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Submerged freshwater plant communities do not show species complementarity effect in wetland mesocosms

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It is a generally accepted theory that ecological functions are enhanced with increased diversity in plant communities due to species complementarity effects. We tested this theory in a mesocosm study using freshwater submerged plant beds to determine if increasing species number caused overyielding and species complementarity. We applied a maximum of four species in the plant beds corresponding to the typical species number in natural freshwater plant beds. We found no clear effects of species number (1–4) on biomass production and thus no conclusive overyielding and complementarity effect. This may be explained by low species differentiation among the four species in plant traits relevant for resource acquisition in freshwater, or that other species interactions, e.g. allelopathy, were inhibiting overyielding. The existing knowledge on species complementarity in aquatic plant communities is sparse and inconclusive and calls for more research.

1. Introduction

A general theory in ecology is that ecological functions are enhanced with increased species diversity [1]. Previous work in species-rich grasslands (greater than 20 plant species in 9×9 m plots) has supported the theory by showing positive relationships between species richness and ecological functions such as nutrient uptake rates and primary production [2,3]. For submerged plant communities, only three studies have tested this theory [4–6] with mixed results. These three experiments tested for overyielding (i.e. mixture of species performs an ecosystem function better than monocultures) in biomass production in two to four species plant beds (less than $1.7\,\mathrm{m}^2$) compared to single-species communities including altogether 11 submerged plant species, but where [5] did verify the theory, studies [4,6] did not. All studies were based on manipulated experiments where [5] was conducted as a transplanting study in the field, and [4,6] were conducted in mesocosms. However, the low number of studies and the mixed results among studies calls for more studies to create general conclusions on overyielding for aquatic plant communities.

Several causes, working synergistically or separately, may explain overyielding in multi-species communities [7]. Among the most-verified causes is the complementarity effect (CE) stating that increased diversity leads to enhanced functional differences between species, which can lead to greater and more effective exploitation and acquisition of resources, thus overyielding in, e.g. productivity [8,9]. The other important cause is the selection effect (SE) stating that higher biodiversity increases the probability that one or a few dominant species specifically adapted to the surroundings are present in the community [8,9], thereby enhancing the community's ecological function. In addition, other species interactions such as competitive exclusion, facilitation,

and release of pathogens have been suggested as being responsible for overyielding at high plant diversity [10,11].

Submerged plants growing in multi-species beds may complement each other in several ways which could lead to overyielding in productivity. First, nutrient uptake in submerged species occurs over both roots and shoots allowing for complementarity between species with different ratios of above- and below-ground biomass. Second, light interception may be more efficient if several species with different morphologies are present. Third, temporal complementarity may occur among species such that total acquired resources may increase over the scale of days or seasons.

We tested whether increasing species richness in submerged freshwater plant beds containing one to four species caused overyielding in plant bed biomass productivity through CE or SE. We hypothesized that the presence of more species would enhance the resource uptake with a concomitant overyielding in biomass productivity. We applied a maximum of four plant species in the plant beds corresponding to natural and constructed wetlands where species richness is similarly low.

2. Methods

A mesocosm experiment was conducted (56°13′42.8″ N, 10°07′34.0″ E) in summer using partially buried plastic tubs (1.4 m^2 and 53 cm high) with 5 cm sand sediment and 25 cm tap water simulating submerged plant communities. A drip irrigation system provided each mesocosm with 1.2 l water per hour (retention time 2 days) and mesocosms were aerated. Nutrients were added three times weekly to ensure concentrations of 1.0 mg PO$_4$–P l^{-1}, 1.0 mg NH$_4$–N l^{-1} and 3.0 mg NO$_3$–N l^{-1}, which simulates nutrient concentrations in freshwater streams and wetlands in agricultural areas [12].

Four common submerged plant species, *Potamogeton perfoliatus* L., *Potamogeton obtusifolius* Mert. & Koch, *Ranunculus aquatilis* L. and *Elodea canadensis* Michx. were collected from nearby lakes. Apical shoots (20 cm) were acclimatized in tap water for 3 days before planting. Thirteen species combinations were established (four 1-species beds, four 2-species beds, four 3-species beds and one 4-species) in a fully randomized experiment with four replicates. The species in the four 2-species beds were chosen randomly. In each mesocosm, 50 g fresh weight was planted, shared among one to four species, such that each species contributed equally to initial bed biomass. Shoots from the same species were planted adjacently to simulate natural plant beds. After eight weeks above- and below-ground biomass in each mesocosm was harvested, separated by species and dried for 5 days at 60°C before weighing.

