Reply to Cao et al.'s comment on “Does the responses of Vallisneria natans (Lour.) Hara to high nitrogen loading differ…

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Reply to Cao et al.'s comment on "Does the responses of *Vallisneria natans* (Lour.) Hara to high nitrogen loading differ between the summer high-growth season and the low-growth season? Science of the Total Environment 601–602 (2017) 1513–1521"

We thank for reflecting on our result (Yu et al., 2017). Cao et al. (2017) find that our conclusion that shading effects induced by high phytoplankton biomass together with toxicity of high ammonium contributed to the loss of submersed macrophytes is debatable. They argue that species-specific features of macrophytes, pH and light attenuation scenarios should have been included in the study of the influence of high N on submerged macrophytes.

We agree with Cao et al. (2017) that the effects of high ammonium on submersed macrophytes are species specific. Ammonium-related physiological stress on submersed macrophytes depends on light condition, for instance, and is aggravated at low light as suggested by Cao et al. (2011). Species (e.g. of different growth forms) may therefore differ in their response to high ammonium levels because of species-specific tolerance to low-light conditions. However, in our study, the purpose was to compare the effects of high ammonium between the growing season and the low-growth season on one plant species (i.e. *Vallisneria natans*) and not to study species-specific differences in tolerance.

With regard to the toxicity of ammonia (NH_3)/ammonium (NH_4^+) caused by pH, Cao et al. (2017) argue that toxicity of NH_3 and NH_4^+ is pH dependent and that the toxicity of NH_3 is not negligible in our experiment. We agree. In Yu et al. (2017) NH_3 and NH_4^+ were commonly expressed as total ammonium. When the concentration of un-ionized NH_3 in our experiments was calculated according to the widely used method by Wood (1993) and Emerson et al. (1975), NH_3 shows a significant increase along the treatment gradient ($r = 0.94$, $n = 9$, $p < 0.001$ in growing season; $r = 0.99$, $n = 9$, $p < 0.001$ in low-growth season) being much higher in the growing season (medians between 0.10 and 6.04 mg L^{-1}) than in the low-growth season (0.03–1.23 mg L^{-1}). Highly significant positive correlations occurred, however, between NH_3 and NH_4^+ for both the growing season ($r = 0.90$, $n = 9$, $p < 0.001$) and the low-growth season ($r = 0.96$, $n = 9$, $p < 0.001$), reflecting that variation in NH_3 resulting from the N treatment was much larger than the variation in NH_3 induced by variation in pH. All the high concentrations of NH_3 were therefore accompanied by high NH_4^+ concentrations and appeared in the high nitrogen treatments (N100 a, b and N20 a, b). Consequently, the concentration of total ammonium in our experiment is, in our opinion, a valid indicator of the toxicity of both NH_3 and NH_4^+. We however, fully agree that studies of the specific role of NH_3 and NH_4^+ are interesting and relevant.

Cao et al. (2017) also suggest that light attenuation scenarios should have been considered in the study. Exploration of the potential shading effects from phytoplankton and periphyton on plants was one of the main purposes of our study based on correlative analysis. Chlorophyll *a* concentrations of phytoplankton and periphyton were carefully measured and related to the growth of macrophytes. Weak or even significant positive relationships were found between the growth variables of macrophytes and periphyton chlorophyll *a*, suggesting a negligible effect of periphyton shading. The highly significant negative relationships between the growth variables of macrophytes and phytoplankton chlorophyll *a* suggested that phytoplankton shading or factors related to phytoplankton growth play a vital role in regulating macrophyte growth. Cao et al. (2017) suspect that 60 μg L^{-1} phytoplankton Chl *a* may not be strong enough to cause shading effects at water depths of 40 cm and 80 cm based on the observations that such effects were not significant at 100 cm with 40–50 μg Chl *a* L^{-1} in Li et al. (2008) and Zhang et al. (2016) at similar NH_4^+ levels. They suggest that resuspension of the sediment play a role in our study. We did measure the concentration of suspended solids, which averaged 9.3 mg L^{-1} and ranged between 3 and 20.4 mg L^{-1}. We calculated various components of suspended particles according to the method by Jeppesen et al. (2003). The results showed that the contributions of inorganic, algal, and non-algal organic suspended solids were 27.8% (6.8–67.2%), 18.0% (9.4–23.6%), and 58.6% (50.2–74.8%), respectively, for the summer experiment and 51.6% (26.2–75.0%), 7.0% (2.7–11.9%), and 41.4% (20.9–68.8%), respectively, for winter-spring experiment (unpublished data). This concurs with the analyses based on 50 lake-year data on 28 shallow lakes in the mid-lower Yangtze Basin, demonstrating a mean algal contribution of 11% (0.5–47%) (Wang et al., 2017). Although phytoplankton contributed with a relative low percent, $Chla_{Phyt}$ showed a significant positive correlation with detritus (summer, $r = 0.87$, $n = 8$, $p < 0.01$; winter-spring: $r = 0.78$, $n = 10$, $p < 0.01$) and inorganic SS (summer, $r = 0.87$, $n = 8$, $p < 0.05$; winter-spring: $r = 0.81$, $n = 10$, $p < 0.05$) in both experiments. Similar relationships were found in the study of Danish lakes referred to above (Jeppesen et al., 2003). These results demonstrate that when phytoplankton increases so do detritus and inorganic suspended matter (for likely reasons for such relationships, see Jeppesen et al., 2003). Phytoplankton can therefore effectively indicate the overall light attenuation conditions, but the $Chla_{Phyt}$ threshold for plant growth may vary, depending on the proportion they represent of total suspended matter. This may help explaining why the $Chla_{Phyt}$ thresholds vary with experimental condition.

