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

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Peter F. Klok and Gerard van der Velde

P. F. Klok · G. van der Velde (✉)

Department of Animal Ecology and Physiology, Institute for Water and Wetland Research, Radboud University, Nijmegen, The Netherlands

e-mail: g.vandervelde@science.ru.nl

P. F. Klok

Department of Particle Physics, Institute for Mathematics, Astrophysics and Particle Physics, Radboud University, Nijmegen, The Netherlands

G. van der Velde

Naturalis Biodiversity Center, Leiden, The Netherlands

Abstract

The initial decomposition of large floating-leaved macrophytes, such as waterlilies, can be studied by following changes in leaf damage and area loss of leaf blades marked in their natural environment. This approach was taken in the present study to examine the initial decomposition patterns of floating leaf blades of *Nuphar lutea* (L.) Sm., *Nymphaea alba* L. and *Nymphaea candida* J. Presl at three freshwater sites differing in nutrient status, alkalinity and pH. Floating leaf blades of the three plant species were marked and numbered within established replicate plots and the leaf length, percentages and types of damage and decay of all marked leaves were recorded weekly during the growing season. We found autolysis, microbial decay, infection by phytopathogenic fungi (*Colletotrichum nymphaeae*) and oomycetes (*Pythium* sp.), feeding by pond snails, and mechanical factors to be the most important causes of leaf damage. Several types of successional patterns reflecting different causes of damage could be distinguished. Young floating leaves were affected by feeding of more or less specialized invertebrate species consuming leaf tissue. Later, non-specialized invertebrate species started feeding on the floating leaves. In the two investigated more alkaline lakes the seasonal patterns of initial decomposition differed between *Nymphaea* and *Nuphar*.

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Introduction

The decomposition of leaf blades of floating-leaved macrophytes commences when the leaves are still connected to the parent plant. The usual approach to study this process is to place detached or harvested plant material in litter bags (Brock et al., 1982; Wieder & Lang, 1982; Cotrufo et al., 2010; Lecerf, 2017; Laanbroek et al., 2018; Taketani et al., 2018). Much less attention has been paid to the initial decomposition of aquatic macrophytes before detachment. Decomposition in these natural conditions involves a complex set of interacting processes (Fig. 1), which can be broadly classified into internal (physiological) and external (abiotic or biotic) processes (Van der Velde *et al*., 1982). Often, various stages of decomposition occur on one plant or even on a single leaf.

During initial decomposition macrophyte tissue can be used by herbivores and phytopathogenic and saprotrophic microorganisms. Before death, the plant tissue senesces during which further decomposition and fragmentation is initiated by weak pathogens and facultative herbivores, leading to the production of debris and faecal pellets. The chemical composition of plant tissue also changes during senescence due to the hydrolysis of macromolecules like DNA and proteins and the resorption of nutrients like N and P as well as carbon compounds such as starch. This leads to a loss of tissue structure, sometimes also to a loss of secondary compounds. Subequently, leaves are colonized by microorganisms, which makes the tissue more attractive for detritivorous macroinvertebrates (Rogers & Breen, 1983).

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The phases of initial decomposition can be studied well in the floating leaf blades (laminae) of large leaved plants such as waterlilies (Nymphaeaceae). Their turnover is low (P/B_{max} 1.35-2.25 yr^{-1}) and the leaves exist for a relatively long time, on average 38-48 days, which allows weekly recordings to follow the damage and the fate of the leaves (Klok & Van der Velde, 2017). The study of waterlilies has several other advantages.

Waterlilies occur worldwide (Conard, 1905; Wiersema, 1987; Padgett, 2007) and in many types of water bodies differing in the physico-chemical conditions of water, sediment or both (Van der Velde, Custers & De Lyon, 1986). They occupy a fixed position in the plant zonation in the littoral zone of lakes between emergent and submerged macrophytes. The nymphaeid growth form combines floating leaves with rooting in the sediment (Luther, 1983; Den Hartog & Van der Velde, 1988). In addition, waterlilies produce thin underwater leaves and aerial leaves when crowding occurs at the water surface or water levels are lowered (Glück, 1924; Van der Velde, 1980).

