

1 **Initial decomposition of floating leaf blades of *Nymphoides peltata* (S.G. Gmel.) O.**

2 **Kuntze: causes, impacts and succession**

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17

18 **Abstract**

19

20 **Background.** During a study on the outdoor floating leaf blade production of *Nymphoides peltata*
21 (S.G. Gmel.) O. Kuntze (Fringed Water Lily), ~~their~~ initial leaf blade decomposition was studied
22 simultaneously by measuring infected, damaged and lost area of floating leaf blades.

23 **Methods.** Data ~~of-on~~ initial decomposition over time were collected for all leaves during one growth
24 season in four plots: two in outdoor mesocosms and two in an oxbow lake. Each leaf was tagged
25 uniquely upon appearance in a plot. The vegetation in the mesocosms differed with respect to plant
26 species, one contained a mono-culture of *N. peltata* and the other *N. peltata* associated with *Glyceria*
27 *fluitans* (L.) R. Br. and *G. maxima* (Hartm.) Holmb. The lake plots were situated within a monospecific
28 *N. peltata* stand, differing in depth and position within the stand. Leaf length, visually estimated
29 percentages of damaged area for each damage type, and decay of the tagged leaves were recorded bi-
30 weekly. When the leaf blades sunk under the water surface or disappeared completely, they were no
31 longer followed. Under water the leaf-leaves decayed and were consumed by snails completely, so
32 contributing to the detritus food chain.

33 **Results.** The observed causes of damage on floating leaves were consumption and/or damage by
34 waterbirds (*Fulica atra*), pond snails, caterpillars (*Elophila nymphaeata*, *Cataclysta lemnata*),
35 chironomid larvae (*Cricotopus trifasciatus*), infection by a phytopathogenic fungus (*Septoria*
36 *villarsiae*), senescence by autolysis, and microbial decay. The impact on leaf loss and succession is
37 described for the causes found.

38

39 Keywords: Enclosure, Herbivores, Laminae, Mesocosm, Nymphaeid growth form, Senescence,
40 Succession of decomposition causes

41

Comment [C1]: I did not understand this wording. Do you mean something like "Successional changes in causes of leaf decomposition and impacts of different causes are discussed"?

42

43 **Introduction**

44

45 Aquatic macrophytes can be considered as the basic frame of wetland ecosystems, called macrophyte-
46 dominated systems (Den Hartog & Van der Velde 1988, Jeppesen, Søndergaard & Christoffersen
47 1998). The nymphaeid macrophyte, a growth form represented by species of Nymphaeaceae,
48 Menyanthaceae, Potamogetonaceae, Polygonaceae and Aponogetonaceae, forms the base of
49 nymphaeid-dominated systems (Van der Velde 1980). This growth form is characterized by floating
50 leaves, flowers on or elevated above the water surface and roots in the sediment of shallow open waters
51 or littoral borders (Luther 1983, Van der Velde 1981).

52 In shallow freshwater lakes, *Nymphoides peltata* can cover large areas (e. g. Brock, Van der Velde &
53 Van de Steeg, 1987), where submerged plants disappeared by accumulation of organic matter on the
54 sediment during succession, eutrophication and acidification (Arts et al. 1990; Wiik et al. 2015).

55 Information about distribution, growth form, importance of floating leaves, habitat and environmental
56 conditions of *N. peltata* (Fig. 1) is given in Klok & Van der Velde (2022).

57

58

59 **Decomposition of floating leaf blades**

60 Decomposition of leaves in natural conditions (Fig. 2) involves a complex set of interacting processes,
61 which can be roughly classified into internal (physiological) and external (abiotic or biotic) processes
62 (Van der Velde et al. 1982, Kok 1993). At first only host specific species – more or less specialized and
63 often restricted to particular plant taxa – are able to break through the defense system to consume fresh
64 plant tissue. At a later stage, when the defense system has been weakened, other species colonize the
65 leaves.

Comment [C2]: I did not understand this part of the sentence. Why would submerged plants disappear due to accumulation of organic matter on the sediment? Do you mean that submerged plants died due to shading by *Nymphoides*, resulting in accumulation of dead plant matter on the sediment surface? Or do you mean that dead leaves of *Nymphoides* dropped onto submerged aquatic vegetation, smothering it and resulting in build up of organic matter on the sediment?

