Initial decomposition of floating leaf blades of waterlilies: causes, damage types and their impact

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Abstract

Initial decomposition (i.e. leaf damage and loss) of large floating leaved macrophytes, such as waterlilies, can be studied well: the turnover of floating leaf blades is low and leaves can persist for a relatively long time. In the present study, the initial decomposition patterns of floating leaf blades of *Nuphar lutea* (L.) Sm., *Nymphaea alba* L. and *Nymphaea candida* Presl, were examined at three freshwater sites differing in ~~water quality, such as~~ nutrient status, pH and alkalinity. Floating leaf blades of each species were tagged and numbered within established replicate plots and the leaf length, percentages and types of damage ~~to~~ and decay of leaves were measured and estimated weekly throughout the growing season.

The m~~M~~ost important damage causes found in this study ~~with respect to~~~~Only a few damage causes had a significant impact on leaf damage and leaf loss~~ were autolysis, phytopathogenic fungi (*Colletotrichum nymphaeae*, *Pythium* sp.), pond snails and mechanical damage, although other sources were also registered. The floating leaves offer food for a series of specialized insects consuming leaf area from below the water surface, from the upper surface or by mining the leaf tissue. Several forms of succession of damage could be distinguished on the leaves and during the season. In alkaline waters the seasonal patterns of initial decomposition differed between *Nymphaea* and *Nuphar*.

Introduction

Plant leaves are, already during their development, exposed to abiotic~~ factors,~~, such as weather conditions, ~~-~~as well as biotic factors,~~.~~ such as infection by fungi and viruses, herbivores, and other animals, that cause initial damage to ~~using these~~ leaves~~ in various ways)~~. This exposure is well-known for crops and ornamental plants as it causes economic damage.

Initial decomposition, when the leaves are still connected to the plant, precedes the breakdown cycle~~process of leaf material entering the decomposition cycle~~ later occurring ~~o~~in the soil, in open air or in water. In ecological studies much attention is paid to the latter process because these soil processes are important for the biogeochemical cycles. However, with the exception of agriculture, horticulture and forestry for which phytopathology is a main discipline, much less attention is paid to the first process, in particular for aquatic macrophytes.

Initial decomposition of floating leaves of aquatic macrophytes such as waterlilies includes both leaf damage and loss (Lammens & Van der Velde, 1978 ;Van der Velde *et al*., 1982; Van der Velde & Van der Heijden, 1985), occurring even before a leaf unrolls at the water surface. A classification of the various causes of initial decomposition of floating leaves (~~was proposed earlier ((~~Van der Velde *et al*., 1982) separates ~~. Herein a primary division is made~~them in internal and external causes, the internal due to physiological factors (autolysis), the external due to either abiotic or biotic factors. Roweck (1988) added water level fluctuations as abiotic factor and mass starvation as a result of stress factors under internal factors to this classification.

Decomposition of aquatic macrophyte tissue consists of a complex series of interacting processes (Kok, 1993; Fig. 1), and ~~. O~~often various stages of the decomposition process can be found on one plant or even on one leaf. During initial decomposition macrophyte tissue can be used by herbivores,~~ and~~ phytopathogenic and saprotrophic microorganisms. Before the plant material dies away, the plant tissue goes through the senescence phase d~~. D~~uring ~~senescence~~ which further decomposition and fragmentation by weak pathogens, facultative herbivores, grazers and scrapers occur, leading to the production of debris and fecal pellets. The (bio)chemical composition of plant tissue also changes during senescence due to hydrolysis of macromolecules like DNA and proteins and due to resorption of soluble nutrients like N and P. This leads to a loss of tissue structure, sometimes to a loss of secondary chemical compounds and to the colonization of the tissue by microorganisms, making senescent, microbial~~ly~~ enriched tissue more attractive for ~~facultative~~ detritivore~~ous~~ macroinvertebrates (Rogers & Breen, 1983).

