**Interactive comment on "Aquatic macrophytes can be used for wastewater polishing, but not for purification in constructed wetlands" by Yingying Tang et al.**

Referee 4

For final publication, the manuscript should be accepted as is

This is a very good paper and worthy to be published. I do not find any problems to accept it as it is.

**Response**

We would like to thank the referee for her/his very positive comments.

**Interactive comment on "Aquatic macrophytes can be used for wastewater polishing, but not for purification in constructed wetlands" by Yingying Tang et al.**

Referee 5

Overall this study is very nice, novel, and useful and should be published after minor revisions (see comments).

**Response**

We would like to thank the referee for her/his positive and constructive comments.

**General comments**

This paper focusses on improving water quality by using aquatic plants systems in combination with different sediment types. In the study, the authors use several aquatic plant with contrasting growth forms and three different sediment types. They assessed how these systems perform in the removal of nutrients (focus on N&P) and where these nutrients end up in the system, with a focus on the plants and sediment. They furthermore studied how many of the nutrients coming into the system (loading) can be removed by harvesting the plant biomass. The fact that they combine different nutrient loading, different species with contrasting growth forms, and different sediment type makes this study very interesting, useful and novel and will be of interest to scientists (ecological and biotechnological), ecosystem managers and wastewater treatment specialists.

The authors conclude that the selected species can be used to remove free N and P from the water up to a certain nutrient loading, above which the plants only sequester a very small portion of the nutrients introduced to the water. The amount of nutrients removed from the water not only depends on species, but also interacts with sediment characteristics. This stresses the importance to consider the whole system, but in particular the plant and sediment characteristics in successfully designing a sustainable CWS.

Overall the quality of the research and presentation is very good and should be published after mainly textual revisions. Some parts could be presented in a bit more detail or more concisely for clarity, as I was confused about some sentences and terms used. (See specific comments.) For example, the title does fit the content very well, providing the reader know the difference between water polishing and water treatment. To me this was not clear, so perhaps the authors could changes this for something like: plants can be used to remove nutrients from surface water up to certain nutrient loading levels.

The paper also cites many relevant references, but could be improved by adding a few more recent ones and adding a few more references of other research where plants have been used to remove nutrients. This will then allow for more discussion on the general applicability of this study and the influence of plant growth form in nutrient removal. (Also see specific comments.)

I've added quite some specific comments and suggestions to aid the authors in revising the manuscript. Most points concern sentences / terms that were not completely clear to me, while a few are about questions I had about the methods, results and discussion. I hope this will help in a swift revision and publication.

**Response to general comments**

We thank the referee for the positive comments and for the constructive suggestions to improve our manuscript. Please find below a point-to-point reply to all specific comments raised by this referee, including those on our title and using more recent references. All page and line numbers refer to the revised manuscript without tracked changes. We feel that the changes made to the revised manuscript, based on this referee's comments, have improved readability and clarity. We also thank the reviewer for pointing out some technical issues with our manuscripts, all of which we corrected in the revised manuscript.

**Specific comments**

Title: I'm not sure that the difference between polishing and purification are clear to all readers. Perhaps change for low and high loading (See general comments).

Line 25-26; here you make it very clear what you mean with polishing VS purifying, perhaps also include this in the introduction or just use the loading terms instead of polishing / purifying. Additionally, in the title you mention polishing vs treatment. I think it would be good to choose the same terms throughout the text or leave them out.

**Response**

We agree that clarifying the difference between wastewater polishing and purification helps readers understand the purpose and conclusion of our manuscript. The referee commented that explaining these definitions in the abstract and introduction would help readers understand the title better. Therefore, we have added the following text in the introduction [P5, L81], and kept the title as it was:

'By studying the resulting distribution of P and N among the different sediment, macrophyte and water compartments, we aimed to determine the nutrient removal efficiency by floating or submerged aquatic macrophytes from wastewater at low (polishing) or high (purification) loading rates, and the interacting role of sediment type.'

Line 27: you only mention the importance of soil, but I think that the effect of plant species / plant growth form is also an important conclusion which could be added here.

**Response**

We agree with the reviewer that this should be mentioned explicitly. To emphasize the importance of plant species, we have now changed the text [L26] in the summary section to:

'The outcome of this controlled study not only contributes to our understanding of nutrient dynamics in constructed wetlands, but also shows the differential effects of wetland sediment types and plant species.'

Line 40: I'm confused about the definition of the terms 'free surface flow systems' and 'subsurface flow systems' you use. Is the only difference between the two systems that the soil can also take up nutrients in the subsurface systems? The name suggest that in the subsurface systems, the water doesn't flow over the sediment, but rather through it (helophyte CWS). If this is true, than the incorporation of sediment is not the only difference. Because you do not mention these terms in the rest of the text you could also remove them to avoid confusion.

**Response**

We agree with the referee. For clarity, we have now deleted "(free surface flow systems)" and "(subsurface flow systems)".

Line 46-47: You mention that low maintenance leads to a saturated system. I think the reason why a low maintenance system is the same as an easily saturated system may not be evident to all readers, please elaborate. I think you mean that if you do not remove P from the sediment, the binding capacity will decrease and no additional P will be taken up, thus no water quality increase.

**Response**

We have now modified the text [P2, L47] to:

‘As a result of low maintenance, however, these systems easily become saturated with P and other nutrients, which decreases their nutrient binding capacity. As a result, they only work efficiently for a limited amount of time (Drizo et al., 2002).’

Line 47-48: I think seasonality is also an important limiting factor for these systems. You mention this in the discussion, but perhaps you can also include it here. If you want a system with plants to remove nutrients year round in the temperate regions of the world, you will need to add energy in the form of light and heating in the cold seasons, thereby increasing energy consumption and perhaps making it not such a ’low energy requiring system’, as you mention before.

**Response**

We agree with the referee. We have now added this information to the text [P2, L49]:

‘Furthermore, at higher latitudes seasonality is an important factor for these systems because additional energy will be needed during cold seasons (e.g. the use of warmed greenhouse facilities) to remove nutrients by macrophytes growth year-round (Wittgren & Mæhlum, 1997).’

Line 50: You mention few studies have been performed, could you shortly give their main results?

**Response**

We have now added [P3, L55]:

'Although these studies showed that submerged or floating macrophytes can be used to remove nutrients from wastewater due to their high growth rates, they did not elaborate on nutrient removal efficiencies under different nutrient loadings (Vymazal, 2007; Gao et al., 2009).'

Line 57: with adsorption, do you only mean adsorption to sediment, or more in general (also to waterborne particles?)

**Response**

We have clarified this by changing the text to [P4, L63]:

'There is a suite of mechanisms involved in the processes of nutrient removal and recovery in natural and constructed wetlands, including sediment adsorption, phosphate ($PO_4^{3-}$) adsorption by aluminium (Al), iron (Fe) or calcium (Ca), precipitation, plant absorption, volatilization, and microbial processes such as iron oxidation, nitrification, DNRA (dissimilatory nitrate reduction to ammonium) and anammox (anaerobic ammonium oxidation) (Van Loosdrecht & Jetten, 1998; Van Dongen et al., 2001; Kadlec & Wallace, 2008; Wu et al., 2014).'

Line 60: How are the mechanisms affected? Is their speed affected, or efficiency? Please clarify.

**Response**

For clarity, we have now added [P4, L68]:

'Rates and removal efficiencies by these mechanisms are generally affected by factors such as nutrient loading, plant species and sediment type (Gale et al., 1994; Tanner, 1996; Jampeetong et al., 2012).'

Line 70: could you explain why you report the nutrient loading levels in unit per square metre? As the system is 3-dimentional, would it not be more logical to provide loading in units per litre or per cubic metre?

**Response**

Since fluxes (here nutrient loading rates) are per definition expressed as units per square meter per time, we have used loading per square meter throughout the text.

Line 74: If you want to use these terms (polishing / purifying), please specify the difference here as you did in the abstract.

**Response**

For clarity, we have now changed the text to [P5, L81]:

'By studying the resulting distribution of P and N among the different sediment, macrophyte and water compartments, we aimed to determine the nutrient removal efficiency by floating or submerged aquatic macrophytes from wastewater at low (polishing) or high (purification) loading rates, and the interacting role of sediment type.'

Line 74-76: I miss the sediment in your aim / main research question.

**Response**

We agree with the referee. We have now added this information to the text [P5, L81]:

'By studying the resulting distribution of P and N among the different sediment, macrophyte and water compartments, we aimed to determine the nutrient removal efficiency by floating or submerged aquatic macrophytes from wastewater at low (polishing) or high (purification) loading rates, and the interacting role of sediment type.'

Line 81: did you mix the sediment before adding to the mesocosm?

**Response**

We carefully mixed the sediment before adding it to mesocosm. We have now modified the text to [P6, L88]:

‘All mesocosms were filled with 20 cm (135 L) of carefully homogenized clay (originating from Lalleweer, 53°16' N, 6°59' E; n=9), peaty clay (originating from De Deelen, 53°01' N, 5°55' E; n=9) or peat (originating from Ilperveld, 52°27' N, 4°56' E; n=9), after which they received a layer of 50 cm of Nijmegen tap water ($NH_4^+$ < 0.03 mg $L^{-1}$, $NO_3^-$: 16.40 mg $L^{-1}$, $PO_4^{3-}$ < 0.03 mg $L^{-1}$, pH: 7.7, total inorganic carbon (TIC): 30 mg C $L^{-1}$).’

Line 82: could you summarize the basic characteristics for tap water, as you did for sediment?

**Response**

We have now added this information to the text [P6, L88], see above.

Although tap water contained relatively high $NO_3^-$ concentrations, this did not influence our results. As we started our experiment one year after filling the basins, denitrification had taken place and $NO_3^-$ was lost via denitrification, as measured.

Line 90-92: Did you add the nutrients to the surface water? I was also wondering why you included the natural deposition, generally it’s very good to include it! But it seems that they are negligible compared to your real treatment. Also, you used relatively old references to determine the amount of background nutrient deposition, are they still relevant? I think you can also just report your loading treatment as is.

**Response**

We have now changed the text to [P6, L100]:

‘To create these, treatment solutions were added three times a week to the surface water to enable loading rates of 0.43, 21.4 and 85.7 mg P $m^{-2}$ $d^{-1}$ (added as $NaH_2PO_4.H_2O$ and atmospheric deposition of 0.1 kg P $ha^{-1}$ $y^{-1}$) (Furnas, 2003) and 1.3, 62 and 249 mg N $m^{-2}$ $d^{-1}$ (added as $NH_4NO_3$ and atmospheric N deposition of 35 kg N $ha^{-1}$ $y^{-1}$ in this part of the Netherlands) (RIVM, 2014).’

Atmospheric N deposition of 35 kg N $ha^{-1}$ $y^{-1}$ (9.59 mg N $m^{-2}$ $d^{-1}$) is a very important N input, especially at low N loading rates. Therefore, it is necessary to include nutrient deposition to calculate the nutrient budget.

For natural N deposition, we have now used a reference that covered the N deposition during the experimental period instead, and found that it was higher than anticipated based on average values for the Netherlands:

RIVM, 2014. Concentration and deposition maps of the Netherlands: Total Nitrogen (2014). Available at: http://geodata.rivm.nl/gcn/.

Line 96: What do you mean by environmentally relevant densities? Typical densities for this species in lakes, ditches? Please add a bit more detail or a reference. Could you also explain why you used different amounts per species? Would your results have been different if you've started with the same amount for all species? I feel that this section needs a bit more argumentation.

**Response**

As biomass production depends on plant density, we adopted typical plant densities for each species as found in the field, instead of similar densities for all species. We have added the following reference for this:

'De Lyon, M. J. H., Roelofs, J. G. M., 1986. Waterplanten in relatie tot waterkwaliteit en bodemgesteldheid. Deel 1 and 2. Laboratorium voor Aquatische Oecologie, Katholieke Universiteit Nijmegen, Nijmegen.'

Line 97: Why do you mention Chara hispida here? It's not mentioned in line 68 of your introduction. If you want to include it, perhaps just mention that you also tested chara, but that it was outcompeted so is not a suitable species for water purification under your experimental conditions and will therefore be disregarded in the rest of this study.

**Response**

At the beginning of our experiment, we planned to include *Chara hispida* as one of our submerged species, but since it was outcompeted in almost all experimental units, we had to exclude it from our analyses. To avoid confusion over why we divided our mesocosms in four equal parts and only used three species, we decided to mention the species in our manuscript.

To clarify why we did not include it in our results section, we have now changed the text to [P7, L113]:

'As *C. hispida* was completely outcompeted by spontaneously developing vegetation, the quarters with this species were excluded from the rest of this study.'

Line 103: Were rooted species harvested including roots, or just the shoots?

**Response**

We have now modified the text to [P7, L115]:

'During the experimental period, 20 % of the total plant biomass (for rooted macrophytes aboveground biomass only) was harvested when vegetation reached 100 % cover, to avoid space limitation.'

Line 110: Because pH can vary over time, please provide information on the time of measurement.

**Response**

To specify the time of measurement, we have now changed the text to [P8, L122]:

'pH of water samples was measured between 12:00 PM and 2:00 PM using a combined Ag/AgCl electrode (Orion, Thermo Fisher Scientific, Waltham, MA, U.S.A.) with a TIM840 pH meter (Radiometer Analytical, Lyon, France).'

Line 116: Please provide information why it was important to measure Al, Fe and Ca (They can bind P, but I'm not sure all readers will know). You can for example add this information near line 56/57.

**Response**

We have now included the importance of Al, Fe and Ca in nutrient removal in our introduction [P4, L63]:

'There is a suite of mechanisms involved in the processes of nutrient removal and recovery in natural and constructed wetlands, including sediment adsorption, phosphate ($PO_4^{3-}$) adsorption by aluminium (Al), iron (Fe) or calcium (Ca), precipitation, plant absorption, volatilization, and microbial processes such as iron oxidation, nitrification, DNRA (dissimilatory nitrate reduction to ammonium) and anammox (anaerobic ammonium oxidation) (Van Loosdrecht & Jetten, 1998; Van Dongen et al., 2001; Kadlec & Wallace, 2008; Wu et al., 2014).'

Line 133: Do you mean total P or inorganic P (PO4) here, please specify.

**Response**

To specify which P fraction we used to calculate the P budget, we have now modified the text to [P10, L144]:

'Furthermore, nutrient changes in surface water and pore water were calculated from changes of N ($NO_3^-$ and $NH_4^+$) and total P concentrations (end minus start).'

Line 135-136: could you provide a reference for these processes to explain why this assumption is valid. I also wondered if you couldn't quantify (or at least estimate) the amount stored in the sediment, based on your sediment measurements In that way, you don't have to make this assumption, but can provide proof.

**Response**

We have now added the following reference for coupled nitrification/ denitrification:

'Wetzel, R. G., 2001. Limnology: lake and river ecosystems. *3rd ed*. Academic Press, San Diego, California.'

