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Peer Review File

Physicochemical Properties of Cattail (*Typha*) Bioproducts as Substitutes for Commercial Horticultural Growing Media

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Round 1

Associate Editor, Eric Roy

- Line 38 Correct spelling is "phosphorus", not "phosphorous"
 - Resolved, see FINAL_Boutin_mlo.docx, line 46.
- Lines 51-52 "a product with negative repercussions for wetlands and the global climate" Please add a citation or two for this statement a review paper would be suitable. Some citations are provided in the subsequent paragraph, but any statement of fact should include a supporting citation at first mention.
 - Resolved. The Cleary et al. paper originally cited later in the manuscript supports claims regarding climate impact. I added two new references to the paper (Poulin et al. 1999 and Mazerolle 2003) which discuss the impact of peat mining on vegetation and amphibian communities, respectively. See FINAL_Boutin_mlo.docx, line 60.
- Line 65 typo extra parenthesis
 - Resolved. See FINAL_Boutin_mlo.docx, line 73 .
- Line 69 typo "gasses" should be "gases"
 - Resolved. See FINAL Boutin mlo.docx, line 77.
- Lines 75-78 Has anyone estimated what proportion of GHG emissions are associated with transport for these materials? For many food products, transport even when long-distance is not the dominant source of GHG emissions (see, for example: <u>https://ourworldindata.org/food-choice-vs-eating-local</u>). This fact makes me wonder how accurate this statement on Lines 75-76 is: "The carbon footprints of both peat and coir are magnified by the shipping inherent in their distant origins..." Perhaps one of the cited references provides more information?
 - The Cleary et al. article that is referenced reports that transport accounts for 10% of the GHG emissions of Canadian sphagnum peat moss. Since the word "magnify" can connote multiplication, I replaced "magnified" with "increased." See FINAL_Boutin_mlo.docx, line 84.
- Lines 87-88 "This study measures" and "will be evaluated" are used here, whereas past tense is used in other places. Please make sure to use consistent tense in the revision. I suggest using "This study measured" and "were evaluated" here and consistently using past tense for methods and results generally.
 - In response to comments by reviewers A and C, I removed the latter half of this original paragraph and distributed it through the Methods section, and added new

paragraphs describing the study objectives at the end of the Introduction. This addition is all in past tense. See FINAL_Boutin_mlo.docx, lines 106-120.

- Lines 131-137 Is any additional information available related to the pyrolysis step? Temperature? Duration? Etc. Please add any details available.
 - I added an approximation of the average pyrolysis duration for each batch, see FINAL_Boutin_mlo.docx, lines 150-151. Temperature was not measured due to problems with our infrared thermometer, so I am reluctant to report an estimate in the Methods section. In the discussion (See FINAL_Boutin_mlo.docx, line 318) I state that our pyrolysis temperature was likely between 962 and 1000 C. This range comes from a paper (Maggetti et al. 2011) which reviews temperatures measured in surface bonfires.
- Line 190 and Figure 1 note: technically, arithmetic mean should not be used for pH. See, e.g.,: <u>https://www.journalofdairyscience.org/article/S0022-0302(82)82165-6/pdf</u> Please adjust presentation of pH data accordingly (several options exist, including presenting boxplots of pH with nonparametric stats and/or use of hydrogen ion concentration).
 - I attempted the pH analyses in the manner that you requested, but realized that once transformed to H+ ion concentrations, the data became skewed, thus requiring a log transformation prior to a statistical analysis using a parametric test. A log transformation of H+ ion concentration was ostensibly a reinvention of pH, which did not seem to be a proper solution to your request. My co-author advised that- if possible- the statistically correct course of action is always to transform raw data to achieve a normal distribution to enable the use of parametric tests. When I brought up the possibility of using a non-parametric test on the H+ ion concentrations, he informed me that to do so would be statistically unsound, as the data could be easily normalized through a log transformation and then subjected to a higher-power parametric test. We believe that this is the reason that every paper we have cited in this manuscript analyzed their pH data without reverting to H+ concentrations. For this reason, we chose to stick with our original method of analysis, working with anti-log transformed H+ concentrations, i.e. pH.
 - Comment from Otte: Concentrations are never normally distributed. Normality of distributions is a requirement for parametric statistical analysis. This can usually be addressed by log-transforming the data. In the case of proton concentrations, they are typically reported as pH, which are therefore already log transformed. Other transformations might be needed when geospatial distribution plays a role in the analysis as well, see Reimann C, Filtzmoser P, Garrett R, Dutter R (2008) Statistical data analysis explained. Applied environmental statistics with R. Chichester, UK: Wiley. ISBN: 978-0-470-98581-6. When transformation is not possible, then non-parametric analysis may do the trick, but such tests have much less power. For an example where we used that approach, see Werkmeister C, Jacob DL, Cihacek L, Otte ML (2018) Multi-element composition of Prairie

