

1 **Appendix 1: Development of the vegetation over the growing season**

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3 Plant cover of emergent and submerged macrophytes increased over the growing season. This  
4 increase was much stronger inside the enclosure compared to the control plot. We observed  
5 strong herbivore effects on plant cover of different species groups already one year after placing  
6 the enclosures (Table A1.1, Fig. A1.1).

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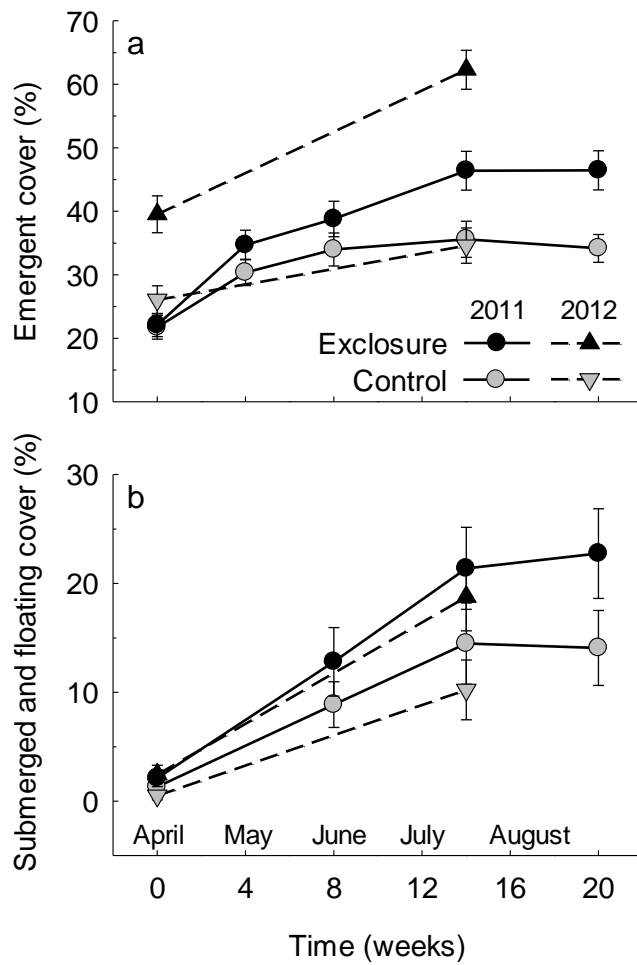
8 **Table A1.1.** Effects of time and enclosure treatment on cover of emergent species and  
9 submerged and floating species over the growing season in 2011 and 2012. Chi-squares ( $\chi^2$ ) and  
10 significance ( $P$ ) calculated with general linear mixed models with paired plot nested within study  
11 area and month as random factor (See methods in the main manuscript). Degrees of freedom  
12 (d.f.) are 1 for all analyses, because  $P$  values were obtained by comparing two models: with and  
13 without the term of interest.

14

	<b>Emergent cover</b>		<b>Submerged and floating cover</b>	
	$\chi^2$	$P$	$\chi^2$	$P$
17 <b>2011</b>				
18 Time (T)	14.41	<0.001	14.76	<0.001
19 Enclosure treatment (E)	36.92	<0.001	23.94	<0.001
20 Interaction (T*E)	12.73	<0.001	9.64	0.002
21 <b>2012</b>				
22 Time (T)	15.44	<0.001	10.21	0.001
23 Enclosure treatment (E)	67.21	<0.001	22.11	<0.001
24 Interaction (T*E)	7.26	0.007	4.06	0.044

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27

28 **Figure A1.1:** Cover of a) emergent plant species and b) submerged and floating species in  
 29 exclosures and control plots over the growing season of 2011 (starting end March, early April till  
 30 the end of August) and the growing season of 2012 (starting mid-March till mid-July, which was  
 31 week 14 of the previous year).

## 32 **Appendix 2: Herbivore identity**

33 Herbivore density itself is often hard to quantify, and previous attempts to relate herbivore density  
34 to the effect size of grazing have found a weak or no correlation (Marklund and others 2002),  
35 which may explain the absence of a direct correlation between aquatic herbivores and vegetation  
36 characteristics. However, our areas contained different herbivore types and some are more and  
37 others are less likely to be the potential main herbivore.