It is unfortunately not possible to calculate nutrient amounts stored in the sediment, due to the fact that the amounts of nutrient release or adsorption by the sediment are very small compared to total nutrient contents in the sediment. Therefore, no differences in nutrient concentrations in the sediment existed between the start and the end of the experiment.

Line 145: please clarify what you mean with: ’except for treatments also including time as main effect’, which are they?

**Response**

For clarity, we have now modified the text to [P11, L157]:

‘The main effects (including nutrient loading, sediment type, plant species, and time) and interactions of treatments on N ($NO_3^-$ and $NH_4^+$) and P concentrations in surface water were also tested by linear mixed models.’

Line 148: Please mention which R packages/statistical tests you used for the regression models.

**Response**

We have now changed the text to [P11, L160]:

‘We analyzed the influence of nutrient loadings on P and N sequestration (uptake plus adsorption to plants) rates using linear and logistic regression models with the summary function.’

Line 148: you introduce the term ’sequestration’ here. I’m not sure everyone will be familiar with this, could you explain or swap for a term you’ve mentioned before, such as nutrient uptake or absorption?

**Response**

We have now modified the text to [P11, L160]:

‘We analyzed the influence of nutrient loadings on P and N sequestration (uptake plus adsorption to plants) rates using linear and logistic regression models with the summary function.’

Line 152-153: I think you mean that there is a main effect; however, looking at the graphs not all treatments show an increase. Perhaps you could provide some more information on this. You performed a full-factorial experiment and especially the interactions are very interesting I think!

**Response**

To provide more information, we have now added the following sentence to the text [P12, L166]:

‘There were significant interactions between time and plant species ($X^2$=10.18; $P < 0.01$) for surface water P, and between time and nutrient loadings ($X^2$=8.92; $P < 0.05$) for surface water N.’

Line 158: Because you don’t give the data, perhaps it’s informative if you just provide the mean +/- SE values.

**Response**

We have now changed the text to [P13, L171]:

‘Peat sediments had the highest P concentrations in the pore water, whereas the lowest were found in clay sediments ($X^2$=20.20; $P < 0.001$; 4.65 ± 0.15 mg $L^{-1}$ and 0.71 ± 0.05 mg $L^{-1}$ for peat and clay, respectively), even though total P and Olsen P concentrations were much higher in clay than in the other two sediments (Table 1).’

Paragraph 3.3 seems a bit long and sometimes unstructured; perhaps not all information is needed. e.g. is line 186-187 really relevant for your story? Also lines 183-184 may fit better at the end of the paragraph.

**Response**

We agree with the referee and have now corrected this paragraph according to the referee's suggestions.

Results: I expected information on Fe, Al and Ca in the results, as you mention them in the M&M.

**Response**

We only used Fe, Al and Ca concentrations in the sediment in this manuscript, and we think that the way we wrote about the measurements of Fe, Al and Ca in material and methods section may indeed be confusing to readers. Therefore, we have now modified the text to [P8, L128]:

'Concentrations of total P were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES; IRIS Intrepid II, Thermo Fisher Scientific, Franklin, MA, U.S.A.).'

and the text to [P9, L133]:

'Furthermore, 200 mg of dry sediment was digested in a microwave oven (MLS-1200 Mega, Milestone Inc., Sorisole, Italy) with 4 mL 65 % $HNO_3$ and 1 mL 30 % $H_2O_2$, after which digestates were analyzed and concentrations of total Al, Fe, Ca and P in sediments were determined by ICP-OES (see above).'

Results: I also expected fig. 6 to be mentioned here, for example in section 3.3.

**Response**

We have now moved Fig 6 from discussion section to results section 3.3, and added two sentences to the text [P16, L205]:

'For *C. demersum*, nutrient sequestration rates increased linearly with increased nutrient loading, while for *M. spicatum* there was a logistic response to external nutrient loading (Fig. 6). *A. filiculoides* showed linearly increasing P sequestration rates upon increased P loading and a logistic response to external N loading.'

Line 203: perhaps add that N removal also depends on species.

**Response**

We have now added one more sentence to the text [P18, L222]:

"Furthermore, this study also shows that N removal efficiency of macrophytes strongly depends on plant species involved."

Line 206: are these averages calculated over all nutrient loading treatments?

**Response**

For clarity we have now changed the text to [P19, L225]:

'With average biomass production rates of 3.4 and 1.0 g DW $m^{-2}$ $d^{-1}$, respectively, *A. filiculoides* and *M. spicatum* showed the highest growth rates, regardless of sediment type and nutrient loading, and therefore have the best potential for being used to remove nutrients in constructed wetlands.'

Line 235-237: Could you comment a bit more on how the different growth forms impact nutrient removal and which species you would recommend under what loadings? Do you expect similar results for other floating / submerged plants? Perhaps you can use the 'few references' you mention are available on this topic.

**Response**

In our study we only determined specific nutrient removal efficiencies of the floating or submerged macrophytes we tested, and therefore the results cannot be simply extrapolated to general plant growth forms. We strongly feel that the removal efficiency depend on the plant species involved due to their specific biomass production and nutrient uptake rates, and not necessarily on plant growth forms. We have therefore now changed the corresponding parts according to the specific comments and suggestions of the referee, as explained in detail below.

Line 237-238: this sentence is hard to read, perhaps start with: "Low O2 mobilizes PO4 [...]"

**Response**

We have now modified the text to [P21, L255]:

'As low $O_2$ concentrations, induced by the coverage of floating macrophytes or dense growth of submerged macrophytes, can mobilize P from the sediment, *A. filiculoides* and *M. spicatum* did not only take up all P being discharged into the system by both their roots and shoots, but additionally took up mobilized P (Wetzel, 2001).'

Line 240: do they only take up nutrients by their roots or also via their shoots when nutrients are mobilized and leach into the water?

**Response**

We have now changed the text to [P21, L255]:

'As low $O_2$ concentrations, induced by the coverage of floating macrophytes or dense growth of submerged macrophytes, can mobilize P from the sediment, *A. filiculoides* and *M. spicatum* did not only take up all P being discharged into the system by both their roots and shoots, but additionally took up mobilized P (Wetzel, 2001).'

Line 241-242: please add reference to support this.

**Response**

We have now added the following reference:

'De Lyon, M. J. H., Roelofs, J. G. M., 1986. Waterplanten in relatie tot waterkwaliteit en bodemgesteldheid. Deel 1 and 2. Laboratorium voor Aquatische Oecologie, Katholieke Universiteit Nijmegen, Nijmegen.'

Line 245: How does this seasonality affect your maximum loading you can remove with plants? Should we divide the results of your study by 2 to get the year round maximum nutrient loading to account for the winter influx of nutrients, assuming that they are then bound to the sediment? Please elaborate on this.

**Response**

To specify the effect of seasonality on nutrient uptake rates of plants, we have now added the following sentence to the text [P22, L261]:

Under low external loading, sediments will take up most of the P during winter. Since submerged plants have N and P accumulation rates that are higher than the low nutrient loading, they heavily rely on uptake of nutrients from the sediment. Thus, the nutrients stored in the sediment in winter can be mobilised and taken up by macrophytes in summer, creating an efficient and sustainable constructed wetland for water polishing in temperate climates. Furthermore, predicted climate change will lead to higher temperatures and thus longer growing seasons in temperate regions, indicating that these systems may be operational longer and longer every year.

Line 247-248: how do you come to 6-24mg? In fig. 6 I don't see a maximum.

**Response**

We have now modified the text to [P23, L269]:

'When P loading in the treatment water increases, uptake rates of *A. filiculoides* double or even triple, to rates 7.87 or 17.64 mg P $m^{-2}$ $d^{-1}$.'

Line 248-249: You mention that your results are comparable to the results of Reddy & de Busk, but according to you, they found values 2 times higher than yours, which sounds much higher to me. Could this be because you were not near the maximum potential of Azolla, as suggested by the linear relationship between loading and uptake (fig6)?

**Response**

We have now changed the text to [P23, L270]:

'The highest value is lower than results of Reddy and DeBusk (1985), who reported P uptake rates of 43 ± 15 mg P $m^{-2}$ $d^{-1}$ by *A. filiculoides* grown in an N-free, 3 mg $L^{-1}$ P medium which, however, had much higher $PO_4^{3-}$ concentrations in the surface water than our high nutrient loading treatment.'

Line 264: Very nice that you mention N-fixation in Azolla! Could you add references of a study that researched the N-fixation capacity of Azolla to assess how much they can fix N and thus argue if this amount could have affected your results. N was still removed from the water, so I see no reason why this would be very detrimental to your story.

**Response**

We have now modified the text to [P24, L288]:

'Although it can be estimated that $N_2$-fixation rates by *Azolla* grown in an N-free medium were in the range of 1.4 - 2.7 kg N $ha^{-1}$ $d^{-1}$ (Reddy & DeBusk, 1985), in our study we added N to the surface water which may affect $N_2$ fixation. Therefore it was difficult to calculate N removal rates for *A. filiculoides*, as the unknown $N_2$ fixation rates lead to an overestimation of N uptake rates by *A. filiculoides*.'

Line 263-265: This argument needs a bit more information, could you provide some mechanisms why you conclude that senescence is more important than soil leaching? I'm not sure the information provided is sufficient.

**Response**

To provide more information we have now changed the text to [P24, L295]:

‘At the end of the growing season, dissolved N concentrations increased under high nutrient loading, similar to P concentrations. This increase may result from a combination of reduced plant uptake, nutrient leaching from senescing plants and reduced denitrification rates as a result of lower temperatures. Due to the different available pathways for nitrogen removal from the sediment, sediment saturation of N seems unlikely.’

Line: 271- 274: Here you extrapolate your results to plant growth forms. Only your data does not allow for this, please provide literature on other species with the same growth form or on the mechanisms which will show why we can assume that other species with the same growth form with have similar nutrient removal rates.

**Response**

Although we can see how our statement may be confusing, we did not mean to extrapolate our results to plant growth form (see above). In order to avoid confusion, we have now changed the text to [P25, L301]:

‘We showed that in macrophyte-dominated CWS, both the submerged and the floating macrophytes we tested are able to remove most of the added nutrients at low P and N loadings, whereas at higher nutrient loadings, floating or submerged macrophytes could only remove 20-45 % and 10-25 % of the external P loads for 21.4 and 85.7 mg P $m^{-2}$ $d^{-1}$, respectively.’

Line 279: explain how the creation of anoxic conditions removes P from the sediment.

**Response**

To specify the mechanism regulating P removal, we have now modified the text to [P25, L309]:

‘While aquatic macrophytes are able to remove this P from the sediments by either creating anaerobic conditions to trigger high P mobilization (Smolders et al., 2006) or through both root and shoot uptake, the external load will have to be reduced for this process to occur efficiently.’

DISCUSSION: Do you have any idea why C. demersum performed so poorly?

**Response**

To provide more information about the reason for the poor performance of *C. demersum*, we have now changed the text to [P19, L228]:

‘*C. demersum*, on the other hand, appeared to be less suitable, since this species was easily outcompeted for light by other species, such as floating algae and *Zanichellia spp*.’

Discussion: Could you compare your results to other studies about CWS with floating or submerged plants or with plant nutrient uptake studies? This will enable you to give more general recommendations and conclusions. Perhaps also shortly compare your results with more traditional emergent CWS, as you’ve mentioned there’s a lot of literature about those and readers will be more familiar with those.

**Response**

To compare nutrient uptake rates of floating or submerged macrophytes with nutrient uptake rates of emergent macrophytes, we have now added the following sentence to the text [P23, L272]:

‘P uptake rates of *A. filiculoides* in this study are similar to, or even lower than, results of Brix (1994), who reported P uptake rates of 8 - 41 mg P $m^{-2}$ $d^{-1}$ by emergent macrophytes. The main advantage of using floating macrophytes instead of emergent macrophytes is, however, that they can be harvested multiple times a year and that they take up nutrients from both the water layer and sediment.

Line 283-286: Perhaps remove the species specific information here, as the effects on N are different (Azolla is always better). Furthermore, looking at fig. 6 M. spicatum is only better in P uptake between your low and medium nutrient treatment, because you’ve fitted a log.-line, at 22mgP input azolla is already better (although not sign.) It’s important to keep in mind how reliable your regression is when only using 3 points on the x-axis, especially when making these kinds of general statements and using the regression line to determine thresholds. I would tone this down a little bit, or at least acknowledge the uncertainty.

**Response**

We agree with the referee. We have now modified the text to [P26, L316]:

'At a low nutrient loading *M. spicatum* and *A. filiculoides* performed equally well for P removal whereas at loads ≥ 22 mg P $m^{-2}$ $d^{-1}$, *A. filiculoides* removes P more efficiently.'

and the text to [P20, L237]:

'Our results indicate that at a low nutrient loading *M. spicatum* and *A. filiculoides* performed equally well for P removal whereas at loads ≥ 22 mg P $m^{-2}$ $d^{-1}$, *A. filiculoides* removes P more efficiently (Fig. 6a).'

**Technical comments**

Line 54: change 'drained' to 'removed'

**Response**

We have now corrected this issue.

Line 55: add recent reference, several recent studies that have looked into this as well.

**Response**

We have now added one recent reference:

'Biswas, S., Sarkar, S., 2013. *Azolla* cultivation: a supplementary cattle feed production through natural resource management. Agriculture Update 8, 670-672.'

Line 63: you mention 'soil type'. Do you mean the same with this as with 'soil characteristics' in line 60? If so, it's better to use the same term throughout the whole text.

**Response**

We have corrected this.

Line 68: because you specifically use macrophyte species with contrasting growth forms, I would stress this here.

**Response**

We have now changed the text [P5, L75] to:

‘Using a full-factorial outdoor mesocosm experiment, we studied the nutrient uptake rates of three different aquatic macrophytes with contrasting growth forms, *Azolla filiculoides*, *Ceratophyllum demersum* and *Myriophyllum spicatum*, growing on peat, peaty clay or clay sediments.’

Line 70: Please provide reference of ’environmentally relevant nutrient levels’.

**Response**

We have now added one reference:

‘Lamers, L. P. M., Smolders, A. J. P., Roelofs, J. G. M., 2002. The restoration of fens in the Netherlands. Hydrobiologia 478, 107-130.’

Line 79: please check your calculation for volume. I’m not sure that 20cm in a 185cm diameter cylinder is 135 L.

**Response**

The calculation is correct, as 135 L is the soil volume for each quarter.

Line 143-146: Long, complex sentence. Could you abbreviate this or split in 2 sentences?

**Response**

We have now split this sentence into [P11, L155]:

'Linear mixed models were used to test the main effects and interactions of treatments on sediment characteristics, biomass production rates, the ratios between N and P, and nutrient budgets with mesocosm number as a random effect, by using R package nlme.'

And [P11, L157]:

'The main effects (including nutrient loading, sediment type, plant species, and time) and interactions of treatments on N ($NO_3^-$ and $NH_4^+$) and P concentrations in surface water were also tested by linear mixed models.'