66 Decomposition of young leaf blades of *N. peltata* already starts under water before they unroll with
67 consumption and/or damage. During initial decomposition, when leaves are still floating, macrophyte
68 tissue is used by herbivores and by phytopathogenic and saprotrophic microorganisms. Various causes
69 and stages of decomposition can be recognized on a single leaf. Damage of leaves can induce the
70 leaching of soluble carbohydrates such as oligosaccharides and starch, proteinaceous and phenolic
71 compounds, some of which can be rapidly metabolized by microorganisms. Finally, plant tissue
72 senesces by autolysis, colouring yellow by the resorption of chlorophyll, followed by further
73 decomposition initiated by weak pathogens and facultative herbivores, leading to the production of
74 debris and fecal pellets. The chemical composition of plant tissue also changes during autolysis due to
75 hydrolysis of macromolecules (which may weaken tissue structure), resorption of nutrients (like N and
76 P, as well as carbon compounds such as starch) and loss of secondary compounds. Furthermore, leaves
77 are colonized by microorganisms, causing microbial enrichment which makes the tissue more attractive
78 for detritivorous macroinvertebrates. Decayed floating leaves sink underwater to the bottom, where
79 they provide a resource for detritus-based benthic food webs during further decomposition (Klok &
80 Van der Velde 2019). To study the latter decomposition process, the litterbag method is commonly
81 used (Brock et al. 1982, Brock 1985, Wieder & Lang 1982). As *N. peltata* only grows in alkaline water
82 (Van der Velde, Custers & De Lyon 1986, Smits et al. 1988) and development of floating leaves is
83 dependent on calcium uptake of this plant, decomposition is studied only in alkaline water (Smits,
84 Schmitz & Van der Velde 1992).

85

86 **Research questions**

87 The present study covers initial decomposition of floating leaves of *Nymphoides peltata* (S.G. Gmel.)
88 *O. Kuntze* in different water bodies by investigating the effects of damage causes found on floating
89 leaves. Together with the complementary study about the production of floating leaves (Klok & Van

Comment [C3]: How is this known? References should be cited for previous work on this topic.

Comment [C4]: Cite reference

Comment [C5]: Reference?

Comment [C6]: It seems that this general topic was already studied by Van der Velde G, Van der Heijden L.A. 1985. That previous study should be introduced here and here is should be explained what further new information will be presented in the present study.

90 der Velde 2022) it describes the life and plasticity of floating leaves of *Nymphoides peltata* under
91 different environmental circumstances. This study deals with the following questions:

- 92 1) What are the causes and patterns of initial decomposition of floating leaves?
93 2) What is their impact on leaf properties?
94 3) How does initial decomposition progress during the season and is there a succession of causes?
95 4) Which factors make the leaves susceptible for decomposition?

Comment [C7]: This is an important question but was not addressed by your experiment. This question should not be listed among the others which were addressed by your student. This question could still be discussed in the Discussion section although your study makes no concrete contribution towards addressing this question.

97 **Materials and Methods**

98 99 **Sites**

100 Research took place in four plots two in outdoor mesocosms and two in an oxbow lake, each with a
101 size of 50x50 cm (Klok & Van der Velde 2022) (Table 1).

102

103 **Potential, actual and photosynthetic leaf area**

104 A distinction was made between potential, actual and photosynthetic leaf area. The potential area is
105 defined as the area of an entirely intact leaf, the actual area as the potential area minus the area missing
106 and the photosynthetic area as the remaining green part of the actual area.

107

108 **Regression equation for calculating leaf area**

109 The potential leaf area was calculated from leaf length and leaf width and is described
110 mathematically by equation (1), which has been determined by previous research (Van der
111 Velde et al. 1982):

$$112 \quad A(L, W) = 1.028 * \pi * \left(\frac{L+W}{4}\right)^2 \quad (1)$$

113 where: $A(L, W)$ = potential leaf area at length L and width W (mm²)

114 L = leaf length (mm)

115 W = leaf width (mm)

116 1.028 = correction factor (the leaves are not circular)

117

118 **Field data**

119 The initial leaf decomposition of *N. peltata* floating leaf blades ~~has been~~ studied in the same plots
120 and at the same time as the leaf production as described in Klok & Van der Velde (2022). Initial
121 decomposition data included visually estimating both leaf damage and decomposition per cause as
122 percentage of the potential leaf area of each leaf. Several types of damage and their causes have been
123 distinguished and described earlier (Lammens & Van der Velde 1978, Van der Velde 1979).