Floating leaf blades of waterlilies develop under water and subsequent unroll at the water surface, are attacked by microorganisms, phytopathogens and herbivorous animals, both from the underside and at the surface exposed to air (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; herbivores reduce plant growth in the long term (Marquis, 1992; Stenberg & Stenberg, 2012).

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Responses of waterlilies to attacks include replacing old leaves by new ones, shifting from floating leaves to underwater leaves (Kouki, 1993), producing hydrophobic epicuticular wax layers (Riederer & Müller, 2006; Aragón *et al*., 2017) (Fig. 2), spines (Zhang & Yao, 2017), sclereids containing calcium oxalate crystals (Brock & Van der Velde, 1983; Franceschi & Nakata, 2005), tough tissue (Kok *et al*., 1992; Mueller & Dearing, 1994), and plant secondary metabolites 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 are able to attack the fresh plant tissue. These species are more or less specialized and often restricted to particular plant species, genera or families. Other species colonize the leaves at later stages after autolysis, microbial decay and other factors have weakened the defense system (Kok *et al*., 1992). Damage of leaves can induce the leaching of soluble carbohydrates such as oligosaccharides and starch, proteinaceous and phenolic compounds, some of which can be rapidly metabolized by microorganisms (Brock, Boon & Paffen, 1985). Partially decayed floating leaves sinks to the bottom, where they provide a resource fuelling detritus-based benthic food webs and continue being decomposed (Brock, 1985; Van der Velde & Van der Heijden, 1985; Kok & Van der Velde, 1991; Kok *et al*., 1992; Kok, 1993).

The present study summarizes causes and patterns of initial decomposition of floating leaves of three species of waterlilies in three water bodies differing in pH, alkalinity, nutrient levels and surrounding land use. Data from previous studies were compiled to answer three

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questions: 1) What are the causes and patterns of initial decomposition of floating leaves? 2) What is the impact of each cause? 3) How does initial decomposition progress during the season?

Materials and Methods

Sites

Field research took place in dense, nearly mono-specific stands of waterlilies in three different water bodies located in The Netherlands: Haarsteegse Wiel (HW), Oude Waal (OW) and Voorste Goorven (VG) (Table 1). Three plots were laid out in stands of *Nuphar lutea* (L.) Sm. (HW and OW in 1977; VG in 1988), two plots in stands of *Nymphaea alba* L. (OW in 1977; VG in 1988) and one plot in a stand of *Nymphaea candida* Presl (HW in 1977). The plots were accessed with a small zodiac, which was navigated by gently paddling. Otherwise no boating or navigation occurred in the water bodies, which prevented damage of the plants by propellers etc.

Haarsteegse Wiel located in the Province of Noord-Brabant originates from two connected ponds created by dike bursts along the River Meuse. This is an isolated eutrophic water body with low alkalinity (Table 1). The water level fluctuates, depending on precipitation, groundwater seepage and evaporation. Stratification of the water column occurs during summer. The lake bottom consists of sand and an organic layer with increasing thickness towards the littoral zone. The waterlily beds are situated in the wind-sheltered part of the lake.

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Oude Waal in the Province of Gelderland is a highly eutrophic oxbow lake in the forelands of the River Waal. Depth during the growing season is shallow, except for three connected breakthrough ponds (Table 1). The water level is dependent on precipitation, upward groundwater seepage, overflow of the River Waal in winter and/or spring, which strongly influences water chemistry and quality, as well as evaporation. The bottom consisting of clay and sand is covered by an organic layer of varying thickness in the nymphaeid beds.

Finally, Voorste Goorven in the Province of Noord-Brabant is a shallow, oligotrophic, isolated, culturally acidified moorland pool with very low alkalinity. It is surrounded by forests stocking on poorly buffered sandy soils. The hydrology is mainly dependent on precipitation, upward groundwater seepage and evaporation.