38

### 39 *Herbivore densities over time*

40 Whereas Mallard and Mute swan densities remained stable over the past ten years in the  
41 Netherlands, the number of Graylag geese breeding pairs increased with 21% and their total  
42 population increased with ca 8% per year (Boele and others 2012; Hornman and others 2013).  
43 Hunting of Graylag geese is restricted and may only occur between 15 august and 30 september.

44 Muskrat is an invasive species which arrived in the Netherlands around 1950. After an  
45 initial fast increase, large efforts have been taken to decrease population densities by  
46 muskratcatchers. Since around 2000 the number of caught muskrats per year have decreased  
47 considerably whereas the catching efforts (hours of hunting) remained the same or increased. In  
48 the central catching district in the netherlands (where most of our study areas were located), the  
49 total number of muskrats caught decreased from 174 000 in 2004 to about 24 000 in 2010, 19  
50 000 in 2012 and nearly 16 500 in 2013 (Anouk Prins, Muskrat control agency, Huizen, the  
51 Netherlands).

52 If livestock was present in the area, it was only allowed on the meadows in late summer  
53 (august), at the end of the bird breeding season. When conditions become too cold or too wet in  
54 autumn they were taken from the fields.

55

### 56 *Aquatic herbivores*

57 Visual inspection of the grazing damage on stems matched the feeding behavior of swans and  
58 goose as scars were found mostly between 10 and 50 cm above the water (van den Wyngaert  
59 and others 2003). Besides, the number of stems with signs of herbivory was highest on the

60 vegetation fringe (data not shown) and vegetation cover of both emergent and floating and  
61 submerged plants was decreased outside the exclosures (Fig. A2.1), which points towards  
62 herbivores coming from the water. We therefore assume that waterfowl can be responsible for the  
63 observed effects.

64 In our multiple stepwise regression (See appendix 3), we summed the aquatic herbivores  
65 per area which did not result in inclusion of herbivore in any of the models. When waterfowl,  
66 muskrats and livestock were entered as separate variables together with the other variables,  
67 muskrat density was included as an explanatory variable for i) the emergent cover difference  
68 between control plots and exclosures in 2012, ii) the emergent cover increase between 2011 and  
69 2012 in the control plots, iii) the proportion of species colonising the exclosures and iv) species  
70 disappearing from the control plots. Our study, in line with the results of a correlative field survey  
71 (Sarnecki and others 2011), therefore adds (correlative) evidence of a top down control of this  
72 invasive species, which still awaits experimental testing.

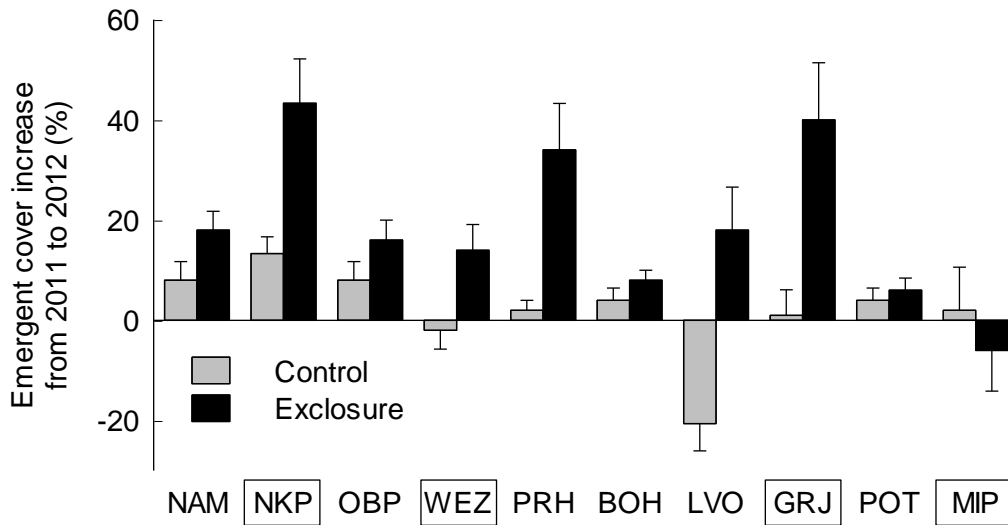
73 Because grazing effects decreased with increasing water depth, we, in line with other  
74 studies on the role of fish (Marklund and others 2002; Gayet and others 2011), suspect that  
75 grazing by fish played a minor role.