These phases of initial decomposition can be studied well in the floating leaf blades (laminae) of large leaved plants such as waterlilies in which the turnover ~~of floating leaf blades, further indicated in this paper as floating leaves or leaves,~~ is low (P/B_{max} 1.35-2.25) and the leaves exist for a relatively long time, on average 38-48 days (Klok & Van der Velde, 2017). The study of waterlilies has several other advantages. Waterlilies occur worldwide (Conard, 1905; Wiersema,

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1987; Padgett, 2007) in many types of water bodies with different ~~physico~~physic-chemical conditions of water and sediment (Van der Velde, Custers & De Lyon, 1986). Furthermore, they have a fixed position in the vegetation zonation along water bodies between helophytes and submerged macrophytes. The nymphaeid growth form is characterized by the combination of ~~possessing~~ floating leaves and rooting in the sediment (Luther, 1983; Den Hartog & Van der Velde, 1988) and thus~~.Thes~~ the plants will not float away as ~~other f~~the free floating-leaved plants ~~which are free floating~~. Besides floating leaves, waterlilies also produce thin underwater leaves and at crowding at the water surface and at lowered water level aerial leaves (Glück, 1924; Van der Velde, 1980).

~~When developed, t~~The leaves have defense mechanisms against damage and decay which slow down decomposition processes. Because of their development under water and subsequent ~~occurrence~~ unrolling at~~on~~ the water surface, floating leaf blades of waterlilies are attacked by microorganisms, phytopathogenic fungi and herbivorous animals such as folivores, both from the surrounding water below and from the air above (Lammens & Van der Velde, 1978; Van der Velde *et al.*, 1982; Van der Velde & Van der Heijden, 1985; Martínez & Franceschini, 2018). Young leaves can already be attacked under water before they unroll. Longterm effects of folivores on plant growth are reported as negative (Marquis, 1992) by reducing leaf density (Stenberg & Stenberg, 2012). Defenses of waterlily leaves against attacks include replacing old floating leaves by new ones, shifting from floating leaves to underwater leaves (Kouki, 1993), hydrophobic epicuticular wax layers (Riederer & Müller, 2006; Aragón *et al*., 2017), spines (Zhang & Yao, 2017), sclereids with calcium oxalate crystals (Brock & Van der Velde, 1983; Franceschi & Nakata, 2005), tough tissue (Mueller & Dearing, 1994; Kok *et al*., 1992), and plant secondary metabolic chemical compounds such as alkaloids (Hutchinson, 1975) and phenolics (Kok *et al*., 1992; Vergeer & Van der Velde, 1997; Smolders *et al*., 2000; Martínez & Franceschini, 2018). This means that only specific species can break through the defense and can use the fresh plant tissue, while other species have to wait for autolysis and decay or other factors to weaken~~ing~~ the defense system (Kok *et al*., 1992). In the first case the attacking species are more or less specialized and often restricted to plant species, genus or family. Damage of leaves can ~~cause a~~ leach out ~~of~~ soluble carbohydrates such as oligosaccharides and starches, proteinaceous material and phenolic compounds which are metabolized at high rates by microorganisms during the initial decomposition (Brock, Boon & Paffen, 1985).

Fully decayed floating leaf material that sinks to the bottom makes a significant contribution to the detritus food chain by further decomposition processes (~~-~~Brock, 1985; Van der Velde & Van der Heijden, 1985; Kok & Van der Velde, 1991; Kok, 1993). They reach the bottom as debris, decayed leaves, leaf fragments and fecal pellets which fuel the benthic communities serving as food for detritivores and saprophytes (Kok *et al*., 1992).

Brock, Boon & Paffen (1985) used harvested laminae of the waterlily *Nymphaea alba* in litter bags situated on the bottom in the field and in the laboratory and showed that weight loss during decomposition was low under acid conditions in a moorland pool (Voorste Goorven) and fast in an eutrophic alkaline oxbow lake (Oude Waal) with similar results under laboratory conditions mimicking a comparable water quality as in the field. During the first 10-30 days a pronounced weight loss and a rapid change in organic matter composition was observed, after that period changes ~~are~~ were small and an accumulation of structural carbohydrates such as cellulose, hemicellulose and lignin from the cell wall fraction could be observed. The disappearance of that fraction was dependent on the water quality of the water body (Brock, Boon & Paffen, 1985).

The present study focusses on patterns and causes of initial decomposition of floating leaves of three species of waterlilies in plots in three water bodies differing in pH, buffering capacity, nutrient levels and surroundings. Data was collected to answer the following research questions:

- What are the causes and the patterns of initial decomposition of floating leaves?
- What is the effect of each cause on initial decomposition?
- How does decomposition progress during the season?