Leaf area

Potential and actual leaf area were distinguished to quantify leaf area loss. The potential area refers to 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 using a quadratic regression to relate it to leaf length (Van der Velde & Peelen-Bexkens, 1983; Klok & Van der Velde, 2017). Specifically, undamaged, fully green floating leaves randomly sampled outside the plots were taken to the laboratory where both length and area were measured to establish relationships of the form:

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$$A(L) = c_i L^2 \quad (1),$$

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 = regression coefficient of species i

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

Study design and data collection

Six representative plots of 1 m^2 were laid out in the center of mono-specific stands, each containing one rhizome apex per plot. A non-destructive leaf-marking method was used to mark all floating leaves individually within the plots (REF). Newly unrolled leaves were marked with uniquely numbered Rotex tape fixed around the petiole just under the leaf blade. This enabled us to collect data during the full life-span of the leaves. Each plot was bordered by 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. This set-up does not affect the unrolling of floating leaves in the plots. All leaves having their petioles within the frame were counted and measured. A leaf was considered present as long as, after partial degradation and fragmentation, tissue of the lamina was connected to the petiole in the case of OW and HW. In VG a leaf was considered gone when it was completely brown, dead and submerged, or when it had disappeared.

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All leaves within the plots were inspected and measured at weekly intervals during the growing season, typically from April until November. Site visits involved marking newly unrolled leaves, counting the number of leaves, measuring leaf length from the leaf tip to one of the basal lobe tips and visually estimating different types of initial decomposition expressed as percentage of the potential leaf area of each leaf. Leaves showing several types of damage were photographed in the field and harvested outside the plots to be studied and photographed in the laboratory.

Results

Loss of leaf tissue tended to increase during the vegetation season (Table 2). In the more alkaline lakes (OW and HW), leaf area loss by damage of *Nuphar lutea* and *Nymphaea alba* was less than 20% of the total potential leaf area until mid-September, but increased to more than 50% thereafter. Leaf area loss by damage of *Nymphaea candida* (HW) was less than 10% of the potential area in the beginning and increased to almost 20% in September-October. In the acidic moorland pond (VG) leaf area loss was minimal as these leaves did not fragment (Fig. 3).

Causes and impacts of initial decomposition

The causes of damage classified in the present study were autolysis, frost, hailstones, dehydration, mechanical damage, bird scratches, feeding waterfowl (*Fulica atra* L. and *Gallinula*

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chloropus L., Rallidae), pond snails (*Lymnaea* sp., Lymnaeidae, Gastropoda), reed beetles (*Donacia crassipes* F., Chrysomelidae, Coleoptera), adults and larvae of the water-lily beetle (*Galerucella nymphaeae* L., Chrysomelidae, Coleoptera), a weevil (*Bagous rotundicollis* Bohemann, Curculionidae, Coleoptera), larvae of the aquatic moth brown china mark (*Elophila nymphaeata* (L.), Lepidoptera), larvae of a dung fly (*Hydromyza livens* (Fabricius), Scathophagidae, Diptera), chironomid larvae (Chironomidae, Diptera), including *Endochironomus* spp. and *Tribelos intextus* (Walker), a phytopathogenic fungus (*Colletotrichum nymphaeae* (Pass.) Aa) and oomycete (*Pythium* sp. Jacobs), and microbial decay (Fig. 4, Table 3). In some cases, specific causes could not be identified.

Autolysis. The influence of autolysis reached its maximum towards the end of the growing season. In October the percentage of affected leaves was 100%, however, the surface area affected by autolysis was generally around 10%. Leaf area affected by autolysis decreased over time, since microbial decay became predominant on leaf areas affected by autolysis (Fig. 5).

Frost. Frost in early spring can damage the tips of young leaves sticking out of the water. As a result, such leaves can lose up to one third of their area (Fig. 6). However, the effect on the total leaf surface area was less than 5%.

Hailstones. Occasional hailstone showers damage the floating leaves by penetrating the leaf and leaving typical Y-shaped scars (Fig. 7). However, leaf area damage by hail was minimal overall.

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Dehydration. High winds often lift floating leaves above the water surface and may flip them over. Subsequently, those leaves are exposed to air, particularly the leaf margins, leading to leaf desiccation (Fig. 8). Overall, the effect of desiccation stress on leaf surface area was generally less than 5%.