76

### 77 *Terrestrial grazers*

78 In 4 of the 10 areas, livestock was grazed on the meadows bordering the water bodies in which  
79 the control plots and exclosures were placed (Fig. A2.1; MID, on average 287 kg cows ha<sup>-1</sup>; NKP,  
80 313 kg cows ha<sup>-1</sup>; GRJ, 84 kg sheep ha<sup>-1</sup> and WEZ, 152 kg sheep ha<sup>-1</sup>. For explanation of area  
81 codes see the legend of Fig. A3.1). Signs of livestock grazing (leaf and stem damage high above  
82 the soil level (up to ca 1 m), hoof prints) were found in 9 of the 50 replicates (MID = 3 accessible  
83 replicates, NKP = 2, GRJ = 3 and WEZ = 1) and we removed 2 of those replicates (in NKP) from  
84 our analysis because the cattle destroyed the fences of the exclosures. The placement of the  
85 other plots was as such that they most likely could not be easily reached by the sheep and cows  
86 (See Fig. 1.b) as a dense *Phragmites* stand at the bankward plot end and intermediate to deep  
87 water at the open water plot end refrained livestock from entering the plot. As we observed clear

88 herbivore effects at both sites with and without livestock (both regionally; over all areas, and  
 89 locally, over the gradient from the water to land), we conclude that livestock likely did not play a  
 90 large role in our study.  
 91



92  
 93 **Figure A2.1:** Emergent cover increase (%;  $\pm$ S.E.) from July 2011 to July 2012 in the control plots  
 94 and exclosures per study area. Area order along the x-axis indicates an increasing muskrat  
 95 density. Squares around the area name indicate the presence of large grazers in those areas.  
 96 Abbreviations of area names are Naardermeer (NAM), Nieuw Keverdijkse polder (NKP),  
 97 Oostelijke Binnenpolder (OBP), De Westbroekse Zodden (WEZ), Polder Ronde Hoep (PRH),  
 98 Botshol (BOH), Loenderveen Oost (LVO), Groene Jonker (GRJ), Polder Tienhoven (POT),  
 99 Middelpolder (MIP). See Table 1 in the main manuscript for more site descriptions and muskrat  
 100 densities.

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102 **References**

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122 **Appendix 3: Correlation between environmental variables**

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124 **Methods**

125 We first correlated all measured and calculated environmental variables with each other using  
126 Pearson correlation in SPSS (SPSS 19.0; IBM Corporation, Armonk, New York). We selected a  
127 set of variables that were not correlated and provided representative measures of relevant growth  
128 conditions (Table A3.1). Subsequently, we performed multiple stepwise regressions (in SPSS)  
129 with these selected independent variables to determine which of them affected vegetation  
130 development and species turnover (Table A3.2).

131 Because water depth and vegetation N:P ratio appeared most often as determining  
132 vegetation characteristics, those were selected for further analyses.

133 **Table A3.1.** Pearson correlation coefficient between the environmental variables. Variables in the first row and first column follow the same order  
 134 and abbreviations are indicated in the first column. Methods are described in the main text. Variables in the gray area were included in the  
 135 regression analysis. Bold numbers are significant at the 0.05 level and underlined numbers are significant at the 0.01 level

