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MACROPHYTES AND NUTRIENT DYNAMICS: PROCESS AND FIELD STUDIES IN THE UPPER REACHES OF LOWLAND RIVERS "MANUDYN II"

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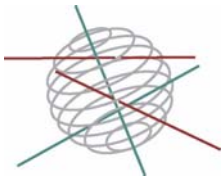

Terrestrial Ecosystems

FINAL REPORT PHASE 1

MACROPHYTES AND NUTRIENT DYNAMICS: PROCESS AND FIELD STUDIES IN THE UPPER REACHES OF LOWLAND RIVERS

"MANUDYN II"

SD/TE/04A

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SUMMARY

Introduction

Due to the high urbanisation of Flanders water quality is reduced resulting in turbid system where plant growth is inhibited due to the poor light conditions. This lack of light has resulted in the disappearance of macrophytes. Because efforts have increased to reduced nutrient loadings towards our rivers light conditions have increased with a return of freshwater macrophytes. Because nutrient loading of our rivers is still high macrophyte growth is even stimulated. This high biomass results in reduced drainage during summer with high water levels and possible flooding as result. From the previous MANUDYN I project we know that macrophytes have a crucial role in the nutrient cycle of rivers. They seem to prefer ammonium as nitrogen source instead of nitrate which is available in higher amounts.

The general objective of this project is to develop a numerical tool allowing the quantitative description of the growth and decay of macrophytes, and of their interactions with nutrients from the water column and the sediments. For this purpose we will study in detail the growth, decay, and nutrient uptake, release and allocation processes of macrophytes in response to their various physical, chemical and biological controlling factors. These include light intensity, temperature, water quality, sediment quality, stream velocities and macrophyte or macro-algae species composition. Experiments will be performed at various spatial and temporal scales in order to develop integrated models describing the kinetics of growth and decomposition of river macrophytes.

Methodology

Three work packages were created with an increasing spatial complexity (individual, plant patch and river stretch). WP1 and 2 were all performed ex-situ to keep certain environmental variables constant. A schematic overview of the work packages is given in the following figure.

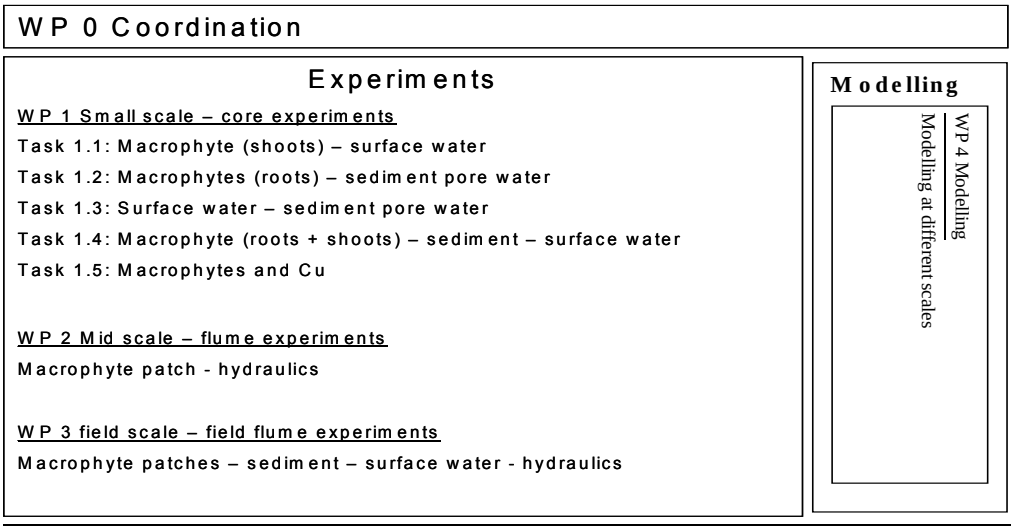


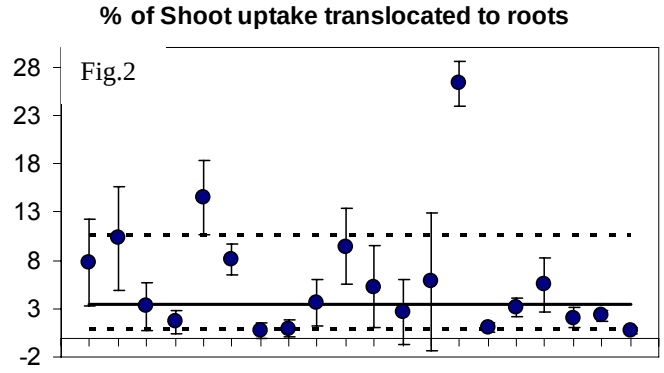
Fig. 1: General overview of the Manudyn II project structure

This project has started with the work package dedicated to small scale experiments at the level of a single individual of a macrophyte (WP1). The uptake mechanisms of *P natans, C platycarpa* and *R fluitans* were quantified under a range of variable conditions of light intensity, temperature, water quality, sediment quality and stream velocities. In the other packages a spatial up scaling was performed. In this report the the results of WP1 and 2 will be summarized.

Results

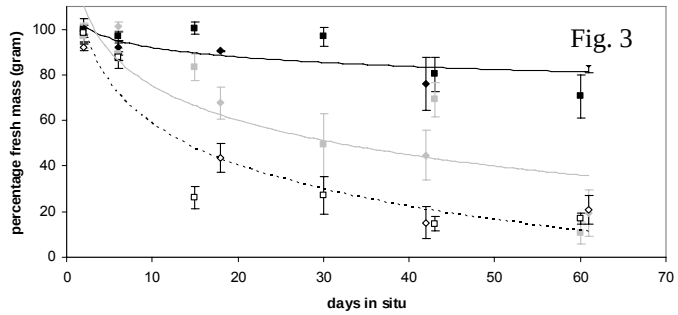
Nutrient uptake and release

On individual plant scale and during short time periods P natans and C platycarpa were not able to satisfy the nitrogen need trough the uptake of nitrate. Their nitrogen need had to be completed by ammonium uptake. From our results it is clear that ammonium is taken up in excess and stored in their biomass allowing them to fixate dissolved inorganic carbon (DIC) in the absence of ammonium and nitrate. From the same experiments was clear that nitrogen and carbon uptake took place through the stems. Between 1 and 10 % of the ammonium uptake of the roots was explained by translocation from the stem (Fig 2). The uptake of dissolved inorganic nitrogen (DIN) for *C platycarpa* was strongly dependent on the ammonium concentration and temperature but for *P natans* only dependent of temperature. Light did influence the DIC uptake but had no effect on the DIN uptake.



In general we therefore can say that, with the exception of temperature, both macrophytes have similar DIC and DIN uptake.

However, at longer exposure times (several weeks) ammonium became toxic for the plants with a reduction in biomass. As suggested by Li et al (2007) this could be the result of consumption of plant carbon to store the ammonium as protein in the plant. On the mid long term (days) plants rather preferred nitrate as a nitrogen source but were able to use ammonium after an adaptation period. The storage of nutrients in the plant was also species dependent.



black = 6 °C
grey = 15 °C
white = 20 °C

diamond = C platycarpa
square = R fluitans

Temperature was not only an important parameter during the accumulation of nutrients but also during decay of plant material (Fig below). With increasing temperature plant decay was much faster and primarily the result of bacterial decomposition. The contribution of fungi in this decomposition process was negligible.

Hydraulic influence

Because nutrient uptake is strongly determined by the existing hydraulic conditions the effect of macrophyte resistance is investigated. From these experiments it was clear that friction was generated (60%) primarily by the leaves. For the tested species a clear trade-off was seen between the ratio photosynthetic surface/ total friction and stream velocity.

Optimalization of in-situ macrophyte measuring

Because estimation of macrophyte growth at river scale is a time consuming activity three different (manual transect method, DGPS method and aerial photography) methods of macrophyte mapping were compared. All three methods gave comparable results allowing us to choose the most efficient method for the measurements of macrophyte patches. This aerial photography method will therefore be used in the second phase of this project.

INTRODUCTION

Context

Recently, different authors have shown that nutrient retention within river catchments can not be neglected (Behrendt & Opitz 2000). Behrendt and Opitz (2000) report significant retention of nutrients (nitrogen and phosphorus) for about 100 different river basins in Europe.

Macrophytes are one of the important actors in the use of nutrients in smaller streams. The role of macrophytes within the biogeochemical cycle of carbon, nitrogen and phosphorus has been studied in great details for standing freshwater systems (Bloemendaal & Roelofs, 1988; Best, 1982; Collins & Wlosinsky, 1989; Hoostmans & Vermaat, 1991; Weisner and Al, 1997). In contrast, studies on the role of macrophytes in streaming waters are limited. Nutrient concentrations are important controlling factors in streaming waters, but due to the continuous input of fresh material, especially by diffuse sources, they never become limiting in eutrophic systems. The water column is a more important source for nutrients in streaming waters than in standing water, because the water flow brings in a continuous supply of new nutrients. Species adapted to streaming water can take up inorganic nitrogen and phosphorus very efficiently (Schwoerbel & Tillmanns 1964, Vincent & Downes 1980). Submerged macrophytes take up nutrients from the sediments via roots and from the water via shoots (Sytsma and Anderson 1993, Carr & Chambers 1998). The relative importance of both will depend on the nature of the element and on its concentration in the interstitial and ambient water (Carignan & Kalff 1980). Moreover, different strategies of nutrient uptake exist among the macrophytes (Haury & Aïdara, 1999). However most of the studies done so far rely on measurements of nutrients in plant tissues, water and sediment pore water, which only gives an indirect information about the nutrient uptake fluxes by roots and shoots, and about nutrient translocation between roots and shoots. One of the most powerful tools to study the N uptake and translocation pathways inside the plant can be obtained by performing experiments with enriched ^{15}N stable isotopic tracers ($^{15}N\text{-}NH4^{+}$ and $^{15}N\text{-}NO3^{-}$) (Bouma et al. 2002).

Macrophytes can also play an important role in the detoxification of metal contaminated sediments. Indeed, some river sediments comprise high metal concentrations, e.g. copper, through historical contamination activities (Santschi et al., 2001) and this is also the case in the Nete catchment (Vandecasteele et al. 2005). Macrophytes seem to survive high concentrations of metals such as copper and they can incorporate very high amounts in their tissues (Samecka-Cymerman & Kempers 2004, Demirezen & Aksoy 2004). However, the mechanisms of uptake and the interaction sediment-macrophyte-surface water are not clear.

Furthermore, other factors determining the distribution and growth of macrophytes include stream velocity, depth, light availability, temperature and water quality (Cloern 2001). The degradation process depends on the structure and chemical composition of the macrophytes and on environmental conditions such as temperature and oxygen.

Macrophytes and especially submerged ones alter the water flow pattern drastically (Sand-Jensen & Pedersen, 1999). Stream velocity in vegetation patches can decline towards very low values with high Manning's n as a consequence. This increase in resistance is complicated due to vegetation characteristics like flexibility (Kouwen and Unny 1973, Tsujimoto et al. 1996).

In some tributaries of the Scheldt, macrophytes have become dominant over large parts of the streams. This is especially the case in the Nete and Dijle basins, two tributaries in the eastern part of the Scheldt basin. Due to the high nutrient input mainly by diffuse sources, excessive developments of macrophytes are frequently observed during summer.

Another example of excessive macrophyte development in rivers concerns the Semois River, one of the tributaries of the Meuse. Some sections of the Semois river are colonized by invasive species as *Ranunculus fluitans*. Even if a significant number of other species is growing in the river, (Thoen et al., 1996), *Ranunculus fluitans* is preponderant and represents more than 80 % of the total aquatic plant biomass (Roussel, 1995). So, in some zones, *Ranunculus fluitans* concentration can reach almost 500 g of dry matter per square meter (Gommes, 1978).

Although the presence of macrophytes can be considered as beneficial in terms of nutrient and metal retention of stream, several drawbacks can also appear when excess biomasses develops. In the case of rivers serving important recreational functions such as the Semois, invasion of the bathing places, interference with canoeing and odour emissions due to the decomposition of the plants strongly reduce the tourist attractiveness, with the resulting economic consequences. Moreover, in rivers from the Dijle and Nete catchment, excess developments of macrophytes causes a limitation of the hydraulic capacity of

the systems and an increase of the water levels in the upstream zones, which can result in flooding. Additionally excess macrophyte biomass can result in the induction of high amplitude oxygen and pH daily cycles. Combined with high ammonium concentration, this can lead to fish mortalities as a result of ammonia conversion. Finally, by the predominance of one species, a reduction of the biodiversity can also be obtained. For these reasons, control of excess macrophyte growth is one of the priorities of integrated management policy of river catchments. Powerful tools allowing the correct management of macrophyte growth are river models. However, only few modelling studies on rivers include the macrophyte dynamics as ruled by multiple environmental factors. Some modelling attempts of the macrophyte development and growth were performed during the last two decades (Rutherford 1977, Asaeda et al. 2000, Best et al. 2001, Herb & Stefan 2003, van Nes et al. 2003). The frameworks of macrophyte productivity models are usually quite similar, but different environmental factors are included and diverse algorithms are used to describe the relationships (Carr et al., 1997). Moreover, the majority of these models are considering macrophytes in lakes and stagnant waters, excluding the effect of flow velocity (Carr et al. 1997, Barendregt & Bio 2003). A principal reason for this lies in the difficulty for modelling the growth, development and effects of a heterogeneous plant population on the water quality. Indeed the ecological and physiological characteristics of the various plants can be highly different and the relations between the substratum, the liquid phase and the plant are very complex. For example, the model of Rutherford (1977) is exclusively devoted to daily dissolved oxygen concentration variations. Besides these modelling attempts, most of the studies available on macrophytes focus on physiological aspects, limiting factors for growth and activity and their impact on the aquatic ecosystems (Kelly at al 1983, Carpenter & Lodge 1986, Madsen & Adams 1988, Carr & Goulder 1990, Carr & Chambers 1998).
A great deal of models apply to the highly eutrophic ecosystems and neglect nutrients (nitrogen and phosphorus) as possible limiting factors, considering they are sufficiently abundant for an optimal growth of the plant biomass. Then the main controlling factors are temperature and light, possibly limited by self-shading (Best 1982). These simplified descriptions of the behaviour of macrophytes prevent the use of such models for non- or less eutrophic ecosystems, where nutrients can act as controlling factors for plant growth. However these models are useful allowing the identification and quantification the main factors which control the growth of macrophytes.
More recently, the experimental research (laboratory and field) led to the development of more sophisticated models taking into account a larger number of processes. Moreover they reduce model calibration based only on field data sets. Indeed the current approach in the field of the ecosystems modelling is based on a sharp comprehension of the occurring phenomena and on the experimental determination of the corresponding biokinetics. For example, a study on influence of nutrients on the growth of macrophytes in small rivers (Wright and Mc Donnel, 1986b) led to the development of a model aiming to simulate and demonstrate the influence of phosphorus loading as growth limiting factor of a heterogeneous macrophyte population, mainly composed of *Elodea canadensis, Potamogeton crispus* and *Potamogeton foliosus*. They (1986a) also developed a model based on field measurements performed on various sections of a single river with variable nutrients concentrations (10 to 1350 μg P/l). They used the differences observed in macrophyte growth to draw the relations existing between water quality and aquatic plants yields.
These examples show that growth modelling for aquatic rooted plant has been mainly restricted to the macrophyte compartment itself, as set in a given river section. The development of general models using macrophytes as local state variable (biomass) but also for their contribution on the whole basin scale is not known very well. However, they seem the only way to approach the subject as a whole.
The development of such models must rely on experiments designed to describe in a quantitative way the growth and decay of macrophytes in response to physical, biological and chemical external factors. Important factors are species composition, patch densities, water flow velocities, nutrient concentrations in the water and sediments, temperature and light.