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Mechanical damage. This type of damage is caused by wind and wave action resulting in cracks in the leaf tissue or lost leaves when the petiole breaks (Fig. 8). For *Nuphar lutea* at HW, *Nymphaea alba* at OW and *Nymphaea candida* at HW, the percentage of leaves affected over the whole vegetation period ranged from 60-80%. *Nuphar lutea* at OW showed peaks of 90% in spring, 70% in autumn and 10% in summer. In contrast, *Nuphar lutea* at VG and *Nymphaea alba* at VG showed no mechanical damage.

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Bird scratches. Scratches are often caused by the claws of birds, mostly coots (*Fulica atra*) but also the common moorhen (*Gallinula chloropus*), as they walk or run over the leaves (Fig. 9). In general, the scratches are straight and affect only the epidermis of the leaf, but angle-shaped cuts due to claws penetrating the leaf tissue also occur. The affected leaf surface area was low, generally below 5%, although a high percentage of leaves was affected, sometimes up to 100% for all plots at HW and OW. In contrast, the plots at VG showed no scratches.

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Consumption by coots. Consumption of leaf tissue by coots can be recognized by missing parts in the form of triangular areas at the edge of leaves. Sometimes major parts of leaves are consumed. Generally, prints of the beak are visible around the consumed areas (Fig. 10).

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Neverthless, the overall effect on total leaf surface area was minimal. The plots at VG showed no damage by coot consumption.

Consumption by pond snails. A major cause of damage on fresh leaf tissue is caused mainly by *Lymnaea stagnalis* L., to a lesser extent also by other lymnaeids. Pond snails consume folded leaves still under water. Rows of holes can then be seen in the unrolled floating leaves, large near the edge and smaller towards the center of the leaf (Fig. 11). In general, lymnaeid snails show a preference for decaying leaf material, such as areas infected by fungi. Damage by these snails was generally an important cause of damage during the whole period for both *Nuphar lutea* and *Nymphaea alba*, contributing 20-40% to the total leaf area loss.

Consumption by reed beetles. Both *Nuphar* and *Nymphaea* spp. are host plants of the reed beetle *Donacia crassipes*. The adult beetles live on the upper side of floating leaves where they feed on leaf tissue (upper epidermis, parenchyma and lower epidermis). The leaf areas removed as a results of tissue consumption by reed beetles are round to oval. Eggs are deposited in two or three rows on the leaf underside. To this end, the beetle gnaws a round or oval hole in the leaf, then sticks its abdomen through the hole to reach the leaf underside and oviposit (Fig. 12). The percentage of leaf area damaged by reed beetles was minimal.

Consumption by water lily beetles. The waterlily beetle (*Galerucella nymphaeae*) completes its full life cycle on the upper surface of floating leaves. Both adult beetles and larvae feed on the upper epidermis and palisade and sponge parenchyma. The larvae, which can be considered half

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miners, create irregular trenches on the surface, leaving the lower epidermis of the leaf intact and depositing their faeces in the trenches. The resultin pattern of leaf tissue damage is easily recognized. The adult beetles consume smaller areas (Fig. 13). Damage was only found in *Nymphaea alba* at VG, where leaves started to be affected in mid-June, rising to damage levels of 30-40% between August and October and reaching a sharp peak of 60% in mid-October.

Consumption by weevils. The adults of *Bagous rotundicollis* scrape off areas of leaf tissue (ca. 1 cm diameter) from the underside of floating leaves near the margin. Only the lower epidermis and sponge parenchyma is consumed, whereas the palisade parenchyma and upper epidermis remain intact (Fig. 14). Damage by weevils was found only in *Nymphaea alba* at VG, with up to 30% of these leaves being affected.

Consumption and damage by the brown china mark. The caterpillar of the **aquatic** moth *Elophila nymphaeata* damages floating leaves in two ways, by leaf tissue consumption and by cutting out oval leaf patches that they can attach to the underside of a floating leaf to make a shelter. They can also spin two patches together to construct a floating shelter (Fig. 15). The effect of these activities on leaf surface area was low, at most 5%. *Nymphaea candida* at HW, *Nuphar lutea* at VG and *Nymphaea alba* at VG were not damaged by the moth.