Overyielding, CE and SE for total, above- and below-ground biomass production were calculated using the additive partitioning method [9,13]. Multiple variables with ecological importance were determined: D_{max} (transgressive overyielding), NE (net effect of diversity), RY (relative yield), CE and SE (following [13]). A positive D_{max} implies that multi-species beds have a higher biomass compared with single-species beds with the highest biomass, and it is therefore a measure of strong complementarity [13]. A negative D_{max} indicates that the most productive single-species bed has higher biomass than any multi-species beds. NE measures whether multi-species beds produce more than expected from the biomass (NE > 0), and if so, whether this is due to CE, SE or a combination. The sum of CE and SE results in NE. CE and SE are measured as the RY of biomass production in mono-species beds and the deviation from expected RY of the species in multi-species beds. A positive CE indicates that resource partition or facilitation has occurred,

and a positive effect appears when the RY of species is greater than expected in multi-species compared with single-species beds. Negative CE indicates direct interference between species. By contrast, positive SE indicates that one (or more) competitive and dominant species is present in multi-species beds, producing more biomass than expected and negative SE indicates the presence of a competitive species producing less biomass than expected. Complementarity dominates in multi-species beds if CE > SE and, likewise, selection dominates in multi-species beds if CE < SE.

Statistical tests were conducted using JMP (13.0). One-way ANOVAs were conducted to compare the effect of species richness on biomass accumulation. Data were tested for variance homogeneity with Levene's test and log-transformed if necessary. *Post-hoc* Tukey HSD tests were applied to significant results from ANOVAs. One-sample *t*-tests were used to test for diversity variables being significantly different from zero. Linear regressions tested for relations between species richness and response variables (NE, CE and SE) for biomass data. For more details on data analysis, see the electronic supplementary material.

3. Results

Although we observed differences in biomass among plant bed treatments, we did not detect significant overyielding in multi-species compared to single-species plant beds (table 1), neither for total biomass or when separated into above- and below-ground biomass. Furthermore, we did not find transgressive overyielding in any treatments ($D_{max} < 0$; table 2), meaning that the performance of multi-species beds were not exceeding the most efficient single-species beds (*R. aquatilis*; table 1). Only in two out of nine multi-species beds did we observe a significant positive CE (table 2). However, none of those treatments had a significant positive NE of species diversity (table 2). SE was not positive in any treatments. Either NE, CE or SE varied significantly with species number for either total, above- or below-ground biomass (figure 1), indicating that there was no effect of increasing species richness.

4. Discussion

As opposed to our hypothesis, we did not find that an increase from one to four species in submerged freshwater plant beds enhanced biomass productivity through CE or SE. There was no difference in total biomass among beds, thus no overyielding, and no change in CE in response to number of species in plant beds for either above- or below-ground biomass. These results followed the negative findings from studies [4,6] but was contrary to the theory verification in [5], although all of these studies were also testing CE on species poor and relative small aquatic plant beds. Furthermore, no change in SE was present with increasing species number in our study, suggesting that no single species was dominating and causing overyielding in multi-species plant beds [9]. Although *R. aquatilis* was the most productive species in single-species beds, this did not result in higher biomass production when this species was part of multi-species beds. Thus, *R. aquatilis* produced less in multi-species beds than expected from the single-species beds hence no positive SE with increasing species number.

Evidence for CE in our multi-species study was lacking, whereas study [5] observed CE. The difference between these two studies may be linked to the low species number

Table 1. Biomass production over eight weeks (g DW, mean values $\pm$ 1 s.d.). Ec, *Elodea canadensis*; Po, *Potamogeton obtusifolius*; Pp, *Potamogeton perfoliatus*; Ra, *Ranunculus aquatilis*. Letters indicate significant differences. One-way ANOVA results shown below in the table (d.f. = 12, 39).