Materials and Methods

Sites

Field research took place in 1977 and in 1988 in three different water bodies in The Netherlands: Haarsteegse Wiel (HW), Oude Waal (OW) and Voorste Goorven (VG) where~~. In these water bodies,~~ dense, nearly mono-specific waterlily stands occurred. Three plots were laid out in stands of *Nuphar lutea* (HW and OW, 1977; VG, 1988), two plots in stands of *Nymphaea alba* (OW, 1977; VG, 1988) and one plot in a stand of *Nymphaea candida* (HW, 1977).

The Haarsteegse Wiel (Province of Noord-Brabant; 51^{0}43'05" N, 5^{0}11'07" E) originates from two connected breakthrough ponds created by dike bursts along the river Meuse in the past. It is an isolated eutrophic water body with low alkalinity. The water level depends on precipitation, seepage and evaporation. During the summer period stratification occurs. The bottom consists of sand and a detritus layer with increasing thickness towards the littoral border. The waterlily beds are situated in the wind-sheltered part of the lake.

The Oude Waal (Province of Gelderland; 51^{0}51'13" N, 5^{0}53'35" E) is a shallow highly eutrophic, alkaline oxbow lake in the forelands of the river Waal. The depth during the growth season is shallow, except for three connected breakthrough ponds. The water level is dependent on precipitation, upward seepage, overflow of the River Waal in winter and/or spring (which strongly influences water chemistry and quality), and evaporation. The bottom consists of clay and sand, covered by a detritus layer of varying thickness in the nymphaeid beds.

The Voorste Goorven (Province of Noord-Brabant; 51^{0}33'53" N, 5^{0}12'26" E) is a shallow, oligotrophic, isolated, culturally acidified moorland pool, showing very low alkalinity values. The hydrology is mainly dependent on precipitation, upward seepage and evaporation. The lake has a poorly buffered sandy soil and is surrounded by forests.

Characteristics of the investigated water bodies are listed in Table 1. Chemical characteristics were derived from Brock, Boon & Paffen (1985) and Kok, Van der Velde & Landsbergen (1990). In none of these water bodies boating or navigation occurred, and thus ~~which is important to mention as in that case floating leaves can also be~~ no damage~~d~~ by boat propellers, etc., occurred. For the present study we used a small zodiac with peddles to gently reach the plots.

Potential and actual leaf area

To quantify leaf loss, a distinction was made between potential and actual leaf area. The potential area was defined as the area of the intact leaf. The actual area was defined as the potential area minus the area that was missing.

The potential leaf area was calculated by correlation with the leaf length, using a quadratic regression equation (Van der Velde & Peelen-Bexkens, 1983; Klok & Van der Velde, 2017). Randomly harvested undamaged, fully green leaves sampled outside the plots were taken to the laboratory and both length and area using a planimeter were measured in order to determine equation coefficients between leaf length and area. With the aid of these equations the potential areas of floating leaves in the plots were calculated. Mathematically, the equation is described by:

$$A(L) = c_i L^2 \qquad (1)$$

where:

$A(L)$ = potential leaf area at length L (cm^2)

L = leaf length from the leaf tip to a basal lobe tip (cm)

c_i = correlation coefficient of species i

i = species (*Nuphar lutea*, *Nymphaea alba*, *Nymphaea candida*)

Collected plot data

To collect data on initial decomposition during the growing season, six representative plots of 1 m^2 were laid out in the center of mono-specific stands, surveying one rhizome apex per plot. A non-destructive leaf-marking method was used to mark all floating leaves within a plot, which enabled data collection during the complete life-span of the leaves. A square perforated PVC tube frame, held approximately 15 cm below the water surface by cork floaters and anchored to the bottom by four bricks, bordered a plot. In this way the unrolling of floating leaves in the plot was not hindered and all leaves having their petioles within the frame were counted and measured. A leaf was considered still present as long as, after fragmentation, tissue of the lamina

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was connected to the petiole in the case of OW and HW. In VG the leaf was considered gone when it was completely decayed and sunk under the water surface or when it disappeared.

Measurements and observations of all leaves within a plot took place weekly during the growing season, in general from April until November. It included tagging newly unrolled leaves with uniquely numbered Rotex tapes (fixed around the petiole just under the leaf), counting the actual number of leaves, measuring leaf length ~~in mm (~~from the ~~leaf~~ tip to ~~a~~ the basal lobe tip~~)~~ and visually estimating the different types of initial decomposition as percentage of the potential leaf area of each leaf. During the whole growing season, undamaged leaves were harvested at random a few meters outside the plots at each location to measure length ~~(mm)~~ and area ~~(cm^2)~~ to ~~eventually~~ determine the coefficients of equation (1).