Mining by a dung fly. Larvae of the dung fly *Hydromyza livens* only occurred in *Nuphar* leaves, where they mine and consume leaf tissue. Eggs are laid at the underside of the leaves. For that purpose the fly goes underwater, following the dichotomous veins on the underside of the leaves

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Mining by chironomids. Larvae of some Chironomidae mine the leaf tissue and dig or eat their way through the leaf tissue. Typical damage on *Nuphar* leaves is caused by larvae of *Tribelos intextus*. These larvae mine leaves still folded underwater, resulting in rows of small holes that become visible when the floating leaves unroll at the water surface (Fig. 17). Other miners observed at the study sites are larvae of *Cricotopus trifasciatus* (Meigen in Panzer, 1813) (Fig. 18), which is a half miner. The species was observed to cause some damage at the leaf margins of *Nuphar lutea* in the neighbourhood of *Nymphoides peltata* (Gmel.) O. Kuntze in OW. Overall, however, the impact of these chironomid species on floating leaves was minimal.

Mining by *Endochironomus* spp. Larvae of these midges mine in floating leaves. The mines of could clearly be distinguished from those of other Chironomidae described above, since they appear on the upper side of the floating leaves as straight dark stripes (Fig. 19). The total effect on the decomposition of floating leaves was minimal.

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Infection by phytopathogens. The leaves of *Nuphar lutea* were infected by the oomycete *Pythium* “type F” (Fig. 20) and the leaves of *Nymphaea alba* and *Nymphaea candida* by the fungus *Colletotrichum nymphaeae*, the causative agent of leaf spot disease (Fig. 21). The percentage of damaged surface area was about 15% for *Pythium* and up to 55% for *Colletotrichum*.

Microbial decay. The resistance of a leaf against microbial infection disappears quickly due to autolysis, facilitating microbial decay (Fig. 5). The effect on the affected surface area ranged from 15 to 25%, with an exceptional level of damage reaching 60% in *Nymphaea candida* at HW at the very end of the growing season.

Unknown causes. Missing leaves or parts thereof can result from animal consumption or damage; however, in many cases, the cause could not be determined. Leaves disconnected from their petioles were scattered by wind and wave action and could not be traced back to their parent plant. Damage occasionally rose to 60% for *Nuphar lutea* at HW, *Nymphaea alba* at OW and *Nymphaea candida* at HW; however, this type of damage was hardly found for *Nuphar lutea* at OW and VG and *Nymphaea alba* at VG.

Discussion

Senescence and autolysis. Newly unrolled leaf blades of waterlilies are fully green and hydrophobic due to a thick epicuticular wax layer. This waxy layer gradually erodes during senescence and as cellulolytic and other bacteria and fungi colonize the leaf tissue (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 throughout the growth period. During autolysis the leaves turn from green to yellow, and subsequently to brown. Concomitant microbial decay softens the leaves. The list of causes identified in the present study (Table 3) clearly indicates that autolysis followed by microbial decay had the greatest impact on the initial decomposition of floating leaves. Leaves developed during 53 to 73 % of the vegetation period (Klok & Van der Velde, 2017) of 135 to 199 days (Table 2).

Infection by phytopathogens and microbial decay. In *Nuphar* both microbial decay and infection by the phytopathogenic oomycete *Pythium* sp. "type F" were important from the start of the season. In *Nymphaea*, infection by the phytopathogenic fungus *Colletotrichum nymphaeae* also started early and increased in importance towards the end of the season. In general, microbial decay and phytopathogenic infection gradually increased in importance, whereas most other causes of damage diminished over time.