124

125 **Results**

126

127 **Floating leaf data**

128 Floating leaf information, comprising total number of leaves, total potential leaf area, leaf life span,
129 growth period and vegetation period, is shown per plot in Table 2. The combination over time of total
130 number of leaves and total potential leaf area is shown per plot (Fig. 3).

131 Unfortunately, data of the first weeks in 1978 were missing for CT1 and CT2. Fortunately, the growing
132 season of *N. peltata* started on the same date both in 1978 and in 1979, so data of the first weeks of
133 1979 for CT1 and CT2 have been used to give an indication of number of leaves and leaf area at the
134 start of the season.

135

136 **Causes and patterns of initial decomposition**

137 All causes and stages of initial decomposition found in this study are described below:

Comment [C8]: I think it is important to include this, otherwise you would need to describe in Methods how you determined the fungus species as well as the invertebrate species. I assume this is described in the references cited here.

138 *Consumption and damage by waterfowl.* Consumption of leaf tissue by coots (*Fulica atra* L., Rallidae)
139 occurred in the lake plots only and can be recognized by missing parts in the form of triangular areas at
140 the margin of leaves. Sometimes major parts of leaves are consumed. Prints of the beak are visible
141 around the consumed areas.

142 | *Consumption by pond snails.* A major cause of damage on fresh leaf tissue in all plots ~~is~~ was caused by
143 *Lymnaea stagnalis* (L.) (Lymnaeidae, Gastropoda) by consuming parts of young leaves under water,
144 resulting in rows of holes in the unrolled leaf blades, large near the edge and smaller towards the center
145 | of the leaf. To a lesser extend other lymnaeid species and other freshwater pulmonate snails ~~are~~ were
146 involved, showing a preference for decaying leaf material, in particular areas infected by fungi.

147 *Consumption and damage by aquatic caterpillars of moths.* The caterpillars of the moths *Elophila*
148 *nymphaeata* (L.), Crambidae, Lepidoptera (brown china-mark, lake plots only) (Gaevskaya 1969,
149 Lammens & Van der Velde 1978) and *Cataclysta lemnata* (L.), Crambidae, Lepidoptera (small china-
150 | mark, tank plots only) damaged d floating leaves in two ways: by leaf tissue consumption and by cutting
151 out oval leaf patches that are used for shelter (Van der Velde 1979). Floating shelters are created either
152 by attaching a patch to the underside of a floating leaf or by spinning two patches together (*Elophila*),
153 or by constructing a floating case by various materials, in particular small leaf pieces (*Cataclysta*).

154 *Mining by chironomid larvae.* Larvae of the midge *Cricotopus trifasciatus* Mg., Chironomidae,
155 | Diptera, were observed to mine their way through the leaf tissue by consuming particular tissue layers
156 while leaving the lower epidermis unaffected (halfminer) (Lammens & Van der Velde 1978). They
157 occurred in the lake plots only (Fig. 4).

158 *Infection by phytopathogens.* Leaves were infected in all plots by the fungus *Septoria villarsiae* Desm.,
159 Mycosphaerellaceae, Capnodiales, the causative agent of a leaf spot disease (Fig. 5). Asexual spores
160 (conidia) are produced in conidiomata (black spherical structures) (Fig. 6).

161 *Autolysis.* Autolysis occurred in all plots and is visible by the change in leaf color from green to yellow,
162 indicating that chlorophyll is degraded.

163 *Microbial decay*. The resistance of a leaf against microbial infection disappears quickly due to erosion
164 of the wax layer and autolysis, facilitating microbial decay in all plots, which ~~is~~was indicated by a
165 change in leaf color from yellow to brown and the softening of the leaf tissue by maceration. ~~By~~
166 During microbial decay, ~~they sink~~leaves sunk under the water surface.

167 In tank plots CT1 and CT2 loss and damage by *Cataclysta lemnata*, pond snails, *Septoria villarsiae*,
168 autolysis and microbial decay occurred. Bemmelse Strang lake plots BS1 and BS2 suffered from loss
169 and damage by *Fulica atra*, pond snails, *Elophila nymphaeata*, *Cricotopus trifasciatus*, *Septoria*
170 *villarsiae*, autolysis and microbial decay. Fig. 7 shows the relative contributions to initial
171 decomposition of all causes in all plots.