Results

Vegetation period, total number of leaves produced and total potential area of leaves of the species in the plots are presented in Table 2. Data on damage to leaves are presented per damage cause for all plots in Table 3.

Initial decomposition tended~~s~~ to increase in time during the vegetation season with the exception of the acid Voorste Goorven (with *Nuphar lutea* and *Nymphaea alba*). In the alkaline waters (Oude Waal and Haarsteegse Wiel) leaf damage ~~for~~ of *Nuphar lutea* was less than 20% related to the total potential leaf area in the plot until half September, but afterwards increased to more than 50%. ~~For~~ Leaf damage of *Nymphaea alba* and *Nymphaea candida* ~~it~~ was less than 10% with an increase to almost 20% in October (Fig. 2 and Fig. 3).

~~Vegetation period, total number of leaves produced and total potential area of leaves of the species in the plots are presented in Table 2. Data on damage to leaves are presented per damage cause for all plots in Table 3.~~

~~Leaf loss by external causes per plot in time is given in Fig. 2 and percent contributions to leaf damage by external causes per plot per cause in time are given in Fig. 3.~~

Causes of initial decomposition and their impact

The damage causes found in this study were autolysis, frost, hail stones, dehydration, mechanical damage, scratches, the bird *Fulica atra*, pond snails, the beetle *Donacia crassipes*, imagines and larvae of the beetle *Galerucella nymphaeae*, the beetle *Bagous rotundicollis*, larvae of the moth *Elophila nymphaeata*, larvae of the fly *Hydromyza livens*, larvae of Chironomidae, larvae of *Endochironomus* ~~spec~~spp., phytopathogenic fungi, microbial decay and unknown causes.

Autolysis. The newly unrolled floating leaves are green and hydrophobic ~~by~~ due to an epicuticular wax layer (Fig. 4). During senescence this wax layer erodes by colonization of bacteria and fungi. In this stage the leaf tissue can be attacked by cellulolytic bacteria (Howard-

Williams *et al*., 1978; Robb *et al*., 1979; Rogers & Breen, 1981; Barnabas, 1992). Autolysis starts shortly after the first leaves are fully grown and continues through the whole floating leaf vegetation period. The leaf turns from green to yellow, which leads at the end of the existence of the floating leaf to total microbial decay, the leaf turning brown. Autolysis is controlled by the plant ~~itself by~~ hormones (e.g. Osborne, 1963). ~~As expected, t~~The influence of autolysis reached its maximum towards the end of the growing season. In October the percentage of affected leaves ~~rose t~~was~~o~~ 100%, however, the surface area affected by autolysis was ~~quite stable and~~ generally around 10%. For separate leaves the area affected by autolysis ~~may~~ decreased in time, since microbial decay ~~will take~~took over part of the area (Fig. 5).

Frost. Frost in early spring may damage the tips of young leaves sticking out of the water. Frost ~~does~~ did not occur frequently, but when it did, ~~because of frost individual~~ leaves ~~may lose~~lost up to one third of their area (Fig. 6). The effect on surface area was less than 5%.

Hail stones. Occasional hail stone showers damage the floating leaves by penetrating through the leaf and making typical Y-shaped scars ~~on the leaves~~ (Fig. 7). Leaf damage area due to hail was minimal.

Dehydration. Due to hard wind floating leaves are lifted from the water, flip over and ~~subsequently~~ the air exposed parts, ~~(~~ in particular the leaf margin, ~~)~~ dry out (Fig. 8). The effect on leaf surface area was generally less than 5%.

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Mechanical damage. This damage is caused by wind and wave action, and consists of cracks in the leaves or lost leaves by breaking of the petiole (Fig. 8). The percentage of affected leaves was quite high during the whole data taking period for ~~plots (~~*Nuphar lutea* at~~,~~ HW~~)~~, ~~(~~*Nymphaea alba*~~,~~ at OW~~)~~ and ~~(~~*Nymphaea candida*~~,~~ at HW), ranging ~~about~~ 60-80%. ~~Plot (~~*Nuphar lutea* at~~,~~ OW~~)~~ showed peaks of 90% in spring and 70% in autumn with a dip of 10% in summer. ~~Plots (~~*Nuphar lutea*~~,~~ at VG~~)~~ and ~~(~~*Nymphaea alba*~~,~~ at VG~~)~~ showed no mechanical damage~~ at all for this cause~~.