Weather conditions. Minor causes of leaf impairment occurring once during spring were frost damage of the first newly unrolled leaves and hailstones, which hardly affected leaf area loss but contributed to leaf fragmentation. Moreover, high solar radiation and air temperature dehydrated leaves that had been flipped over, with the impact being high in HW and OW but not in the wind sheltered VG. Furthermore, prolonged cloudy and wet weather imposes stress on waterlilies by

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Damage by animals. Causes of damage by insects were similar for *Nymphaea* and *Nuphar* with the exception of *Hydromyza livens* and *Tribelos intextus*, which appear to be specific for *Nuphar* (Brock & Van der Velde, 1983; Van der Velde & Hiddink, 1987). Some species such as *Bagous rotundicollis* (Van der Velde et al., 1989) and *Donacia crassipes* (Gaevskaya, 1969) exclusively feed on Nymphaeaceae. Other species such as *Galerucella nymphaeae* and *Elophila nymphaeae* feed on floating-leaved macrophytes but also consume emergent macrophytes (Gaevskaya 1969; Lammens & Van der Velde, 1978; Pappers et al., 2001). *Cricotopus trifasciatus* causes damage on leaves of *Nymphoides peltata* (Lammens & Van der Velde, 1978) and was also observed to damage *Nuphar lutea* leaves (Van der Velde & Hiddink, 1987).

In VG, damage was mainly caused by phytophagous insects consuming floating leaf tissue, particularly herbivorous beetles, fly larvae and mining chironomid larvae. Leaf fragmentation was hardly observed in the acidic VG, which was also the site most sheltered against wind and wave action by a surrounding forest. This allowed the waterlily beetle *Galerucella nymphaeae* to cause extensive damage. Herbivory by this species removes the lower epidermis of their tracks, which makes the leaf tissue vulnerable to subsequent microbial attack (Wesenberg-Lund, 1943;

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Roweck, 1988). Although leaf area loss is mainly caused by fungi and bacteria, the process is initiated by the beetle (Wallace & O'Hop, 1985). The damaged areas characterized by regular margins made by adult *Galerucella nymphaeae* are distinct from those made by adult *Donacia crassipes* where the margins of damaged areas are rather irregular (Roweck, 1988). *Galerucella nymphaeae* was absent in the two water bodies which are frequently exposed to strong wind. In winter the adults of this beetle hide in remains of dead emergent macrophytes, under the bark of trees or in ground litter and reappear in spring when the floating leaves begin to develop. In acidic water, leaves were consumed by specialized insect species causing only minor leaf area loss.

Consumption by snails was only found in the more alkaline lakes, since they require calcium to build their shells. Snails at those sites prefer consuming decaying, microbially colonized parts of the leaves (Kok, 1993).

Nymphaea candida (HW) showed an increase in scratches by bird claws towards the end of June, which may have been due to young coots. High densities of waterfowl at HW and OW are facilitated by the surrounding meadows where birds graze during winter.

pH and alkalinity. Decomposition of leaves was slowed at the acidic site (VG) because of low alkalinity and high Al concentrations of the water, as well low pH and high phenolic contents of the plant tissue. The inhibition of cell wall degradation slows fragmentation, increases concentrations of phenolics and reduces softening of the leaf tissue, resulting in a low-quality

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food resource for detritivores, which are also inhibited by high Al concentrations and low pH (Kok, 1993).

Harvested green leaf blades of *Nymphaea alba* placed in litter bags in the field and laboratory and showed lower leaf area loss under acidic conditions in a moorland pool (VG) than in a eutrophic, more alkaline oxbow lake (OW), and results in laboratory conditions mimicking differences in water chemistry were similar (Brock, Boon & Paffen (1985). Depending on water chemistry, mass loss was pronounced and organic matter chemical composition changed rapidly during the first 10-30 days, followed by an accumulation of structural plant polymers such as cellulose, hemicellulose and lignin. The disappearance of those fractions was dependent on the water quality of the water body (Brock, Boon & Paffen, 1985).

The decomposition pattern of *Nuphar lutea* was similar in the two more alkaline waters, and differed from the patterns of *Nymphaea alba* and *N. candida*. In the acidic VG, the effect of leaf damage on leaf area loss was minimal for both *Nuphar lutea* and *Nymphaea alba* (Fig. 2).

Conclusions

Overall patterns of initial decomposition of floating waterlily leaves were influenced by plant species, water chemistry and local conditions such as the extent of wind exposure. Fresh floating leaves offer food for a suite of more or less specialized insects, consuming leaf tissue under water, on the upper surface or by mining in the tissue. Coots also consumw floating leaves.

