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1 **Mixotrophy in aquatic plants, an overlooked ability**

2

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13

14 **Keywords:** aquatic plant, mixotrophy, heterotrophy, dissolved organic carbon

15 **Glossary**

16 **Absorbotrophy:** (also called osmotrophy) the uptake of low-molecular-weight substances by way of passive
17 or active transport at cell membrane level.

18 **Axeny:** experimental conditions free of microbes, competitors and facilitators. Often this does not include
19 internal microbiota such as endophytes in plants.

20 **Biotrophic mixotrophy:** refers to the organic carbon gained by an autotrophic organism living with another
21 organism without killing it, in a parasitic or mutualistic interaction.

22 **Autotrophy:** nutrition mode involving inorganic carbon fixation via photosynthesis (mainly).

23 **Heterotrophy:** nutrition mode involving the consumption of carbon organic compounds from plant and
24 animal sources for example.

25 **Dissolved organic carbon (DOC):** the carbon fraction of organic matter soluble in water, composed of parti-
26 cles of less than 0.45 µm.

27 **Embryophyte:** terrestrial multicellular plants, including angiosperms, that repeatedly recolonized freshwa-
28 ter and marine ecosystems.

29 **Phagocytosis:** cellular process bringing prey or particles into the cell in a vesicle formed by a budding of the
30 cell membrane, mostly in the framework of cellular nutrition.

31 **Endophyte:** any microbe (especially fungi or bacteria) that inhabits the internal tissues of plants without
32 causing negative consequences (endosymbiosis in the case of reciprocal benefits).

33 **Mixotrophy:** nutrition mode combining autotrophy and heterotrophy, in which organic matter is obtained
34 by photosynthesis and by direct uptake from the environment.

35 **Necrotrophic mixotrophy:** refers to the carbon gained by autotrophic organisms from prey that they kill,
36 such as insects in the case of carnivorous plants, or small crustaceans for aquatic plants like *Utricularia* sp.

37 **Phagotrophy:** nutrition mode by engulfment of prey or particles in the cell by phagocytosis.

38

39 **Abstract**

40 **Aquatic Embryophytes play a key role in the proper functioning of aquatic ecosystems, where**
41 **carbon (inorganic and organic forms) is pivotal in biogeochemical processes. There is growing awareness**
42 **that mixotrophy, the direct use of exogenous organic carbon by autotrophs, is a widespread phenomenon**
43 **and has emerged recurrently in the evolution of many autotrophic lineages. Despite living in an environ-**
44 **ment providing organic matter and presenting many favorable predispositions, aquatic plants from the**
45 **Embryophytes, except carnivorous ones, have never been deeply investigated for mixotrophy.**
46 **Here, we address the possibility that aquatic plants may exhibit mixotrophy, a prospect overlooked by**
47 **research until now, and that this may be much more widespread than imagined under the conventional**
48 **paradigm of plants considered as strict autotrophs.**

49 **Aquatic ecosystems, a favorable environment for opportunistic plants with mixotrophic abilities.**

50 Aquatic **Embryophytes** (hereafter called aquatic plants) are terrestrial plants that moved back secondarily
51 in their evolution to the aquatic environment. In freshwater aquatic ecosystems, on which we focus, they
52 provide many services. They act as a shelter for various species of epiphytic microorganisms, macroinverte-
53 brates and fish [1,2]. They are one of the main primary producers and they regulate water flow and mitigate
54 high temperatures by providing shade [3].

55 In addition to these many important aspects, we suggest in this paper that another crucial facet of their
56 metabolism has been overlooked until now. Except in the germination stage, it is considered that they rely
57 on photosynthesis (autotrophy) for their carbon nutrition. However, many terrestrial Embryophytes are
58 partially **heterotrophic**, meaning that they count on both organic carbon resources and photosynthesis
59 (mixotrophy;[4]). Furthermore, aquatic ecosystems contain **dissolved organic carbon (DOC)** originating
60 from animal or plant residues and are increasingly contaminated by organic-rich discharges from human
61 activities such as wastewater and agricultural leachates. In this context, could **mixotrophy** based on DOC be
62 widespread in aquatic plants?

63 Here we review the evidence for mixotrophy in aquatic plants. We present mixotrophy, three main hy-
64 potheses concerning its emergence in this plant group, and its ecological relevance.