treatment	total biomass (g DW)	above-ground biomass (g DW)	below-ground biomass (g DW)
1-species beds			
Ec	22.6 ($\pm$1.5)^{de}	19.0 ($\pm$1.5)^{c}	3.6 ($\pm$0.2)^{de}
Po	19.5 ($\pm$2.8)^{e}	19.2 ($\pm$2.9)^{c}	0.2 ($\pm$0.1)^{f}
Pp	24.3 ($\pm$3.6)^{bcde}	19.0 ($\pm$2.9)^{c}	5.9 ($\pm$0.8)^{cd}
Ra	56.7 ($\pm$4.2)^{a}	40.6 ($\pm$3.3)^{a}	16.1 ($\pm$1.4)^{a}
2-species beds			
Po + Ec	23.0 ($\pm$3.4)^{cde}	20.9 ($\pm$3.2)^{bc}	2.1 ($\pm$0.5)^{e}
Pp + Ec	27.2 ($\pm$2.8)^{bcde}	22.1 ($\pm$2.5)^{bc}	5.1 ($\pm$0.3)^{cd}
Ra + Ec	34.0 ($\pm$7.7)^{bcd}	26.5 ($\pm$5.6)^{bc}	7.5 ($\pm$2.7)^{bc}
Pp + Ra	32.8 ($\pm$3.9)^{bcd}	25.1 ($\pm$2.2)^{bc}	7.6 ($\pm$2.1)^{bc}
3-species beds			
Pp + Po + Ec	23.7 ($\pm$2.0)^{cde}	20.8 ($\pm$1.2)^{bc}	3.2 ($\pm$0.4)^{de}
Pp + Po + Ra	35.9 ($\pm$5.2)^{b}	30.0 ($\pm$5.6)^{b}	5.9 ($\pm$1.4)^{cd}
Pp + Ra + Ec	29.3 ($\pm$4.3)^{bcde}	23.9 ($\pm$3.7)^{bc}	10.1 ($\pm$1.2)^{ab}
Ra + Po + Ec	26.1 ($\pm$4.5)^{bcde}	22.8 ($\pm$3.9)^{bc}	3.3 ($\pm$0.7)^{de}
4-species beds			
Pp + Po + Ra + Ec	34.4 ($\pm$3.5)^{bc}	28.9 ($\pm$3.4)^{b}	5.6 ($\pm$1.2)^{cd}
F-ratio	16.59	9.13	43.25
p-value	<0.0001	<0.0001	<0.0001

Table 2. Average transgressive overyielding (D_{max}), NE, CE and SE for total biomass in polycultures (treatments with two to four species). Pp, *Potamogeton perfoliatus*; Ra, *Ranunculus aquatilis*; Ec, *Elodea canadensis*; Po, *Potamogeton obtusifolius*. Asterisks indicate significant difference from zero (based on one-sample *t*-test).

treatment	D_{max}	NE	CE	SE
2-species beds				
Pp + Ra	−0.4 ($\pm$0.1)*	−7.8 ($\pm$4.5)*	−5.5 ($\pm$3.5)	−2.2 ($\pm$2.2)
Pp + Ec	−0.5 ($\pm$0.1)*	3.7 ($\pm$3.2)	3.9 ($\pm$3.2)*	−0.1 ($\pm$0.2)
Ra + Ec	−0.4 ($\pm$0.2)*	−5.6 ($\pm$8.9)	−2.3 ($\pm$9.4)	−3.3 ($\pm$1.1)*
Po + Ec	−0.6 ($\pm$0.1)*	2.0 ($\pm$3.9)	1.9 ($\pm$4.0)	0.0 ($\pm$0.1)
3-species beds				
Pp + Po + Ec	−0.6 ($\pm$0.0)*	1.5 ($\pm$2.3)	1.5 ($\pm$2.1)	0.0 ($\pm$0.3)
Pp + Po + Ra	−0.4 ($\pm$0.1)*	2.4 ($\pm$6.0)	4.5 ($\pm$8.2)	−2.1 ($\pm$3.5)
Pp + Ra + Ec	−0.5 ($\pm$0.1)*	−5.3 ($\pm$5.0)	−1.3 ($\pm$5.3)	−4.0 ($\pm$0.7)*
Ra + Po + Ec	−0.5 ($\pm$0.1)*	−6.8 ($\pm$5.2)	−3.7 ($\pm$4.2)	−3.1 ($\pm$1.5)*
4-species beds				
Pp + Po + Ra + Ec	−0.4 ($\pm$0.1)*	3.7 ($\pm$4.1)	6.9 ($\pm$5.8)*	−3.3 ($\pm$1.9)*

*$p < 0.05$.

in the two studies and the selected species. When the maximum species number is only four, every single-species interaction affecting biomass production will be of great importance to the final biomass production in each experimental plant bed. In [6], the lack of CE in submerged plant communities was suggested to be a result of allelopathy. The strength and effect of such interaction can vary greatly among species and therefore result in non-conclusive results in and between experiments with few but different species between experimental beds. A possible effect of low species number is supported by the studies from grasslands showing that overyielding and species complementary only occurred in communities exceeding eight plant species [2,3].

