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MACROPHYTES AND NUTRIENT DYNAMICS IN THE UPPER REACHES OF THE SCHELDE BASIN (MANUDYN I)

P. MEIRE, J.P. VANDERBORGHT, F. DEHAIRS



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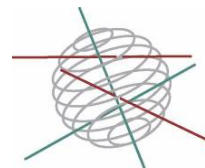
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FINAL REPORT

**MACROPHYTES AND NUTRIENT DYNAMICS IN THE UPPER
REACHES OF THE SCHELDE BASIN
(MANUDYN I)**

EV/33

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October 2006



D/2007/1191/37

Published in 2007 by the Belgian Science Policy

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TABLE OF CONTENTS

Summary	5
I. Introduction.....	9
II. Aim of the project.....	11
III. Description of the study area.....	13
IV. Estimating macrophyte biomass in a lowland river: a critical analysis of different methods	17
IV.1. Introduction.....	17
IV.2. Material and method.....	17
IV.2.1. Site description.....	17
IV.2.2. Data collection.....	18
IV.2.3. Statistics.....	19
IV.3. Results.....	20
IV.4. Discussion	26
V. Macrophyte distributions in the Nete catchment.....	29
V.1. Introduction	29
V.2. Material and methods.....	29
V.2.1. Macrophyte inventory.....	29
V.2.2. Abiotic variables.....	29
V.2.3. Analysis: initial ordinations.....	30
V.3. Results	31
V.4. Discussion.....	36
VI. The role of macrophytes on the C, N, P and O₂ cycling.....	39
VI.1. The role of macrophyte biomass in nutrient assimilation in the lowland river Aa.	39
VI.1.1. Introduction.....	39
VI.1.2. Material and methods.....	39
VI.1.3. Results	40
VI.1.4. Discussion.....	46
VI.2. Nutrient mass balances in the Aa, a nutrient rich lowland river	50
VI.2.1. Introduction.....	50
VI.2.2. Material and methods.....	51
VI.2.3. Results	55
VI.2.4. Discussion.....	59
VI.3. C, N and O ₂ cycling in the Aa as assessed from stable isotope biogeochemistry	62
VI.3.1. Introduction.....	62
VI.3.2. Methods.....	63
VI.3.3. Results and discussion	63
VI.3.3.1. Seasonal variations	63
VI.3.3.2. Spatial variation in the particulate organic material pool	66
VI.3.3.3. Diurnal variations	67
VII. The assimilation of ¹⁵N by three different macrophytes under enforced stream conditions in a flume	71
VII.1. Introduction.....	71
VII.2. Material and Methods.....	71
VII.2.1. Experimental setup	71
VII.2.2. Velocity profiles and bending angles	74
VII.2.3. Analysis.....	74

VII.2.4.	<i>Calculation of uptake rates and relative growth rate (RGR)</i>	74
VII.3.	Results	75
VII.3.1.	<i>Vertical velocity profiles</i>	75
VII.3.2.	<i>Macrophytes</i>	78
VII.3.3.	<i>¹⁵N-uptake</i>	79
VII.4.	Discussion	84
VIII.	A one-dimensional model of the oxygen dynamics in a vegetated stream and its application to nutrient mass balance analysis	89
VIII.1.	Study site	89
VIII.2.	Materials and methods	90
VIII.2.1.	<i>Hydraulic characterisation</i>	90
VIII.2.2.	<i>Water quality</i>	91
VIII.2.3.	<i>Other field measurements</i>	92
VIII.3.	Description of the model	92
VIII.4.	Results and discussion	96
VIII.4.1.	<i>Hydraulics</i>	96
VIII.4.2.	<i>Oxygen concentration</i>	98
VIII.4.3.	<i>Model parameters</i>	103
VIII.4.4.	<i>Process rates</i>	104
VIII.4.5.	<i>Nitrogen budgets</i>	107
VIII.5.	Conclusions and management perspectives	111
IX.	Overall conclusions	115
X.	Management alternatives: suggestions for management policy: translation to the practice	117
XI.	Acknowledgements	119
XII.	References	121

Summary

The improving water quality in Flemish streams has created better conditions for the development of macrophytes. Reduced organic load and thus improved light conditions combined with still high nutrient concentrations resulted in a dominance of macrophytes in large parts of the Nete catchment, a subcatchment of the Scheldt. This massive macrophyte growth has an impact on the nutrient balance in the system by the uptake and release of the nutrients. However it is not clear how big this impact is. This study aimed at an improved understanding of the relation between the macrophyte community and the nutrient dynamics within the river basin.

Biomass and diversity of the macrophytes differs a lot within the river basin. To try to understand the determining factors for the presence of macrophytes an inventory is made at 108 unshaded locations in the Nete catchment. At each location abundance of all macrophyte species was estimated, both in spring and autumn over a length of 50m. Physical parameters, such as width, depth, orientation, current velocity and grain size distribution were measured once. Chemical parameters, such as nutrients, heavy metals, oxygen and pH, and conductivity were measured six times a year.

A large number of macrophyte species was found in the Nete catchment although no endangered species were found. Also, the abundance of most of the species was very low. In contrast, a few of them (especially generalist species like *Stuckenia pectinatus*, *Potamogeton natans*, *Potamogeton trichoides*, *Callitriche platycarpa*, *Sparganium emersum*, *Sagittaria sagittifolia*, *Glyceria fluitans* and *Phalaris arundinacea*) were dominant. These species covered sometimes more than 50% of the site. There is also a significant difference in macrophyte distribution and diversity between the three subbasins of the Nete.

Several environmental variables might explain these variations in macrophyte diversity in the Nete catchment. The main abiotic variables explaining the macrophyte diversity in the Nete catchment are morphological ones (width and depth). The other factors that explain the occurrence of several dominant macrophyte species were suspended material, pH, ammonium, phosphates, oxygen and temperature. However, oxygen and temperature vary significantly during the day and six single measurements over a year might not reflect the temperature and oxygen range well enough. Sediment characteristics, namely the percentage of fine sand and ammonium and phosphate in the pore water, play a minor role in explaining the macrophyte diversity in the rivers of the Nete catchment.

The evolution of macrophyte biomass and the role of these macrophytes in nutrient storage and dynamics were studied in a small part of the river Aa in the Nete catchment. The study section has a length of approximately 1,5 km and is comprised between two level-regulating gates. This section has not been mowed since the late nineties. First, a sampling design to measure the macrophyte biomass in a representative way was tested. To obtain high reliability, a variance of less than 10%,

more than 200 samples of 15 by 15 cm are needed, which is impossible to handle on a routine basis. Our monitoring strategy in the unmanaged study section of the Aa however, consists of sampling monthly 30 plots of 15 by 15 cm, namely 10 at the upstream gate, 10 in the middle and 10 at the downstream gate. The real biomass can be approximately 40% dry biomass more or less compared to the in this way estimated biomass. Differences between monthly sampling should differ more than 40% dry biomass to indicate a significant change.

It reveals that biomass increased significantly over the period 2003-2005. One of the reasons of this increase might be due to the fact that the study section is not mowed for several years. Especially some dominant macrophyte species with an apical growth meristem, like e.g. *Potamogeton natans* and *Callitriche platycarpa*, take advantage of the absence of management and are likewise responsible for the increase in total macrophyte biomass. Other dominant species with a basal growth meristem, e.g. *Sagittaria sagittifolia* and *Sparganium emersum*, are of minor importance in the last year of the period concerning biomass. Peak biomass in the growing season differs a lot from year to year and is dependent on the temperature and the precipitation in the respective year: the peak moment of biomass can be situated between May and August.

The nutrient content of the dominant macrophyte species from the study section were determined for two years (2003 and 2004). The average nutrient content of all macrophyte species during all periods were relatively constant. Compared to literature, both N- and P-contents in macrophytes are very high, namely well above the suggested saturating growth values. Macrophytes in rivers always have higher N- and P-content compared to macrophytes in lakes, but the tissue N- and P-content of the macrophytes in our study were even higher compared to the values for river macrophytes found in the literature. This indicates that the Aa is a very eutrophic river where nutrients in the surface water are not limiting. As a result, the nutrient standing stock in the macrophyte biomass is also high in the peak of the growing season. However the continuous load of N and P is so large that mowing once or twice a year is almost useless in the light of nutrient removal.

In the same study section, mass balance studies were done during six 24-hour campaigns at different seasons. For each campaign, the most important water quality parameters (i.e. N-NH_4^+ , N-NO_2^- , N-NO_3^- , P-PO_4^{3-} , O_2 , pH, temperature, conductivity and suspended matter) were measured every two hours at the upstream gate, at an intermediate point and at the downstream gate. Velocity profiles were also measured in order to calculate discharge at that period. Those measurements allow us to compare the concentrations in a specific water mass at different locations within the study section. Results show that in the growing season, the nitrogen and phosphorus concentration is lower at the downstream part. A mixing-dilution model for this section showed that this difference could not only be explained by a dilution of the

inflow of water from the Slootbeek. The model indicated the occurrence of non-conservative processes especially during the summer months (July, August and September). Label experiments showed that macrophytes are the major consumers of available nitrogen in the surface water with a preference for ammonium. Also, but in a lesser extent, the sediment can act as a sink for ammonium and as a source for nitrates. Phytoplankton plays a minor role in the mass balance as well for nitrates as for ammonium.

Flume experiments using ^{15}N labelled ammonium or nitrate in the surface water confirm that most dominant macrophytes take up nutrients by their stems and leaves and that ammonium is the nitrogen source of preference. The flume is an artificial streaming system, which was used to test the uptake of N-NH_4^+ and N-NO_3^- by three different macrophyte species, *Potamogeton natans*, *Callitriche platycarpa* and *Sparganium erectum*, with different stream velocities and at different locations within a macrophyte patch. The flume has a test section where a macrophyte patch is created by placing 20 pots of one macrophyte species. These experiments, each for a duration of 3 hours, is carried out in a controlled situation: controlled light regime, controlled stream velocity, one macrophyte species at a time, nutrient free sediment and known nutrient concentrations in the surface water. Surface water samples were taken just before the start and at the end of an experimental run to check the nutrient status of the surface water. After each run, 3 macrophyte pots were taken out of the flume, weighed, dried and analysed for ^{15}N . Results were compared to ^{15}N values of unexposed macrophyte species. All species took up nitrogen via their stems and leaves and gave preference to ammonium as a nitrogen source. These experiments also showed a distinction between fast growing species like *Potamogeton natans* and *Callitriche platycarpa* in a lesser extent and slower growing species like *Sparganium erectum*. Stream velocities did not influence directly the overall uptake of available nitrogen in a macrophyte patch, but given a certain stream velocity, the uptake of available nitrogen is dependent of the location within a patch.

Based on a one-year monitoring of the oxygen dynamics into the study section of the river Aa using two *in situ* OTC (oxygen-temperature-conductivity) sensors, a simple, one-dimensional, steady-state oxygen model describing the main transport and reaction processes has been built. It has been used to quantify the respective role of the production and respiration reactions in the overall oxygen budget, and by extension, in the carbon and nitrogen balance.

In the model, the advection term is deduced from the water residence time within the study section, as computed from the conductivity profiles at the upstream and downstream boundaries. Measurements show that the mean water velocity decreases by a factor of 6-7 between March and May, with a parallel increase in the slope of the energy grade line (from $<1.0 \cdot 10^{-4}$ to $>3.5 \cdot 10^{-4} \text{ m m}^{-1}$). Although the mean water discharge is 4 times less in summer than in winter, the very large increase in

the resistance to flow associated with macrophyte growth is thus responsible for a very significant drop in the water head.

Given the oxygen time-series at the upstream gate and the variation of PAR (photosynthetically active radiation, also measured on a continuous basis), the model is able to reproduce very satisfactorily the evolution of oxygen with time at the downstream gate. This requires the adjustment of two parameters only (maximum production rate, respiration rate) for each model run. The other two model parameters (saturation irradiance, rate of oxygen transfer at the interface) are found to be constant throughout the year. Model results show that the study section is most of the time, a net oxygen sink and a net nitrogen source. This can only be sustained if a continuous input of organic carbon and nitrogen is available, probably supplied by the input of vegetation debris originating from the upper catchments, and its subsequent trapping into the vegetation mat. The role of vegetation sloughing thus seems particularly important, and is evidenced both by its impact on the hydraulic behaviour (a permanent, very significant decrease of the resistance to flow after a storm) and on the biological response (a concomitant decrease of the oxygen production and respiration rate linked to the decrease of the macrophyte biomass in the system).

It can be concluded that the macrophyte growth has a significant effect on the nutrient concentrations in the water, but due to the high load coming from diffuse and point sources during the time of the project the absolute effect on the total flux of nutrients downstream is minimal. Mowing and removing the macrophyte biomass from the system therefore will not really affect the nutrient flux, but the organic material that is transported downstream might have an effect on the oxygen balance. Finally it is clear that not mowing results in a shift of the macrophyte community, from opportunistic species to more specific species. Mowing in patterns might therefore enhance the diversity of the macrophyte community.

I. Introduction

Eutrophication of aquatic ecosystems is directly linked to the increased urbanisation and fertilisation of river catchments. This process is characterised by a massive production of organic biomass and may lead to a variety of negative consequences such as toxic algal blooms, reduced biodiversity, and the occurrence of anoxic conditions due to the degradation of this excessive amount of organic material. However, various studies have shown that no simple relationship can be established between the anthropogenic release of nutrients within the river catchments and their input into the coastal zone (Howarth et al., 1996; Billen & Garnier, 1997; Cloern, 2001). A number of processes are indeed responsible for the retention and/or elimination of nutrients within the riverine and estuarine systems. The Scheldt basin is a good example of such behaviour: it is submitted to a very high input of nutrients, but a significant fraction of these is retained within the basin, as attested by the large difference between the estimated nutrient loads from domestic, industrial and agricultural origin and the fluxes reaching the North Sea. According to Billen (cited in Howarth et al., 1996), about 50% of the nitrogen input to the Scheldt basin is removed, mainly by denitrification, before reaching the North Sea. For phosphorus similarly, about 50% of the input is probably removed by sedimentation (Van Damme et al., submitted).

Beside nutrients, the Scheldt basin is also submitted to a very high organic load. The input of organic matter into the estuary results in intense oxygen consumption through respiration and to large emissions of carbon dioxide to the atmosphere (Frankignoulle et al., 1998). The overall budget of organic matter at the basin scale is not well established, but following the recent efforts devoted to sewage purification, an increasing part of organic carbon seems to be internally produced in the tributaries of the system, as a result of eutrophication. During the last decade, the improvement of the water quality with respect to organic matter and suspended solids from urban and industrial origin, combined with the still elevated nutrient load, has resulted in an enhanced primary production of macrophytes and phytoplankton in the upper catchments. This is particularly well demonstrated by the spectacular development of macrophytes in the upper part of the Scheldt tributaries.

Up to now, considerable efforts have been devoted to the understanding of nutrient behaviour (especially nitrogen) in the Scheldt estuary. However, less attention has been paid to the study of nutrient fluxes in the upper catchments of the basin, where retention and loss processes are also important. Recently, different authors have shown that retention can not be neglected (e.g. Behrendt and Opitz, 2000; Peterson et al., 2001). Behrendt and Opitz (2000) report significant retention of nutrients (nitrogen and phosphorus) for about 100 different river basins in Europe. Shallow depths and high surface to volume ratios of headwater streams would favour short uptake lengths (a function of discharge) for headwater streams, relative to larger

streams. Budget studies of every compartment within these catchments are thus necessary to identify and quantify the processes controlling the transport of nutrients and organic carbon to the estuarine and coastal zones. Furthermore, a better understanding of nutrient cycles and the belonging nutrient budgets in the whole river basin are needed to support good management practices aimed at improving the water quality of the aquatic continuum.

The improving water quality in Flemish streams has created better conditions for the development of macrophytes. 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 (Van Steen, 1999; Verbessem, 2000). Due to the high nutrient input mainly by diffuse sources, excessive developments of macrophytes are frequently observed during summer. The hydraulic capacity of the systems becomes limited and water levels rise in the upstream zones, which can result in flooding. A better understanding of the factors determining the excessive biomass production of macrophytes can help in solving this problem. On the other hand, this large fixed biomass acts as a biological filter with respect to dissolved and particulate matter, and may significantly affect the biogeochemistry of the downstream zones.

Macrophytes play an important role in the ecology of river water systems. Their presence influences the direct environment. They strongly modify the chemical and physical features of the water and the sediment. In rivers, rooted macrophytes strongly modify the water flow in water systems. The velocity steeply declines when submerged macrophytes occur in patches (Sand-Jensen et al, 1999). Besides, sedimentation takes place within and especially after the macrophyte patch, lowers the turbidity, leading to nutrient retention and clearing of the surface water (Sand-Jensen and Madsen, 1992; Schulz et al., 2003). In this way, macrophyte seeds can germinate in these newly created habitats. Macrophytes attract macro-invertebrates which on their turn attract different fish populations (Armitage et al., 1994; unpublished data). Macrophytes play also a major role in nutrient cycling. They take up a high amount of nutrients of the surface water (Madsen and Cedergreen, 2002) and the sediment (Carr and Chambers, 1998), and release their nutrients partly in late autumn when they decay (Bloemendaal & Roelofs, 1988).

II. Aim of the project

The objectives of this project are to understand and quantify:

- (1) The impact of various environmental factors on the presence of macrophytes in river basins.
- (2) The role of primary producers (macrophytes and phytoplankton) on the budget of carbon, oxygen, nitrogen and phosphorus in the upper catchments of the Scheldt basin.

Prior to all field experiments, an appropriate method was developed and tested to quantify macrophyte biomass in lowland rivers (Chapter IV).

Additionally, experiments were performed at 3 different scales:

First, the appearance of macrophytes in relation with various environmental factors was studied at the scale of the whole Nete catchment area (Chapter V).

Second, a test river stretch with known hydrological features was selected on the Aa river, a tributary of the Nete to study the seasonal variations of macrophyte growth and related nutrient, oxygen and carbon dynamics (Chapter VI).

Third, the use of nitrogen by macrophytes was studied in controlled conditions using a flume incubator (= artificial river) (Chapter VII).

Finally, the results were used for developing an “output algorithm”, as a first step to link estuarine models to a description of the upstream freshwater system. (Chapter VIII)

III. Description of the study area

The distribution and dynamics of macrophytes and their role in the C, N, O₂ and P budgets of rivers was studied in the Nete catchment area.

The Nete basin in Flanders, a sandy region which is located in the northern part of Belgium, consists of typical lowland rivers (Figure 1). However, the river systems are far from natural. In early sixties, reallocation of land had taken place and in consequence, rivers have been straightened. Human constructions such as dikes and weirs have been built to ensure adequate discharge of the river. From then on, agriculture has been heavily modified and intensified. River water became organically loaded and highly turbid, surface water quality and ecological quality of the Nete basin declined rapidly (Bosmans et al., 1989). In early eighties, when purification treatment plants became operational, turbidity in rivers started to decline (Bosmans et al., 1989) and macrophytes started germinating widely. However, rivers were still very eutrophic due to the high nutrient loads and a huge biomass of macrophytes developed in these aquatic systems. In summer, mowing strategies are applied to avoid inundation in the upstream areas. Nevertheless, the water of the Nete basin is the purest of Flanders (VMM, 2004). Gradually, the surface water quality of the Nete basin has ameliorated (Yseboodt et al., 1997; Yseboodt et al., 2005) and more macrophyte species appeared in the Nete basin.

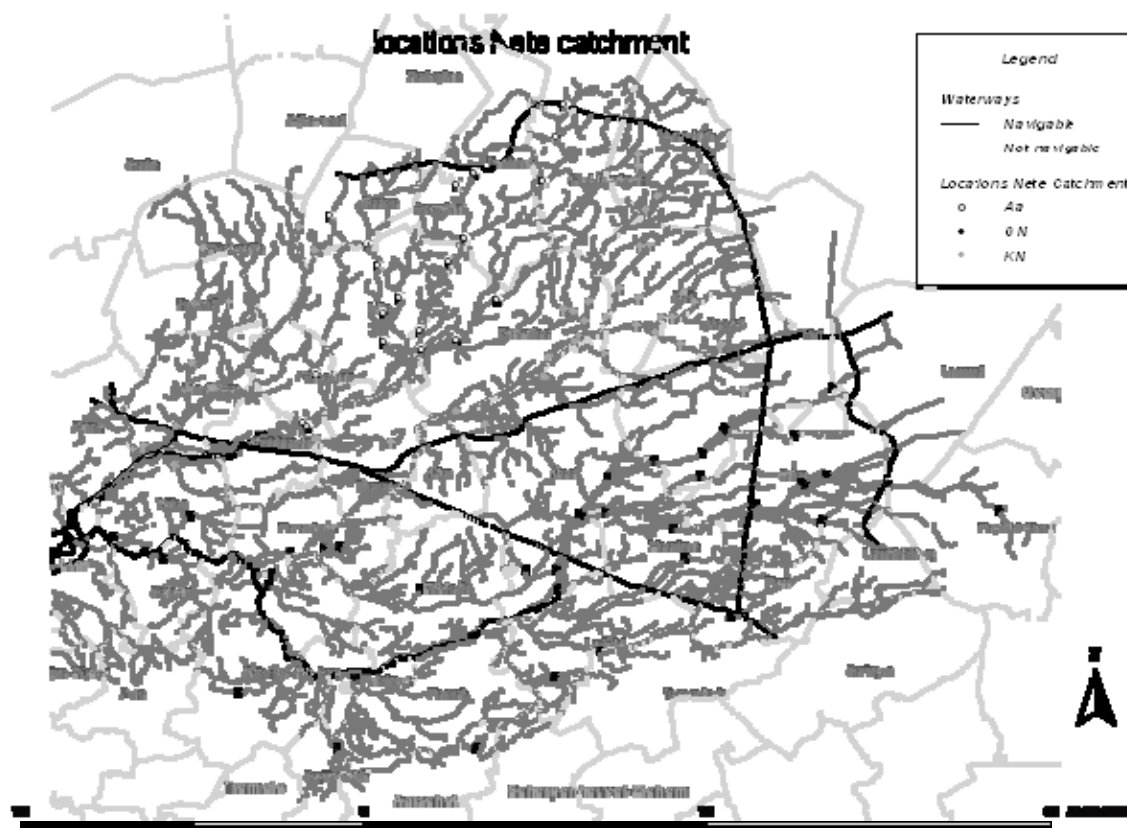


Figure 1: Overview of the Nete catchment. GN = 'Grote Nete' subbasin (subbasin of the Nete catchment); KN = 'Kleine Nete' subbasin (subbasin of the Nete catchment); Aa = 'Aa' subbasin (subbasin of the Kleine Nete subbasin).

Within this basin, a test river stretch with known hydrological features was selected on the Aa river, a tributary of the Nete. The river Aa (Figure 2) is mainly rain fed, has its origin in very low pastures at a height of 30m and comprises a basin of 25.054 ha (Brosens, 1965). The canalized study section has a length of approximately 1,5km and is bordered with two adjustable weirs. Mean river width of the study section is about 18m and mean depth about 2,2m. Mean water depth is about 1,5m. Stream velocities vary between 0,024 m/s and 0,200 m/s. The investigated section of the Aa is mainly bordered with crop fields and pastures and is situated downstream of a water purification system (Lichtaart 25000 eqinh). The river bed of the Aa consists primarily of iron rich sandy soils.

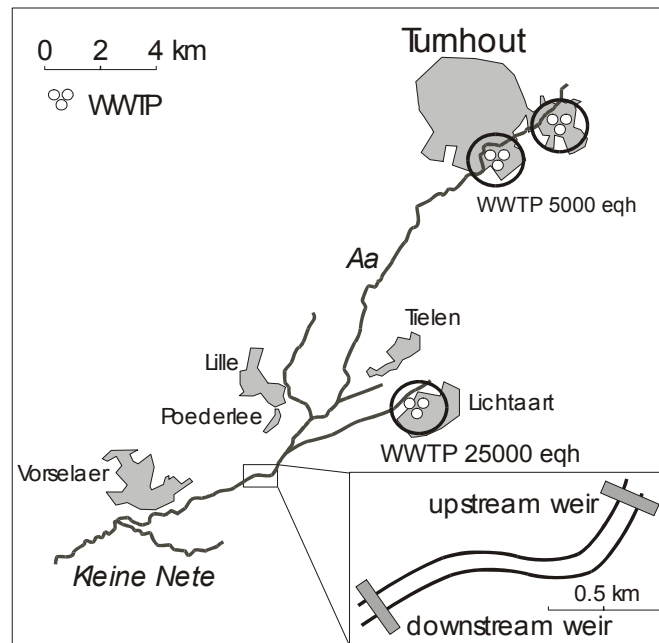


Figure 2: The Aa River downstream Turnhout with the test river stretch. Upstream weir also called gate 3 and downstream weir also called gate 4.

IV. Estimating macrophyte biomass in a lowland river: a critical analysis of different methods

IV.1. Introduction

Recently, macrophytes are incorporated as one of the criteria to describe the ecological status of water systems (2000/60/EC). The ecological status implies surface water quality as well as sediment quality. Macrophytes are very appropriate indicators because they comprise both water and sediment quality. Macrophytes have been used to predict the trophic status of river and associated channel systems. Several indices based on macrophyte species assemblages were developed to predict the trophy level. Nowadays, three main methods are developed to assess eutrophication: Mean Trophic Rank in UK (MTR) (Dawson et al., 1999a) and refined MTR (Ali et al., 1999; Holmes et al., 1999; Szoszkiewicz et al., 2002), Trophic Index with macrophytes in Germany (TIM) (Schneider and Melzer, 2003) and the Biological Index for Rivers in France (IBMR) (Haury et al., 2003). However, next to these qualitative methods, very little is known about quantitative methods to assess water quality, although biomass of macrophytes also can be used as indicator of river trophy (Roelofs & Bloemendaal, 1988; Sarnelle et al., 1998). Macrophyte biomass estimations can give very useful and additional information to the qualitative methods to assess the river quality. In oligotrophic systems, a low biomass of rooted, submerged macrophyte species is expected. In waters with nutrient rich sediments (especially P), a higher biomass of rooted, submerged macrophyte species will be developed. Finally in eutrophic systems, where surface water is the main supplier of nutrients, floating macrophyte species become dominant (Roelofs & Bloemendaal, 1988). However, this is true when biomass is sampled in the peak season. There is less known about sampling biomass throughout the season. Does seasonality play a major role in biomass estimations? Is the variation between two successive sample periods really due to seasonality or is it due to internal variation?

This chapter will focus on the methodology and generated variation of biomass sampling in rivers. The aim of the study is to determine the amount of samples needed to obtain an allowable quantitative estimation of the biomass within a river system. This is done throughout the season and with various plot sizes.

IV.2. Material and method

IV.2.1. Site description

Measurements were carried out in the river Aa (Figure 2). Since 1989, macrophytes appeared in the Aa due to the improvement of the water purification system located upstream of the studied section. Since then, a huge biomass of water vegetation develops in the Aa and moreover macrophyte biodiversity increases annually. Main species present in the Aa are submerged macrophytes such as *Callitriche platycarpa* Kütz., *Ceratophyllum demersum* L., *Elodea nuttalli* (Planch) St John, *Sparganium*

emersum Rehm. and *Potamogeton pectinatus L.*, floating macrophytes such as *Potamogeton natans L.* and emerged macrophytes such as *Rorippa amphibia (L.) Besser* and *Sagittaria sagittifolia L.*

IV.2.2. Data collection

In August 2003, five iron grids with equal squares of 225 cm² were laid down manually, next to each other, on the bottom of the river Aa, downward the downstream weir (Figure 2). The grid consists of five rows (A, B, C, D and E) parallel to the width of the river and 75 columns parallel to the stream direction. They cover a total area of 0,75m x 11,25m (the length multiplied by the width of the river). In each square, all macrophytes were cut just above the river bed and collected by hand, starting from the most downstream row and from the left side to the right side of the river. Biomass of each square was put into a plastic bag and placed in a cooled box before transporting to the lab. All fieldwork was done during the same day. In the lab, the fresh weight of every species was measured to the nearest 0,01g after drying the plants with tissue paper. Dry weight of macrophyte species has been determined after drying for 48 hours by 70°C to the nearest 0,001g. Fresh weight of the macrophytes in the grid is visually illustrated by a contour plot generated by SURFERTM for windows.

Samples in the section between the weirs are taken by hand with a kind of instrument, called the 'surber' (Figure 3). It consists of a metal square of 15x15cm and a metal framework, welded to it, with stick that allows holding the instrument above the water level. A net is attached to the framework and is made of rustproof steel. It is orientated to the downstream direction and catches the picked macrophytes.



Figure 3: the surber, instrument for sampling macrophytes of 15cmx15cm.

IV.2.3. Statistics

Bootstrapping was used to analyse this dataset. Bootstrapping is a method for estimating generalization error based on "resampling" (Weiss and Kulikowski 1991; Efron and Tibshirani 1993; Hjorth 1994; Shao and Tu 1995). The interest in this study is not obtaining a point estimate of a statistic, but the variation in the point estimate and the confidence intervals for the true value of the parameter. If the normality assumption does not hold, the traditional methods may be inaccurate. Resampling techniques such as the bootstrap provide estimates of the standard error, confidence intervals, and distributions for any statistic.

In our case, the mean value of the dataset is the statistic of interest. Bootstrapping the dataset was done on fresh and dry weight biomass per plot and fresh and dry weight biomass per plant species. Furthermore, the bootstrapping method was executed on the biomass upstream the weir, taken in different seasons. The output was compared with the results of the bootstrapped dataset downstream the weir. In addition, biomass averages between successive months and between every other month were analysed with ANOVA to detect significant biomass changes during these periods.

By adding adjacent plots together, new plots of 15x30 cm and plots of 30x30 cm were created. Bootstrapping was also done on this new made datasets to compare the coefficients of variation of different plot sizes. In addition, the earlier mentioned datasets are also analysed based on the Central Limit Theorem (CLT). In this case, the assumption of a normal distributed dataset is hold. These datasets include a high amount of sample sizes so that they meet the normality assumption (Legendre et al., 1989). Besides, two program scripts similar to that of bootstrapping were written. The first program script has an additional condition factor so that there is no fully ad random sampling. The script divides the width of the river in five equal subblocks, parallel to the stream direction (Figure 4). While running the program script, 10000 datasets are created, each consisting of samples that are drawn by taking an equal number of samples within each subblock, and with replacement. In this way, border samples (first en fifth subblock) as well as bulk samples (other subblocks) of the river are equally counted in over the five subblocks.

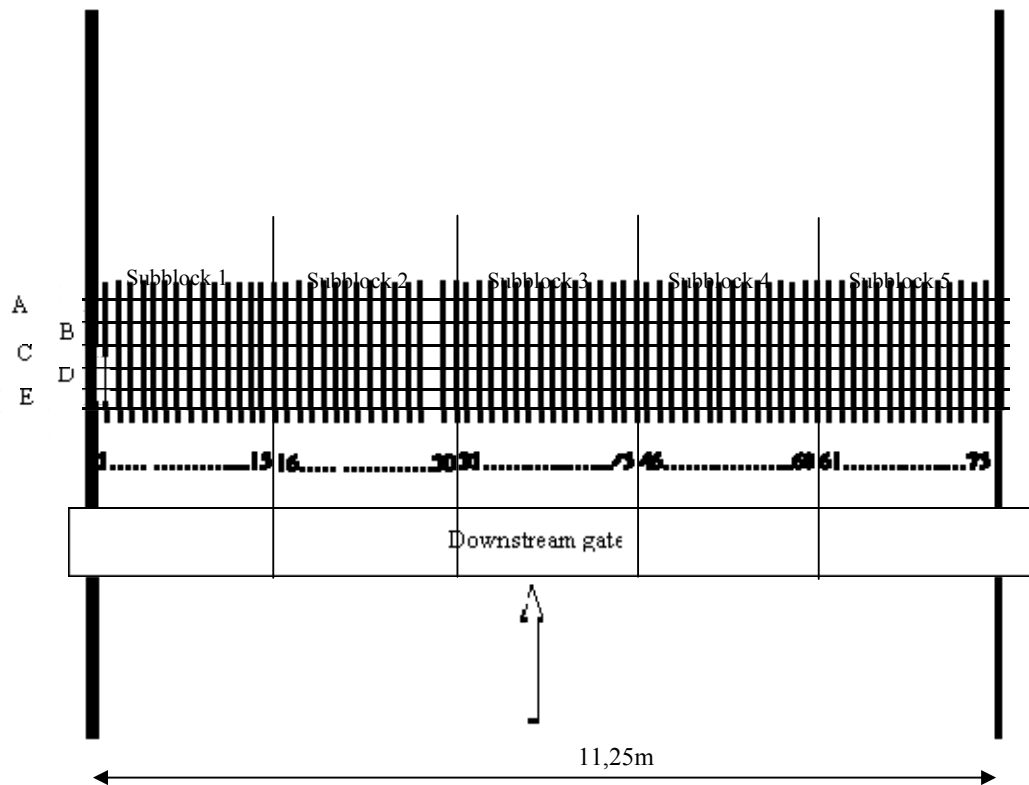


Figure 4: Scheme of the method test section, virtually divided in 5 equal subblocks, where a not fully ad random sampling occurs. There is the extra condition of equal sampling within each subblock.

IV.3. Results

A global sight of the fresh weight biomass standing in the grid area is illustrated in figure 5. The presence of macrophytes is concentrated at the left side of the river. Seven species are found in the dataset: *Callitriche platycarpa*, *Ceratophyllum demersum*, *Elodea nuttallii*, *Potamogeton natans*, *Potamogeton pectinatus*, *Ranunculus penicillatus* and *Rorippa amphibia*.

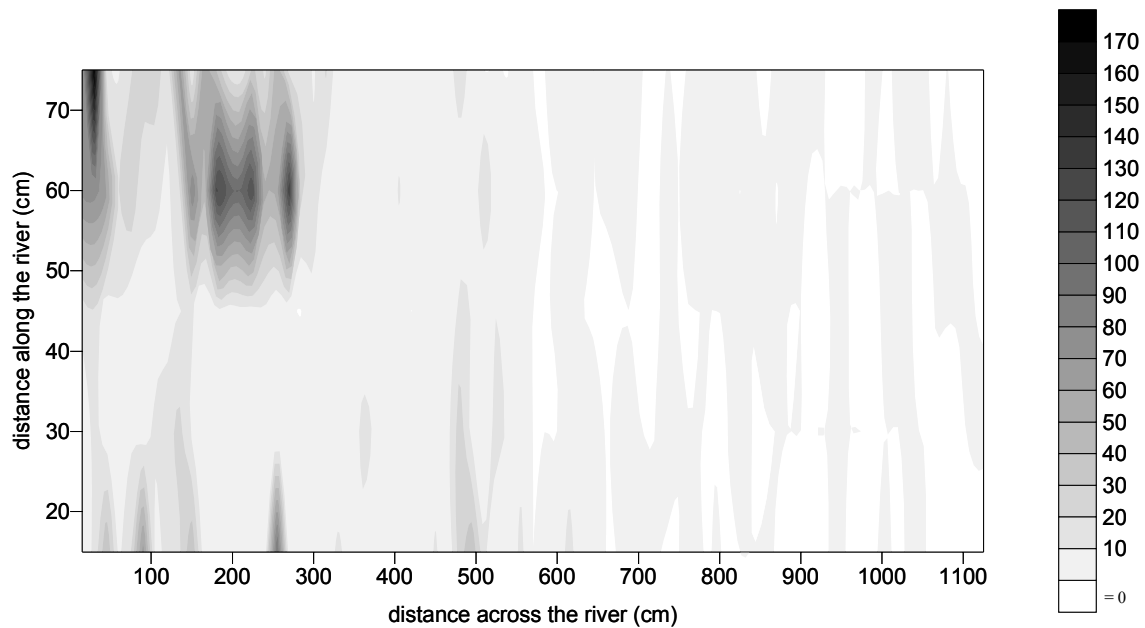


Figure 5: Fresh weight total biomass (expressed in g/225cm²) in the method test section: surfer diagram (top view).

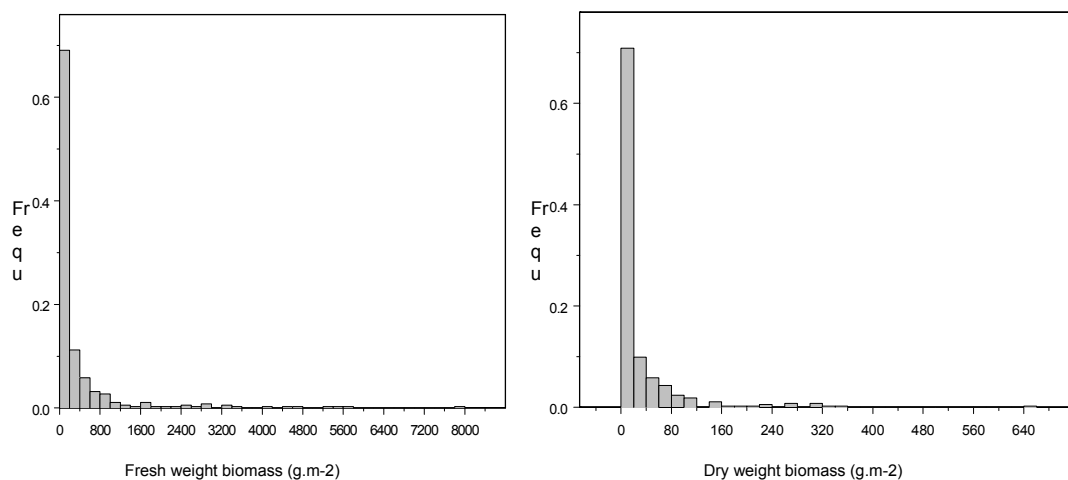


Figure 6: Frequency distribution of fresh and dry weight biomass within the dataset.

Summary statistics of fresh and dry weight biomass per plot are shown in table 1 and figure 6. The data within the grid are not normally distributed according to the Shapiro-Wilkinson Normality Test (Table 1).

Table 1: Summary statistics and normality distribution test (Shapiro-Wilkinson test) of the dataset.

summary statistics	FW (g/225cm ²)	DW (g/225cm ²)	Shapiro-Wilkinson test	FW	DW
mean	7.95	0.615	w-value	0.5433	0.5674
SE	1.04	0.074	p-value	0	0
CV (%)	254	232			

In the graphs, bootstrap and CLT outputs are only shown for fresh weight biomass because there is a strong positive relationship between fresh and dry weight biomass. R^2 amounts to 0,94. Bootstrap output of fresh weight biomass and of dry weight biomass of macrophytes reveals that at least 248 respectively 232 samples are necessary to have a variation of 10% within the dataset. If 30 samples are taken, the coefficient of variation amounts 45% for fresh weight biomass and 41% for dry weight biomass (Figure 7). The central limit theorem (CLT) gives slightly higher coefficients of variation but similar results due to the high amount of sample sizes (Table 2, Figure 7).

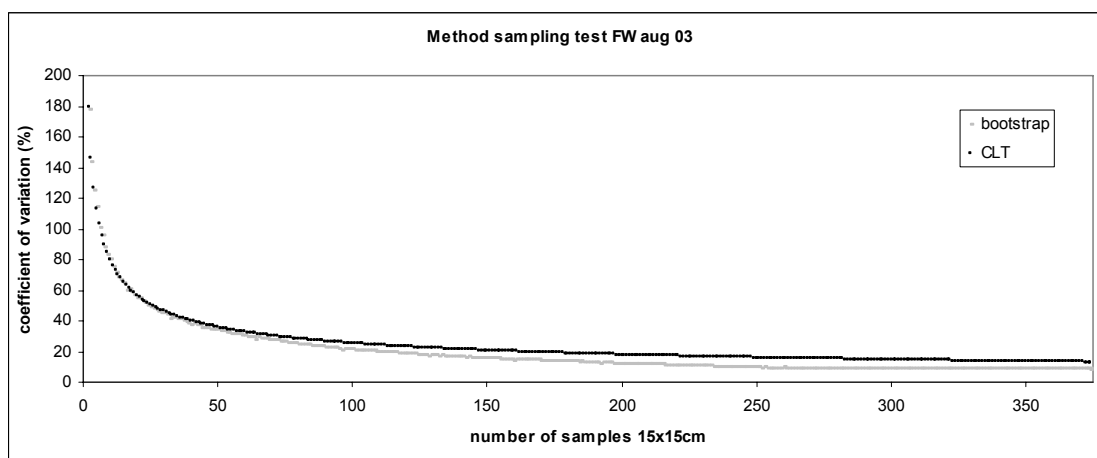


Figure 7: The coefficient of variation in relationship to the number of samples taken within the dataset: bootstrap output and output obtained by the central limit theorem of fresh weight biomass (FW) within the method test section.

Table 2: Results of bootstrap and CLT for fresh and dry weight biomass. The method test dataset of 375 samples in august 2003 has been compared with the smaller datasets in different seasons. Results are based on the total biomass per plot, possibly containing more than 1 macrophyte species. CV = coefficient of variation (%); CLT = central limit theorem; downstream = samples taken downstream the weir; upstream = samples taken upstream the weir.