Line 155 and further: you often write p<0.000, which means that p is negative. I believe you mean p<0.001.

**Response**

We have corrected this issue.

Line 158-161: To remove some ambiguity, perhaps rewrite as: [...] even though total P and Olsen P concentrations were much higher in clay than in the other two soils [...].

**Response**

We have corrected this issue.

Line 177: I think you mean Azolla OR Spicatum. Now it seems like they were both inside one quarter.

**Response**

We have now corrected it.

Line 179: 'on the other hand' seems to contradict the previous sentence where M. spicatum also didn't take up more than 20%.

**Response**

We have now deleted 'on the other hand'.

Line 188: Here you use 'absorbed', I was wondering if you mean the same with words like: take up nutrient, sequester nutrients and absorb nutrients. If so, it is more clear to choose one term and use that one throughout the whole text for clarity.

**Response**

We have now corrected this issue.

Line 195: 'As P loading [...]' can be removed as the first 2 lines of the paragraph provides the same information.

**Response**

We have now deleted this line.

Line 251: It's hard to compare values in mols and grams, please choose one unit and use it throughout the text.

**Response**

We have now used grams throughout the whole text.

Line 257: add '[..] in our study.' to the end of the sentence to make sure that readers know your talking about your results.

**Response**

We have now added this.

Line 271: change beginning to: '[...] CWS, both submerged and floating plants are [...]'

**Response**

We have now corrected this.

Line 274-276: Too many dependent clauses, please reformulate. (Also check the rest of the text for these sentences, they are often hard to understand.)

**Response**

We have now corrected this.

Line 278: change with: [...] resulting in saturated soil and thus leading to an increase in water nutrient levels under continued nutrient input.

**Response**

We have now corrected this.

General: I'm not sure the term 'soil' is used often when referring to aquatic systems, sediment may be more appropriate in the context of your study

**Response**

We now use the term 'sediment' throughout the entire text.

# Aquatic macrophytes can be used for wastewater polishing, but not for purification in constructed wetlands

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**Abstract**

The sequestration of nutrients from surface waters by aquatic macrophytes and sediments~~soils~~ provides an important service of both natural and constructed wetlands. While emergent species take up nutrients from the ~~soil~~sediment, submerged and floating macrophytes filter nutrients directly from the surface water, which may be more efficient in constructed wetlands. It remains unclear, however, whether their efficiency is sufficient for wastewater purification, and how plant species and nutrient loading affects nutrient distribution over plants, water, and ~~soil~~sediment. We therefore determined nutrient removal efficiencies of different vegetation (*Azolla filiculoides*, *Ceratophyllum demersum* or *Myriophyllum spicatum*) and ~~soil~~ sediment types (clay, peaty clay and peat) at three nutrient input rates, in a full factorial, outdoor mesocosm experiment. At low loading (0.43 mg P $m^{-2}$ $d^{-1}$), plant uptake was the main pathway (100 %) for phosphorus (P) removal, while ~~soils~~ sediments showed a net P release. *A. filiculoides* and *M. spicatum* showed the highest biomass production and could be harvested regularly for nutrient recycling, whereas *C. demersum* was outcompeted by spontaneously developing macrophytes and algae. Higher nutrient loading only

stimulated *A. filiculoides* growth. At higher rates (≥ 21.4 mg P m$^{-2}$ d$^{-1}$) 50-90 % of added P ended up in ~~soils~~sediments, with peat ~~soils~~ sediments becoming more easily saturated. For nitrogen (N), 45-90 % was either taken up by the ~~soil~~ sediment or lost to the atmosphere at loadings ≥ 62 mg N m$^{-2}$ d$^{-1}$. This shows that aquatic macrophytes can indeed function as an efficient nutrient filter, but only for low loading rates (polishing), not for high rates (purification). The outcome of this controlled study not only contributes to our understanding of nutrient dynamics in constructed wetlands, but also shows the differential effects~~the importance of wetland~~ of wetland ~~soil~~ sediment ~~characteristics~~types and plant species. Furthermore, the acquired knowledge may benefit the application of macrophyte harvesting to remove and recycle nutrients from both constructed wetlands and nutrient-loaded natural wetlands.

**Keywords: Eutrophication, nutrient removal, macrophytes, nutrient budgets, purification, water management**

## 1. Introduction

Excess loading of phosphorus (P) and nitrogen (N) from domestic, agricultural and industrial wastewaters is the main cause of eutrophication of aquatic ecosystems, damaging their ecological quality and functioning (Kronvang et al., 2005; Kantawanichkul et al., 2009). Surface water eutrophication can lead to algal and cyanobacterial blooms, die-off of indigenous vegetation and serious decrease in biodiversity (Pretty et al., 2003; Conley et al., 2009). In recent decades, wetlands have been constructed to mitigate eutrophication of watercourses, lakes and seas by reducing the nutrient loads in discharge water of wastewater treatment plants, farmlands, households or industries (Brix & Arias, 2005; Mitsch et al., 2005).

Constructed wetland systems (CWS) use macrophytes ~~(free surface flow systems)~~ or a combination of macrophytes and ~~soil~~sediment, ~~(subsurface flow systems),~~ to remove nutrients from the water (Brix, 1994; Vymazal, 2007). These systems are either used as stand-alone water purification systems

(Vrhovšek et al., 1996; Jing et al., 2001) or as a polishing method of pre-treated wastewater (Kaseva, 2004; Greenway, 2005). The most commonly used macrophyte species are emergent genera such as *Typha, Phragmites, Scirpus, Phalaris* and *Iris* (Vymazal, 2011). Advantages of CWS include utilization of natural processes, low cost and energy requirements, and easy operation and maintenance (Brix, 1999; Konnerup et al., 2009). As a result of low maintenance, however, these systems easily become saturated~~, especially~~ with ~~especially~~ P and other nutrients, which ~~a~~ decreas~~ed~~ ~~ing~~es their nutrient binding capacity~~ of the sediment over time,~~. ~~and t~~As a result~~therefore~~, they only work efficiently for a limited amount of time (Drizo et al., 2002). Furthermore, at higher latitudes seasonality is an important factor for these systems because additional energy will be needed during cold seasons (e.g. the use of warmed greenhouse facilities) to~~would have~~ remove nutrients by macrophytes growth year-round (Wittgren & Mæhlum, 1997).

Although much research has focused on the optimal design of CWS with respect to the most efficient macrophyte species (Lin et al., 2002; Scholz & Xu, 2002), only few have investigated the possibility of using floating or submerged aquatic macrophytes in treatment systems. Although these ~~research findings~~studies showed that submerged or floating macrophytes can be used to remove nutrients from wastewater due to ~~its~~their high growth rates~~, .~~,~~wherea~~ they ~~but~~ did ~~s did~~ not ~~elaborate on~~~~focus~~elaborate on nutrient removal efficiencies under different nutrient loadings (Vymazal, 2007; Gao et al., 2009). While helophytes mainly take up nutrients from the ~~soil~~sediment, floating and submerged aquatic macrophytes, such as *Azolla* spp. or *Myriophyllum* spp., can also take up nutrients from the water layer (Best & Mantai, 1978; Van Kempen et al., 2012). By regularly harvesting these plants, nutrients may be ~~drained~~ removed from the system. The aquatic biomass can then be used in various bio-based applications, for instance, as a bio-fertilizer or as fodder for livestock (Hauck, 1978; Biswas & Sarkar, 2013).

There is a suite of mechanisms involved in the processes of nutrient removal and recovery in natural and constructed wetlands, including sediment adsorption, phosphate ($PO_4^{3-}$) adsorption by

~~Al~~aluminium (Al), ~~Fe~~iron (Fe) or ~~Ca~~calcium (Ca), precipitation, plant absorption, volatilization, and microbial processes such as iron oxidation, nitrification, DNRA (dissimilatory nitrate reduction to ammonium) and anammox (anaerobic ammonium oxidation) (Van Loosdrecht & Jetten, 1998; Van Dongen et al., 2001; Kadlec & Wallace, 2008; Wu et al., 2014). Rates and removal efficiencies by ~~These~~ these mechanisms are generally affected by factors such as nutrient loading, plant species and ~~soil~~ sediment ~~characteristics~~ type~~s~~ (Gale et al., 1994; Tanner, 1996; Jampeetong et al., 2012). So far, most studies have focused on the effects of only one or two of these factors on nutrient retention in wetlands, whereas little information is available on interactions among plant species, ~~soil~~ sediment type and nutrient loading. Only by including all interactions, however, can nutrient sequestration efficiency of wetland plants and ~~soils~~ sediments under different loads be assessed.

Here, we studied the effects of plant species, nutrient loading and ~~soil~~ sediment type on nutrient uptake rates of aquatic macrophytes and nutrient retention rates of ~~soils~~sediments. Using a full-factorial outdoor mesocosm experiment, we studied the nutrient uptake rates of three different aquatic macrophytes with contrasting growth forms, *Azolla filiculoides*, *Ceratophyllum demersum* and *Myriophyllum spicatum*, growing on peat, peaty clay or clay ~~soils~~sediments. Three different, environmentally relevant, nutrient loadings of P (0.43, 21.4 and 85.7 mg P m$^{-2}$ d$^{-1}$) and N (1.3, 62 and 249 mg N m$^{-2}$ d$^{-1}$) were applied to the mesocosms, representing pre-treated (low nutrient loading), and eutrophic and hypertrophic wastewater input (medium and high nutrient loading) (Lamers et al., 2002). By studying the resulting distribution of P and N among the different sediment, macrophyte and water compartments, we aimed to determine the nutrient removal efficiency by floating or submerged aquatic macrophytes from wastewater at low (polishing) or high (purification) loading rates, and the interacting role of sediment type.~~By studying the resulting distribution of P and N among the different sediment, macrophyte and water compartments, we aimed to determine the nutrient removal efficiency by floating or submerged aquatic macrophytes from wastewater at low (polishing) or high (purification) loading rates, and the interacting role of sediment type.~~

~~2. By studying the resulting distribution of P and N among the different soil~~~~sediment, macrophyte and water compartments, we aimed to determine whether floating or submerged aquatic macrophytes can effectively remove nutrients from wastewater removal by floating or submerged aquatic macrophytes may be an efficient approach for atat low loading rates (polishing) or forat high loading rates (purifying purification)wastewater., and taking into accountand the interacting role of sediment type.~~

## ~~3.~~2. Materials and methods

### *~~3.1.~~2.1. Experimental set-up*

Twenty-seven mesocosms (185 cm Ø, 90 cm depth) were sunk into the ground outside the greenhouse facility at Radboud University (Nijmegen, The Netherlands). All mesocosms were filled with 20 cm (135 L) of carefully homogenized clay (originating from Lalleweer, 53°16' N, 6°59' E; n=9), peaty clay (originating from De Deelen, 53°01' N, 5°55' E; n=9) or peat (originating from Ilperveld, 52°27' N, 4°56' E; n=9), after which they received a layer of 50 cm of Nijmegen tap water ($NH_4^+ < 0.03$ mg $L^{-1}$, $NO_3^-$: 16.40 mg $L^{-1}$, $PO_4^{3-} < 0.03$ mg $L^{-1}$, pH: 7.7, total inorganic carbon (TIC): 30 mg C $L^{-1}$ ~~data from Vitens Laboratory~~). ~~Soil~~ Sediment characteristics are displayed in Table 1, expressed per unit volume to enable comparison among ~~soil~~ sediment types with respect to nutrient exchange and plant nutrient availability. In all mesocosms, crossed transparent carbon fiber plates were used to create four fully isolated quarters. We did not include non-vegetated treatments because: 1) our focus was on complete ecosystems in constructed and natural wetlands, i.e. including ~~soil~~ sediment and vegetation; 2) bare ~~soils~~ sediments always show spontaneous vegetation development if light and nutrient conditions suffice (see section 2.2); 3) continuous plant removal would lead to significant ~~soil~~ sediment disturbance; and 4) dark conditions would affect ~~soil~~ sediment biogeochemistry. Mesocosms were randomly assigned to “low”, “medium” or “high” nutrient loading treatment (n=3 for all). To create these, treatment solutions were added three times a week to the surface water to enable loading rates of 0.43, 21.4 and 85.7 mg P $m^{-2}$ $d^{-1}$ (added as $NaH_2PO_4 \cdot H_2O$ and atmospheric deposition of 0.1 kg P $ha^{-1}$ $y^{-1}$) (Furnas, 2003) and 1.3, 62 and 249 mg N $m^{-2}$ $d^{-1}$ (added as $NH_4NO_3$ and atmospheric N deposition of ~~20~~ 35 kg N $ha^{-1}$ $y^{-1}$ in this part of the Netherlands~~; TNO~~) (RIVM, 2014). In the results and discussion sections, treatments will be ~~called~~ referred to as 0.43 (low), 21.4 (medium) and 85.7 (high) mg P $m^{-2}$ $d^{-1}$, according to their respective P loading.

### ~~3.2.~~2.2. *Plant measurements*

In July 2013, environmentally relevant densities (based on personal field observations) (De Lyon & Roelofs, 1986) of *Ceratophyllum demersum* (5.03 ± 0.24 g DW $m^{-2}$; rigid hornwort, submerged macrophyte), *Chara hispida* (8.66 ± 0.69 g DW $m^{-2}$; bristly stonewort, submerged macroalga) and *Myriophyllum spicatum* (5.31 ± 0.60 g DW $m^{-2}$; Eurasian water-milfoil, submerged macrophyte) were planted randomly in each of three quarters of every mesocosm to establish. In April 2014, patches of *Azolla filiculoides* (28.39 ± 0.88 g DW $m^{-2}$; water fern, floating macrophyte) were added to the water layer of the remaining quarter. Apart from these four introduced species, other species colonized the quarters, including *Zanichellia* spp. and floating algae. As *C. hispida* was completely outcompeted by spontaneously developing vegetation, the quarters with this species were excluded from the rest of this study~~results.~~ During the experimental period, 20 % of the total plant biomass (for rooted macrophytes aboveground biomass only) was harvested when vegetation reached 100 % cover, to avoid space limitation. During the final harvest, biomass of all present species was harvested separately and dried (48 h at 60 °C), after which they were weighed, ground and homogenized. ~~As *C. hispida* was completely outcompeted by spontaneously developing vegetation, the quarters with this species were excluded from the results.~~

### ~~3.3.~~2.3. *Chemical analyses*

Surface water samples were collected every week between May and October 2014, whereas pore water samples were collected anaerobically every month using ceramic soil moisture ~~cups~~ samplers

(SMS rhizons, Eijkelkamp, Giesbeek, Netherlands). pH of water samples was measured between 12:00 PM and 2:00 PM using a combined Ag/AgCl electrode (Orion, Thermo Fisher Scientific, Waltham, MA, U.S.A.) with a TIM840 pH meter (Radiometer Analytical, Lyon, France). Total inorganic carbon (TIC) of water samples was measured using an Infra-red Gas Analyzer (IRGA; ABB Analytical, Frankfurt, Germany). Concentrations of $PO_4^{3-}$, $NO_3^-$ and $NH_4^+$ in the surface water and pore water were measured colorimetrically on an Auto-Analyzer III system (Bran & Luebbe, Norderstedt, Germany) by using ammonium molybdate (Henriksen, 1965), hydrazine sulphate (Kamphake et al., 1967) and salicylate (Grasshoff & Johannsen, 1972), respectively. Concentrations of total ~~Al, Fe, Ca, and~~ P were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES; IRIS Intrepid II, Thermo Fisher Scientific, Franklin, MA, U.S.A.).