However, other species interactions not tested for such as facilitation or pathogen release may also stimulate overyielding in plant communities [10,11] causing inconsistency between
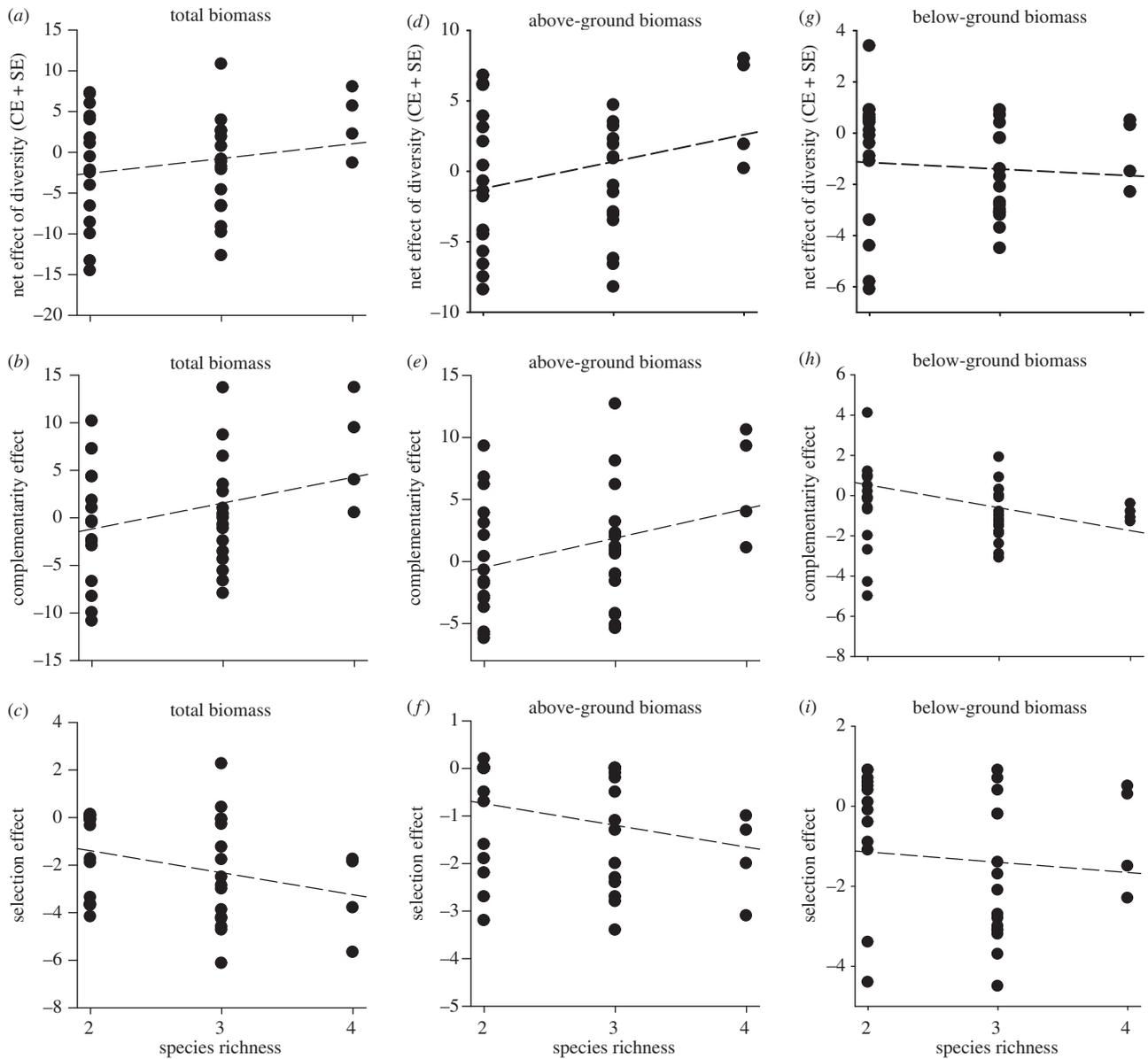
Figure 1. NE, CE and SE. *a,b,c* = total biomass; *d,e,f* = above-ground biomass; *g,h,i* = below-ground biomass. Each dot represents a mesocosm. No significant relationships present but trend-line is shown (d.f. = 1, 34).