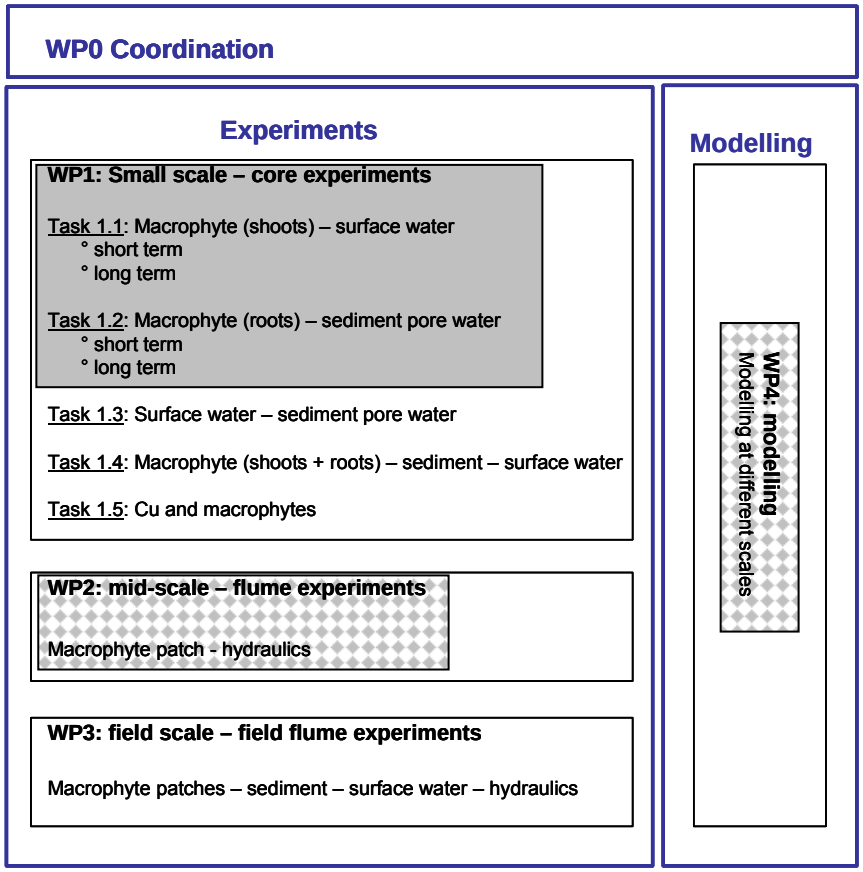
Objectives

The general objective of this project is to develop a numerical tool allowing the quantitative description of the growth and decay of macrophytes, and of their interactions with nutrients from the water column and the sediments. For this purpose we will study in detail the growth, decay, and nutrient uptake, release and allocation processes of macrophytes in response to their various physical, chemical and biological controlling factors. These include light intensity, temperature, water quality, sediment quality, stream velocities and macrophyte species composition. Experiments will be performed at various spatial and temporal scales in order to develop integrated models describing the kinetics of growth and decomposition of river macrophytes. Once integrated into stream ecosystem models, this might serve as an efficient tool to

explore various scenarios of macrophyte biomass controls. For example the effects of biomass removal (mowing) on in-stream nutrient retention can be evaluated, with the possible consequences for the downstream (estuary, coastal zone) ecosystems.
The expected results of the small and mid-scale experiments will provide an improved insight in the detailed processes of nutrient uptake in macrophytes. Both the increased understanding and the associated modelling will be essential for incorporating the role of macrophyte in the scope of integrated water management. When deeper insight is achieved, the results can be used to manage the occurrence of macrophytes. The results will indicate at what time the highest nutrient storage can be found in the macrophyte biomass and when the lowest nutrient fluxes downstream are expected. Moreover, they will verify whether removing these plants has a significant impact on the nutrient retention capacity of the headwaters. The results will allow designing a management plan indicating e.g. the period during which weed cutting would have the largest impact on nutrient retention capacity.
The better understanding of the detailed uptake and growth processes concerning freshwater macrophytes and of the link between the catchment and the estuary can greatly improve the water quality management. It will be possible to evaluate the impact of different strategies on macrophyte populations and on the water quality at the interface between the headwaters and the estuarine system. Although the models will be at first a research tool, its results will be important to disseminate the knowledge gained towards all interested end-users.

METHODS AND APPROACHES

As suggested from literature, the study of macrophyte growth and degradation processes, and of nutrient, light and temperature effects is a difficult task to be performed directly in the field, and this because of the heterogenic and complex interactions between streaming water, sediments and macrophyte patches. Therefore, in order to fulfil our objectives, experiments were planned to be conducted at different scales taking into account an increasing complexity (see table below): from the simplest system taking into account a single macrophyte specimen to the most complex system considering several macrophyte patches within rivers. In parallel with the experiments, modelling will follow each of the scale steps.



In the first phase of the MANUDYN project, work was concentrated around small scale and mid-scale experiments (see the grey areas in the table above),

The uptake mechanisms of different macrophyte species at the scale of single specimens (WP1) were studied under a range of variable conditions of light intensity, temperature, water quality, sediment quality, stream velocities and for different macrophyte species or algae, etc.... On the one hand, this was done on the short term (from a few hours to a few days) with isotopic techniques (for ^{15}N and ^{13}C) or based on oxygen budgets. On the other hand, growth and decomposition were followed from nearby by means of experiments on the long term (weeks to months). Also, the influence of bacteria and fungi on the decomposition of macrophytes was investigated. All compartments were studied: the sediment, macrophyte shoots and roots and the flowing water.

The second work package was dedicated to medium scale experiments at the level of a macrophyte patch. We studied the influence of the hydraulics on the nutrient uptake and breaking strength of different macrophyte patch densities. This was carried out in the flume facility of the NIOO-CEME enabling us to do measurements of i) hydrodynamics, ii) uptake kinetics and iii) exchange processes under different current velocity regimes.

Finally, some preliminary field scale studies were conducted in order to i) check out the variability of nutrient contents in aquatic plants and ii) test out several methods to determine macrophyte biomasses in rivers. This was done for *Ranunculus fluitans* in different stretches of the Semois River.

WP1 –EXPERIMENTS AT THE SCALE A A MACROPHYTE SPECIMEN

1.1 Short term experiments (a few hours)

The uptake of ammonium, nitrate and carbon by macrophytes were studied over a few hours for various conditions of substrate concentration, light and temperature using isotopic tracers. This was done on 2 macrophyte species dominating the upper reaches of the Scheldt river basin: *Potamogetan natans* and *Callitriche platycarpus* (see picture below)

1.1.1 Plant collection

In July 2007 macrophyte specimens of 2 morphological different species (*P natans and C platycarpa*) were collected in the Grote Caliebeek (Nete Catchment). The plants were hand picked by gently removing parts of the plant roots (*P natans*) from the sediments. The collection of *C. platycarpa* was done by collecting 30 by 30 cm mats of unrooted floating plants. Plant material was transported submerged in river water towards the laboratories where they were rinsed and placed in aerated 15L aquaria filled with artificial mineral water (AMW, Kilham et al. 1998) amended with 14 mg/l $N\text{-}NO_3^-$ and 2.5 mg/l $N\text{-}NH_4$ for 2 to 14 days before being used in incubation experiments.
Additionally, at the end of May 2008, 16 specimens of *P. natans*, a macrophyte with a well developed root system, were hand picked by gently removing part of their roots from the sediments. Each specimen was then rooted in a plastic bag containing 550 g of sand soaked with river water containing initially 0.5 mg/l of $N\text{-}NO_3^-$, 0.01 mg/l of $P\text{-}PO_4^{3-}$, and 0.6 mg/l of $N\text{-}NH_4^+$. The bags were placed and fixed in a container at the bottom of the river and left in the field for 2 weeks to allow re-growth of roots. All plants in their bags were then transported submerged in river water to the lab and were kept one day until further experimentation the next morning.

C. platycarpa

P. natans

1.1.2 Incubations with tracers

Incubators design

Eight cylindrical incubators (Fig. 1) were specially designed and build by the Institute of Biology (Dr Marianne Holmer) from the University of Southern Denmark and kindly send to Brussels for experimental use. These incubators are made by the superposition of a grey PVC bottom sediment-holder cylinder (12 cm height) and a top transparent plexi-glass water-holding cylinder (40 cm height) of about 10 cm diameter (Fig. 1). The bottom cylinder is filled with sand to root the macrophyte specimen and is closed on top by a perforated red PVC lid that is assembled around the shoot (see Fig. 1). The hole in the lid (0.5 cm diameter) around the shoot is then filled with a fast (85 sec) hardening polivinylsiloxane paste (President fast putty soft, Coltène ®). Leaks at interfaces are prevented by the use of silicone grease. The upper cylinder is filled with water (2L) to hold the shoots of the macrophyte specimen. On top, a rubber stopper perforated by 2 aeration holes is placed. One of the holes holds a glass pipette that is connected to an aquarium air-pump that bubbles to ensure the mixing of the water column during incubation.
The advantage of this system is that it allows the separate labeling of water column and sediment interstitial water allowing to trace eventual translocation between shoots and roots.

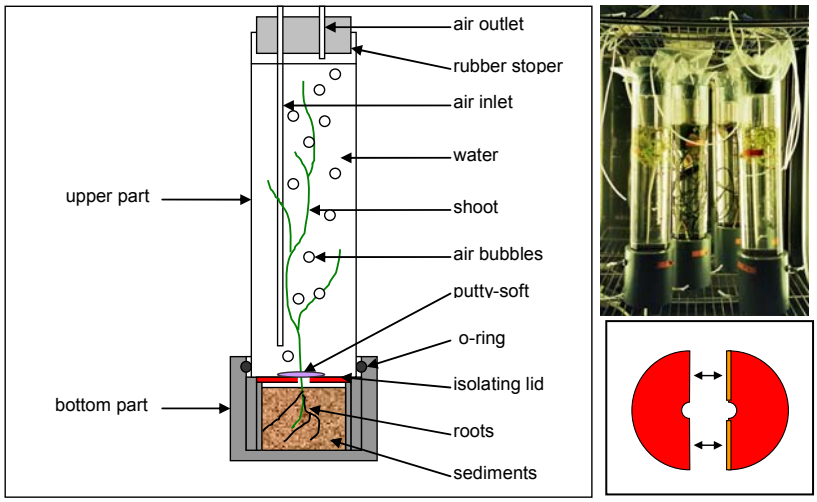


Fig. 1 The whole plant incubator for the very short term experiments

Incubations

At the start of the experiment, individual macrophyte specimens were placed in the incubators: *P. natans* were rooted in artificial sediment (sand soaked with AMW enriched with 1.75 mg/l N-NH_4^+) while *C platycarpa* were allowed to float in the water solution. Plants were incubated for 4 to 6 hours with ^{15}N (as NH_4^+ or NO_3^-) and ^{13}C-HCO_3^- tracers in different conditions of light (0 to 175μmol Quanta/m²/s), temperature (5 to 25°C) and NH_4^+ (0.06 to 1.8 mgN/l) or NO_3^- (0.07 to 2.8 mgN/l) concentrations. For *P. natans*, ^{15}N was either added in the water column or in the sediment interstitial water, and ^{13}C was always added in the water column.
After incubation, the specimens were removed from the incubators, rinsed with distilled water and paper towel dried. Roots and shoots (for *C platycarpa*) and new leaves, old leaves, stems (including leaf peduncle) and roots (including stolons) (for *P. natans*) were separated with scissors and placed in Petri-dishes. They were dried in an oven at 60°C for 3 days to 1 week (depending on the biomass) and reduced to powder using scissors and pestle. Each sample was analyzed for its N and C content and ^{15}N and ^{13}C content using an elemental analyzer (Flash series 1112) coupled to a Finnigan Delta plusXL isotope ratio mass-spectrometer (IRMS) according to Nieuwenhuize et al (1994). C and N uptake rates were computed according to the modified Dugdale and Goering (1967) equations.

1.1.3 Results

Comparison *P. natans* vs. *C platycarpa* in terms NH_4^+, NO_3^- and DIC uptake:

P. natans and *C platycarpa* displayed very similar ranges of NH_4^+, NO_3^- and DIC uptake rates (Fig. 2), whatever the conditions considered. This shows that despite very different morphological characteristics, both species are potentially important considering their production and their DIN use.

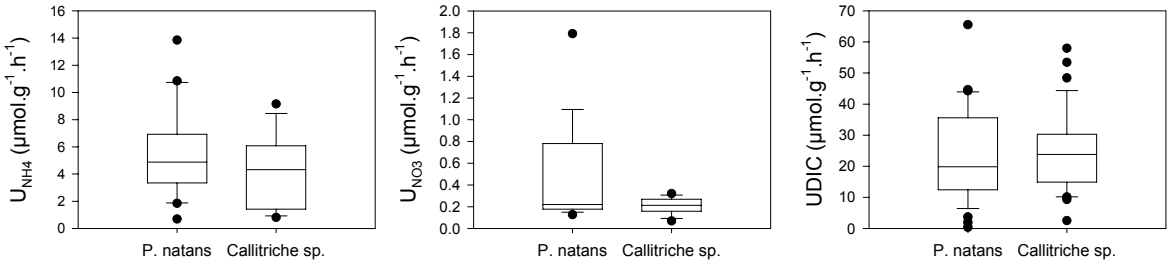


Fig. 2. Box-plot showing the importance of *P. natans* and *C platycarpa* for the uptake of NH_4^+, NO_3^- and DIC in various conditions of light, temperature, and DIN concentration.

Preference for NH_4^+ or NO_3^-:

The uptake of NH_4^+ and NO_3^- by *P. natans* and *C. platycarpa* was measured in various conditions of light, temperature, and DIN concentration (see methods). Whatever the conditions, both macrophyte species displayed higher ammonium uptake rates than nitrate uptake rates (Fig. 3) showing their preference for NH_4^+, as has been largely documented for many freshwater and marine algae (Lotze & Schramm 2000) and aquatic plants (Bouma et al. 2002, Touchette and Burkholder, 2000) as well as for foliar uptake of terrestrial plants (Wilson 1992, Peuke et al. 1998, Ignatova & Dambrine 2000).

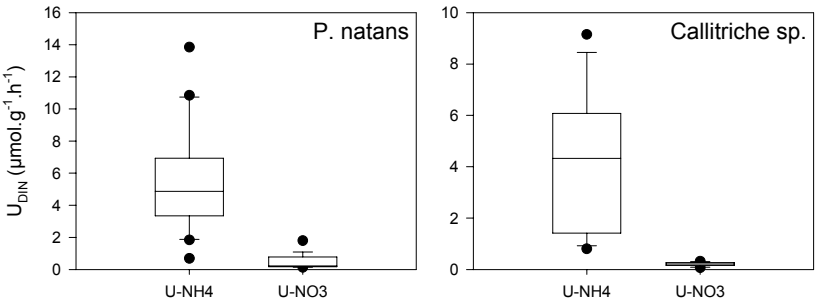


Fig. 3. Box-plot showing the importance of NH_4^+ and NO_3^- uptake by *P. natans* and *C. platycarpa* in various conditions of light, temperature, and DIN concentration.