~~Soil~~ Sediment samples were collected at the end of the experiment, and subsequently volume weighted and dried for 48 h at 60 °C to determine bulk density. Dry ~~soil~~ sediment samples were heated for 4 h at 550 °C and re-weighed to determine organic matter content. Furthermore, 200 mg of dry ~~soil~~ sediment was digested in a microwave oven (MLS-1200 Mega, Milestone Inc., Sorisole, Italy) with 4 mL 65 % $HNO_3$ and 1 mL 30 % $H_2O_2$, after which digestates were analyzed and concentrations of total Al, Fe, Ca and P in sediments were determined by ICP-OES (see above). Plant available P was determined by extraction according to Olsen et al. (1954), whereas an NaCl-extraction was performed to determine exchangeable N ions ($NO_3^-$ + $NH_4^+$) as described in Tomassen et al. (2004). Total P concentrations in plants were determined by digestion of 200 mg of dry plant material and analyzed as described above. Furthermore, 3 mg of dry plant material was combusted to determine C and N content using an elemental analyzer (Carlo Erba NA 1500, Thermo Fisher Scientific, Waltham, MA, USA).

### ~~3.4.~~2.4. *Budget calculations*

For both N and P, nutrient budgets were calculated to determine the distribution among biomass, ~~soil~~ sediment and other components. Cumulative biomass production and nutrient content of submerged or floating macrophytes (target species and others) were used to calculate plant uptake rates of N and P. Furthermore, nutrient changes in surface water and pore water were calculated from changes of N ($NO_3^-$ and $NH_4^+$) and total P concentrations (end minus start). After subtracting the N and P uptake of plants and water components from the external loading, we assume that the remainder is either stored in the ~~soil~~ sediment or, in case of N, lost through coupled nitrification/denitrification ~~or anammox~~ (Wetzel, 2001)~~ or anammox (ref)~~.

### *~~3.5.~~2.5. Statistical Analyses*

All analyses were performed using the software program R (version 3.2.1; R development Core Team, 2015). The effects were considered significant if $P$ < 0.05. In order to meet the assumption that residuals fit a normal distribution and homogeneity of variance, we transformed ~~soil~~ sediment characteristics, N ($NO_3^-$ and $NH_4^+$) and P concentrations in surface water, biomass production rates, N: P ratios in macrophytes, N and P budgets and N and P sequestration rates (response variables) by log (response variable) or log (response variable+1) in case the lowest value of a variable was below one. Linear mixed models were used to test the main effects and interactions of treatments on ~~soil~~ sediment characteristics, biomass production rates, the ratios between N and P~~, N ($NO_3^-$ and $NH_4^+$) and P concentrations in surface water (except for treatments also including time as a main effect in this model)~~, and nutrient budgets with mesocosm number as a random effect, by using R package nlme. The main effects (including nutrient loading, sediment type, plant species, and time) and interactions of treatments on N ($NO_3^-$ and $NH_4^+$) and P concentrations in surface water were also tested by linear mixed models. Tukey tests were used to find differences between treatments by using R package multcomp. We analyzed the influence of nutrient loadings on P and N sequestration (uptake plus adsorption to plants) rates using linear and logistic regression models with the summary function. All graphs were plotted using R package ggplot2.

## ~~4.~~3. Results

### *~~4.1.~~3.1. Surface water and pore water quality*

Over time, surface water P and N ($NH_4^+$+$NO_3^-$) concentrations increased (Figs. 1 and 2; $X^2$=~~4~~3.~~26~~44; *P* < 0.05 and $X^2$=~~35~~23.~~61~~63; *P* < 0.~~000~~ 001 for P and N respectively), especially towards the end of the growing season. There were significant interactions between time and plant species ($X^2$=10.18; *P* < 0.01) for surface water P, and between time and nutrient loadings ($X^2$=8.92; *P* < 0.05) for surface water N. When macrophytes were growing on peat or peaty clay ~~soils~~sediments, P concentrations in the surface water increased with increasing external P loading ($X^2$=~~115~~99.~~87~~80; *P* < 0.~~000~~ 001 and $X^2$=~~88~~59.~~94~~40; *P* < 0.~~000~~ 001 for peat and peaty clay ~~soils~~ sediments respectively).

Porewater nutrient concentrations depended on ~~soil~~ sediment type. Peat ~~soils~~ sediments had the highest P concentrations in the pore water, whereas the lowest were found in clay ~~soils~~ sediments ($X^2$=~~12~~20.~~07~~20; *P* < 0.0~~0~~01; ~~data not shown~~ 4.65 ± 0.15 mg $L^{-1}$ and 0.71 ± 0.05 mg $L^{-1}$ for peat and clay, respectively), ~~-~~even though ~~their~~ total P and Olsen P concentrations were much higher in clay than in the ~~for the~~ other two ~~soils~~ sediments (Table 1). In addition, mesocosms filled with peat ~~soils~~ sediments had higher N concentrations in the pore water than those with peaty clay and clay ($X^2$=7.13; *P* < 0.05; data not shown). Surface water and porewater together never contained more than 12 % of total P and N added to the system at P loadings ≥ 21.4 mg P $m^{-2}$ $d^{-1}$ (Figs. 4 and 5).

### *~~4.2.~~3.2. Macrophyte productivity and nutrient ratio*

Due to their high biomass production rates, *A. filiculoides* and *M. spicatum* could be harvested weekly and biweekly, respectively. *A. filiculoides* had the highest biomass production rates of all three macrophyte species ($X^2$= 55.45, *P*<0.~~000~~001), whereas *C. demersum* grew best on peaty clay ~~soils~~ sediments ($X^2$=10.67, *P* < 0.01), but almost disappeared when growing on clay and peat ~~soils~~ sediments due to competition with algae and other non-target species (Fig. 3). Biomass production rates of *A. filiculoides* were significantly higher at high nutrient loading than at low nutrient loading

($X^2$=11.39, $P$ < 0.01), whereas no effect of nutrient loading was found for the other macrophytes. In quarters with *C. demersum* there was a higher production rate of non-target species than in quarters with *A. filiculoides* and *M. spicatum* ($X^2$=6.28, $P$ < 0.05). *A. filiculoides* showed high N: P ratios (> ~~24~~ 11 ~~mol~~ g ~~mol~~g$^{-1}$) when grown at ≤ 21.4 mg P m$^{-2}$ y$^{-1}$ ($P$ < 0.~~000~~001), whereas all other species generally showed N: P ratios ranging from ~~8~~ 4 to ~~17~~ 8 ~~mol~~ g ~~mol~~g$^{-1}$, without an effect of ~~soil~~ sediment type (Table 2).

### ~~4.3.~~3.3. *Plant nutrient uptake*

*A. filiculoides* and *M. spicatum* accumulated much more P than *C. demersum* ($X^2$=23.66, $P$ < 0.~~000~~001; Fig. 4). At a P loading of 0.43 mg m$^{-2}$ d$^{-1}$ around 100 % of added P and N were accumulated by the targeted macrophytes (Figs. 4 and 5). For the quarters with *A. filiculoides* ~~and~~ or *M. spicatum,* around 20-40 % and 10-20 % of the P added was taken up by target species at P loadings of 21.4 and 85.7 mg m$^{-2}$ d$^{-1}$, respectively, regardless of ~~soil~~ sediment types. *C. demersum*~~, on the other hand,~~ never took up more than 20 % of the P added at these loadings. Still, at a loading of 85.7 mg P m$^{-2}$ d$^{-1}$, removal rates by macrophytes were significantly higher than at 0.43 mg P m$^{-2}$ d$^{-1}$ ($X^2$=7.22, $P$ < 0.05; Fig. 4). The average P sequestration rates by *A. filiculoides* and *M. spicatum* were 3 to 9 mg m$^{-2}$ d$^{-1}$ at P loadings ≤ 21.4 mg m$^{-2}$ d$^{-1}$. At a high P loading of 85.7 mg m$^{-2}$ d$^{-1}$, the average P removal rates by *A. filiculoides* and *M. spicatum* were 16 to 20 and 6 to 14 mg m$^{-2}$ d$^{-1}$, respectively. ~~In addition, *C. demersum* had higher P and N uptake rates in mesocosms with peaty clay compared to mesocosms with clay ($X^2$=10.50, $P$ < 0.01; $X^2$=10.43, $P$ < 0.01).~~ In quarters with *C. demersum*, more P was taken up by other, spontaneously developing species than in quarters with *A. filiculoides* and *M. spicatum* ($X^2$=6.89, $P$ < 0.05). ~~In addition, these non-target plants in *C. demersum* quarters had lower P uptake rates on peaty clay than on peat and clay soils ($X^2$=6.92, $P$ < 0.05).~~ *A. filiculoides* and *M. spicatum* ~~absorbed~~ sequestrated much more N than *C. demersum* and the final biomass of *A. filiculoides* had the highest N content (including $N_2$ fixed) among all macrophyte species ($X^2$=10.28, $P$ < 0.01; Fig. 5). At high N loadings, less than 21 % of added N was removed by the targeted macrophytes. In addition, *C. demersum* had higher P and N uptake rates in mesocosms with peaty clay compared to mesocosms with clay ($X^2$=10.50, $P < 0.01$; $X^2$=10.43, $P < 0.01$).

For *C. demersum*, nutrient sequestration rates increased linearly with increased nutrient loading, while for *M. spicatum* there was a logistic response to external nutrient loading (Fig. 6). *A. filiculoides* showed linearly increasing P sequestration rates upon increased P loading and a logistic response to external N loading.

### *~~4.4.~~3.4. Mobilization and adsorption of nutrients by the ~~soil~~sediment*

At a P loading of 0.43 mg $m^{-2}$ $d^{-1}$, ~~soils~~ sediments were sources of P, whereas ~~soils~~ sediments became P sinks at P loading ≥ 21.4 mg $m^{-2}$ $d^{-1}$ (Fig. 4). On average, 50 to 80 % and 70 to 90 % of P added accumulated in ~~soils~~ sediments at medium and high nutrient loadings, respectively (Fig. 4). In quarters with *C. demersum*, more P accumulated in the ~~soil~~ sediment than in quarters with *A. filiculoides* ($X^2$=11.25, $P < 0.01$). ~~As P loading increased, more P accumulated in the soils ($X^2$=566.40, $P < 0.000$).~~ At medium and high N loads, 45 to 90 % and 80 to 90 %, respectively, was either taken up by the ~~soil~~ sediment or lost to the atmosphere through coupled nitrification/denitrification~~denitrification/anammox~~ (Wetzel, 2001).

## ~~5.~~4. Discussion

In our mesocosm experiment, we show that at low nutrient input (≤ 0.43 mg P $m^{-2}$ $d^{-1}$), 100 % of external loading could be removed through macrophyte uptake, whereas with loadings ≥ 21.4 mg P $m^{-2}$ $d^{-1}$, 50 to 90 % of added P ended up in ~~soils~~sediments. Differences exist, however, between binding abilities of ~~soils~~sediments, with clay ~~soils~~ sediments being able to immobilise P better than peaty clay or peat ~~soils~~sediments. Apart from P, macrophytes were able to remove no more than 65 % and 21 % of added N at loadings of 62 mg $m^{-2}$ $d^{-1}$ and 249 mg $m^{-2}$ $d^{-1}$, respectively, while the remaining N was either stored in the ~~soil~~ sediment or lost to the atmosphere through coupled nitrification/denitrification~~denitrification and/or anammox~~. Furthermore, this study also shows that N removal efficiency of macrophytes strongly depends on plant species involved.

### *~~5.1.~~4.1. Growth and nutrient uptake of macrophyte species in constructed wetlands*

With average biomass production rates of 3.4 and 1.0 g DW $m^{-2}$ $d^{-1}$, respectively, *A. filiculoides* and *M. spicatum* showed the highest growth rates, regardless of sediment type~~s~~ and nutrient loading~~s~~, and therefore have the best potential for being used to remove nutrients in constructed wetlands. Due to their high growth rates, these species could be harvested biweekly or even weekly. *C. demersum,* on the other hand, appeared to be less suitable, since this species was easily ~~readily~~ outcompeted for light by other species, such as floating algae and *Zanichellia spp*. P was removed most efficiently by *A. filiculoides,* followed by *M. spicatum and C. demersum*. Although a high P load (85.7 mg $m^{-2}$ $d^{-1}$) resulted in increased uptake rates of 6 to 14 and even 16 to 20 mg P $m^{-2}$ $d^{-1}$ for *M. spicatum* and *A. filiculoides*, respectively, these rates were not sufficient to efficiently filter all added P from the system.

~~DFor *C. demersum*, nutrient sequestration rates increased linearly with increased nutrient loading, while for *M. spicatum* there was a logistic response to external nutrient loading (Fig. 6). *A. filiculoides* showed linearly increasing P sequestration rates upon increased P loading and a logistic response to external N loading. These d~~Different response types between species to external nutrient loading ~~between species~~ most likely resulted from differences in main nutrient sources and nutrient limitation (Fig. 6). For rooted *M. spicatum,* plants mainly rely on ~~soil~~ sediment uptake (Best & Mantai, 1978; Barko & Smart, 1980; Carignan & Kalff, 1980), whereas for non-rooted *A. filiculoides* and *C. demersum* water is the main nutrient source (Denny, 1987; Mjelde & Faafeng, 1997). Our results indicate that at a low nutrient loading *M. spicatum* and *A. filiculoides* performed equally well for P removal ~~at loads ≤~~ ~~low loadings~~ ~~22 mg P $m^{-2}$ $d^{-1}$, *M. spicatum* is the most efficient P remover,~~ whereas at loads ≥ 22 mg P $m^{-2}$ $d^{-1}$, *A. filiculoides* ~~is more efficient~~removes P more efficiently (Fig. 6a). In addition, the effective thresholds for P purification (100 % removal) of *C. demersum, A. filiculoides*, and *M. spicatum* are 1.9, 4.8 and 6.8 mg P $m^{-2}$ $d^{-1}$, respectively (Fig 6a). Threshold values for complete N removal are 8.6 and 31.4 mg N $m^{-2}$ $d^{-1}$ for *C. demersum* and *M. spicatum*, respectively (Fig. 6b). *A. filiculoides*, on the other hand, hardly ever becomes N limited due to its symbiosis with a diazotrophic microbial community (Handley & Raven, 1992). Under low external P loadings, *A. filiculoides* therefore displayed very high N: P ratios indicating P limitation at P loadings ≤ 21.4 mg P $m^{-2}$ $d^{-1}$. *C. demersum*, on the other hand, having no access to ~~soil~~ sediment or atmospheric N, probably showed N limitation in these systems, as indicated by their low N: P ratios. For all species, N: P ratios decreased with increasing P load.