In summary, some points raised by Cao et al. (2017) help to move this topic forward. Particularly, disentangling the roles of NH_3 and NH_4^+ would allow for a closer scrutiny on their ecological effects. However, our additional analyses demonstrate that total ammonium and $Chla_{Phyt}$ are sufficient ecosystem indicators for revealing the different responses of our focus species (*Vallisneria natans* (Lour.) Hara) to high nitrogen between growing and low-growth seasons. Our results are therefore robust to the concerns noted by Cao et al. (2017).

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References

Cao, T., Ni, L.Y., Xie, P., Xu, J., Zhang, M., 2011. Effects of moderate ammonium enrichment on three submersed macrophytes under contrasting light availability. Freshw. Biol. 56:1620–1629. https://doi.org/10.1111/j.1365-2427.2011.02601.x.

Cao, Y., Wang, W., Li, W., 2017. Letter to the Editor Re: Yu Qing, Wang Hai-Jun, Wang Hong-Zhu, Li Yan, Liang Xiao-Min, Xu Chi, Jeppesen Erik. Does the responses of *Vallisneria natans* (Lour.) Hara to high nitrogen loading differ between the summer high-growth season and the low-growth season? Sci. Total Environ. 601–602: 1513–1521. https://doi.org/10.1016/j.scitotenv.2017.08.022.

Emerson, K., Russo, R.C., Lund, R.E., Thurston, R.V., 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. J. Fish. Res. Board Can. 32, 2379–2383.

Jeppesen, E., Jensen, J.P., Søndergaard, M., Hansen, K.S., Møller, P.H., Rasmussen, H.U., et al., 2003. Does resuspension prevent a shift to a clear state in shallow lakes during reoligotrophication? Limnol. Oceanogr. 48:1913–1919. https://doi.org/10.4319/lo.2003.48.5.1913.

Li, W., Zhang, Z., Jeppesen, E., 2008. The response of *Vallisneria spinulosa* (Hydrocharitaceae) to different loadings of ammonia and nitrate at moderate phosphorus concentration: a mesocosm approach. Freshwater Biol. 53 (11):2321–2330. https://doi.org/10.1111/j.1365-2427.2008.02053.x.

Wang, H.J., Wang, H.Z., Pan, B.Z., Liang, X.M., 2017. Variations of algal and non-algal turbidity among the Yangtze subtropical shallow lakes. Acta Hydrobiol. Sin. 41, 414–419 (In Chinese with English abstract).

Wood, C.M., 1993. Ammonia and urea metabolism and excretion. In: Evans, D.H. (Ed.), The Physiology of Fishes. CRC Press, Ann Arbor, MI, pp. 379–425.

Yu, Q., Wang, H.J., Wang, H.Z., Li, Y., Liang, X.M., Xu, C., Jeppesen, E., 2017. Does the responses of *Vallisneria natans* (Lour.) Hara to high nitrogen loading differ between the summer high-growth season and the low-growth season? Sci. Total Environ. 601–602:1513–1521. https://doi.org/10.1016/j.scitotenv.2017.05.268.

Zhang, Z., Cao, Y., Jeppesen, E., Li, W., 2016. The response of *Vallisneria spinulosa* (Hydrocharitaceae) and plankton to pulse addition of inorganic nitrogen with different loading patterns. Hydrobiologia 767, 175–184.

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