Relative importance of different plant organs in the uptake of DIN and DIC:

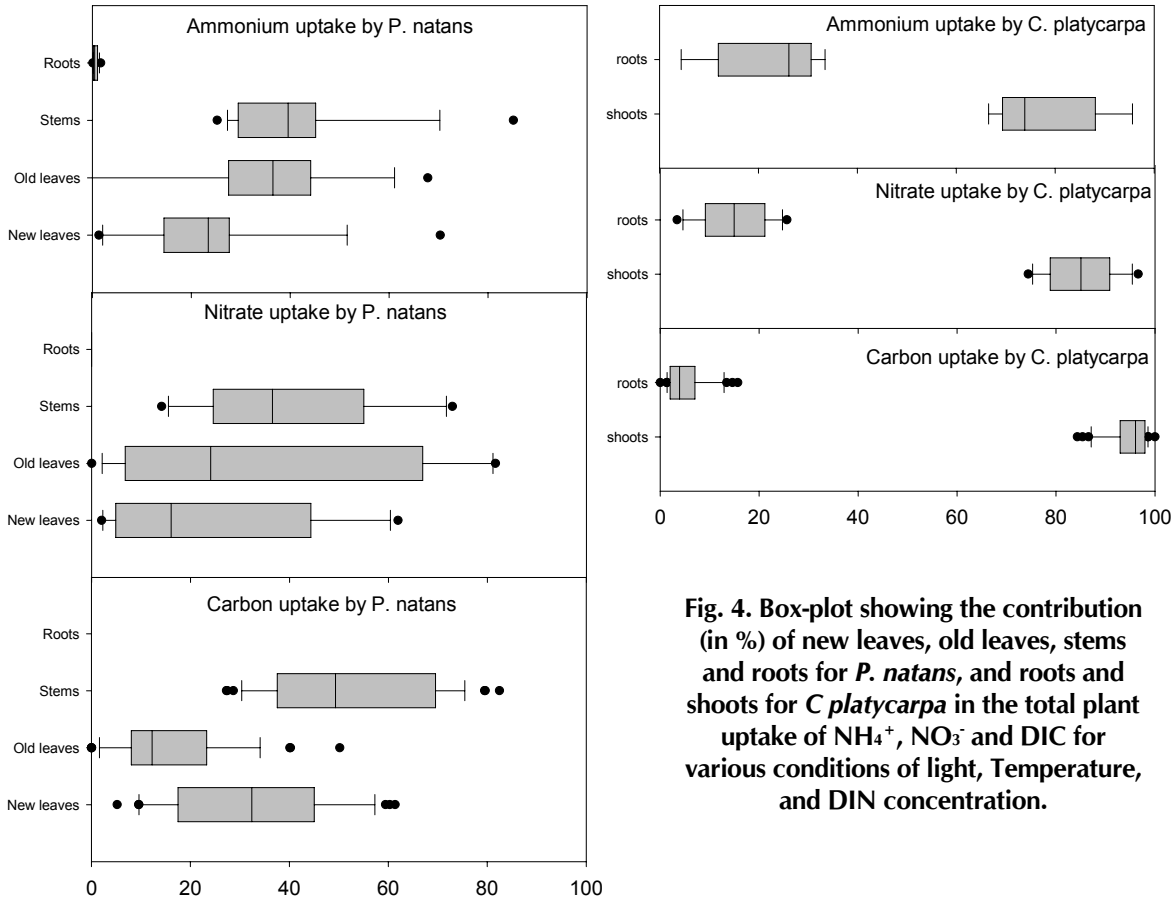


Fig. 4. Box-plot showing the contribution (in %) of new leaves, old leaves, stems and roots for *P. natans*, and roots and shoots for *C platycarpa* in the total plant uptake of NH_4^+, NO_3^- and DIC for various conditions of light, Temperature, and DIN concentration.

For *P natans*, all plant parts of the shoot are of similar importance for N uptake, and C uptake is highest for stems and new leaves (Fig. 4). Ammonium uptake by *P. natans* roots is insignificant (Fig. 4), however this result may be biased by the fact that only a small part of the root system of this macrophyte, forming dense below-ground networks of stolons, was sampled and used in the incubations. A true estimation of the below-ground vs above-ground biomass of these plant is needed for final conclusion. Similarly, for *C platycarpa*, most of the DIN and DIC fixation occurs in the shoots (Fig. 4). A study by Madsen and Cedergreen (2002) had

similar results for 4 freshwater macrophyte species (*Elodea canadensis, Callitriche cophocarpa, Ranunculus aquatilis and Potamogeton crispus*) growing in nutrient rich rivers: all species were able to satisfy their demand for mineral nutrients by leaf nutrient uptake alone.

Translocation between shoots and roots:

The translocation of N taken up by the shoot to the roots in *P. natans* could only be estimated for NH_4^+ as the biomass labeling with $^{15}N\text{-}NO_3^-$ and $^{13}C\text{-}HCO_3^-$ was not significant enough to estimate transfer rates correctly. Translocation of NH_4^+ taken up by the shoot to the roots varied between 1 and 10% of whole shoot N uptake with a median value of 3.4% (Fig. 5 A). On the average, translocation of NH_4^+ taken up by the shoot to the roots were 8 times larger that direct roots uptake (Fig.5 B).

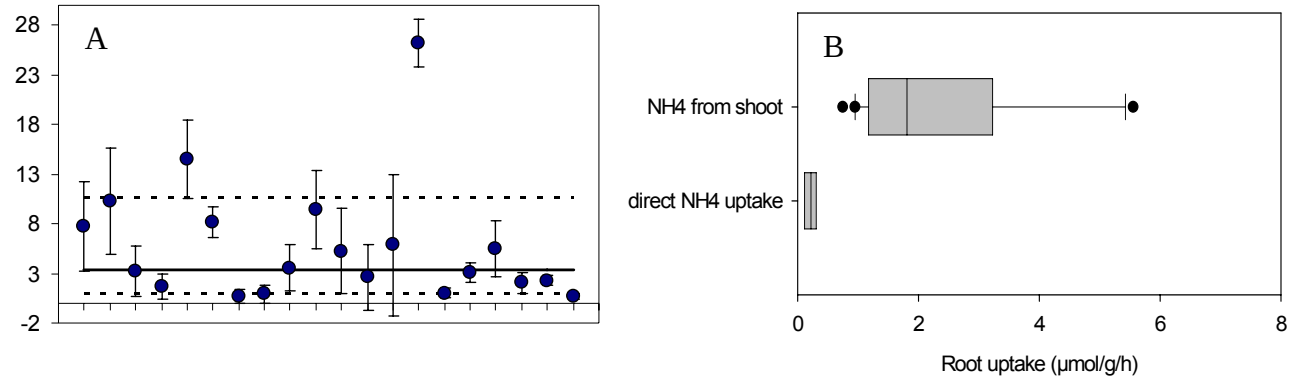


Fig. 5. A. Translocation of NH_4^+ taken up by *P. natans* shoots to roots (as % shoot uptake), under various conditions of light, temperature and DIN levels. B. Direct and translocated NH_4^+ uptake by the roots of *P. natans*.

Translocation of NH_4^+ taken up by the roots to the shoot could also not be determined because of insignificant biomass labeling.

C:N uptake ratio vs. C:N ratio of the plant biomass:

The ratio of DIC uptake vs. NH_4^+ and NO_3^- uptake under optimal conditions is compared to the C:N ratio of the macrophyte biomass. The DIC:NO_3^- uptake ratio is much higher than the biomass C:N, while the DIC:NH_4^+ uptake ratio is much lower (Fig. 6). This shows that both macrophyte species are not able to respect their biomass requirements in N with NO_3^- as the only DIN source. On the contrary, both macrophytes use excess amounts of NH_4^+ compared to their biomass requirements. This "surge uptake" of NH_4^+ by aquatic macrophytes has been demonstrated for many marine macrophytes as being linked to a a strong diffusive component (e.g., D'Elia and DeBoer, 1978, Fujita, 1985, Friedlander and Dawes, 1985 and Smit, 1998). The possibility of a surge uptake of ammonium enables its assimilation at a lower energetic cost, as described by Lobban and Harrison (1994) and could be of great advantage. A recent study (Li et al 2007) conducted on the Asian *Potamogeton maackianus* species demonstrated that NH_4^+ taken up was very rapidly converted to proteins which accumulated in plant tissue – constituting a reserve for periods of low N availability. However, in case of prolonged exposure to ammonium, excessive amounts of protein accumulation were observed by the authors, increasing the C:N ratio of plant tissues by 2. As photosynthetic C fixation was not able to sustain the C demand for protein synthesis, the plant started to use C reserves (starch) and growth was slowed down or even stopped.

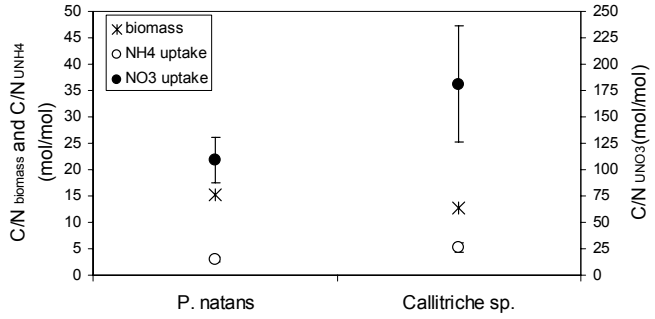
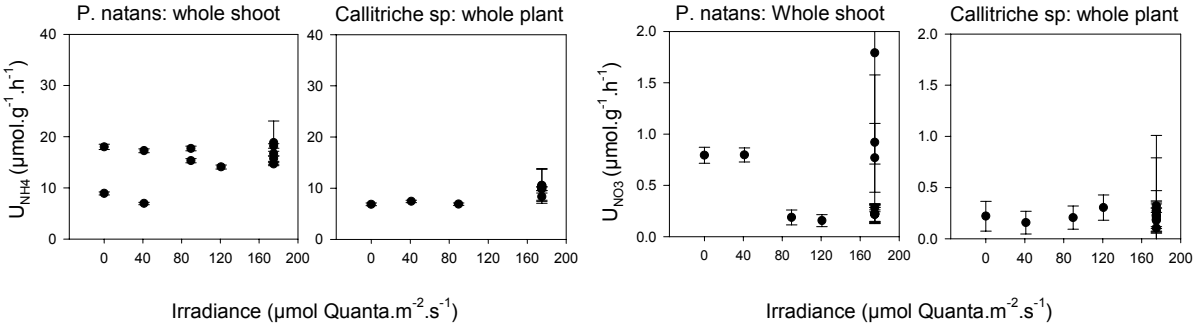


Fig. 6. DIC:NH_4^+ and DIC:NO_3^- uptake ratio (take attention to different scales) compared to the C:N ratio of macrophyte biomass

Effect of Light:

The uptake of NH_4^+, NO_3^- and DIC were measured at light intensities between 0 and 175 μmol Quanta/m²/s, at 20°C and at saturating DIN levels. Carbon uptake rates varied as expected according to a hyperbolic function although parameter estimates are poor because of a lack of values at low light intensities. NH_4^+ and NO_3^- uptake rates were variable and not significantly different one from each other whatever the plant species considered (Fig. 7). The uptake of inorganic N nutrients is thus independent from the available light showing the uncoupling of DIN uptake and photosynthesis, at least for short periods. Similar results were obtained for the NH_4^+ uptake by marine Rhodophyta species by Nishihara et al (2005).



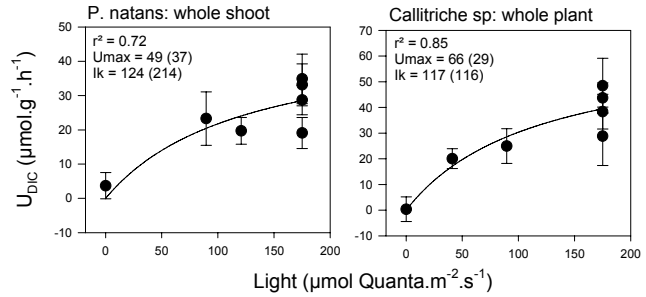


Fig. 7. Effect of light irradiance on the NH_4^+, NO_3^- and DIC uptake of *P. natans* and *C. platycarpa*. shoots . The U_{DIC} has been related to light with a hyperbolic function U = Umax * (1/(1 + Ik)). Correlation coefficient (r^2) and parameter values with errors under brackets are displayed.

Effect of Temperature:

The uptake of NH_4^+, NO_3^- and DIC were measured at temperatures between 5 and 20°C (Fig. 8) for constant light conditions (175 μmol Quanta/m²/s) and at saturating DIN levels. Temperature had only a significant effect on NH_4^+ and DIC uptake by *P. natans* and could be modeled with an exponential function ($U = a * e^{b.T°}$) with similar values for the exponential parameter (b). The NH_4^+ and DIC uptake by *C platycarpa* were not significantly affected by temperature changes between 5 and 25°C, showing its ability to grow over a wide range of environmental conditions. No significant temperature effect could be detected on the NO_3^- uptake of both species which stayed low throughout the tested temperature range, although variations could have been masked by the large error bars characterizing low rate determinations. Pilon and Santamaria previously studied the effect of temperature on photosyntheses for 3 freshwater macrophyte species (P. pectinatus, P. perfoliatus and C. obtusangla) and found similar curve shapes (exponential increase) in the range of temperatures between 5 and 25°C, with optimal temperatures between 30 and 37°C, however the

slope of the curves were lower with maximum a doubling of the photosynthetic O_2 production between 5 and 25°C, compared to our results for *P. natans*.