136

	N:P	Mass	Depth	Fetch	Graz.	Algae	Secchi	Rat	Bird	L.St.	N	P	
137													
138	N:P - Vegetation N:P ratio (gg <sup>-1</sup> )	1											
139	Mass - Standing biomass (kgm <sup>-2</sup> )	0.198	1										
140	Depth - Water depth (m)	0.04	0.092	1									
141	Fetch - Wind fetch (m)	0.24	0.246	-0.199	1								
142	Graz. - Aquatic grazers (kgha <sup>-1</sup> )	-0.153	0.107	0.172	0.135	1							
143	Algae - Algae density (µgL <sup>-1</sup> )	<b>-0.306</b>	-0.086	<u><b>-0.375</b></u>	0.102	-0.102	1						
144	Secchi - Secchi depth (cm)	<b>0.342</b>	0.115	<u><b>0.453</b></u>	0.244	0.011	<b>-0.407</b>	1					
145	Rat - Muskrat density (kgha <sup>-1</sup> )	0.225	0.238	0.046	<b>0.304</b>	-0.238	0.11	<b>0.477</b>	1				
146	Bird - Waterfowl density (kgha <sup>-1</sup> )	-0.153	0.107	0.172	0.135	<u><b>1.000</b></u>	-0.102	0.011	-0.238	1			
147	L.St - Livestock density (kgha <sup>-1</sup> )	<b>-0.314</b>	0.054	-0.145	-0.029	-0.037	<b>0.335</b>	<b>-0.397</b>	<b>-0.325</b>	-0.037	1		
148	N - N in biomass (%DW)	-0.056	-0.202	<b>-0.297</b>	-0.014	-0.28	0.155	-0.232	0.049	-0.28	0.065	1	
149	P - P in biomass (%DW)	<u><b>-0.834</b></u>	<u><b>-0.336</b></u>	-0.102	-0.284	-0.068	<u><b>0.403</b></u>	<u><b>-0.431</b></u>	-0.195	-0.068	<b>0.305</b>	<u><b>0.425</b></u>	1
150	Water level fluctuations (m)	<b>-0.331</b>	-0.15	<u><b>-0.609</b></u>	-0.058	-0.167	0.28	<u><b>-0.593</b></u>	<u><b>-0.391</b></u>	-0.167	<u><b>0.587</b></u>	<u><b>0.411</b></u>	<u><b>0.398</b></u>



151 **Table A3.2.** Results of stepwise regression analyses on vegetation characteristics (emergent cover differences between control plots (C) and  
 152 exclosures (E), species richness, species turnover and changes over time). For each significant model, the goodness of fit ( $R^2$ ),  $F$  and  $P$ -value of  
 153 significant model selected, the independent variables included and their regression coefficients (between brackets) are given. Independent  
 154 variables were: N:P ratio of the vegetation in 2012, aboveground biomass  $m^{-2}$  2012, water depth averaged over the transect (cm), wind fetch (m),  
 155 and density of aquatic grazers ( $kg\ ha^{-1}$ ). Ns indicates when no model could be fitted.