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Subsequently, the damaged leaves offer food for non-specialized insects, pond snails, fungi, oomycetes and microbes. Tissue loss as a consequence of microbial decay is particularly prominent.

Floating-leaved macrophyte stands in wind-sheltered locations were colonized by partially different insect species and this led to different impacts than in wind-exposed plots, where mechanical damage by wind and wave action was significant. Impacts on floating leaves in acidic and more alkaline waters were also different.

Of the causes of initial decomposition found in th studies summarized here, only a few have significant impact on leaf damage and leaf loss. Identified causes with high impact were autolysis, microbial decay, infection by phytopathogens, consumption by pond snails and mechanical damage.

Several types of succession of damage could be distinguished. Erosion of the wax layer and damage by phytophagous insects were followed by cellulolytic bacteria, a fungus and oomycete, followed by snails and mechanical damage, followed by biotic causes or decay or autolysis, followed by phytopathogens or microbial decay, followed by tissue removal by snails. In the more alkaline waters, this was followed by leaves breaking up or, in acidic water, by intact dead leaves sinking to the bottom.

Acknowledgements

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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 modelling, W.J. Metzger for English language corrections and Manuela Abelho and one anonymous reviewer for constructive comments that improved the manuscript.

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Table 1. Characteristics of the three study sites in The Netherlands to investigate the initial decomposition of floating-leaved macrophyte leaves.

Characteristic	Haarsteegse Wiel (HW)	Oude Waal (OW)	Voorste Goorven (VG)
Type of water body	Breakthrough lake	Oxbow lake with three breakthrough ponds	Moorland pool
Location	51^{0}43'05" N, 5^{0}11'07" E	51^{0}51'13" N, 5^{0}53'35" E	51^{0}33'53" N, 5^{0}12'26" E
Area (ha)	18	25	5
Maximum depth	17 m	1.5 m and 6-7 m	2 m
Water level fluctuations	Low	High in winter and spring	Low
Stratification	In summer, thermocline at 4-6 m	No	No
Hydrology	Precipitation, evaporation, groundwater seepage	Precipitation, evaporation, upward groundwater seepage, river overflow	Precipitation, evaporation, upward groundwater seepage
Surrounding vegetation	Trees, shrubs, reeds	Grassland	Forest
Wind and wave action	Low	Moderate	Moderate
Bottom	Sand, organic (sapropel)	Sand, clay, organic (sapropel)	Sand, organic (sapropel)
Trophic state	Eutrophic	Highly eutrophic	Oligotrophic
Alkalinity (mmol L^{-1})*	1.5	4.3-6.7	<0.01-0.07
pH*	7.1-8.5	6.7-8.3	4.7-5.5
Sampling year	1977	1977	1988
Macrophyte species (water depth of plot)	*Nuphar lutea*, (1.5 m) *Nymphaea candida* (2.5 m)	*Nuphar lutea* (1.5 m) *Nymphaea alba* (1.5 m)	*Nuphar lutea* (2 m) *Nymphaea alba* (2 m)

*From Brock, Boon & Paffen (1985) and Kok, Van der Velde & Landsbergen (1990).

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Table 2. Summary characteristics of floating-leaved macrophyte stand in three small water bodies of The Netherlands.

Site	Species	Year	Vegetation period		Growth period		Total number	Total potential
			Time span	Days	Time span	Days	of leaves.(m^{-2})	leaf area (cm^2)
HW	*Nuphar lutea*	1977	May 10 – Nov 24	199	May 10 – Sep 13	127	77	49674
OW	*Nuphar lutea*	1977	May 11 – Nov 1	175	May 11 – Sep 7	120	59	39898
VG	*Nuphar lutea*	1988	Apr 28 – Oct 27	183	Apr 28 – Sep 8	134	22	8440
HW	*Nymphaea candida*	1977	Jun 7 – Oct 19	135	Jun 7 – Aug 16	71	43	11185
OW	*Nymphaea alba*	1977	May 11 – Nov 6	180	May 11 – Sep 7	120	108	53035
VG	*Nymphaea alba*	1988	Apr 28 – Oct 27	183	Apr 28 – Sep 8	134	80	23053

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