. Please edit careful the article a. Line 249, line
 415, 416, 417, 4)
- 9) The reference to the R package is a bit strange R/ggpubr etc. Please modify in: "we use the R package
 ggpubr (Reference)". But it is very nice that the authors cite all the packages. This is important and often
 overlooked.
- 206 10) Please report "Model 1 and 2" in a less R script style. Please use mathematical notation
- 207 11) I think the breakpoint analysis was achieved with the "segmented" package and not dplyr", correct?
- 208 12) Line 464-465 this is interesting, please elaborate more this point.
- 209 Dear Anonymous Referee 2,
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Thank you for your review and suggestions. We will respond here to your comments:

213 1) The Referee asks whether it is possible that the reduction of soil moisture in the glasshouse 214 experiment was not enough to trigger the physiological response inducing earlier senescence. 215 He therefore questions whether an increased VPD can trigger the upregulation of ABA. 216 Literature shows that ABA, which is known to control earlier senescence, is indeed upregulated 217 as a response to the stomatal changes corresponding to changing vapor pressure deficit levels 218 (McAdam and Brodribb, 2016;McAdam et al., 2016;Bauerle et al., 2004;Xie et al., 2006). We 219 agree that the treatments $+0^{\circ}C$ and $+3^{\circ}C$ did not result in large differences in soil water 220 content. However, we will test this statistically, as suggested (for example, see the supplementary file 'TEST_SWC_markdown' and Rose et al. (2012) for additional information 221 222 on the possible statistical methodology). On the other hand, it is likely that larger differences 223 were present between the reference plots and the treatments, as the reference plots were

224 irrigated more (L. 160), and such irrigation regime showed values of soil water content of up 225 to ca. 0.25 m^3/m while the values of 0.05 m^3/m^3 were reached in the treatments (see Fig 1; 226 unfortunately, sensor malfunctioning did not allow us to gather soil water content data for 227 the reference plots). Given that we observed a high mortality, it might have been the case that 228 our +3 °C treatment was too extreme, triggering necrosis instead of earlier senescence 229 (Munné-Bosch and Alegre, 2004). We have commented extensively on the different 230 interpretation of the treatment effects and the lack of soil water content measurements in the 231 reference plots in L. 169 – 179.

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- 233 2) The Referee suggests we talk about heat stress rather than drought stress. As mentioned 234 above, the reference plots were irrigated more than the treatments plots (L. 148 - 149; 159 -235 160). Therefore, the more appropriate definition would be "treatment based on warming, less 236 irrigation and increased atmospheric aridity". We could use this definition (although longer 237 and somewhat impractical it is the closest to reality). In reference to L. 469 - 470 ("...the 238 drought of 2019, which coincided with several heat waves, might have been less damaging for 239 late summer leaf dynamics, than the drought of 2018..."), a more detailed comparison 240 between experimental manipulation and mature trees in years 2018 and 2019 would have 241 required a factorial approach separating drought and warming, while our design was more 242 basic. In addition, as shown in figure 3, the rainfall deficit was high in all years. It is true that 243 the rainfall deficit was extremely high in 2018 – 2019, but the rainfall deficit was also high in 2017 – 2018 and 2019 – 2020. Likely, more site specific measurements on the soil water 244 245 content would indeed have been useful. Note, however, that figure 2 and table 1 also indicate 246 that there was not only little precipitation but also that this precipitation fell in irregular 247 patterns, making potential droughts more likely. We have commented on the interpretation 248 of the treatment effect in L. 171 – 179. In addition, we have adapted our terminology to heat 249 stress and increased atmospheric aridity were appropriate (e.g. see L. 178; L. 491; L. 580; L. 250 585; L. 588: L. 620; L. 662 and L 691 – 693). Finally, we improved the discussion about the 251 treatments (L. 589 - 594). We also added data on the volumetric soil water content measured at the flux tower in Brasschaat (see L. 204; fig. 2; L. 219 – 231 and L. 244 - 252) and commented 252 253 on this data (L. 477 – 479 and L. 612 - 618).
- 255 3) The Referee asks to comment on the effect of the 20% reduction in light due to the colorless polycarbonate roof in the glasshouses (L. 162 – 163; "A draw-back of the experiment is that 256 257 the saplings in the reference plots received more incoming light (i.e. \pm 20%) than the saplings 258 in the glasshouses (Van den Berge et al., 2011)"). The Reviewer raises an interesting point: can 259 a reduction / change in the light affect the photoperiod? Preliminary tests suggested that the ratio of light in different wavelengths (e.g. R/FR) during civil twilight (i.e. what is required for 260 261 phytochrome to detect the photoperiod) does not change seasonally significantly in our study 262 area. This provide indirect evidence for us to believe that our light reduction (limited to 20%), 263 combined with the fact that very low light intensities are needed for plants to detect 264 photoperiod (Legris et al., 2019; Poorter et al., 2019; Franklin and Quail, 2010), would not have 265 caused significant changes in photoperiod. We agree that it could be interesting to test the 266 effect of the roof alone. However, this is not feasible in the short term. The effect of the roof is also partly captured by the results on the saplings in the +0 $^{\circ}$ C treatment glasshouses. We 267

have added extensive comments on the (potential) effect of the polycarbonate roof in L. 125 – 126; L. 184 – 189 and L. 650 – 659)

4) The Referee asks to consider restructuring section 2.2 and 2.1.2. We will consider this in the revision. This part has been improved. For clarity, measurements of CCI and loss of canopy greenness are not mentioned anymore when describing sites and climate (2.1.2) but only later on (in 2.2).