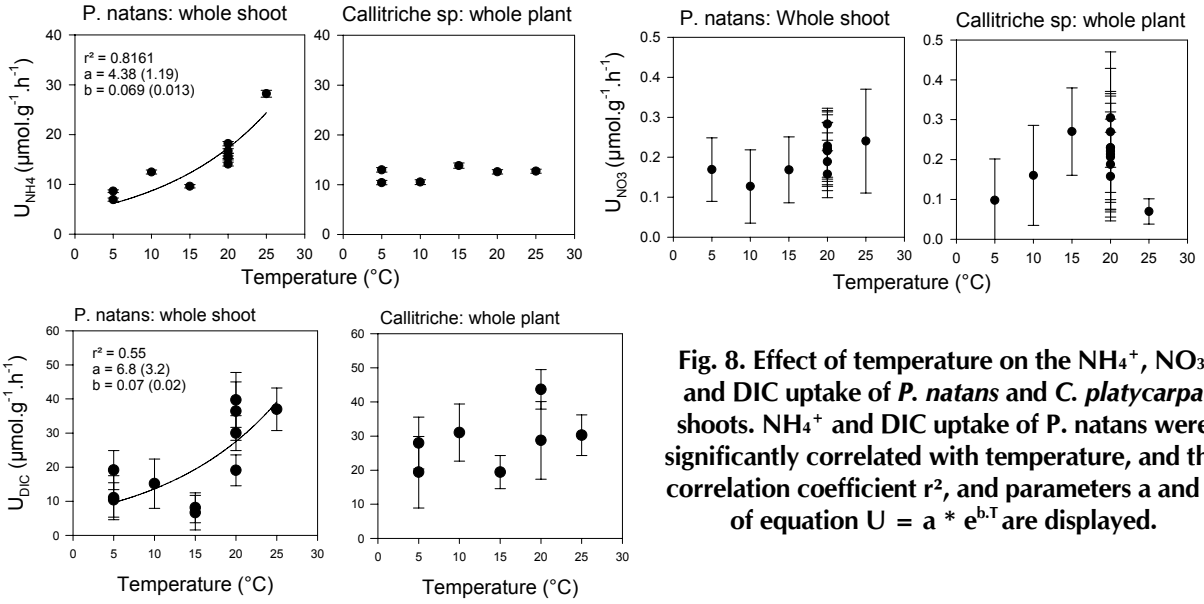


Fig. 8. Effect of temperature on the NH_4^+, NO_3^- and DIC uptake of *P. natans* and *C. platycarpa* shoots. NH_4^+ and DIC uptake of P. natans were significantly correlated with temperature, and the correlation coefficient r², and parameters a and b of equation $U = a * e^{b.T}$ are displayed.

Effect of NH_4^+ and NO_3^- concentration:

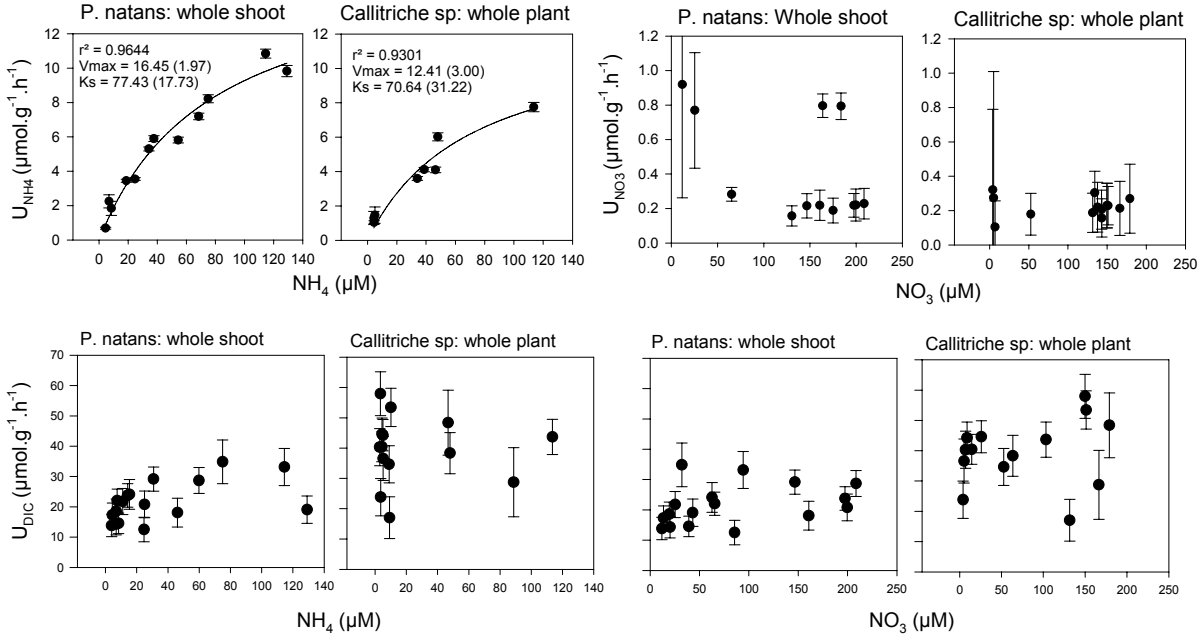


Fig. 9. Effect of NH_4^+ concentration on the NH_4^+ uptake of *P. natans* and *C platycarpa*. shoots (new leaves, stems, shoots, floating roots, and whole shoot). Half-saturation constant (Ks), maximum uptake rates (Vmax), and correlation coefficients (r²) are presented. Values under brackets are the estimated errors on parameters)

The uptake of NH_4^+ and DIC were measured at NH_4^+ concentrations between 5 and 130 μM, and the uptake of NO_3^- and DIC were measured at NO_3^- concentrations between 10 and 200 μM at 20°C and in constant light conditions (175 μmol Quanta/m²/s). Ammonium concentrations have a very strong effect on the ammonium uptake of both studied macrophyte species (Fig. 8) and could be modeled with a classical Monod equation: $U = V_{max}/(1 + K_s)$; with U: uptake rate, V_{max}: maximum uptake rate and K_s: half saturation constant. Both species had similar affinities for ammonium with Ks values around 75 μM NH_4^+ (around 1 mgN/l) and similar V_{max} values (around 14 μmol/g/h). On the contrary, nitrate uptake rates were low whatever the level of nitrate considered (Fig. 9) and they were not affected by the presence of ammonium (not shown). Similarly,

the NH_4^+ and NO_3^- concentration showed no significant effects on the DIC uptake of both macrophytes. Even at very low levels of DIN, both macrophyte species were able to continue to fix DIC. Probably these macrophytes grown under relatively high NH_4^+ concentrations and able to perform surge uptake of NH_4^+ (see fig. 6) had sufficient N reserves in their cells to continue growing.

1.1.4 Conclusions

- Both macrophyte species displayed very low NO_3^- uptake rates, and were unable to respect biomass N needs with only NO_3^- as a DIN substrate. So they are forced to use NH_4^+.
- Both macrophyte species displayed a preference for NH_4^+ and they take up excess NH_4^+ compared to their biomass needs. This surge uptake of ammonium gives them the ability to store N in their biomass and allows them to fix DIC in the absence of NH_4^+ and NO_3^- . However, on the long term, this excess ammonium uptake could result in reduced growth rates for the plants (see results in 1.3 for the long term experiments).
- Nitrogen and carbon uptake occurs mainly through the shoots of both species, and translocation of DIN from shoot to root could account for 1 to 10% of *P. natans* shoot uptake. Translocation of NH_4^+ from the shoot to the roots was 8 times higher than direct root uptake, confirming the predominant importance of dissolved N from the water column for the studied macrophytes.
- Factors controlling the DIN uptake are NH_4^+ concentration and, in the case of *P. natans* only, temperature. Factors controlling DIC uptake are light, and in the case of *P. natans* only, temperature.
- There were no effects of light on the DIN uptake and there were no effects of DIN levels on the DIC uptake.
- Except for their dependence on temperature, both macrophyte species displayed very similar DIN and DIC uptake behavior in spite of their very different morphology.

1.2 Medium term experiments (a few days)

The growth and respiration of macrophytes in various conditions of N and P nutrient levels, and light were determined at the scale of a few days (2 to 3) using respirometry techniques. This was done for the species *Ranunculs fluitans* (fig.11), the most common one present in large biomasses in the Semois River (fig. 10).

Fig. 10: The Semois River with macrophytes patch

Fig. 11: Ranunculus fluitans

1.2.1 Development of equipment

Our first prototype of respirometer was of a classical design with a volume of 1 litre and with no gas phase. We experienced problems of oversaturation in the liquid with the formation of bubbles leading to inaccurate oxygen measurements. Its was therefore decided to develop a bigger respirometer with a greater gas phase and a volume of 50 l of water and 65 litres of air, to avoid the bubbles problems.
This special L(G)SS-type respirometer (photobioreactor) allowing the incubation of whole aquatic macrophytes specimens was first designed and constructed. The **L(G)SS** respirometer contains measurement probes in the **L**iquid and in the **G**as phase, and is entirely closed, meaning the liquid and gas phase are **S**tatic (see fig. 12 and 13)

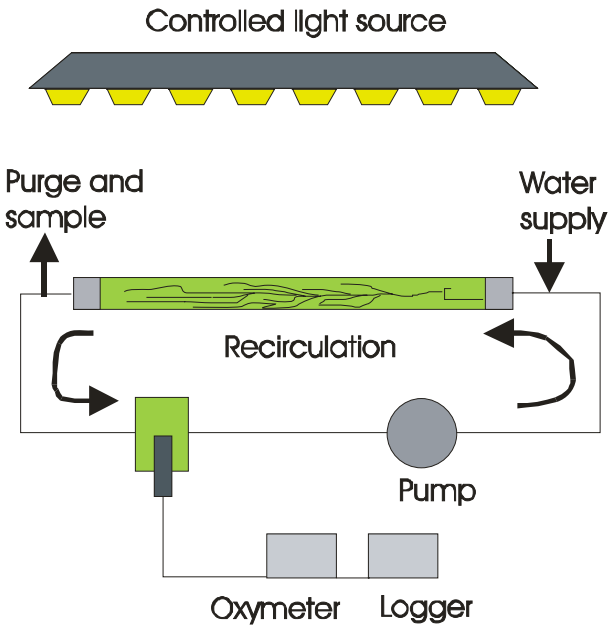


Fig. 12 : Schema of L(G)SS respirometer with a programmable light source, a pipe with the plant inside, a pump for water circulation and sensors.

Fig. 13 : The photobioreactor –respirometer

Some features of the respirometer : L = 3 m, diameter = 250 mm, volume of liquid phase close to 50 L (measured exactly at each test) , volume of gas phase ± 65 L; circulating flow rates : in the range 2-14 m^3/h, Equipped with O_2, pH, temperature and flow rate sensors on the liquid phase and gas pressure sensor on the gas phase.

This system allows to vary and to control the flow rate (and thus the water velocity in the wetted section), the light (intensity and cycles). Moreover the main state variables as pH, temperature, dissolved oxygen, gas pressure, light, flow rates are recorded continuously during the experiment.
Data are stored automatically in a dedicated data logger, and the PLC system controls and regulates the liquid flow rates and the cycles for light.
We have studied influence of light intensity, NH_4^+ and NO_3^- concentrations. So far for each experience, we setup a 2 hours photoperiod i.e. , 2 hours light followed by 2 hours dark, in order to study oxygen production-consumption associated to the measured biomass production. Taking into account the sampling of macrophytes in the river, the setup of the respirometer, and the measurements at the end of a test, such a test takes a week but we collect many cycles (photosynthesis/respiration) in the same time.

1.2.2 Results

Effect of NH4 concentration:

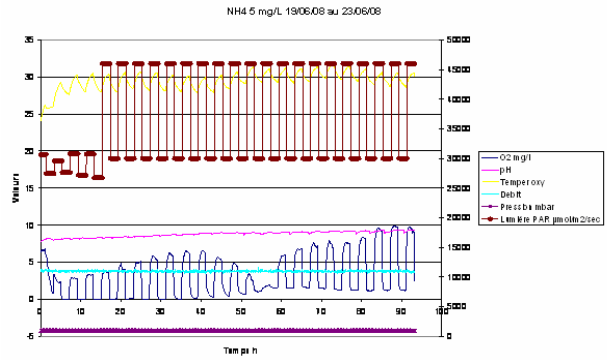


Fig. 14 : Respirogram for a N-NH_4^+ concentration of 5 mg/l.

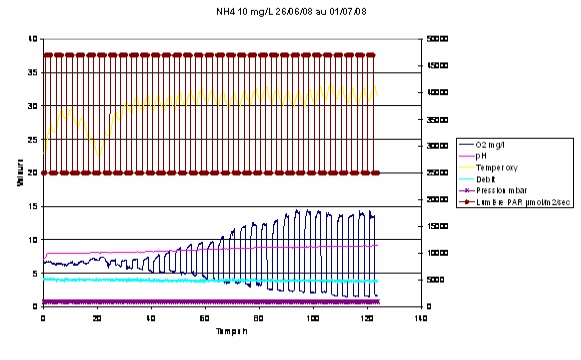


Fig. 15 : Respirogram for a N-NH_4^+ concentration of 10 mg/l.

Figures 14 and 15 show all measured variables: O_2, pH, light, flowrate, temperature and pressure for 2 levels of ammonium (5 and 10 mgN/l). Oxygen production starts slowly and reaches a maximum after 90 h. All the data of an experiment are recorded in a dataset that will be used for model calibration.

Effect of N-NO3 concentration:

A similar experiment was performed with N-NO_3 as a DIN source. The results are presented in figure 16

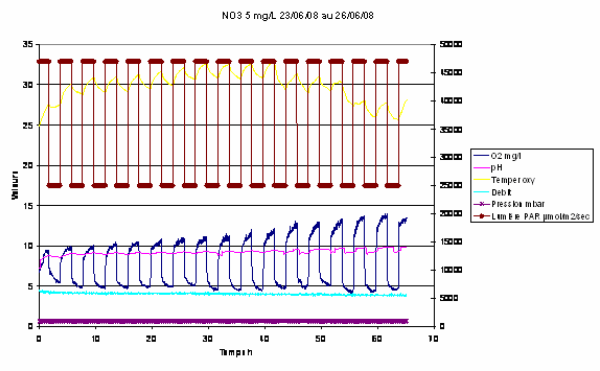


Fig. 16: Respirogram for a N-NO3 concentration of 5 mg/l.

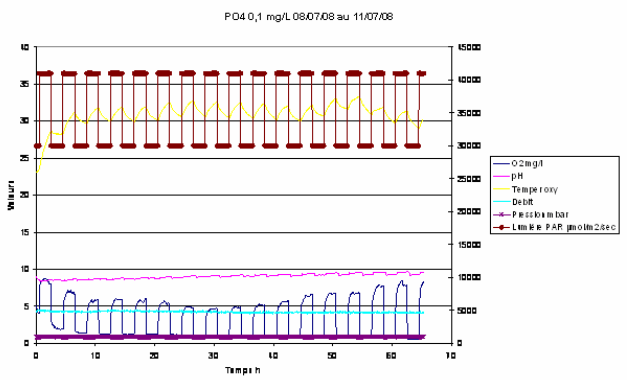


Fig. 17: Respirogram for a P-PO4 concentration of 5 mg/l.

We can compare this graph with the precedent, NH_4^+ concentrations, we can clearly see the different curves for oxygen (in blue). In the presence of nitrate, plants are able to immediately produce oxygen during light periods while with NH_4^+ as their N source, an adaptation period with almost no O_2 production is observed for a few cylcles.

Effect of P-PO4:

Finally we looked at the variability of O_2 production in an experiment with initial phosphate at 5 mg/L (close to what plants experience in the field). Oxygen production cycles remained relatively constant all over the experiment (Fig. 17) although PO_4^{3-} levels presumable decreased. There was thus no very important effect of the phosphate concentration. Maybe we could use lower concentrations but it would be far from the river actual concentrations.