Pothole Wetland Soils along depth profiles reflects past disturbance to a depth of at least one meter. WETLANDS 38: 1245–1258 https://doi.org/10.1007/s13157-018-1032-7

- Line 293 I think this is an important point. Even if one of these Typha materials can offset a portion of the peat moss in a mixture, that could likely provide meaningful environmental benefits. Total replacement is ideal, but partial replacement should also be considered as a worthy goal. [Update I now see this is mentioned in the Conclusion. Text on Lines 424-432 is an excellent way to end the article.]
 - Thank you!
- Line 323 I think this is probably accurate, but a seedling germination & bioassay experiment would be needed to confirm. I suggest recommending such an experiment for future work.
 - Resolved. See FINAL_Boutin_mlo.docx, lines 451-453.

Production Editor, Aimee Diehl

- The authors have used CSE name-year style correctly for in-text citations. However, the end references list does not conform to CSE name-year style requirements. This may be a simple fix using a Zotero plug-in, but the authors should be made aware that changes will be required before publication.
 - o Resolved. See FINAL_Boutin_mlo.docx, lines 501-616.

Reviewer A, David Tilley

- Abstract needs more detail. it should be a stand alone doc that relates the major findings and implications.
 - I supplemented the abstract with implications from the Conclusion. See FINAL_Boutin_mlo.docx, lines 38-43.
- line 30 Clearly state that results refer to the characteristics listed above
 - Resolved. See FINAL_Boutin_mlo.docx, lines 29-30.
- line 32 Clearly label physical vs chemical in list above.
 - Resolved. See FINAL_Boutin_mlo.docx, lines 25-27.
- 100: You need to clearly articulate the aims at end of Intro and before M&M: Here is a suggestion:

The primary aim of this study is to evaluate the horticultural viability of Typha domingensis bioproducts as sustainable alternatives to conventional growing media, with

a focus on their physicochemical properties relevant to horticulture. By achieving this aim, the study seeks to address the following objectives:

Assess the pH, electrical conductivity (EC), and nitrogen drawdown index (NDI) of Typha-based substrates to determine their chemical suitability for plant growth. Determine the water holding capacity (WHC) and dry bulk density (DBD) of Typha bioproducts to evaluate their physical properties and practicality in horticulture. Compare the performance of Typha bioproducts against commercially available growing media to contextualize findings and validate their potential as sphagnum peat moss substitutes.

Explore the feasibility of implementing Typha harvest as a nutrient export strategy from eutrophic wetlands, which may simultaneously offer an economically viable and eco-friendly solution for the horticultural industry.

- Thank you for your suggestion. I borrowed heavily from it while re-writing the end of my introduction. In accordance with comments from other reviewers, I used the past tense in this section. See FINAL_Boutin_mlo.docx, lines 106-120.
- 115 How did you identify typha to species? How do you know it wasn't latifolia?
 - One of the plants in the harvested area was already flowering (see photo below), and the large gap between the male and female flowers indicated that these plants were *T. domingensis*. See





• 137 Describe where and time this was done.

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I added the date of pyrolysis and the general location, see
FINAL_Boutin_mlo.docx, lines 146-147. I also amended the previous paragraph

to indicate that dried, shredded Typha was sent to North Dakota before pyrolysis. See FINAL_Boutin_mlo.docx, lines 142-143.