Scratches. Damage by scratches is caused by the fingernails of birds, mostly Coot (*Fulica atra* L.) and also Moorhen (*Gallinula chloropus* L.), as they are walking or running over the leaves (Fig. 9). In general the scratches are straight and effect only the epidermis of the leaf, but angle-shaped cuts due to nails penetrating the leaf tissue also occur (Lammens & Van der Velde, 1978). The impact on leaf surface was low, generally below 5%, despite the high percentage of affected leaves, sometimes up to 100% for ~~plots (~~*Nuphar lutea*~~,~~ at HW and OW~~), (*Nuphar lutea*, OW)~~, ~~(~~*Nymphaea alba*~~,~~ at OW~~)~~ and ~~(~~*Nymphaea candida* at~~,~~ HW~~)~~. ~~Plots (*Nuphar lutea*, VG)~~ and ~~(~~*Nymphaea alba*~~,~~ at VG) showed no damage ~~at all~~by scratches.

Consumption by *Fulica atra* L. (Rallidae). Damage by consumption of leaf tissue by the Coot (*Fulica atra*) can be recognized by ~~omissions~~ missing parts in the form of triangular areas at the edge of a leaf. Sometimes a major part of the leaf has been consumed. Generally prints from the beak are visible around the consumed areas (Fig. 10). The total effect on leaf surface area was

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minimal; ~~, while plots (~~*Nuphar lutea*~~, VG)~~ and ~~(~~*Nymphaea alba*~~,~~ at VG) showed no damage ~~at all~~by *Fulica atra* consumption.

Consumption by pond snails (Lymnaeidae). Damage on fresh leaves is caused mainly by *Lymnaea stagnalis* L. and to a lower extent by other lymnaeids. ~~Since snails grow best and become larger by eating soft fresh leaf material.~~ Pond snails consume f~~F~~olded leaves still under water ~~are the victim of consumption~~, and the ~~which can be seen from~~ rows of holes can be seen in the unrolled floating leaves, large near the edge and becoming smaller towards the center of the leaf (Fig. 11). In general snails have a preference for decaying leaf material, e.g. consuming areas that were infected by fungi. ~~Van der Aa (1978) noticed small holes in the center of many spots and suggested that an arthropod has been active. Possibly he observed the result of grazing by snails on the spots.~~ Damage by snails was generally ~~is~~ an important cause of damage during the whole period ~~of data~~ for both *Nuphar lutea* and *Nymphaea alba*, with a contribution of 20-40%.

Consumption by *Donacia crassipes* F. (Chrysomelidae). Both *Nuphar* spp. and *Nymphaea* spp. are h~~H~~ost plants of the beetle *Donacia crassipes* ~~are waterlilies (*Nuphar* spp. and *Nymphaea* spp.)~~. The imagines live on the floating leaf upper side where they feed on leaf tissue (upper epidermis, parenchym till the under epidermis). The lost areas by consumption are round to oval. ~~Around t~~These damaged areas are starting points for later decay ~~starts after some time~~. Eggs are deposited in two rows on the underside of leaves; ~~. For that purpose~~ the beetle gnaws a round to oval hole in the leaf by which it can stick the abdomen for oviposition at the floating leaf

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underside (Fig. 12). Hatched larvae sink to the bottom and feed on roots. After ~~a~~ three-years ~~life cycle~~, they overwinter as pupae in cocoons attached to roots (Bienkowski, 1996)~~)~~. The percentage of damaged leaf area ~~is~~ was minimal.