Coefficients of variation for fresh / dry weight biomass in the Aa											
location dataset	dataset	number of samples within the dataset		CV (%) for 30 samples		number of samples needed to obtain a CV < 10%		number of samples needed to obtain a CV < 20%		number of samples needed to obtain a CV < 30%	
				bootstrap	CLT	bootstrap	CLT	bootstrap	CLT	bootstrap	CLT
downstream	aug/03	375		45 / 41	46 / 42	248 / 232	> 375 / > 375	111 / 98	170 / 142	63 / 53	75 / 62
upstream	aug/03	30		38 / 32	41 / 34	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30
upstream	mei/03	30		23 / 22	24 / 23	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30	19 / 16	20 / 18
upstream	jun/03	30		25 / 54	27 / 56	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30	23 / 30	24 / 30
upstream	jul/03	30		21 / 21	23 / 23	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30	17 / 16	18 / 17
upstream	sep/03	30		52 / 45	54 / 46	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30
upstream	okt/03	30		39 / 37	42 / 40	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30
upstream	nov/03	21	CV for 21	50 / 48	50 / 48	> 21 / > 21	> 21 / > 21	> 21 / > 21	> 21 / > 21	> 21 / > 21	> 21 / > 21
upstream	jan/04	25	CV for 25	- / 97	- / 102	- / > 25	- / > 25	- / > 25	- / > 25	- / > 25	- / > 25
upstream	mrt/04	25	CV for 25	69 / 70	72 / 73	> 25 / > 25	> 25 / > 25	> 25 / > 25	> 25 / > 25	> 25 / > 25	> 25 / > 25
upstream	apr/04	30		33 / 34	34 / 35	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30
upstream	mei/04	30		22 / 22	22 / 23	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30	18 / 17	17 / 18
upstream	jul/04	30		26 / 34	26 / 36	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30	22 / 30	24 / 30
upstream	aug/04	30		20 / 45	20 / 48	> 30 / > 30	> 30 / > 30	> 30 / > 30	> 30 / > 30	13 / 30	14 / 30
upstream	sep/04	30		17 / 16	17 / 16	> 30 / > 30	> 30 / > 30	23 / 20	24 / 21	10 / 9	11 / 9
upstream	okt/04	30		12 / 11	13 / 12	> 30 / > 30	> 30 / > 30	12 / 11	13 / 12	6 / 5	6 / 6
upstream	nov/04	24	CV for 24	29 / 27	30 / 29	> 24 / > 24	> 24 / > 24	> 24 / > 24	> 24 / > 24	21 / 20	> 24 / 23
upstream	jan/05	20	CV for 20	30 / 31	32 / 33	> 20 / > 20	> 20 / > 20	> 20 / > 20	> 20 / > 20	> 20 / > 20	> 20 / > 20

30 plots have been sampled every month upstream the weir (Figure 8). These datasets are treated as full datasets on their own. The coefficients of variation by bootstrapping and CLT for fresh weight as well as for dry weight biomass within the datasets of different seasons are given in table 2. The graphical presentation of the bootstrap output is shown in figure 9, only for fresh weight biomass. The coefficients of variation vary between the seasons. There is a relatively small difference between the coefficients of variation for fresh and dry weight biomass within the same month, except for those in the months of peak biomass of each year, namely August 2004 and June 2003.

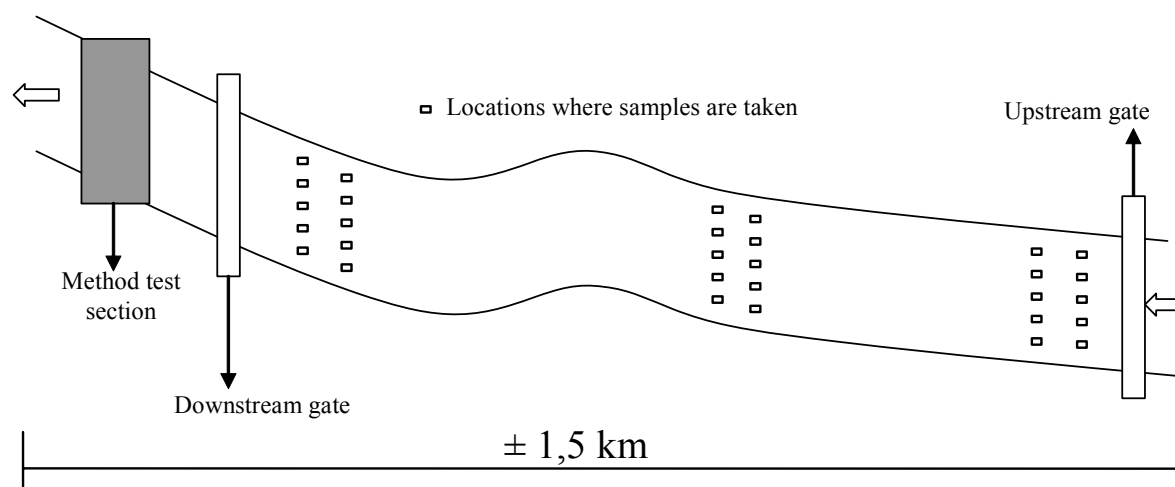


Figure 8: Schematic presentation of the section between the two adjustable weirs on the river Aa.

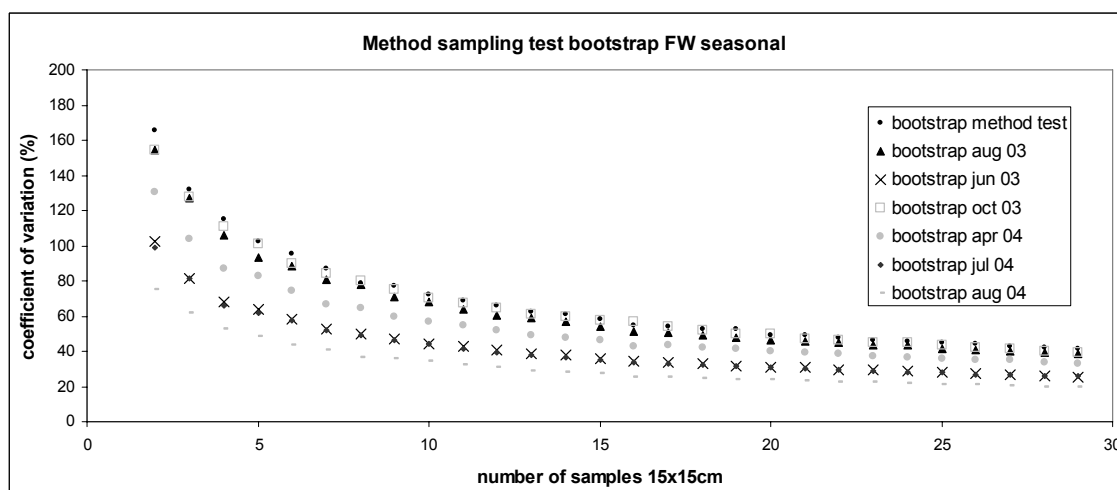


Figure 9: Coefficients of variation generated by bootstrapping fresh weight standing biomass. Results of the method test section are compared with the monthly taken 30 samples of biomass in different seasons.

Comparing the coefficients of variation between the method test section and the samples taken upstream the weir in the same month (august 2003), the maximal difference in coefficients of variation for 30 samples is 9%. The smaller dataset upstream the weir shows a smaller coefficient of variation, namely 38% compared to 45% for fresh weight biomass and 32% compared to 41% for dry weight biomass (by bootstrapping).

Because macrophytes are not equally divided over the width of the river, two scripts without fully ad random sampling are created in S-plus. The output of the script, that divides the river stretch in 5 subblocks, shows that for fresh weight biomass as well as for dry weight biomass, more than 375 samples are needed to obtain a coefficient of variation less than 10% if we assume the dataset is not normally distributed (according to the Shapiro-Wilkinson Normality Test) (Figure 10). 30 samples picking out of the dataset gives a coefficient of variation of 41% for fresh weight biomass and of 42% for dry weight biomass. Selecting the plots in this way does not give a lower coefficient of variation.

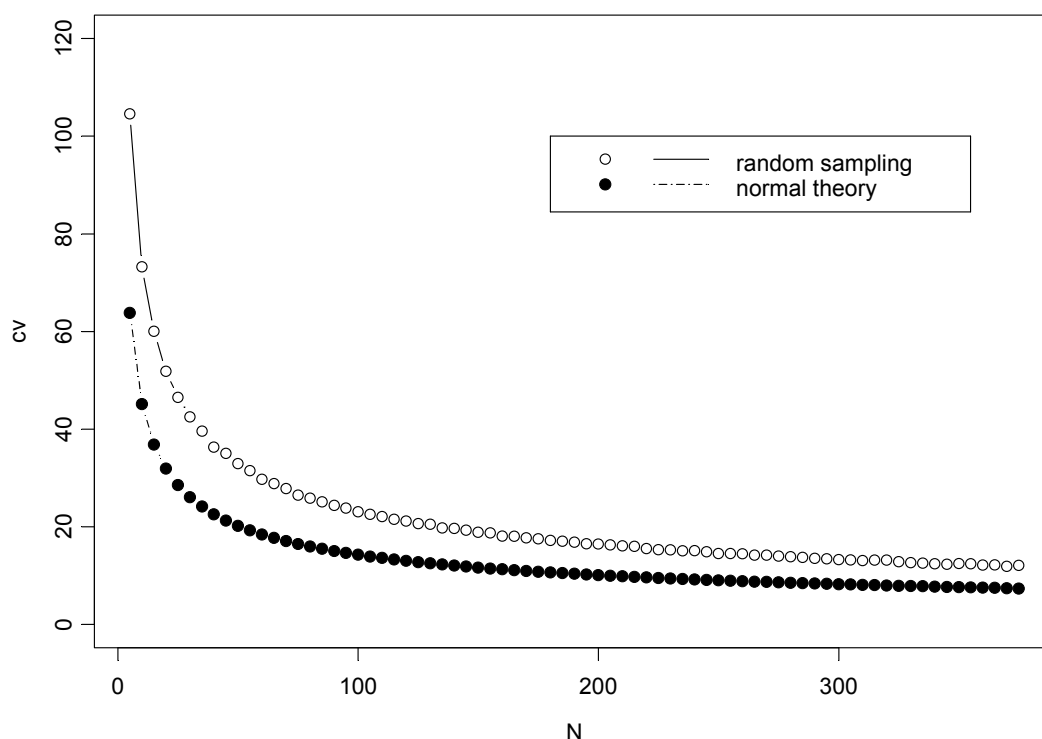


Figure 10: Bootstrap outputs with extra condition factor. Relationship between coefficient of variation in % (CV) and number of samples (N) for fresh weight biomass within the method test section. (◇) "Random sampling": random sampling occurs with equal sampling within the five subblocks. (●) "Normal theory": random sampling with equal sampling within the five subblocks, assuming data are normally distributed.

The dataset was also analysed by bootstrapping and CLT on the different macrophyte species present, namely *Callitriche platycarpa*, *Ceratophyllum demersum*, *Elodea nuttalli*, *Potamogeton natans*, *Potamogeton pectinatus* and *Rorippa amphibia*. Bootstrap and CLT outputs for fresh and dry weight biomass are shown in table 4. *Callitriche platycarpa* never reached a coefficient of variation less than 45%, even if all 375 samples are taken into account. Notably, only for *Callitriche platycarpa*, the CLT gives much higher coefficients of variation than the bootstrapping method (Table 3). *Potamogeton natans* is the macrophyte species with the highest reliability for biomass estimation. Yet, there is a coefficient of variation of 39% if 30 samples are taken within the dataset, which is comparable with the coefficient of variation of the total biomass per plot (Table 2).

Table 3: results of bootstrap and CLT for fresh and dry weight biomass of the different macrophyte species in the method test dataset of 375 samples in august 2003. CV = coefficient of variation (%); CLT = central limit theorem.

CV (%) for fresh and dry weight of macrophytes					dataset aug 03 containing 375 samples			
species	CV (%) for 30 samples		number of samples needed to obtain a CV < 10%		number of samples needed to obtain a CV < 20%		number of samples needed to obtain a CV < 30%	
	bootstrap	CLT	bootstrap	CLT	bootstrap	CLT	bootstrap	CLT
<i>Callitriche platycarpa</i>	241 / 218	249 / 228	> 375 / > 375	> 375 / > 375	> 375 / > 375	> 375 / > 375	> 375 / > 375	> 375 / > 375
<i>Ceratophyllum demersum</i>	74 / 71	77 / 74	> 375 / > 375	> 375 / > 375	210 / 204	> 375 / > 375	130 / 124	203 / 188
<i>Elodea nuttalli</i>	110 / 103	112 / 106	> 375 / > 375	> 375 / > 375	> 375 / > 375	> 375 / > 375	225 / 221	> 375 / > 375
<i>Potamogeton natans</i>	38 / 38	39 / 40	222 / 224	> 375 / > 375	93 / 95	121 / 124	47 / 48	53 / 55
<i>Potamogeton pectinatus</i>	74 / 77	77 / 80	> 375 / > 375	> 375 / > 375	203 / 210	> 375 / > 375	129 / 135	206 / 223
<i>Rorippa amphibia</i>	171 / 171	178 / 178	> 375 / > 375	> 375 / > 375	> 375 / > 375	> 375 / > 375	> 375 / > 375	> 375 / > 375

Table 4: results of bootstrap and CLT for fresh and dry weight biomass of different plot sizes in the method test dataset of 74 samples in august 2003. The number of samples within the dataset is reduced because of the creation of bigger plots. CV = coefficient of variation (%); CLT = central limit theorem.

CV (%) for fresh/dry weight for different plot sizes					dataset aug 2003 (reduced to 74 samples)			
plot size	CV (%) for 30 samples		number of samples needed to obtain a CV < 10%		number of samples needed to obtain a CV < 20%		number of samples needed to obtain a CV < 30%	
	bootstrap	CLT	bootstrap	CLT	bootstrap	CLT	bootstrap	CLT
15x15cm	40 / 38	41 / 39	> 74 / > 74	> 74 / > 74	> 74 / > 74	> 74 / > 74	52 / 43	59 / 48
15x30cm	31 / 27	32 / 28	> 74 / > 74	> 74 / > 74	70 / 57	> 74 / 63	32 / 26	35 / 28
30x30cm	30 / 27	31 / 28	> 74 / > 74	> 74 / > 74	72 / 57	> 74 / 61	32 / 26	34 / 27

Every plot or sample has a magnitude of 15cm by 15cm. Imaginary plots with a size of 15cm by 30cm were made by adding the biomass of adjacent plots such that a new dataset is created. Also, a dataset with imaginary plots of 30cm by 30cm has been created. Doing this, the number of samples has declined compared to the original dataset. Datasets with bigger, imaginary plots show lower coefficients of variation than the original dataset (Table 4).

The coefficients of variation between two successive months and between every other month are summarized in table 5a. Coefficients of variation between two successive months are not always higher than the internal variation (Table 2) and coefficients of variation between every other month are not always higher than the variation between two months. Table 5b summarizes the p-values obtained by pairwise comparison of biomass averages between two successive months and between every other month. There are few significant changes in biomass in two successive months except in the outset of the growing season. Looking at p-values of every other month, more significant changes in biomass can be observed, although not spectacular.

Table 5: (a) Coefficients of variation (CV) of mean biomass between different months in 2003, 2004 and January 2005 for fresh weight (FW) as well as for dry weight (DW) between (a1) two successive months and (a2) every other month. (b) P-values obtained by pairwise comparison (ANOVA) of mean biomass between different months in 2003, 2004 and January 2005 for fresh weight (FW) as well as for dry weight (DW) between (b1) two successive months and (b2) every other month. P-values in bold point out a significant difference in biomass between the respective months with $\alpha = 0,05$.

(a1) CV FW/DW (%)	2003	2004
MARCH - APR	-	32 / 40
APR - MAY	-	103 / 93
MAY - JUNE	70 / 83	4 / 20
JUNE - JULY	32 / 71	-
JULY - AUG	2 / 24	34 / 53
AUG - SEP	5 / 2	24 / 62
SEP - OCT	88 / 76	19 / 10
OCT - NOV	11 / 6	10 / 10
(a2) CV FW/DW (%)	2003	2004
JAN - MARCH	-	141 / 127
FEB - APRIL	-	141 / 117
MARCH - MAY	-	85 / 65
APRIL - JUNE	-	105 / 103
MAY - JULY	43 / 17	4 / 20
JUNE - AUG	34 / 87	34 / 53
JULY - SEP	7 / 26	11 / 12
AUG - OCT	91 / 78	42 / 70
SEP - NOV	94 / 72	9 / 1
NOV - JAN	141 / 113	64 / 58

(b1) p-value FW/DW	2003	2004
MARCH - APR	-	< 0,01 / 0,01
APR - MAY	-	< 0,01 / 0,09
MAY - JUNE	0,03 / 0,14	0,90 / 0,72
JUNE - JULY	0,39 / 0,27	-
JULY - AUG	0,44 / 0,22	0,06 / 0,13
AUG - SEP	0,56 / 0,57	0,35 / 0,21
SEP - OCT	0,11 / 0,11	0,53 / 0,80
OCT - NOV	0,79 / 0,96	0,70 / 0,68
(b2) p-value FW/DW	2003	2004
JAN - MARCH	-	0,85 / 0,80
FEB - APRIL	-	< 0,01 / 0,01
MARCH - MAY	-	< 0,01 / < 0,01
APRIL - JUNE	-	< 0,01 / 0,11
MAY - JULY	0,13 / 0,51	0,90 / 0,72
JUNE - AUG	0,14 / 0,07	0,06 / 0,13
JULY - SEP	0,17 / 0,08	0,25 / 0,53
AUG - OCT	0,02 / 0,02	0,12 / 0,16
SEP - NOV	0,12 / 0,16	0,42 / 0,57
NOV - JAN	0,03 / 0,03	0,02 / 0,03

IV.4. Discussion

There is little knowledge in which way macrophytes can be sampled appropriately to obtain reliable biomass data although many researchers work with it as a tool for in situ research experiments in rivers (Carr & Chambers, 1998; Kaenel et al, 2000; Owens et al., 2001) and ponds or lakes (Chambers et al., 2001; Woolf & Madsen, 2003; Strand & Weisner, 2001). To assess the biomass density, one must have an idea of all growing macrophytes in a river system. This comprises macrophytes

rooted immediately next to the banks, macrophytes that grow in deeper parts of the river but also the stream gullies where no macrophyte species are found.

Analysis of biomass of macrophytes has been done in a lot of lakes in Florida (Canfield et al, 1990). The biomass was sampled between July and September. They arrive typically at more than 200 samples to estimate the mean macrophyte standing crop with a permissible error of 10%. Our results for a river are in the same order, namely 227 and 212 samples for respectively fresh and dry weight standing biomass. This research project has tried to quantify biomass amounts of macrophytes in order to obtain reliable estimation of a river stretch. The method analysis shows that a high number of samples of 15cm by 15cm are needed to obtain a coefficient of variation less than 10%. In practice, this kind of monitoring is infeasible. Even if a coefficient of variation less than 20% would be accepted, monitoring would still be impracticable. Dividing the method test section in five subblocks does not ensure a lower coefficient of variation. A precise estimation of biomass in a river is only possible if a numerous number of samples will be taken.

Biomass density also differs a lot according to the different macrophyte species. Some species prefer to occur in dense patches like *Callitriche* sp. (Sand-Jensen et al, 1999), others (*Elodea nuttallii*, *Ceratophyllum demersum* & *Potamogeton pectinatus*) comprise a large volume of the water and still others (*Potamogeton natans*) form a big carpet of floating leaves on the water surface which results in a lower biomass. Compared to the number of samples for total biomass, still, a larger number of samples is needed to have an idea of the density of each species separately. The only macrophyte species in the lowland river Aa that provides similar results as the total biomass is *Potamogeton natans*. 222 and 224 samples of respectively fresh and dry weight are needed to get a coefficient of variation < 10%. This is due to its high abundance in the Aa.

The way of monitoring the river stretch by taking 30 samples of 15cm by 15cm each month, as it is done in the Aa, doesn't seem to be very useful, especially from June on (Table 2 and 5). The variation within each month is quite high so that it is impossible to distinguish whether the variation between two successive months is due to the variation within a month or between two months. Besides, significant difference in mean biomass of macrophytes was only discovered in the beginning of the growing season of both 2003 and 2004. If a significance level of $\alpha = 0,1$ is accepted, just one additional significant difference of mean fresh weight biomass between July 2004 and August 2004 could be detected.

Coefficients of variation are much higher every other month. Still, it is not always clear which kind of variation is the dominant factor. Also, the p-values didn't indicate significant changes in macrophyte biomass. Taking samples every other month in this way is still not relevant enough to follow up the biomass of growing macrophytes. However, by sampling biomass every other month, the time of peak biomass – which

can vary between June and August – could be missed. Anyway, it is useful to know the month of peak biomass in a year. Within this period, sampling every month can be very advantageous.

Taking bigger plot sizes of biomass can offer a possible solution. In table 4, it's obvious that plot sizes of 15cm by 30cm already provide a 10% reduction in variation. Still bigger plot sizes, like 30cm by 30cm did no longer contribute to lower coefficients of variation.

V. Macrophyte distributions in the Nete catchment

V.1.Introduction

The occurrence of macrophytes is natural and widespread, both in lakes (Cook, 1974; Ward et al., 1987; Makarewicz & Dilcher, 1988; Kiersch et al., 2004) and in rivers (Cook, 1974; Haslam, 1978; Madsen & Adams, 1989). Macrophytes exist in different forms: species can be totally submerged, floating or emergent (Haslam, 1978). Their presence is determined by a combination of environmental factors such as habitat (lake or river), width, depth, available nutrients in surface water and sediment, substratum, stream velocity, competitive factors, etc. (Roelofs & Bloemendaal, 1988; Cedergreen, 1999; Heegaard et al., 2001; Takamura et al., 2003). Investigation of the variables explaining the occurrence of macrophytes has mainly focused on lakes. Nevertheless, knowledge about environmental factors influencing the presence of macrophytes in rivers will be required by the Water Framework Directive (WFD) (Council, 2000) to develop a method of measuring the ecological status of surface waters. Alkalinity seems to be an important factor in determining macrophyte communities in a lot of studied areas (Wiegand, 1984; Robach et al., 1996; Sabbatini et al., 1998; Riis et al., 2000; Dodkins et al., 2005). Also width and depth are considerable environmental variables. This positive evolution is the main reason why we want focus on the distribution of macrophyte species in the Nete basin and more specifically, on the environmental conditions needed for the most abundant macrophyte species recorded.

V.2.Material and methods

V.2.1. Macrophyte inventory

The Nete catchment, a subcatchment of the Schelde basin, is located in the north east of Belgium (Figure 1). Within this catchment, hundred eleven non-shaded sections from smaller as well as bigger rivers were selected. Each section of a river was divided into 5 parts with a length of 10 m where a list of macrophyte species was made. This was done by wading in the water – if feasible – and searching carefully for possible species. In half of the 100 locations, an inventory was made as well as in springtime (June 2003) as in autumn (October 2003). The other half of the inventory was made in May-June 2004 and in October 2004. The cover percentage of each macrophyte species was estimated in each section of 10 m. 44 locations belong to the Grote Nete subbasin, 47 locations belong to the Kleine Nete subbasin and 20 locations belong the Aa subbasin.

V.2.2. Abiotic variables

Water quality on the hundred locations was measured six times in the same year as the inventory, namely every other month in 2003 (January, March, May, July, September and November) for the first half of the locations and in the same months

in 2004 for the other half. For each sample, a bucket with a rope is used to sample the surface water of the river of interest. Oxygen, temperature, pH and conductivity were directly measured in the bucket with a WTW multimeter. After that, a Winkler bottle and a normal bottle was filled and transported to the laboratory. BOD was analysed using the Winkler procedure. N-NH_4^+ , N-NO_2^- , N-NO_3^- , P-PO_4^{3-} , Cl^- , SO_4^{2-} , SiO_2 and CO_2 were analysed with a SKALAR segmented flow analyser whereas cations (Na^+ , K^+ , Ca^{2+} and Mg^{2+}) and metal ions (Al^{3+} , As^{2+} , Ba^{2+} , Cd^{2+} , Co^{2+} , Cr^{2+} , Cu^{2+} , Fe^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} and Zn^{2+}) were measured on an ICP THERMO type iris/CID with radial plasma.

Stream velocities at all locations were measured with an electromagnetic flow meter and were carried out two times before the growing season, namely in March 2004 and in March 2005.

In March and April 2005, 4 sediment core samples of the top 20 cm were taken at each location with a Wildcore sediment sampler. These fresh samples were analysed in the laboratory the day after sampling for the parameters pH, N-NH_4^+ , N-NO_2^- , N-NO_3^- and P-PO_4^{3-} . After drying the samples at 104°C for 48 hours, grain size, organic content, total N, total P and percentage dry weight were determined. Grain size of the samples is expressed in percentage silt ($< 63 \mu\text{m}$), fine sand ($63 \mu\text{m} < x < 250 \mu\text{m}$) and coarse sand ($> 250 \mu\text{m}$). Grain size was analysed with a laser diffractor system (Mastersizer S, Malvern Instruments).

V.2.3. Analysis: initial ordinations

The species table (94 sites $\times$ 50 species) was processed to a centred Principal Component Analysis (PCA) (Pearson, 1901) which allows to conserve own species variability on ecological gradients so that to better take account for species specific responses to environmental features.

Potentially influential environmental variables were selected by Pearson's correlations with the first significant axis of the flora analysis. Then, the selected set of variables was ordinated with a standardized PCA (Hotelling, 1933).

After describing the different ecological gradients in the two compartments, a co-inertia analysis (Dolédéc & Chessel, 1994) was performed to investigate the relationship between environmental and floristic structures. This analysis constructs a system of axis maximizing the sum of square covariances between the variables of the two transformed data tables (co-inertia), and by this way, axes maximize the common ecological parts between the two tables. A randomisation procedure was performed to test the significance of the relationship between the two tables, which is assessed by the vectorial correlation (R_v) (Robert & Escoufier, 1976). Based on 9999 permutations of the rows (sites) of the two tables, total co-inertia is computed at each iteration. The significance of the relationship is assessed by the probability to

observe a greater co-inertia than the observed one under the null hypothesis (out of a total of 9999 simulated + 1 observed replicates).

Computations and associated graphical representations were implemented using the *ade4* package available in *R* freeware (Ihaka & Gentleman, 1996).

V.3.Results

Figure 11 and table 6 show the macrophyte species present in the Nete catchment and in its three subbasins for two different inventarisation periods, namely spring and autumn.

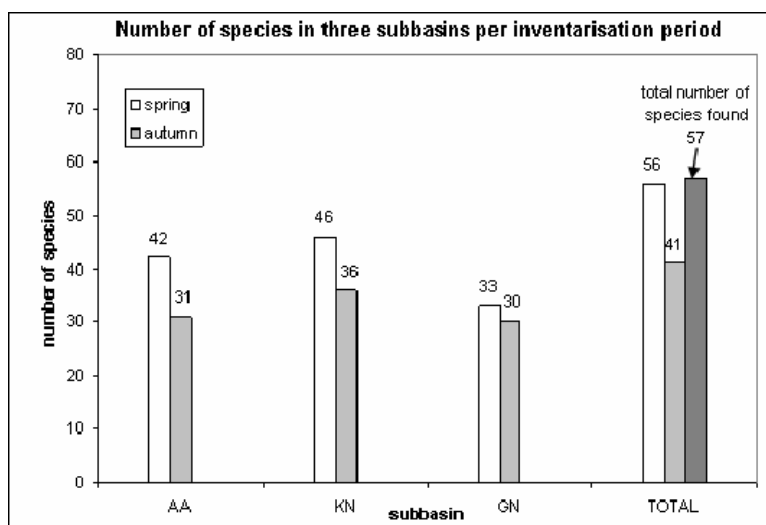


Figure 11: number of species in the three subbasins per season. AA = subbasin of the Kleine Nete subbasin; KN = Kleine Nete subbasin; GN = Grote Nete subbasin; Total = total number of species in the whole Nete catchment in spring and autumn.

The Kleine Nete basin comprised the highest diversity for macrophyte species whereas the Grote Nete basin has the lowest diversity, both in spring and in autumn. Total number of species found in the Nete catchment was higher than in each subbasin separately which might indicate that the three subbasins hold different macrophyte species. Total number of species found in both seasons was 57 but no endangered species were found. Species diversity was always lower in autumn than in spring.

Table 6: list of macrophyte species in the Nete catchment

Code	Macrophyte species in the Nete Catchment	
ALISMPLA	<i>Alisma plantago-aquatica</i>	L.
ALOPEGEN	<i>Alopecurus geniculatus</i>	L.
ALOPEPRA	<i>Alopecurus pratensis</i>	L.
BERULERE	<i>Berula erecta</i>	(Hudson) Coville
CALLIPLA	<i>Callitriche platycarpa</i>	Kuetzing
CALLISTA	<i>Callitriche stagnalis</i>	Scopoli
CARDAMA	<i>Cardamine amara</i>	L.
CAREXRIP	<i>Carex riparia</i>	Curtis
CERATDEM	<i>Ceratophyllum demersum</i>	L.
ELEOCPAL	<i>Eleocharis palustris</i>	(L.) Roemer & Schultes
ELEOCP-P	<i>Eleocharis palustris subsp. palustris</i>	
ELODECAN	<i>Elodea canadensis</i>	Michaux
ELODENUT	<i>Elodea nuttallii</i>	(Planchon) St.John
EPILOHIR	<i>Epilobium hirsutum</i>	L.
EQUISFLU	<i>Equisetum fluviatile</i>	L.
EQUISPAL	<i>Equisetum palustre</i>	L.
EUPATCAN	<i>Eupatorium cannabinum</i>	L.
GLYCE-SP	<i>Glyceria sp.</i>	R.Br.
GLYCEMAX	<i>Glyceria maxima</i>	(Hartman) Holmberg
HOLCULAN	<i>Holcus lanatus</i>	L.
HOTTOPAL	<i>Hottonia palustris</i>	L.
HYDRORAN	<i>Hydrocotyle ranunculoides</i>	
IRIS_PSE	<i>Iris pseudacorus</i>	L.
JUNCUEFF	<i>Juncus effusus</i>	L.
LEMNAMIN	<i>Lemna minor</i>	L.
LYCOPEUR	<i>Lycopus europaeus</i>	L.
LYSIMNUM	<i>Lysimachia nummularia</i>	L.
LYSIMVUL	<i>Lysimachia vulgaris</i>	L.
LYTHRSAL	<i>Lythrum salicaria</i>	L.
MENTHAQU	<i>Mentha aquatica</i>	L.
MYOSOL-C	<i>Myosotis laxa (subsp. cespitosa)</i>	(Schultz) Nordh.
MYRIOSPI	<i>Myriophyllum spicatum</i>	L.
NUPHALUT	<i>Nuphar lutea</i>	(L.) J.E.Smith
NYMPHALB	<i>Nymphaea alba</i>	L.
PEUCEPAL	<i>Peucedanum palustre</i>	(L.) Moench
PHALAARU	<i>Phalaris arundinacea</i>	L.
PHRAGAUS	<i>Phragmites australis</i>	(Cavanilles) Steudel
POLYNAMP	<i>Polygonum amphibium</i>	L.
POLYNHYD	<i>Polygonum hydropiper</i>	L.
POLYNPER	<i>Polygonum persicaria</i>	L.
POTAMCRI	<i>Potamogeton crispus</i>	L.
POTAMLUC	<i>Potamogeton lucens</i>	L.
POTAMMUC	<i>Potamogeton mucronatus</i>	Sonder
POTAMNAT	<i>Potamogeton natans</i>	L.
POTAMTRI	<i>Potamogeton trichoides</i>	Chamisso & Schlechtendal
RANUNP:H	<i>Ranunculus peltatus var. heterophyllus</i>	(Cosson & Germ.) Meijden
RANUNREP	<i>Ranunculus repens</i>	L.
RANUNSCE	<i>Ranunculus sceleratus</i>	L.
RORIPAMP	<i>Rorippa amphibia</i>	(L.) Besser
RUMEXHYD	<i>Rumex hydrolapathum</i>	Hudson
SAGITSAG	<i>Sagittaria sagittifolia</i>	L.
SOLANDUL	<i>Solanum dulcamara</i>	L.
SPARGEME	<i>Sparganium emersum</i>	Rehmann
SPARGERE	<i>Sparganium erectum</i>	L.
STUCKPEC	<i>Stuckenia pectinatus</i>	(L.) Boerner
SYMPHOFF	<i>Symphytum officinale</i>	L.
TYPHALAT	<i>Typha latifolia</i>	L.

Macrophyte species with the highest average abundance in spring and with at least an average abundance of 5 % were *Stuckenia pectinatus* > *Sparganium emersum* > *Potamogeton natans* > *Callitriche platycarpa* > *Potamogeton trichoides* > *Sagittaria sagittifolia* > *Phalaris arundinacea* > *Ranunculus peltatus var. heterophyllus* > *Glyceria maxima* and other *Glyceria* species (Figure 12). In autumn, following order of abundance of macrophyte species was recorded: *Potamogeton natans* >

Callitriche platycarpa > *Phalaris arundinacea* > *Glyceria maxima* > *Sparganium emersum* > *Phragmites australis* > *Sparganium erectum* > *Ranunculus peltatus* var. *heterophyllus* > *Ceratophyllum demersum* > *Elodea nuttallii* > *Sagittaria sagittifolia* and *Glyceria* species (Figure 12). So, typical spring macrophytes are *Stuckenia pectinatus*, *Potamogeton trichoides*, *Sagittaria sagittifolia* and *Sparganium emersum*. In autumn, other species like *Ceratophyllum demersum*, *Elodea nuttallii* and *Polygonum hydropiper* came into prominence. *Potamogeton natans*, *Callitriche platycarpa*, *Phalaris arundinacea* and *Glyceria maxima* are species with similar abundance in both seasons.

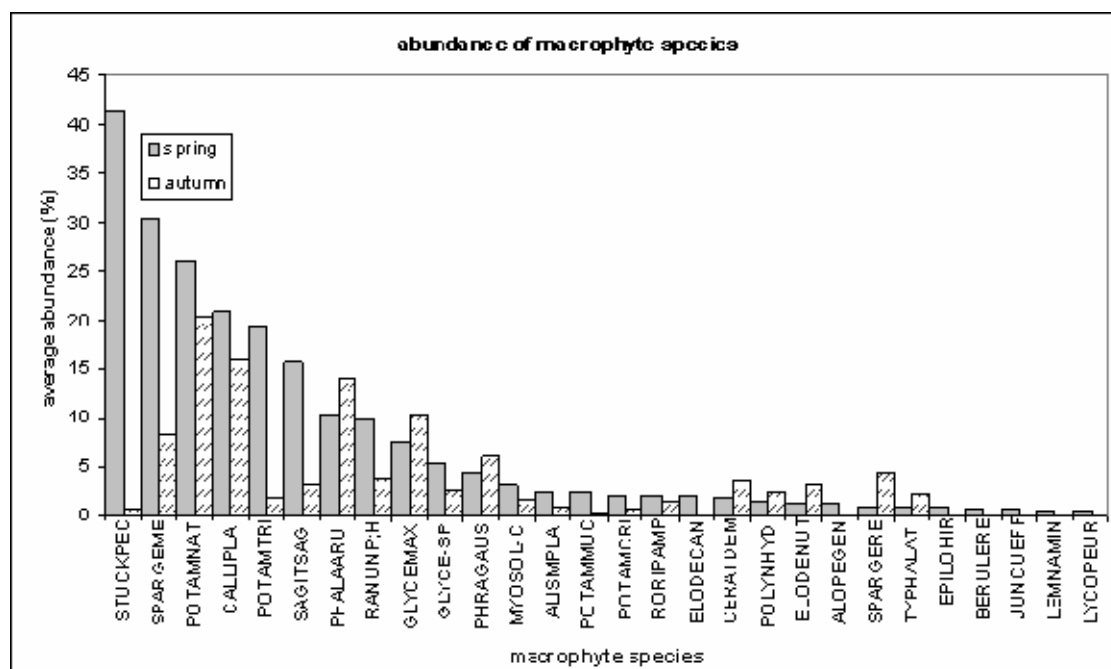


Figure 12: average abundance (expressed in %) of the main macrophyte species in the Nete catchment for two inventarisation periods (i.e. spring and autumn)

However, vegetation analysis with Twinspan resulted in a similar output for spring and autumn, so macrophyte vegetation was grouped irrespective of the inventarisation period. A centred PCA-output is shown in figure 13. Most species are located in the centre of the graph due to the dominance of some macrophyte species which are *Stuckenia pectinatus*, *Potamogeton natans*, *Potamogeton trichoides*, *Sagittaria sagittifolia*, *Sparganium emersum*, *Callitriche platycarpa*, *Ranunculus peltatus* var. *heterophyllus*, *Myosotis laxa* (subsp. *Cespitosa*), *Glyceria maxima*, *Phalaris arundinacea* and other *Glyceria* species.

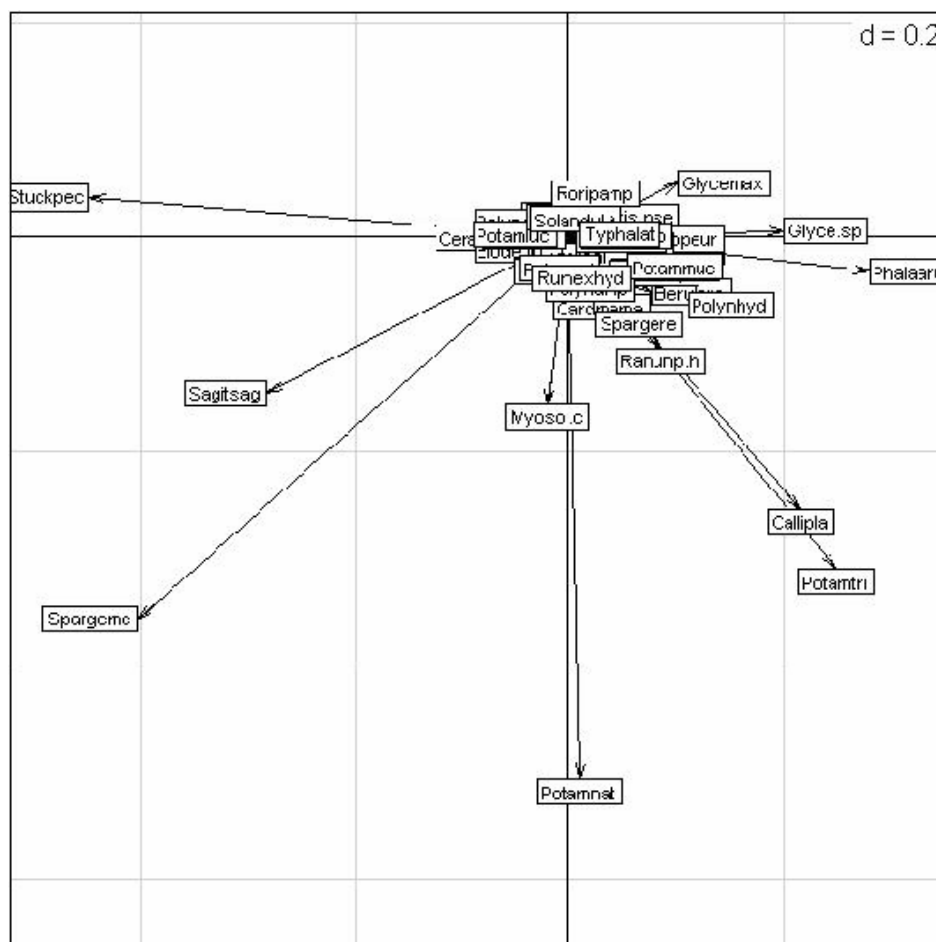


Figure 13: PCA-analysis (centred) of macrophyte vegetation.

Codes of species can be found in table 6.

Table 7 showed all Pearson correlation coefficients of the measured abiotic variables with the three species related PCA axes. Variables with significant correlations were used for co-inertia analysis, i.e. to find maximum correlation between species ordination and ordination of abiotic variables. Almost three quarters of the variation was explained by the first two axes (62% by the first axis, 12% by the second axis). Coinertia R_v statistic amounts 0.43 and was significant with a p -value < 0.0001 . From table 7, it is remarkable that sediment related variables were less explicative than water and morphology related variables to explain the macrophyte species occurrence. Depth and width of the river play a major role. Also heavy metal concentrations in surface water (Al, As, Ba, Cr, Cu, Fe, Ni, Pb and Zn) might play an important role as they present high correlations with the first (and/or the second) species PCA-axis. Except for Cr and Ni, all other heavy metals were positively correlated with the first and/or second axis.