### *~~5.2.~~4.2. Using aquatic macrophytes for polishing of pre-treated wastewater*

Due to regular harvesting of *A. filiculoides* and *M. spicatum*, P and N were removed at rates of around 3 to 9 mg P $m^{-2}$ $d^{-1}$ and 31 mg N $m^{-2}$ $d^{-1}$ at loadings of 0.43 mg P $m^{-2}$ $d^{-1}$ and 1.3 mg N $m^{-2}$ $d^{-1}$. These results are comparable to those found by Van Kempen (2013) who found uptake rates of 3.7 mg P $m^{-2}$ $d^{-1}$ (13.4 kg $ha^{-1}$ $year^{-1}$) and 13.7 mg N $m^{-2}$ $d^{-1}$ (50 kg $ha^{-1}$ $year^{-1}$) in summer, and 4.8 mg P $m^{-2}$ $d^{-1}$ (17.5 kg $ha^{-1}$ $year^{-1}$) and 69.3 mg N $m^{-2}$ $d^{-1}$ (253 kg $ha^{-1}$ $year^{-1}$) in early fall for *A. filiculoides* grown in N-free water with ~~25~~ 2.38 ~~µmol~~ mg $L^{-1}$ $PO_4$. For *M. spicatum*, our results are in the same range as those reported by Smith and Adams (1986) and N uptake rates of 0.05-1.26 g N $m^{-2}$ $d^{-1}$ by *Myriophyllum aquaticum* reported by Nuttall (1985). ~~Due to~~ As low $O_2$ concentrations, induced by the coverage of floating macrophytes or dense growth of submerged macrophytes, can mobilize P from the sediment, *A. filiculoides* and *M. spicatum* did ~~lowering of the $O_2$ concentration in the water layer, similar to other floating or densely growing submerged macrophytes (Caraco et al., 2006), these plants~~ not only take up all P being discharged into the system by both their roots and shoots, but additionally ~~mobilize and~~ t~~ake~~ook up mobilized P ~~from the soil by their roots and the creation of anaerobic conditions~~ (Wetzel, 2001).

Since uptake of nutrients by aquatic macrophytes depends on their biomass production and thus on macrophyte photosynthesis, these systems would only function optimally during the growing season

(Wetzel, 2001). Under low external loading, sediments will take up most of the P during winter. Since submerged plants have N and P accumulation rates that are higher than the low nutrient loading, they heavily rely on uptake of nutrient~~-~~s from the sediment. Thus, the nutrients stored in the sediment in winter can be mobilised and taken up by macrophytes in summer, creating an efficient and sustainable constructed wetland for water polishing in temperate climates. Furthermore, predicted climate change will lead to higher temperatures and thus longer growing seasons in temperate regions, indicating that these systems may be operational longer and longer every year.

~~Under low external loading, soils sediments will take up most of the P during winter, which can subsequently be mobilised and taken up by macrophytes in summer, creating an efficient and sustainable constructed wetland for water polishing in temperate climates. In addition, an increase in temperature induced by climate change could potentiallycan can contribute to the increase in nutrient uptake rates of plants during the growing season.~~

### ~~5.3.~~4.3. *Using aquatic macrophytes for wastewater purification*

When P loading in the treatment water increases, uptake rates of *A. filiculoides* double or even triple, to rates ~~around 6~~7.87 or 17.64~~-24~~ mg P $m^{-2}$ $d^{-1}$. The highest value is ~~comparable to~~lower than results of Reddy and DeBusk (1985), who reported P uptake rates of 43 ± 15 mg P $m^{-2}$ $d^{-1}$ by *A. filiculoides* grown in an N-free, 3 mg $L^{-1}$ ~~$PO_4^{3-}$~~P- medium which, however, had ~~has~~ much higher $PO_4^{3-}$ concentrations in the surface water ~~than our concentrations~~than our high nutrient loading treatment. ~~Although~~ P uptake rates of *A. filiculoides* in this study are similar to, or even lower than, results of Brix (1994), who reported P uptake rates of 8 - 41 mg P $m^{-2}$ $d^{-1}$ by emergent macrophytes The main advantage of using floating macrophytes instead of emergent macrophytes is, however, that they can be harvested multiple times a year and that they take up nutrients from both the water layer and sediment. ~~, floating macrophytes are still more suitable for wastewater purification than emergent macrophytes, due to the fact that they are easy to harvest and need less maintenance.~~ Although plants could not take up all P at medium or high external P loadings, overall surface water quality remained around or below ~~12~~ 0.37 ~~μmol~~ mg $L^{-1}$ when clay sediments were used for the construction of the wetland. At the end of the growing season, however, plant uptake decreased and P availability in surface waters above peaty clay and peat ~~soils~~ sediments increased strongly to concentrations around ~~60~~ 1.86 and ~~72~~ 2.23 ~~μmol~~ mg P $L^{-1}$, respectively, indicating not only inactivity of aquatic macrophytes but probably also P saturation of ~~soils~~sediments. Due to the 7-8 times higher Fe and Al contents (~~400~~ 22.6 vs. 2.6-~~50~~3.3-~~60~~ ~~mmol~~ g $L^{-1}$ FW, ~~450~~ 11.9 vs. ~~60~~1.5-~~70~~ 1.8 ~~mmol~~ g $L^{-1}$ FW for Fe and Al, respectively) of clay ~~soils~~sediments, P was most probably immobilized more efficiently by clay (Reddy & DeLaune, 2008), which resulted in lower P concentrations in surface water above clay ~~soils~~sediments in our study.

More than 98 % of added N was removed from the surface water during the run of the experiment. As nutrient loading increased, the amount of added N that was removed by plant uptake decreased. Harvested biomass of target plants contained 31 mg N $m^{-2}$ $d^{-1}$ for *M. spicatum*, whereas in the quarters with *C. demersum*, non-target macrophytes or algae ~~absorbed~~ sequestrated most N. ~~For *A. filiculoides* it was difficult to calculate N removal rates due to unknown $N_2$ fixation rates leading to an overestimation of N uptake rates by *A. filiculoides*~~Although it can be estimated that $N_2$-fixation rates by *Azolla* grown in an N-free medium were in the range of 1.4 - 2.7 kg N $ha^{-1}$ $d^{1}$ (Reddy & DeBusk, 1985), in our study we added N to the surface water which may affect $N_2$ fixation. Therefore it was difficult to calculate N removal rates for *A. filiculoides*, as the unknown $N_2$ fixation rates lead to an overestimation of N uptake rates by *A. filiculoides*. ~~.~~ N that was not taken up by plants, but was still removed from the water layer most likely ended up in the ~~soil~~ sediment or was released to the atmosphere by coupled nitrification/denitrification (Wetzel, 2001)~~and/or anammox~~. On average, inorganic N ($NH_4^+$+$NO_3^-$) concentrations in the surface water were below ~~8~~ 0.11 ~~μmol~~ mg $L^{-1}$ with external loadings ≤ 62 mg N $m^{-2}$ $d^{-1}$ and around ~~20~~ 0.28 ~~μmol~~ mg $L^{-1}$ when receiving 249 mg N $m^{-2}$ $d^{-1}$. At the end of the growing season, dissolved N concentrations increased under high nutrient loading,~~;~~ similar to P concentrations~~,~~. ~~suggesting~~ This increase may result from a combination of reduced plant uptake, ~~lower denitrification rates. This suggests that~~ nutrient leaching from senescing plants and reduced denitrification rates as a result of lower temperatures ~~is more important than soil sediment saturation~~. Due to the different available pathways for nitrogen removal from the sediment, sediment saturation of N seems unlikely.

### ~~5.4.~~4.4. *Implications for management*

We showed that in macrophytes-dominated CWS, both the submerged ~~or~~ and the floating macrophytes we tested are able to remove most of the added nutrients at low P and N loadings, whereas at higher nutrient loadings, floating or submerged macrophytes ~~can~~ could only remove 20-45 % and 10-25 % of the external P loads for 21.4 and 85.7 mg P m$^{-2}$ d$^{-1}$, respectively. For water management~~, using fast growing aquatic macrophytes, such as *A. filliculoides* or *M. spicatum*~~ regular mowing of ~~-~~fast growing aquatic macrophytes, such as *A. filliculoides* or *M. spicatum* allows complete removal of added nutrients at relatively low nutrient loading (≤ 4.8 mg P m$^{-2}$ d$^{-1}$ or ≤ 6.8 mg P m$^{-2}$ d$^{-1}$, respectively). Although *A. filiculoides* still extracted P and competed with ~~soil~~ sediment adsorption at higher P loads (≥ 21.4 mg P m$^{-2}$ d$^{-1}$), most external P ended up in the ~~soil~~sediment, eventually resulting in saturated sediments and thus leading to an increase in water nutrient levels under a continued nutrient input~~saturation~~. While aquatic macrophytes are able to remove this P from the ~~soils~~ sediments by either creating anaerobic conditions to trigger high P mobilization (Smolders et al., 2006) or through both root and shoot uptake, the external load will have to be reduced for this process to occur efficiently. Consequently, at these higher P and N loads, the macrophyte stage can only be used as an additional polishing step after a major part of the nutrients have been removed by other ways of water treatment.

## ~~6.~~5. Conclusions

Here, we show that aquatic macrophytes can be used for polishing, but not as a stand-alone purification treatment for nutrient removal from wastewater. ~~At loads ≤ 22 mg P m$^{-2}$ d$^{-1}$,~~ At a low nutrient loading *M. spicatum* and *A. filiculoides* performed equally well for P removal~~. is the best~~

~~option,~~ whereas at loads ≥ 22 mg P $m^{-2}$ $d^{-1}$, *A. filiculoides* removes P more efficiently. Furthermore, we have shown that ~~soil~~ sediment type is a previously underestimated factor influencing the efficiency of nutrient removal and immobilization. Especially at higher P loads, ~~soils~~ sediments form highly important sinks and the saturation potential of the ~~soil~~ sediment is therefore important. Clay ~~soils~~ sediments should be preferred, as these take longer to become saturated than more organic ~~soils~~sediments.


## Acknowledgements

The authors would like to thank Moni Poelen and Peter Cruijsen for their practical assistance and Ankie De Vries-Brock, Germa Verheggen, Jelle Eygensteyn, Paul Van der Ven, Roy Peters and Sebastian Krosse for their assistance with the chemical analyses. SFH, MMLvK and LMJML were funded by the European Union, GO EFRO 2007-2013 (Water-Rijk, Rich Water World). YT was funded by the China Scholarship Council (CSC; file number 201206140019).


## Author Contributions

Conceived and designed the experiment: J.G.M.R., A.J.P.S., L.P.M.L. and M.M.L.V.K.; Performed the experiment: E.J.H.V., L.M.J.M.L. and M.M.L.V.K.; Analysed the data: S.F.H., Y.T. and E.J.H.V.; Wrote the paper: S.F.H., Y.T., A.J.P.S., L.P.M.L. and M.M.L.V.K.

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Table 1 ~~Soil~~ Sediment characteristics of peat, peaty clay and clay ~~soils~~ sediments used in the experiment (±SE; n=36). pH and Total inorganic carbon (TIC) are derived from porewater analyses, whereas all other analyses were performed using fresh or dry ~~soil~~ sediment (see Sect. 2.3.).

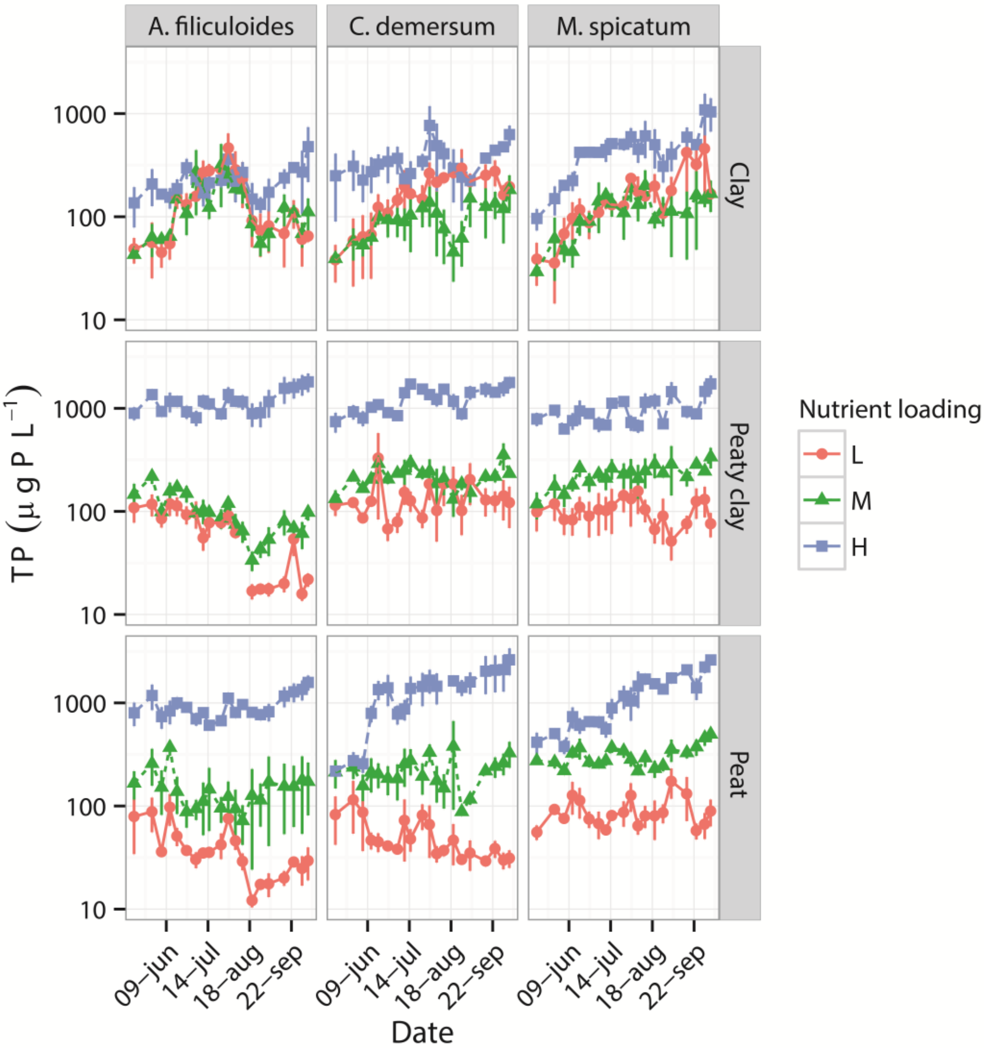
| Sediment ~~Soil~~ | Bulk density (kg DW.L$^{-1}$ FW) | Organic matter % | pH | TIC (~~µmol~~ mg C L$^{-1}$) | Salt extractable N ($NO_3^-$+ $NH_4^+$) (~~µmol~~ mg N L$^{-1}$ FW) | Olsen-P (~~umol~~ mg L$^{-1}$ FW) | Total-P (~~mmol~~ mg L$^{-1}$ FW) | Total-Fe (~~mmol~~ g L$^{-1}$ FW) | Total-Al (~~mmol~~ g L$^{-1}$ FW) | Total-Ca (~~mmol~~ g L$^{-1}$ FW) |
|---|---|---|---|---|---|---|---|---|---|---|
| Peat | 0.15 (0.00)$^{C}$ | 43.73 (0.80)$^{A}$ | 7.20 (0.02)$^{A}$ | ~~8825~~105.~~84~~91 (~~120~~1.~~3~~~~6~~44)$^{A}$ | ~~551~~7.72 (~~58~~0.~~71~~82)$^{B}$ | ~~269~~8.35~~41~~ (~~13~~0.~~16~~41)$^{B}$ | ~~4~~154.~~98~~38 (~~0~~5.~~19~~89)$^{B}$ | ~~47~~2.~~15~~64 (0.~~92~~05)$^{B}$ | ~~55~~1.~~4~~~~3~~50 (~~1~~0.~~80~~05)$^{B}$ | ~~65~~2.6~~05~~ (~~1~~0.~~06~~04)$^{B}$ |
| Peaty clay | 0.23 (0.01)$^{B}$ | 34.39 (1.63)$^{B}$ | 6.92 (0.03)$^{B}$ | ~~5892~~70.~~89~~71 (~~240~~2.~~5~~~~6~~89)$^{B}$ | ~~494~~6.~~11~~92 (~~70~~0.~~17~~98)$^{B}$ | ~~153~~4.~~90~~77 (~~13~~0.~~98~~43)$^{C}$ | ~~3~~105.~~39~~09 (~~0~~5.~~19~~89)$^{C}$ | ~~58~~3.~~72~~29 (~~4~~0.~~32~~24)$^{B}$ | ~~67~~1.~~8~~~~4~~83 (~~5~~0.~~37~~14)$^{B}$ | ~~62~~2.~~1~~~~4~~49 (~~5~~0.~~02~~20)$^{B}$ |
| Clay | 1.00 (0.01)$^{A}$ | 5.07 (0.24)$^{C}$ | 7.18 (0.04)$^{A}$ | ~~10189~~122.~~53~~27 (~~537~~6.~~6~~~~7~~45)$^{A}$ | ~~1063~~14.~~66~~89 (~~123~~1.~~98~~74)$^{A}$ | ~~1104~~34.24~~4~~~~8~~ (~~18~~0.~~69~~58)$^{A}$ | ~~22~~689.~~25~~75 (~~0~~12.~~41~~71)$^{A}$ | ~~402~~22.~~74~~55 (~~5~~0.~~26~~29)$^{A}$ | ~~438~~11.~~77~~85 (~~8~~0.~~05~~22)$^{A}$ | ~~101~~4.~~85~~07 (~~1~~0.~~31~~05)$^{A}$ |