- 145-7 Standard practice to give enough info on purchased materials so someone can buy them easily. Maybe give purchase city, store, site.
 - o Resolved. See FINAL_Boutin_mlo.docx, lines 160-161.
- 170 You lost me on the contextualization. Please explain in more detail why you analyzed a subsample.
 - I moved lines 94-95 of the original manuscript to this section to add clarity. See FINAL_Boutin_mlo.docx, lines 188-191. My primary reason for looking at PSD was to be able to explain differences in WHC between the media investigated in our study, and also those between our WHC results and literature values, namely those of Leiber-Sauheitl et al. 2021. In the final sentence of section 4.4, I attribute our higher WHC for shredded Typha with our much higher share of particles <2mm, when compared to Leiber-Sauheitl et al. 2021.
- 174 Please spell out DBD
 - Resolved. See FINAL_Boutin_mlo.docx, line 194.
- 180 It would be helpful to reiterate the number of replicates of each variable.
 - Resolved. See FINAL_Boutin_mlo.docx, line 202.
- Solid overall study and publication. Congrats!
 - Thank you!

Reviewer B, Katherine Porterfield

- I think it would be helpful to include a figure that aggregates all the physicochemical characteristics in one place. This would enable readers to quickly ascertain which of the materials performed the best across all parameters (the Conclusion section seems to be the first place where it is spelled out explicitly that the composted Typha performed best overall). This could be accomplished by combining Figures 1-6 into a single multi panel figure. Alternatively, the data presented in Figures 1-6 could be collapsed into a single table (e.g., example attached). Since Figure 5 does not include a suitability range like the other parameters, it could be moved to the Supplemental Materials.
 - I removed Figure 5 from the manuscript and moved it to Supplemental Materials as Figure S1. I also created a new table similar to the example that you provided. See FINAL_Boutin_mlo.docx, line 294. In addition, I added colored regions to indicate sub-optimal (yellow) and/or extreme (red) values in figures that

previously contained only an optimal (green) range. See FINAL_Boutin_mlo.docx, lines 217, 272, 287.

- Abbreviations should be defined on first mention in figure captions (e.g., Line 209, Line 267)
 - o Resolved. See FINAL_Boutin_mlo.docx, lines 236, and 288.
- Reference list format needs some slight alterations to match the requested Council of Science Editors Name-Year citation style (e.g., journal names should be in abbreviated form and not italicized).
 - Resolved. See FINAL_Boutin_mlo.docx, lines 501-616.
- Authors should consider selecting an accessible color palette for readers with red/green color deficiency (a divergent or sequential palette could work well). There are many resources available online to help with palette selection (e.g., https://www.simplifiedsciencepublishing.com/resources/best-color-palettes-for-scientific-figures-and-data-visualizations
 - I replaced the red, yellow, and green used in my original figures with Med Pink, Light Orange, and Light Green from the Alternating Light/Dark Pairs Eight Color Combination for Charts in the resource that you provided. I ran the new figures through the BYU Colorblind Image Tester (https://bioapps.byu.edu/colorblind_image_tester), which rated them as "Friendly" with 99.77% confidence. See FINAL_Boutin_mlo.docx, lines 217, 235, 250, 272, 287, and 294.
- A seedling bioassay with a revised Typha product blend seems like a logical next step. If this is part of the future work plan, a sentence to that effect might be included in the Conclusions section.
 - Resolved. See FINAL_Boutin_mlo.docx, lines 451-453.

Reviewer C, Anonymous

- Title Title may suggest that the alternative product was used to grow some horticultural crops. However, the research is limited to evaluating physico-chemical properties. A title better reflective of the content may be helpful. Suggested Physicochemical properties of Cattail (Typha) bioproducts as substitutes for commercial growing media for horticultural crops.
 - Thank you! My new title closely resembles your suggestion. See FINAL_Boutin_mlo.docx, lines 3-4.
- Some of the suggestions for the future could have been investigated in this study. For examples, rinsing, mixing PT and CT, lowering pyrolysis temperature for PT, etc.