172

173 **Impact of causes**

174 The impact of initial decomposition causes on leaves for all plots is shown in Table 3. Autolysis and
175 microbial decay are the main decomposition causes. Generally, initial decomposition caused by
176 animals was a very small part of the total potential floating leaf area in all plots, since *N. peltata* leaves
177 disappear under water rather soon after autolysis and cell death ~~for further decomposition~~ and ~~are~~were
178 thus lost for measurement of further decomposition. The combination of number of leaves and total
179 potential leaf area clearly shows that in the second half of the growth period the tank plots produced
180 smaller leaves, in contrast to the lake plots, as described in Klok & Van der Velde (2022).

181 For all plots the absolute and relative loss by damage causes of photosynthetic leaf area over time is
182 shown (Fig. 8) with the impact of damage causes (Table 3).

183 The order of impact of damage causes from high to low, based on average percentages of the total
184 potential area affected, is per plot:

- 185 - CT1: autolysis, *Septoria*, microbial decay, snails, *Cataclysta*,
- 186 - CT2: autolysis, microbial decay, *Septoria*, snails, *Cataclysta*,
- 187 - BS1: *Cricotopus*, microbial decay, autolysis, *Fulica*, snails, *Elophila*, *Septoria*,

188 - BS2: microbial decay, *Cricotopus*, autolysis, *Fulica*, snails, *Septoria*, *Elophila*.

189 The impact of initial decomposition damage (= loss of photosynthetic leaf area) per cause over time for
190 the plots is shown in Fig. 9 (plot CT1), Fig. 10 (plot CT2), Fig. 11 (plot BS1) and Fig. 12 (plot BS2).

191 All figures use the same scale for proper comparison.

192 The minor damage causes *Cataclysta*, *Elophila*, *Septoria* and snails are also shown with an enlarged Y-
193 scale to be able to see display more details (Fig. 13). For *Cataclysta*, *Cricotopus*, *Elophila* and *Septoria*
194 the damage increments are shown along with the total damage over time (Fig. 14, Fig. 15, Fig. 16, Fig.
195 17). The development of several large generations of midge and moths can be seen (Fig. 9 through Fig.
196 17). Not all generations exist parallel in time. For *Cataclysta*, *Cricotopus*, *Elophila* and *Septoria* the
197 summation of the increments is shown for all plots (Fig. 18).

198 The percentage of leaves affected in the tank plots had some variation (91.0 and 95.6 %), which was
199 slightly lower than in the lake plots (96.4 and 97.0 %). The average percentage of potential leaf area
200 affected was stable: high for the tank plots (84.15 and 84.53 %) and low for the lake plots (23.23 and
201 23.75 %).

202

203 Succession of causes

204 The succession of damage causes, based on first occurrence, is per plot:

- 205 - CT1 (mixed): autolysis, microbial decay, snails, *Septoria*, *Cataclysta*,
- 206 - CT2 (monospecific): autolysis, microbial decay, snails, *Septoria*, *Cataclysta*,
- 207 - BS1 (center): *Cricotopus*, microbial decay, autolysis, snails, *Elophila*, *Fulica*, *Septoria*,
- 208 - BS2 (border): *Cricotopus*, snails, autolysis, microbial decay, *Elophila*, *Septoria*, *Fulica*.

209 The above succession lists show that the tank plots (CT1, CT2) have the same succession order, while
210 the lake plots (BS1, BS2) have quite a different order.

211

212 Influences on susceptibility

Comment [C9]: Does this paragraph refer to only the minor causes of damage listed in the previous paragraph or all causes of damage? Please specify at the start of this paragraph.

Comment [C10]: This is Discussion not Results. Please integrate into the Discussion section

213 Prolonged cloudy and wet weather are supposed to impose stress on *Nymphoides* by weakening the
214 defense of leaves due to reduced solar radiation, and thus promoting heavy infection and damage by
215 phytopathogens (Van der Aa 1978). Leaf blades contain phenolics with fungistatic properties
216 (Smolders et al. 2000) and poor light conditions reduce the content of phenolics in leaf tissue (Vergeer
217 & Van der Velde 1997), which makes mature leaves vulnerable to infection.

218

219 **Discussion**

220

221 Unfortunately, due to the fact that the four locations did not contain multiple plots, it is useless to apply
222 statistics for firm conclusions.