Table 7: Pearson correlation coefficients of the environmental variables with the PCA-axis of the macrophyte species ordination. S- in front indicates a sediment related variable, whereas W- indicates a surface water related variable. DM = dry matter; S-fs = fine sand; S-cs = coarse sand; S-M = median of grain size variable; S-N = total nitrogen; S-OM = organic material; S-P = total phosphorus; S-sl = silt; S-W-H₂O = proportion wet over dry material; W-T = water temperature; SM = suspended material

	*** p < 0,0001	** p < 0,001	* p < 0,05
	PCA-AXIS 1 Pearson correlation	PCA-AXIS 2 Pearson correlation	PCA-AXIS 3 Pearson correlation
depth	-0.65 ***	-0.10	0.19
width	-0.54 ***	-0.31 *	0.08
velocity	-0.03	-0.25 *	-0.15
DM (%)	0.06	-0.09	-0.13
S-fs	-0.25 *	-0.30 *	0.07
S-cs	0.12	0.01	-0.25 *
S-M	0.01	-0.11	-0.16
S-N	-0.09	0.01	0.09
S-N-(NO ₂ ⁻ + NO ₃ ⁻)	-0.12	-0.07	0.25 *
S-N-NH ₄ ⁺	-0.02	0.34 **	0.11
S-OM	-0.14	0.07	0.23 *
S-P	-0.08	0.23 *	0.17
S-pH H ₂ O	0.05	0.13	-0.07
S-pH KCl	0.13	0.03	-0.08
S-P-PO ₄ ³⁻	-0.02	0.22 *	-0.07
S-sl	-0.05	0.11	0.28 *
S-W-H ₂ O	-0.11	0.06	0.11
W-BOD	0.29 *	0.30 *	0.00
W-Ca	0.00	0.14	-0.29 *
W-Cl ⁻	-0.11	0.06	-0.21 *
Alkalinity	-0.06	0.11	-0.31 *
W-Cond	-0.10	0.10	-0.30 *
W-K ⁺	0.14	0.18	-0.17
W-Mg ²⁺	-0.01	-0.11	-0.18
W-N-(NO ₂ ⁻ + NO ₃ ⁻)	-0.11	-0.17	-0.22 *
W-Na ⁺	-0.25 *	-0.03	-0.25 *
W-N-NH ₄ ⁺	0.31 *	0.42 ***	0.10
W-N-NO ₂ ⁻	-0.01	0.21 *	-0.16
W-N-NO ₃ ⁻	-0.16	-0.18	-0.22 *
W-O ₂	-0.36 **	-0.49 ***	0.02
W-O ₂ %	-0.43 ***	-0.47 ***	-0.01
W-pH	-0.47 ***	-0.16	-0.22 *
W-P-PO ₄ ³⁻	0.27 *	0.34 **	-0.03
W-SiO ₂	0.50 ***	0.38 **	0.14
W-SO ₄ ²⁻	0.02	0.02	-0.07
W-T	-0.60 ***	0.03	-0.19
SM	0.47 ***	0.36 **	0.23 *
W-Al	0.43 ***	-0.09	0.05
W-As	0.54 ***	0.08	0.01
W-Ba	0.51 ***	0.31 *	0.05
W-Cd	-0.20	0.06	0.04
W-Co	0.11	-0.18	0.05
W-Cr	-0.60 ***	-0.01	-0.28 *
W-Cu	0.45 ***	0.35 **	-0.02
W-Fe	0.33 **	0.32 *	0.30 *
W-Mn	0.12	0.14	0.14
W-Ni	0.03	-0.32 *	0.07
W-Pb	0.44 ***	0.12	0.31 *
W-Zn	0.41 ***	0.31 *	0.08

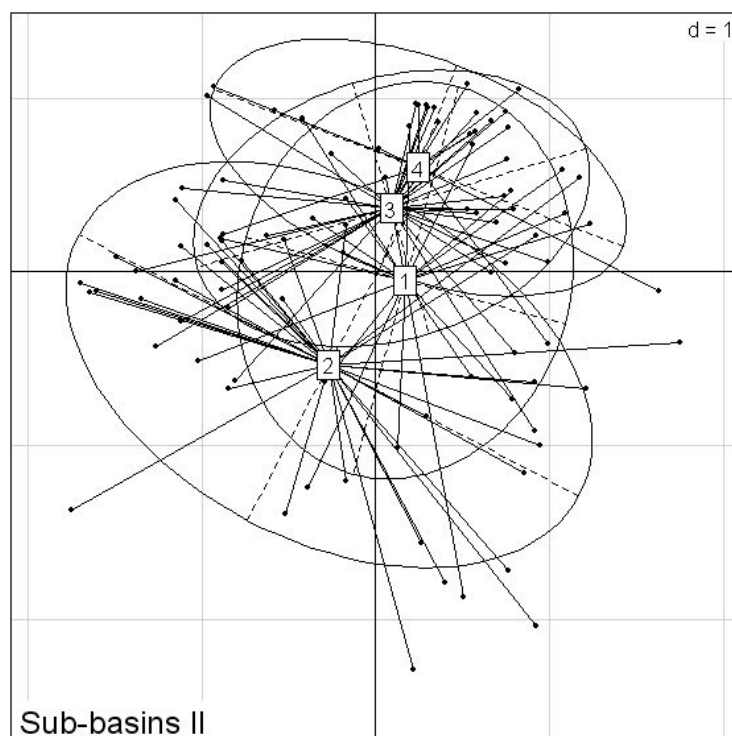


Figure 14: classification of the subbasins based on macrophyte species occurrence. 1 = subbasin of the Kleine Nete (upstream of the mouth of the Aa in the Kleine Nete); 2 = subbasin of the Aa; 3 = subbasin of the Grote Nete; 4 = subbasin of the Kleine Nete (downstream of the mouth of the Aa in the Kleine Nete). Numbers are put in the centre of the corresponding ellipses.

Ellipses in figure 14 mark out the different subbasins which are superposed according to macrophyte species orientation. The Aa subbasin has the highest range of macrophyte species whereas the Kleine Nete subbasin downstream of the mouth of the Aa and the Grote Nete subbasin consist of other vegetation groups with a lower range. The Kleine Nete subbasin upstream of the mouth of the Aa is comparable with the Aa subbasin.

V.4.Discussion

The Nete basin is still a quite eutrophic system which might be the reason that no endangered species were found. Some species, which are very dominant in the Nete catchment, are also very dominant in other eutrophic European lowland rivers in Denmark (Madsen & Adams, 1989; Riis et al., 2000; Baattrup-Pedersen et al., 2002; Pedersen et al., 2006), in the UK (Demars & Harper, 2005) and in France (Bernez et al., 2004). These species are *Potamogeton natans*, *Stuckenia pectinatus*, *Sparganium emersum*, *Sagittaria sagittifolia*, *Elodea* species and *Callitriche* species. No significant differences could be detected in species communities between spring and autumn, which might indicate that no drastic changes occur in macrophyte

evolution during a season. However, there are some indications that e.g. *Stuckenia pectinatus*, *Potamogeton trichoides* and *Sparganium emersum* are typical abundant in spring whereas species like e.g. *Elodea nuttallii* and *Ceratophyllum demersum* become abundant from September onwards (Figure 12).

From the 49 environmental variables measured, width and depth are some of the most descriptive variables in rivers. This is in accordance with several other authors (Riis et al., 2000; Barendregt & Bio, 2003; Williams et al., 2003). Next to that, other variables play a role in predicting macrophyte occurrence. Surprisingly, heavy metal concentrations in the surface water were remarkable determinators for species occurrence. Our study reveals also that pH, oxygen level and water temperature are highly correlated with the PCA-axes. However, caution should be taken to interpret oxygen and water temperature measurements as these variables were successively taken on the different locations and these can fluctuate considerably during the day. Nevertheless, oxygen, pH and water temperature were also found to be predictors by other authors (Dawson & Szoszkiewicz, 1999). Finally, the second gradient of the PCA axis is determined by some nutrient related variables (W-N-NH_4^+ , W-P-PO_4^{3-} , S-N-NH_4^+ , S-P , S-P-PO_4^{3-} and W-N-NO_2^-). Our study indicates that phosphorus in the surface water was more strongly associated with macrophyte species then sediment related phosphorus which is consistent with (Dawson & Szoszkiewicz, 1999) but inconsistent with (Ali et al., 1999; Demars & Harper, 2005). Nitrogen related variables were less important then phosphorus related variables. This hints that the lowland rivers of our study are phosphorus limited which is mostly the case in river ecosystems (Mainstone & Parr, 2002). Only N-NH_4^+ , from both water and sediment, plays a minor role, probably because water plants have an uptake preference for N-NH_4^+ uptake.

Sediment composition, soil organic matter (SOM), conductivity and alkalinity turned out to be not important to clarify species occurrence in the Nete catchment. Because the basin is a non-calcareous lowland area, this might indicate that carbon availability is not limiting in contrast to other European lowland areas (Dawson & Szoszkiewicz, 1999; Riis et al., 2000; Demars & Harper, 2005).

Some dominant macrophyte species have clear preference habitat characteristics. *Stuckenia pectinatus* e.g., is closely correlated with the first PCA-axis which is translated in following attributes: *Stuckenia* prefers deeper and wider lowland rivers and seems to be sensible for most metals (Al, As, Ba, Cu, Pb, Zn and Fe) except for chrome. Occurrence of *Stuckenia* is also determined by high oxygen, high pH, low suspended material, a high percentage of fine sand, low BOD, low W-P-PO_4^{3-} and low N-NH_4^+ . *Potamogeton natans* is mainly associated with the second PCA-axis, but still a lot of its correlated environmental variables are the same as the first axis. As a result, *Potamogeton natans* also seems to be sensible for some metals (Ba, Cu, Zn and Fe) except for nickel. Just as *Stuckenia*, this species is determined by a high

oxygen level, low suspended material, a high percentage of fine sand, low BOD, low $W-P-PO_4^{3-}$ and low $N-NH_4^+$. Additionally, it also prefers low nutrient levels of $S-N-NH_4^+$, $S-P$, $S-P-PO_4^{3-}$ and $W-N-NO_2^-$. *Sparganium emersum* and in a lesser extent *Sagittaria sagittifolia* exhibit the same features from both former species which is confirmed by (Pedersen et al., 2006). *Glyceria species* and *Phalaris arundinacea* are amphibious macrophyte species which are strongly associated with smaller streams (more narrow and less deep). For the rest, they have opposite habitat preferences compared to real water plants such as *Stuckenia pectinatus*, *Sparganium emersum*, *Potamogeton natans* and *Sagittaria sagittifolia*.

VI. The role of macrophytes on the C, N, P and O₂ cycling

VI.1. The role of macrophyte biomass in nutrient assimilation in the lowland river Aa.

VI.1.1. Introduction

In this paper, the evolution of biomass and different macrophyte species was evaluated monthly during a three year survey (2003-2005) in an unmanaged 1.5 km long section of a Belgian lowland river (the Aa). Secondly, nutrient assimilation (N, P and C) by macrophyte species and total nutrient stock in the study section was followed up between seasons during the first two years.

VI.1.2. Material and methods

Site description

Measurements were carried out in the test river stretch of the river Aa described previously (Figure 2).

Biomass sampling and nutrient analysis

Every month from May 2003 till November 2005, 30 samples of aboveground biomass were taken in the studied section except for December 2003, February 2004, December 2004, February 2005, March 2005, December 2005 where no samples were taken because of too cold water temperatures and/or high water levels. For the same reasons, less than 30 samples were taken in some months, namely November 2003 (21 samples), January 2004 (25 samples), March 2004 (25 samples), November 2004 (24 samples) and January 2005 (20 samples). In case of 30 samples, one third was taken at the upstream weir, one third in the middle and one third at the downstream weir (Figure 2). In the other case, some or all samples in the middle were skipped. Samples were taken by diving and cutting the aboveground biomass in a plot of 15 x 15 cm, marked out by the surber and dry weight was determined for each species as described in chapter IV. Additionally, samples were mixed and N and P content in the plant tissue were analysed following the procedure of (Walinga et al., 1989). C concentrations were measured with a C/N analyser. Mean water quality parameters of the Aa are known from monitoring campaigns during the period 2003-2004 and are described in Chapter VI.3.

Statistical analysis

Normally distributed (with or without transformation) data were analysed with ANOVA followed by a post-hoc Tuckey-test. Kruskal-Wallis ANOVA was used as non parametric test and when significant, it was followed by an unpaired Mann-Whitney U test comparing sample groups two by two.

VI.1.3. Results

Biomass

In figure 15, the monthly biomass evolution is presented during the three year survey in the study section. Dry weight biomass in the study section increases significantly from 210 g m⁻² in 2003 to 283 g m⁻² in 2005. Mann-Whitney U test showed differences in dry weight biomass between all years (table 8).

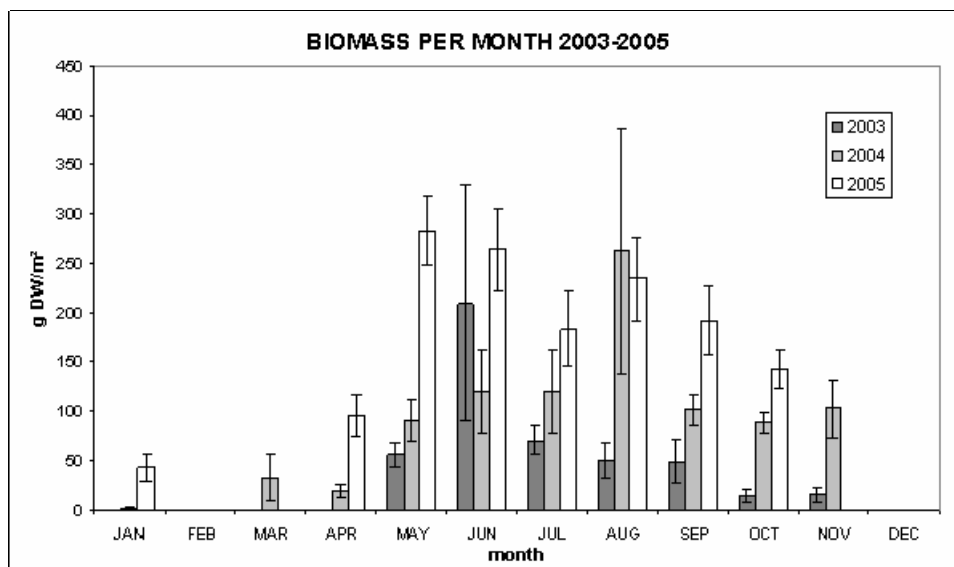


Figure 15: Average biomass per month (g m⁻²) starting in May 2003 until November 2005 with bars indicating standard errors. Due to high water levels or too cold water temperatures, no samples were taken in December 2003, February 2004, December 2004, February 2005 and March 2005.

Table 8: mean dry weight (DW) in g m⁻² per year and standard error. On the right: p-values between years obtained by the Mann-Whitney U-test

	mean DW (g m ⁻²)	St. Error	Mann-Whitney U-Test		
			2003	2004	2005
2003 (N=201)	69.01	18.71	2003	-	0,0059***
2004 (N=284)	93.42	15.00	2004	-	< 0,0001***
2005 (N=230)	185.74	12.62	2005	-	-

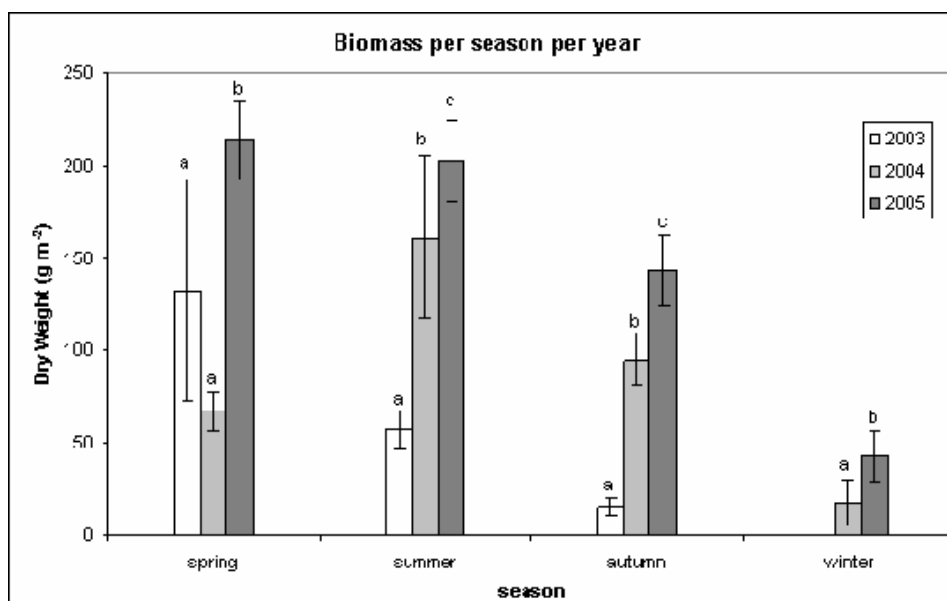


Figure 16: Dry weight biomass in g m^{-2} per season per year with bars indicating standard errors. Dry weight biomass of the same season was compared with each other over the survey years. Significant differences are indicated with letters above the bars.

Mann-Whitney U test also showed significant differences in aboveground dry weight biomass between the same seasons of different years (Figure 16). Spring biomass (i.e. biomass of April, May and June) between 2003, 2004 and 2005, summer biomass (July, August and September) between 2003, 2004 and 2005, autumn biomass (October, November and December) between 2003, 2004 and 2005 and winter biomass (January, February and March) had all significant different biomass except for spring biomass of 2003 compared to spring biomass of 2004.

The increase of biomass was mainly due to the significant increase of some macrophyte species, mainly *Potamogeton natans* and *Callitriche platycarpa* (Figure 17). Biomass was almost equally divided over the study section during the survey years. Only in autumn 2004 and 2005, dry weight biomass was significantly lower in the middle compared to the upstream weir ($p < 0.01$ and $p < 0.05$ respectively) and in spring 2004, biomass was significantly lower than both upstream and downstream weir ($p < 0.0001$ and $p < 0.0001$ respectively).

Nutrients

Average nutrient concentrations and nutrient ratio's in all aboveground biomass of the study section are given in table 9. Nutrient ratios (N/P and C/N) in the dominant species are shown in figure 18.

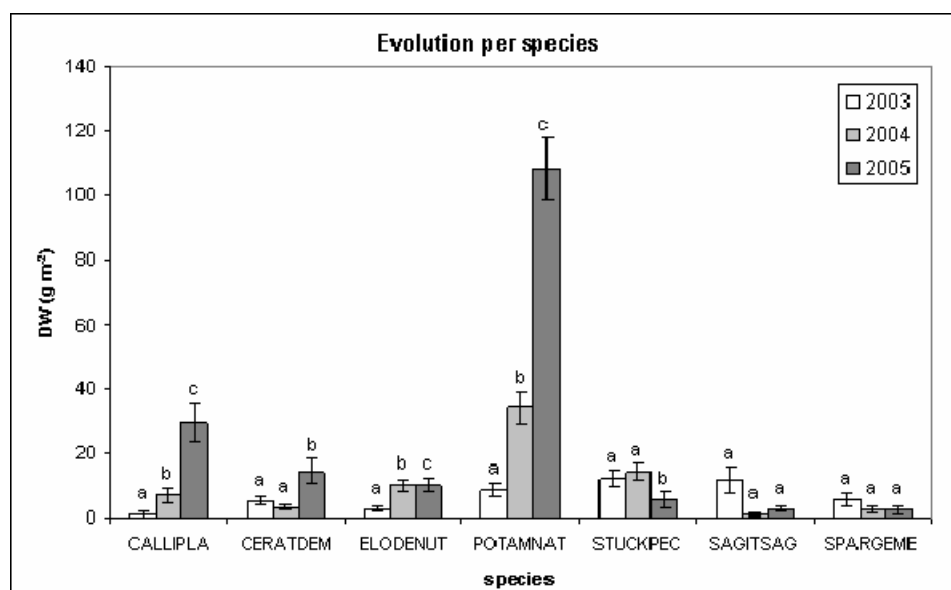


Figure 17: Evolution of dry weight biomass (g m^{-2}) of the dominant macrophyte species in the study section during the period May 2003 – November 2005. Bars indicate standard errors. Dry weight biomass of the same species was compared during the three survey years. Significant differences are indicated with letters above the bars. CALLIPLA = *Callitriche platycarpa*; CERATDEM = *Ceratophyllum demersum*; ELODENUT = *Elodea nuttallii*; POTAMNAT = *Potamogeton natans*; STUCKPEC = *Stuckenia pectinatus*; SAGITSAG = *Sagittaria sagittifolia* and SPARGEME = *Sparganium emersum*.

No significant difference could be detected between the C-concentrations of different species and as a consequence, variations in C/N ratios are fully due to variation in N-concentrations. The variation in N/P ratios is due to the combination of variations in N and P concentrations. *Potamogeton natans* has the lowest N-concentration and is significantly lower than all species except *Sagittaria sagittifolia* and *Sparganium emersum* (table 10). *Stuckenia pectinatus* and also *Potamogeton natans* have low P-concentrations compared to other species. Both N/P and C/N ratio in the plants did not differ between 2003 and 2004. Between seasons however, C/N ratio is lower in spring, and significantly lower than in summer (Figure 19). This observation was true for almost all dominant species and is ascribed to both a lower C and a higher N-concentration in spring macrophytes compared to the concentrations in summer and autumn macrophytes. $P < 0.0001$ and $p < 0.05$ were recorded for C concentrations between spring and summer and between spring and autumn respectively. For N concentrations between spring and summer and between spring and autumn, $p < 0.01$ and $p < 0.05$ were recorded respectively. Macrophyte N/P ratio does not show any difference between the seasons as well as the P concentration.

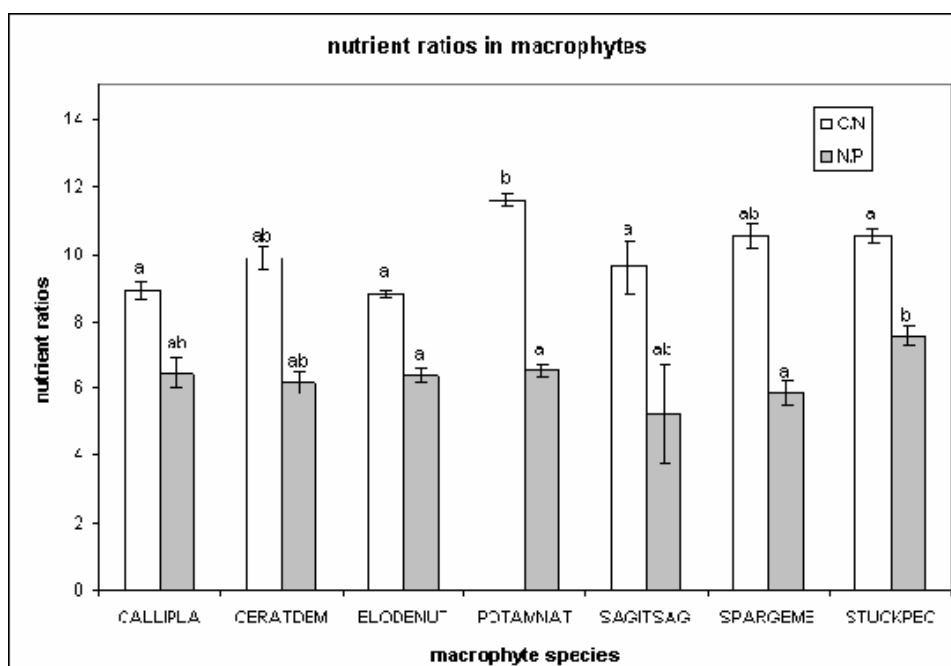


Figure 18: C/N and N/P ratio's with bars indicating standard errors within 6 dominant macrophyte species of the study section. Significant differences are indicated with letters above the balks. CALLIPLA = *Callitriche platycarpa*; CERATDEM = *Ceratophyllum demersum*; ELODENUT = *Elodea nuttallii*; POTAMNAT = *Potamogeton natans*; STUCKPEC = *Stuckenia pectinatus*; SAGITSAG = *Sagittaria sagittifolia* and SPARGEME = *Sparganium emersum*.

Table 9: average nutrient concentration and nutrient ratio's with standard deviation in aboveground biomass in the study section.

	AVERAGE ± stdev
N-conc (mg/g DW)	32,4 ± 8,6
P-conc (mg/g DW)	5,4 ± 2,0
C-conc (mg/g DW)	326,5 ± 49,5
N/P	6,7 ± 3,2
C/N	10,4 ± 3,1

Multiplying dry weight biomass (g DW m^{-2}) with nutrient concentration ($\text{mg g}^{-1} \text{DW}$) gives an idea of how many nutrients are stocked in the macrophyte biomass. The nutrient stock of the aboveground biomass (mg m^{-2}) of 2003 and 2004 is shown in figure 20. Higher macrophyte biomass in 2004 yields higher nutrient stocks. Total stock of nutrients in the river study section of 15 x 1450 m is shown in table 11.

Table 10: P-values indicating significant differences of N- and P-concentrations in 6 macrophyte species. P- values are obtained by one-way ANOVA and $\alpha = 0.05$.

N-conc	CALLIPLA	CERATDEM	ELODENUT	POTAMNAT	STUCKPEC	SAGITSAG	SPARGEME
CALLIPLA	***	1.000000	1.000000	0.02175	0.999979	0.996482	0.228551
CERATDEM		***	0.999984	0.00153	1.000000	0.998316	0.149670
ELODENUT			***	0.00007	0.999300	0.984226	0.055283
POTAMNAT				***	0.00006	0.270503	0.999999
STUCKPEC					***	0.999063	0.101904
SAGITSAG						***	0.698845
SPARGEME							***
P-conc	CALLIPLA	CERATDEM	ELODENUT	POTAMNAT	STUCKPEC	SAGITSAG	SPARGEME
CALLIPLA	***	0.924974	0.999969	0.053258	0.04399	0.776564	0.999544
CERATDEM		***	0.438121	0.362003	0.311018	0.050078	0.577828
ELODENUT			***	0.00006	0.00005	0.790255	0.999994
POTAMNAT				***	1.000000	0.00005	0.00414
STUCKPEC					***	0.00004	0.00322
SAGITSAG						***	0.965483
SPARGEME							***

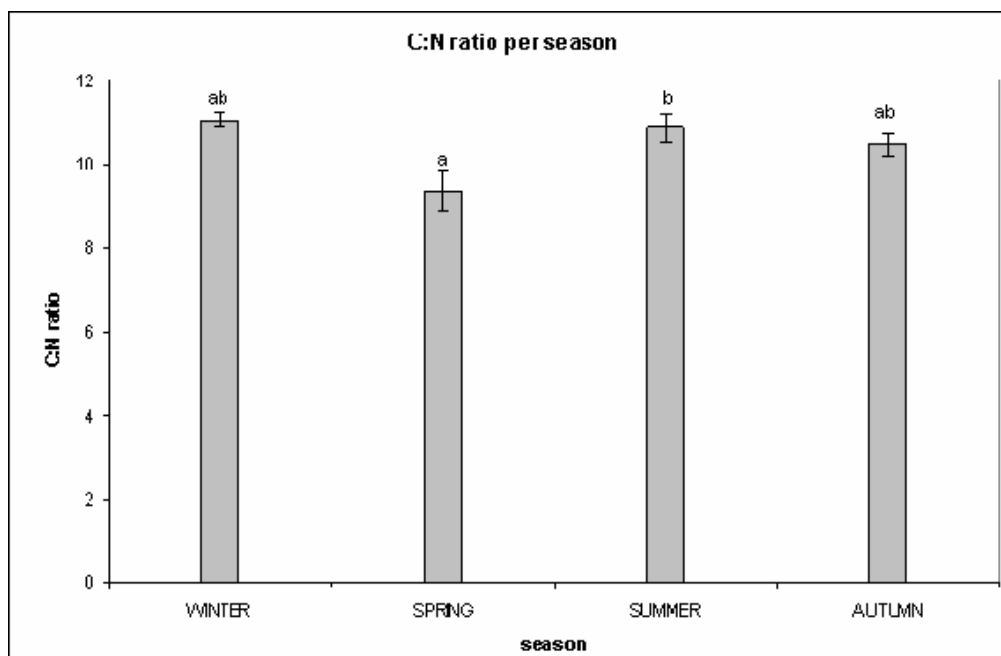


Figure 19: C/N ratio of all aboveground biomass in different seasons with bars indicating standard errors.

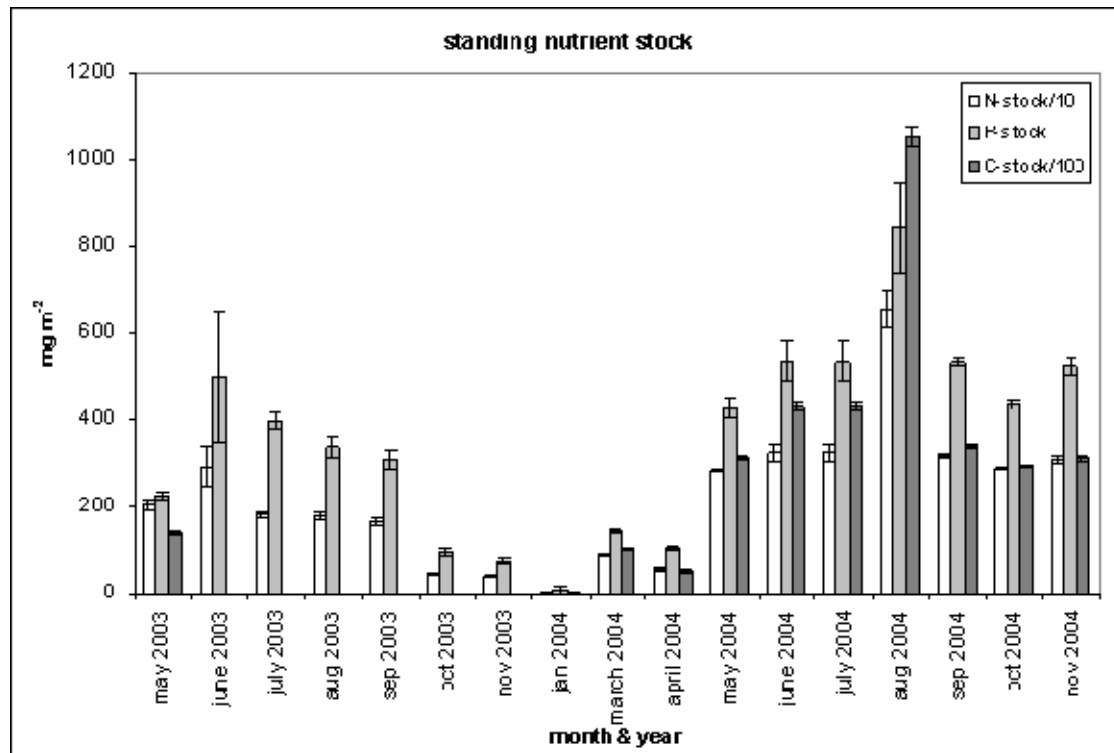


Figure 20: Monthly standing stock of N (divided by 10), P and C (divided by 100) in mg m^{-2} with bars indicating standard deviation. No measurements were available for C from June 2003 until November 2003.

Table 11: Monthly standing nutrient stock with standard deviation in the whole study section for 2003 and 2004.

Standing nutrient stock in study section												
	2003						2004					
	kg N	stdev	kg P	stdev	kg C	stdev	kg N	stdev	kg P	stdev	kg C	stdev
JAN	-	-	-	-	-	-	1.0	0.2	0.2	0.2	10.5	0.2
MARCH	-	-	-	-	-	-	19.8	0.4	3.1	0.1	220.2	3.1
APRIL	-	-	-	-	-	-	12.0	0.8	2.3	0.1	113.6	5.3
MAY	44.3	2.8	4.9	0.2	300.8	10.4	61.8	1.3	9.3	0.5	677.2	9.8
JUNE	63.9	10.0	10.9	3.3	-	-	70.6	4.1	11.6	1.0	939.6	22.3
JULY	38.8	1.7	8.6	0.5	-	-	70.6	4.1	11.4	1.0	929.9	22.1
AUG	37.9	1.7	7.3	0.5	-	-	142.5	9.5	18.3	2.3	2284.0	48.8
SEP	35.9	1.8	6.7	0.5	-	-	68.8	0.9	11.6	0.2	737.3	11.2
OCT	9.7	0.5	2.1	0.2	-	-	62.9	0.7	9.5	0.3	642.4	4.1
NOV	9.1	0.6	1.6	0.2	-	-	67.1	1.4	11.4	0.4	675.7	10.8

VI.1.4. Discussion

Biomass

In this unmanaged study section, dry weight biomass of all years were in the same size order compared to other researchers in river systems (Madsen & Adams, 1989; Flynn et al., 2002). Also, dry weight biomass of the same seasons of different years was always highest in 2005. Most of the dominant species increased in biomass during the years and two species, *Potamogeton natans* and *Callitriche platycarpa*, showed a remarkable rise in biomass development. *Stuckenia pectinatus* growth decreased in 2005 while *Sagittaria sagittifolia* and *Sparganium emersum* didn't show a clear evolution over time, although a decreasing trend could be observed. Weed cutting favours the growth of *Sagittaria sagittifolia* and *Sparganium emersum* because shoots can be continuously replaced by their basal meristem (Sand-Jensen et al., 1989). The stop of weed cutting might be beneficial for *Potamogeton natans* and *Callitriche platycarpa* as they don't lose their apical meristem (Deschamp & Cooke, 1985; Madsen & Breinholt, 1995; Baattrup-Pedersen et al., 2002). However, no explanation could be found for the increase of *Stuckenia pectinatus*.

The overall observed increase in biomass could be ascribed to the end of weed cutting since the late nineties, because a high amount of nutrients are still present in the Aa resulting in fast growing macrophyte species. However, long term research showed no difference in macrophyte biomass between managed and unmanaged lowland streams in Denmark (Baattrup-Pedersen et al., 2002).

Nutrients

Average nutrient concentrations within macrophyte species are quite variable and our ranges are relatively high compared to the ranges taken from literature (table 19) (Reddy et al., 1987; Shardendu & Ambasht, 1991; Madsen & Breinholt, 1995; Carr & Chambers, 1998; Madsen et al., 1998; Fernández-Aláez et al., 1999; Thiébaud & Muller, 2001; Wigand et al., 2001; Madsen & Cedergreen, 2002; Cronin & Lodge, 2003; Marion & Paillisson, 2003; Garbey et al., 2004; Andersen et al., 2005; Boedeltje et al., 2005; Chaiprapat et al., 2005). Variations are mainly species specific and with higher nutrient status of the water column and the sediment, a higher nutrient content in macrophyte species might be observed provided that both N and P are not limiting (Madsen & Breinholt, 1995; Madsen & Cedergreen, 2002). Indeed, at N:P surface water ratios (i.e. total inorganic nitrogen divided by SRP) above eight, nitrogen is always in surplus relative to phosphorus (Mainstone & Parr, 2002). In the study section of the Aa, N:P ratios of surface water always exceeded 26, so one might argue that SRP could be the limiting factor. However, P-concentrations of all species remained high above critical concentrations ($1.3 \text{ mg g}^{-1} \text{ DW}$, value suggested by Gerloff & Kromholz, 1966 or $2.6 \text{ mg g}^{-1} \text{ DW}$, value suggested by Colman et al.,

1987, indicating that this nutrient did not limit macrophyte growth. Both N and P in the Aa are quite high, so the system might be seen as an eutrophic one.

C:N ratios obtained by our research differed among species but were restricted to average values between 8 and 13 (figure 18). Especially *Potamogeton* species and *Stuckenia pectinatus* seem to have higher C:N ratios than other species which is mainly due to a lower N-assimilation. This is confirmed by other researchers (table 12). The rank order of C:N ratios from the dominant species is *Potamogeton trichoides* > *Potamogeton natans* > *Stuckenia pectinatus* > *Sparganium emersum* > *Ceratophyllum demersum* > *Sagittaria sagittifolia* > *Callitriche platycarpa* > *Elodea nuttallii*. Nevertheless, other researchers reported higher overall C:N ratios for aquatic macrophytes (table 12). This could be due to the excess of nitrogen present in our river section. Also in table 12, the N:P ratios in macrophyte species are shown. The highest N:P ratios (10 – 19) were recorded in species originating from lakes containing very low SRP-concentrations, which indicate P-limitation (Shardendu & Ambasht, 1991; Fernández-Aláez et al., 1999). Researchers who worked with an experimental setup enriching the water and/or the sediment with both nutrients, achieved lower N:P ratios (2 – 7) (table 12) (Reddy et al., 1987; Carr & Chambers, 1998; Wigand et al., 2001; Madsen & Cedergreen, 2002). In the lowland river Aa, the average N:P ratios of macrophytes (5 – 10) are laid in between the former two N:P ratio ranges. In the surface water of the Aa, SRP-concentrations are higher than reported in the lakes but lower than the concentrations used in the experiments which could clarify this specific range of N:P ratios. The rank order of N:P ratios in species is as follows: *Potamogeton trichoides* > *Stuckenia pectinatus* > *Ceratophyllum demersum* > *Callitriche platycarpa* > *Potamogeton natans* > *Elodea nuttallii* > *Sparganium emersum* > *Sagittaria sagittifolia*. These ranking is due to variations of both N and P-content in the species, so no clear relationship could be established. Only for *Stuckenia pectinatus*, it is for sure that the high N:P ratio is ascribed to its low P-content.

Although average spring C:N ratios in species seemed to be lower than C:N ratios of all seasons, only a significant difference could be detected between spring and summer C:N ratios. The lower C:N ratio in spring might be due to the rapid growth of fresh material with still a low content of organic material, which is a similar strategy to land plants. (Royer & Minshall, 1997) also didn't find a significant difference in C:N ratio between species in spring and autumn, although a slightly higher C:N ratio was detected in autumn.

Nutrient standing stock followed the dry weight biomass pattern during the two years, so nutrient standing stock is the highest in summer. Other research work reported mainly lower nutrient standing stock (in mg N or P m⁻²) when comparing the same months with each other, e.g. standing stock in October in a Danish lake (Andersen et al., 2005). This is because nutrient content in macrophytes of our study section is

mainly higher than reported elsewhere. Multiplying nutrient concentrations in macrophytes with dry weight biomass, which is mainly in the same size order of other researchers, reveals higher nutrient standing stock.

Table 12: Nutrient concentrations of different macrophyte species by other research workers.