65 **Mixotrophy in freshwater ecosystems**

66 *DOC as a source of carbon for photosynthetic organisms*

67 Dissolved organic carbon (DOC) is the fraction of organic matter that is water-soluble and represents 67
68 to 90 % of the total organic carbon in water [5,6]. DOC is composed of particles of less than 0.45 μm [6] and
69 it is found at concentrations from 0.1 to more than 300 $\text{mg}\cdot\text{L}^{-1}$ [7]. DOC is composed of a wide variety of
70 molecules, mainly humic acids (50-75 %) as well as carbohydrates (mono-, di-, oligo- and polysaccharides),
71 polyols, lipids, amino acids, small organic acids, etc. [5,6].

72 DOC is partly autochthonous, formed from residues of aquatic organisms [6,8], and mostly allochthonous
73 from riverbanks and watersheds, including anthropogenic sources [6,9]. In a global warming context, storms
74 and droughts are increasingly prevalent. Because of altered river flow regimes – leading to severe flooding
75 or lower water levels – combined with changes in land use, periods of high DOC concentration are more
76 frequent [8,10]. Moreover, global changes in pH and UV-B radiations affect the degradation, concentration,
77 and bioavailability of DOC, as well as its autochthonous *versus* allochthonous origin [6]. Finally, the devel-
78 opment of densely populated areas leads to high levels of DOC of widely varying concentrations and nature
79 [10]. DOC is therefore available and, under specific limiting environmental conditions (low light and/or inor-
80 ganic carbon resources), aquatic plants may be selected for their mixotrophy capacities.

81 *The notion of mixotrophy*

82 By definition, the use of exogenous carbon sources such as DOC by organisms that are otherwise auto-
83 trophic (relying on photosynthesis) is called mixotrophy [4,11–13]. More specifically, plant metabolisms
84 display a continuum between autotrophy, mixotrophy (Figure 1A), and strict heterotrophy (Figure 1B). Even
85 in globally autotrophic plants, heterotrophic activity occurs, based on endogenous resources, in tissues
86 subject to permanent darkness (e.g. roots and rhizomes) and at night in the case of aerial tissues. This abil-
87 ity suggests that most autotrophs are metabolically preadapted to use organic carbon sources, if their car-
88 bon source evolves from endo- to exogenous [14].

89 Mixotrophy is a complex of mechanisms of organic carbon uptake that has been classified in different
90 ways [11,15,16]. In this paper we follow the categorization by Selosse *et al.* (2017) that defines three mech-
91 anisms: **necrotrophic**, **biotrophic** and **absorbotrophic** mixotrophy (Box 1) [4]. Our hypothesis is that aquatic
92 plants present some of these mechanisms, which are already well known and studied in microalgae.

93 *Ecology of mixotrophy*

94 Mixotrophy is often a response to a lack of nutrients in the environment [13,17]. Plants in low light
95 conditions favor mixotrophy for carbon supply. Some forest plants have adapted to the shade thanks to
96 mixotrophy, using the carbon from the fungus associated with their roots (biotrophic mixotrophy based on
97 mycorrhizal fungi; [18]). Mixotrophy also enables plants to obtain organic nitrogen and phosphorus in nutri-
98 ent-poor conditions: for example, carnivorous plants like *Nepenthes* adapt to nutrient-poor soils by recover-
99 ing nitrogen and phosphorus from their prey [4] in a necrotrophic mixotrophy, and carbon compounds are
100 also transported with these elements. Furthermore, absorbotrophic mixotrophy has often been used for *in*
101 *vitro* culture on sugar-enriched media, especially for callus and seeds [19,20].

102 Absorbotrophic mixotrophy exists in autotrophic microalgae. In the case of freshwater microalgae, mixo-
103 trophy is mostly reported in oligotrophic systems [21] and in eutrophic, DOC-rich but poorly illuminated
104 estuaries [9], where it probably plays a role in carbon supply. In microalgae, absorbotrophic or necrotrophic
105 mixotrophy by **phagocytosis** is mainly associated, respectively, with high levels of DOC and concomitant

106 light limitation [22,23]. Significant organic pollution is also favorable [24], suggesting that mixotrophic mi-
107 croalgae seize trophic opportunities. There is an emerging consensus about the frequency and diversity of
108 mixotrophy for algae, protists, and terrestrial plants [4,16].