- I was working with a limited amount of *Typha* materials, so once I finished the initial analyses I did not have enough left over to investigate the impact of rinsing. Also, when this work was being completed I did not have the resources for labscale pyrolysis, so we were limited to the very high temperatures of wood fires. Finally, in order to be potentially viable, a PT and CT blend would have needed alterations to the processing of its components (e.g. rinsing and lower-temp pyrolysis), which did not occur for reasons previously mentioned.
- Add implications in the abstract
 - I supplemented the abstract with implications from the Conclusion. See FINAL_Boutin_mlo.docx, lines 38-43.
- Line 87-89 use past tense
 - In response to comments by reviewers A and C, I removed the latter half of this original paragraph and distributed it through the Methods section, and added new paragraphs describing the study objectives at the end of the Introduction. This addition is all in past tense. See FINAL_Boutin_mlo.docx, lines 106-120.
- Add research questions or objectives or hypotheses in the introduction
 - See prior response
- Some contents from the last two paragraphs of the introduction are a better fit for the methods
 - I removed lines 87-97 of my original manuscript and split the information throughout section 2.3 such that the relevance of each characteristic is mentioned right before I describe the methods used to measure those characteristics. See FINAL_Boutin_mlo.docx, lines 165-166, 171-172, 181-182, 188-189, and 194-195.
- Conclusion- add some numbers from the results and implications.
 - I have now added the corresponding value from the results wherever I made a claim about a substrate's physicochemical characteristics. See FINAL_Boutin_mlo.docx lines 443-449.
 - I am not sure what you mean by "and implications:" whether to add numbers to my implications, or to add implications generally. My conclusion ends with implications, and I couldn't find a good place in these lines to add numbers. I made an attempt to address this by clarifying the phrase "refinement in processing" by adding "to improve chemical characteristics" immediately afterwards, thus reiterating what improvements are necessary. See FINAL_Boutin_mlo.docx lines 458-459.

Additional New Proposed Edits

Associate Editor, Eric Roy

- 1. The authors add a statement indicating the likely temperature range in the Methods section (Section 2.2.3).
 - a. Resolved. See lines 157-160 (all references to line numbers are for Final version).
- 2. Furthermore, the authors better clarify how this temperature range compares to optimum temperatures for pyrolysis of similar feedstocks reported in the literature.
 - a. I'm reluctant to do this because "optimum" depends on the purpose of the biochar, and biochar as a main component of potting media is not a purpose that has been adequately pursued: I am not aware of any study that has investigated horticulturally relevant physicochemical characteristics of biochar along a temperature gradient. While I could point to studies that suggest a likely temperature at which pH would be optimized, those pyrolysis conditions might cause unacceptable impacts on equally-important characteristics such as NDI or water holding capacity. Research planned for the spring of 2025 will explore the horticulturally and environmentally relevant attributes of *Typha domingensis* biochars along a temperature gradient from 300 to 800 C. Only when this is complete will I be able to give a confident answer to this question.
- 3. Remove the ANOVA and post-hoc Tukey comparisons for all parameters
 - a. Resolved. All figures have been updated. See lines 225, 240, 254, 276, and 288, as well as the first figure in the Supplemental Materials (Figure S1).
- 4. It is not appropriate to formally report statistical differences between mixture characteristics based on repeated measures, rather provide assessment of differences in narrative form. Simply rely on the mean (or median) and variance (stdev or range) for assessment of each material relative to optimal ranges. I suggest median and range for pH, but the authors can decide.
 - a. Resolved. All references to ANOVA, Tukey Tests, and statistical significance were removed from section 2.4, figure captions, the results, and the discussion. No new text was added.
- 5. State that future work is needed to replicate this preliminary study for other Typha stands and processing methods (e.g., lower pyrolysis temperature) to better inform design.
 - a. Resolved. See lines 442-444. Also, the preliminary nature of this study was specified in lines 23 and 67.