Significant differences among ~~soil~~ sediment types are indicated by different capital letters (A, B and C).

Table 2 Plant tissue ratios between N and P for different macrophytes subjected to different nutrient loadings (0.43, 21.4 and 85.7 mg P $m^{-2}$ $d^{-1}$) at the end of the experiment. Average N: P ratios of target species are given with standard error.

| Species | Soil type | N : P (~~mol~~ g : ~~mol~~g) | | |
|---|---|---|---|---|
| | | 0.43 | 21.4 | 85.7 |
| | Clay | ~~34~~15.~~77~~ 70 (±~~1~~0.~~03~~47)[a] | ~~42~~19.~~88~~ 36 (±1.~~12~~86)[a] | ~~17~~8.~~87~~ 07 (±~~1~~0.~~28~~58)[b] |
| *A. filiculoides* | Peaty clay | ~~49~~22.~~21~~ 22 (±~~3~~1.~~66~~65)[a] | ~~24~~10.~~10~~ 88 (±0.~~64~~29)[b] | ~~11~~5.~~23~~ 07 (±0.~~32~~14)[c] |
| | Peat | ~~41~~18.94 (±0.~~23~~10)[a] | ~~24~~10.~~17~~ 92 (±~~1~~0.~~95~~88)[b] | ~~12~~5.~~84~~ 80 (±0.~~75~~34)[c] |
| | Clay | ~~8~~4.~~92~~ 03 (±~~1~~0.~~36~~61) | ~~9~~4.~~16~~ 14 (±~~1~~0.~~12~~50) | NA |
| *C. demersum* | Peaty clay | ~~9~~4.~~33~~ 21 (±0.~~97~~44) | ~~9~~4.~~04~~ 08 (±~~1~~0.~~59~~72) | ~~8~~3.~~04~~ 63 (±0.~~84~~38) |
| | Peat | ~~16~~7.66~~9~~ 5(±~~4~~1.~~29~~94)[a] | ~~9~~4.~~43~~ 26 (±0.~~69~~31)[ab] | ~~7~~3.~~52~~ 40 (±0.~~93~~42)[b] |
| | Clay | ~~10~~4.71~~43~~ (±~~1~~0.~~39~~63) | ~~9~~4.~~80~~ 42 (±0.~~53~~24) | ~~9~~4.~~22~~ 16 (±~~1~~0.87~~92~~) |
| *M. spicatum* | Peaty clay | ~~13~~6.~~31~~ 01 (±~~1~~0.~~80~~81)[a] | ~~10~~4.~~24~~ 63 (±0.~~56~~25)[ab] | ~~8~~3.~~40~~ 80 (±0.~~74~~34)[b] |
| | Peat | ~~10~~4.~~14~~ 58 (±~~1~~0.~~18~~53) | ~~9~~4.~~66~~ 36 (±0.~~38~~17) | ~~8~~3.~~34~~ 77 (±0.~~78~~35) |

Significant differences among different nutrient loadings are indicted by different lower case letters (a, b and c); there were no significant differences among ~~soil~~ sediment types. Note that NA means that there were no replicates for this treatment.



A. filiculoides
C. demersum
M. spicatum
Clay
Peaty clay
Peat
TP (µg P L⁻¹)
1000
100
10
09–jun
14–jul
18–aug
22–sep
Date
Nutrient loading
L
M
H

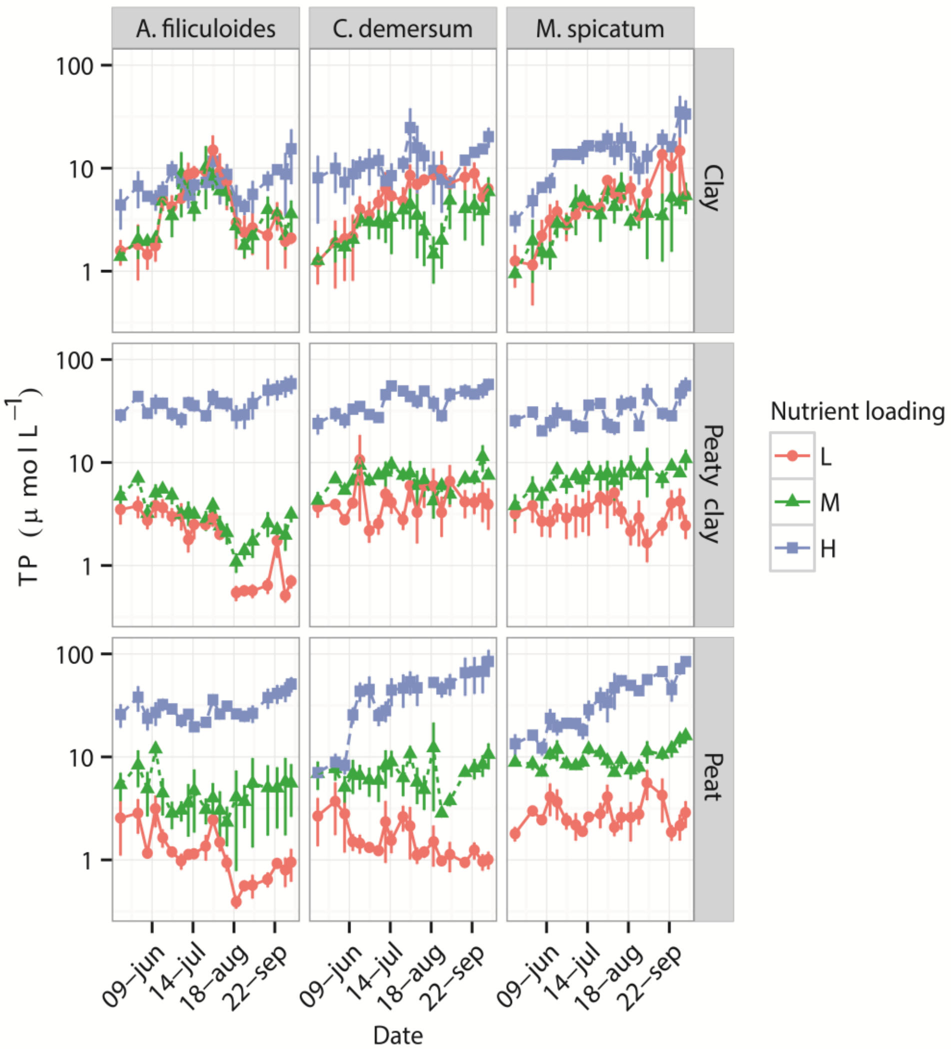
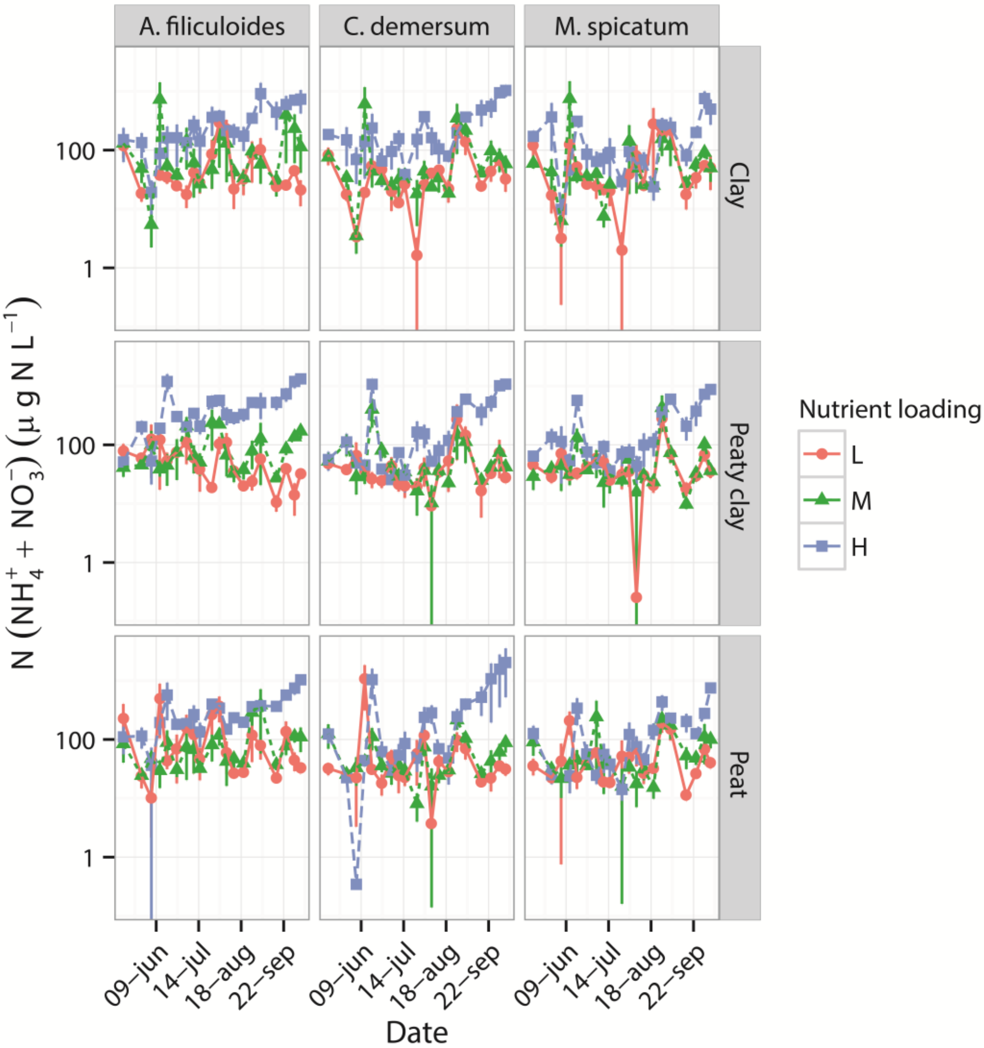
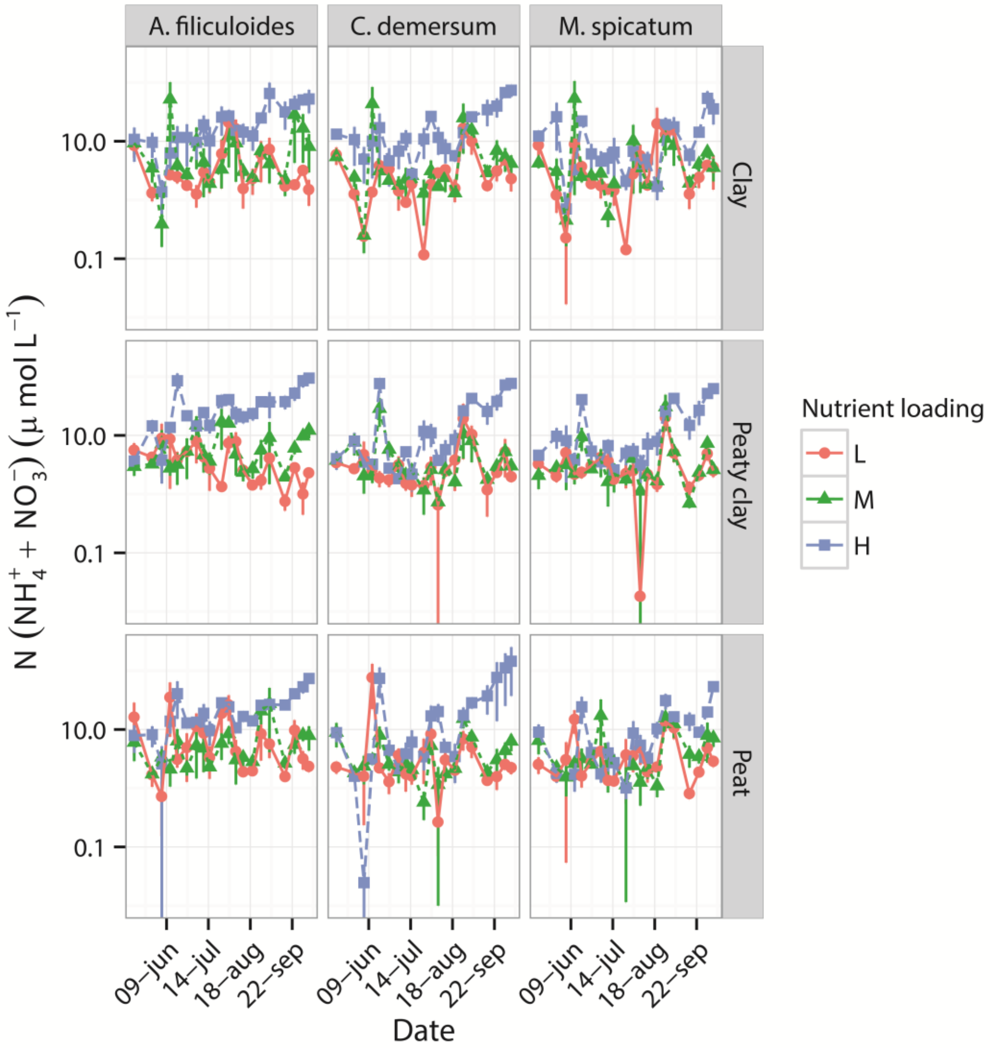



Figure 1. Surface water TP concentrations subjected to different nutrient loadings (L = 0.43 mg P $m^{-2}$ $d^{-1}$; M = 21.4 mg P $m^{-2}$ $d^{-1}$; H = 85.7 mg P $m^{-2}$ $d^{-1}$) in mesocosms with different plant species (vertical panels) on clay, peaty clay or peat ~~soils~~ sediments (horizontal panels) during the experiment. Average TP concentrations are given with SEM. Note the $log_{10}$ scale for the y-axis.