109 *Diversity of mixotrophy in aquatic microalgae*

110 Absorbotrophic mixotrophy (Box 1) reported in microalgae from different phylogenetical backgrounds
111 points to the relevance of mixotrophy in aquatic ecosystems. For example, the growth of *Chlorella sorokin-*
112 *iana* is improved by acetate in high light conditions or by glucose in low light conditions [25]; *Chlamydomo-*
113 *nas sp.* can be mixotrophic [26,27] and its growth is boosted by glucose [28]; absorbotrophic mixotrophy is
114 also reported in diatoms, such as *Phaeodactylum tricornutum* [29]. Many flagellated microalgae from a
115 wide variety of clades are also necrotrophic mixotrophy by phagotrophy, i.e. by capturing smaller preys
116 [12,17,30], including green microalgae [31].

117 Certain aquatic heterotrophic protists and symbiotic algae form consortia, which combine photosynthesis
118 and necrotrophic mixotrophy by phagotrophy [21,23]. Unlike the majority of organisms that are autotrophic
119 and become mixotrophic through secondary heterotrophy, these protists have acquired the capacity to
120 perform photosynthesis through symbiosis [21,32,33]. From an evolutionary point of view, mixotrophy can
121 therefore derive from both autotrophy and heterotrophy. It indicates that the evolution to mixotrophy is a
122 response to a widespread opportunity in ecosystems [4]. However, as far as aquatic environments are con-
123 cerned, research has mostly focused on microalgae rather than aquatic plants.

124 **Mixotrophy in aquatic plants, old and new evidence**

125 *A gap in research*

126 We propose several hypotheses to explain the currently limited research on mixotrophy in aquatic plants.
127 First, the belief that plants are purely autotrophic remains dominant, in spite of the emerging view of a con-
128 tinuum between auto- and heterotrophy. Second, aquatic autotrophs have been far less studied than ter-
129 restrial ones over the last 20 years (a quick search on March 16, 2021 produced 12.3% entries for “*plant*
130 *AND physiology AND ecology*” with the complementary keyword “*aquatic*” on the WebOfScience, KJD, Med-
131 line, RSCI and SCIELO databases). Yet, some examples below show pioneering evidence for mixotrophy in
132 aquatic plants. Third, microorganisms, bacteria in particular, are very efficient in absorbing carbon. This is
133 one reason why research might have neglected the fact that plants could efficiently compete for DOC [28].
134 However, locally and chronically high DOC concentrations suggest that significant amounts of DOC remain
135 available for aquatic plants, at least temporarily, and allow mixotrophy.

136 Mixotrophy has been poorly studied in Embryophytes, with the exception of predation in the case of car-
137 nivorous plants with dedicated organs such as *Utricularia sp.* [34,35]. The question here is therefore to see
138 if mixotrophy can be widespread in aquatic plants, beyond mechanisms used in carnivory.

139 *Arguments in favor of mixotrophy in aquatic plants*

140 As early as 1961, glucose and sucrose were reported to increase the growth of aquatic Araceae under low
141 light conditions [36]: sucrose, glucose and fructose had significant effects on the growth of *Lemna sp.* and
142 *Spirodela sp.* unlike maltose, ethyl alcohol, glycerol and mannitol [37]. The optimum amount of sucrose was
143 5 to 20 g.L⁻¹ [38–42]. Surprisingly, there were no further studies nor questions about the ecological implica-
144 tions for the next fifty years; perhaps because these results were considered as artifactual (substrate con-
145 centrations were indeed too high to be ecologically relevant).