Effect of light

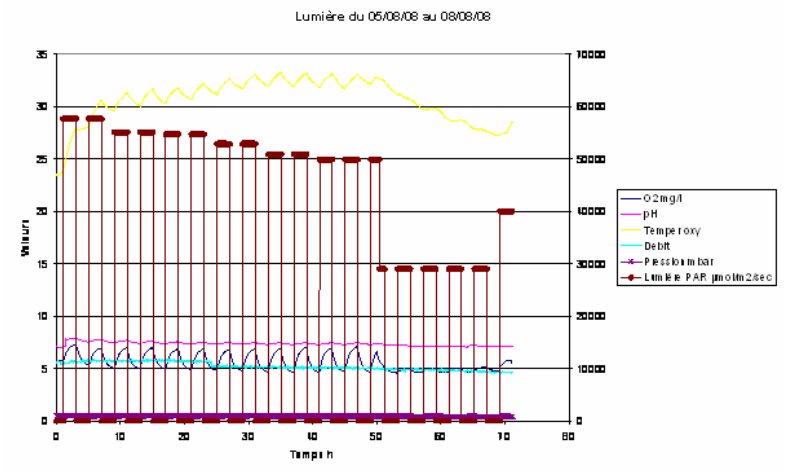


Fig. 18: Influence of light

In this test, we decreased the light energy on the tube every 8 h, in order to have a least 2 cycles (light/dark) for calculation. As we can see (Fig. 18) there is an important decrease in oxygen productivity when the light is decreased of 40 %. This confirms the role of shading in control of macrophytes in river.

Effect of temperature:

As it can be seen in the previous curves (Figs 17 and 18), the temperatures of the experiments are rather high. Of course this is partly due to the heating effect of lights but also related to summer conditions. As there is no air-conditioning in the laboratory, we postponed the tests at lower temperatures to the end of the growth season, but even at this time there is still a problem to maintain the temperature as low as 15 °C. We have thus to consider the use of the first version of respirometer or to use a chiller to maintain the water temperature around 15°C and lower.

1.2.3 Conclusions

- Ranunculus exposed to ammonium first showed an inhibition of its O_2 production lasting from a few hours (NH_4^+ of 5 mgN/l) to severall days (NH_4^+ of 10 mgN/l). This inhibition is not observed with nitrate, which seemed more appropriate as a N source. After a few hours (days) of adaptation however, the plants diplayed similar O_2 production patterns whith NH_4^+ and NO_3^-.
- There is almost no influence of phosphate concentration in the tested range, we are probably far away of the limiting concentrations.
- The light influence is very important on the growth of ranunculus, with a decrease of light of 40 % there is no oxygen production.

1.3. Long term experiments (several weeks, months)

1.3.1 Incubation experiments

For the long term experiments plant material (*P natans, R fluitans*) was incubated in 40 liters boxes with different nitrate (25, 10 and 5 mg l^{-1}) and ammonium concentrations (10, 4 and 2 mg l^{-1}). Decline in biomass was monitored during two months. From the monitoring of the biomass could be seen that both tested species reacted differently on the nutrient concentrations. When *P natans* was grown under a concentration of 25 mg NO_3^- per litre an average positive growth rate of 0.06 g fresh weight per day was observed. When lower concentrations of nitrate were presented (between 10 and 5 mg l^{-1}) growth rates were respectively 0.05 and 0.07 g day^{-1}, which is the same as under the high nitrate conditions. However, this growth rate was not constant along the measured time period. Initially growth was even higher with values up to 0.3 g fresh weight day^{-1} (see fig 19).
When however nitrogen was given under the form of ammonium a clear negative growth was seen for all used concentrations (2, 4 and 10 mg l^{-1}) and species. This is quite remarkably because the very short term experiments have clearly shown that there is a preference for ammonium when available. A possible explanation for this difference is the toxicity of ammonium above certain concentration (NH_4^+ levels in the short term experiments did not exceed 1.8 mg/L). Additionally, the negative growth may be explained by a similar mechanisms as the one described for *P. maackianus* in Li et al. (2007). These authors showed that in case of prolonged exposure to ammonium, excessive amounts of protein accumulated in plant tissues and that the photosynthetic C fixation was not able to sustain the C demand for protein synthesis. As a consequence, the plants started to use its C reserves (mainly stored as starch) and growth was slowed down or even stopped.

When ammonium is available for the plant they will preferentially take this nitrogen form with an increase of the concentration in the plant cells (as observed by Li et al, 2007). However, this can only be confirmed when the nutrient content of the remaining litter is known. Analysis of the nutrient content of the remaining biomass is in progress and will be available during the coming months.

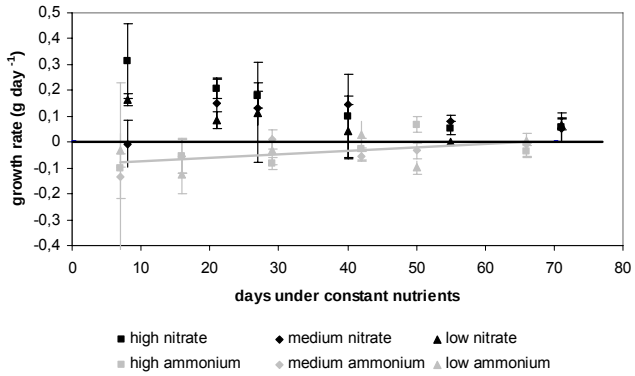


Fig. 19: Growth rate of two macrophyte species (*P natans* and *R fluitans*) under various nitrate and ammonium concentrations. Black dots represent nitrogen available as nitrate, grey dots represent nitrogen available as ammonium.

1.3.2. Effect of temperature:

The effect of temperature on decay of freshwater macrophytes (*P. natans*, *R. fluitans* and *C. platycarpa*) was investigated by incubating aquatic plants at 6, 15 and 20 °C. At 15 °C biomass declined gradually until less than 20 % is remaining after 60 days (Fig. 20). For P natans the biomass seemed to increase until day 15. This increase in fresh mass is not the result of increased growth but due to higher water content (23%) of the remaining biomass.
At a temperature of 6 °C almost no decay took place resulting in more than 70 % of the fresh mass remaining after 60 days. However, with increasing temperature biomass decay was much higher (*R. fluitans and C. platycarpa*) (Fig.21)

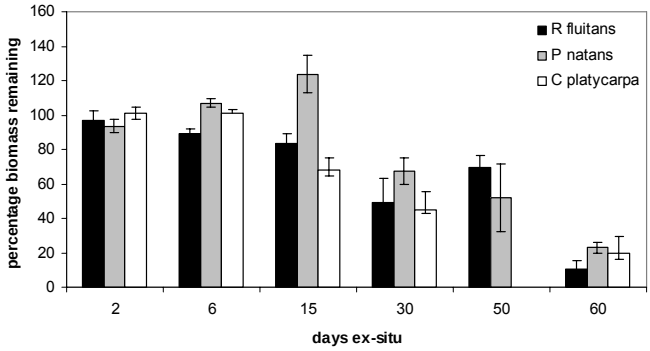


Fig. 20: Decay of *R fluitans* (black), *P natans* (grey) and *C. platycarpa* (white) at 15°C

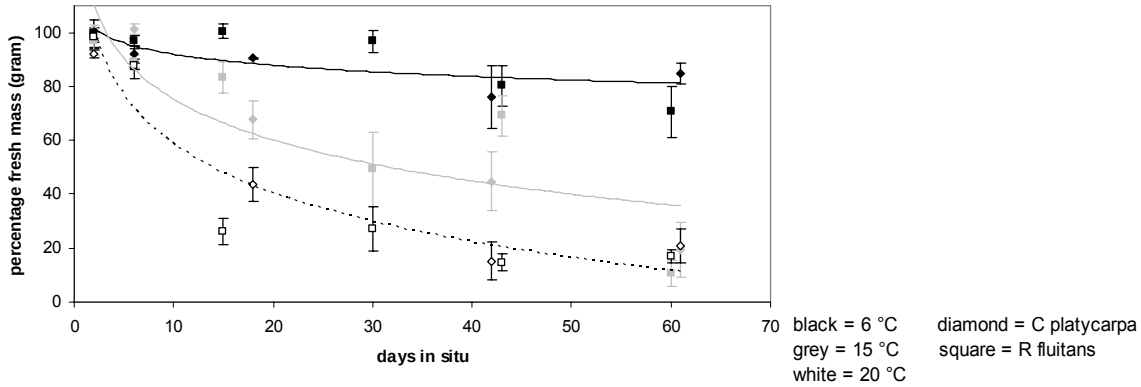


Fig 21: Decay of R. fluitans and C. platycarpa at different temperatures

1.3.3. Effect of bacteria and/or fungi:

Bacteria and fungi are important decomposers of aquatic litter (Webster & Benfield 1986, Hieber and Gessner 2002). In this experiment the importance of fungi and bacteria in the breakdown of aquatic plant species was tested ex-situ. For each experiment a series of 3 replicates with 3g of fresh vegetation litter was incubated in 3 L of 2.5 µm filtered water (to avoid macro-invertebrates) of the Aa and the Semois. To inhibit fungal growth Amphotericine B was added regularly (every 10 days) and macrophytes (*R. fluitans* and *P. natans*) were allowed to decompose at a constant temperature of 15°C. In a second set of experiments bacterial growth was inhibited with the use of a broad spectrum antibiotic. During all experiments temperature and light conditions (dark) were kept constant to avoid interference with the investigated parameters. When amphotericine was added fresh mass declined comparable with the blanco treatment (less than 20 % remaining after 60 days). When antibiotics were added more than 80 % of the biomass remained indicating that bacterial decay is much more important than fungal decay (Fig. 22). Further analysis of the nutrient tissue contents is in progress but the first results indicate that the concentration of nitrogen and phosphorus in the remaining litter increases strongly.

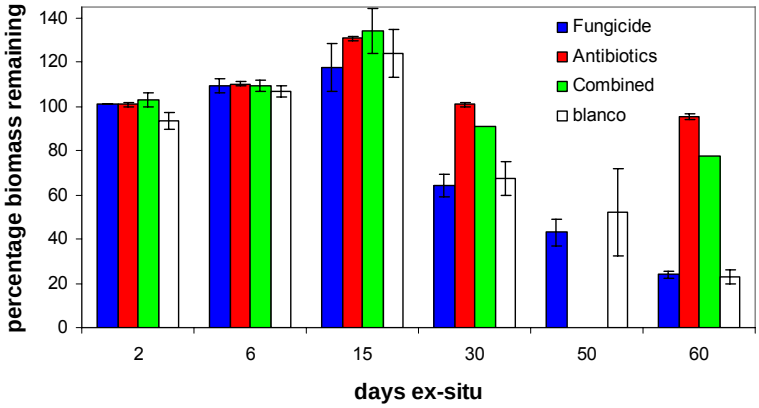


Fig. 22: Decay of the fresh mass of *P. natans* under different treatments (fungicide, antibiotics, fungicide + antibiotics) during 60 days

1.3.4. Conclusions

- Growth was on the long run negatively influenced by high ammonium concentrations.
- Macrophyte decay accelerated with increasing temperature and decay was mostly done through bacterial decomposition. Fungal decay was very small compared with bacterial decay

WP2 – EXPERIMENTS AT THE SCALE OF A MACROPHYTE PATCH

2.1 Influence of hydraulics on macrophyte bending.

The drag imposed on macrophytes in a stream current was measured as the force present at the base of the stem, while exposing plants of known dimensions to a range of velocities from 50 to 800 mm s^{-1} in a unidirectional flow flume (Fig .23) The base of the macrophyte stems were connected to the attachment point of a force transducer (cf. Bouma et al. 2005)

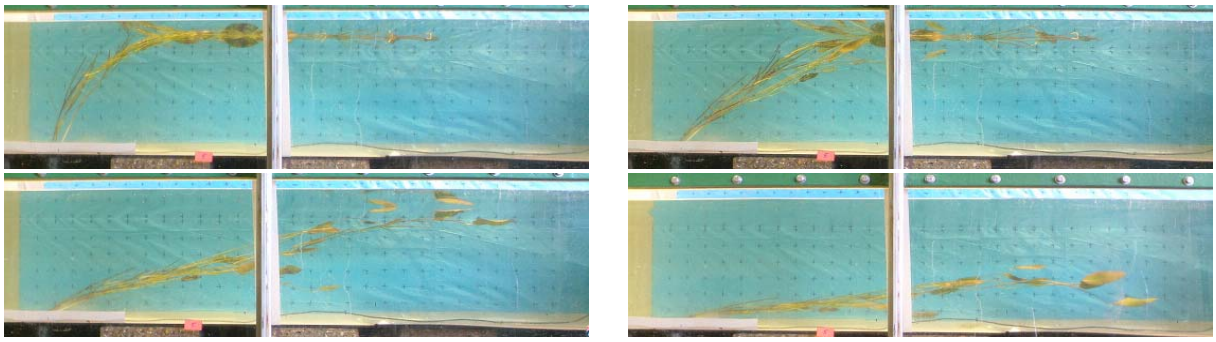


Fig. 23: Side view pictures of the bending capacity of P natans at 4 different flow velocities

The flume is equipped with an ADV to measure velocity and turbulence. A programmable robot arm automatically moves the ADV towards the desired places within and around the vegetation. The flume fits vegetation patches with a size of up to 2 m long and 0.6 m wide. The flume can generate flows up to 0.4 m/s when using a water column of 0.4 m high and 0.6 m wide; this velocity can be doubled when using a water column of 0.4 m high and 0.3 m wide. Detailed information and pictures on the racetrack flume can be found at www.nioo.nl/science/facilities/flume/flume.html or in Bouma et al (2005) and Hendriks et al. (2006).

For the hydraulic part individual plant species were mounted in the flume. When exposed to flow, these plants experience drag forces which may cause parts of the plants to break loose. This drag can be divided in profile or form drag, which is due to the low pressure that develops behind an object due to separation of flow as the fluid moves around the object, and skin or friction drag, which results from viscous shear as a fluid moves over (bent) plant tissues or wetted surface (Denny 1988, Ennos 1999). For a given surface area friction drag as present when this surface area is oriented parallel to the flow, is often negligible compared to profile drag as present if this surface area is oriented perpendicular to the flow. The ability of flexible plants to alter their shape in order to reduce drag by reducing the cross-sectional area perpendicular to the flow, is often referred to as reconfiguration. The drag imposed on the plants was measured as the force present at the base of the stem, while exposing plants of known dimensions to a range of velocities from 50 to 800 mm s-1 in a unidirectional flow flume. The base of the stem was connected to the attachment point of a force transducer (cf. Bouma et al. 2005). The force transducer consists of a stiff solid platform, carried by 2 steel cantilever beams, with 4 temperature corrected strain gauges mounted in pairs on opposite sides of both steel cantilevers (cf. Denny 1988, Carrington 1990). The construction of the force transducer compensates for moment, so that the voltage output of the force transducer was linear with forces up to 10 N. The force transducer was easily calibrated using weights (Bouma et al. 2005).

To asses the trade-off between the maximization of light interception and the reduction of its drag, drag and light intercepting surface measurements on aquatic vegetation plants with different leaf morphologies were performed. Not surprisingly, absolute drag forces imposed on the various aquatic vegetations increased with current velocity (Fig. 24a) (two way Anova, $p < 0.001$, $F = 7.5$) and were significantly different between the investigated species ($p < 0.001$, $F = 7.8$). A post-hoc Tukey test showed that *S. erectum*, with absolute drag forces up to 1.2 N, was significantly higher than for all other species ($p < 0.05$). Drag was three to four times higher than for the submerged species (*R. penicillatus* and *C. platycarpa*) and surficial or floating leaved species (*P. natans* and *S. pectinatus*; Fig. 24a). Because the

used species had different morphological features drag was also expressed per unit of total surface area. The drag experienced by the relatively flexible floating and submerged species was comparable when expressed per unit of total frontal area (Scheffe post hoc test, $p > 0.95$) (Fig. 24b). The stiffer emergent species *S. erectum* had a significant ($p < 0.05$) higher increase in relative drag with velocity than all other species (up to 28 N m^{-2} vs. 7 to 10 N m^{-2} for the other species).