experimental plant beds and between studies. In addition, the presence of more functional plant groups in grasslands (e.g. C3 and C4 grasses or nitrogen fixers and non-fixers) has shown positive CE [7,14]. In our experiment, all plants were submerged plants with rather similar traits adapted for enhancing resource acquisition at reduced light and inorganic carbon availability and thus making CE more unlikely. Lastly, the study described in [5] was an *in situ* experiment establishing plant communities in a field site, whereas our study as well as [4,6] were mesocosm studies. This indicates that mesocosms should not be chosen for future tests of CE in aquatic plant communities, but more ideally should be tested in natural communities. This could also provide the opportunity to measure any temporal complementarity among species which is not included in a short-term meso-cosm study.

In conclusion, multi-species submerged plant beds did not show overyielding and no CE or SE, which may be explained by low differentiation in species traits among sub-merged plant species or by species interactions not tested for

such as allelopathy. Only few studies have tested species complementarity in aquatic plant beds and the studies are inconclusive. Future studies should test for overyielding in aquatic plant communities consisting of more than four species to test if higher species number result in more consist-ent response between experimental plant beds, and also include tests for other species interactions than CE and SE that could affect overyielding.

References

1. Díaz S, Cabido M. 2001 Vive la difference: plant functional diversity matters to ecosystem processes. *Trends Ecol. Evol.* **16**, 646–655. (doi:10.1016/S0169-5347(01)02283-2)

2. Weisser WW *et al.* 2017 Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: patterns, mechanisms, and open questions. *Basic Appl. Ecol.* **23**, 1–73. (doi:10.1016/J.BAAE.2017.06.002)

3. Tilman D, Wedin D, Knops J. 1996 Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* **379**, 718–720. (doi:10.1038/379718a0)

4. Engelhardt KAM, Ritchie ME. 2002 The effect of aquatic plant species richness on wetland ecosystem processes. *Ecology* **83**, 2911–2924. (doi:10.1890/0012-9658(2002)083[2911:TEOAPS]2.0.CO;2)

5. Gustafsson C, Boström C. 2011 Biodiversity influences ecosystem functioning in aquatic angiosperm communities. *Oikos* **120**, 1037–1046. (doi:10.1111/j.1600-0706.2010.19008.x)

6. Choudhury MI, McKie BG, Hallin S, Ecke F. 2018 Mixtures of macrophyte growth forms promote nitrogen cycling in wetlands. *Sci. Total Environ.* **635**, 1436–1443. (doi:10.1016/j.scitotenv.2018.04.193)

7. Loreau M. 2000 Biodiversity and ecosystem functioning: recent theoretical advances. *Oikos* **91**, 3–17. (doi:10.1034/j.1600-0706.2000.910101.x)

8. Fargione J, Tilman D, Dybzinski R, Lambers JHR, Clark C, Harpole WS, Knops JM., Reich PB, Loreau M. 2007 From selection to complementarity: shifts in the causes of biodiversity–productivity relationships in a long-term biodiversity experiment. *Proc. R. Soc. B* **274**, 871–876. (doi:10.1098/rspb.2006.0351)

9. Loreau M, Hector A. 2001 Partitioning selection and complementarity in biodiversity experiments. *Nature* **412**, 72–76. (doi:10.1038/35083573)

10. Hille Ris Lambers J, Harpole WS, Tilman D, Knops J, Reich PB. 2004 Mechanisms responsible for the positive diversity–productivity relationship in Minnesota grasslands. *Ecol. Lett.* **7**, 661–668. (doi:10.1111/j.1461-0248.2004.00623)

11. Cardinale BJ, Srivastava DS, Duffy, JE, Wright JP, Downing AL, Sankaran M, Jouseau C 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature* **443**, 989–992 (doi:10.1038/nature05202)

12. Kjærgaard C, Hoffmann CC. 2013 *Konstruerede vådområder til målrettet reduktion af næringsstoffer i drænvand.* [*In Danish.*] Note from DCE and DCA; Aarhus University.

13. Petchey OL. 2003 Integrating methods that investigate how complementarity influences ecosystem functioning. *Oikos* **101**, 323–330. (doi:10.1034/j.1600-0706.2003.11828.x)

14. Van Ruijven J, Berendse F. 2005 Diversity–productivity relationships: initial effects, long-term patterns, and underlying mechanisms. *Proc. Natl Acad. Sci. USA* **102**, 695–700. (doi:10.1073/pnas.0407524102)