146 In the 1990s, it was demonstrated that *Lemna minor* grew faster with 20 g.L⁻¹ of sucrose under light or
147 dark conditions than without organic input (1.6x and 29.0x, respectively; [43,44]). *Spirodela punctata* exhib-
148 ited a 2.8x and 57.0x higher growth rate in the presence of 20 g.L⁻¹ of sucrose under light and dark condi-
149 tions respectively [43,44]. With 20 g.L⁻¹ of galactose, the trend was similar (growth rates 2.4 and 58.0 times
150 higher respectively). However, *L. minor* and *Wolffia brasiliensis* developed less upon addition of galactose
151 [39]; it therefore appears that the nature of the sugar and substrate and their interaction with the plant
152 species are essential. Although these results were obtained with considerably higher sugar concentrations
153 than found in the environment, they showed that plants use sucrose as a source of carbon in absor-
154 botrophic mixotrophy. This research even produced an achlorophyllous mutant strain of *L. gibba* on a sugar
155 medium with the same growth rate as the original chlorophyllous strain under light conditions [39]. In the
156 last decade, a publication showed an enhancement of *L. minor* growth by sucrose (from 5 to 10 g.L⁻¹) under
157 low light conditions [45] and various sugars were reported to further the growth of *Spirodela polyrhiza* [46].

158 A major caveat is that increased amounts of CO₂ from microbial respiration of DOC may enhance photo-
159 synthesis and therefore growth, since CO₂ concentration is normally limited in water. Consequently, sterile
160 conditions are required to rigorously demonstrate mixotrophy, to show the mixotrophic potential of the
161 plant and rule out growth fueled by CO₂ from microbes. But such artificial conditions will not allow to show
162 the ecological effects of the presence of microbes competing for DOC. Indeed, outside of the Araceae fami-
163 ly, **axenic** growth of the milfoil *Myriophyllum spicatum* from the Haloragaceae family is enhanced by su-
164 crose, even in the light [47]. This suggests that organic matter and not microbial respiration was causal. We
165 observed similar results on non-axenic coontail *Ceratophyllum demersum* (Ceratophyllaceae) in the light
166 (unpublished data) with a 1.23x significant growth increase at a more ecologically-relevant concentration of
167 10 mg.L⁻¹ of sucrose.

168 Carbohydrates appear to be the best opportunity for aquatic plant mixotrophy, but it has been hypothe-
169 sized that low-molecular weight organic acids are also good candidates [48,49]. These results show that the
170 response of aquatic plants depends on their physiology and phylogenetic position and would lead to very
171 specific behaviour depending on the environment. Indeed, DOC concentrations are variable in space and
172 time. *In situ*, a constant supply of DOC at low concentrations could be more suitable for some species, while
173 peaks at high concentrations could be more suitable for others. The frequent renewal of this enriched water
174 might be necessary to allow a real gain in carbon (and thus of biomass).

175 Phytoremediation of organic pollutants can represent a final metabolic clue in favour of mixotrophy in
176 aquatic plants [24,50]. Indeed, in addition to *Lemna* sp., *C. demersum* and *M. spicatum* are used in phy-
177 toremediation to decontaminate nitrogen-containing organic pollutants [51]. These compounds are ab-
178 sorbed by the plant and can be sequestered or integrated into metabolic detoxifying chains (e.g. Royal
179 Demolition Explosive (RDX) compounds metabolized into methanol and formaldehyde) [51]. It represents
180 an important argument for heterotrophic capabilities, as the process provides carbon as well as nitrogen
181 from molecules not initially foreseen in the plant's metabolism.

182 *Mixotrophic mechanisms are questioned in very few researches*

183 Despite this, recent research has concentrated more on the indirect effects of DOC, notably its role as a
184 trace metal element carrier, with a focus on its ecotoxicological effect rather than on mixotrophy. A recent
185 review stressed the indirect effects of DOC, such as the attenuation of UV-B radiation, harmful trace metal
186 element binding and CO₂ produced by microbial respiration [6]. Similarly, *in vitro* culture protocols are
187 based on DOC-enriched media, but the possibility of direct DOC utilization is hardly considered, even
188 though it could be a clue to the use of DOC *in situ* [52–54]. While many studies address the link between
189 DOC and aquatic plants, very few of them acknowledge the possibility of mixotrophy [6,54].

190 **Toward a renewed consideration of mixotrophy in aquatic plants**

191 *A favorable physiology*

192 Various factors favor mixotrophy in aquatic plants (Figure 2). First is the actual physiology of aquatic
193 plants which have no bark nor cuticle barriers, leaving them more open to exchanges with the environment
194 [55]. In addition, the fluid mechanics of the plants, which no longer need to maintain turgidity, eases trans-
195 fers between cells [56]. The exchange surface with water is also enhanced by the extensively dissected
196 shape of aquatic plants' leaves (Figure 2), considered as an adaptation to capture mineral nutrients and
197 light. Organic compounds can be easily transported from the apoplast to the cell via symporters [16,57]:
198 this mechanism, already observed in tissues of terrestrial plants under dark conditions, is an evolutionary
199 predisposition.