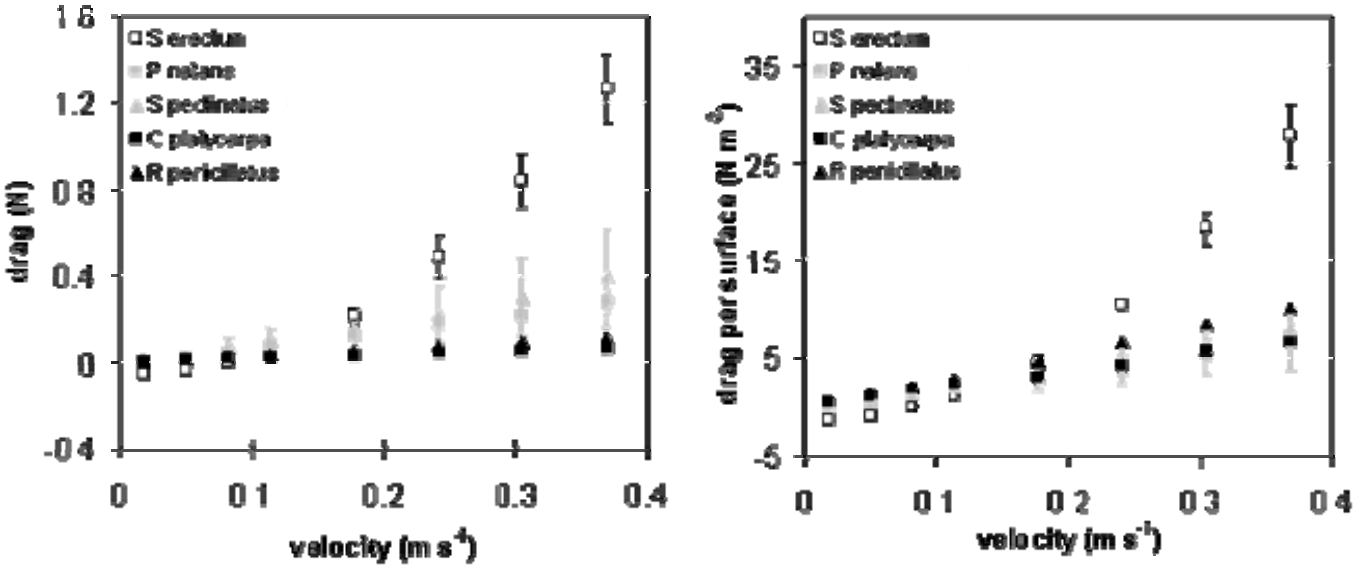


Fig 24 Absolute (a) and relative drag (b) of various macrophyte groups (white = emergent, grey = surficial or floating leaved, black = submerged) in function of increasing velocity. Standard deviations (n = 2) are shown, but not all visible, within the graph.

To asses to which extend leaves contribute to the absolute drag experienced by aquatic species with contrasting morphological strategies, drag was determined before and after leaf removal. Removal of the leaves showed that for floating leaved and submerged species, the ratio drag with leaves (N) versus drag without leaves (N) was much higher then 1 (Fig. 25a) indicating that the leaves of submerged and floating leaved species contributed 60% (± 6%) of the absolute drag during all measured velocities. The emergent species *S. erectum* had the same total drag (N) with or without leaves, indicating no trade-off between leaf development and drag. Calculating the ratio of the drag per surface area in the presence of leaves (N m^{-2}) versus the drag per surface area without leaves (N m^{-2}) gave in most cases values less than 1 (Fig. 25b).

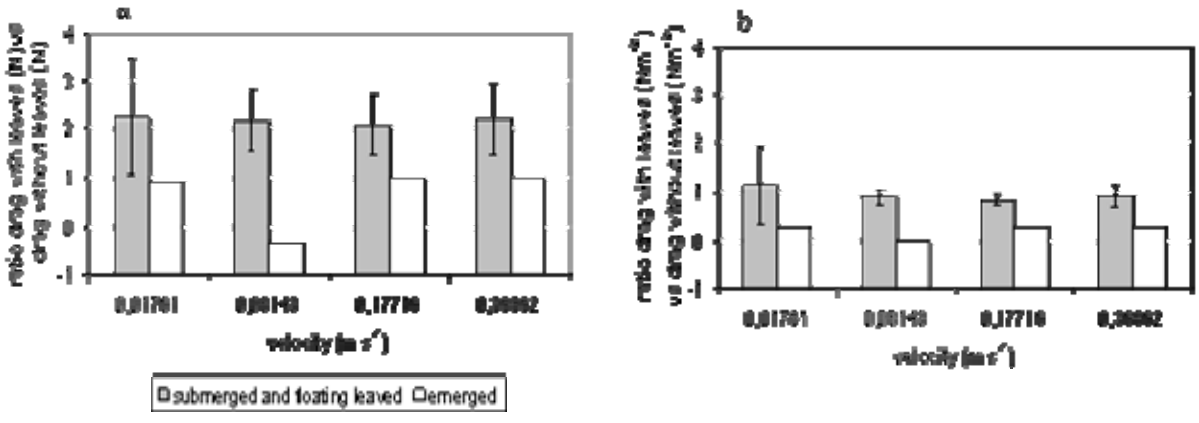


Fig. 25 a en b The ratio absolute drag with leaves versus absolute drag without leaves (a) and the ratio relative drag with leaves versus relative drag without leaves (b). Error bars show the standard deviation between the different species.

Understanding the effect of current velocity on drag and photosynthetic surface also requires insight in to which extend vegetation reconfigures by bending. Bending and thus flexibility increased in the following

order: *S. erectum* (emergent) < *P. natans* (surficial or floating leaved) = *R. penicillatus* (submerged) < S. pectinatus (surficial or floating leaved) = *C. platycarpa* (submerged) (Fig. 28a) (Anova with x as sorting variable $p < 0.001$, $F = 59$). No significant differences were detected (Scheffe post hoc test) between *P. natans* and *R. penicillatus* on the one hand and *C. platycarpa* and *S. pectinatus* on the other hand ($p > 0.95$). Our results indicate that emergent species fall in a different group than the floating and submerged species with respect to flexibility. The bending effect of the different species was significantly influenced by current velocity ($p < 0.001$, $F = 13$), with increasing velocity stimulating bending angles up to a maximal level (Fig. 25a). For *C. platycarpa* and *S. pectinatus* a constant level of 10 degrees was reached at 0.18 m s^{-1}. With removal of the leaves the angles with the horizontal plane increased slightly (two way anova, $p = 0.05$, $F = 4$) resulting in stems remaining longer in an upright position with increasing velocity. This lowered capacity to bend with velocity was observed for all submerged species.

Increased bending allows drag to remain constant with increasing flow velocities. However, as soon as we approached maximal bending, drag started to increase quickly for both submerged and floating leaved species (Fig. 26b) at higher current velocities. At low horizontal angles drag was slightly higher for the surficial and floating leaved species then for the submerged species. For the emergent species (*S. erectum*) drag increased dramatically due to the absence of a flexible stem. Relative drag in all submerged species was between 7 and 10 N m^{-2} at lowest horizontal angles. *S. erectum* on the other hand experienced drag up to 25 N m^{-2} (Fig 24b).

In the floating leaved species P. natans increasing current velocity caused a logarithmical decline ($R^2 = 0.93$, $p < 0.001$, $F = 77$) of the available leaf area from approximately 200 towards 100 cm^2 (Fig. 26a), whereas S. pectinatus, only experienced a 10% reduction of its photosynthetic surface at the highest current velocity (Fig. 26a). For the submerged species *C. platycarpa* the available photosynthetic surface area declined logarithmically with velocity towards 70 % ($R^2 = 0.56$, $p < 0.05$, $F = 8$), whereas for the other submerged species *R. penicillatus* a linear decline was observed towards 85 % ($R^2 = 0.53$, $p < 0.05$, $F = 7$). Photosynthetic surface area did not decline for the non flexible emergent species *S. erectum*. When the photosynthetic surface area was expressed as a function of the bending angle of the plant, a linear relation was seen for P. natans, *R. penicillatus* and *C. platycarpa* (Fig. 26b). For *S. pectinatus* this relation remained constant until the angle with the horizontal was smaller than 13° from which point a decline in area was seen.

When the ratio photosynthetic surface area over drag was plotted against current velocity, the three different growth strategies were easily distinguished (Fig. 27). For all submerged and floating leaved species, the ratios were significantly related with velocity (two way Anova, $p < 0.005$, $F = 15$) and species (two way Anova, $p = 0.002$, $F = 5.6$). *S. erectum* was not added in the anova due to negative values at the lowest velocity. These initial negative values were the result of the inflexible curved stem causing a force in the opposite direction of the flow. A post hoc Scheffe test indicated that. *P. natans* and *S. pectinatus* significantly ($p = 0.1$) differed from *C. platycarpa/R. penicillatus* and vice versa

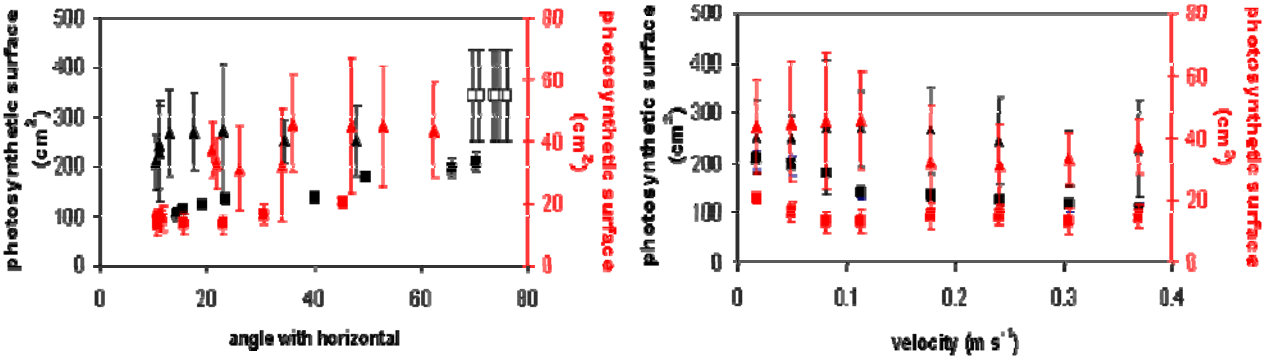


Fig 26 a and b The amount of available photosynthetic light intercepting surface in function of stream velocity for all submerged and floating leaved species (a) and bending angles with the horizontal (b) for all tested species. Standard errors (n = 2 or 3) are shown.

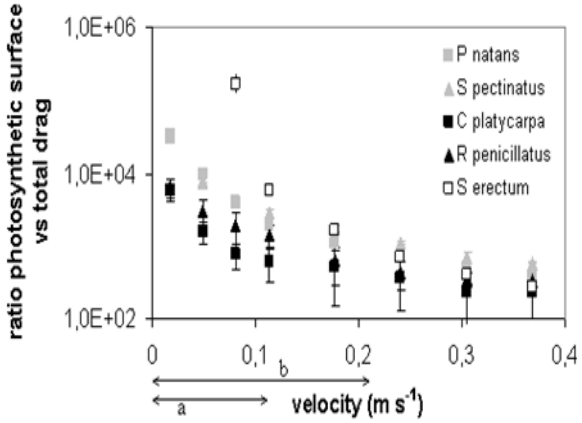


Fig 27 The trade-off between photosynthetic surface area and absolute drag (cm^2 N^{-1}) in function of the stream velocity (m s^{-1}) for plants with leaves. Error bars are shown. The arrows below the x-axis indicate the velocity range of emergent (a) and broad leaved macrophytes (b) according to Newson et al. (1998), Kemp et al.(1999) and Clifford et al. (2006).

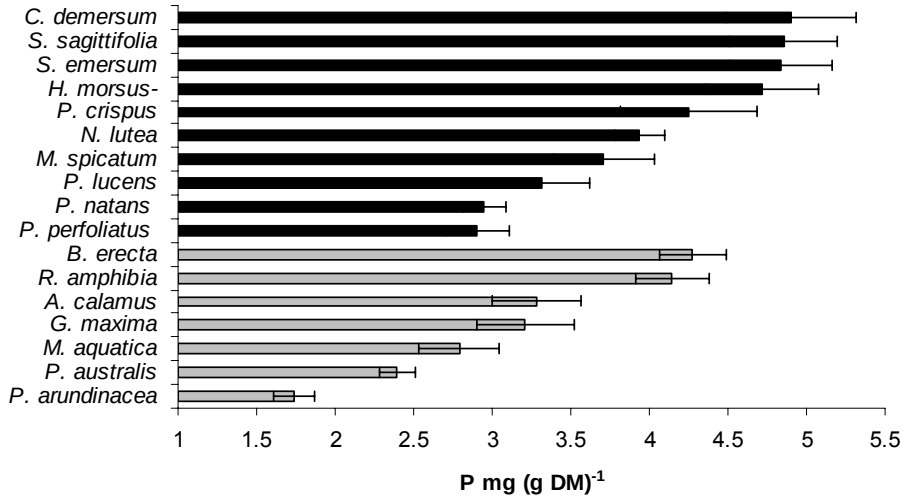
2.2 Conclusions

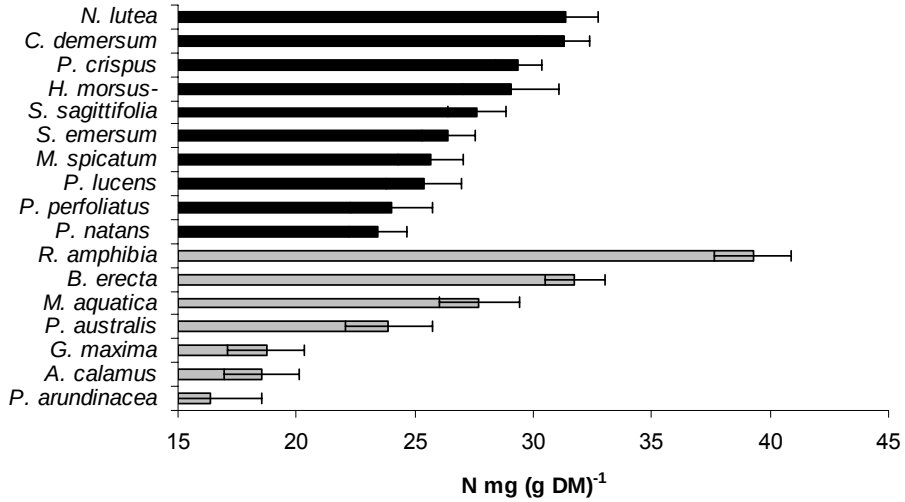
- Drag and bending capacity of the stems was mainly generated by the leaves. Between various morphological growth strategies a clear difference in photosynthetic surface vs total drag was shown. The first group, with emergent species, was characterized by a high ratio at velocities beneath 0.1 ms-1.
- At higher velocities the more submerged species reduced their total drag enabling them to survive in these harsh conditions. Within these submerged species a clear distinction was seen between those concentrating their leaf area on or just beneath the water surface (*P. natans* and *S. pectinata*) and those with more or less evenly distributed biomass (*C. platycarpa* and *R. penicillatus*).