		N (mg g ⁻¹ DW)	P (mg g ⁻¹ DW)	C (mg g ⁻¹ DW)	N:P	C:N	research method	additional info
<i>Elodea densa</i>	Reddy et al., 1987	35,6 - 40,3	12,8 - 13,7		2,8 - 2,9		experiment	nutrient enriched: 10,5 mg N-NH ₄ ⁺ l ⁻¹ , 10,5 mg N-NO ₃ ⁻ l ⁻¹ and 3,0 mg P-PO ₄ ³⁻ l ⁻¹
<i>Elodea canadensis</i>	Madsen and Cedergreen, 2002	43,4 - 49	6,2 - 17,98		2,7 - 7,0		experiment	nutrient enriched: up to 0,25 - 0,37 mg P-PO ₄ ³⁻ l ⁻¹ and to 21 - 28 mg N-NO ₃ ⁻ l ⁻¹
<i>Elodea canadensis</i>	Madsen et al., 1998	7 - 28					experiment	N levels: 0,07 - 7 mg available N l ⁻¹ and 0,16 mg P-PO ₄ ³⁻ l ⁻¹ ; CO ₂ levels: 0,75 - 18,92 mg l ⁻¹
<i>Elodea canadensis</i>	Bastviken et al., 2005					7 - 12	ponds	total available N: 5 - 15 mg l ⁻¹
<i>Elodea canadensis</i>	Thiébaud and Muller, 2001		2,9 - 7,5				stream	
<i>Elodea nuttallii</i>	Garbey et al., 2004		5,3 - 8				stream	N-NO ₃ ⁻ (0,27 - 0,94 mg l ⁻¹), N-NH ₄ ⁺ (0,03 - 0,05 mg l ⁻¹) and P-PO ₄ ³⁻ (0,018 - 0,074 mg l ⁻¹)
<i>Elodea nuttallii</i>	Thiébaud and Muller, 2001		3 - 8				stream	
<i>Elodea nuttallii</i>	Our study	29,2 - 41,0	4,4 - 8,2	261,1 - 354,9	5,0 - 6,6	8,7 - 8,9	stream	N-NO ₃ ⁻ (1,2 - 7,3 mg l ⁻¹), N-NH ₄ ⁺ (0,08 - 3,01 mg l ⁻¹) and P-PO ₄ ³⁻ (0,04 - 0,14 mg l ⁻¹)
<i>Callitriche cophocarpa</i>	Madsen and Cedergreen, 2002	42 - 47,6	8,37 - 14,26		3,3 - 5,0		experiment	nutrient enriched: up to 0,25 - 0,37 mg P-PO ₄ ³⁻ l ⁻¹ and to 21 - 28 mg N-NO ₃ ⁻ l ⁻¹
<i>Callitriche cophocarpa</i>	Madsen et al., 1998	10,5 - 46,2					experiment	N levels: 0,07 - 7 mg available N l ⁻¹ and 0,16 mg P-PO ₄ ³⁻ l ⁻¹ ; CO ₂ levels: 0,75 - 18,92 mg l ⁻¹
<i>Callitriche cophocarpa</i>	Madsen and Breinholt, 1995	41,5 - 46,10					experiment	enriched to 4,2 mg N-NH ₄ ⁺ l ⁻¹ , 4,2 mg N-NO ₃ ⁻ l ⁻¹ and 1,86 mg P-PO ₄ ³⁻ l ⁻¹
<i>Callitriche platycarpa</i>	Garbey et al., 2004		2,5 - 5,5				stream	N-NO ₃ ⁻ (0,27 - 0,94 mg l ⁻¹), N-NH ₄ ⁺ (0,03 - 0,05 mg l ⁻¹) and P-PO ₄ ³⁻ (0,018 - 0,074 mg l ⁻¹)
<i>Callitriche platycarpa</i>	Thiébaud and Muller, 2001		2,7 - 6,7				stream	
<i>Callitriche platycarpa</i>	Our study	25,0 - 41,4	3,5 - 8,7	223,8 - 361,2	4,8 - 7,1	8,7 - 9,0	stream	N-NO ₃ ⁻ (1,2 - 7,3 mg l ⁻¹), N-NH ₄ ⁺ (0,08 - 3,01 mg l ⁻¹) and P-PO ₄ ³⁻ (0,04 - 0,14 mg l ⁻¹)
<i>Ranunculus peltatus</i>	Garbey et al., 2004		5 - 8				stream	N-NO ₃ ⁻ (0,27 - 0,94 mg l ⁻¹), N-NH ₄ ⁺ (0,03 - 0,05 mg l ⁻¹) and P-PO ₄ ³⁻ (0,018 - 0,074 mg l ⁻¹)
<i>Ranunculus peltatus</i>	Thiébaud and Muller, 2001		3 - 9				stream	
<i>Potamogeton natans</i>	Fernández-Aláez et al., 1999	16	1,2	430	13,3	26,9	lake	low P-PO ₄ ³⁻ (3 à 5 µg/l)
<i>Potamogeton natans</i>	Our study	24,5 - 33,3	3,2 - 6,8	285,1 - 378,5	4,9 - 7,7	11,3 - 11,6	stream	N-NO ₃ ⁻ (1,2 - 7,3 mg l ⁻¹), N-NH ₄ ⁺ (0,08 - 3,01 mg l ⁻¹) and P-PO ₄ ³⁻ (0,04 - 0,14 mg l ⁻¹)
<i>Potamogeton trichoides</i>	Fernández-Aláez et al., 1999	23	2,3	410	10	17,8	lake	low P-PO ₄ ³⁻ (3 à 5 µg/l)
<i>Potamogeton trichoides</i>	Our study	27,9 - 30,7	2,9 - 3,9	362,1 - 389,7	7,9 - 9,6	12,7 - 13,0	stream	N-NO ₃ ⁻ (1,2 - 7,3 mg l ⁻¹), N-NH ₄ ⁺ (0,08 - 3,01 mg l ⁻¹) and P-PO ₄ ³⁻ (0,04 - 0,14 mg l ⁻¹)
<i>Potamogeton alpinus</i>	Boedeltje et al., 2005	44,4	4,5 - 5,4		8,2 - 9,9		experiment	N-NO ₃ ⁻ levels (0 - 7 mg/l) and muddy or sandy sediment
<i>Potamogeton amplifolius</i>	Cronin and Lodge, 2002	13,1 - 29,9		410,2 - 420,8		14,1 - 31,3	lake + experiment	difference in light availability
<i>Potamogeton crispus</i>	Shardendu et al., 1990	25	2,3		10,9		lake	low nutrient status (N-NO ₃ ⁻ max = 0,16 mg l ⁻¹ and P-PO ₄ ³⁻ max = 0,013 mg l ⁻¹)
<i>Stuckenia pectinatus</i>	Shardendu et al., 1990	19	1,03		18,4		lake	low nutrient status (N-NO ₃ ⁻ max = 0,16 mg l ⁻¹ and P-PO ₄ ³⁻ max = 0,013 mg l ⁻¹)
<i>Stuckenia pectinatus</i>	Wigand et al., 2001	12 - 15	3,4 - 4,7		3,2 - 3,5		experiment	water with low P-PO ₄ ³⁻ (0,00098 mg l ⁻¹), NH ₄ ⁺ -N (0,02 mg l ⁻¹) and N-NO ₃ ⁻ (3,5 mg l ⁻¹)
<i>Stuckenia pectinatus</i>	Carr and Chambers, 1998	24 - 31	2,1 - 6,2		5,0 - 11,4		experiment	enriched sediment (0,04 - 0,95 mg exchangeable P g ⁻¹ DW and 0 - 0,36 mg exchangeable N g ⁻¹ DW)
<i>Stuckenia pectinatus</i>	Royer and Minshall, 1997					10 - 11	stream	eutrophic: N-NO ₃ ⁻ + N-NO ₃ ⁻ (0,82 - 2,02 mg l ⁻¹), N-NH ₄ ⁺ (0,02 - 0,05 mg l ⁻¹) and P-PO ₄ ³⁻ (0,01 - 0,16 mg l ⁻¹)
<i>Stuckenia pectinatus</i>	Spencer et al., 1997	11,4 - 30,0		377,3 - 396,5		13 - 35	experiment	total available N: culture concentration or added N: 4,2 mg l ⁻¹
<i>Stuckenia pectinatus</i>	Our study	26,1 - 36,1	3,4 - 6,6	282,3 - 365,3	5,5 - 7,7	10,1 - 10,9	stream	N-NO ₃ ⁻ (1,2 - 7,3 mg l ⁻¹), N-NH ₄ ⁺ (0,08 - 3,01 mg l ⁻¹) and P-PO ₄ ³⁻ (0,04 - 0,14 mg l ⁻¹)
<i>Littorella uniflora</i>	Andersen et al., 2005	23,8 - 36,4	1,9 - 3,4		10,7 - 12,5		experiment	difference in light availability and CO ₂ concentrations
<i>Sagittaria sagittifolia</i>	Our study	25,2 - 43,6	4,6 - 9,2	288,3 - 346,9	4,7 - 5,5	8,0 - 11,4	stream	N-NO ₃ ⁻ (1,2 - 7,3 mg l ⁻¹), N-NH ₄ ⁺ (0,08 - 3,01 mg l ⁻¹) and P-PO ₄ ³⁻ (0,04 - 0,14 mg l ⁻¹)
<i>Sparganium emersum</i>	Our study	23,3 - 39,1	3,8 - 9,0	262,1 - 381,5	4,3 - 6,1	9,8 - 11,2	stream	N-NO ₃ ⁻ (1,2 - 7,3 mg l ⁻¹), N-NH ₄ ⁺ (0,08 - 3,01 mg l ⁻¹) and P-PO ₄ ³⁻ (0,04 - 0,14 mg l ⁻¹)
<i>Ceratophyllum demersum</i>	Royer and Minshall, 1997					8 - 9	stream	eutrophic: N-NO ₃ ⁻ + N-NO ₃ ⁻ (0,82 - 2,02 mg l ⁻¹), N-NH ₄ ⁺ (0,02 - 0,05 mg l ⁻¹) and P-PO ₄ ³⁻ (0,01 - 0,16 mg l ⁻¹)
<i>Ceratophyllum demersum</i>	Our study	30,8 - 40,0	3,6 - 7,6	318,7 - 372,1	5,3 - 8,6	9,3 - 10,3	stream	N-NO ₃ ⁻ (1,2 - 7,3 mg l ⁻¹), N-NH ₄ ⁺ (0,08 - 3,01 mg l ⁻¹) and P-PO ₄ ³⁻ (0,04 - 0,14 mg l ⁻¹)
<i>Hydrilla verticillata</i>	Shardendu et al., 1990	19,7	1,08		18,2		lake	low nutrient status (N-NO ₃ ⁻ max = 0,16 mg l ⁻¹ and P-PO ₄ ³⁻ max = 0,013 mg l ⁻¹)
<i>Trapa natans</i>	Marion et al., 2003	21,7 - 31	3,30 - 5,80		5,3 - 6,6		lake	eutrophic
<i>Nymphoides peltata</i>	Marion et al., 2003	27,3 - 32,3	6,84		4,0 - 4,7		lake	eutrophic
<i>Nuphar advena</i>	Cronin and Lodge, 2002	24,9 - 29,4		408,7 - 423,7		14,4 - 16,4	lake + experiment	difference in light availability
<i>Nymphaea alba</i>	Marion et al., 2003	21,6 - 23,4	2,96 - 4,17		5,6 - 7,3		lake	eutrophic
<i>Vallisneria spiralis</i>	Wigand et al., 2001	14 - 18	3,6 - 4,1		3,9 - 4,4		experiment	water with low P-PO ₄ ³⁻ (0,00098 mg l ⁻¹), NH ₄ ⁺ -N (0,02 mg l ⁻¹) and N-NO ₃ ⁻ (3,5 mg l ⁻¹)
<i>Lemna minor</i>	Chaiyaprat et al., 2005	19,7 - 59,7	6,8 - 14,8		2,9 - 4,0			swine wastewater

This also indicates that our study was conducted in a eutrophic lowland river. Nevertheless, the proportion of nutrients stocked in macrophyte biomass compared to the continuous supply of nutrients is very low. From this point of view, mowing macrophyte biomass in the lowland river Aa is not efficient at all.

To conclude, the river studied is a eutrophic system with high biomass of 7 dominant macrophyte species that contain quite high levels of nutrients (N and P). Nutrient standing stock is the highest when dry weight biomass is highest, namely at the end of the summer. From this point of view, (partly) mowing could be an option to remove the nutrients at that time. However, because of the continuous supply of nutrients, the amount of nutrients removed is very low.

VI.2. Nutrient mass balances in the Aa, a nutrient rich lowland river

VI.2.1. Introduction

Within aquatic ecosystems, sediments, surface water, macrophytes and phytoplankton are the compartments playing a major role in the nutrient dynamics of lakes (Bini et al., 1999; Asaeda et al., 2000; Asaeda et al., 2001; Marion & Paillisson, 2003; Rooney & Kalff, 2003), rivers and estuaries (Michel et al., 2000; Wigand et al., 2001; Magalhães et al., 2002; Nielsen, 2003). However, the role and abundance of macrophytes in lakes may be quite different compared with rivers. Unlike in lakes, current velocity is an important factor which may reduce the growth and biomass density of macrophytes (Chambers et al., 1991). On the other hand, the sediment compartment in rivers is less important as a nutrient source for macrophytes as they can use the continuous supply of nutrients via the surface water (Haslam, 1978). Indeed, increased nutrient uptake by macrophyte shoots from the surface water is a fact in eutrophic rivers (Madsen & Cedergreen, 2002), also because of the periodic stripping of the boundary layer by passing waves (Stevens & Hurd, 1997). Nutrient dynamics and mass balance studies in lakes (Asaeda et al., 2001; Marion & Paillisson, 2003; Rooney & Kalff, 2003) and estuaries (Nielsen, 2003) are quite well reported, sometimes throughout one or more seasons (Michel et al., 2000; Magalhães et al., 2002). Nonetheless, nutrient budget studies in freshwater rivers are relatively scarce, although it might be important to know the relationship between anthropogenic release of nutrients within the river catchments and their input into the coastal zone (Billen & Garnier, 1997; Cloern, 2001). A number of processes are indeed responsible for the retention and/or elimination of nutrients within the riverine and estuarine systems. The Scheldt basin is a good example of such behaviour: it is submitted to a very high input of nutrients, but a significant fraction of these is retained within the basin as attested by the agricultural origin and the fluxes reaching the North Sea (Howarth et al., 1996). Less attention has been paid to the study of nutrient fluxes in the upper catchments of the basin, where retention and loss processes probably cannot be neglected (Behrendt & Opitz, 2000; Peterson et al.,

2001). Budget studies of every compartment within the river catchments are necessary to identify and quantify the processes controlling the transport of nutrients to the estuarine and coastal zones. Furthermore, a better understanding of nutrient cycles and nutrient budgets for the whole river basin are needed for implementing appropriate management practices.

In this study, we focus on daily nutrient fluxes (mainly N and P) through a 1.5 km section of a macrophyte-dominated lowland river during different seasons. A mass balance integrating the incoming nutrient sources (upstream river, tributaries and groundwater inflow), the sediment, phytoplankton and macrophyte compartment was set up.

VI.2.2. Material and methods

Site description

The river Aa is a typical lowland river within the Nete catchment, a part of the Scheldt basin (Belgium) (figure 1). The river Aa is mainly rain fed, has its origin in very low pastures at a height of 30m and comprises a basin of 25054 ha (Brosens, 1966). A tributary of the Aa, the Grote Caliebeek, receives the treated sewage from a 55000 eqh wastewater treatment plant (WWTP). Since 1989, macrophytes appeared in the Aa. This is due to the improvement of the water purification system located about 3 km upstream of the studied section. Since then, a huge biomass of water vegetation develops in the Aa and moreover macrophyte biodiversity increases annually. Main species present in the Aa are submerged macrophytes such as *Callitriche platycarpa* Kütz., *Ceratophyllum demersum* L., *Elodea nuttalli* (Planch) St John, *Sparganium emersum* Rehm., *Potamogeton trichoides* Cham. et Schlecht., *Potamogeton crispus* L. and *Stuckenia pectinatus* (L.) Boerner, floating macrophytes such as *Potamogeton natans* L. and emerged macrophytes such as *Rorippa amphibia* (L.) Besser and *Sagittaria sagittifolia* L..

The canalized study section has a length of approximately 1.5 km and is bordered with two adjustable weirs (figure 2). Mean river width of the study section is about 15 m and mean depth about 2.2 m. Mean water depth is about 1 m. Stream velocities vary between 0.024 m s^{-1} and 0.200 m s^{-1} . The investigated section of the Aa is mainly bordered with crop fields and pastures. The sediment bed of the Aa consists primarily of iron rich sandy soils and dense macrophyte beds develop in spring and summer. In table 13, mean water quality parameters of this section are given from 1965 until now which indicate an apparent amelioration of the parameters N-NH_4^+ , P-PO_4^{3-} and BOD. Yet, N-NO_3^- concentration has increased over this period due to more available oxygen in the surface water.

Table 13: mean water quality parameters of the lowland river Aa during the period 1964-2004.

water quality	1964-1965	1985-1986	1993-1994	1999-2000	2003-2004
$N-NH_4^+$ (mg/l)	2,20 (+/- 2,13)	4,23 (+/- 0,78)	3,01 (+/- 1,78)	1,61 (+/- 1,08)	0,65 (+/- 0,69)
$N-NO_2^-$ (mg/l)	0,24 (+/- 0,17)	0,19 (+/- 0,14)	0,24 (+/- 0,13)	0,24 (+/- 0,20)	0,18 (+/- 0,10)
$N-NO_3^-$ (mg/l)	0,58 (+/- 1,51)	1,35 (+/- 0,75)	2,33 (+/- 0,91)	3,96 (+/- 1,90)	2,92 (+/- 1,52)
$P-PO_4^{3-}$ (mg/l)	-	0,48 (+/- 0,10)	0,24 (+/- 0,15)	0,13 (+/- 0,05)	0,08 (+/- 0,02)
Cl^- (mg/l)	45 (+/- 10)	68 (+/- 10)	45 (+/- 12)	50 (+/- 8)	61 (+/- 17)
pH	6,90 (+/- 0,25)	6,80 (+/- 0,23)	7,05 (+/- 0,28)	7,08 (+/- 0,10)	7,33 (+/- 0,21)
BOD	-	17,5 (+/- 13,7)	10,8 (+/- 7,8)	6,7 (+/- 5,3)	4,7 (+/- 3,8)

24 hour experiments and monitoring parameters

Between August 2003 and October 2004, six 24 hour experiments were held. In following order, the measurements took place on

- 21st of August 2003 at 8am until 22nd of August 2003 9am
- 5th of November 2003 at 13pm until 6th of November 2003 18pm
- 3rd of March 2004 at 13pm until 4th of March 2004 18pm
- 17th of May 2004 at 17pm until 18th of May 2004 18pm
- 5th of July 2004 at 17pm until 6th of July 2004 18pm
- 28th of September 2004 at 17pm until 29th of September 2004 18pm

To estimate discharges during the campaigns, velocity profiles were measured at the upstream weir, in the middle and at the downstream weir at the start of the campaigns. In all of the campaigns, the weather was clear and no rainfall occurred. These computed discharge measurements can be compared with those from the hydrologic information centre (HIC).

In each campaign, two data probes – one at the upstream and one at the downstream weir – measured the following variables each five minutes: oxygen, temperature, conductivity, pH and turbidity.

Every two hours, surface water samples were taken with a bucket from the bridges at the up- and downstream weirs, and from a boat at mid-distance between the two weirs. A subsample was taken from the bucket, placed in a cooled box and transported to the lab for analysis.

In the campaigns of 2004, water was also sampled on the Sloopbeek, a small tributary that comes into the Aa located 5 meters downstream from the upstream weir. This was done every six hours of each campaign and water samples were taken and transported in a cooled box to the lab for analysis.

All samples were analysed for available $N-NH_4^+$, $N-NO_2^-$, $N-NO_3^-$ and $P-PO_4^{3-}$ on a SKALAR segmented flow analyser. The half of the samples (from every 4 hours)

were also analysed for chloride as a conservative tracer on a SKALAR segmented flow analyser.

Eight groundwater tubes spread along the sides of the river were monitored during the campaigns. The level of the groundwater was recorded and groundwater samples were taken with a hand pump in a bucket from which a subsample was taken, filtered with 45 µm filters, acidified and transported in a cooled box to the lab for analysis on available N and P. These measurements were necessary to take into account groundwater inflow in the river.

Macrophyte biomass

Every campaign, 30 samples of aboveground biomass were taken in the studied section except for November 2003 (21 samples) and March 2004 (25 samples) because of too cold water temperatures and/or high water levels. In case of 30 samples, one third was taken at the upstream weir, one third in the middle and one third at the downstream weir. In the other case, samples in the middle were skipped. Samples were taken by diving and cutting the aboveground biomass in a plot of 15 x 15 cm, marked out by the surber. Whilst cutting, the biomass was caught by the net of the surber. Macrophyte samples were placed in plastic bags, transported to the lab where they were sorted out at species level. Every species per plot was weighed and dried for at least 48 hours at 75°C. Hereafter, dry weight of the samples was determined and biomass in the study section was estimated.

Process measurements

At 3 occasions during the 2004 macrophyte growing season (May, July and September 2004) process studies were conducted in order to estimate dissolved inorganic nitrogen (ammonium and nitrite + nitrate) fluxes between the water column and sediments, phytoplankton and macrophyte shoots.

Ammonium and nitrate uptake rates by phytoplankton and macrophyte shoots were determined using ^{15}N -enrichment techniques according to Dugdale and Goering (1967). For phytoplankton, 250 ml glass incubation bottles were filled with river water sampled at the upstream weir in the morning. For macrophytes, shoots from 2 dominant species (*Potamogeton natans* and *Ceratophyllum demersum*, representing between 12 and 66% of the total macrophyte biomass) were taken with scissors close to the upstream weir. The shoots were gently rinsed with deionised water and were placed individually in glass incubation bottles with 250 ml of filtered (GF/F glass-fibre filter) point water. Each incubation bottle was spiked with $^{15}\text{N-NH}_4^+$ (99%) or $^{15}\text{N-NO}_3^-$ (98%) (final abundance between 1 and 10 %) and incubated for 4 hours at the *in-situ* temperature and at both natural light intensity and in the dark. At the end of incubation, the water from the phytoplankton incubation bottles was filtered through combusted GF/F filters and the filters were dried for 12h at 50°C in an oven.

Macrophyte shoots were removed from the bottles, gently rinsed with deionised water and dried for at least 48h in the oven at 50°C. Dried organic material from the filters and macrophytes shoots were analysed for organic N content (PN) and ^{15}N using a C-N elemental analyser (Flash series 1112) coupled to an isotope ratio mass spectrometer (Thermo-Finnigan Delta plus XL) as described by Nieuwenhuize et al. (1994).

The uptake rates were calculated according to the simplified Dugdale and Goering

(1967) model:
$$U = \frac{(A_{pf} - A_n)}{(A_{di} - A_n) \times dt} \times PN_f$$

With U = uptake rate, A_{pf} = ^{15}N abundance in the PN pool after incubation, A_n = natural ^{15}N abundance = 0.365%, A_{di} = initial ^{15}N abundance in the ammonium or nitrate pool after adding the spike, dt = incubation time, PN_f = PN concentration after incubation.

For macrophytes shoots, the term PN_f was replaced by the fraction of N per dry weight of plant material.

Sediment cores were collected in triplicate close to the upstream weir with a Beeker sampler (Eijkelkamp). Each core consisted of a 30 cm plastic transparent tube with a diameter of 8 cm. The tube contained approximately a sediment layer of 15 cm with a supernatant water phase of around 20 cm. Net fluxes at the sediment water interface were evaluated by incubating the cores at *in situ* temperature and following the nutrient (ammonium and nitrite + nitrate) concentrations in the supernatant water over time for 8 hours. Sediment water exchange fluxes were computed for each of the cores by taking the initial slope of the concentration variations with time.

Set up of the mass balance

For each campaign, the measured flow of nutrients at the upstream, the middle and the downstream weir were compared with the results of a simple mixing-dilution model. This model simulates the conservative variation of nutrient concentration induced by the mixing of the entering river water (with known nutrient concentrations) with the waters of the Sloopbeek and groundwater (with known nutrient concentrations).

The Sloopbeek and groundwater flows in the model were calibrated using the chloride profiles at the up-, middle and downstream stations and knowing the chloride content in both ground and Sloopbeek water. It is assumed that in the model, the river section had a length of 1450 m, a constant depth of 1 m and a constant width of 15 m which is acceptable.

Practically, the curves of nutrients (N-NH_4^+ , N-NO_2^- , N-NO_3^- and P-PO_4^{3-}) at the three stations were plotted against a time axis relative to the time at the upstream weir. This allowed to compensate for the transport time between the 3 stations so that the characteristics of a same water mass can be compared at a given moment.

Transport times were based on the velocity measured in the Aa and on discharges available from the Flemish Hydrologic Information Centre.

Finally comparing modelled middle and downstream profiles with the measured ones allow us to calculate a reactivity term by difference. This term represents the influence of active biogeochemical processes on the nutrient balance of the river stretch.

When available, the phytoplankton, macrophyte and the sediment compartments are incorporated in the nitrogen mass balance.

The ammonium and nitrate uptake rate by phytoplankton in the whole river stretch was calculated as: $N_{up} = U_{N-light} \times (t_{light}/24) + U_{N-dark} \times (t_{dark}/24)$.

With N_{up} = quantity of N (nitrate or ammonium) taken up (in $\text{mg N m}^{-3} \text{ h}^{-1}$) ; $U_{N-light}$ and U_{N-dark} = N uptake rate in the light and the dark (in $\text{mg N m}^{-3} \text{ h}^{-1}$) ; t_{light} and t_{dark} = duration of the day and the night in a 24 hour period (hours).

The ammonium and nitrate uptake rate by macrophytes in the whole river stretch was calculated as: $N_{up} = (U_{N-light} \times (t_{light}/24) + U_{N-dark} \times (t_{dark}/24)) \times B/V$.

With N_{up} = quantity of N (nitrate or ammonium) taken up (in $\text{mg N m}^{-3} \text{ h}^{-1}$) ; $U_{N-light}$ and U_{N-dark} = N uptake rate in the light and the dark (in $\text{mg N kg}^{-1} \text{ DW h}^{-1}$) ; t_{light} and t_{dark} = duration of the day and the night in a 24 hour period (hours) ; B = macrophyte biomass in the studied river stretch (kg DW) and V = volume of the river stretch (= 21750 m^3).

The ammonium and nitrate exchange rate with the sediments in the whole river stretch was calculated as: $N_{exch} = F_N d^{-1}$

With N_{exch} = quantity of N (nitrate or ammonium) exchanged (in $\text{mg N m}^{-3} \text{ h}^{-1}$) ; F_N = nitrate or ammonium flux at the sediment water interface ($\text{mg N m}^{-2} \text{ h}^{-1}$) ; d = mean depth of the water column in the river (= 1 m).

VI.2.3. Results

Chloride profiles

The behaviour of the tracer element chloride during the six campaigns is shown in figure 21. Chloride pulses were quite variable during a time period of 24 hours in all seasons and values were always highest at the upstream weir whereas the difference between the middle and the downstream weir were negligible. At the same time chloride measured in the Sloopbeek varied between 17 and 29 mg l^{-1} which is significantly lower than in the main river channel.

Ammonium, nitrate and phosphate profiles

The behaviour of the major nutrients during the six campaigns is shown in figure 22. As for chloride, the nutrient profiles showed extremely large variations over 24 h periods. Ammonium and nitrate variations were extremely irregular within the 24h periods with large peaks and valleys. This highlights the irregular sources of

ammonium and nitrate for the river, probably linked to the management of the wastewater discharge located upstream from our studied section. At one occasion, in July 2004, a clear failure of the WWTP was reported to us and the effects of this were clearly seen in the ammonium profiles (until $4 \text{ mg l}^{-1} \text{ N-NH}_4^+$). But considering the ammonium profiles from other campaigns, it is clear that similar events were responsible for the large variability observed.

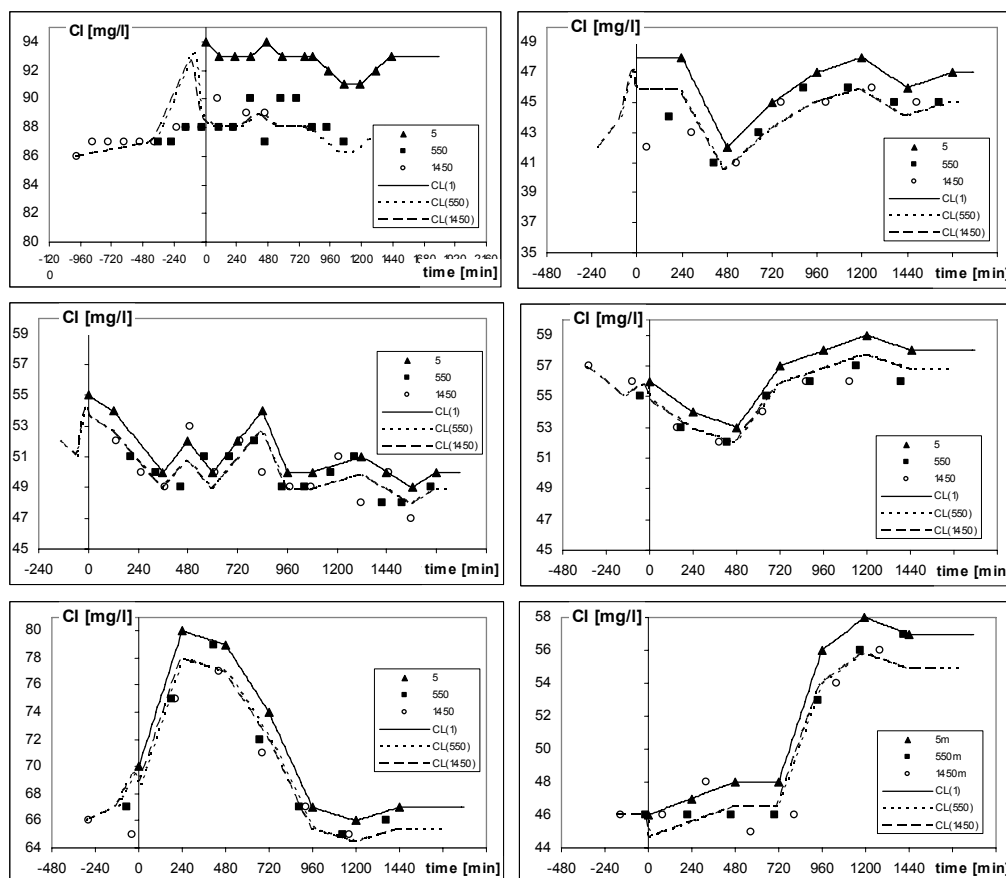


Figure 21: Tracer chloride values [mg l^{-1}] at the upstream weir (▲), in the middle (■) and at the downstream weir (○) for the six campaigns plotted against relative time [min]. Accordingly, the behaviour of one water package is shown. Dotted lines represent chloride values obtained by modelling the dilution originating from the Sloopbeek only in the middle (..... CL(550)) and at the downstream weir (— CL(1450)).

P-PO_4^{3-} profiles also showed irregular patterns during a 24 h period which can also be attributed to the WWTP upstream the section. However, patterns were less clear compared to the ammonium and nitrate patterns in the water package.

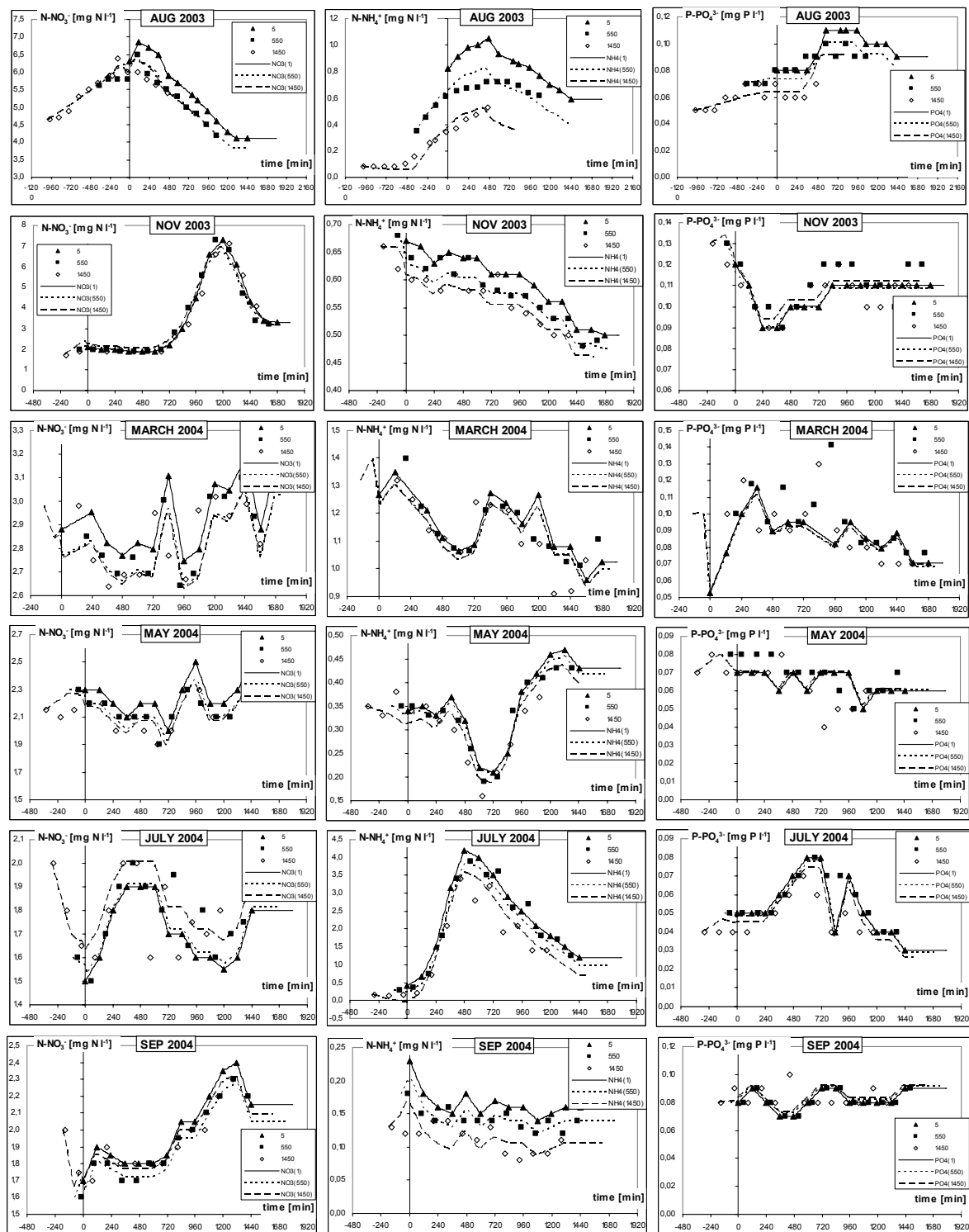


Figure 22: N-NH₄⁺, N-NO₃⁻ and P-PO₄³⁻ values [mg l⁻¹] at the upstream weir (▲), in the middle (■) and at the downstream weir (○) for the six campaigns plotted against relative time [min].

Accordingly, the behaviour of one water package is shown. Dotted lines represent values obtained by modelling the dilution originating from the Slootbeek and reaction terms in the middle ("....." CI(550)) and at the downstream weir (—"—" CI(1450)).

Macrophyte biomass

Macrophyte biomass in the six campaigns is presented in figure 23. Highest biomass was found in the growing season of 2004 with a peak in July. Biomass in August 2003 was remarkably lower than biomass in May, July and September 2004. Although biomass was still there in November 2003 and March 2004, it was almost not active and consisted mainly of brown stems of *Potamogeton natans* without leaves.

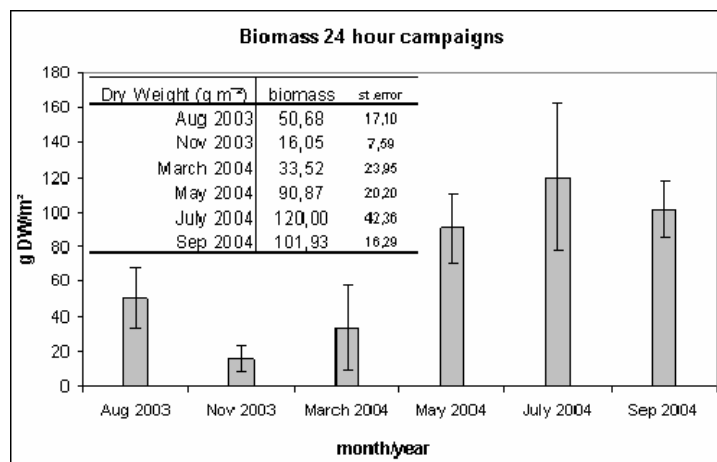


Figure 23: Macrophyte aboveground biomass standing in the 1,5 km long study section of the Aa for all 24 hour campaigns. Values are expressed in gram dry weight per square meter (g DW/m²) with bars indicating standard errors.

Processes

Results obtained from the process measurements done in May, July and September 2004 are presented in table 14. For ammonium, uptake by phytoplankton was only of minor importance as was the exchange with the sediments in spring and summer. Most important was the uptake by macrophytes. For nitrite + nitrate, phytoplankton is again of minor importance. In spring, macrophyte uptake of nitrate is the dominant process, in summer sediments and macrophytes are of similar importance as nitrate sinks, and in fall, sediments are the most important nitrate sink.

Table 14: Net exchange of ammonium and nitrate between the water and the sediments (SED), the macrophytes (MAC) and the phytoplankton (PHY) in May, July and September 2004. Values correspond to the average rate measured in 3 cores. Negative values correspond to net consumptions.

mg m ⁻³ h ⁻¹	SED _{NH4}	SED _{NO2 + NO3}	MAC _{NH4}	MAC _{NO3}	PHY _{NH4}	PHY _{NO3}
MAY	0.006	0.12	-3.6 ± 0.5	-2 ± 0.3	-0.57	-0.19
JULY	0.21	-7.4	-30 ± 29	-8 ± 7	-0.57	-0.19
SEPTEMBER	3.6	-8.8	-15 ± 2	-0.3 ± 0.2	-0.15	0

VI.2.4. Discussion

Nutrient budget

It was shown that groundwater contributions are of minor importance in the studied river stretch since Anibas et al (unpublished: FWO project VUB-UA-RUG) concluded that only at the first part of the upstream weir, some seepage could be detected. Therefore, the observed diluted chloride signal in the middle and downstream stations must be linked to the inflowing water of the Sloopbeek holding lower Cl^- concentrations. The dilution factor was modelled via the measured chloride values of both the Sloopbeek and the Aa. From this, the discharge of the Sloopbeek could be estimated which gave almost equal result as the measured values. This dilution factor was included in further modelling calculation of the nutrient patterns as described in “methods”.

Downstream nutrient profiles resulting from the mixing-dilution model compared to the measured ones highlight the occurrence of non-conservative processes especially during the summer months (July, August and September) where we observe lower N-NH_4^+ concentrations and higher N-NO_3^- concentrations than expected from mixing-dilution.

For each of the situations, a reaction rate term representing the non-conservative processes can be computed by taking the difference between modelled mixing-dilution curve and observed profiles (table 15). Modelled fits which incorporate a consumption or production factor between the upstream and downstream weir showed good results for most N-DIN components (figure 22).

Table 15: consumption or production rates of phosphate, ammonium, nitrite + nitrate and dissolved inorganic N (DIN) in $\text{mg m}^{-3} \text{ h}^{-1}$ calculated from the modelled curves of the nutrients of all 24 hour campaigns. Negative values indicate a “disappearance” or consumption and positive values indicate a “generation” or production of the respective nutrients between the upstream and the downstream weir.

consumption/production in $\text{mg m}^{-3} \text{ h}^{-1}$	P-PO_4^{3-}	N-NH_4^+	$\text{N-(NO}_2^- + \text{NO}_3^-)$	N-DIN
August 2003	-1,0	-30	-4	-34
November 2003	2,0	-10	102	92
March 2004	0,5	-1	-8	-9
May 2004	0,1	-6	-11	-16
July 2004	-1,2	-100	42	-58
September 2004	0,5	-22	21	-1

Obtained consumption rates (table 15) show that in all campaigns, N-NH_4^+ disappeared through the river section and this was highest in the macrophyte growing season (summer). On the other hand, there was a net production of N-(NO_2^-

+ NO_3^-) in November 2003, July 2004 and September 2004. In the oxygen rich study section, we may assume that a part of the N-NH_4^+ present is converted to N-NO_2^- and N-NO_3^- (Scholz & Trepel, 2004), but still a considerable amount of N-NH_4^+ has disappeared during the flow time, especially in the growing season and it is clear that the river macrophytes do play a role in the uptake of N-NH_4^+ in the growing season while in late autumn and winter, their role is negligible. Also, a net inorganic N consumption rate was observed for all campaigns except in November 2003 where net N-NO_3^- was produced.

Non-conservative processes also occurred in the downstream regions for P-PO_4^{3-} in August 2003 and July 2004, yet in a lesser extent. Because the disappearance of P-PO_4^{3-} is most clear in the middle of the growing season, it might be that macrophytes – just as for N-DIN – take up a part of the P-PO_4^{3-} . However, consumption involving P-PO_4^{3-} are more complex: sediment sorption (as an abiotic process) has been shown as a substantial factor in P retention (Fox et al., 1989 ; House & Warwick, 1999). Furthermore, the organic matter content of sediment also influences the ability of sediments to adsorb P (Smith et al., 2005) just as present concentrations of iron and calcium (House & Denison, 2002).

For all nutrients, the consumption rates were highest at the peak of the growing season, namely in August 2003 and July 2004. In September 2004, the consumption rate of N-NH_4^+ and N-NO_2^- compensate almost for the production rate of N-NO_3^- .

Not surprising, in this small river, phytoplankton was the less important DIN user. Indeed, it was demonstrated (Billen et al., 1994) that in small rivers from the heads of a watershed, phytoplankton could never develop large biomasses as their growth rates were always smaller than the river dilution rate (i.e. the dilution induced by lateral effluents and groundwater seepage in the main river channel).

Sediments represented an important net source for N-NH_4^+ and sink for N-NO_3^- in the summer situations (July and September 2004) showing the importance of benthic ammonification and denitrification processes.

Most important DIN sink is represented by macrophyte uptake which had a preference for ammonium. Preference for ammonium by macrophytes was previously shown by several studies (Nichols & D.R., 1976; Reddy et al., 1987; Cedergreen & Madsen, 2003; Scholz & Trepel, 2004).

Uptake by macrophytes was estimated by incubating shoot cuts and may therefore not be representative of the whole rooted plant uptake. Recently, (Bouma et al., 2002) have shown that incubating plant parts of *Spartina anglica* (a seagrass) to determine N uptake rates underestimated whole plant uptake rates by a factor 2 to 3. As no study of this kind was done for freshwater species considered in this work, we tried to compare our measurements with a recent study done by Van Belleghem et al (submitted). In this study, whole freshwater macrophyte plants from *Potamogeton*

natans collected in June 2005 (middle of the growing season) in the studied section of the river Aa, were incubated under controlled light, flow and temperature conditions with ^{15}N tracers in a flume (Van Belleghem et al., in prep). Results also revealed a net preference for N-NH_4^+ uptake and an average uptake rate ranging from 0.037 to $0.059 \text{ g N-NH}_4^+ \text{ kg}^{-1} \text{ DW h}^{-1}$ and from 0.0029 to $0.0059 \text{ g kg}^{-1} \text{ DW h}^{-1}$ for N-NO_3^- . By applying this rate to the standing macrophyte biomass in May and July we have an N-NH_4^+ uptake of 3.4 to $7.1 \text{ mg N m}^{-3} \text{ h}^{-1}$ and a N-NO_3^- uptake of 0.3 to $0.7 \text{ mg N m}^{-3} \text{ h}^{-1}$. These values are generally lower but in the same range than what we measured with shoot incubations and we will thus assume that our measurements are in an acceptable range for budgeting purposes.