A. filiculoides
C. demersum
M. spicatum
Clay
Peaty clay
Peat
100
1
N $(NH_4^+ + NO_3^-)$ ($\mu$ g N L$^{-1}$)
09–jun
14–jul
18–aug
22–sep
Date
Nutrient loading
L
M
H


1280




~~Figure~~ 2. Surface water N ($NH_4^+$+$NO_3^-$) concentrations subjected to different nutrient loadings (L = 0.43 mg P $m^{-2}$ $d^{-1}$; M = 21.4 mg P $m^{-2}$ $d^{-1}$; H = 85.7 mg P $m^{-2}$ $d^{-1}$) in mesocosms with different plant species (vertical panels) on clay, peaty clay or peat ~~soils~~ sediments (horizontal panels) during the experiment. Average N ($NH_4^+$+$NO_3^-$) concentrations are given with SEM. Note the $\log_{10}$ scale for the y-axis.

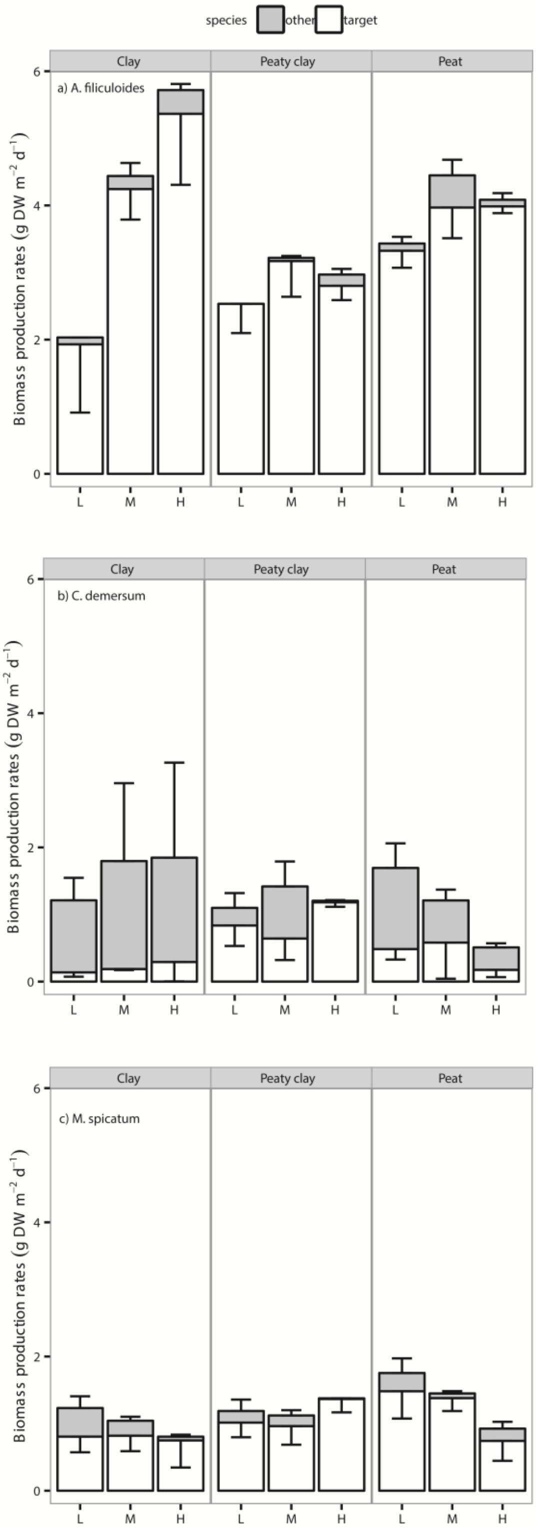
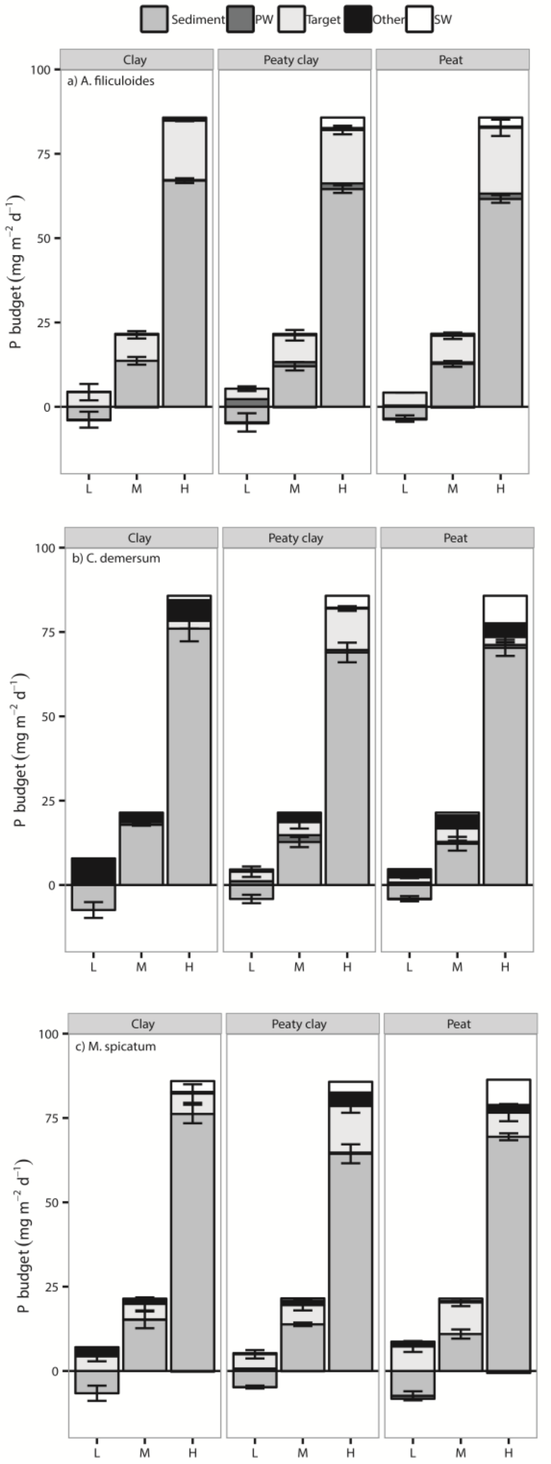



Figure 3. Biomass production rates (in g DW $m^{-2}$ $d^{-1}$) of *A. filiculoides* (a), *C. demersum* (b), *M. spicatum* (c) and other, non-target plants (e.g. floating algae, *Zanichellia* spp and other plants) grown on different ~~soil~~ sediment types and subjected to different nutrient loadings (L = 0.43 mg P $m^{-2}$ $d^{-1}$; M = 21.4 mg P $m^{-2}$ $d^{-1}$; H = 85.7 mg P $m^{-2}$ $d^{-1}$). Average biomass production rates of target species (-SEM) and other plants (+SEM) are given.



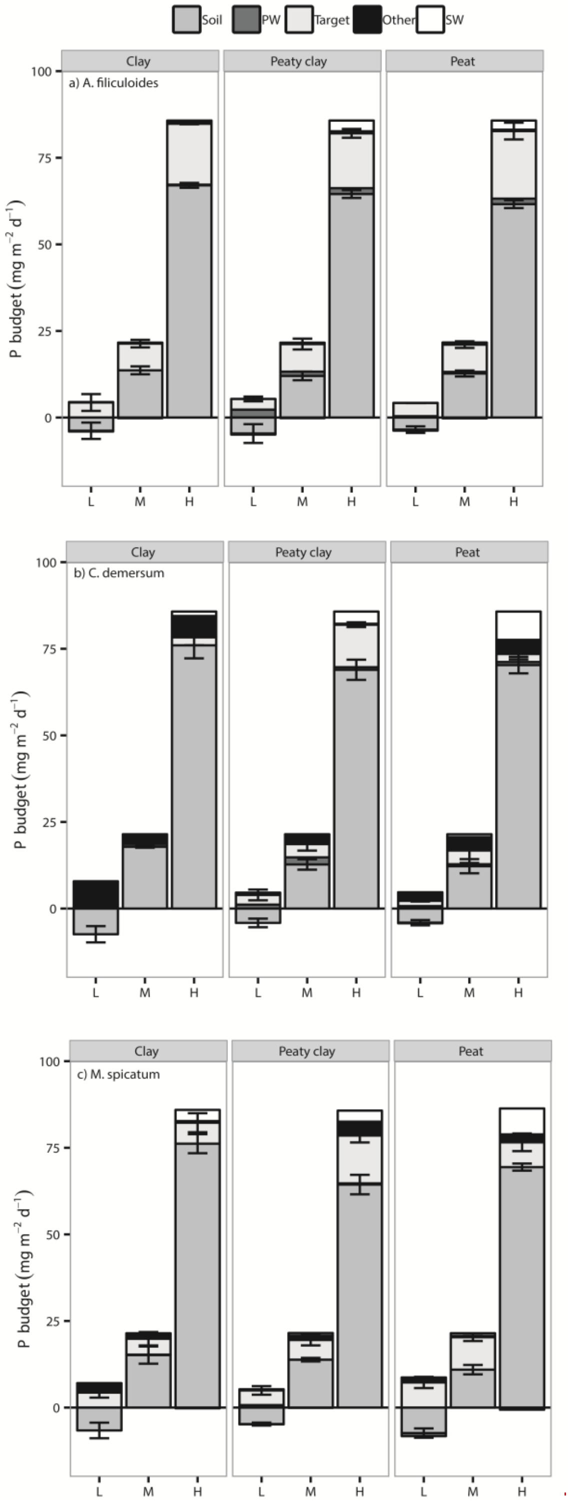




Figure 4. P budgets of ~~soil~~sediment, surface water, pore water, target species and other plants subjected to different nutrient loadings (L = 0.43 mg P m$^{-2}$ d$^{-1}$; M = 21.4 mg P m$^{-2}$ d$^{-1}$; H = 85.7 mg P m$^{-2}$ d$^{-1}$) for (a) *A. filiculoides*, (b) *C. demersum*,

and (c) *M. spicatum*. Standard errors are given only for ~~soil~~ sediment and target species. PW = pore water, SW = surface water. Positive values represent P accumulation in relative parts; negative values represent P release from respective compartments.

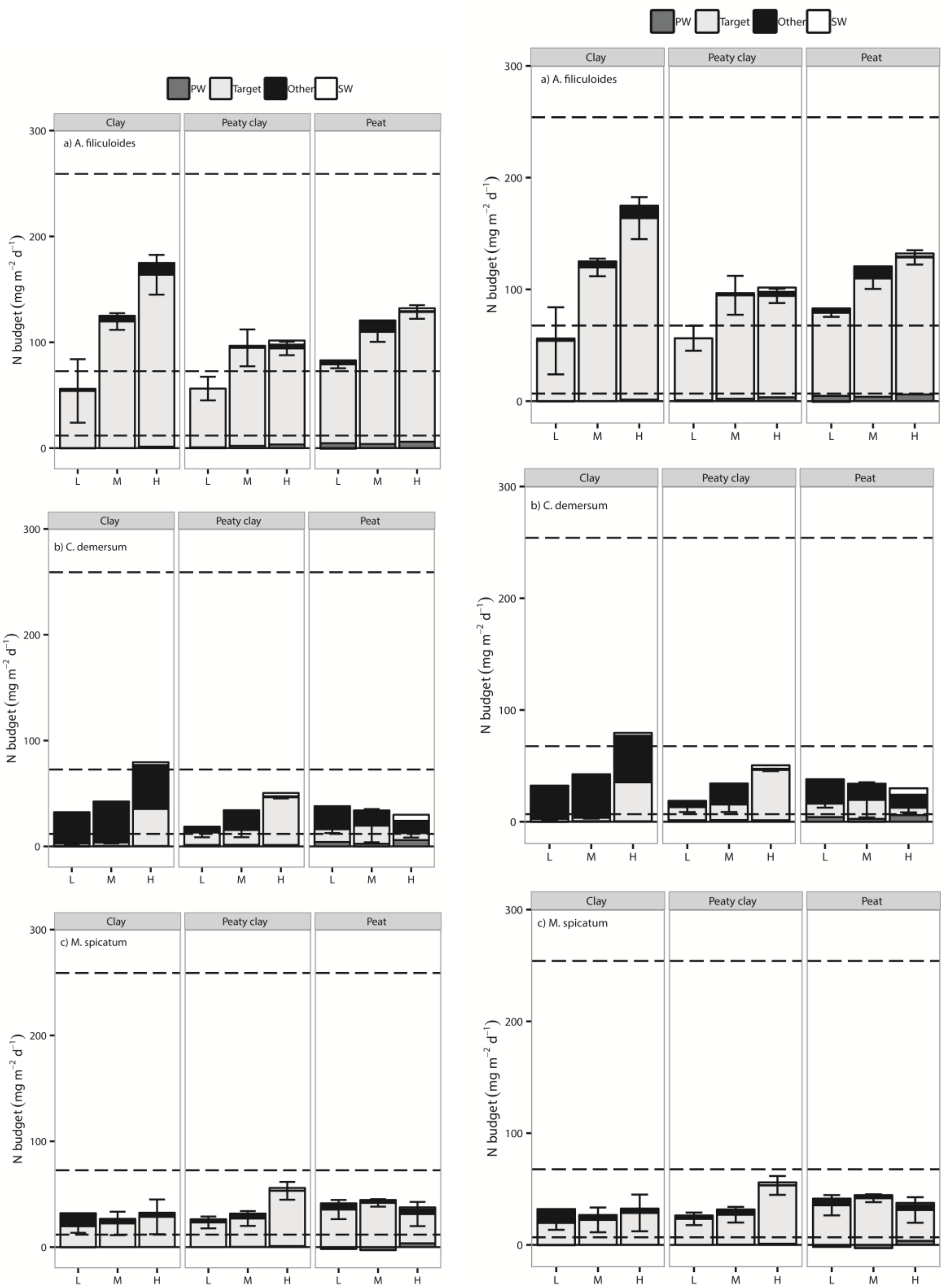


Figure 5. N distribution in surface water, pore water, target species and other plants subjected to different nutrient loadings (L = 0.43 mg P $m^{-2}$ $d^{-1}$; M = 21.4 mg P $m^{-2}$ $d^{-1}$; H = 85.7 mg P $m^{-2}$ $d^{-1}$) from (a) *A. filiculoides*, (b) *C. demersum* and (c) *M. spicatum* macrophyte systems. Standard errors are given only for target plants. PW = pore water, SW = surface water. Positive values represent N accumulation in relative parts; negative values represent N release from respective compartments. The lowest, medium and highest dashed lines represent external N input at low, medium and high N loadings (including actual atmospheric N deposition), respectively.