WP 3 FIELD SCALE EXPERIMENTS

3.1 Nutrient content of various macrophyte species

Aquatic plants have both below- and aboveground adaptations to enable establishment and living in dynamic and harsh environments (Usherwood, Ennos & Ball, 1997). For example under conditions of high pelagic nutrient availability, nutrient demand can be satisfied by foliar uptake (Maitai & Newton, 1982). To have an idea of the variability in nutrient storage of freshwater plants 17 plant species, of different morphological growth strategies, collected along a downstream gradient on a lowland river were analysed for their nitrogen, phosphorus, carbon, silica and potassium content. An overview of the results is shown in Fig 28.





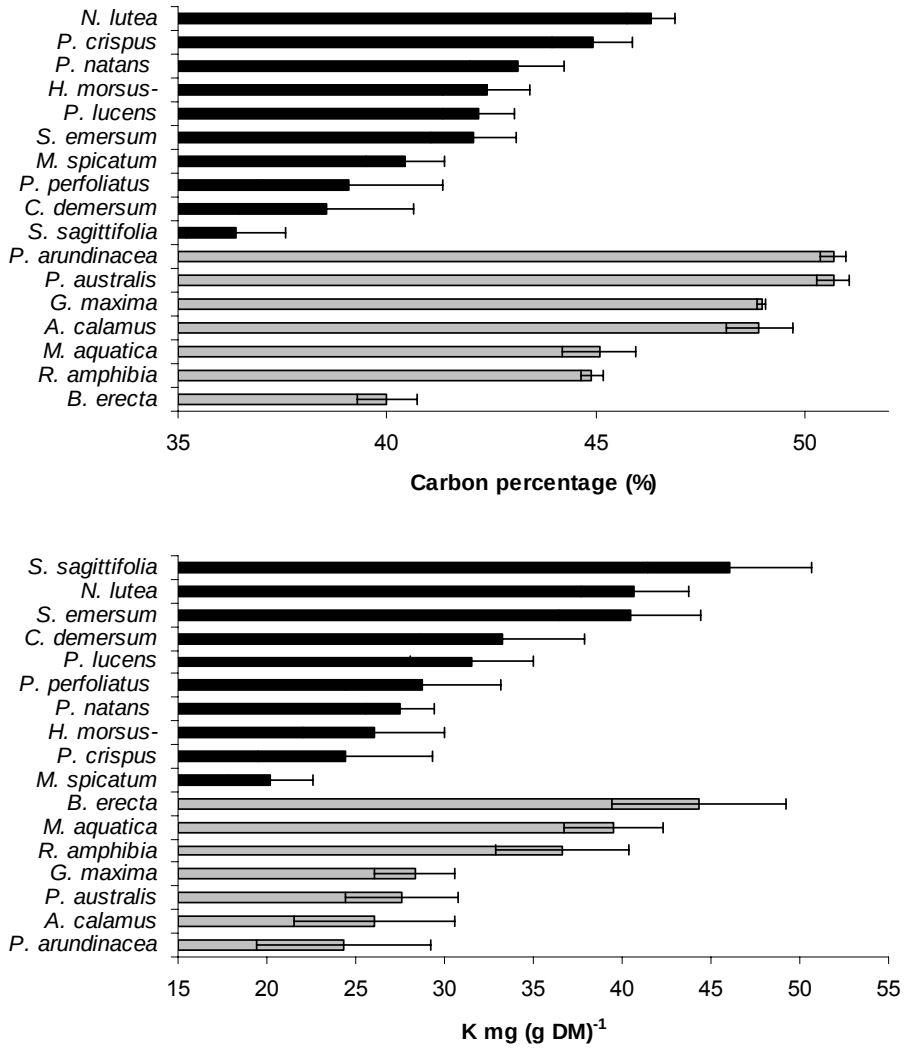


Fig. 28. An overview of the N, P, K and C content of 17 different aquatic macrophyte species. Black bars represent pure aquatic species and grey bars represent near-riverbank species.

If we look at the biogenic silica (BSi) content (Fig. 29), a parameter often neglected in water plants, very high concentrations are seen in some macrophyte species. Because not all collected plant species were submerged a separation was made between submerged macrophytes and emerged helophytes. When both plant groups were compared significant differences ($p < 0.001$; Wilcoxon Rank Sum test) in silica content were observed, respectively 12.7 (SD = 9.3, n =135) and 8.4 mg per gram dry mass (SD = 7.7, n = 79) for macrophytes and helophytes. When morphological and architectural differences within the group of submerged macrophytes were taken into account as a second objective, two significant ($p = 0.0001$) different groups were identified. In the first group those species (*M. spicatum, P. perfoliatus, C. demersum, P. crispus, P. lucens, S. emersum, S. sagittifolia*) were grouped which have a horizontal growth strategy. This group had an average silica content of 15.9 mg g per dry mass (SD= 9.1, n= 75). In the second group those species (*P. natans, N. lutea, H. morsus-ranae*) were grouped which concentrate their leaves floating on or just beneath the water surface (7.9 mg BSi per gram dry mass, SD = 7.0, n = 60). From the same statistical test it was also seen that this second group had comparable biogenic silica values as the emerged species.

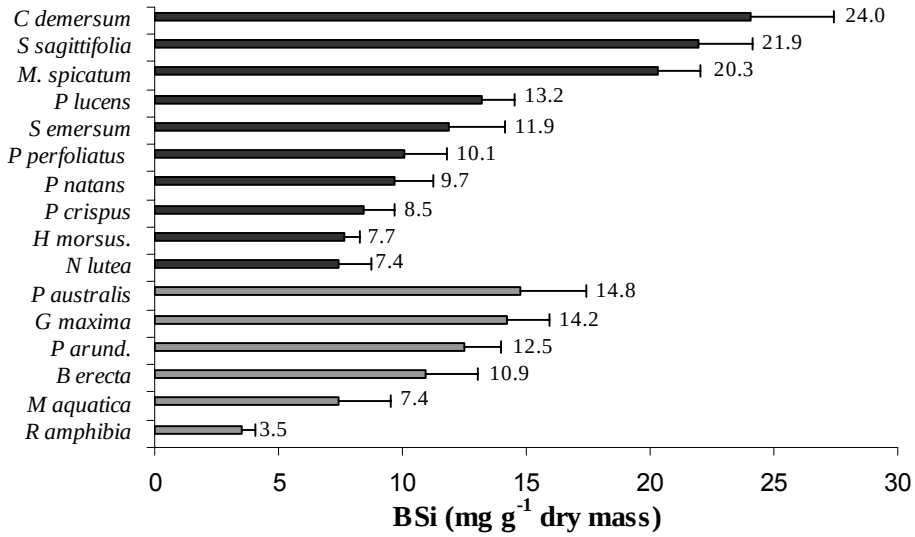


Fig. 29 Mean BSi concentrations (mg g^{-1} dry mass + SE) of 16 macrophyte species from a two year sampling period (2006-2007).

Because river vegetation experiences constant hydrodynamic forces, up to 25 times higher than terrestrial vegetation (Denny & Gaylord 2002), increasing the structural rigidity also potentially benefits macrophytes. This structural rigidity is often accomplished by energetically expensive compounds like lignin (6957 kcal kg^{-1} (Jung *et al*. 1999)) and cellulose (4000 kcal kg^{-1} (Jung *et al*. 1999)). BSi could be a cheap alternative to these molecules for river vegetation, as it has a similar effect on the cell wall as these complex compounds but with a lower energetic cost (Raven 1983). Therefore in this second part the existence of an antagonistic relation between cellulose/ lignin and BSI was investigated.
From the results it was seen that BSi and cellulose content were significantly related in both aquatic and wetland species: the relation was opposite for both groups of species. For aquatic species, lower cellulose content was associated with increasing BSi accumulation ($F = 4.45$, $p < 0.0001$, $R^2 = 0.27$). In wetland species, an opposite relation was observed, as cellulose percentages increase with increasing BSi content ($F = 4.65$, $p < 0.0001$, $R^2 = 0.38$) (Fig. 30)

Similar results were observed for the BSi-lignin data (figure 31). There is a significant ($F = 2.73$, $p < 0.01$, $R^2 = 0.15$) opposite effect as compared to cellulose: increasing BSi content was associated with increasing lignin content. Similarly, the BSi-lignin relation for wetland species is opposing what was found for the cellulose data. The logarithmic function indicates slightly lower lignin concentrations with increasing BSi concentrations ($F = 2.50$, $p < 0.05$, $R^2 = 0.27$).

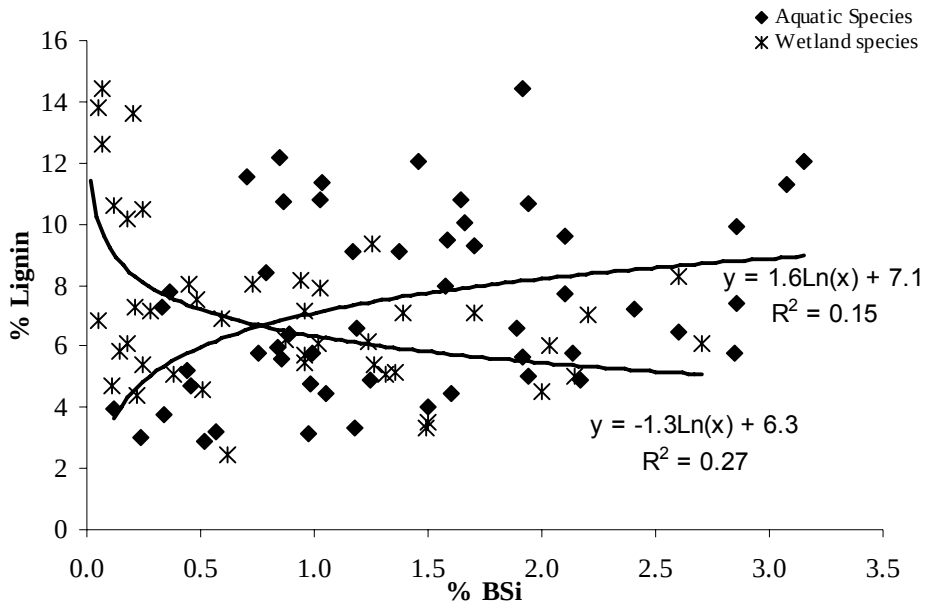


Fig. 30: Antagonistic relations between the BSi concentration (%) and the cellulose concentration (%) for aquatic (n = 56) and wetland species (n = 42). Each data point represents the concentrations of an n = 5 mixture of replica's found on the same location along a downstream gradient. Both correlations are significant.

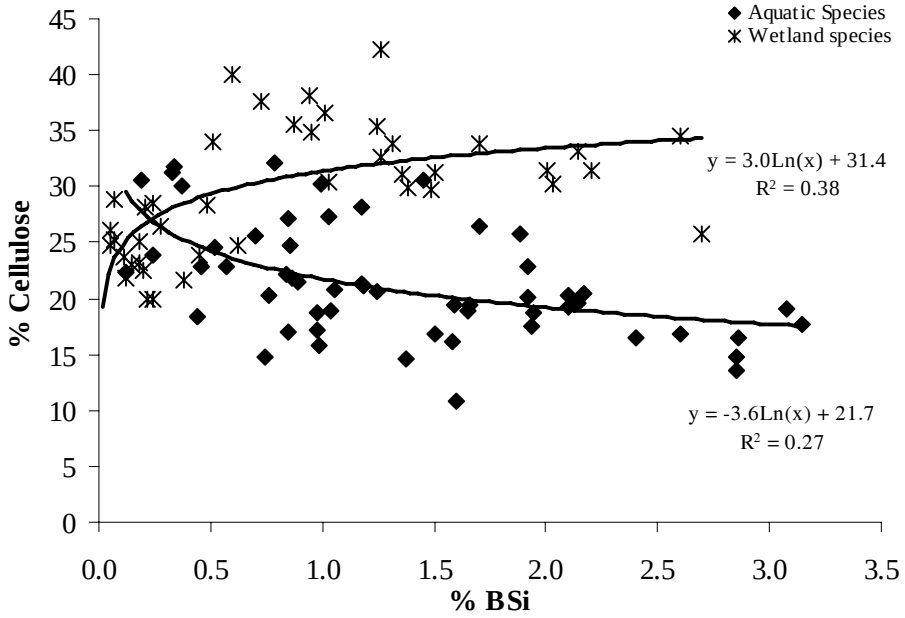


Fig. 31: Antagonistic relations between the BSi concentration (%) and the lignin concentration (%) for aquatic (n = 52) and wetland species (n = 44). Each data point represents the concentrations of an n = 5 mixture of replica's found on the same location along a downstream gradient. Both correlations are significant.

Conclusions

- Macrophyte plants had a high variability in BSi content. BSi was especially high in *C. demersum*, *S. sagittifolia* and *M. spicatum*.
- There seems to exist an antagonistic relation between BSi concentration and the cellulose concentration of both aquatic and wetland species.

3.2 Validation of techniques to determine field macrophyte biomass

Three different techniques were tested out the determine macrophyte biomasses in whole river stretch reaches: a traditional method based on measurements of surface water cover along transects, a DGPS method and aerial photography image analysis. For this, 5 sites of measurements were selected along the Semois River (Fig. 32), and the 3 techniques were compared at site "Izel".

Fig. 32 : Sites to survey.

3.2.1. Traditional method : transect

The principle (see for example the PhD thesis of Olivier Dufayt is based on the interception measurements on a group of transects with a 10 m inter-distance (fig. 33). We have chosen one of our 5 selected measurements sites to validate our measurements (Izel on the Semois river – Fig. 34).
When the coverage percentage is determined, we collect a 1 m^2 sample of macrophytes, for wet and dry biomass measurements for calibration. The biomass is deducted for the whole spot from the covered area. In the same time river water is also sampled for the usual analysis (N and P compounds, pH, O2, SS, COD…)

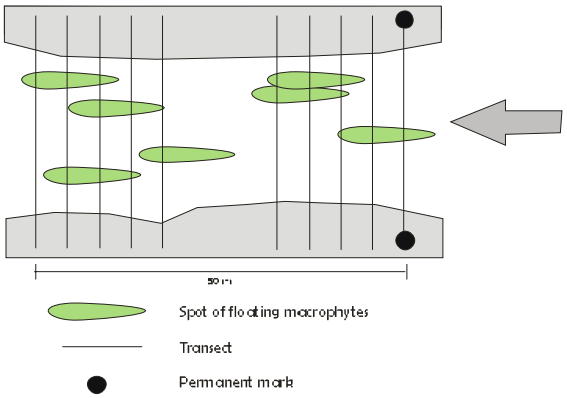


Fig. 33 : Principle of transect measurements

Fig. 34: Picture of manual transect in the Semois river.

The problem of this method is that it is men/time consuming; it needs 3 persons with decameters to realize transects. And the progression in the river is really difficult because of the dense plant mats.