A global insight on the DIN cycling can be obtained by comparing calculated net ecosystem consumption rates (table 15) with measured process rates (table 14).

For ammonium (Figure 24A), we can clearly see that sediments and phytoplankton only play a minor role compared to the uptake by macrophytes. The measured consumption rates represented 70 % of the net ecosystem ammonium depletion in May and September. However, it was only 30 % in July. Cumulated errors linked to each rate estimation could partly be responsible for this “missing” ammonium sink. However, especially in July, it is clear that another, un-quantified, ammonium sink seems to be important in this small river. Two possibilities could be important: adsorption of ammonium to suspended particles and nitrification. Adsorption of ammonium to particles is included in the estimation of phytoplankton uptake, as this rate corresponds to the total transfer of dissolved N to particulate N (Dugdale & Goering, 1967). As this rate was very small, adsorption must also be negligible. The most likely un-quantified N-NH_4^+ sink is thus nitrification in the water column. Generally, this process is believed to be of minor importance in the water column of small rivers because of the very slow growth rates of nitrifying bacteria. However, higher rates have been observed in wastewater contaminated rivers because of the seeding of active nitrifying micro-organisms (Brion and Billen, 2000). Accordingly, our studied river stretch is located downstream a WWTP which showed regularly treatment failures as was the case in July 2004 (see the ammonium profiles in figure 22 showing a massive contamination). We will thus make the hypotheses that nitrification is the missing ammonium sink which is transformed in $\text{N-(NO}_2^- + \text{NO}_3^-)$. This $\text{N-(NO}_2^- + \text{NO}_3^-)$ source will be included in the $\text{N-(NO}_2^- + \text{NO}_3^-)$ mass balance.

For $\text{N-(NO}_2^- + \text{NO}_3^-)$ (Figure 24B), macrophyte and sediments seem both to be important according to the season: macrophytes in spring (May), both in summer (July) and sediments in fall (September). In July, the very high apparent nitrification rate determined by N-NH_4^+ budgeting allows to explain the overall net N-NO_3^- production observed in the river stretch. However, for all seasons, an additional N-NO_3^- sink (May and July) or source (September) is needed to close the budget. As to our knowledge all major processes were estimated for the budget, it seems likely that

these imbalance rates represent the accumulation of errors on the different rate estimations. Most probably, estimations of sediment-water fluxes suffer from the fact that rates could only be measured on bare sediments and not inside macrophyte patches. Therefore, extrapolating bare sediment rates to the entire river bed may be not appropriate.

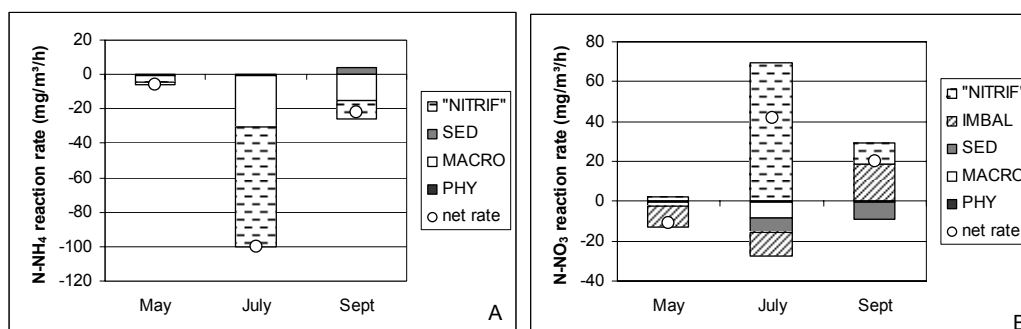


Figure 24: Mass balance for N-NH₄⁺ and N-NO₃⁻ in the studied river stretch of the Aa. Negative rates correspond to consumptions. SED, MACRO and PHY represent the measured N uptake or release by sediments, macrophyte and phytoplankton respectively. Net rate is the net N consumption rate calculated from nutrient profiles (see methods). "NITRIF" is an apparent nitrification rate calculated by balancing the N-NH₄⁺ budget (sum of all rates = net rate). IMBAL, is an imbalance rate calculated by closing the N-NO₃⁻ budget (sum of all rates = net rate).

Another important factor of error could be the estimation of macrophyte uptake as only 2 species were considered. Indeed in May and July, these 2 species only represented 29% and 12 % of the total biomass respectively, while in September they were 62 %. Clearly for both May and July, uptake by dominant species as *Callitriche platycarpa*, *Elodea nuttallii* and *Stuckenia pectinatus* should be assessed.

VI.3. C, N and O₂ cycling in the Aa as assessed from stable isotope biogeochemistry

VI.3.1. Introduction

The natural stable isotopic composition of carbon, nitrogen and oxygen has been shown to be a powerful tool to understand their cycling in a variety of aquatic systems. Such studies use the variation in the stable isotopic composition of N and C in particulate organic matter, dissolved inorganic C (DIC) and Oxygen (O₂) induced by a process-specific level of discrimination against the heavy or light isotope, to reveal the dominant processes acting on the N, C and O pools (Cifuentes et al. 1989; Horrigan et al. 1990; Montoya et al. 1990; 1991; Velinsky et al. 1991; Ostrom et al. 1997; Wu et al. 1997; Sigman et al. 1999; Lehmann et al. 2004).

VI.3.2. Methods.

Samples were taken every 2 hours at the upstream and downstream weir of the Aa river stretch (Figure 2) during 24h sampling campaigns as described in chapter VI.3. Immediately after sampling, two 50 ml and one 25 ml penicillin flask were filled for the measurement of ^{18}O of dissolved oxygen (DO) and ^{13}C of dissolved inorganic carbon (DIC), respectively. The samples were subsequently poisoned with mercuric chloride to inhibit microbial activity and sealed air-tight. A known volume of water was filtered in duplicate through pre-weighted and pre-combusted Whatman GF/F filters for analysis of suspended matter (SPM) concentration, ^{13}C and ^{15}N of SPM, C/N ratios, particulate organic carbon (POC) and particulate organic nitrogen (PON) concentrations.

In all cases, ^{15}N and ^{13}C of SPM and PON and POC concentrations are measured simultaneously using an elemental analyzer (EA, Carlo-Erba C/N analyzer) coupled via a conflo-interface to an isotope ratio mass spectrometer (IRMS, Finnigan Delta-Plus XL) (Nieuwenhuize et al., 1994). ^{13}C of DIC and ^{18}O are measured using automated methods where CO_2 or O_2 samples are directly injected in the EA-RMS. First, He is injected in the penicillin bottles to create a headspace. Additionally, the bottles for ^{13}C -DIC analysis receive 250 μl of pure ortho-phosphoric acid to convert all DIC to CO_2 . All bottles are then shaken overnight at room temperature to allow equilibration. Gas from the headspace is then injected in the EA-IRMS system for ^{13}C and ^{18}O analysis.

VI.3.3. Results and discussion

VI.3.3.1. Seasonal variations

Suspended particulate organic matter

$^{15}\text{N}_{\text{SPM}}$ was higher during the summer months than during the other seasons (Figure 25). During summer, when macrophyte biomass is highest, macrophytes and phytoplankton can become enriched in ^{15}N when source ^{15}N (nitrate or ammonium) increases as a result of increased consumption of N source. The increase in $^{15}\text{N}_{\text{SPOM}}$ could thus be an indication of net removal of N nutrients from the water column. ^{13}C of SPOM showed a clear minimum in spring (May 2004) followed by a gradual increase during summer (Figure 26). Such pattern probably reflects the incorporation of isotopically light CO_2 during spring after which CO_2 with gradually increasing ^{13}C is used to build new plant biomass. The net removal of isotopically light CO_2 from the DIC pool is also reflected in the increase in $^{13}\text{C}_{\text{DIC}}$ during the spring and summer (Figure 27).

Dissolved inorganic carbon and oxygen

^{13}C of dissolved inorganic carbon (DIC) showed a cyclic seasonal pattern with lowest values found during late autumn (November; $\delta^{13}\text{C}_{\text{DIC}} = -16.3 \pm 0.4\text{‰}$) and

highest during late spring (May; $\delta^{13}\text{C}_{\text{DIC}} = -11.6 \pm 0.9\text{‰}$) (Figure 27 and 28). When all water column DIC originates from the dissociation of limestone (CaCO_3), $\delta^{13}\text{C}_{\text{DIC}}$ values equal 1‰. However, inputs of CO_2 produced during the respiration of organic matter can decrease water column DIC because of the low $\delta^{13}\text{C}$ value of organic matter (e.g. $\delta^{13}\text{C} = -32.4 \pm 3.4\text{‰}$ for macrophytes in the Aa River, $\delta^{13}\text{C} = -28.2\text{‰}$ for organic carbon in sand from the rivers banks of the Aa). The low winter $\delta^{13}\text{C}_{\text{DIC}}$ signature indicates that respiratory CO_2 inputs are important. The subsequent increase in $\delta^{13}\text{C}_{\text{DIC}}$ toward May is a consequence of an increased productivity of the system. Primary producers preferentially assimilate $^{12}\text{CO}_2$ during photosynthesis, leaving the remaining CO_2 enriched in $^{13}\text{CO}_2$. Highest $\delta^{13}\text{C}_{\text{DIC}}$ values are found in May suggesting that the CO_2 demand is highest in May. Macrophyte biomass peaks in July but $\delta^{13}\text{C}_{\text{DIC}}$ values indicate that net CO_2 demand is lower in July, probably because of the increased respiration exerted by the macrophyte biomass.

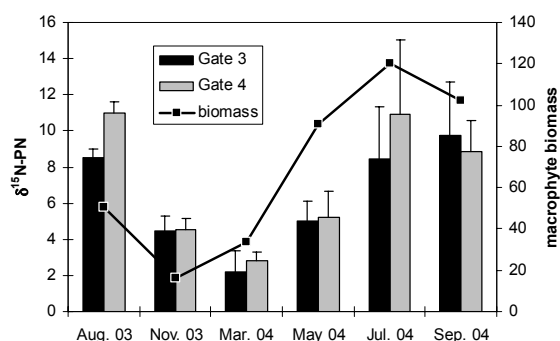


Figure 25. Seasonal variation in $\delta^{15}\text{N-SPM}$ (‰) and macrophyte biomass (g dry weight/m²) (data UA) in the Aa River 2003-2004. Error bars = 1 standard deviation.

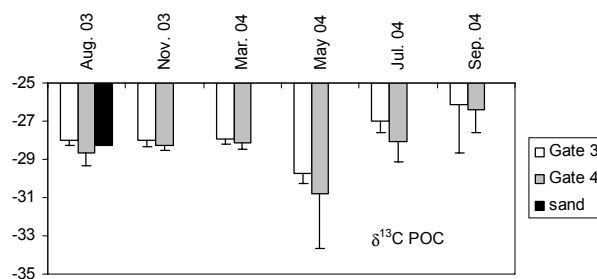


Figure 26. Seasonal variation in $\delta^{13}\text{C-SPOM}$ (‰) in the Aa River 2003-2004. Error bars = 1 standard deviation.

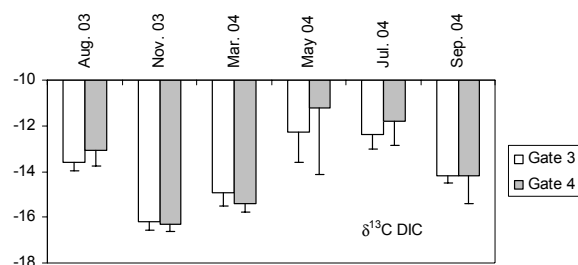


Figure 27. Seasonal variation in $\delta^{13}\text{C}_{\text{DIC}}$ (‰) in the Aa River 2003-2004.
Error bars = 1 standard deviation.

$\delta^{18}\text{O}$ of dissolved oxygen (DO) were relatively stable from late autumn till early spring, but they became increasingly variable toward summer (Figure 28). In addition, mean $\delta^{18}\text{O}_{\text{DO}}$ values decreased toward late spring and summer (Figure 28-insert). In November and March, $\delta^{18}\text{O}$ were generally higher than 24.2‰, the characteristic $\delta^{18}\text{O}$ value for DO in equilibrium with air O_2 . Values higher than 24.2‰ indicate that respiratory processes dominate in the water column (heterotrophy), while values below 24.2‰ indicate that photosynthetic O_2 production dominates (autotrophy). Thus, the overall decrease in $\delta^{18}\text{O}$ suggests that the system changes from a heterotrophic system in winter to an increasingly autotrophic system in summer. The large range of $\delta^{18}\text{O}_{\text{DO}}$ values observed in July and May, with values above and below 24.2‰, suggest, however, a strong variability in the relative importance of respiration and photosynthesis in the system (see below).

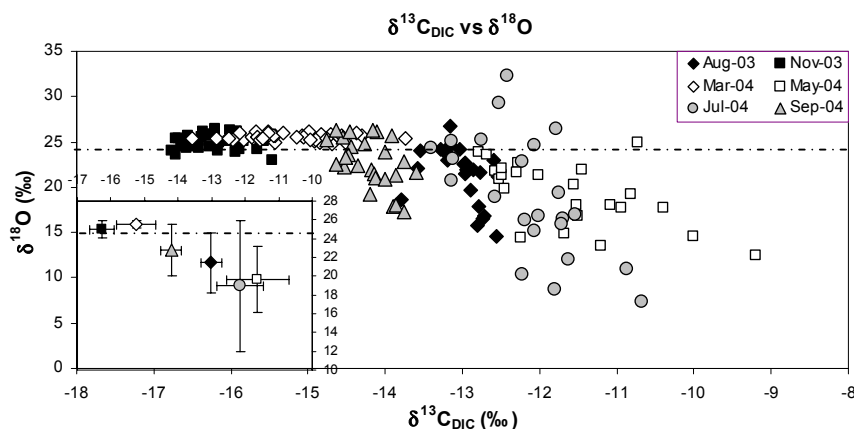


Figure 28. Seasonal variation in the $\delta^{13}\text{C}_{\text{DIC}}$ and $\delta^{18}\text{O}_{\text{DO}}$ composition in the Aa River 2003-2004. The insert shows the mean ($\pm$ SD) of all months. The dashed line represents the $\delta^{18}\text{O}_{\text{DO}}$ value for a situation where DO is in equilibrium with air O_2 (24.2‰).

VI.3.3.2. Spatial variation in the particulate organic material pool

Suspended particulate organic matter

There were no marked changes in the total concentration of SPM between gates 3 and 4 (Figure 29). However, there was a slight change in the C and N isotope composition of SPM during the summer months, with $^{13}\text{C}_{\text{SPM}}$ decreasing and $^{15}\text{N}_{\text{SPM}}$ increasing between gate 3 and 4 (Figure 25, 26). The decrease in ^{13}C and the increase in ^{15}N between the gates probably reflects an increase in the relative contribution of plant and/or phytoplankton material to the SPOM pool, since macrophytes and algae typically have lower ^{13}C and higher ^{15}N (between $-37.3 \pm 3.7\text{‰}$ and $-32.4 \pm 3.4\text{‰}$ for ^{13}C and between $7.1 \pm 3.8\text{‰}$ and $12.1 \pm 3.9\text{‰}$ for ^{15}N) than soil organic matter (^{13}C of -28.2‰ ; ^{15}N of 4.0‰). Such increase in macrophyte and algal C and N contribution is also reflected in an increase in Chl-a/POC and Chl-a/PN (Figure 30). In addition, the isotopic composition of phytoplankton and macrophyte material in the SPOM pool can have changed when C sources with decreasing ^{13}C and N sources with increasing ^{15}N were utilized.

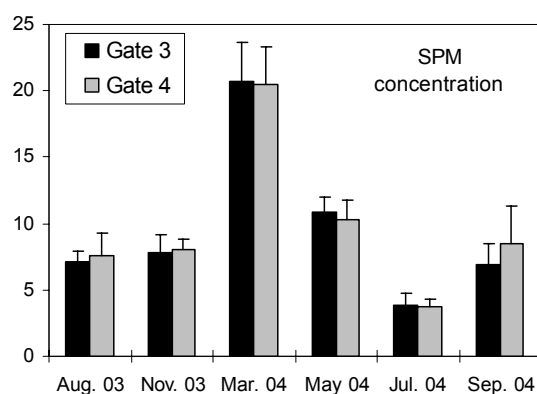


Figure 29. Seasonal variation in total SPM concentration in the Aa River 2003-2004. Error bars = 1 standard deviation.

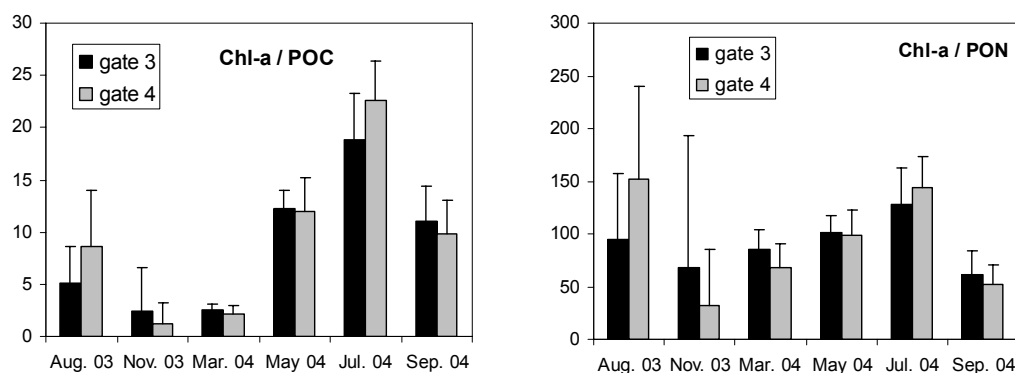


Figure 30. Seasonal variation in Chl-a/POC and Chl-a/PN in the Aa River 2003-2004. Error bars = 1 standard deviation.

VI.3.3.3. Diurnal variations

Both $\delta^{13}\text{C}_{\text{DIC}}$ and $\delta^{18}\text{O}$ values show considerable diurnal variation at gate 3 and 4. Variations at the upstream gate 3 are assumed to reflect biological processes occurring in the river section upstream of the gate and are therefore not discussed here. After a period equal to the transit time between the gates, variations at the downstream gate 4 will reflect those at gate 3 on which changes due to biological processes occurring in the river section between gate 3 and 4 are superimposed. Thus, any change in $\delta^{13}\text{C}_{\text{DIC}}$ and $\delta^{18}\text{O}$ signatures between gate 3 and 4 will be a consequence of biological activity in the river section under study.

We first estimated the transit time between the gates and compared samples take at gate 3 at time $t_{(x)}$ and at gate 4 at time $t_{(x + \text{transit time})}$ to ensure that the same water body was sampled. Figures A2 and A3 show the increase/decrease (Δ) in $\delta^{13}\text{C}_{\text{DIC}}$, $\delta^{18}\text{O}$ and DO for the different months except August, for which we did not have the necessary data to calculate the transit time. For all other months, transit times were calculated by estimating the time lag between two marked peaks in conductivity (data ULB), eventually corrected for variations in flow velocity (July).

Two major processes that can affect the $\delta^{13}\text{C}_{\text{DIC}}$ in the water column are heterotrophic respiration and photosynthetic CO_2 uptake. In general, heterotrophic respiration decreases the $\delta^{13}\text{C}_{\text{DIC}}$ by adding $^{12}\text{CO}_2$ to the DIC pool while photosynthesis increases the $\delta^{13}\text{C}_{\text{DIC}}$ by preferentially removing $^{12}\text{CO}_2$ from the DIC pool. Thus, negative $\Delta\delta^{13}\text{C}_{\text{DIC}}$ values indicate that respiration dominates over photosynthesis, while positive $\delta^{13}\text{C}_{\text{DIC}}$ values indicate that photosynthesis dominates over respiration in the water column.

$\Delta\delta^{13}\text{C}_{\text{DIC}}$ values were generally negative during the dark sampling hours (shaded bars) and during the day in November and March. During May, July and August, $\Delta\delta^{13}\text{C}_{\text{DIC}}$ values were generally positive during the daylight sampling hours (Figure 31).

Living organisms continually produce CO_2 as a byproduct of cell metabolism (respiration). Primary producers also use CO_2 during the daylight hours, when sunlight provides the necessary energy for photosynthesis. During the night, respiration is the main biological process in the water column and $\delta^{13}\text{C}_{\text{DIC}}$ values will thus decrease. During the day, photosynthetic CO_2 uptake can balance or exceed the input of respiratory CO_2 and overall $\delta^{13}\text{C}_{\text{DIC}}$ values will increase. Such increase is, however, most prominent during the growth season, when plant biomass is sufficiently high to offset the CO_2 input by photosynthetic CO_2 uptake.

The main processes affecting the $\delta^{18}\text{O}_{\text{DO}}$ in the water column are heterotrophic respiration and photosynthetic O_2 production. Heterotrophic respiration increases the $\delta^{18}\text{O}_{\text{DO}}$ signature by preferentially removing ^{16}O from the DO pool. Photosynthetic O_2

production adds O_2 with a $\delta^{18}O$ signature equal to that of oxygen in water ($\delta^{18}O_{H_2O}$), which has an annual average of $-6.4 \pm 0.4\text{‰}$ in the Aa River. $\delta^{18}O_{DO}$ values in “biologically dead” aquatic systems are typically 24.2‰ , the characteristic $\delta^{18}O$ value for DO in equilibrium with air O_2 . Values higher than 24.2‰ are an indication of respiratory removal of O_2 from the water column, while values below 24.2‰ are an indication of photosynthetic O_2 input.

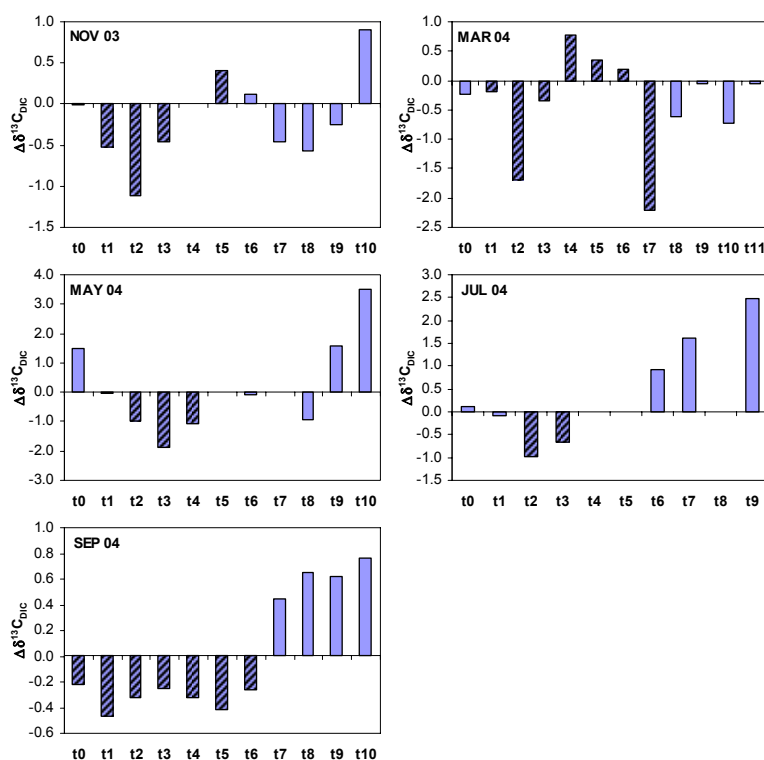


Figure 31: Seasonal variation in the diurnal change in $\delta^{13}C_{DIC}$ ($\Delta\delta^{13}C_{DIC} = \delta^{13}C_{DIC(gate\ 4)} - \delta^{13}C_{DIC(gate\ 3)}$) between gate 3 and gate 4 in the Aa River 2003-2004. The shaded bars denote samples taken between sunset and sunrise. Note that the amplitude of the changes are largest during May and July 2004.

$\Delta\delta^{18}O_{DO}$ values were always positive during the night (shaded bars), which is consistent with respiration dominating over photosynthesis at night, when photosynthesis stops because of a lack of solar energy to carry out the reaction (Figure 32). At the same time ΔDO values are negative, indicating net consumption of DO in the river section between gate 3 and gate 4. During daylight hours, $\Delta\delta^{18}O_{DO}$ were negative (grey bars) in November 2003, May and September 2004, while ΔDO values were positive. This suggests that net O_2 production by photosynthesis dominates during these periods. In July 2004, $\Delta\delta^{18}O_{DO}$ were also negative with positive ΔDO values during the day, but during twilight hours, positive $\Delta\delta^{18}O_{DO}$ values

coincided with negative ΔDO values. Positive $\Delta\delta^{18}\text{O}_{\text{DO}}$ values co-occurring with negative ΔDO values were also observed in March during daylight hours. These periods are probably characterized by low solar irradiance and thus low photosynthetic activity, so that respiration still dominates over photosynthesis.

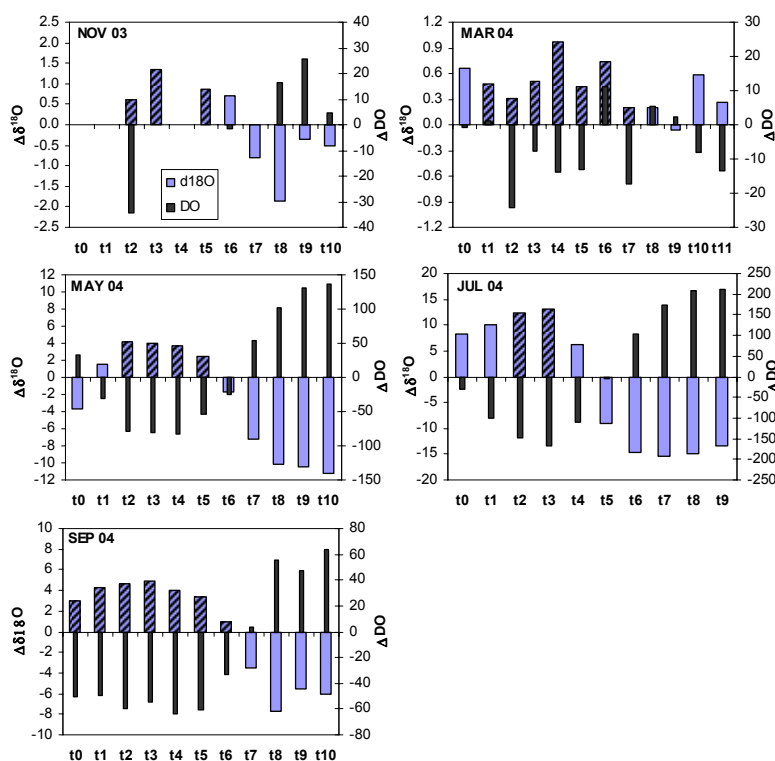


Figure 32. Seasonal variation in the diurnal change in $\delta^{18}\text{O}$ ($\Delta\delta^{18}\text{O}$) and DO (ΔDO , data UA, ULB) between gate 3 and gate 4 in the Aa River 2003-2004. The shaded bars denote samples taken between sunset and sunrise. Note that the amplitude of the changes are largest during May, July and September 2004.

To conclude, the results of the diurnal variations in $\delta^{13}\text{C}_{\text{DIC}}$ and $\delta^{18}\text{O}_{\text{DO}}$ show that during the night, the river section between gate 3 and 4 acts as a source of CO_2 and a sink of O_2 . During the day, the river section acts as a sink of CO_2 and a source of O_2 . Photosynthesis carried out by macrophytes is a key factor in determining if the system will act as a source or sink for CO_2 and O_2 . During the growth season (May till September), macrophytes induce large shifts in the oxygenation status and the CO_2 concentration of the water column. Only during the winter and spring months, when primary producer biomass is low, the river is a continuous source of CO_2 and a continuous sink of O_2 .

VII. The assimilation of ^{15}N by three different macrophytes under enforced stream conditions in a flume

VII.1. Introduction

In nutrient-rich streams, N and P supply is mainly delivered by the surface water (Madsen & Cedergreen, 2002). Periodic stripping of the boundary-layer by passing waves can increase nutrient uptake by macrophytes by a factor 10 (Stevens & Hurd, 1997). This is true for plant species forming open stands and macrophytes having streamlined leaves which permit the water to pass through the vegetation rather than being deflected around it (Sand-Jensen, 1998). Throughout a closed vegetation patch however, hydrodynamics are quite different, considering the unidirectional flow of streams. Hot-wire anemometry has shown a steep reduction of flow velocity in positions in dense canopy of macrophytes, while flow is accelerated along and above the patches (Sand-Jensen & Mebus, 1996). A relative high intensity of turbulence behind the canopy is shown by Hurd et al. (Hurd et al., 1994). Also within the canopy, turbulence has been observed (Sand-Jensen & Pedersen, 1999). Flow and turbulence inside and behind the plant canopies differ as mean stream velocity varies. Flow and turbulence are important for both the residence time of water and the exchange rate of solutes (e.g. N-NH_4^+ , N-NO_3^- and P-PO_4^{3-}) between the plant surfaces and the surrounding water (Sand-Jensen & Pedersen, 1999).

Therefore, one might expect that N-NH_4^+ and/or N-NO_3^- uptake will vary at different locations within a vegetation patch with different current velocities. This was investigated by performing N-uptake experiments under controlled conditions in a flume. The experiments attempted to quantify how N-uptake by macrophytes is affected by the following parameters: nitrogen source (N-NH_4^+ versus N-NO_3^-), current velocity and the location within a vegetation patch. Three macrophyte species with strongly contrasting growth forms were considered.

VII.2. Material and Methods

VII.2.1. Experimental setup

Three macrophyte species were used separately for the ex-situ experiments: a totally submerged species (*Callitriche platycarpa* Kütz.), a submerged species with floating leaves (*Potamogeton natans* L.) and an emergent one (*Sparganium erectum* L.). They were gathered from the Aa, a lowland river in the Northern part of Belgium. The plant individuals, needed for each experiment, were collected approximately 3 days before the beginning of the experiments. Intact *C. platycarpa*, *P. natans* and *S. erectum* species were taken out of the river and transported in transparent recipients to the lab. Macrophyte individuals were planted in small pots of 20 cm by 30 cm. Nutrient-free white sand was used to anchor the plants in the pots. After planting, the small pots were put in transparent boxes filled with tap water in order to adapt to the

lower nutrient levels. In total, 70 pots were planted with *C. platycarpa* and with *P. natans* and 55 pots with *S. erectum*. Macrophytes were exposed to a day-night cycle of 14/10 with 4 daylight spectrum lamps.

The study was carried out in the Dutch Institute for Ecological Research in the Netherlands in June 2005, the middle of the growing season. The ex-situ experiments were carried out in a circular flume, which can be seen as a controlled river situation (Figure 33A) (Vogel & LaBarbera, 1978). A complete description can be found in (Bouma et al., 2002)). In total, 18 experiments were done. Each macrophyte species was exposed at three stream velocities to labelled ammonium and to labelled nitrate.

First, the flume was filled with nearly 10 m³ of tap water comprising low nutrient concentrations. Mean concentration of N-NO₃⁻ was 0.37 mg l⁻¹. The concentrations of N-NH₄⁺, N-NO₃⁻ and P-PO₄³⁻ were lower than the detection limits of 0.08 mg l⁻¹, 0.01 mg l⁻¹ and 0.02 mg l⁻¹ respectively. In order to investigate the uptake of ammonium by the macrophytes, ¹⁵N labelled NH₄Cl was added to the flume water, increasing the concentration up to 0.08 mg l⁻¹ N-NH₄⁺. The labelled salt solution was distributed all over the flume length and mixed very well – by creating a high velocity – through the water. After stabilizing, the pots with *C. platycarpa* were placed in the test section in a regular pattern (Figure 33B(1)). The velocity for the first experiment was regulated at 5 cm s⁻¹ and after three hours, the velocity was set to zero. At the same time, macrophytes at position 2, 5 and 9 were taken out of the test section (Figure 33B(1)). The aboveground biomass at the three positions was cut, plants were rinsed very well, weighed and frozen at -20 °C. New pots with *C. platycarpa* were added into the respective positions in the test section, the velocity was regulated this time at 10 cm s⁻¹ and after three hours of running, the above procedure was repeated. In the third experiment with *C. platycarpa*, velocity was set at 30 cm s⁻¹ and again, the above procedure was repeated. Before adding label to the flume, after mixing the label and after each experiment, water samples were collected and analysed. Depletion of ¹⁵N-NH₄⁺ in the surface water did not occur during three successive experiments. At the end, another set of new pots with *C. platycarpa* were placed in the flume and detailed velocity profile measurements were performed (see below).

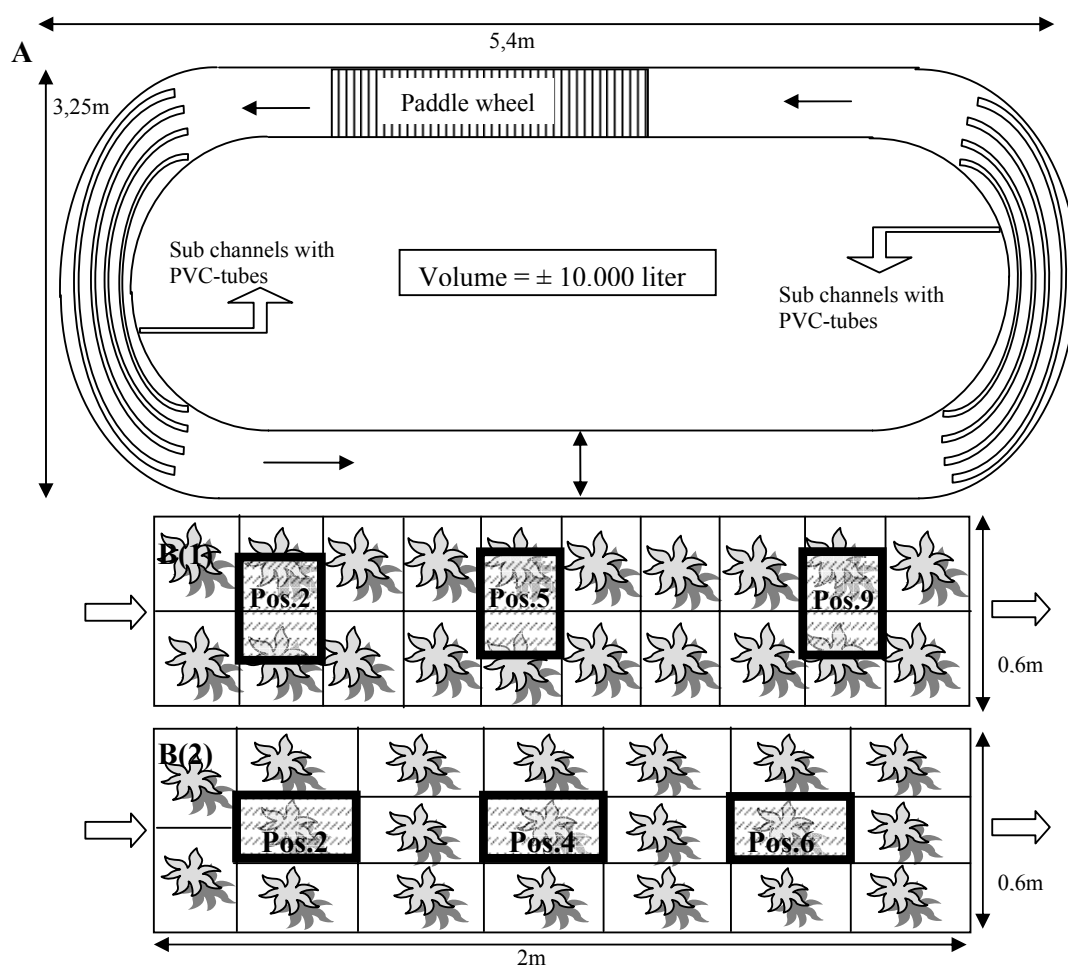


Figure 33: (A) schematic presentation of the flume (top view) and (B) schematic presentation of the arrangement of 20 pots with macrophytes in the test section for (1) *Callitriche platycarpa* and *Potamogeton natans* and for (2) *Sparganium erectum*. $\star$ = macrophytes in each pot. The black rectangles with biomass were selected to analyse ^{15}N in the biomass standing in the different locations in a patch.

The same experiments were repeated for the macrophyte species *P. natans* (Figure 33B(1)) and *S. erectum* (Figure 33B(2)). Before a new species was placed in the flume test section, the water in the flume was refreshed by new tap water and $^{15}\text{N}\text{-NH}_4^+$ was added, increasing the concentration up to $0.08 \text{ mg l}^{-1} \text{ N-NH}_4^+$. The whole procedure remains the same as for *C. platycarpa*, except that leaves and stems of *P. natans* were separated while taking the aboveground biomass.

In the second set of experiments, $^{15}\text{N}\text{-NO}_3^-$ label was added to the refreshed water in the flume increasing the concentration up to $0.53 \text{ mg N-NO}_3^- \text{ l}^{-1}$. The uptake experiments for all three macrophytes were carried out in the same way as described above.

VII.2.2. Velocity profiles and bending angles

For each macrophyte species and for each enforced flume velocity (5 cm s⁻¹, 10 cm s⁻¹ and 30 cm s⁻¹) vertical velocity profiles were measured at four positions: 10 cm upstream the patch, in the upstream margin of the patch, in the middle and at the downstream margin of the patch. All positions were located along the centre line of the flume and the velocity measurements were performed with an Acoustic Doppler Velocimeter (ADV; Nortek field version), mounted on a 3D positioning system that can be placed anywhere along the length of the working section. Prior to a set of velocity measurements the flume was seeded with very fine deep-sea clay sediment or artificial seeding particles, which stay in suspension, even at very low flow velocities. At each location a time series was measured with a frequency of 25 Hz over a period of 60 s, yielding 1500 data points. The ADV provided measurements for the individual velocity compounds *u*, *v* and *w*. For each compound mean and standard deviation of a time series were registered, and used to calculate the relative

turbulence intensity, according to the following equation:
$$TI = \frac{\sqrt{u_{stdev}^2 + v_{stdev}^2 + w_{stdev}^2}}{\sqrt{u_{av}^2 + v_{av}^2 + w_{av}^2}}$$

The bending angles, i.e. the angle between the vertical axis and the plant species, vary with velocity. A transparent plastic sheet was placed at the side of the test section where the profile of each macrophyte patch at each velocity was drawn. Bending angles were measured with a setsquare using the drawings.

VII.2.3. Analysis

Oven dried or lyophilized well grinded plant samples are combusted at high temperature (1010 °C). After drying over a water trap, the formed CO₂ and N₂ gases are separated on a GC-column and carried to the IRMS in a helium flow for analyzing the ion-ratios corresponding to the isotopic composition (in this case δ¹⁵N). This is done on a Fisons NA-2500 elemental analyzer with auto sampler and a Hasep-Q column 80-100 mesh I.D. 2mm followed by the Isotope Ratio Mass Spectrometer Finnigan MAT Delta S or Delta Plus using the Conflo II interface. Detailed information about the methods and analysis can be found in books and articles (Böhlke & Coplen, 1995; Werner & Brand, 2001; Carman & Fry, 2002).

A destruction analysis to determine total N- and P-concentrations was done using digestion tubes with H₂SO₄-salicylic acid-H₂O₂ (Novozamsky et al., 1983).

One way factorial ANOVA was used to test differences between uptake rates, relative growth rates and total N concentrations.

VII.2.4. Calculation of uptake rates and relative growth rate (RGR)

The N-NH₄⁺ and N-NO₃⁻ uptake rate per (part of) species expressed in mg N g⁻¹ DW h⁻¹ was calculated as follows:

$Up = \% \text{ }^{15}\text{N}_{\text{exp}} - \% \text{ }^{15}\text{N}_{\text{control}} \times [N]_{\text{total}} / (3 \times 100) \times ([N\text{-NXy} + \text{}^{15}\text{N-NXy}]_{\text{water}} / [\text{}^{15}\text{N-NXy}]_{\text{water}})$
 with $[N\text{-NXy} + \text{}^{15}\text{N-NHy}]_{\text{water}} / [\text{}^{15}\text{N-NXy}]_{\text{water}} = 1$ for N-NH_4^+ , and $= 3.3$ for N-NO_3^- .

$\% \text{ }^{15}\text{N}_{\text{exp}}$ = relative ^{15}N concentration within the (part of) species after the experiments.

$\% \text{ }^{15}\text{N}_{\text{control}}$ = relative ^{15}N concentration within an unlabelled (part of) species.

$[N]_{\text{total}}$ = total N concentration in the (part of) species expressed in mg N g^{-1} DW.

$[N\text{-NXy}]_{\text{water}}$ = unlabelled N-NH_4^+ or N-NO_3^- concentration in the surface water.

$[\text{}^{15}\text{N-NXy}]_{\text{water}}$ = labelled N-NH_4^+ or N-NO_3^- concentration in the surface water.

The RGR that could be maximally supported by the N-uptake from the water, as measured during the flume experiment (RGR_{max}) expressed in day^{-1} was calculated as follows: $(\text{N-uptake rate} / [N]_{\text{total}}) \times 24$.