Additional New Comments

Associate Editor, Eric Roy

- 1. The two short paragraphs on Lines 110-115 should be formatted as bullet points.
 - a. Resolved: see lines 116 and 119.
- 2. Please remove unnecessary hyphens ("environmentally-friendly")
 - a. I removed all grammatically incorrect hyphenation, which primarily occurred in the terms "environmentally friendly" and "horticulturally relevant." See lines 12, 27, 31, 32, 44, 78, 85, 110, 125, 341, 441, 467.

Editor in Chief, Marc Beutel

- 1. In the highlight statement, work in the biomass reuse/harvesting theme somehow to better highlight the link between natural/treatment wetland design/management.
 - a. Resolved. See lines 13 and 14.
- 2. Include a sentence or two and/or 1-2 references related to constructed treatment wetlands and significance of Typha growth/harvesting (e.g., potential for net nutrient removal).
 - a. I added a number and a reference for likely TN and TP harvest potential. See lines 55-58.
- 3. In the conclusion, move closing statement related to importance of biomass harvesting to start of conclusion and better highlight linkage to wetland/treatment wetland design and management.
 - a. Resolved. See lines 438-442 and 466-468.

Dear Mr. Boutin,

You have done a nice job addressing reviewer comments. The revised manuscript is an improvement on the initial submission and very close to being ready for publication in JEED.

However, I do have a few more comments, detailed below. I have also included some comments from EIC Dr. Marc Beutel.

Our comments require additional Minor Revisions.

Details on pyrolysis:

From the **Response to Reviewers** document:

AE Original Comment: Lines 131-137 – Is any additional information available related to the pyrolysis step? Temperature? Duration? Etc. Please add any details available.

Author Response: I added an approximation of the average pyrolysis duration for each batch, see FINAL_Boutin_mlo.docx, lines 150-151. Temperature was not measured due to problems with our infrared thermometer, so I am reluctant to report an estimate in the Methods section. In the discussion (See FINAL_Boutin_mlo.docx, line 318) I state that our pyrolysis temperature was likely between 962 and 1000 C. This range comes from a paper (Maggetti et al. 2011) which reviews temperatures measured in surface bonfires.

AE New Proposed Edits: The authors add a statement indicating the likely temperature range in the Methods section (Section 2.2.3). Furthermore, the authors better clarify how this temperature range compares to optimum temperatures for pyrolysis of similar feedstocks reported in the literature.

Re: analysis of pH data:

AE Original Comment: Line 190 and Figure 1 – note: technically, arithmetic mean should not be used for pH. See, e.g.,: https://www.journalofdairyscience.org/article/S0022-0302(82)82165-6/pdf Please adjust presentation of pH data accordingly (several options exist, including presenting boxplots of pH with nonparametric stats and/or use of hydrogen ion concentration).

Author Response:

- I attempted the pH analyses in the manner that you requested, but realized that once transformed to H+ ion concentrations, the data became skewed, thus requiring a log transformation prior to a statistical analysis using a parametric test. A log transformation of H+ ion concentration was ostensibly a reinvention of pH, which did not seem to be a proper solution to your request. My co-author advised that- if possible- the statistically correct course of action is always to transform raw data to achieve a normal distribution to enable the use of parametric tests. When I brought up the possibility of using a non-parametric test on the H+ ion concentrations, he informed me that to do so would be statistically unsound, as the data could be easily normalized through a log transformation and then subjected to a higher-power parametric test. We believe that this is the reason that every paper we have cited in this manuscript analyzed their pH data without reverting to H+ concentrations. For this reason, we chose to stick with our original method of analysis, working with anti-log transformed H+ concentrations, i.e. pH.
- Comment from Otte: Concentrations are never normally distributed. Normality of distributions is a requirement for parametric statistical analysis. This can usually be addressed by log-transforming the data. In the case of proton concentrations, they are typically reported as pH, which are therefore already log transformed. Other transformations might be needed when geospatial distribution plays a role in the analysis as well, see Reimann C, Filtzmoser P, Garrett R, Dutter R (2008) Statistical data analysis explained. Applied environmental statistics with R. Chichester, UK: Wiley. ISBN: 978-0-470-98581-6. When transformation is not possible, then non-parametric analysis may do the trick, but such tests have much less power. For an example where we used that

approach, see Werkmeister C, Jacob DL, Cihacek L, Otte ML (2018) Multi-element composition of Prairie Pothole Wetland Soils along depth profiles reflects past disturbance to a depth of at least one meter. WETLANDS 38: 1245–1258 https://doi.org/10.1007/s13157-018-1032-7