Example of a measure by the transect method in Izel:

Table 1 : Example of results from the transect method at Izel station

Transects on 25/06/08 (Izel)			
N° Transect	L with ranunculus (m)	Total length of the river (m)	% coverage
1	22.8	28	81.43
2	29.4	32.1	91.59
3	31	35.7	86.83
4	33.6	35.1	95.73
5	33	36.1	91.41
6	31.9	35.1	90.88
7	28.9	34.6	83.53
8	28.9	33.1	87.31
9	28.5	32.6	87.42
total coverage (average)			88.46

3.2.2. DGPS Method

In order to solve the time consuming problem of the transect method we decided to use a DGPS system (fig. 35 and 36) with a +- 1 cm precision to measure the surface of patches in situ. The system is composed of 2 GPS, one located on a defined point is used to calculate errors and the second is moved around the spot to measure directly its area (fig. 37, 38). The two GPS communicate by radio modem (RTK configuration).

An example of results for the Izel station is given in figures 39. In this case the coverage percentage is 86 %.

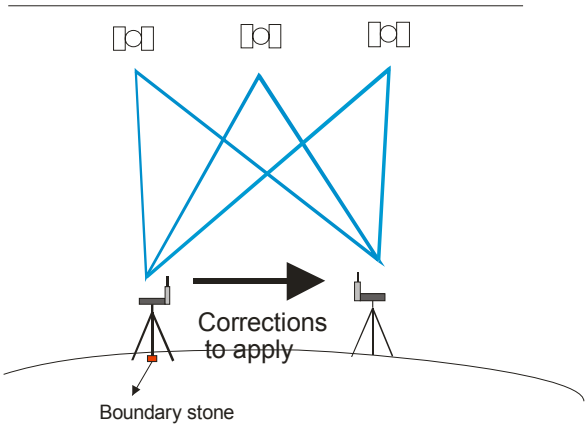


Fig. 35 : Principle of DGPS measurements

Fig. 36 : DGPS system 1200 Leica

Theoretically it is possible to measure the total area of a patch with the DGPS with centimetric precision however when the coverage is high (as in the example) we recommend the faster and easier method of transect with the DGPS.

Fig. 37 : DGPS systems on the field: setup of reference station

Fig. 38 : DGPS systems on the field: measurements in the Semois river with the rover

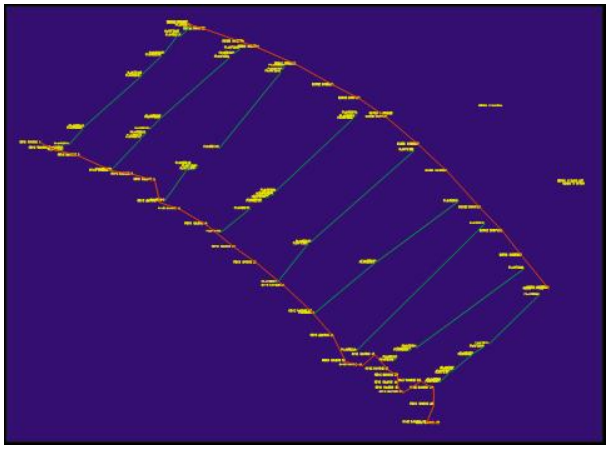


Fig. 39 : Example of results obtained with DGPS (Izel 2008)

The advantage of the DGPS technique is theoretically a better accuracy and surely rapidity.

3.2.3. Aerial photography

Finally, we developed a third tool in order to go faster in the survey of our 5 sites. We decided to work by aerial photography and the determination of coverage percentage is obtained by image analysis. We must thank Air Loisir (St-Hubert) for their participation to this study, and for the quality of their pictures. The pictures were taken by a EOS 400 camera, placed on a plane flying at an altitude of 300 feets

An example of photography is showed hereafter (Fig 40 and 41); it's an assembly of 8 numerical pictures. In order to get the scale for the pixels we previously located on the ground a scale indicator (a colored square of 1m*1 m – Fig. 40). The Image Analysis software tools give us finally the coverage percentage as we can see in the second illustration (Fig. 42). In fact it's easier to take into account the water surface without plants. With our image analysis software, we can calculate the coverage percentage, in this case about 54 % for the whole site. Making a zoom on the patch to get the same spot as in Fig. 39 we get finally a coverage of 87%.

Fig. 40 : Ground scale 1m X 1m

Fig. 41 : Our test site (Izel) from the sky. Picture taken on june 2008. The white spot is the calibration system (Fig. 40)

Fig. 42 : Result of Image analysis on figure 41

If we compare the methods (taking for the transect method the date closer to the aerial pictures) we got :

Aerial picture: 87 %
DGPS : 86 %
Transects : 89 %

We can consider that the methods are validated and yield similar results.
We will continue measurements on this site until the end of the season and the method will be ready for the 5 sites in the second phase.

3.2.4. Conclusions

- The 3 techniques of plants growth measurements, transects, DGPS and aerial photography have been tested and validated.
- Results obtains on the test site selected percentage of coverage are very similar : Aerial picture: 87 %, DGPS : 86 % and finally transects : 89 %

3.3 Validation of techniques to perform in field dragging measurements

Finally, we developed a method to estimate in situ dragging. This is done by two nets, one in upstream to avoid contamination by the river and the other one in downstream (Fig. 43 and 44). And we thus recover the product of dragging at the downstream net. A simultaneous velocity measurement is realised by a doppler flowmeter in the middle of the patch.

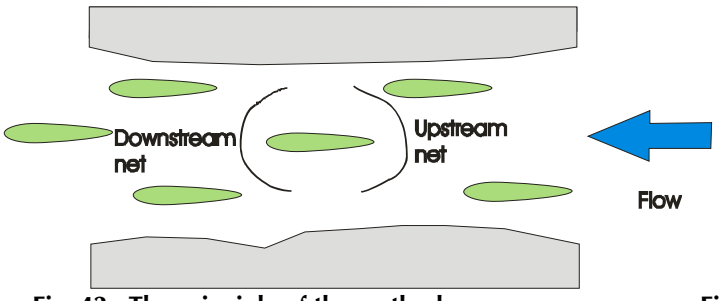


Fig. 43 : The principle of the method

Fig. 44 : Picture of the system in the River.

The dragging measurement method has been tested and is ready for the next campaigns

General conclusions and recommendations

In this first phase of the MANUDYN II project, attention was focused on:

1- The interaction between the macrophyte shoot and the water column at different time scales: from a few hours to several months.

Not all results are yet available to draw final conclusions but the following findings can already be summarized:

- The tested macrophyte species displayed large differences in dissolved inorganic nitrogen (DIN) upake, growth and respiration in the presence of NH_4^+ and NO_3^-. Very short term experience demonstrated that all macrophytes had a clear preference for ammonium as nitrogen source for N-NH_4^+ concentrations below 1.8 mg/L.. In comparison with their biomass need they even take up excess ammonium and it could be that they store NH_4^+ when available in the field in order to use it when NH_4^+ is not available. The ability of macrophytes to fix DIC in the absence of NH_4^+ and NO_3^- confirms this hypothesis. On the other side very low NO_3^- uptake rates were demonstrated on the short run making it unable to grow when only nitrate is available. So plants are forced to take up ammonium as a nitrogen source. Similarly, respirograms obtained from short term experiments with a photo-bioreactor with NH_4^+ and NO_3^- nutrients showed clear differences although growth and respiration have still to be calculated. Finally, when macrophytes experience long periods of high ammonium concentrations (2 to 10 mgN/L) their growth was negative resulting in the die back of vegetation. This seems somewhat contradictory but an explanation should be sought in the toxic capacity of ammonium above certain thresholds. Therefore to maintain healthy vegetations in a river ammonium levels in the water column have to be controlled.
- All macrophyte species tested out used the N from the flowing water through shoot uptake rather than the one available in the sediments through root uptake.
- Among all parameters tested out, NH_4^+ concentration and temperature were the major factors controlling the uptake of NH_4^+, while light and temperature controlled the C uptake.
- Short term experiment also showed that macrophyte species with very different morphological characteristics displayed very similar shoot DIN and DIC uptake characteristics.
- Long term decomposition experiments of macrophyte biomass of *P. natans* and *R. fluitans* with bacteria or fungi, showed that after 60 days, 80% of the biomass had been decomposed.
- Analysis of elemental composition of various macrophyte species showed interesting correlations between the Si content and the morphological characteristics of plants. Helophytes had lower Si content than submerged macrophytes, and submerged plants with a horizontal growth strategy had higer Si content than plants with floating leaves.

2- The effect of hydraulic forcing on macrophyte bending.

For individual macrophytes hydraulic resistances was highest for emerged species (three to four times higher than for submerged species). For water managers this means that emergent species have to be prevented when water levels have to be low to prevent flooding.

3- The comparison of different methods to determine the macrophyte biomass at the scale of a whole river stretch.

The 3 methods tested out (aerial picture, DGPS and transects) displayed very similar results and showed that in a portion of the Semois river, R. fluitans covered up to 89 % of the river in summer conditions.

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PERSPECTIVES FOR THE SECOND PHASE.

WP1 –EXPERIMENTS AT THE SCALE OF A SINGLE SPECIMEN OF MACROPHYTE.

Task 1.3 Material exchange between the sediment and the water ***(year 3)***

The uptake of nutrients by the roots of macrophytes is determined by the flux of nutrients to the rooting zone in the sediment. This flux is determined by the input of dissolved nutrients into the sediment and the release of nutrients from organic matter present in the sediment. The transport of dissolved nutrients from the overlying water into the sediment will be studied along with estimation of mineralization rates of organic matter.

The exchange of nutrients across the sediment-water interface is determined by sediment core incubations. Sediment cores with and without macrophytes are sampled and brought to the laboratory. For controlled conditions of temperature and mixing, the change in concentration of NO_3, NH_4, PO_4, Cu and others are followed in the overlying water. From this the exchange, which can be influx into the sediments or efflux, can be calculated. Along with the exchange measurements pore water profiles are determined to obtain the concentrations of dissolved compounds in the sediments. The gradient in concentrations can also be analyzed to derive fluxes across the sediment-water interface and compared with the direct measurements. Analysis is performed with diagenetic models. The role of upwelling of (shallow) groundwater is expected to be of minor importance, but this will be verified with tracer (chloride) profiles found in the sediment pore water.

The fluxes and concentrations are further determined by the mineralization processes in the sediments. The amount and reactivity of organic matter should be known, so it can be compared to the amount roots might take up.

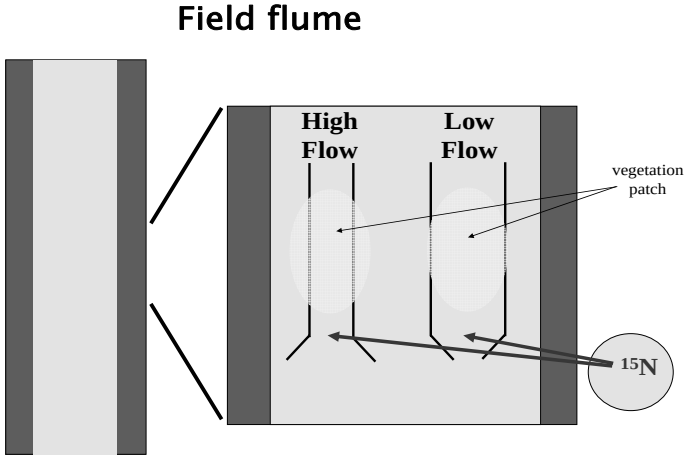
Therefore, slices of sediment from different depths are taken and incubated in slurries. The initial nutrient composition and organic matter content is measured. The release of nutrients is followed in the liquid phase. The increase in concentrations can be used derive the reactivity of the matter present by analyzing the slope, along with the amount of reactive matter based on the asymptote.

Combining the results of both experiments will give the data needed to describe the nutrient behaviour in sediments and captured in a model that will be linked to macrophyte dynamics.

The above experiments and measurements will be performed 6 times to cover the seasonal cycle. The first sampling activity is just before the growing season to capture the starting conditions and after that, 3 times within the growing season of macrophytes. The fifth will be in early autumn at the point that the plant biomass dies off. The last sampling period will be in mid winter.

WP3 –EXPERIMENTS AT THE SCALE OF A RIVER STRETCH WITH MACROPHYTE PATCHES ***(year 3)***

To validate the use of small and mid-scale measurements on a larger scale, we will measure uptake also in the field. Because it is impossible to label a whole river segment, we will use field flumes for this purpose. A field flume is a small channel that will be built inside the river (see figure below). The channel will be constructed by placing metal pipes 1 m apart in the river bottom. These metal pipes will be put in place before the growing season starts. During the experiment, we will mount transparent plates on the pipes to obtain a small channel. By varying the width of the inlet of the channel, we will create 2 parallel field flumes that will differ a factor 2 in current velocity. Label will be added at the inlet of the channel, using a constant pumping rate. We will simultaneously apply label ($^{13}HCO_3^-$ + $^{15}NO_3^-$ or $^{13}HCO_3^-$ + $^{15}NH_4^+$) to two parallel field flumes (see figure below) so that the nutrient concentration is similar in both flumes. During the labelling, current velocities within the field flumes will be measured with 4 x electro magnetic velocity meters (i.e., two EMV's per flume). These instruments are ideal to measure within vegetation patches. To be



able to use two different nitrogen forms ($^{15}NO3^-$ vs. $^{15}NH_4^+$), we will move the field flume upstream when we shift from the $^{13}HCO_3^-$ + $^{15}NO_3^-$ to the $^{13}HCO_3^-$ + $^{15}NH_4^+$ treatment.

WP4 –MODELLING

Task 4.2. Macrophyte-sediment model

In this workpackage a sediment-macrophyte model is developed. It captures the growth dynamics of the macrophyte species and related uptake or release of compounds to sediment processes. The models are first constructed to analyse the experiments on macrophyte dynamics and sediment processes (work package 1). The integration delivers a generic macrophyte submodel for the larger scale models directed towards patches and ecosystem.

2.1. macrophyte growth model for different species
2.2. sediment model
2.3. Integrated macrophyte-sediment model

Task 4.3. Macrophyte patch model

In here the processes that determine patch behaviour are described. A distinction is made between processes affecting flow dynamics such as flow-resistance due to macrophytes presence, and processes related to nutrient uptake and release kinetics.

Models are first developed to assist design of the experimental set-up, in this case on flume experiments (laboratory and field). Both to create the right experimental dimensions and tracer application settings, as again the data analysis.

In the FEMME-modelling environment an example model for the laboratory flume is available that will be used after adapting it to include macrophyte related effects. Furthermore, the 2D or 3D models developed in the FWO-project on "Exchange processes in river ecosystems", will be used to describe transfer and exchange of matter as accurately as possible (Delft3D, Fortran models developed in FEMME).

The result will be the patch behaviour against flow and mass transfer as a function of hydrodynamics and plant density. The submodel of task 4.2 is implemented and responds to local conditions within the patch.

Task 4.5. Occurrence of macrophytes in function of environmental factors

In the previous MANUDYN-project a huge data-set is constructed on the presence of macrophyte species and biomass in relation to environmental properties. This workpackage is directed towards the development of probability relationships for the occurrence of macrophyte species in streams. It should become a submodule in the generic management model