Consumption by *Galerucella nymphaeae* L. (Chrysomelidae). The Waterlily Beetle (*Galerucella nymphaeae*) completes its full life cycle on the upper surface of floating leaves. In winter the adults hide in remains of dead helophytes, under the bark of trees or in ground litter. Simultaneous with the development of floating leaves the beetles appear. Eggs are attached in clusters to the upper surface of floating leaves. Hatching of eggs is followed by three larval stages and pupation, taking 15-29 days. Both imagines and larvae feed on the upper surface of floating leaves by grazing epidermis and palisade and sponge parenchyma. The larvae, which can be considered halfminers, create irregular trenches on the surface leaving the under epidermis of the leaf intact. In the trench they deposit their feces which leads to decay. The under epidermis decays and disappears, which makes the leaves vulnerable to fungal and microbial attacks (Wesenberg-Lund, 1943; Roweck, 1988). So leaf disappearance is finally caused by fungi and bacteria, but the process is initiated by the beetle (Wallace & O'Hop, 1985). The pattern of damage to the leaves is easily recognized~~.~~ (Fig. 13). Imagines consume smaller areas in contrast to the larvae. ~~(Fig. 13).~~ These damaged areas with regular margins made by *Galerucella nymphaeae* imagines can be distinguished from those made by *Donacia crassipes* of which the margins are more ragged (Roweck, 1988). Damage was only found in ~~plot (~~*Nymphaea alba* at~~,~~

VG, ~~) only~~ with affected leaves starting half June going up to 30-40% in August until October and a sharp peak of 60% ~~half~~ in mid October.

Consumption by *Bagous rotundicollis* Bohemann (Curculionidae). The beetle *Bagous rotundicollis* feeds on waterlily leaves (Van der Velde *et al.*, 1989). The adult scrapes off areas with a diameter of ca. one cm from the underside of the floating leaf near its margin in which way the lower epidermis and sponge parenchyma are consumed, leaving the palisade parenchyma and upper epidermis intact (Fig. 14). Damage was found only in ~~plot (~~*Nymphaea alba* at ~~,~~ VG) ~~only~~ with up to 30% affected leaves.

Damage and consumption by *Elophila nymphaeata* (L.) (Crambidae). The caterpillar of the moth *Elophila nymphaeata* damages the leaf in two ways. The larva consumes leaf tissue and cuts out oval patches from the floating leaf. It can attach a patch to the underside of a floating leaf to make a shelter below the leaf or it spins two patches together to make a floating shelter (Fig. 15). ~~Life cycle and behavior of *E. nymphaeata* are described by Reichholf (1970).~~ The effect on leaf surface was low, at most 5%; ~~, while leaves in plots (~~*Nymphaea candida* at~~,~~ HW~~)~~, ~~(~~*Nuphar lutea* and ~~, VG) and (~~*Nymphaea alba* at~~,~~ VG~~)~~ showed no damage.

Mining by *Hydromyza livens* (Fabricius) (Scatophagidae). The larvae of the fly *Hydromyza livens* only occurs in *Nuphar* leaves~~. The autecology of this fly species is extensively described in~~ (Brock & Van der Velde, ~~(~~1983), where they ~~. The larvae of *Hydromyza livens*~~ mine and consume ~~in~~ the leaf tissue~~ which they consume~~. The eggs of this fly are laid at the underside of the leaves.

For that purpose the fly goes via the margin under water and follows the dichotomous nerves till it reaches the midrib of the leaf, where it lays an egg. From the egg the larva immediately starts to mine in the leaf tissue. The mine track shows a very characteristic shape as the larvae first mine towards the margins of the leaf, then bend and mine parallel to the leaf margin, bend again towards the midrib and mine further into the petiole where they pupate (Fig. 16). Since they also mine the petiole, they create a weak breaking point where the leaf can break off and float away. The total effect on decomposition of floating leaves was less than 8%. With translucent light it appeared that the real damage was higher due to leakage, etc.

Mining by Chironomidae. Larvae of some Chironomidae mine in the leaf tissue and dig/eat their way through the leaf tissue. Typical damage on *Nuphar* leaves is caused by larvae of *Tribelos intextus*. The larvae mines the leaves when they are still folded and below the water surface ~~and thus damage the rolled leaf. S~~so when the floating leaves unroll at the water surface rows of small holes become visible (Van der Velde & Hiddink, 1987) (Fig. 17). Other miners observed are larvae of *Cricotopus trifasciatus* (Meigen in Panzer, 1813) (Fig. 18), which is a half miner intensively damaging the floating leaves of *Nymphoides peltata* (Gmel.) O. Kuntze (Lammens & Van der Velde, 1978). It wa~~i~~s observed to cause some damage at the leaf margins ~~on floating leaves~~ of *Nuphar lutea* in the neighbourhood of *Nymphoides* in OW (Van der Velde & Hiddink, 1987). The total effect on the decomposition of floating leaves was minimal.