AE Response to Authors:

The treatment of pH data in the literature is inconsistent and multiple options exist. That said, a few points:

(1) Presentation of arithmetic mean pH can be problematic because:

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average(pH) \neq -log(average[H+])
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In cases where pH measurements exhibit little variability, the difference between average(pH) and -log(average[H+]) becomes very small and essentially meaningless (Boutilier and Shelton 1980). Therefore, if the triplicate pH measurements for each material were very close (which appears to be the case based on the reported standard deviations), I think the authors have a good case for keeping the presentation of average(pH) in Figure 1 and Table 1 as is.

Boutilier, R. G., & Shelton, G. (1980). The statistical treatment of hydrogen ion concentration and pH. Journal of Experimental Biology, 84(1), 335-340. [link]

(2) The above paper also provides discussion of standard deviation for pH. Note that asymmetry can exist depending on the dataset.

(3) Boutin and Otte make an argument for their current approach to comparing pH across materials (post-hoc Tukey test using pH data). I disagree with some points included in the Response to Reviewers document.

(a) The authors state, "if possible-the statistically correct course of action is always to transform raw data to achieve a normal distribution to enable the use of parametric tests." I respectfully disagree. It depends on the dataset and hypothesis. Using a nonparametric test can be a valid option. See pages 97-100 in the USGS Statistical Methods in Water Resources (link).

(b) The authors express concern that using a nonparametric test will result in an unacceptable loss of statistical power. However, the authors are here comparing 8 groups with n=3 per group (note: I missed that n=3 in my initial review when I recommended boxplots). Therefore, I would expect statistical power to be low to begin with (plus see next point on pseudo-replication). Fortunately, determining pH differences is not the primary objective of this study. Instead, pH data serve as one line of evidence for material suitability in horticulture.

(c) Furthermore, upon closer investigation of the statistics used in the paper, it became clear that the current statistical approach is flawed due to pseudo-replication. The study does not include true replication across treatments. Instead, repeated measures (3) were made for either (a) a single batch of each material (with the possible exception of the biochar, which included separate

batches of the pyrolysis step) – all derived from a single harvested plot of Typha, or (b) single purchased commercial product batches. Therefore, the 3 observations for each material are not independent and ANOVA and post hoc Tukey tests are not appropriate. I think the paper is still publishable, but the current statistical approach inherently oversells the confidence in differences across treatments.

AE New Proposed edits: I recommend the authors:

(1) Remove the ANOVA and post-hoc Tukey comparisons for all parameters

(2) Simply rely on the mean (or median) and variance (stdev or range) for assessment of each material relative to optimal ranges. I suggest median and range for pH.

(3) State that future work is needed to replicate this preliminary study for other Typha stands and processing methods (e.g., lower pyrolysis temperature) to provide more information to inform design.

Additional New Comments

Associate Editor, Eric Roy

- The two short paragraphs on Lines 110-115 should be formatted as bullet points.
- Please remove unnecessary hyphens ("environmentally-friendly")

Editor in Chief, Marc Beutel

On a more general note, one theme that is missing from this paper is the key aim of the journal informing ecological engineering design. The word design does not appear in the paper. There is a link - if we can find a sustainable use for Typha biomass, then wetlands and treatment wetlands could be designed and/or managed in new ways to support net nutrient removal from systems. This theme is a bit underplayed in the paper. Please do the following:

- 1. In the highlight statement, work in the biomass reuse/harvesting theme somehow to better highlight the link between natural/treatment wetland design/management.
- 2. Include a sentence or two and/or 1-2 references related to constructed treatment wetlands and significance of Typha growth/harvesting (e.g., potential for net nutrient removal).
- 3. In the conclusion, move closing statement related to importance of biomass harvesting to start of conclusion and better highlight linkage to wetland/treatment wetland design and management.