VII.3. Results

VII.3.1. Vertical velocity profiles

The vertical velocity profiles measured upstream of the vegetation patch showed similar shapes for the different macrophyte species and enforced flume velocity conditions, although a closer look revealed some important differences (Figure 34 I – 42 I). Most upstream profiles were U-shaped with a nearly constant velocity down to 10 cm above the bottom surface. Nevertheless, the constant flow velocity in the upper water layer and the velocity measured near the bottom were found to be different among the macrophyte species and the effect was more obvious at higher flow conditions. For *Callitriche platycarpa* the U-shaped profile was even deformed at 30 cm s^{-1} (Figure 36 I). The observed influence of species on the velocity profile upstream of the vegetation patch can be attributed to the structure and configuration of the plants, which is decisive for the flow resistance exerted by the macrophyte stand (Nepf & Vivoni, 2000; Sand-Jensen, 2003; Green 2005).

The patches of *Callitriche platycarpa* showed a dense structure; plants tightly joined and bent together at increasing flow conditions, resulting in a skimming flow with low velocities inside the canopy and high velocities above the canopy (Figure 34 II, III, IV - 36 II, III, IV). As the dense vegetation stand was partly blocking the cross section area, water was forced to flow over the canopy, reaching flow velocities up to twice the enforced flume velocity. At the upper border of the canopy, the sharp drop in velocity was coinciding with an increase of turbulence intensity (Figure 34). In that turbulent layer, flapping of the vegetation was observed and as flume velocity increased, the turbulent zone was compressed.

The patches of *Sparganium erectum*, on the other hand, were hardly altered by the enforced flow conditions in the flume. Consequently, the shape of the velocity profile at a specific location in the patch was hardly changing with increasing flow conditions, except for a shift towards higher velocity ranges (Figure 40 II, III, IV – 42

II, III, IV). Comparing the velocity profiles measured at different locations in the stand, however, revealed that the local arrangement and structure of the plants were strongly affecting the velocity profile. At the upstream margin and in the middle of the patch, two flow layers could be distinguished in the velocity profile (Figure 40 II, III – 42 II, III): a fast flow in the upper layer (15-35 cm), where the cylindrical structured leaves were less closely packed together, and a reduced flow in the lower layer (0-15 cm), where the plant structures were resembling a stand of rigid cylinders. Due to the stiffness and strong resistance of *Sparganium erectum*, the flow velocities reached in the fast flowing upper layer were steeply decreasing as the water was penetrating deeper into the vegetation stand. At the downstream margin of the patch, the flow stratification had completely vanished and along the entire profile flow velocities were low and variable in direction, causing increased turbulence intensity (Figure 40 III – 42 III), especially at the lower flume velocities (5 cm s^{-1} and 10 cm s^{-1}).

Considering the velocity profiles measured in the patches of *Potamogeton natans*, a clear distinction could be made between the floating situation at the lower flume velocities (5 cm s^{-1} and 10 cm s^{-1}) and the fully submerged situation that occurred at the highest flume velocity (30 cm s^{-1}). In the former situation (Figure 37 II, III, IV – 38 II, III, IV), the flow was penetrating the vegetation and passing through the stand of flexible stems underneath the floating leaves. Along the profile, flow velocities were varying with the local density of the vegetation. In the latter situation (Figure 39), the buoyancy of the leaves and the plants' resistance against bending were exceeded by the force of the flowing water, immersing the entire vegetation patch and reconfiguring the stand to a streamlined submerged volume, hardly 10 cm high at the upstream margin and up to 25 cm high at the downstream border. As shown by the velocity profiles (Figure 39 II, III, IV), the water was no longer passing through the vegetation in this case, but merely flowing over the suppressed macrophyte stand (skimming flow). Moving downstream the vegetation patch, the skimming flow velocity was increasing due to reduced free cross section area above the canopy. And at the downstream margin of the patch, a turbulent zone developed just below the overhanging layer of submerged leaves (Figure 37-39).

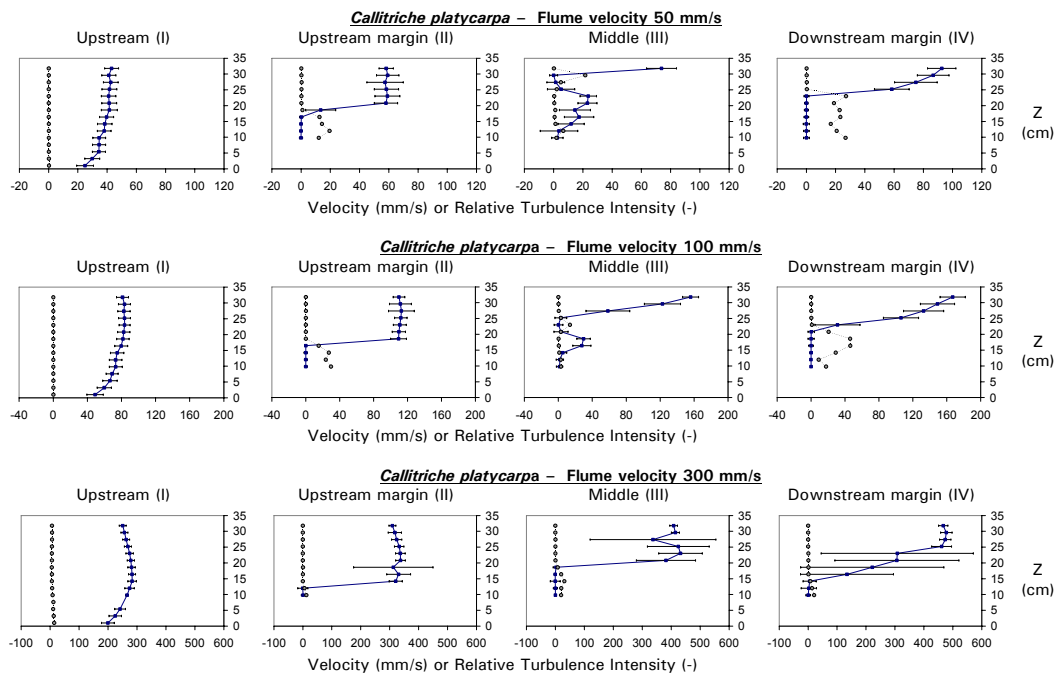


Figure 34-36 Real velocity (mm s^{-1}) and Turbulence Intensity (-) patterns for *Callitriche platycarpa* at three enforced velocities (50, 100 and 300 mm s^{-1}) upstream the patch (I), in the upstream margin of the patch (II), in the middle of the patch (III) and at the downstream margin of the patch (IV).

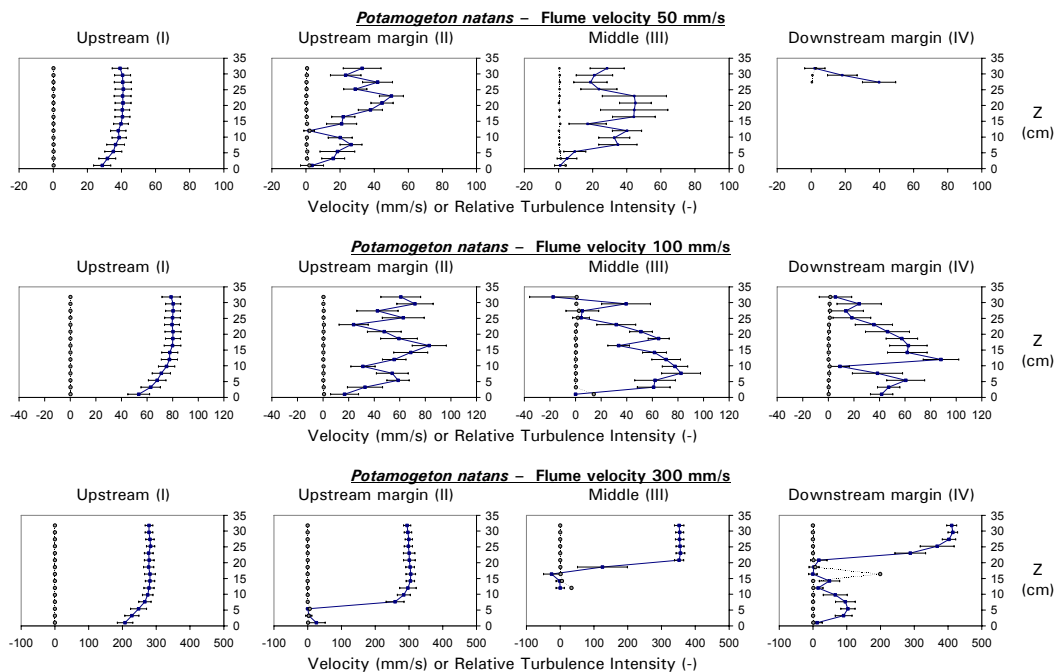


Figure 37-39: Real velocity (mm s^{-1}) and Turbulence Intensity (-) patterns for *Potamogeton natans* at three enforced velocities (50, 100 and 300 mm s^{-1}) upstream the patch (I), in the upstream margin of the patch (II), in the middle of the patch (III) and at the downstream margin of the patch (IV).

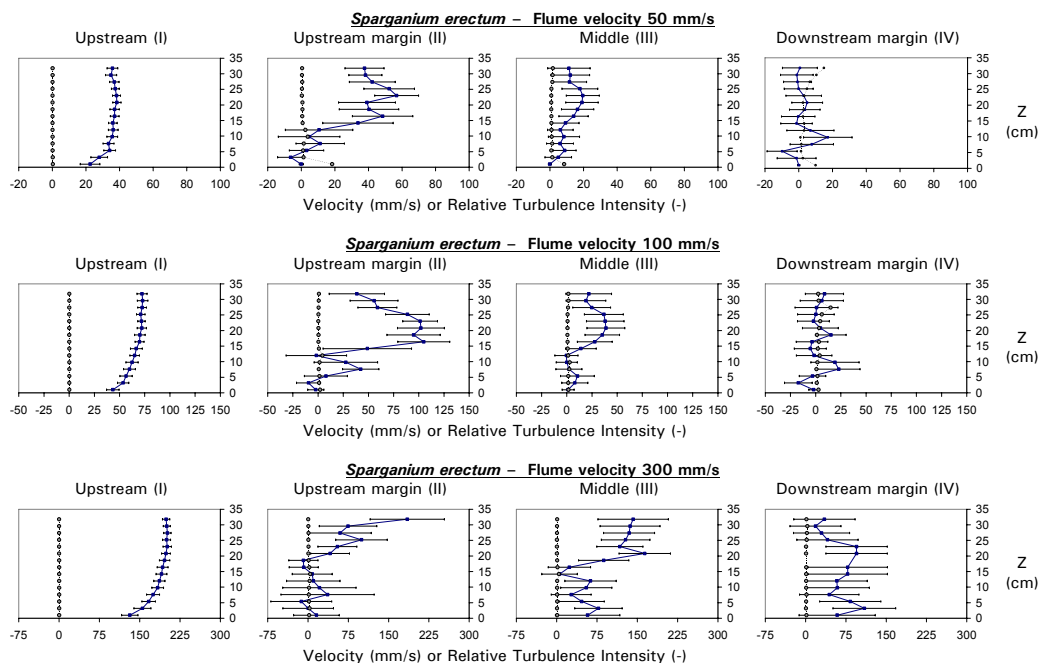


Figure 40-42: Real velocity (mm s^{-1}) and Turbulence Intensity (-) patterns for *Sparganium erectum* at three enforced velocities (50, 100 and 300 mm s^{-1}) upstream the patch (I), in the upstream margin of the patch (II), in the middle of the patch (III) and at the downstream margin of the patch (IV).

VII.3.2. Macrophytes

The bending angle of the macrophyte species along with the current velocity is plotted in figure 43. *Sparganium erectum* is not plotted because still no bending occurred at the highest velocity of 30 cm s^{-1} . An inverse pattern is observed in the plot: at the lowest current velocity of 5 cm s^{-1} , *Callitriche platycarpa* showed a higher bending then *Potamogeton natans*. At 10 cm s^{-1} , both species have the same bending angle of 45° whereas *Potamogeton natans* exhibit a higher bending at 30 cm s^{-1} .

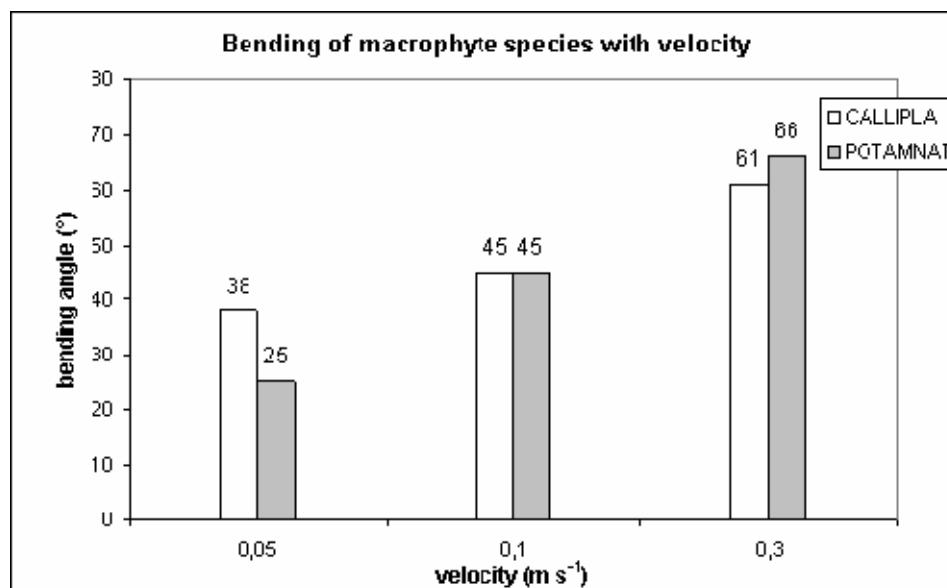


Figure 43: Bending angle of *Callitriche platycarpa* and *Potamogeton natans* with three current velocities. Bending angles of *Sparganium erectum* are not plotted on the graph because no bending could be observed.

VII.3.3. ¹⁵N-uptake

Figure 44 shows that all three macrophyte species prefer N-NH_4^+ as a nitrogen source, although uptake by *Sparganium erectum* is very small. *Callitriche platycarpa* and *Potamogeton natans* have significant higher percentage of ^{15}N after the experiments with $^{15}\text{N-NH}_4^+$ labelled water then with $^{15}\text{N-NO}_3^-$ labelled water ($p < 0,001$ and $p < 0,001$ respectively). This is not the case for *Sparganium erectum* ($p = 0,122$). *Potamogeton natans* has the highest incorporation of $^{15}\text{N-NH}_4^+$. Uptake rates of all species and different parts of species are given in table 16. All species have higher uptake rates for $^{15}\text{N-NH}_4^+$ then for $^{15}\text{N-NO}_3^-$ during the 3 hours of the experiment. *Sparganium erectum* has the lowest uptake rate for both $^{15}\text{N-NH}_4^+$ and $^{15}\text{N-NO}_3^-$. *Potamogeton natans* is the species that has the highest uptake rate for both nitrogen sources. The uptake rate of $^{15}\text{N-NH}_4^+$ by *Callitriche platycarpa* is significantly lower than the uptake rate of leaves and stems of *Potamogeton natans* (Table 17). However, absolute concentrations of N stored within the species are significantly higher in *Callitriche platycarpa* then in *Potamogeton natans*, both for leaves and stems (Table 18). *Sparganium erectum* has significantly lower N-concentrations than both *Callitriche platycarpa* and *Potamogeton natans* (Table 18).

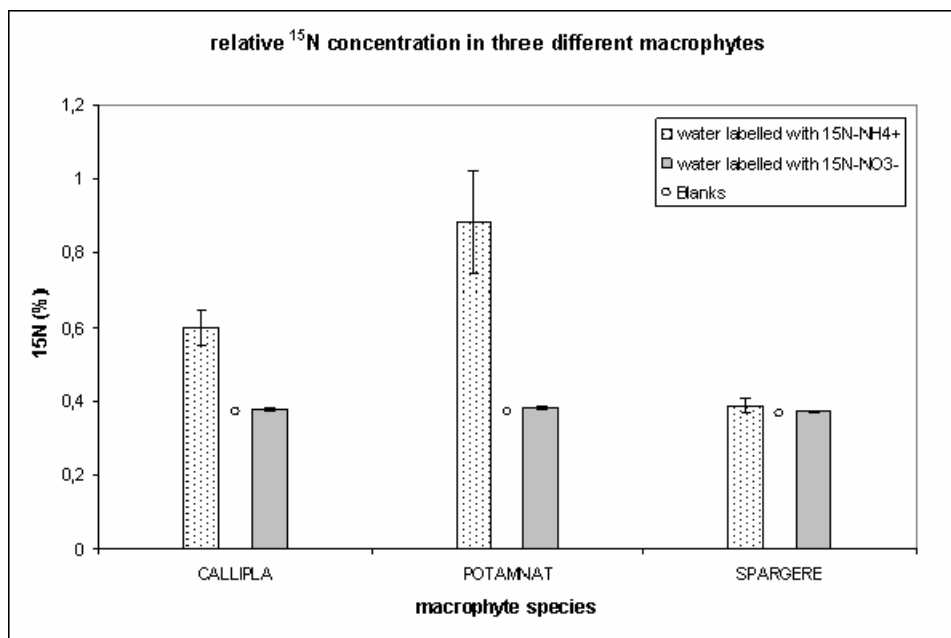


Figure 44: relative ¹⁵N-concentration in the aboveground biomass of three macrophyte species with standard deviation after 3 hours of running in a flume with ¹⁵N-NH₄⁺ labelled water and with ¹⁵N-NO₃⁻ labelled water. Blanks are indicated with o. CALLIPLA = *Callitriche platycarpa*; POTAMNAT = *Potamogeton natans* and SPARGERE = *Sparganium erectum*.

Table 16: average uptake rates of ¹⁵N-NH₄⁺ and standard deviation in three macrophytes or parts of macrophyte species. Rates are expressed in mg N/g DW/h. DW = dry weight; h = hour; CALLIPLA = *Callitriche platycarpa*; POTAMNAT-LEAVE = leaves of *Potamogeton natans*; POTAMNAT-STEM = stem of *Potamogeton natans*; Avg POTAMNAT = average of leaves and stems of *Potamogeton natans*; SPARGERE-ROOT = roots of *Sparganium erectum*; SPARGERE-LEAVE = leaves of *Sparganium erectum*; Avg SPARGERE = average of roots and leaves of *Sparganium erectum*.

Uptake rates ¹⁵ N-NH ₄ ⁺	mg N/gDW/h	Stdev	Uptake rates ¹⁵ N-NH ₄ ⁺	mg N/gDW/h	Stdev
CALLIPLA	0.0295	0.0067	CALLIPLA	0.0036	0.0012
POTAMNAT – LEAVE	0.0529	0.0114	POTAMNAT – LEAVE	0.0050	0.0018
POTAMNAT – STEM	0.0429	0.0070	POTAMNAT – STEM	0.0038	0.0009
Avg POTAMNAT	0.0479	0.0105	Avg POTAMNAT	0.0044	0.0015
SPARGERE – ROOT	0.0008	0.0015	SPARGERE – ROOT	0.0005	0.0005
SPARGERE – LEAVE	0.0020	0.0007	SPARGERE – LEAVE	0.0007	0.0004
Avg SPARGERE	0.0014	0.0014	Avg SPARGERE	0.0006	0.0004

Table 17: p-values (one way ANOVA) for N-NH₄⁺ uptake rates between *Callitriche platycarpa* and leaves and stems of *Potamogeton natans* at significance level $\alpha = 0.05$. CALLIPLA = *Callitriche platycarpa*; POTAMNAT-L = leaves of *Potamogeton natans*; POTAMNAT-S = stems of *Potamogeton natans*

Uptake rate N-NH ₄ ⁺	CALLIPLA	POTAMNAT-L	POTAMNAT-S
CALLIPLA	***	0,000142	0.008624
POTAMNAT-L		***	0,053536
POTAMNAT-S			***

Table 18: p-values (one way ANOVA) for N concentrations between (parts of) species at significance level $\alpha = 0.05$. CALLIPLA = *Callitriche platycarpa*; POTAMNAT-L = leaves of *Potamogeton natans*; POTAMNAT-S = stems of *Potamogeton natans*; SPARGERER-L = leaves of *Sparganium erectum*; SPARGERER-R = roots of *Sparganium erectum*

N-conc	CALLIPLA	POTAMNAT-L	POTAMNAT-S	SPARGERER-L	SPARGERER-R
CALLIPLA	***	0,0217	0,0001	0,0001	0,0001
POTAMNAT-L		***	0,0001	0,0001	0,0001
POTAMNAT-S			***	0,0006	0,0001
SPARGERER-L				***	0,0001
SPARGERER-R					***

Combining the uptake rates and the absolute total N concentration of (parts of) species might give an idea about the relative growth rates (RGR). Figure 45 and table 19 showed that both leaves and stems of *Potamogeton natans* has significantly higher RGR's compared with *Callitriche platycarpa*, which on his turn has a significantly higher RGR compared with shoots and roots of *Sparganium erectum*. No significant trend in ammonium uptake can be observed with increasing stream velocity (Figure 46). This is the case for both *Callitriche platycarpa* and *Potamogeton natans*, also for its leaves and stems apart.

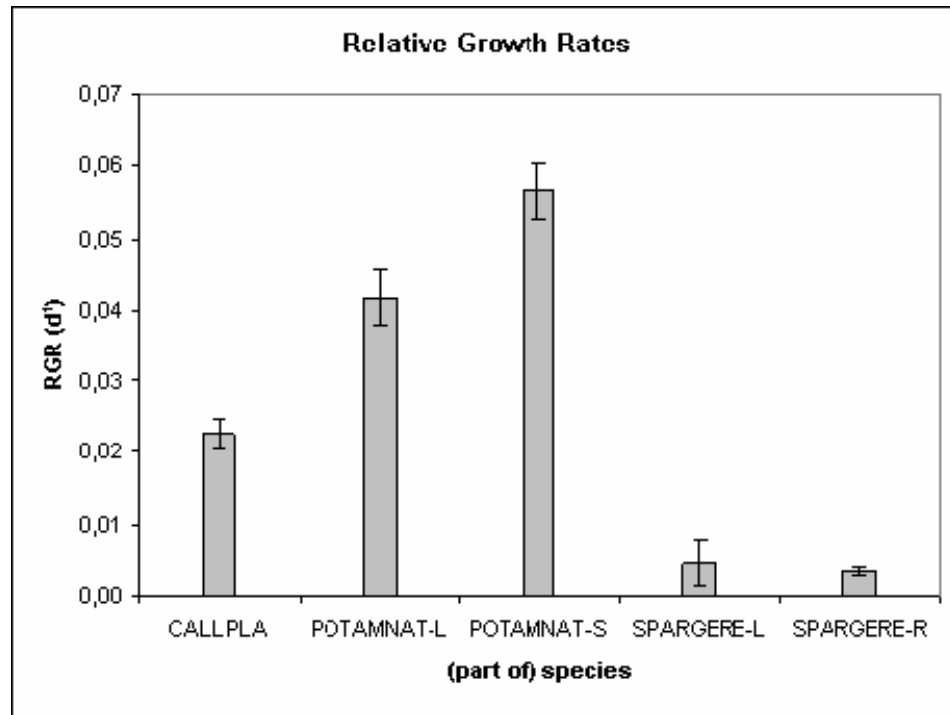


Figure 45: Relative growth rates expressed in d⁻¹ for (parts of) species with standard errors.

CALLIPLA = *Callitriche platycarpa*; POTAMNAT-L = leaves of *Potamogeton natans*;
 POTAMNAT-S = stems of *Potamogeton natans*; SPARGERE-L = leaves of *Sparganium erectum*;
 SPARGERE-R = roots of *Sparganium erectum*

Table 19: p-values (one way ANOVA) for RGR between (parts of) species at significance level $\alpha = 0.05$. CALLIPLA = *Callitriche platycarpa*; POTAMNAT-L = leaves of *Potamogeton natans*;
 POTAMNAT-S = stems of *Potamogeton natans*; SPARGERE-L = leaves of *Sparganium erectum*;
 SPARGERE-R = roots of *Sparganium erectum*

RGR (d ⁻¹)	CALLIPLA	POTAMNAT -L	POTAMNAT-S	SPARGERE-L	SPARGERE-R
CALLIPLA	***	0,000135	0,000126	0,000138	0,000130
POTAMNAT-L		***	0,000248	0,000126	0,000126
POTAMNAT-S			***	0,000126	0,000126
SPARGERE-L				***	0,991754
SPARGERE-R					***

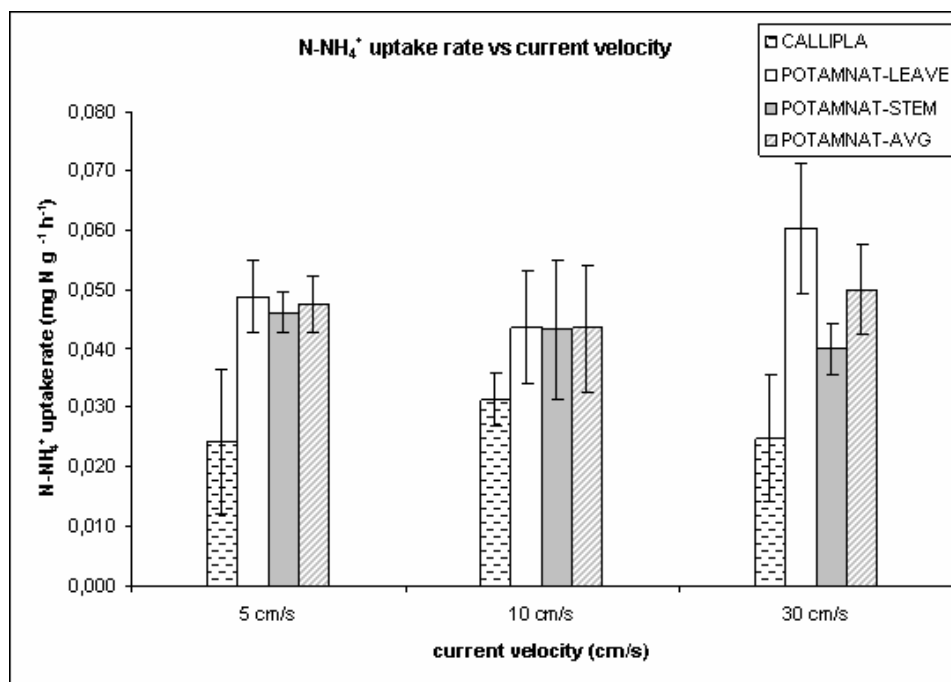


Figure 46: N-NH₄⁺ uptake rates with standard deviation of *Callitriche platycarpa* (CALLIPLA), leaves of *Potamogeton natans* (POTAMNAT-LEAVE), stems of *Potamogeton natans* (POTAMNAT-STEM) and average uptake rate of leaves and stems of *Potamogeton natans* (POTAMNAT-AVG) with increasing stream velocity. No significant differences in N-NH₄⁺ uptake can be detected by the (parts of) species with increasing stream velocity.

In Figure 47, the ¹⁵N-concentration within *Callitriche platycarpa* and within the leaves and stems of *Potamogeton natans* is plotted against the position within the vegetation patch. Figure 15a presents the observed uptake pattern for *Callitriche platycarpa* at 5 cm s⁻¹ and for leaves and stems of *Potamogeton natans* at 5 cm s⁻¹ and at 10 cm s⁻¹. Uptake of ¹⁵N-NH₄⁺ was relatively high in the upstream margin of the patch, lower in the middle of the patch and was again higher at the downstream margin of the patch. At higher velocities (figure 15b), ¹⁵N-NH₄⁺ uptake by *Callitriche platycarpa* and by leaves and stems of *Potamogeton natans* shows an opposite pattern: low uptake in the upstream margin of the patch, higher in the middle and somewhat lower again at the downstream margin of the patch, except for the leaves of *Potamogeton natans* which had the highest uptake of ¹⁵N-NH₄⁺ at the end of the patch.

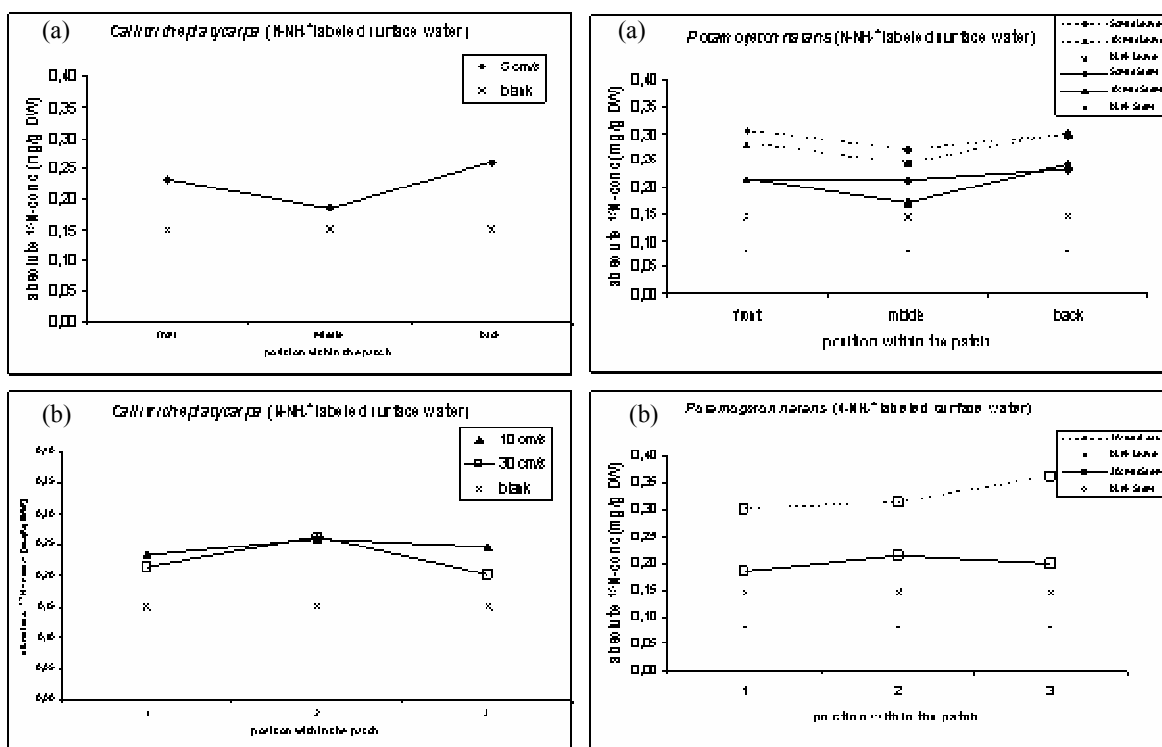


Figure 47: N-NH_4^+ uptake patterns for *Callitriche platycarpa* and *Potamogeton natans* at different locations within a patch (front-middle-back). (a) a typical N-NH_4^+ uptake pattern from the front to the end of the patch [high – low – high uptake] is observed for *Callitriche platycarpa* at 5 cm s^{-1} and for *Potamogeton natans* (leaves and stems) at 5 cm s^{-1} and 10 cm s^{-1} . (b) N-NH_4^+ uptake patterns for *Callitriche platycarpa* and *Potamogeton natans* (leaves and stems) for all velocities. Uptake patterns of the added velocities are also typical [low – high – low uptake] except for the leaves of *Potamogeton natans*.

VII.4. Discussion

Stiff and emergent species with a developed root system, like *Sparganium erectum*, will resist very well against stream velocities ranging from 5 till 30 cm s^{-1} (Bal et al).. In the same velocity range, *Callitriche platycarpa* (fully submerged) and *Potamogeton natans* (submerged with floating leaves) are more flexible and will bend when stream flow is experienced. At low velocities, *Callitriche platycarpa* will bend faster and is somewhat less rigid than *Potamogeton natans* which is in line with results of (Bal et al.). At a velocity of 10 cm s^{-1} , both species have a bending angle of 45° whereas at a velocity of 30 cm s^{-1} , both species bend further down to the bottom. At the highest velocity, leaves of *Potamogeton natans* remain under water (Figure 48). At 10 cm s^{-1} and 30 cm s^{-1} , no clear distinction in bending pattern was observed between these two species unlike the results of (Bal et al.). Bal et al.(in prep) worked with individual species, whereas our work considered whole vegetation patches which may influence the bending characteristics.

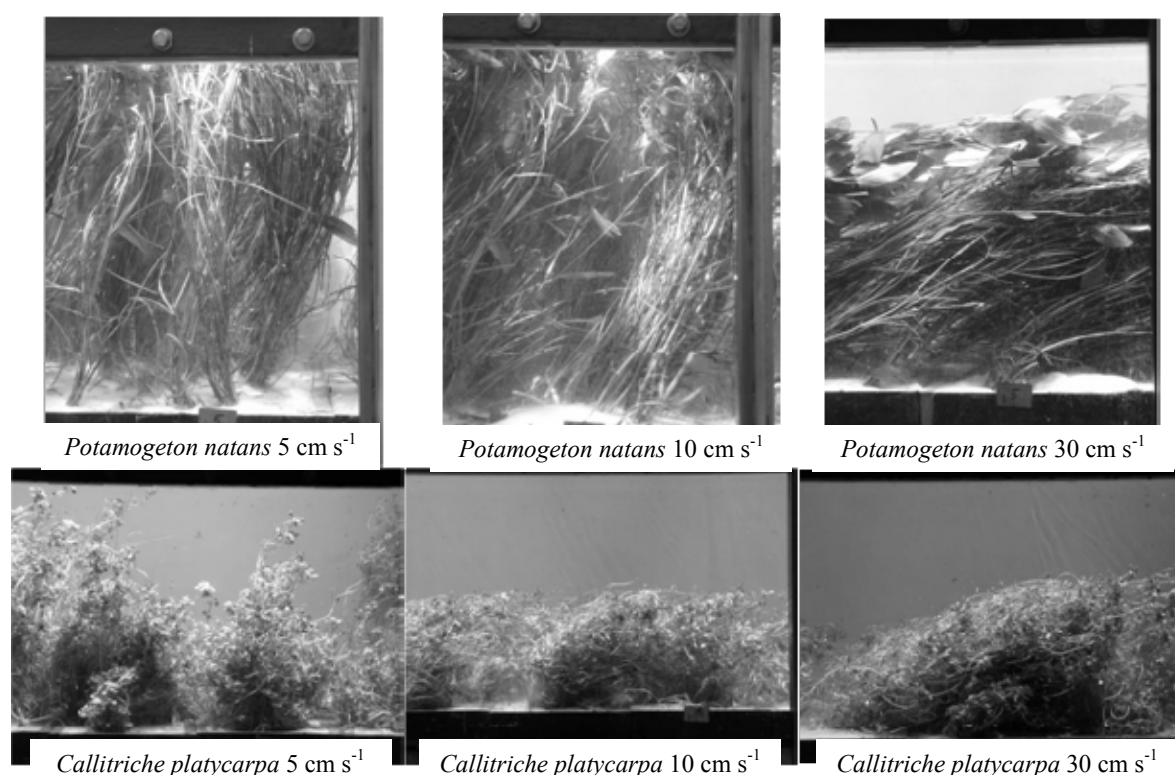


Figure 48: side view of *Callitriche platycarpa* and *Potamogeton natans* in the flume at 3 current velocities.

¹⁵N uptake

It is clear that all three macrophyte species prefer N-NH_4^+ as a nitrogen source instead of $^{15}\text{N-NO}_3^-$. This coincides with other researchers (Nichols & D.R., 1976; Ingemarsson et al., 1984; Thursby & Harlin, 1984; Bernot et al., 2006). Uptake of N-NH_4^+ is energetically preferred because no nitrate reductase activity will be needed (Cedergreen & Madsen, 2003). High concentrations of N-NO_3^- (i.e. 7 mg l⁻¹) would even hamper the growth of macrophytes in contrast to N-NH_4^+ (Boedeltje et al., 2005).

Uptake rates for both nitrogen sources were calculated for all species. Uptake rates for N-NO_3^- in all species are very small. The three hours duration of our experiment was too short to distinguish clear uptake rates for nitrate. The uptake rate obtained for N-NH_4^+ for *Sparganium erectum* was small as well. Leaves of this emergent species have thicker cuticula, which is a typical feature of land plants, and nutrients cannot enter very fast the leaves. The clearly developed root system might be indicating that nutrients are obtained mainly by the sediment.

N-NH_4^+ uptake rate of *Callitriche platycarpa* is significantly lower than that of *Potamogeton natans* (Table 16) which may indicate that *Potamogeton natans* is very efficient in taking up N-NH_4^+ . Leaves of *Potamogeton natans* have significantly higher N-concentrations than stems, but our experiments could not verify whether

leaves take up more N-NH_4^+ or whether the higher N-concentrations are due to allocation from roots and stems to the leaves. Total N-concentration in *Callitriche platycarpa* however, is higher compared with *Potamogeton natans*. Likely, *Potamogeton natans* needs less time to take up the same amount of N-NH_4^+ but cannot stock high amounts of nitrogen compared with *Callitriche platycarpa*. This explains the significantly higher RGR of *Potamogeton natans* and could be of interest for water managers when the aim of nutrient reduction has to be achieved by mowing. RGR values are consistent with other research (Madsen & Cedergreen, 2002). Compared to macrophytes occurring in a pond in Colombia have RGR's which are ten times higher (Cedergreen, 1999).

While hypothesised that with increasing velocity, less nutrients will be taken up, we could not detect any difference for all macrophytes with velocities ranging from 5 to 30 cm s^{-1} . However, repetitive patterns of uptake were found at different locations within a patch for *Callitriche platycarpa* and *Potamogeton natans*. The first pattern was found for *Callitriche platycarpa* at 5 cm s^{-1} and for *Potamogeton natans* (leaves and stems) at 5 and 10 cm s^{-1} and can be linked to velocity patterns found by Hurd et al. (1994) and Sand-Jensen en Pedersen (1999) (Figure 49a). At these velocities, the species do not show a strong bending (Figure 48). Water flow slows down just in front of the patch and due to the continuous supply of N-NH_4^+ and the relative large contact surface with the vegetation, uptake can be relatively high in front of the patch (figure 17a). An accelerated flow passes mainly along and/or above the patch where residence time between water and the top of the macrophytes is low. Hence, the contact surface of the non bending macrophytes with water is low. As a result, N-NH_4^+ uptake will be lower in the middle of the patch. At the end of the patch, water flow will experience higher turbulence because of the sudden height difference between the top of the patch and the bottom. Turbulence eddies can cause a reverse inflow of N-NH_4^+ into the back of the patch which may explain the higher uptake at the end.

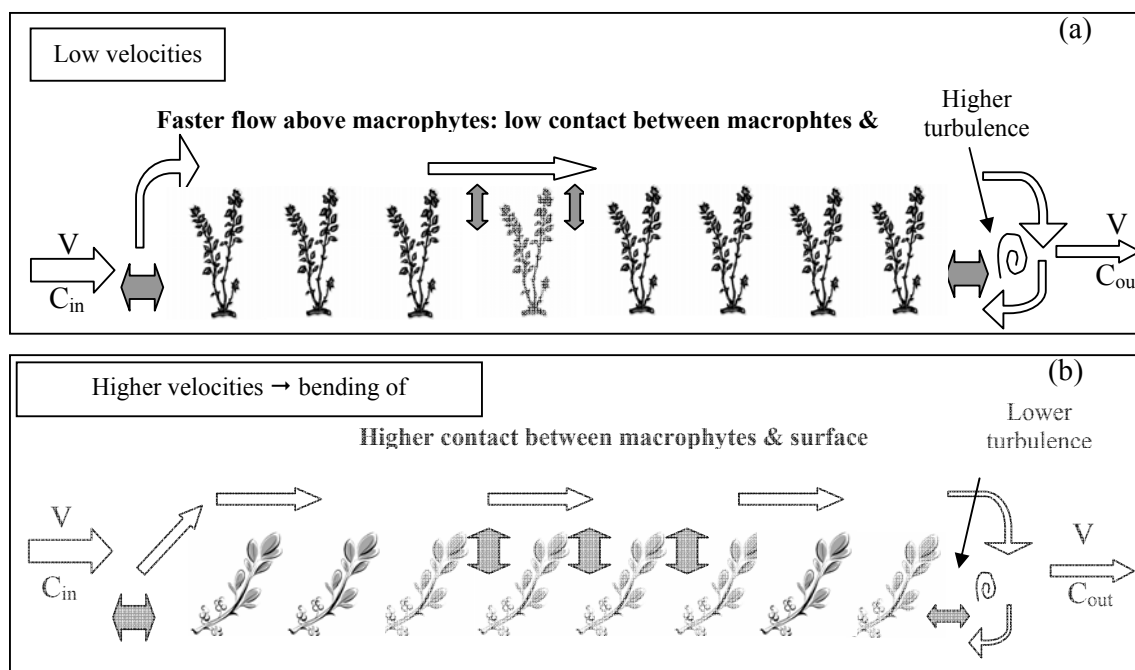


Figure 49: interpretation of the uptake patterns at different locations within a patch. Figure is based on articles from Hurd et al. (1994) and Sand-Jensen and Pedersen (1999).  and  represents macrophytes.  = turbulence eddy. (a) low velocities situation and (b) high velocities situation.