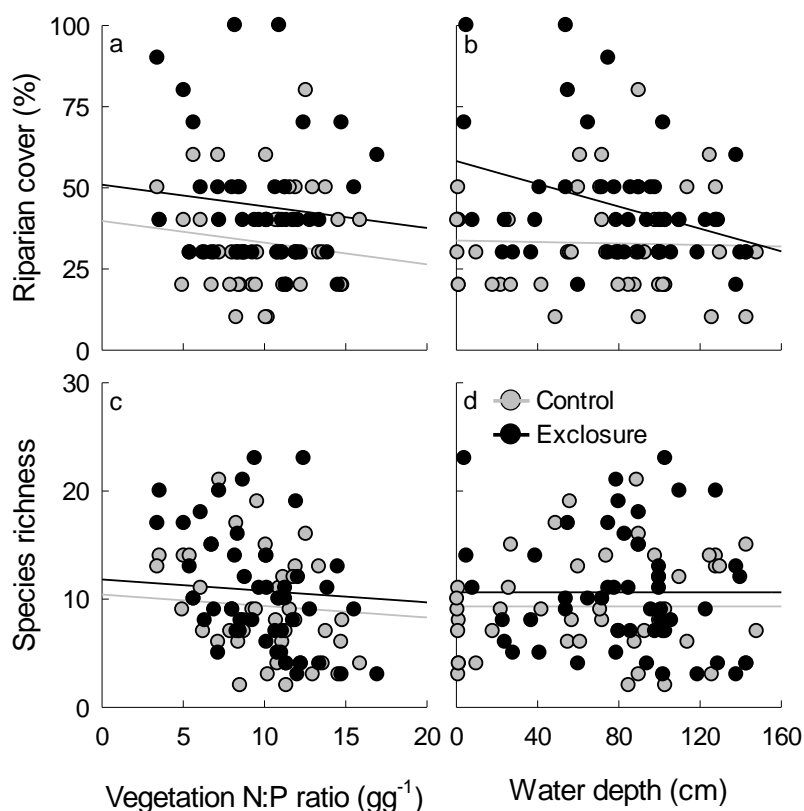
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157	<b>Dependent</b>	<b><math>R^2</math></b>	<b><math>F</math></b>	<b><math>P</math></b>	<b>Variables entered</b>
158	Emergent vegetation cover E	54.0	54.1	<0.001	Water depth (-45.78)
159	Emergent vegetation cover C	10.3	5.28	0.026	Fetch (-0.012)
160	Emergent vegetation cover difference E-C 2012	21.3	13.7	0.001	Water depth (-39.7)
161	Species richness difference E-C 2012			ns	No variables entered
162	Emergent vegetation cover increase 2011 to 2012 E	17.2	8.00	0.007	Water depth (-25.4)
163	Emergent vegetation cover increase 2011 to 2012 C			ns	No variables entered
164	Proportion of species colonised E	21.0	7.24	0.002	Water depth (-27.8) and Vegetation N:P ratio (-2.1)
165	Proportion of species colonised C	7.7	4.91	0.032	Vegetation N:P ratio (-2.6)
166	Proportion of species extinct E	34.6	25.9	<0.001	Water depth (-30.6)
167	Proportion of species extinct C	8.1	5.16	0.028	Wind fetch (0.012)

168

169 **Appendix 4: Relation between environment and species richness in 2011**

170 In 2011, we found a decreasing cover and species richness in both the control and exclosure  
171 plots (Fig. A4A.1), which was consistently higher in the exclosure plots as the interaction between  
172 vegetation N:P ratio and grazing treatment was not significant (Table A4.1). The cover decreased  
173 with water depth in the exclosures but not in the control plots (Fig. A4.1). Species richness in the  
174 plots was not related to water depth, but was affected by grazing (Fig. A4.1; Table A4.1). Similar  
175 trends were observed in 2012.



176  
177

178 **Figure A4.1:** Relation between emergent vegetation cover of the transect (a, b) and number of  
179 species per transect (c, d) with vegetation N:P ratio and Water depth in the control and exclosure  
180 plots for 2011. Vegetation N:P ratio's (gg<sup>-1</sup>) were determined from the biomass samples taken  
181 from the bank side of the plot (see methods). Water depth (cm) was averaged over the entire plot  
182 transect. Gray symbols and lines indicate data for exclosures and black symbols and lines  
183 indicate data for control plots. Each dot represents one measurement.

184 **Table A4.1:**  $\chi^2$  and *P*-values of the mixed linear models on the relation between vegetation characteristics and vegetation N:P ratio (gg<sup>-1</sup>) and  
 185 water depth (m) in 2011.

186

187	188 <b>Variable</b>	<b>Environmental covariable</b>	<b>Exclosure</b>		<b>Environmental</b>		<b>Interaction</b>	
			<b>Treatment</b>		<b>covariable</b>		<b>(Tr x Co)</b>	
			$\chi^2$	<b>P</b>	$\chi^2$	<b>P</b>	$\chi^2$	<b>P</b>
190	Emergent vegetation cover 2011 (%)	Vegetation N:P ratio	13.575	<0.001	76.582	<0.001	0.810	0.368
191		Water depth	13.880	<0.001	61.257	<0.001	4.792	0.029
192	Species richness 2011	Vegetation N:P ratio	9.481	0.002	17.348	<0.001	1.460	0.227
193		Water depth	7.328	0.007	0.409	0.523	1.676	0.195

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195