Mining by *Endochironomus* ~~spec~~spp. (Chironomidae). The larvae of these midges mine in floating leaves. In 1977 there may have been two not so well separated generations. The mines of *Endochironomus* sp. could clearly be distinguished from those of other Chironomidae (previous cause), since the mines are visible on the floating leaf upper side as straight dark stripes (Roweck, 1988) (Fig. 19). The total effect on the decomposition of floating leaves was minimal.

Phytopathogenic f~~F~~ungi. The leaves of *Nuphar lutea* were infected by *Pythium* "type F" (Jacobs, 1982) (Fig. 20) and the leaves of *Nymphaea alba* and *Nymphaea candida* by the leaf spot disease *Colletotrichum nymphaeae* (Van der Aa, 1978) (Fig. 21). The percentage of damage for the surface area was around 15% for *Pythium* "type F" and up to 55% for *Colletotrichum nymphaeae*.

Microbial decay. ~~Due to autolysis the~~ The resistance of a leaf against microbial infection disappears quickly due to autolysis, ~~which gives rise t~~allowing~~o~~ normal microbial decay (Fig. 5). The effect on the affected surface area ranged 15-25%, with an exceptional peak of 60% in ~~plot~~ ~~(~~*Nymphaea candida* at~~,~~ HW~~)~~ at the very end of the growing season.

Unknown causes. Missing (parts of) leaves can be caused by consumption or damage by aquatic animals, however in many occasions the real cause of lacking leaf parts could not be determined. Leaves disconnected from their petioles ~~are~~ were scattered by wind and wave action and could not be followed anymore. Damage occasionally went up to 60% for *Nuphar lutea* ~~in~~ at HW, *Nymphaea alba* ~~in~~ at OW and *Nymphaea candida* ~~in~~ at HW, however, this type of damage was hardly found for ~~for the other plots (~~*Nuphar lutea* at ~~,~~ OW and VG~~), (*Nuphar lutea*, VG)~~ and ~~(~~*Nymphaea alba* at~~,~~ VG~~), this type of damage was hardly found~~.

Discussion

Overall patterns of decomposition of floating leaves ~~in the plots~~ differed for waterlily species, water quality (alkaline vs. acid) and wind exposure (Fig. 3). Leaf fragmentation was hardly observed in the acid water body~~plots. The acid water water body studied~~ (VG) which was also the most sheltered against wind and wave action by a surrounding forest which allowed *Galerucella nymphaeae* to become an important herbivore. This species was lacking in the other ~~plots in~~ water bodies, which ~~with~~ were often subjected to a strong wind exposure. In the acid water plots no consumption by snails was observed in contrast to the alkaline plots. In acid water consumption of leaves occurred by specialized insect species only causing low leaf loss.~~.~~

In the two alkaline waters *Nuphar lutea* showed a similar seasonal decomposition pattern, that differed from that of *Nymphaea alba* and *N. candida*, ~~which showed a pattern that was~~ more similar to each other. In *Nuphar*, there wa~~i~~s an increase ~~in share~~ of normal microbial decay and infection~~s~~ by the phytopathogenic fungi ~~(~~*Pythium* ~~spec~~spp.~~)~~, which both were important for the decomposition from the beginning of the season. In *Nymphaea*, infection by the~~a~~ phytopathogenic fungus ~~(~~*Colletotrichum nymphaeae*~~)~~ started and increased in importance towards

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Also, the discussion should be organized in such a way that you start and finish one subject before passing to the next.

the end of the season. In general, microbial decay and phytopathogenic fungal ~~decay~~ infection increased in ~~relative~~ importance during the season, while unknown causes diminished just as all other damages causes together. Prolonged dark, cloudy and wet weather conditions by rain and/or shadowing are a stress factor –weakening the defenses of waterlily leaves due to reduced availability of sunlight and stimulate heavy infection and decay by phytopathogenic fungi (Van der Aa, 1978). Shading ~~as a stress factor~~ reduces the phenolic content – with fungistatic properties (Vergeer & Van der Velde, 1997), ~~making~~ turning the mature leaves vulnerable to infection by fungi ~~as phenolics have fungistatic properties (Vergeer & Van der Velde, 1997)~~.

From the list of causes of initial decomposition and their impact (Table 3) it is clear that autolysis was the most important for the decomposition of the floating leaves. During the vegetation growth period the development of new floating leaves and the dying ~~off~~ of old leaves continued~~s~~ during a long period. The growing period of leaves comprises 53 to 73 % of the vegetation period (Klok & Van der Velde, 2017).