At higher velocities (10 and 30 cm s⁻¹ for *Callitriche platycarpa* and 30 cm s⁻¹ for *Potamogeton natans*), bending of both species could visually be detected (Figure 48). Water flow slows down at the front of the patch although less abrupt than at lower velocities because species are more streamlined (Figure 49b). Contact surface between water and plants becomes smaller in the front, but still a considerable uptake of N-NH₄⁺ takes place (Figure 47b). Further downstream, flow is accelerated above and along the patch, though contact surface of plants and water increases whereas N-NH₄⁺ uptake could be favoured. Turbulence eddies at the end of the patch may be limited due to the downward press of species and N-NH₄⁺ uptake will be inferior compared with lower velocities. Leaves of *Potamogeton natans* however, showed high uptake at the end of the patch. A plausible explanation is that at 30 cm s⁻¹, leaves remained under water so contact surface between water and leaves considerably increased and higher N-NH₄⁺ uptake could be achieved.

In summary, N-NH₄⁺ is the main nitrogen source for the three macrophytes investigated. No difference in N-NH₄⁺ uptake could be detected with increasing velocity ranging from 5 to 30 cm s⁻¹. *Potamogeton natans* is the most efficient species concerning N-NH₄⁺ uptake, whereby leaves showed higher concentrations, which can be due to either higher uptake or to allocation from stems to leaves. *Callitriche platycarpa* however can store higher concentrations in its tissue than

Potamogeton natans and *Sparganium erectum*. Typical uptake patterns within a vegetation patch are observed for *Callitriche platycarpa* as well as for *Potamogeton natans*.

VIII. A one-dimensional model of the oxygen dynamics in a vegetated stream and its application to nutrient mass balance analysis

VIII.1. Study site

The study site is the Aa river (Figure 2) described in Chapter 3. It is one of the main tributaries of the Kleine-Nete. The river reach used for experimental and modelling purposes represents only a small segment of this watercourse, extending over 1.45 km. It has a maximum water depth of ≈ 1 m under normal flow conditions, a mean annual discharge of $2 \text{ m}^3 \text{ s}^{-1}$ and an average width of 15 m. Accordingly, the mean water velocity is about 0.13 m s^{-1} and the mean water residence time is close to 3 hours, although it can exceed 8 hours at minimum flow. The experimental reach is comprised between two overflow structures, of the type "tilting gates" (also known as "overshot gates"), which are automatically controlled to maintain an almost constant water level at their upstream side, regardless of the water discharge. This is obtained by adjusting the angle of the gate plate, until it ultimately rests on the bottom of the channel, a condition that only occurs in the case of severe storm flow conditions. Two water-level monitoring stations managed by the H.I.C. (Hydrological Information Centre, Flanders Hydraulic Research, Ministry of the Flemish Community) are positioned at the gates (<http://www.lin.vlaanderen.be/awz/waterstanden/hydra/netebekken.htm>, station "Poederlee"). A precise history of the water level variations at both ends of the river reach is thus available, with a time resolution of 1 hour (Figure 50). It shows that the downstream level is almost perfectly regulated at the downstream gate, with a value close to 10.2 m TAW ("Tweede Algemene Waterpassing" is the Belgian levelling reference corresponding to the Mean Low Water in Ostend). In contrast, the upstream water level (immediately after the first gate) displays a strong seasonal influence, which can be put in relation with macrophyte growth: the head loss between the two stations is limited to ≈ 10 cm in the early spring when macrophyte biomass is at the lowest. When biomass peaks are observed in June-July, the head loss may reach more than 50 cm, although minimum discharge values are observed during this period.

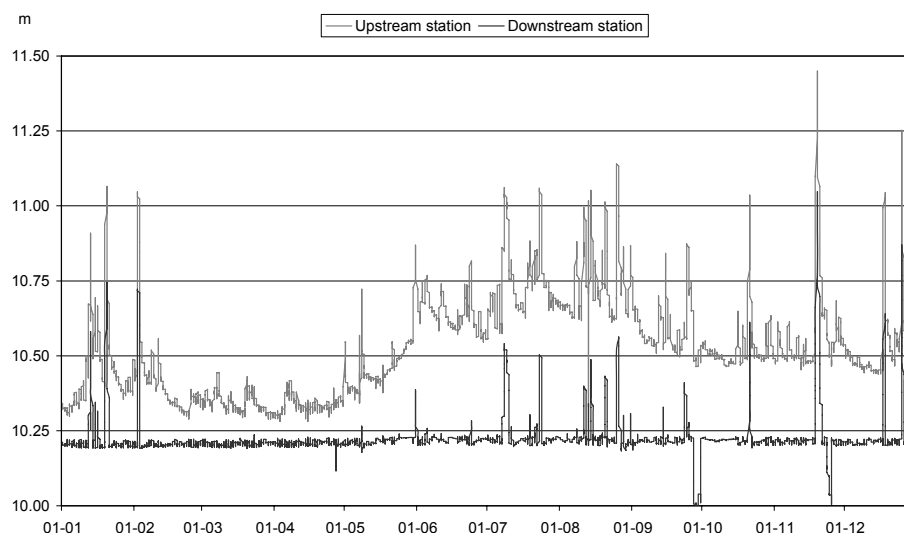


Figure 50: Water surface elevation at the boundaries of the test reach during the year 2004.

Water quality in the Aa-river has been monitored on a regular basis since the late 80's by the V.M.M. (Flemish Environmental Agency, <http://www.vmm.be>). Results of carbon, oxygen and nutrient analysis in the Aa at Poederlee (downstream limit of the test river reach) clearly witness the progress made in wastewater treatment for the last 15 years: the yearly mean values observed in 2003 for BOD ($\approx 3 \text{ mg O}_2 \text{ l}^{-1}$), COD ($\approx 20 \text{ mg O}_2 \text{ l}^{-1}$), NH_4^+ ($< 1 \text{ mg N l}^{-1}$) indicate a concentration decrease of a factor 5, 3 and 10 respectively since 1989. As a consequence, the level of dissolved oxygen has increased on the average from 5 to 9 mg l^{-1} during the same period, but at the same time, the nitrate concentration has been multiplied by a factor of 3 (from 1 to 3 mg N l^{-1}). Total DIN (dissolved inorganic nitrogen) is therefore still elevated (about 3.6 mg N l^{-1} or $250 \mu\text{M}$), as it is also the case for DIP (dissolved inorganic phosphorus), which is close to 0.5 mg P l^{-1} or $15 \mu\text{M}$. Accordingly, the status of the Aa-river may be defined as eutrophic.

The most abundant macrophytes found in the Aa-river are rooted, submerged species as described in Chapter IV.

VIII.2. Materials and methods

VIII.2.1. Hydraulic characterisation

Applying a weir-head / water-discharge relationship to the HIC dataset (time series of water level measured upstream of the tilting gates) was originally considered as the simplest way to estimate the water flow in the test section. Unfortunately, this approach has been found impractical for various reasons: firstly, the gate angle is not monitored and the elevation of the weir crest is therefore not known. Secondly, the overflow structure is rather broad (about 10 m), implying that the head on the weir is

small (on the order of a few cm), and hence difficult to measure with the appropriate resolution. Finally, large accumulation of debris (in particular of decaying macrophyte leaves) along the crest occurs repeatedly, causing a permanent and erratic drift of the weir-head / water-discharge relationship.

An alternative method comes out from the observation that water conductivity of the Aa water is displaying small, yet measurable fluctuations, and that these fluctuations are propagating from the upstream to the downstream boundaries of the experimental reach with almost no deformation. Figure 51 gives an example of this behaviour. It is therefore possible to estimate the water residence time, using conductivity as a natural, conservative tracer submitted to advective transport only. In practice, this requires the evaluation of the time lag between easily discernible events, such as minima and maxima occurring in the conductivity curves. When such events cannot be identified, the transit time can be evaluated by curve superposition. Using one of these methods, it is possible to reconstruct the water velocity evolution as a function of time, although the temporal resolution that can be achieved entirely depends on the frequency of "marker" events.

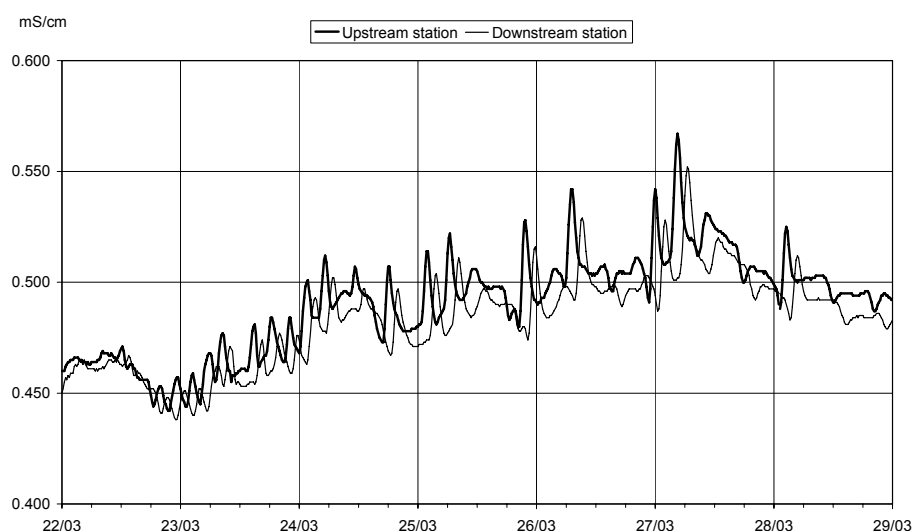


Figure 51: Specific conductivity at the boundaries of the test reach – Week 13, 2004.

VIII.2.2. Water quality

In addition to conductivity, temperature and oxygen concentration have been followed between March and December 2004. All parameters were measured by means of two multi-parameter monitoring system (YSI model 600XLM sensors) installed at both ends of the experimental reach. The upstream monitoring sensor has been positioned along the wall of the gate discharge channel, in the well-mixed zone situated a few meters after the water chute, at a depth of ≈ 50 cm at low flow. Conversely, the downstream sensor has been installed along the wall of the gate inlet channel to avoid the effect of reaeration at the water chute. Although turbulence

was very limited at this position, vertical profiles of oxygen concentration did not reveal the presence of a significant gradient. Both locations were always free of macrophytes. The frequency of data acquisition was first set to 4 measurements per hour until mid-May 2004, and then raised to 6 per hour. Sensor maintenance (data download, cleaning, calibration) was performed every 2 to 3 weeks on the average.

VIII.2.3. Other field measurements

Incident PAR (photosynthetically active radiation) has been monitored from March to December 2004 by means of a terrestrial radiation sensor (LICOR model LI-190SA Quantum Sensor) connected to a data-logger (LI-COR model LI-1400) positioned at the downstream station. Data acquisition occurred at the same frequency as for water quality parameters (4 to 6 h⁻¹). In addition, light transmission in the water column has been occasionally measured during a number of 24-hour experiments. The light attenuation was computed from the scalar irradiance measured at two depths using a pair of underwater spherical radiation sensors (AQUAMATIC model AQPL-UV912 Quantum Sensor).

VIII.3. Description of the model

The oxygen dynamics in the test section of the Aa river is described using a one-dimensional reaction-transport model based on the following assumptions.

a/ The cross-section of the river is supposed to remain identical in shape and dimensions at all coordinates along the longitudinal axis. To establish the geometrical dependence of the surface width, hydraulic radius and cross-section with the water level h , 30 transversal profiles (1 profile every 50 m, source: HIC) have been analysed using a cross section editor routine (Mike11, DHI). Based on this analysis, a polynomial expression has been constructed for each of the above parameters (Table 20).

Table 20: Polynomial description of the cross section geometry (h is the water surface elevation in m TAW).

Cross section Ω	m ²	$1.6029 h^2 - 18.555 h + 35.81$
Width w	m	$3.696527 h^5 - 187.20754 h^4 + 3794.7492 h^3 - 38485.199 h^2 + 195285.07 h - 396643.6$
Hydraulic radius R_h	m	$-0.10401 h^4 + 4.0326 h^3 - 58.560 h^2 + 378.25 h - 918.3$

b/ The water velocity V (m s⁻¹) is computed according to the standard Manning formula for uniform steady flow:

$$V = \frac{1}{n} R_h^{2/3} S^{1/2} \quad (1)$$

where n is the Manning roughness coefficient, R_h is the hydraulic radius (m) and S is the slope of the energy grade line (m m^{-1}). The value of the Manning coefficient at any given time is deduced from the analysis of experimental data: time series of measured water levels and head loss, time series of the velocity obtained from conductivity data. (The seasonal variations of n are presented in details in section 5).

c/ It is also assumed that dispersion processes may be neglected within the short reach length considered (1450 m), which can therefore be described as an ideal plug-flow system. This assumption relies on the low level of turbulence in the water column and on the absence of strong longitudinal and lateral concentration gradients. Even in the case of large variations at the upstream boundary (as displayed for example at Figure 52), dispersion does not seem to play a significant role during the propagation of the concentration front, which remains essentially identical at the exit of the test section. It also implies that the presence of dead zone linked to the accumulation of submerged plants has a negligible impact on the mass transfer properties at the scale of the test section.

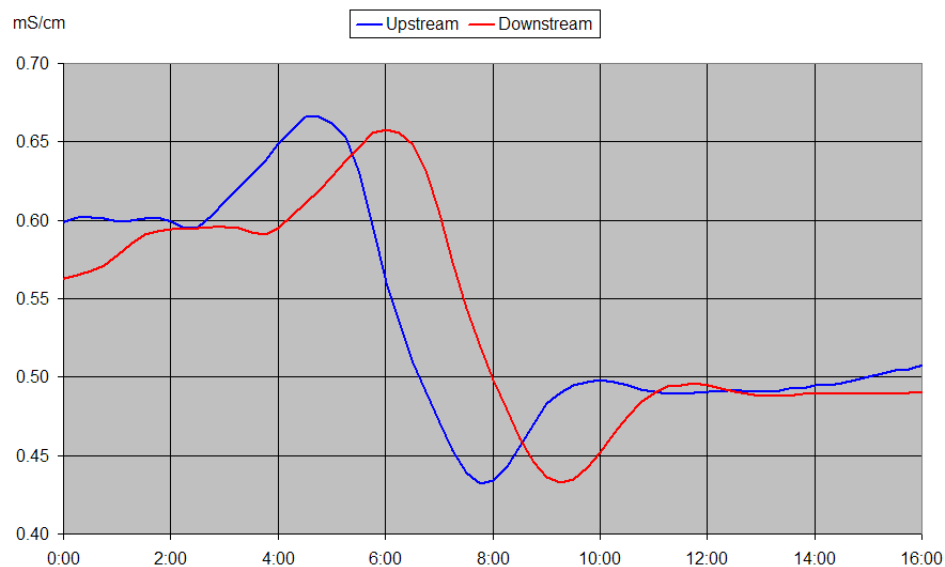


Figure 52: Propagation of a concentration front through the test reach (May 1, 2004)

d/ Three reactive processes are taken into consideration to describe the oxygen dynamics: O_2 production by macrophytes, O_2 consumption by community respiration, O_2 exchange at the air-water interface. In this model, the oxygen production rate is expressed as the product of a maximum production rate per unit surface area μ by a light limitation factor, divided by the mean water depth to convert production per unit surface into production per unit volume:

$$O_2^{\text{prod}} = \mu \left(1 - e^{-\frac{I}{I_k}}\right) \frac{W}{\Omega} \quad (2)$$

where O_2^{prod} and μ are respectively given in $\text{g O}_2 \text{ m}^{-3} \text{ s}^{-1}$ and $\text{g O}_2 \text{ m}^{-2} \text{ s}^{-1}$. The solar irradiance I and the saturation irradiance I_k are expressed in $\mu\text{E m}^{-2} \text{ s}^{-1}$. The mean depth is computed from the ratio between the width w (m) and the cross-section Ω (m^2). It is important to note that, in this model, O_2 production refers to the difference between the rate of oxygen release by photosynthesis and the rate of oxygen uptake due to growth respiration. In terms of carbon equivalent, it is thus intermediate between gross primary production (GPP) and net primary production (NPP). The latter can be computed by subtracting the macrophyte maintenance respiration from the O_2 production. Accordingly, community respiration includes the oxygen demand exerted for maintenance respiration, in addition to the oxygen uptake by heterotrophic organisms in the water column and in the sediments. This community respiration rate is simply expressed as the quotient of a respiration rate k_r (per unit surface area) by the mean water depth:

$$O_2^{\text{resp}} = -k_r \frac{W}{\Omega} \quad (3)$$

where O_2^{resp} and k_r are respectively given in $\text{g O}_2 \text{ m}^{-3} \text{ s}^{-1}$ and $\text{g O}_2 \text{ m}^{-2} \text{ s}^{-1}$.

It should be noted that the rate equations for production and respiration do not include a temperature factor, except for one model run where the respiration rate has been modified, according to:

$$k_r(\theta) = k_r(\theta_{\text{ref}}) \kappa^{(\theta - \theta_{\text{ref}})} \quad (4)$$

In this expression, θ and θ_{ref} are respectively the water temperature and a reference temperature expressed in Celsius and κ is the temperature response factor.

e/ The rate of oxygen transfer at the water surface is described by the classical expression relating the flux at the interface to the oxygen deficit with respect to the saturation concentration. The latter is computed from the mean water temperature:

$$O_2^{\text{aer}} = k_a (C_{\text{sat}} - C) \frac{W}{\Omega} \quad (5)$$

with: $C_{\text{sat}} = -7.63 \cdot 10^{-5} \theta^3 + 7.773 \cdot 10^{-3} \theta^2 - 0.3982 \theta + 14.579$ (6)

in which O_2^{aer} is expressed in $\text{g O}_2 \text{ m}^{-3} \text{ s}^{-1}$ and the piston velocity k_a in m s^{-1} . The oxygen concentration C and the saturation concentration C_{sat} are given in $\text{g O}_2 \text{ m}^{-3}$ or mg l^{-1} . In the above equations, all rate constants are positive; O_2 fluxes are counted positive when directed into the system.

f/ Finally, it is also assumed that the concentration changes within the system are sufficiently slow to allow a quasi-steady state to be reached at any given time.

Our model is thus based on a dynamic oxygen mass budget, following the same approach as McDonnell (1982) or Portielje and Lijklema (1995). The two kinetic

equations describing oxygen production and respiration do not explicitly take into account the biomass density, and the model does not include an explicit macrophyte growth term as it is the case, for example, in the plant growth models developed for shallow streams or ditches by Wright and McDonnell (1986a, 1986b), Davis and McDonnell (1997), Janse (1998) (see Carr et al. (1997) for a detailed review of models that simulate production of rooted macrophytes in aquatic systems). As a consequence, each of our model runs will cover relatively short time periods, allowing us to assume that the active biomass remains constant over the entire simulation.

Taking into account all assumptions made (uniform section, no dispersion, quasi-steady state), the oxygen profile corresponds to the solution of the simple first-order ODE (ordinary differential equation):

$$V \frac{dC}{dx} = \sum_i O_2^i \quad (6)$$

This ODE is numerically solved using a finite difference approximation. More specifically, the longitudinal axis of the river reach is divided into a number of compartments of length Δx . At each time step and for each compartment, Equation 6 is solved using an explicit, forward differencing (Euler's) method. To fully exploit the time resolution of the experimental oxygen data that are used as upstream boundary conditions, the integration time step Δt is taken equal to the time step used for the data acquisition (initially 15, then 10 minutes). Accordingly, Δx is taken equal to $V\Delta t$, where V is the water velocity at the given time of integration. The number of compartments that has to be taken into consideration is obviously equal to the total reach length L divided by the unit compartment length Δx (equivalent to the overall water residence time divided by the time step Δt). Since this is usually not an integer value, the number of compartments is always rounded up and the concentration value at the downstream boundary is estimated by linear interpolation between the last two compartments. Under all circumstances, the integration time step has been found sufficiently short to avoid potential instabilities due to model stiffness.

Boundary conditions: The oxygen concentration at the upstream station is the only boundary condition needed for the ODE integration. Time-dependant model parameters (water levels, irradiance and temperature) are also taken from the experimental dataset, the underlying assumption being that temperature, irradiance and water flow are uniformly distributed along the longitudinal axis. For water level data (available with a one-hour resolution), intermediate values have been estimated by linear interpolation.

Model calibration: Given all experimental data, four model parameters have still to be adjusted, namely μ , I_k , k_r and k_a . The best adjustment is obtained by comparing model results at the downstream boundary to the experimental oxygen data at the same point, using a RMS (root mean square) minimization criteria.

VIII.4. Results and discussion

VIII.4.1. Hydraulics

The impact of macrophyte development on the hydrodynamics of shallow rivers has been the subject of a growing interest since a few years (see for example: Nepf and Vivoni, 2000; Stephan and Gutknecht, 2002; Riis and Biggs, 2003; Choi and Kang, 2004; James et al., 2004; Green, 2005a, b; Armanini et al., 2005). Although it is not the main focus of the present paper, some features of the river hydraulics may contribute to the overall understanding of the biomass budget in the experimental river reach.

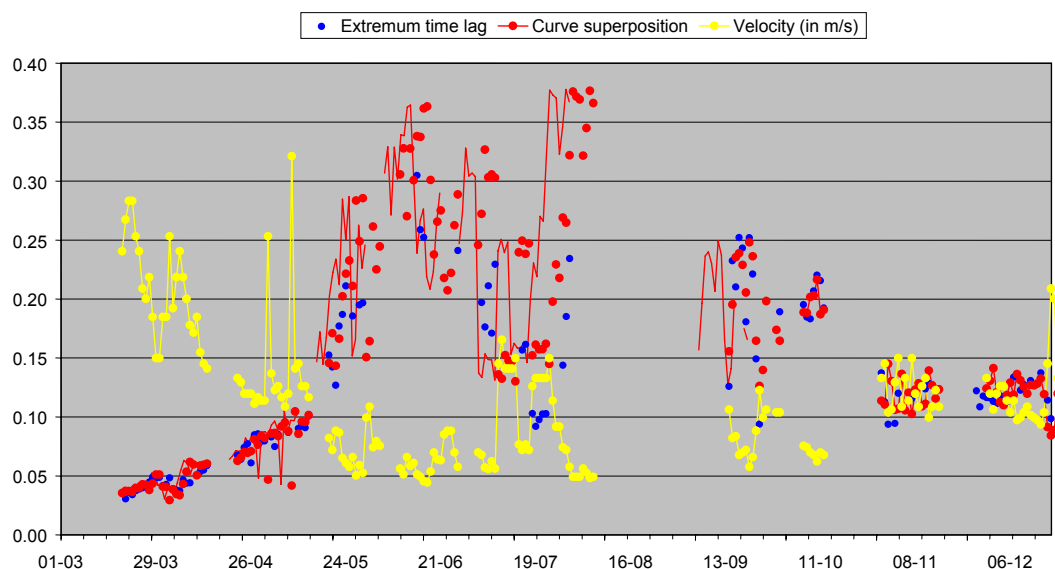


Figure 53: Seasonal evolution of the Manning roughness coefficient in 2004. Values have been obtained using a curve superposition technique (red dots, daily average) or by measuring the time lag between marker events (blue dots, instantaneous values). The mean daily velocity is also shown.

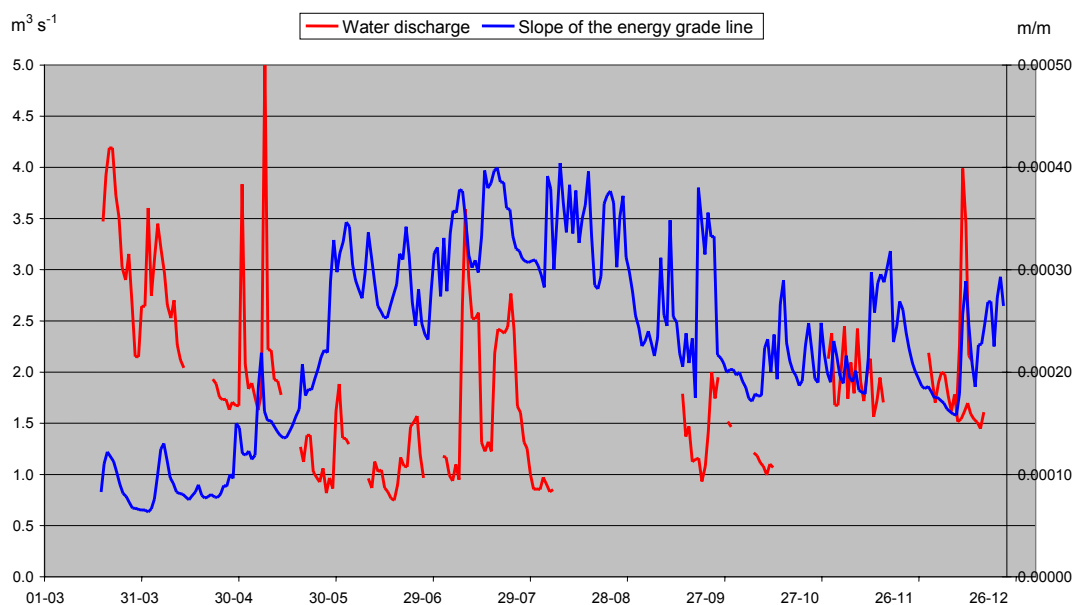


Figure 54: Seasonal evolution of the water discharge and of the slope of the energy grade line (2004)

The seasonal variation of the Manning roughness coefficient n observed during the year 2004 (Figure 53) clearly illustrates the influence of macrophyte growth on the resistance to flow. The Manning coefficient is close to 0.04 at the end of the winter and slowly increases in March/April until it reaches a value of about 0.10. During the same period, the water velocity drops from ≈ 0.30 to $\approx 0.10 \text{ m s}^{-1}$, which corresponds to a decrease in the mean river discharge from ≈ 4.0 to $\approx 1.5 \text{ m}^3 \text{ s}^{-1}$ (Figure 54). Between May and the end of June, the resistance to flow displays a sharp increase, with Manning values reaching a maximum of ≈ 0.35 . Water velocity and river discharge are then at their lowest ($\approx 0.05 \text{ m s}^{-1}$ and $\approx 1 \text{ m}^3 \text{ s}^{-1}$, respectively). During the early summer period (June-July), high Manning values (between 0.25 and 0.38) are regularly reached, although four episodes characterised by a sudden drop in resistance values can be identified. These episodes correspond to the high river discharges associated with the four periods of intense rain that occurred between the 1st of May and the 1st of August and accounted for 76% of the total rainfall depth during this time interval (157 out of 206 mm). It is interesting to observe that, at the end of these storm-flow episodes, the resistance to flow seems to reinstall rapidly, although the initial value is not always fully restored. Two factors may contribute to the very dynamic response of the coefficient n . In a detailed study of the roughness caused by macrophyte growth and its influence on the overall flow field, Stephan and Gutknecht (2002) have concluded: "these plants are highly flexible and behave differently depending on the flow situation. They also react substantially to the flow field and thus, the roughness becomes variable and dynamic". In addition to this

interactive behaviour, it is highly probable that a part of the biomass accumulated within the system (especially the free floating fraction and the decaying material) might be swept away by the enhanced drag linked to the increased current velocity. This process of plant sloughing has been quantified by Hootsmans (1994) in lake Veluwe (The Netherlands), with an estimated 10% of the total aboveground biomass lost when a storm occurs.

Finally, the progressive decay of the macrophyte population during the autumn and early winter period has a clear influence on the resistance to flow: the Manning coefficient drops to 0.25 in September, and remains in the range 0.15 - 0.10 in October / November. It should also be noted that flow surges of short duration are occasionally encountered in early spring, autumn and winter, but the impact on the Manning coefficient is both small and short-lived, probably because the biomass is less dense at these moments.

VIII.4.2. Oxygen concentration

Seven distinct time periods have been selected for model application, on the basis that a complete, validated set of data was available for each given period. In addition to the water velocity, this set must imperatively contain the upstream and downstream oxygen concentrations, the mean river temperature and the solar irradiance. This selection covers a total of 102 days distributed over 7 months (April to October), i.e. about 50% of data recovery. Field data and model results are reported on Figures 55 to 61.

Each graph shows the variation of oxygen concentration measured at the upstream boundary, together with the oxygen saturation concentration, the solar irradiance, and the juxtaposition of the measured and computed oxygen concentration at the downstream boundary. The values of the 4 model parameters (μ , I_k , k_r and k_a) resulting from the model calibration are reported in Table 21 for each selected period. These values are discussed later, after a short description of the model results.

Figures 55 to 57 represent the observed and modelled variations for three 10 to 20 days long periods in April, May and June 2004. They show that the amplitude of the daily oxygen variation at the downstream limit is progressively increasing, from 4 mg l⁻¹ (8 to 12) in April up to 10 mg l⁻¹ (4 to 14) in June - the obvious signature of a eutrophicated system. For all three model runs, the comparison between field data and model results are very satisfactory: the model is able to capture, not only the overall shape and amplitude of the output signal, but also, in a number of cases, small scale features that are linked to the short-term variations of the solar irradiance. Also, for each individual period, only a single set of constant model parameters is needed to calibrate the model.

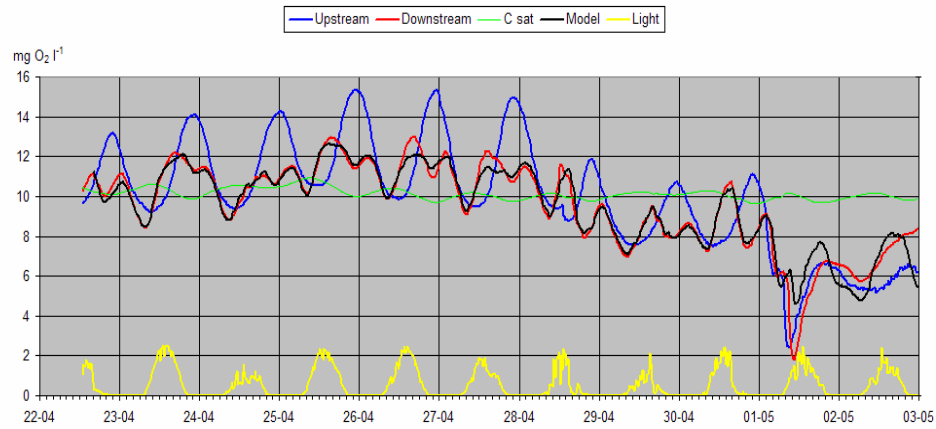


Figure 55: Model results, run 1 (22 April – 3 May 2004) Solar irradiance (Light) is given in arbitrary units, for the purpose of daily comparison only. Csat is the oxygen saturation concentration for the mean temperature observed.

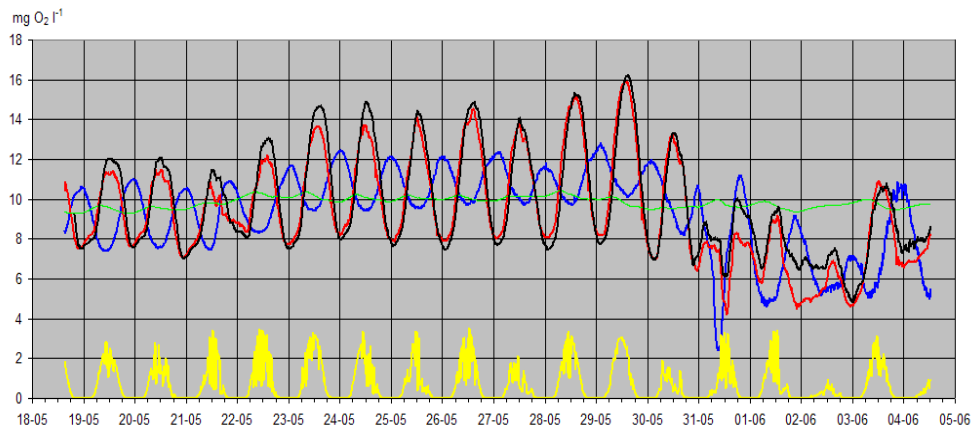


Figure 56: Model results, run 2 (18 May – 5 June 2004)

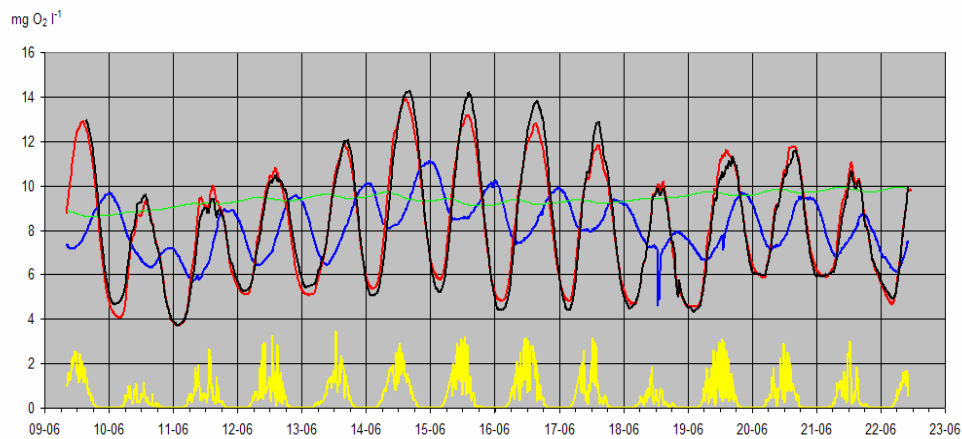


Figure 57: Model results, run 3 (9 June – 23 June 2004)

It should be pointed out that the upstream oxygen signal exhibits a rather unexpected time lag with respect to irradiance, the maximum concentration being reached around midnight and the minimum at noon. This time pattern may be attributed to the spatial distribution of macrophytes in the upstream catchments, i.e. the presence of macrophytes at a distance of ≈ 12 hours (expressed in terms of water travelling time), followed by a river segment where intensive mowing has been carried out and where the residual macrophyte activity is therefore very low. Also, it indicates that the oxygen exchange at the water surface is not a fast process in this river, since significant oxygen deficit and over-saturation can subsist over extended periods of time. In April, when the transit time is short and the community respiration is still sufficiently low, the night maximum at the input can propagate down to the output, giving birth to a second daily oxygen maximum shortly before dawn. This behaviour disappears latter in the season, when the longer transit times associated with lower river discharges tends to reduce the time gap between the two maxima, until they finally coincide.

The graphs also show the effect of a storm flow on the oxygen signal (Figure 55, May 1; Figure 56, May 31). The fast drop in the oxygen content is attributed to the input of reactive organic matter by point and non-point sources such as storm overflows and ditches, and by the release of benthic organic material. When storm flows occur, the assumption of quasi-steady flow ceases to be fulfilled, and the model is not able to correctly reproduce the response of the river reach.

Figures 58 to 60 show the model results for three simulations extending over 16 to 17 days in July and September 2004. All three sequences are characterised by the occurrence of a strong increase in the river discharge near the mid-period. In particular, the first two ones include the two episodes of high flow pointed out earlier, with maximum water velocity observed on July 9 and July 23, respectively. An interesting feature arising from these events is that, in contrast with the previous model runs, a single set of parameters is not able to produce a satisfactory fit over the entire time sequence. This is clearly demonstrated by comparing graphs a and b in Figures 58 to 60, each couple corresponding to a different set of model parameters. For instance, the period comprised between July 18 and July 23 (first five days in Figure 10a) are very well reproduced by the model using a first set of parameters, but the deviation between observed and computed oxygen concentrations progressively increases after the flow surge of July 23. Using a second set of parameters (Figure 59b) restores the model performance. These results demonstrate that storm episodes have a direct, measurable impact on the biology of the system (see later for further discussion).

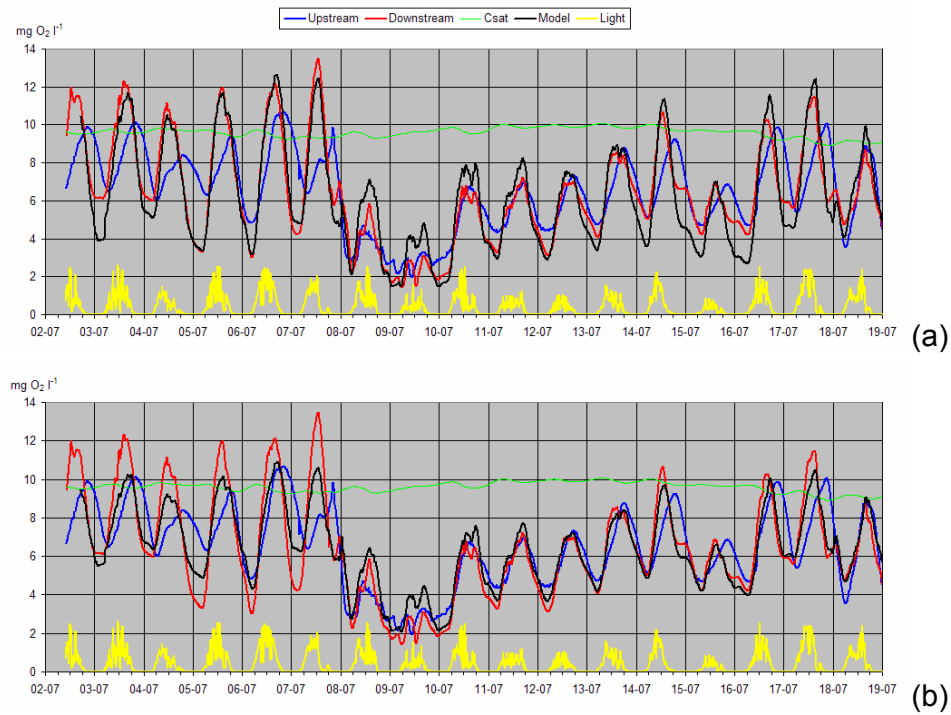


Figure 58: Model results, runs 4 and 5. (a): 3 July – 7 July 2004; (b): 11 July – 18 July 2004

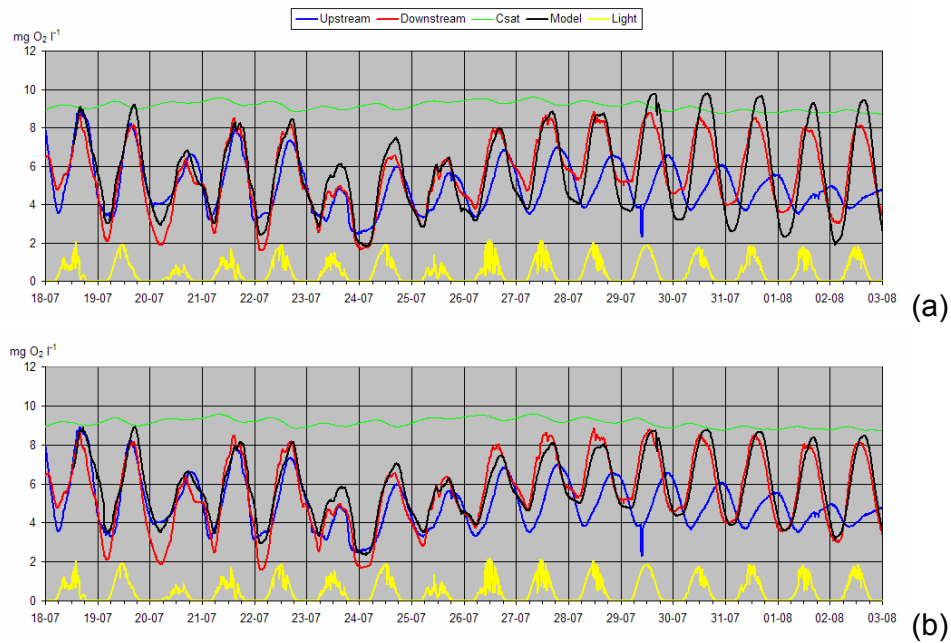


Figure 59: Model results, runs 6 and 7. (a): 19 July – 22 July 2004; (b): 26 July – 3 August 2004

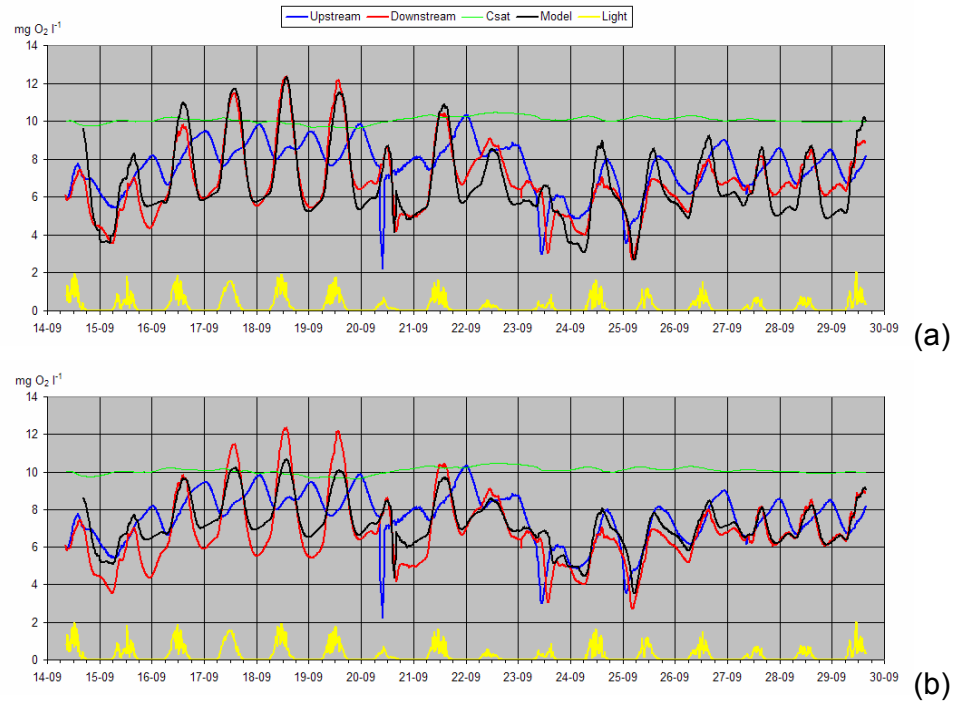


Figure 60: Model results, runs 8 and 9. (a): 16 September – 21 September 2004; (b): 26 September – 28 September 2004

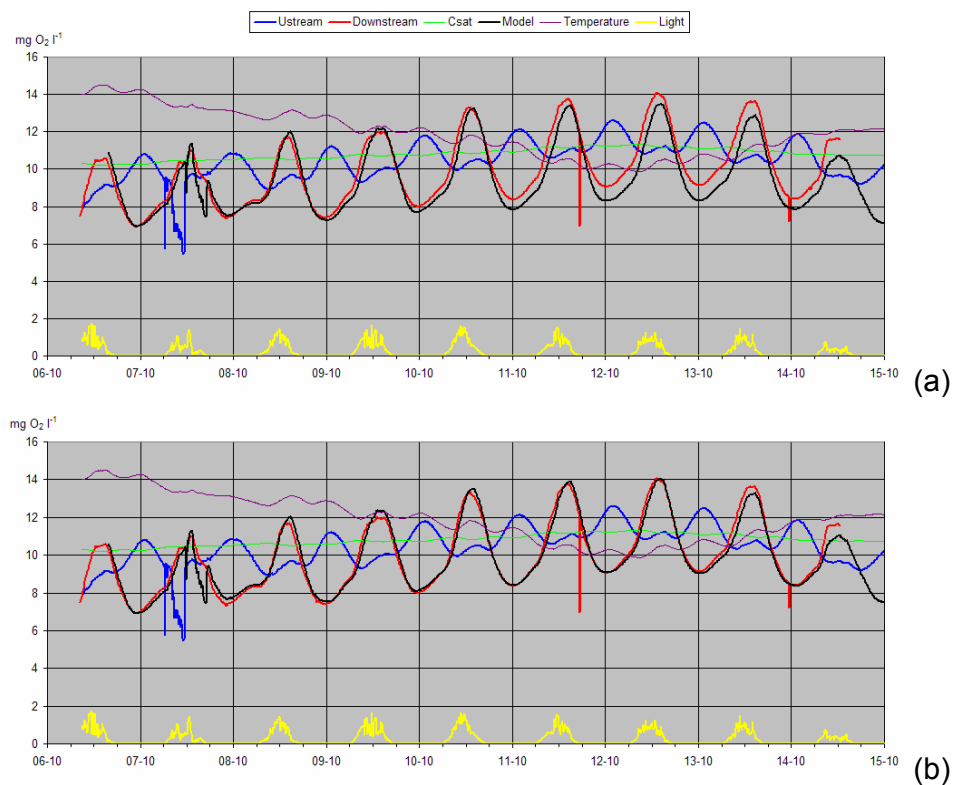


Figure 61: Model results, runs 10 and 11 (6 October – 15 October 2004). (a): without temperature correction; (b) with temperature correction

Finally, a last sequence of 9 days in October 2004 is represented at Figure 61. Although a steady flow is observed during this period, a satisfactory fit is not achieved with a single set of constant model parameters (Figure 61a). In this particular case, the mean water temperature displays a significant decrease (from 14 to 10 °C), and a temperature factor has to be introduced in the respiration term ($\theta_{ref} = 14^\circ$, $\kappa = 1.08$ in Equation 4) to obtain a good model fit (Figure 61b).