200 Unlike for terrestrial plants, drought is not a threat for most aquatic plants and their exchanges are not
201 controlled by stomata. For some species, a developed anchoring root system represents another opportuni-
202 ty for DOC uptake from the substrate [58]. Roots that are buried in the sediment or lying in the water col-
203 umn (e.g. *Lemna* sp., plants with adventitious roots such as *M. spicatum* or *Elodea canadensis*), offer addi-
204 tional absorption possibilities.

205 Mixotrophy is described as evolutionarily metastable in terrestrial plants, since heterotrophically acquired
206 resources are allocated to vegetative development, while photosynthetic carbon supports sexual reproduc-
207 tion [13]. This prevents evolution to full heterotrophy (e.g. by mutational drift on photosynthetic genes;

208 [13,59]). Aquatic plants allocate most resources to vegetative development and sometimes abandon sexual
209 reproduction to increase their growth (e.g. in Araceae such as *Lemna*) [60,61]. High levels of heterotrophy
210 are therefore an opportunity for aquatic plants, with no effect on reproduction (fitness). What is the limit of
211 the evolution of heterotrophy, including the ultimate loss of photosynthesis or even plastids, as in some
212 algal lineages [62]? The presence of achlorophyllous mutants in *Lemna gibba* [39] shows that this is physio-
213 logically possible, but the selection pressure for total loss of photosynthetic capacity in nature does not
214 seem sufficient in comparison with other trait selection. Data are currently lacking on this aspect, but it
215 would be interesting to investigate in COD-rich environments with very limited light and/or CO₂.

216 *Favorable environments*

217 The aquatic environment itself is favorable to mixotrophy (Figure 2). The intensity and quality of the light
218 received by plants vary according to depth, shade and turbidity. Except for species with floating leaves,
219 aquatic plants may undergo a decline in photosynthetic activity. Humic substances and suspended particles
220 have a high impact on light availability, especially during floods or algal blooms [6,9].

221 The concentration of DOC is correlated with the dead biomass produced by surrounding ecosystems and
222 anthropogenic discharges (Figure 2). Demographic pressure and water eutrophication have increased DOC
223 concentration in freshwater ecosystems worldwide. Nutrient over-enriched and light-poor conditions are
224 becoming common features of aquatic ecosystems [9]. In this context, mixotrophy has major evolutionary
225 advantages over pure autotrophy, either for the plants' entire life cycle, or solely during flooding or seasons
226 when DOC levels are high.

227 A final factor is the availability of carbon. Aquatic plants can classically use carbon in different mineral
228 forms: atmospheric (for plants with aerial leaves) or dissolved CO₂ and dissolved bicarbonate HCO₃⁻. In
229 standing waters, the extremely low diffusion of CO₂ is limiting for the submerged aquatic plants, and organ-
230 ic carbon could thus be a providential substitute. Most of the available data on aquatic plant mixotrophy
231 concerns floating plants with partly aerial metabolism (such as *Lemna* sp.), without CO₂ limitation. But for
232 fully submerged plants, under limiting CO₂ concentration and without access to the surface, an even great-
233 er relevance of mixotrophy can be seriously considered.

234 *Possible mixotrophic mechanisms in aquatic plants*

235 Among the three possible mixotrophic mechanisms summarized in Figure 3, we are not aware of bio-
236 trophic mixotrophy in aquatic plants. Yet, the presence of root symbionts has been proven in certain aquatic
237 plants [63,64]. The contribution of the organic carbon in the substrate should be investigated, especially for
238 species living in sediment with a high organic matter content.

239 Necrotrophic mixotrophy by predation, frequent in microalgae, occurs in certain aquatic carnivorous
240 plants with dedicated organs such as *Utricularia* sp. Terrestrial plants without a dedicated organ may also

241 perform **phagocytosis**, as suggested for *Arabidopsis thaliana* and *Hakea* sp. [65], but **phagocytosis** of bacte-
242 ria in plants remains an open question [66].