223

224 **Differences between plots**

225 The number of leaves in the tank plots was considerably higher and the size of leaves considerably
226 smaller compared to the lake plots (Table 2) (Klok & Van der Velde 2022). This can be explained by
227 the limited space and by the limited nutrient availability in the tank plots. At a higher degree of
228 enclosure more and smaller leaves appeared, compared to a low degree of enclosure (Klok & Van der
229 Velde (2022) and literature therein).

230 Compared to the center plot in the lake, the border plot has a higher nutrient availability through
231 continuous supply via water currents, which counts for ~~less~~ fewer, but larger leaves.

232 The tank plots showed a decrease in leaf area over time after a maximum at the start of the season. Due
233 to the sudden inundation of river water in early spring, the reaching of such a maximum was disrupted
234 in the lake plots. This resulted in a later maximum, which was lower for the center plot due to limited
235 nutrients and much higher for the border plot with much less leaves and almost unlimited nutrients.

Comment [C11]: Reword – the reviewer has suggested alternative wording.

Comment [C12]: I didn't understand this wording. Do you mean "promotes the growth of"?

236 | The absence of waterfowl in the tank plots ~~is obvious~~ was expected, since the plots were covered with a
237 | frame with chicken wire. The occurrence of the moth *Cataclysta* in the tank plots ~~is~~ was due to
238 | introduction with *Lemna* in the past, while the moth *Elophila* occurred in the lake plots. The midge in
239 | the lake plots (*Cricotopus*) probably exists in larger water volumes only where wind and wave action
240 | provide the larvae with oxygen. Infection by the fungus (*Septoria*) was very high in the tank plots and
241 | low in the lake plots, which could be caused by the combination of high leaf density and low nutrient
242 | availability in the tank plots.

243 | The average percentage of potential leaf area affected, high for the tank plots and low for the lake plots,
244 | ~~is~~ was caused by the very low infection by *Septoria* in the lake plots compared to the tank plots and
245 | possibly by the high leaf density and the low nutrient availability in the tank plots. Snails had a large
246 | impact in one tank plot.

247 | Incremental damage ~~is~~ was higher for CT1 and BS1 compared to CT2 and BS2, respectively, except for
248 | *Cataclysta*.

249

250 | **Senescence**

251 | Newly unrolled floating leaf blades are fully green and hydrophobic due to a thick epicuticular wax
252 | layer. This layer gradually erodes during senescence and as cellulolytic and other bacteria and fungi
253 | colonize the leaf tissue (Howard-Williams et al. 1978, Robb et al. 1979, Rogers & Breen 1981,
254 | Barnabas 1992). Senescence starts shortly after the first leaves are fully grown and continues
255 | throughout the growth period. During senescence the leaves turn from green to yellow by autolysis, an
256 | orderly physiological process controlled by the plant itself, and ultimately turn to brown. Concomitant
257 | microbial decay softens the leaves.

258 | Van der Velde & Van der Heijden (1985) analyzed the relative increase of different damage and
259 | decomposition types at different age classes (each class 5 days) of the floating leaves of *N. peltata* in
260 | the same plots as used in this study. They compared the results between the concrete tanks (two plots

261 together) and the Bemmelse Strang (two plots together). Patterns of occurrence of the decomposition
262 types over leaf age classes shows clearly that young leaves are damaged already when they appear at
263 the water surface in the case of *Cataclysta lemnata*, (age 1-9, peak at age 3) and *Elophila nymphaeata*
264 (age 1-7, peak at age 1), *Cricotopus trifasciatus* (age 1-13, peak at age 4) snails (age 1-9 CT, peak at
265 age 6 and BS age 1-9, peak at age 1) and Coots (*Fulica atra*) (age 1-10, peak at age 2). *Septoria*
266 *villarsiae* started in the concrete tanks at leaf age 1 with a peak at age 4-5 (leaf age 1-11), while in the
267 Bemmelse Strang it started at leaf age 4 with peak at age 11 leaf age 4-13. Yellow areas started in both
268 waters at age 1 with peak at 5 (CT 1-7, BS 1-9). Decayed areas replaced the yellow ones at leaf age 1-
269 11 with a peak at age 8 in the case of the concrete tanks and at leaf age 1-13 with a peak at age 12 in
270 the case of the Bemmelse Strang.

271 From these results we can conclude that the herbivores prefer young leaves as food. Yellow and
272 decayed areas showed a similar course and peaked after each other. The most clearly clearest difference
273 was the timing of *Septoria*, ~~which~~ infection, which started early in young leaves in the concrete tanks
274 but in the Bemmelse Strang in older leaves.

