

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

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Dear Editor,

We would like to thank you and the reviewers for your time and valuable contributions. We have studied comments carefully and have given a point-to-point response. These comments are all valuable and very helpful for improving our manuscript, as well as the important guiding significance to our researches.

Yours sincerely, On behalf of all authors,

Yingying Tang

C1

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

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this was not clear, so perhaps the authors could change 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.

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#### 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

C4

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?

C5

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<sub>4</sub><sup>3-</sup>) 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-dimensional, would it not be more logical to provide loading in units per litre or per cubic metre?

C6

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,

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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<sub>4</sub><sup>+</sup> < 0.03 mg L<sup>-1</sup>, NO<sub>3</sub><sup>-</sup>: 16.40 mg L<sup>-1</sup>, PO<sub>4</sub><sup>3-</sup> < 0.03 mg L<sup>-1</sup>, pH: 7.7, total inorganic carbon (TIC): 30 mg C L<sup>-1</sup>).

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<sub>3</sub><sup>-</sup> concentrations, this did not influence our results. As we started our experiment one year after filling the basins, denitrification had taken place and NO<sub>3</sub><sup>-</sup> 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<sup>-2</sup> d<sup>-1</sup> (added as NaH<sub>2</sub>PO<sub>4</sub>.H<sub>2</sub>O and atmospheric deposition of 0.1 kg P ha<sup>-1</sup> y<sup>-1</sup>) (Furnas, 2003) and 1.3, 62 and 249 mg N m<sup>-2</sup> d<sup>-1</sup> (added as NH<sub>4</sub>NO<sub>3</sub> and atmospheric N deposition of 35 kg N ha<sup>-1</sup> y<sup>-1</sup> in this part of the Netherlands) (RIVM, 2014).' Atmospheric N deposition of 35 kg N ha<sup>-1</sup> y<sup>-1</sup> (9.59 mg N m<sup>-2</sup> d<sup>-1</sup>) 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.

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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 (National Institute for Public Health and the Environment), "Concentration and deposition maps of the Netherlands"; Total Nitrogen; 2014; <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

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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 re-

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covery in natural and constructed wetlands, including sediment adsorption, phosphate (PO<sub>4</sub><sup>3-</sup>) 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 (PO<sub>4</sub>) 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<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) 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.

C11

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<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) 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

C12

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 ( $X^2=13.77$ ;  $P < 0.01$ ) for surface water P, and between time and nutrient loadings ( $X^2=18.05$ ;  $P < 0.001$ ) for surface water N.'

Line 158: Because you don't give the data, perhaps it's informative if you just provide the mean  $\pm$  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 ( $2=12.07$ ;  $P < 0.01$ ;  $4.65 \pm 0.15$  mg L<sup>-1</sup> and  $0.71 \pm 0.05$  mg L<sup>-1</sup> 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.

C13

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<sub>3</sub> and 1 mL 30 % H<sub>2</sub>O<sub>2</sub>, 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]:

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“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<sup>-2</sup> d<sup>-1</sup>, respectively, *A. filiculoides* and *M. spicatum* showed the highest growth rates, regardless of sediment types and nutrient loadings, 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 O<sub>2</sub> mobilizes PO<sub>4</sub> [...]"

Response

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

C15

‘As low O<sub>2</sub> 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<sub>2</sub> 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

C16

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<sup>-2</sup> d<sup>-1</sup>.'

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  $43 \pm 15$  mg P m<sup>-2</sup> d<sup>-1</sup> by *A. filiculoides* grown in an N-free, 3 mg L<sup>-1</sup> PO<sub>4</sub>-P medium which has much higher PO<sub>4</sub>- concentrations in the surface water than our concentrations.'

C17

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<sub>2</sub>-fixation rates by *Azolla* grown in an N-free medium were in the range of 1.4 - 2.7 kg N ha<sup>-1</sup> d<sup>-1</sup> (Reddy & DeBusk, 1985), in our study we added N to the surface water which may affect N<sub>2</sub> fixation. Therefore it was difficult to calculate N removal rates for *A. filiculoides*, as the unknown N<sub>2</sub> 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 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 will have similar nutrient removal rates.

C18

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<sup>-2</sup> d<sup>-1</sup>, 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 root 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.'

C19

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, L273]:

'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<sup>-2</sup> d<sup>-1</sup> 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  $\geq 22$  mg P m<sup>-2</sup> d<sup>-1</sup>, *A. filiculoides* removes P more efficiently.'

C20

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  $\geq 22$  mg P m<sup>-2</sup> d<sup>-1</sup>, *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.'

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.'

C21

*loides*, *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<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) and P concentrations in surface water were also tested by linear mixed models.'

C22

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.

C23

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,

C24

sediment may be more appropriate in the context of your study

Response

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

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