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- 276 5) The Referee asks to provide more information on the meteorological stations. We will add the 277 following information to the manuscript in the revision. (1) The station of Ukkel is located 278 within a green area in the suburb of Brussels (thus, classifiable as "urban park"). The 279 microclimate is expected to be different than at our study sites. However, data from Ukkel 280 were used to describe the intra-annual variability and long-term trends (Table 1 and Fig. 3), which are less affected by microclimate. (2) The meteorological station of Brasschaat is very 281 282 close to our sampling site in the Park of Brasschaat and in the Klein Schietveld (\pm 3 km and \pm 4 283 km, respectively). The meteorological station in Brasschaat is a 40 m high scaffolding tower, at which measurements are taken at various heights, and stands in a patch of mixed forest 284 covered mainly by Scots pines and deciduous tree species, such as oak and birch (see Carrara 285 286 et al. (2003) for more information). Data of the temperature, precipitation and humidity were 287 taken at the top of the tower. Data from Brasschaat were used to describe the seasonal 288 pattern in 2017, 2018 and 2019, and as input to the models. (3) The station of Woensdrecht is 289 located in an open field at a local airport surrounded by heathland and urban area. It is located 290 near the Markiezaatsmeer, an enclosed swamp ecosystem, within the river mouth of the 291 Schelde. The measurements in both Ukkel and Woensdrecht are taken at a height of 1.5 m. 292 However, these data were only used as gap-filling in case of short term gaps in the long-term 293 Brasschaat series. In terms of differences in the microclimate, it is indeed not ideal that we 294 needed to use data from the meteorological stations of Ukkel and Woensdrecht. However, we 295 are limited here by the availability of the data and the meteorological stations of Ukkel and 296 Woensdrecht are closest (and most representative) for our sampling sites. Note that we added this (and more) information in L. 233 – 255. 297
- 299 6) The Referee comments on the style of the model notation and suggests to better define the 300 variables at first use. We will define the variables further at first use and avoid inconsistencies. 301 However, both the descriptive style and mathematical notation are based on examples and 302 suggested notation in the specific literature (Zuur et al., 2007;Zuur et al., 2010;Zuur et al., 303 2011;Zuur et al., 2016;Simpson, 2018;Pedersen et al., 2019;Wood, 2017) and readers 304 interested in background references might find it easier if style consistency is respected. 305 Perhaps the Editor can comment on the journals preference? We have removed the 306 abbreviations and inconsistencies for the explanatory variables in the model notations (see L. 307 345; L. 356; L. 369 and L.380). Some notation is not significantly changed because it follows 308 similar literature and because there was no agreement on the alternative.
- The Referee notes there is an error in the units of the equation on the vapor pressure deficit.
 Thanks, we will correct this in the revision. The actual and saturation vapor pressure deficit

- are indeed in Pa, while the relative humidity should be noted as a fraction. The data was 312 313 indeed calculated using the correct equation. We have corrected the equation and the kPa to 314 Pa (see L. 142 – 148). 315 8) The Referee points out some typo's. The will be addressed in the revision. All typos have been 316 317 changed (e.g. see L. 316; L. 503- 505; ...) 318 319 9) The Referee suggests to write R packages in a different format. If preferred by the Editor, we 320 will address this in the revision. The editor considered this optional. 321 322 10) The Referee suggests using only the mathematical notation for model 1 and 2. Considering the 323 literature (see the response on comment 6) and the preference of the Editor, we will address 324 this in the revision. The editor considered this optional. 325 326 11) The Referee suggests to remove the reference to the R package "DPLYR" as the breakpoint 327 analysis is done only using the R package "SEGMENTED". While "DPLYR" was used for data 328 wrangling, we agree "SEGMENTED" is indeed the package that is used for the breakpoint analysis. We will remove the reference to "DPLYR" in the revision. We removed the reference 329 330 to the use of this package (see L. 405 – 406). 331 12) The referee asks to elaborate on L. 464. ("For the mature trees, the different drought response 332 of the autumn pattern of chlorophyll (no effect) and the loss of canopy greenness (advanced 333 and enhanced) is probably an important reason of confusion still present today in the literature 334 335 on the relationship between drought and autumn senescence"). We thank the referee for this 336 suggestion and will consider this in the revision. While the detoxification of chlorophyll is a 337 prerequisite for the expression of different coloration values, chlorophyll does not degrade at the same speed as other leaf pigments. In fact, not even all leaf pigments degrade (or are 338 339 formed) at the same velocity throughout the senescence process (Keskitalo et al., 2005). 340 Consequently, observations of changing coloration levels are difficult to interpret. Moreover, 341 note that coloration measurements also take into account leaf yellowing and mortality due to 342 hydraulic failure. We elaborated on the different effect of the drought on the CCI and canopy 343 greenness in the abstract (L. 30 – 33) and text (L. 597 – 605). 344 345 Kind regards, 346 347
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