Table 21: Values of the model parameters for all model runs. Storm episodes occurred at the end of model runs 4, 6 and 8. (*) Biomass data from Chapter IV.

Period mm/dd	Model run #	Figure #	μ	I_k	k_r	k_a	Biomass (*)	Date
			$g\ O_2\ m^{-2}\ s^{-1}$	$\mu E\ s^{-1}\ m^{-2}$	$g\ O_2\ m^{-2}\ s^{-1}$	$g\ O_2\ m^{-2}\ s^{-1}$	$g\ DW\ m^{-2}$	mm/dd
22/04 – 01/05	1	55	$4.0\ 10^{-4}$	470	$1.9\ 10^{-4}$	$2.5\ 10^{-5}$	18.9	20/04
18/05 – 05/06	2	56	$5.0\ 10^{-4}$	470	$1.7\ 10^{-4}$	$2.5\ 10^{-5}$	90.9	18/05
09/06 – 22/06	3	57	$6.0\ 10^{-4}$	430	$2.5\ 10^{-4}$	$2.5\ 10^{-5}$		
03/07 – 07/07	4	58a	$7.5\ 10^{-4}$	430	$3.5\ 10^{-4}$	$2.5\ 10^{-5}$	120.0	06/07
11/07 – 18/07	5	58b	$5.0\ 10^{-4}$	430	$2.5\ 10^{-4}$	$2.5\ 10^{-5}$		
19/07 – 22/07	6	59a	$5.0\ 10^{-4}$	430	$2.5\ 10^{-4}$	$2.5\ 10^{-5}$		
26/07 – 03/08	7	59b	$3.6\ 10^{-4}$	430	$1.8\ 10^{-4}$	$2.5\ 10^{-5}$	261.6	01/08
16/09 – 21/09	8	60a	$6.0\ 10^{-4}$	430	$2.6\ 10^{-4}$	$2.5\ 10^{-5}$		
26/09 – 28/09	9	60b	$3.6\ 10^{-4}$	430	$1.7\ 10^{-4}$	$2.5\ 10^{-5}$	101.9	29/09
06/10 – 15/10	10	61a	$4.8\ 10^{-4}$	430	$1.7\ 10^{-4}$	$2.5\ 10^{-5}$		
	11	61b	$4.6\ 10^{-4}$	430	$1.7\ 10^{-4}$ (at 14°C)	$2.5\ 10^{-5}$		
							88.7	03/11

VIII.4.3. Model parameters

A number of conclusions may be drawn from the analysis of the numerical values reported in Table 21. Firstly, the variations of the model parameters are restricted to rather narrow ranges over the entire period of simulation. The value of the maximum production rate μ remains comprised between 3.6 and $7.5\ 10^{-5}\ g\ O_2\ m^{-2}\ s^{-1}$; for the respiration rate k_r , the variation is limited to the range 1.7 to $3.4\ 10^{-4}\ g\ O_2\ m^{-2}\ s^{-1}$: in both cases, the ratio between maximum and minimum values is on the order of 2 only. For the last two parameters, i.e. the saturation irradiance I_k and the piston velocity for gas transfer k_a , values are (almost or exactly) constant.

Within these limited ranges of fluctuation, some trends can however be pointed out. First of all, a regular increase of μ and k_r is observed between model runs 1 and 4.

For both parameters, this can be put in parallel with the coincident increase in biomass density (also reported in Table 21), although the relationship is far from proportional: during the same time interval, the biomass is multiplied by a factor 7 while the rates are multiplied by a factor 1.8 only. It means that only a fraction of the macrophyte biomass is involved in gross primary production, the other fraction being largely inactive from the photosynthetic point of view, probably because of light limitation under the canopy (self-shading) and of the growing importance of stems and roots in the biomass distribution. It also confirms that the dark respiration by macrophytes only accounts for a part of the total community respiration.

The second important indication arising from Table 21 is the recurrent fall in μ and k_r values that is observed after each storm episode. For μ , decreases of 33, 40 and again 40% are noted after runs 4, 6 and 8, respectively. The corresponding decreases in k_r values are very similar (33, 47 and 35%). The most probable explanation for these sudden drops is that a significant fraction of the vegetation (active, inactive and decaying) is sloughed away by storm flows. This corroborates a previous, similar conclusion based on the reduction of flow resistance at the end of a major storm event.

VIII.4.4. Process rates

To further explore the oxygen dynamics of vegetated flow, a more detailed analysis of transport and reactive fluxes is needed. An example is given in Figures 62 and 63 for the model run #3. Figure 62 shows the evolution with time of the spatially integrated production and respiration rates, expressed in $\text{g O}_2 \text{ s}^{-1}$. Following the model formulation, the production term responds to the highly variable solar irradiance, while the respiration term remains essentially constant. The difference between these two fluxes is equal to the net, instantaneous community production. Time integration of the latter indicates if the system is a net oxygen producer or consumer. In the case illustrated here, the system is clearly a net consumer, with two episodes of net oxygen consumption (10 to 13 June, 18 to 22 June) separated by a period where equilibrium is almost maintained during four days. Over two weeks time, the total community respiration is almost 800 kg larger than the oxygen production by macrophytes. The short period of equilibrium between O_2 production and respiration corresponds to a maximum in daily solar irradiance (Figure 57) with an average value equal to $36 \text{ E m}^{-2} \text{ day}^{-1}$ compared to average values of 23 and $24 \text{ E m}^{-2} \text{ day}^{-1}$ respectively for the previous and following periods. Not surprisingly, solar light appears to be the key factor controlling the day-to-day variations of the trophic status of this system.

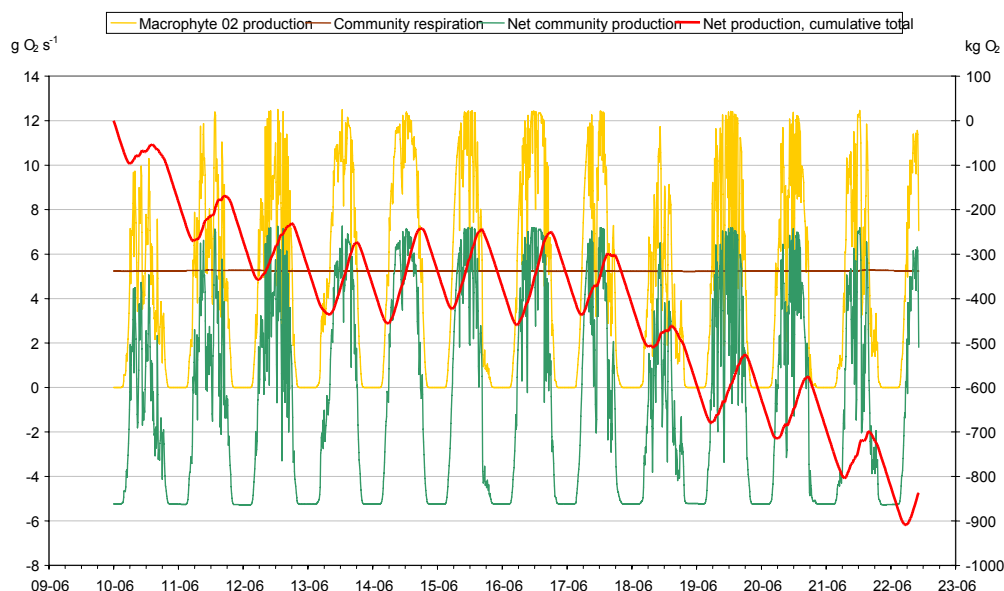


Figure 62: Integrated oxygen production, respiration and net community production (left axis). The cumulative net production is also shown (right axis). Results are given for run 3 (9 June – 23 June 2004).

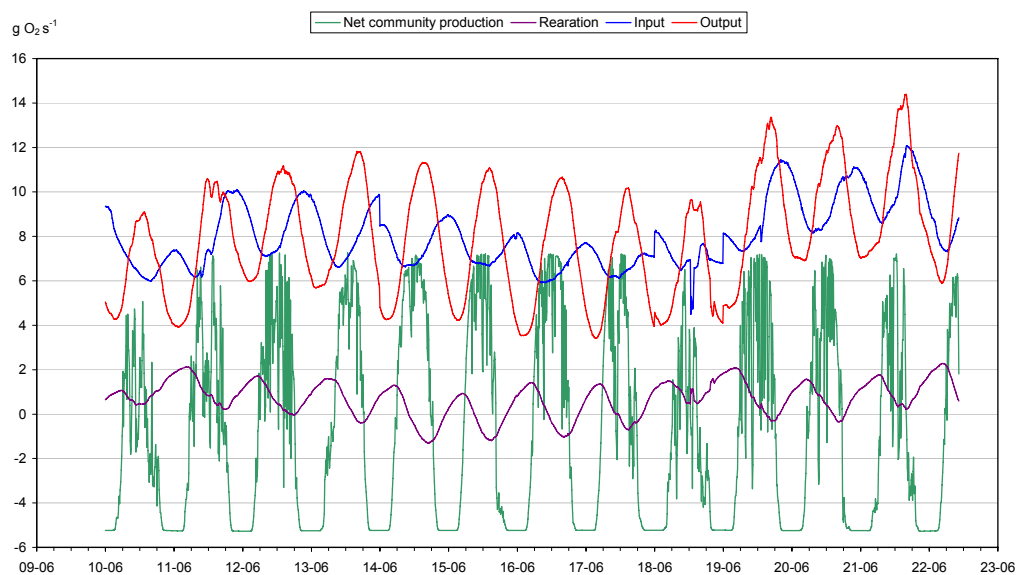


Figure 63: A comparison between reactive fluxes in the river reach (net production, reaeration) and transport fluxes at the upstream and downstream boundaries. Results are given for run 3 (9 June – 23 June 2004).

Complementary information is given on Figure 63, where reaction and transport fluxes are compared for the same model run as above. It shows that, as expected, the net community production is largely dominating the reactive behaviour of the system: in comparison, the physical process of gas exchange is about one order of magnitude lower. But all reactive fluxes remain smaller than the input and output

fluxes at the upstream and downstream limits of the river segment. Table 22 gives an example of the daily variations of the computed fluxes, and also allows an assessment of the model performance in terms of mass balance closure.

Table 22: Daily reaction and transport fluxes for model run #3. P: production, R: Respiration

Date	Irradiance E m ² day ⁻¹	P kg O ₂ day ⁻¹	R kg O ₂ day ⁻¹	Gas exchange kg O ₂ day ⁻¹	Input kg O ₂ day ⁻¹	Output kg O ₂ day ⁻¹	Sum kg O ₂ day ⁻¹	Storage kg O ₂ day ⁻¹	Deviation kg O ₂ day ⁻¹
10/06	13	270	-449	79	609	-547	-38	-43	5
11/06	22	367	-452	98	680	-652	41	46	-5
12/06	27	393	-451	68	730	-731	9	9	-1
13/06	30	414	-450	60	695	-718	1	4	-3
14/06	39	485	-449	-2	656	-675	15	26	-11
15/06	35	417	-449	-3	640	-630	-25	-34	9
16/06	43	475	-449	16	582	-617	7	10	-3
17/06	28	390	-449	31	583	-564	-9	-19	10
18/06	14	271	-449	97	605	-543	-19	-27	8
19/06	33	420	-449	74	774	-777	42	46	-4
20/06	27	385	-449	60	823	-822	-3	-3	0
21/06	22	325	-452	92	874	-864	-25	-21	-5
Total		4610	-5396	670	8250	-8139	-4	-5	1
Daily average		384	-450	56	688	-678	0	0	0

Mass flux analysis reveals a net heterotrophic behaviour in the case of model run #3. The same conclusion holds for all simulations, with only one exception. Table 23 synthesises the values of the net community production for all model runs. The second period (18 May – 5 June) is the only one exhibiting an O₂ production in excess over respiration. For all other simulations, respiration processes are strongly dominating, with daily excess respiration varying from 61 kg O₂ day⁻¹ to a maximum of 180 kg O₂ day⁻¹. Most of the time, the experimental river reach is largely heterotrophic, except during the short period of maximum growth in spring. A significant input of organic material into the system is thus necessary to sustain its heterotrophic status. It is suspected that a large fraction of the organic debris generated in the upper catchments (especially the plants washed out by storm flows or released in the water column by mowing) is actually trapped in the vegetation mat, where it can decompose before being eventually sloughed away.

Table 23: Net community production for all model runs

Model run #	Simulation length	Net community production	Daily community net production
	day	kg O ₂	kg O ₂ day ⁻¹
1	8	-775	-97
2	11	+608	+56
3	12	-786	-65
4	5	-556	-111
5	8	-1260	-157
6	4	-433	-108
7	9	-548	-61
8	6	-1074	-179
9	3	-540	-180
11	7	-520	-74

VIII.4.5. Nitrogen budgets

On the basis of oxygen mass fluxes analysis, it is possible to evaluate some elements of the nutrient cycling within the system. In the next discussion, the uptake and the release of DIN (dissolved inorganic nitrogen) by autotrophic and heterotrophic organisms are estimated using the following procedure.

- All mass fluxes are computed on the basis of daily averaged values, obtained either from field measurements or from the model simulations.
- A statistical correlation between field PAR measurements and the daily solar energy measured at the Belgian Meteorological Institute (Brussels, Belgium) is first established (Figure 64). It allows reconstructing a complete time-series of daily incident PAR from April to November 2004.
- For each simulation (runs 1 to 11), a linear relationship between the measured daily PAR and the computed O₂ production can be obtained. An example is given in Figure 65 for model run 3. These relations are applied to the PAR dataset (from step b) to rebuild a complete set of daily O₂ production values. The resulting time-series is shown on Figure 66, together with the original model values.
- The daily carbon incorporation by the macrophyte population (ΔC_{inc}) is then estimated, using a photosynthetic quotient equal to 1.2. This value is based on a molar C/N ratio (Falkowski and Raven, 1997) equal to 10.2 (see Chapter VI.1. Table 9). The corresponding carbon / oxygen ratio has a value of 0.31 g C per g O₂ produced. It should be recalled here that the "biosynthesis cost", i.e. the amount of

carbon respired to cover the energy requirements for growth, is already accounted for in the computed O_2 production value given by the model.

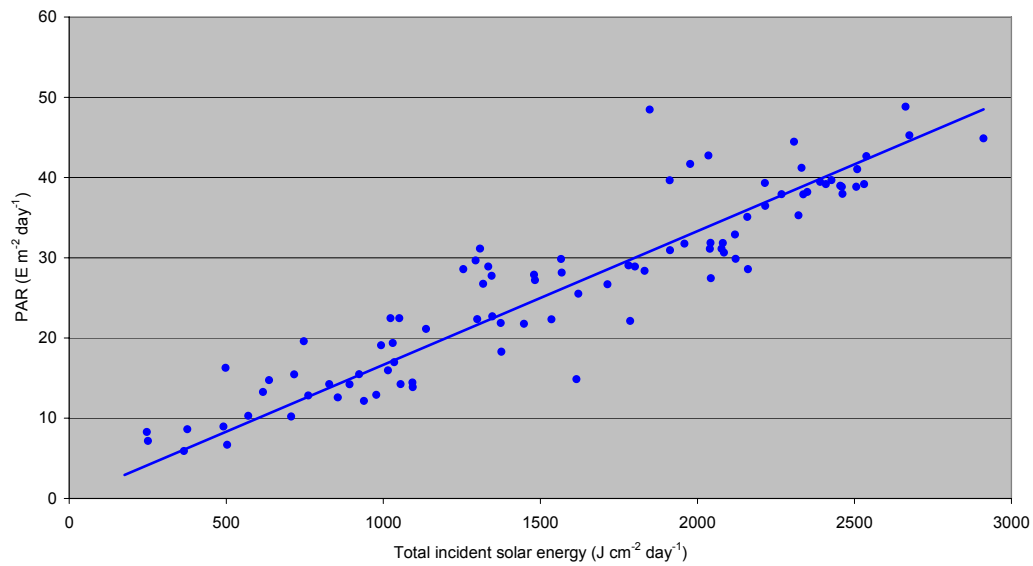


Figure 64: Correlation between measured PAR values and total incident solar energy in Brussels ($r^2 = 0.84$). (Source: IRM 2004)

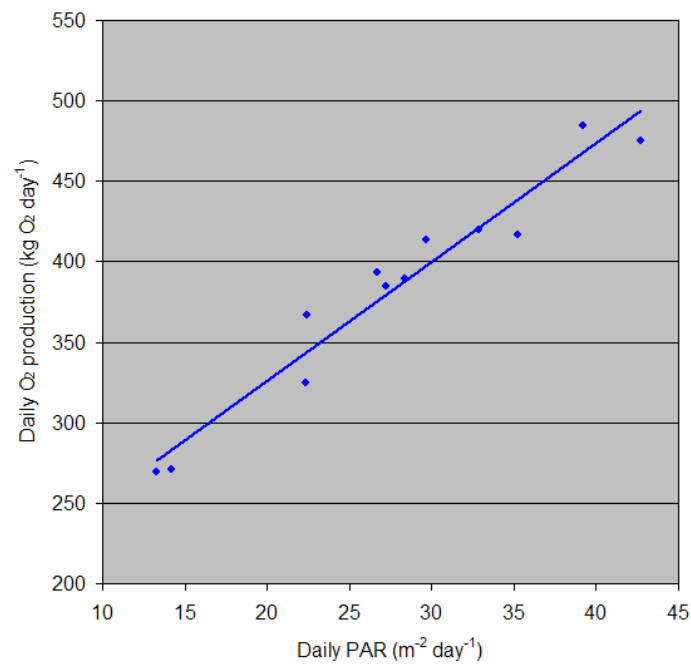


Figure 65: Daily O_2 production as a function of daily irradiance, for run model #3. ($O_2\ prod = 0.0167\ PAR$, $r^2 = 0.95$)

e. From the daily carbon incorporation, the evolution of the macrophyte biomass can be evaluated, using the simple formulation:

$$\Delta B = \Delta C_{\text{inc}} - m B^* \quad (7)$$

In this expression, ΔB is the daily biomass variation (in $\text{g C m}^{-2} \text{ day}^{-1}$); B^* is the mean biomass density for the day considered (in g C m^{-2}), taken equal to the biomass at the end of the previous day increased by a quantity $\Delta B/2$. The parameter m is a first-order kinetics rate factor (in day^{-1}) characterising all processes responsible for the reduction of the plant biomass, namely maintenance respiration, grazing and mortality. In addition, the events that have been identified for their high sloughing impact are also taken into consideration: biomass reductions equal to 33%, 40% and 40% are considered to occur on July 8, July 23 and September 22, respectively. (Although more episodes may have occurred at other dates, they were not monitored and therefore cannot be included in the simulation).

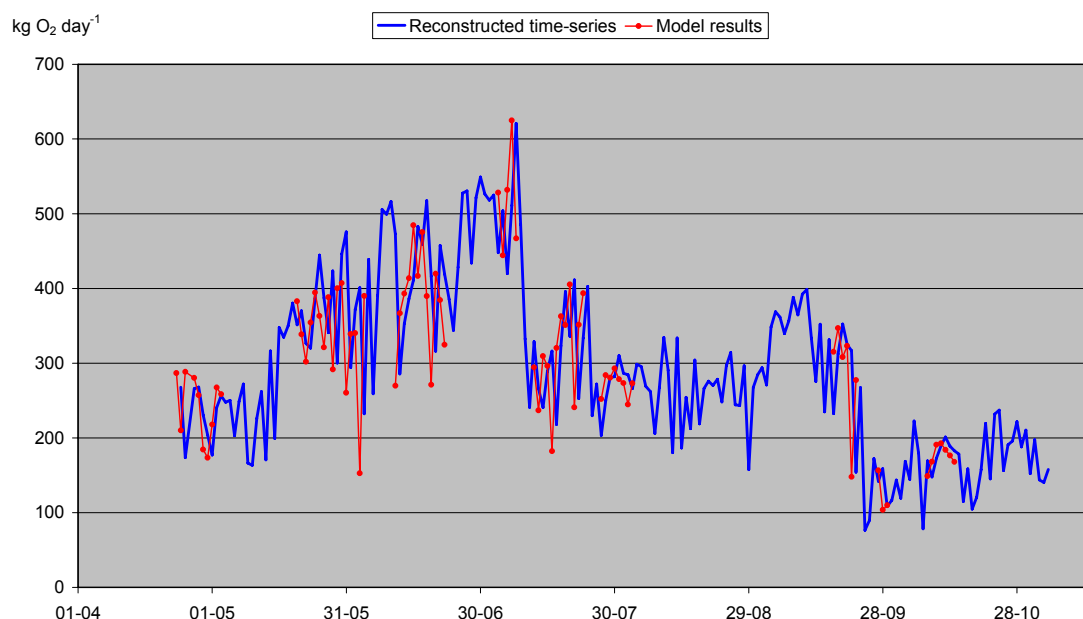


Figure 66: Reconstructed time-series of the daily oxygen production.

The value of the parameter m is then adjusted until a best fit is obtained between the computed and the measured macrophyte biomass density. For the conversion between biomass density in g C m^{-2} and in g DW m^{-2} , a factor equal to 0.314 is applied (Table 9, Chapter VI.1). The result of this fitting is shown on Figure 67, where the predicted evolution of the macrophyte biomass is compared with field determination. It shows that a good agreement can generally be found, except for the highest macrophyte density observed ($261.5 \text{ g DW m}^{-2}$), a value that can only be reached by setting m to 0. All other experimental values can be fairly well reproduced, using m values that remain within a narrow range (0.09 to 0.19 day^{-1}).

f. The respiration by heterotrophic organisms can now be estimated as the difference between community respiration and maintenance respiration. However, it is not possible to isolate maintenance respiration from other contributions to biomass decay. As a consequence, heterotrophic respiration is evaluated under two extreme assumptions: (1) maintenance respiration is equal to 0, and (2) biomass decay is only due to maintenance respiration.

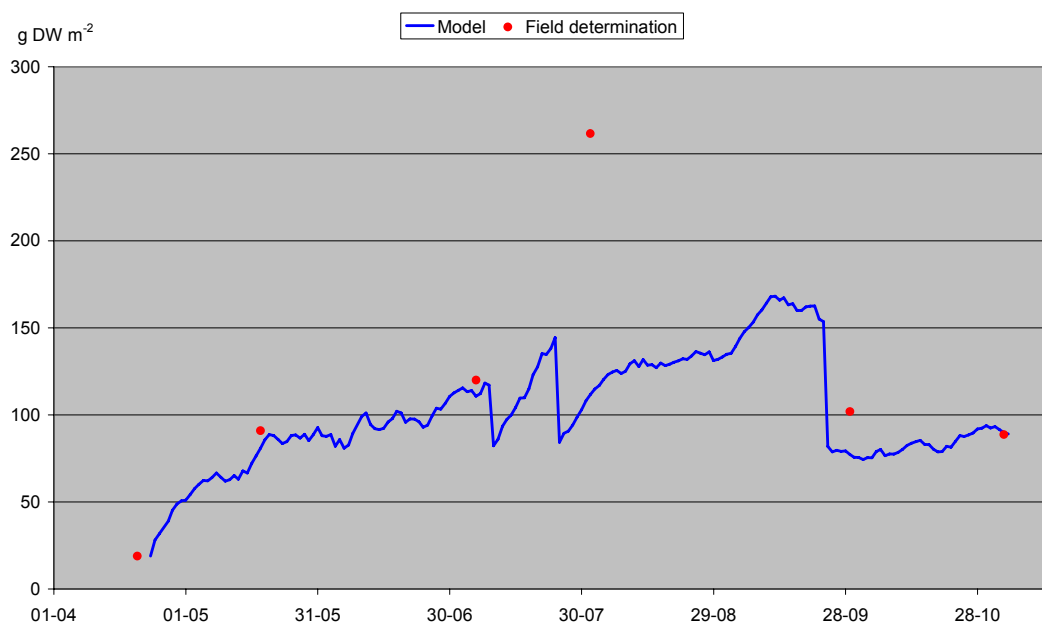


Figure 67: Computed vs. observed macrophyte density.

g. Finally, nitrogen uptake and release can be assessed from the previous results: N uptake is computed from the net biosynthesis (carbon incorporation minus maintenance respiration), using a C/N conversion factor equal to 0.114 g N / g C (Table 9, Chapter VI.1). N release associated to the mineralisation of organic matter is obtained from the value of the heterotrophic respiration, using a conversion factor equal to 0.043 g N / g O₂ respired. This value is derived from the measured macrophyte composition, considering that the organic substrate for heterotrophic respiration is essentially constituted by dead vegetation. It should be noted that the scenario (2) in step f will, on one hand, maximize the respiration of nitrogen-free, carbohydrate compounds by the plants and therefore minimize the nitrogen release by heterotrophic decomposers. On the other hand, it will minimize the estimation of nitrogen uptake by the plants, because a larger part of the incorporated carbon will be used for energy requirements and less carbon will be available for growth. The inverse will apply in the case of scenario (1). However, the net nitrogen flux (uptake minus release) will be identical in both cases. Also, scenario (2) is not always possible: owing to the structure of our model, the maximum value allowed for maintenance respiration is given by the value of carbon incorporation. In this case, it

is assumed that no nitrogen uptake does occur, and maintenance respiration only accounts for a fraction of the total community respiration.

Table 24 gives an overall view of the resulting fluxes for the various simulation periods. (In contrast with O₂ production, which can be related to irradiance, there is no possibility to reconstruct a complete time-series for community respiration.

As a consequence, nutrient budgets cannot be evaluated on a continuous basis, but only for the model runs presented previously). The results clearly demonstrate that, without consideration for denitrification as a potential nitrogen sink, the system always behaves as a source of nitrogen, except for the periods described by model runs #2 and #9, where uptake and release are almost in balance. Depending on the relative importance of maintenance respiration, the release of nitrogen may vary within a factor 2 to 4, but as stated earlier, the net result is not affected. The model predicts a nitrogen export in the range 4 to 10 kg N day⁻¹, except in May and September when a value close to 0 is computed. In addition, sloughing of plant material by storm flow contributes to the net export of N towards the downstream reaches: on the basis of the modelled standing crop and of the estimated biomass losses, the nitrogen export can be estimated to 25, 44 and 52 kg N respectively for the 3 storm events identified in July and August. Of course, the importation of organic debris originating from the upstream catchments must also be considered: obviously, the functioning of the river reach as a net nitrogen source cannot be sustained without the continuous input of an exogenous organic nitrogen supply. This corroborates an earlier conclusion about organic carbon supply to the system, and confirms the heterotrophic status of this particular ecosystem.

VIII.5. Conclusions and management perspectives

Based on a simple 1D oxygen model, the analysis of oxygen, carbon and nitrogen mass fluxes show that the system under study is, most of the time, a net oxygen sink and a net nitrogen source. This can only be sustained if a continuous input of organic carbon and nitrogen is available. It is probably supplied by the input of vegetation debris originating from the upper catchments, and its subsequent trapping into the vegetation mat. The role of vegetation sloughing thus seems very important. It is put in evidence both by its impact on the hydraulic behaviour (a permanent decrease of the resistance to flow after a storm) and on the biological response (a concomitant decrease of the O₂ production and respiration rates).

The functioning of the river reach may be strongly affected by the experimental configuration (a heavily vegetated segment, preceded by a comparatively long watercourse where mowing has only left a minimum of submerged vegetation). The part of the river under study may therefore act as a "first line filter" for particulate organic matter, resulting in an enhanced heterotrophic activity. However, the model

indicates that, even in the case of a more autotrophic system, the maximum N uptake would not exceed about 15 kg N day⁻¹ during short periods.

Table 24: Computed values of N fluxes linked to macrophyte uptake and heterotrophic respiration.. Two scenarios are given for each model run: (1) no maintenance respiration – (2) maintenance respiration equal to the maximum allowed by the model (percentage given in parenthesis gives the fraction of community respiration that is attributed to maintenance respiration). Denitrification is not included in the estimation of net N flux

Run	Date	Macrophyte production	Community respiration	Net community production	Macrophyte C uptake	Maintenance respiration	Heterotrophic respiration	N uptake	N release	Net N
		kg O ₂ day ⁻¹	kg O ₂ day ⁻¹	kg O ₂ day ⁻¹	kg C day ⁻¹	kg O ₂ day ⁻¹	kg O ₂ day ⁻¹	kg N day ⁻¹	kg N day ⁻¹	kg N day ⁻¹
1	23/04-30/04	237	-334	-97	74	0	-334	8.4	-14.4	-
						-120 (100%)	-214	3.3	-9.2	6.0
2	19/05-29/05	359	-303	56	111	0	-303	12.7	-13.0	-
						-238 (100%)	-65	2.5	-2.8	0.3
3	10/06-21/06	384	-450	-76	119	0	-450	13.6	-19.3	-
						-262 (100%)	-188	2.4	-8.1	5.7
4	03/07-07/07	519	-631	-112	161	0	-631	18.4	-27.1	-
						-325 (100%)	-306	4.5	-13.2	8.7
5-6	11/07-22/07	312	-453	-141	97	0	-453	11.0	-19.5	-
						-257 (82%)	-196	0	-8.4	8.5
7	25/07-03/08	264	-325	-61	82	0	-325	9.6	-14.0	-
						-221 (80%)	-104	0	-4.3	4.3
8	16/09-21/09	286	-465	-179	89	0	-465	10.1	-20.0	-
						-236(56%)	-229	0	-9.9	9.9
9	26/09-28/09	123	-303	-180	111	0	-303	12.7	-13.0	-
						-243 (100%)	-60	2.3	-2.6	0.3
11	07/10-13/10	176	-250	-74	54	0	-250	6.2	-10.7	-
						-146 (60%)	-104	0	-4.5	4.5

It is also highly probable that a significant fraction of this fixed nitrogen would have a short residence time in the system, being rapidly exported as plant debris.

At the present time, the excess N flux that is exported downwards may reach 10 kg N day⁻¹. This is only a small fraction of the advective DIN flux, which is close to 500 kg N day⁻¹ on the average.

Finally, it seems difficult to justify a policy of active macrophyte restoration in streams of this size on the basis of nutrient removal only, unless a strict limitation of the impact of sloughing can be achieved. In this case, the head loss caused by macrophyte colonisation will have to be kept under control, for example by selective mowing.

IX. Overall conclusions

A considerable number of macrophyte species was found in the Nete catchment although no endangered species were found. Also, the abundance of most of the species was very low. In contrast, a few of them (especially generalist species like *Stuckenia pectinatus*, *Potamogeton natans*, *Potamogeton trichoides*, *Callitriche platycarpa*, *Sparganium emersum*, *Sagittaria sagittifolia*, *Glyceria fluitans* and *Phalaris arundinacea*) were dominant and covered sometimes more than 50% of the surface. The macrophyte distribution differs between the three subbasins of the Nete. From all the subbasins, the Aa-subbasin has the highest macrophyte habitat diversity.

Several environmental variables might explain these variations in macrophyte diversity in the Nete catchment. The main abiotic variables explaining the macrophyte diversity in the Nete catchment are morphological ones (width and depth), but also suspended material, the pH of the surface water, ammonium and phosphates in the surface water determine the occurrence of several dominant macrophyte species. Sediment characteristics play a minor role in explaining the macrophyte diversity in the rivers of the Nete catchment. The range of these variables within the catchment might be too small to cause differences in macrophyte diversity.

In the first chapter, it turns out that monitoring macrophyte biomass in rivers is not an easy job to do in an appropriate way. To obtain high reliability, more than 200 samples of 15x15 cm are needed, which is impossible to execute for monitoring campaigns. The monitoring strategy used in this study however, consists of sampling 30 plots of 15x15 cm. Interpretation of the macrophyte biomass must be done carefully because variation coefficients can vary between 11 and 30%.

In the studied section of the Aa, it reveals that biomass increased significantly during the last two years between 2003 and 2006. One of the reasons of this increase might be due to the fact that the study section is not mowed for several years. Especially some dominant macrophyte species with an apical growth meristem, like e.g. *Potamogeton natans* and *Callitriche platycarpa*, take advantage of the absence of management and are likewise responsible for the increase in total macrophyte biomass. Other dominant species with a basal growth meristem, e.g. *Sagittaria sagittifolia* and *Sparganium emersum*, are of minor importance in the last year of the period concerning biomass. Peak biomass in the growing season differs a lot from year to year and is dependent on the temperature and the precipitation in the respective year: the peak moment of biomass can be situated between May and August.

The nutrient concentrations of the dominant macrophyte species from the study section were determined for two years (2003 and 2004). The average nutrient concentrations of all macrophyte species during all periods were relatively constant.

C-concentrations are low compared with values from literature. Probably, this is due to a CO₂-deficit in our running waters.

On the other hand, both N- and P-concentrations in macrophytes are very high compared to literature, namely well above the values suggested to be saturating growth. Towards macrophytes originating from lakes, river macrophytes always have higher N- and P-concentrations in their tissue because of the continuous supply of inorganic nutrients. Nevertheless, the tissue N- and P-concentrations of the macrophytes in our study were still much higher compared to values in literature. As a result, the nutrient standing stock in the macrophyte biomass is also high in the peak of the growing season. This indicates that the Aa is a very eutrophic river where nutrients in the surface water are not limited. Besides, the continuous load of available N and P is enormous so that mowing once or twice a year is useless in the light of nutrient removal. However, management will still be needed in some cases, as macrophytes can strongly modify the water flow, thereby enhancing the flooding risk.

In the same study section, mass balance studies are done by six 24-hour campaigns in different seasons. In the growing season, less available nitrogen and phosphorous was observed at the downstream margin of the section which might indicate that different organisms can take up nitrogen and phosphorous. Indeed, macrophytes are the major consumers of available nitrogen in the surface water and especially ammonium is the nitrogen source of preference. Also, but in a lesser extent, the sediment can act as a sink for ammonium and as a source for nitrates. Phytoplankton plays a minor role in the mass balance as well as for nitrates as for ammonium.

Flume experiments using ¹⁵N labelled ammonium or nitrate in the surface water confirm that most dominant macrophytes take up nutrients by their stems and leaves and that ammonium is the nitrogen source of preference. These experiments also showed that there is a distinction between fast growing species like *Potamogeton natans* and *Callitriche platycarpa* and slower growing species like *Sparganium erectum*. Stream velocities do not influence directly the overall uptake of available nitrogen in a macrophyte patch, but given a certain stream velocity, the uptake of available nitrogen is dependent of the location within a patch.

It is clear that macrophyte growth significantly effects nutrient concentrations in the water, but due to a still high load coming from diffuse and point sources the absolute effect on the total flux of nutrients downstream during the project was minimal.

X. Management alternatives: suggestions for management policy: translation to the practice

Macrophyte growth has a significant effect on the nutrient concentrations in the water. However the input of nutrients from diffuse and point sources in the Nete catchment during the period 2003-2006 was high, resulting in a eutrophic status of the surface water. The absolute effect of the macrophyte growth on the total flux of nutrients downstream was therefore minimal. Mowing and removing the macrophyte biomass from the system with this high load of nutrients will not really influence the nutrient flux. Not mowing of the macrophytes results in a shift of opportunistic species to more specific species. It also seems to result in an increase of the biomass over the years and might therefore impact the discharge capacity. However the modelling showed that there is a need to improve the role of macrophytes in hydraulic models. The results of this study have to be combined with results from studies focusing on the effect of macrophytes on the Manning coefficients and the discharge capacity (Bal et al., in pres). More attention will be focused on this also in the second Manudyn project which will start in 2007.

Regarding monitoring water quality it is clear that ammonium as a preferred nitrogen source for the macrophytes is an important factor. In the monitoring programmes all nitrogen sources in the water column should therefore be included. When the nutrient concentrations will decrease in the future, the impact of the macrophytes should be again considered.

Finally it can be said that mowing in patterns is currently the most interesting option. When the patterns are the same over the years, specific species will develop at the not mown sites, while opportunistic species will develop at the other sites. This will result in a higher biodiversity of macrophytes and subsequently a higher biodiversity of macro-invertebrates and fish can be expected.

XI. Acknowledgements

The authors wish to thank Inge Folmer, Susan Strömbom, Michèle Loijens, Nathalie Roevros, Didier Bajura, Michaël Tsagaris and Nicolas Canu (ULB) for their collaboration, support and friendship during the field missions along, over and in the Aa. Thanks are also addressed to E. Cornet, J. Deschutter and H. Vereecken (Hydrological Information Centre, Ministry of the Flemish Community) for their interest and commitment in data retrieval and supply. We also want to thank the Netherlands Institute of Ecology, Centre for Estuarine and Marine Ecology (NIOO-CEME), which allowed us to carry out flume experiments and especially Tjeerd Bouma for his enthusiasm and support during the experiments. The authors further wish to thank the colleagues from the research group Ecobe, especially Dimitri Van Pelt for the essential assistance and friendship on the field and Lieve Clement, Eva De Bruyn and Tom Van der Spiet for the analysis of all samples.

The MANUDYN project has been executed thanks to the funding by the Belgian Federal Science Policy Office in the frame of “Global change, ecosystems and biodiversity SPSP2” (second multi-annual scientific support plan for a sustainable development policy).

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