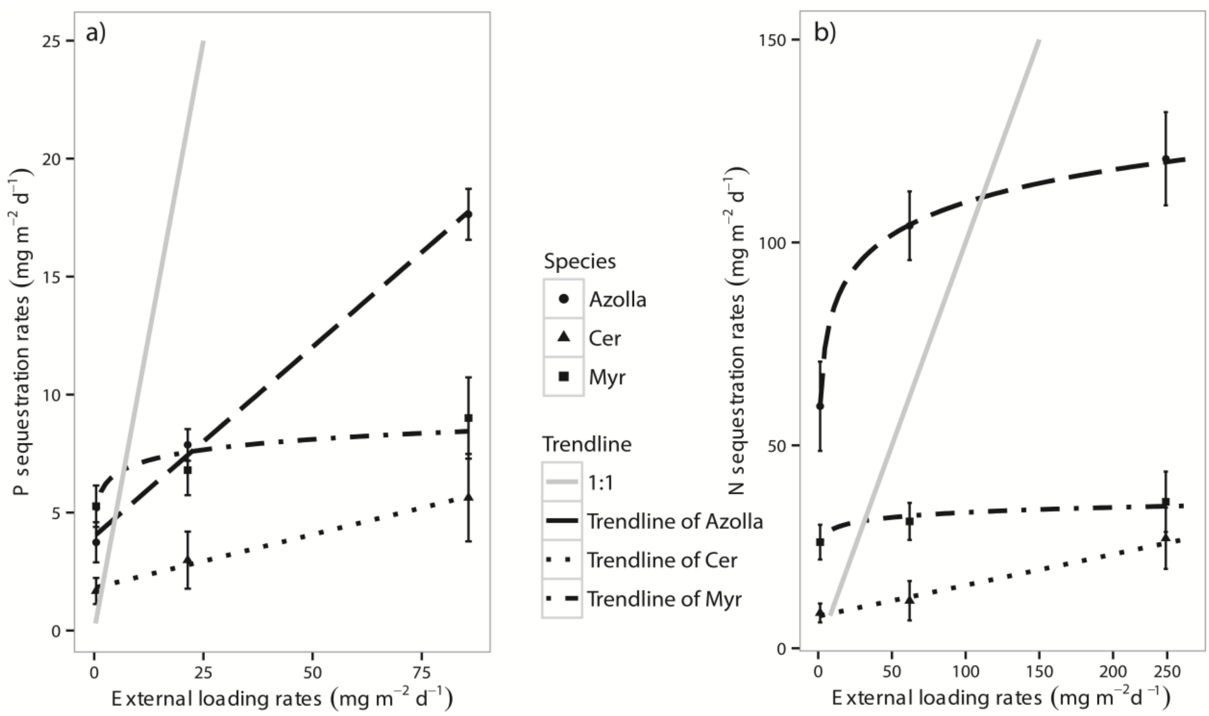


Figure 6. The correlations between external loading and nutrient sequestration rates of P (a) and N (b) by three different aquatic plant species. Standard errors and 1:1 line are given. Note that for *A. filiculoides* $N_2$ fixation is included in the sequestration rates, overestimating the effects of loading.

## Supplementary information

Table S1 Overview of the statistical output from the analyses of the data presented in Figures 1, 2, 3, 4 and 5.

Surface water TP (Figure 1; ANOVA)

| Targeted plants | ~~Soil~~ Sediment types | Time | | | Nutrient loading | | | Time*nutrient loading | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | df | $X^2$ | *P* | df | $X^2$ | *P* | df | $X^2$ | *P* |
| | Clay | 1 | 2.~~57~~06 | 0.~~11~~15 | 2 | ~~1~~2.~~82~~38 | 0.~~40~~30 | 2 | ~~1~~0.98~~44~~ | 0.~~49~~61 |
| *A. filiculoides* | Peaty clay | 1 | ~~8~~4.~~38~~34 | **0.~~00~~04** | 2 | ~~110~~77.~~10~~04 | **0.00** | 2 | ~~80~~89.~~57~~84 | **0.00** |
| | Peat | 1 | ~~7.2~~34.17 | **0.~~01~~04** | 2 | ~~46~~37.~~75~~72 | **0.00** | 2 | ~~25~~30.~~61~~64 | **0.00** |
| | Clay | 1 | ~~12~~10.~~47~~44 | **0.00** | 2 | 12.~~03~~82 | **0.00** | 2 | ~~9~~12.~~32~~87 | **0.~~01~~00** |
| *C. demersum* | Peaty clay | 1 | ~~19~~14.~~35~~20 | **0.00** | 2 | ~~54~~38.~~08~~70 | **0.00** | 2 | ~~9~~8.~~77~~30 | **0.~~01~~02** |
| | Peat | 1 | ~~86~~67.~~75~~32 | **0.00** | 2 | ~~31~~33.~~01~~42 | **0.00** | 2 | ~~76~~71.~~92~~56 | **0.00** |
| | Clay | 1 | ~~46~~33.~~98~~34 | **0.00** | 2 | ~~12~~14.70~~51~~ | **0.00** | 2 | ~~6~~5.~~78~~63 | 0.~~03~~06 |
| *M. spicatum* | Peaty clay | 1 | ~~12~~9.~~59~~03 | **0.00** | 2 | ~~65~~54.~~62~~38 | **0.00** | 2 | ~~10~~9.~~69~~12 | **0.~~00~~01** |
| | Peat | 1 | ~~156~~117.~~93~~02 | **0.00** | 2 | ~~97~~101.~~83~~51 | **0.00** | 2 | ~~85~~64.34~~62~~ | **0.00** |

Surface water N ($NH_4^+$+$NO_3^-$) (Figure 2; ANOVA)

| Targeted plants | ~~Soil~~ Sediment types | Time | | | Nutrient loading | | | Time*nutrient loading | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | df | $X^2$ | *P* | df | $X^2$ | *P* | df | $X^2$ | *P* |
| | Clay | 1 | ~~35~~24.~~44~~68 | **0.00** | 2 | 0.~~22~~75 | 0.69~~90~~ | 2 | ~~17~~9.~~97~~32 | **0.~~00~~01** |
| *A. filiculoides* | Peaty clay | 1 | ~~41~~31.~~43~~10 | **0.00** | 2 | 5.~~24~~12 | 0.~~07~~08 | 2 | ~~43~~37.~~60~~41 | **0.00** |
| | Peat | 1 | ~~34~~26.~~19~~74 | **0.00** | 2 | ~~2~~1.~~58~~34 | 0.~~28~~51 | 2 | ~~21~~14.~~31~~04 | **0.00** |
| | Clay | 1 | ~~31~~19.~~07~~69 | **0.00** | 2 | ~~2~~5.~~75~~20 | 0.~~25~~07 | 2 | ~~9~~3.78 | 0.~~01~~15 |
| *C. demersum* | Peaty clay | 1 | ~~47~~29.~~02~~23 | **0.00** | 2 | ~~2~~0.~~11~~72 | 0.~~35~~70 | 2 | ~~33~~21.~~65~~03 | **0.00** |
| | Peat | 1 | ~~43~~35.~~71~~70 | **0.00** | 2 | 2.~~99~~51 | 0.~~22~~28 | 2 | ~~29~~22.~~46~~01 | **0.00** |
| | Clay | 1 | ~~7~~5.~~88~~26 | **0.~~00~~02** | 2 | ~~1~~2.~~43~~68 | 0.~~49~~26 | 2 | ~~1~~0.~~81~~46 | 0.~~40~~80 |
| *M. spicatum* | Peaty clay | 1 | ~~29~~18.~~16~~72 | **0.00** | 2 | 0.~~16~~47 | 0.~~92~~79 | 2 | ~~16~~10.~~11~~56 | **0.~~00~~01** |
| | Peat | 1 | ~~39~~32.~~52~~80 | **0.00** | 2 | 2.~~41~~44 | 0.30 | 2 | ~~17~~12.~~10~~90 | **0.00** |

Biomass production rates (Figure 3; ANOVA)

| Targeted plants | Position | ~~Soil~~ Sediment type | | | Nutrient loading | | | ~~Soil~~ Sediment type*nutrient loading | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | df | $X^2$ | *P* | df | $X^2$ | *P* | df | $X^2$ | *P* |
| *A. filiculoides* | *A. filiculoides* | 2 | 2.88 | 0.24 | 2 | 11.39 | **0.00** | 4 | 5.65 | 0.23 |
| | Others | 2 | 1.48 | 0.48 | 2 | 1.15 | 0.56 | 4 | 4.23 | 0.38 |
| *C. demersum* | *C. demersum* | 2 | 10.67 | **0.00** | 2 | 0.16 | 0.92 | 4 | 3.89 | 0.42 |
| | Others | 2 | 2.89 | 0.24 | 2 | 0.12 | 0.94 | 4 | 1.60 | 0.81 |
| *M. spicatum* | *M. spicatum* | 2 | 3.63 | 0.16 | 2 | 0.16 | 0.93 | 4 | 4.45 | 0.35 |
| | Others | 2 | 1.62 | 0.45 | 2 | 5.41 | 0.07 | 4 | 3.34 | 0.50 |

P budget (Figure 4; ANOVA)

| Targeted plants | Position | ~~Soil~~ Sediment type | | | Nutrient loading | | | ~~Soil~~ Sediment type*nutrient loading | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | df | $X^2$ | *P* | df | $X^2$ | *P* | df | $X^2$ | *P* |
| *A. filiculoides* | Surface water | 2 | 2.26 | 0.32 | 2 | 0.35 | 0.84 | 4 | 4.00 | 0.41 |
| | Other plants | 2 | 0.53 | 0.77 | 2 | 1.38 | 0.50 | 4 | 2.78 | 0.60 |
| | Targeted plants | 2 | 0.25 | 0.88 | 2 | 13.84 | **0.00** | 4 | 0.20 | 1.00 |
| | Pore water | 2 | 1.07 | 0.58 | 2 | 0.14 | 0.93 | 4 | 0.20 | 1.00 |
| | Soils | 2 | 4.66 | 0.10 | 2 | 792.84 | **0.00** | 4 | 3.14 | 0.54 |
| *C. demersum* | Surface water | 2 | 21.05 | **0.00** | 2 | 1.17 | 0.56 | 4 | 23.61 | **0.00** |
| | Other plants | 2 | 6.92 | **0.03** | 2 | 1.28 | 0.53 | 4 | 3.77 | 0.44 |
| | Targeted plants | 2 | 10.50 | **0.01** | 2 | 0.13 | 0.94 | 4 | 4.04 | 0.40 |
| | Pore water | 2 | 0.88 | 0.64 | 2 | 0.04 | 0.98 | 4 | 1.58 | 0.81 |
| | Soils | 2 | 5.67 | 0.06 | 2 | 728.39 | **0.00** | 4 | 7.47 | 0.11 |
| *M. spicatum* | Surface water | 2 | 11.57 | **0.00** | 2 | 18.76 | **0.00** | 4 | 10.30 | **0.04** |
| | Other plants | 2 | 1.58 | 0.45 | 2 | 1.87 | 0.39 | 4 | 4.81 | 0.31 |
| | Targeted plants | 2 | 6.02 | 0.05 | 2 | 0.38 | 0.83 | 4 | 6.57 | 0.16 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pore water | 2 | 1.75 | 0.42 | 2 | 0.06 | 0.97 | 4 | 0.56 | 0.97 |
| | Soils | 2 | 21.52 | **0.00** | 2 | 1109.54 | **0.00** | 4 | 16.35 | **0.00** |

N budget (Figure 5; ANOVA)

| Targeted plants | Position | ~~Soil~~ Sediment type | | | Nutrient loading | | | ~~Soil~~ Sediment type*nutrient loading | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | df | $X^2$ | *P* | df | $X^2$ | *P* | df | $X^2$ | *P* |
| *A. filiculoides* | Other plants | 2 | 2.20 | 0.33 | 2 | 2.01 | 0.37 | 4 | 4.79 | 0.31 |
| | Targeted plants | 2 | 0.49 | 0.78 | 2 | 7.77 | **0.02** | 4 | 2.95 | 0.57 |
| | Pore water | 2 | 10.17 | **0.01** | 2 | 1.03 | 0.60 | 4 | 1.24 | 0.87 |
| | Surface water | 2 | 2.91 | 0.23 | 2 | 3.90 | 0.14 | 4 | 2.97 | 0.56 |
| *C. demersum* | Other plants | 2 | 5.38 | 0.07 | 2 | 0.66 | 0.72 | 4 | 1.28 | 0.86 |
| | Targeted plants | 2 | 10.43 | **0.01** | 2 | 6.28 | **0.04** | 4 | 9.86 | **0.04** |
| | Pore water | 2 | 14.48 | **0.00** | 2 | 0.04 | 0.98 | 4 | 3.58 | 0.47 |
| | Surface water | 2 | 0.37 | 0.83 | 2 | 10.56 | **0.01** | 4 | 0.26 | 0.99 |
| *M. spicatum* | Other plants | 2 | 3.08 | 0.21 | 2 | 4.53 | 0.10 | 4 | 5.75 | 0.22 |
| | Targeted plants | 2 | 4.00 | 0.14 | 2 | 0.06 | 0.97 | 4 | 3.99 | 0.41 |
| | Pore water | 2 | 4.16 | 0.13 | 2 | 0.02 | 0.99 | 4 | 8.92 | 0.06 |
| | Surface water | 2 | 11.51 | **0.00** | 2 | 14.03 | **0.00** | 4 | 5.73 | 0.22 |