243 Absorbotrophic mixotrophy holds greater potential because of molecules that abound in DOC, and of the
244 physiological traits of aquatic plants mentioned above. However, absorbotrophy could only be adapted to
245 the smallest and most labile molecules of DOC, such as monosaccharides (hexoses and pentoses), disaccha-
246 rides (sucrose, maltose, lactose) and polyols. These molecules of low molecular weight are also naturally
247 involved in metabolic pathways. In contrast, recalcitrant and larger DOC components such as humic or fulvic
248 compounds appear to be more difficult to absorb and to metabolize.

249 In addition to direct use as carbon source in metabolism, DOC compounds, especially carbohydrates, may
250 have indirect functions and effects on metabolism and physiology. Indeed, sugars regulate the expression
251 of genes, including those involved in photosynthesis [20,67], and also act as osmotic regulators [19]. Mixo-
252 trophy could therefore involve direct and indirect effects of absorbed DOC, which should guide future ex-
253 periments towards their understanding.

254 **Concluding remarks and future perspectives**

255 Due to both their physiological capacities and ecological adaptations, mixotrophic aquatic plants might
256 actually be more common than we currently imagine. Among the possible modes of mixotrophy, absor-
257 botrophy holds the greatest potential and is adapted to DOC molecules; this includes mixotrophy by **phago-**
258 **cytosis** of small particles.

259 Mixotrophy can be studied from different angles. By studying growth, it is possible to assess the differ-
260 ences that DOC can induce in realistic conditions (including the competition with microbes). At genomic and
261 biochemical levels, metabolic pathways remain to be discovered, by using isotopic labelling to trace the
262 ingested carbon for example. The search for the intriguing possibility of phagocytosis is also necessary. Ster-
263 ile growth conditions can help rule out the possibility of a microbial loop producing CO₂ that boosts photo-
264 synthesis, while adding labeled CO₂ could serve to assess to what extent the refixation of respired CO₂ en-
265 hances growth. Finally, comparative transcriptomic analysis of plants with or without mixotrophy may help
266 identify the specific genes involved [14,68]. Will this change our view of the functioning of aquatic ecosys-
267 tems and the way we depollute waters? This ecological issue is central in the debate on the mixotrophy of
268 aquatic plants.

269 Finally, mixotrophy in aquatic plants would also further challenge the conventional view that green plants
270 are purely autotrophic [4,17,18,25]. From an evolutionary viewpoint, considering that aquatic plants are in
271 fact terrestrial plants returned to the water [69], mixotrophy may just be an evolutionarily conserved fea-
272 ture.

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407 **Box 1. The three mechanisms of carbon mixotrophy**

408 Mechanisms for mixotrophy can be classified considering the nutrition mechanism and the organic carbon
 409 used [11, 15]. Definitions vary within the scientific community, but here we propose to rely on those given
 410 by Selosse *et al.* (2017) [4]. Absorbotrophic mixotrophy (Figure I,A): in this simplest mechanism, also called
 411 osmomixotrophy [16], the carbon molecules from non-living sources (the gray circles marked "C") are di-
 412 rectly absorbed into cells either by passive pathways (*transfer A*), or by active antiports or symports (*trans-*
 413 *fer B*). Microlocalized membrane internalization mechanisms (phagocytosis) of bigger molecules may also
 414 occur despite the cell wall ([64]; *transfer C*). Absorbotrophic mixotrophy is a mechanism expected in aquatic
 415 plants even in absence of dedicated organ or particular interaction with other organisms. Biotrophic mixo-
 416 trophy (Figure I,B): this refers to the carbon gained by an autotrophic organism living with another organism
 417 without killing it, in a biotrophic parasitic or mutualistic interaction: the carbon resources can be provided
 418 by a fungus (process 3, purple arrows), for example, in some plant-fungal mycorrhizal associations [18] or by
 419 another plant, such as the parasitized host tree in the case of mistletoe [13]. This process can be added to
 420 the standard symbiotic activity: supply of external micro-nutrients (process 2, black arrows) and use of the
 421 plant's carbon output, with carbon dioxide production in return for the benefit of the plant (process 1, red
 422 arrows). We are not aware that this type of mixotrophy exists in aquatic plants, despite the documented
 423 presence of endomycorrhizae [63]. Necrotrophic mixotrophy: some autotrophic organisms can obtain car-
 424 bon compounds from prey that they kill, such as carnivorous plants that feed on insects or aquatic plants
 425 like *Utricularia* sp. that consume small crustaceans [34]. This mixotrophy is very frequent in planktonic algae
 426 that internalize smaller prey in their cells by phagocytosis, a strategy called phagotrophy or phagomixo-
 427 trophy [4], [16], [69]. Phagotrophy uses the same mechanism as the phagocytosis of small particles men-
 428 tioned above, except that in this case the ingested particle is alive and then killed (Figure I,C). Necrotrophic
 429 mixotrophy is expected to be found in carnivorous aquatic plants at least.