N~~Also n~~ormal microbial decay and unknown causes also had~~ve~~ a high impact, except for Voorste Goorven, where the floating leaves showed no fragmentation and/or damage for these causes. Minor causes occurring incidentally ~~at~~ once during the vegetation period, in particular in spring when the first leaves unroll at the water surface, were frost that can cause serious leaf loss and hail stones that hardly have impact with respect to disappeared area, but contribute to further fragmentation of the leaves. Dehydration and mechanical damage are dependent ~~of~~ on wind and wave action. High solar radiation and air temperatures cause the dehydration of the flipped over leaves with a high impact in Haarsteegse Wiel and Oude Waal in contrast with the wind protected Voorste Goorven where the leaves hardly show that type of damage. In the Voorste Goorven damage was mainly caused by specialized consumers of floating leaf tissue in particular herbivorous beetles, fly larvae, mining chironomid larvae ~~(in particular in VG)~~ and the omnivorous Coot. The surrounding biotopes are also important, as meadows are important for Coots to survive winter time by grazing grass in groups in OW and HW. High densities of waterfowl leads to higher damage of the leaves. *Nymphaea candida* (HW) showed an increase ~~for~~ in nail scratches towards the end of June, which may be the influence of young coots.

The difference in leaf damage and leaf loss between acid and alkaline waters was clear (Fig. 2). In the acid VG ~~(with *Nuphar* lutea and *Nymphaea alba*)~~ the effect of leaf damage on leaf loss was minimal both for *Nuphar* lutea and *Nymphaea alba*. Low pH of the water caused a low rate of decomposition of the leaves by several interacting factors such as low HCO_3, high Al concentrations, low pH in the plant tissue, high phenolics stored in the tissue due to inhibition of ~~as~~ cell wall degradation ~~is inhibited~~. Al and low pH cause also a lower number of detritivores leading to low feeding and low leaf fragmentation. Inhibition of cell wall degradation leads to low fragmentation and prevents softening of microbially enriched plant tissue which means that also by high phenolics stored in the tissue the plant tissue has a low resource quality for detritivores (Kok, 1993). Snails are absent under acid conditions ~~because~~ due to the~~of~~ lack of calcium for their shells. Snails prefer to consume decaying, microbially enriched parts of leaves under high pH and alkaline conditions (Kok, 1993).

Conclusions

The floating leaves offer food for a series of specialized insects, consuming leaf area from below the water ~~surface~~ as well as from the upper surface or mining in the tissue, and for birds (Rallidae), which swim around in the neighborhood consuming leaf parts and walk on the leaves scratching the upper surface. Of the causes of initial decomposition of floating leaves that have been found, only a few have significant impact on leaf damage and leaf loss. High impact causes are autolysis, infection by phytopathogenic fungi, consumption by pond snails, mechanical damage and unknown causes. As a consequence of microbial decay, tissue removal is very prominent in some cases.

Other aspects of influence are abiotic conditions and physic~~o~~-chemical characteristics of the water bodies. Wind-sheltered plots showed different insects species with different impact and no mechanical damage by wind and wave action.

Floating leaves in acid and alkaline water also showed different impact of damage causes. Typically, this was the case for the acid Voorste Goorven (*Nuphar lutea* and *Nymphaea alba*), which is sheltered against wind action by trees, in contrast to Haarsteegse Wiel (*Nuphar lutea* and *Nymphaea candida*) and Oude Waal (*Nuphar lutea* and *Nymphaea alba*).

Several forms of succession of damage could be distinguished. Erosion of the wax layer and damage by phytophagous insects were followed by cellulolytic bacteria or fungi, followed by snails or abiotical damage, followed by biotic causes or decay or autolysis, followed by phytopathogenic fungal or normal microbial decay, followed by tissue removal by snails. This was followed by breaking up of leaves in the case of alkaline water or sinking towards the bottom of intact decayed leaves in the case of acid water.

Acknowledgements

We thank M. Ankersmid, R. Kwak, R. de Mooij, H. Peeters, F. Verhoeven, V. Vintges and C.J. Kok for collecting field data, W. Lemmens for help with data modeling, W.J. Metzger for English language corrections and Manuela Abelho and one anonymous reviewer for constructive remarks leading to the improvement of the manuscript.

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