275

276 **Succession of causes**

277 The succession of leaf decomposition causes, based on first occurrence, was exactly the same for both
278 tank plots: autolysis was followed by microbial decay, snails, *Septoria* and finally *Cataclysta*.

279 For the lake plots this succession was different. Both plots started with *Cricotopus*, for the center plot
280 followed by microbial decay, autolysis, snails, *Elophila*, *Fulica* and *Septoria*, and for the border plot
281 followed by snails, autolysis, microbial decay, *Elophila*, *Septoria* and *Fulica*.

282 Primary underwater consumption of young leaves by snails may explain the different succession order
283 in the lake plots for snails, autolysis and microbial decay. The late occurrence of microbial decay in the
284 border plot may be explained by more available nutrients strengthening the leaf condition.

285

Comment [C13]: What does this mean?

Comment [C14]: This seems to contradict your claim in the Introduction (line 63) where you claim that young leaves are only utilized by specialist herbivores but more generalist and facultative herbivores prefer older leaves (line 73, 68)

286 **Comparison with other nymphaeids**

287 The growth period of *N. peltata* in the plots lasted 155 to 171 days, or 85 to 93 % of the vegetation
288 period of 169 to 202 days of the plots. The total loss of photosynthetic area of *N. peltata* was 99.9 %
289 for CT1, 100.0 % for CT2, 53.1 % for BS1 and 60.5 % for BS2 (Table 2, Table 3).

290 From similar research on plots with *Nuphar lutea*, *Nymphaea alba* and *N. candida* (Klok & Van der
291 Velde 2017, Klok & Van der Velde 2019), the growth period lasted 71 to 134 days, or 53 to 73 % of
292 the vegetation period of 135 to 199 days. Percentages of loss of photosynthetic area ranged from 38 to
293 50 % for *Nuphar* and 44 to 54 % for *Nymphaea*.

294 The relatively long growing period for *N. peltata* can be explained by the lasting development of new
295 leaves.

296 Loss of leaf tissue tended to increase during the vegetation period of *Nuphar lutea* and *Nymphaea alba*
297 from less than 20% of the total potential leaf area to more than 50%. Leaf area loss by damage of
298 *Nymphaea candida* (HW) was less than 10% to almost 20%.

299

300 **Conclusions**

301

302 Initial decomposition included the internal cause autolysis and external causes by animals, fungi and
303 microbes. Animals included birds (*Fulica atra*), snails (mainly *Lymnaea stagnalis*), caterpillars of
304 aquatic moths (*Cataclysta lemnata*, *Elophila nymphaeata*) and chironomid larvae (*Cricotopus*
305 *trifasciatus*). The ascomycete *Septoria villarsiae* was the most important fungus. Autolysis and
306 microbial decay were the main causes, while the other causes generally were marginal.

307 From high to low impact the order of initial decomposition causes was autolysis, *Septoria*, microbial
308 decay, pond snails, *Cataclysta* for the tank plots and *Cricotopus*, microbial decay, autolysis, *Fulica*,
309 pond snails, *Elophila*, *Septoria* for the lake plots.

Comment [C15]: This reasoning seems circular. A growing period is defined based on continuing vegetative growth, so it cannot be explained by continuing vegetative growth.

Comment [C16]: Do you mean potential photosynthetic leaf area, as defined in Methods?

310 The succession of causes per plot, based on first occurrence, was autolysis, microbial decay, snails,
311 *Septoria*, *Cataclysta* for the tank plots and it was *Cricotopus*, microbial decay, autolysis, snails,
312 *Elophila*, *Fulica*, *Septoria* for the center lake plot and *Cricotopus*, snails, autolysis, microbial decay,
313 *Elophila*, *Septoria*, *Fulica* for the border lake plot.

314 Factors that influence leaf condition and thus allow initial decomposition are senescence, decrease of
315 nutrient availability and cloudy and rainy weather conditions.

316

317 **Acknowledgements**

318

319 We thank L. van der Heijden, P.M.M. Bexkens and P.A.J. van Grunsven for collecting and working out
320 field data, E.L. Huijser (†) for making the field data digitally available, P.H.M. Charpentier for
321 providing us with all necessary literature and O. Knoppers for checking the english language..

322

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330

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333

Comment [C17]: This finding cannot be a conclusion based on the data presented in this study and therefore this should not be the final concluding statement.

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