430 **Figure legends**

431

432

433 **Figure 1.** Mixotrophy or heterotrophy. (A) Aquatic plants, just like terrestrial plants, produce photosynthe-
 434 tates by photosynthesis (process 1). Plants that are able to additionally use dissolved organic carbon (DOC)
 435 (process 2) are mixotrophic for carbon. (B) Darkness or turbid waters greatly reduce photosynthesis or can
 436 even eliminate it altogether. In this case, the use of internal organic compounds (process 3), and possible
 437 use of dissolved organic carbon (DOC) from the external environment (process 2) are the only way for the
 438 plant to sustain its metabolism. This situation is called heterotrophy. A given plant can be carbon mixotro-

439 phic during the day, and heterotrophic at night. Abbreviations: n, nucleus; v, vacuole. This figure was
440 created using BioRender (<https://biorender.com/>).

441
442 **Figure 2.** Three main factors favor mixotrophy in aquatic plants. First, their capacity to carry out exchanges
443 with the environment (no bark nor cuticle, easy transfers between cells). Second, low light environments:
444 the intensity of light received by plants varies according to depth, shade and turbidity. Consequently, plants
445 in deep water (2a), along a wooded bank or beneath floating vegetation, or even the entire plant popula-
446 tion during flood events causing turbidity (2b), undergo a decrease in photosynthesis, which mixotrophy
447 can compensate. Third, DOC concentration in the water column. The reduced light availability and in-
448 creased presence of DOC offers a double selective advantage for mixotrophy.
449 Abbreviations: cw, cell wall; pm, plasma membrane. This figure was created using BioRender
450 (<https://biorender.com/>).

451
452 **Figure 3.** Various pathways possibly involved in the mixotrophy of aquatic plants. The conventional process
453 of photosynthesis produces photosynthates from carbon dioxide, water, and light energy within chloro-
454 plasts (pathway A, green arrows). Absorbotrophic mixotrophy provides carbon from DOC (pathway B, blue
455 arrows). DOC enters the cell via a passive pathway (1) or via active ports (2), before assimilation within the
456 cell. This pathway could also have a positive regulatory role on photosynthesis, which could be the origin of
457 a second explanation for the strong growth increase observed in light conditions. Biotrophic mixotrophy,
458 with microorganisms housed within (i.e. endophytes) or on the plant is also an alternative carbon source
459 (pathways C and C', red arrows). Two bacteria (in pink) and a fungus (in brown) are represented here: they
460 can also be superficial. The use of DOC by these organisms can lead to the production of metabolites
461 transmitted by diffusion to the neighboring tissues. Endocytosis, the internalization of small prey and parti-
462 cles in the cell (pathway D, purple arrows), is the last pathway that can be expected. It includes a digestion
463 phase within the internalised vesicle, followed by a metabolisation phase resulting in a supply of carbon
464 compounds. Abbreviations: cw, cell wall; n, nucleus; v, vacuole. This figure was created using BioRender
465 (<https://biorender.com/>).

466
467 **Figure I.** Mechanisms of carbon mixotrophy. Abbreviations: c, cytosol; cp, chloroplast; cw, cell wall; n,
468 nucleus; pm, plasma membrane; v, vacuole. These figures were created using BioRender
469 (<https://biorender.com/>).

470
471
472



Water



DOC

Plant

1

2

v

Sucrose

n

1. Photosynthesis
 2. DOC nutrition
- (1) + (2) = **carbon mixotrophy**

A



Water



DOC

Plant

(a)

(b)

2

v

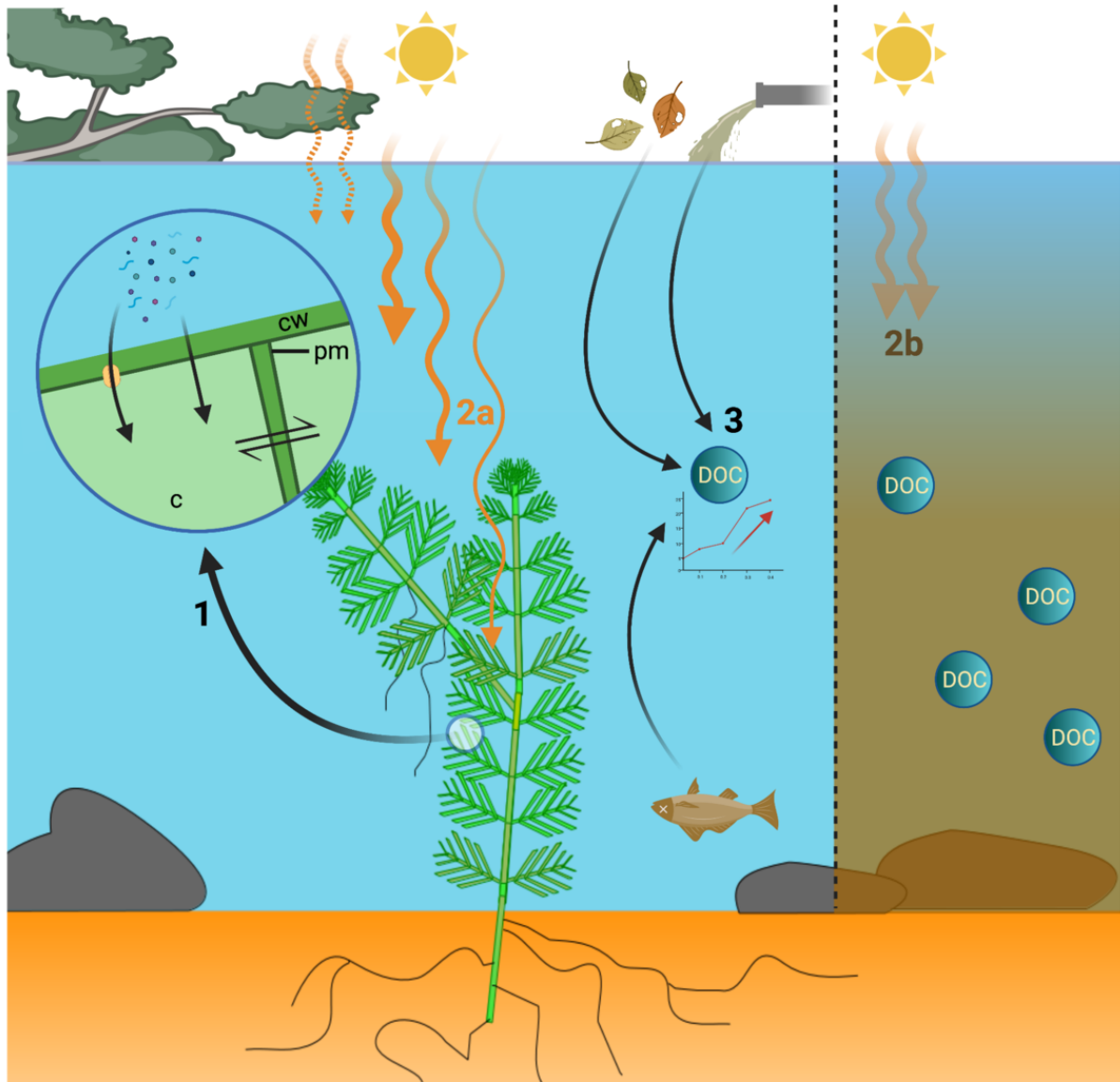
3

n

- Photosynthesis impossible (a: turbid water and/or b: at night), only:
2. DOC nutrition
 3. internal resources
- (2) + (3) = **carbon heterotrophy**

B





Water

