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# *Calliergon megalophyllum* rediscovered in the Netherlands after 50 years: comparison to Swedish habitats

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The moss *Calliergon megalophyllum* is rediscovered in the Netherlands after approximately 50 years of absence, in a location different from before: National Park Weerribben-Wieden. This is a Natura 2000 wetland area, and a Dutch hotspot for rich-fen bryophytes. The species was growing in a fen pool. Plant species composition and water chemistry were compared with Swedish samples collected throughout the country. Water chemistry of *C. megalophyllum* in Sweden was also compared with four other (semi-)aquatic species: *C. giganteum*, *Scorpidium scorpioides*, *Sarmentypnum trichophyllum* and *S. exannulatum*. The species is characteristic for poorly buffered habitats, but has nevertheless relatively high pH, which makes it sensitive to acidification, especially when atmospheric deposition is high. In the Dutch locality, buffer capacity is maintained by input of base-rich ditch water through small channels in the fen. The data further suggest that, like other *Calliergon* species, *C. megalophyllum* is growing in relatively nutrient-rich habitats, especially with respect to P and K. In the Netherlands, plant nutrient concentrations suggest that P is indeed not limiting, which may enhance survival of the species, as P-poor habitats in this country have become very rare.

The (semi-)aquatic moss species *Calliergon megalophyllum* Mikut. was rediscovered in the Netherlands after approximately 50 years of absence, in a location different from before: a fen pool in National Park Weerribben-Wieden, located in the northeastern part of the Holocene, peatland part of the country. National Park Weerribben-Wieden is a Natura 2000 wetland area, and a Dutch hotspot for rich-fen bryophytes (Kooijman 2012).

The last record of *C. megalophyllum* in the Netherlands was back in 1965. *Calliergon megalophyllum* has been recognised as a distinct species for a long time in Fennoscandia (Jensen 1939, Tuomikoski 1940, Nyholm 1965, Tuomikoski and Koponen 1979, Hedenäs 1993a, b, 1997), and was even recognized in the Netherlands (Karczmarz and Touw 1973). However, in the official revision of Rubers (1989), *C. megalophyllum* was included in *C. giganteum* (Schimp.) Kindb., even though there are hardly any problems with their separation, even in the field. When one of us (L. Hedenäs) visited Rijksherbarium in Leiden, the holdings of *C. giganteum* were searched for possible specimens of *C. megalophyllum*. The search

resulted in ten Dutch specimens of *C. megalophyllum*, all from Groningen or Noord-Brabant, and all except one from before 1950 (Appendix 1, van Tooren and Sparrius 2006). All specimens were found in aquatic habitats such as pools and ditches. The species was considered extinct, and when two of us (L. Hedenäs and A. M. Kooijman) visited some of the former localities in 1992, we did indeed not find it.

*Calliergon megalophyllum* has a distinct northern hemisphere distribution pattern, occurring in a belt with a latitude between approximately 60–70°, from Norway, Sweden, Finland, Russia and Canada to the USA, usually in pools and small rivers (GBIF 2015). In northern and northeastern Europe, it is relatively frequent in many areas. However, the species has occasionally been found also at lower latitudes, in Estonia, Latvia, Poland, Germany, Czech Republic, Denmark (Hedenäs 2003, Meinunger and Schröder 2007), and, as mentioned, in the Netherlands.

Although the species is widely distributed in the northern hemisphere, data on habitat conditions are scarce. In

this paper, we will characterize water chemistry and plant species composition in the new Dutch locality of *C. megalophyllum*, and compare them to the general habitat conditions of the species in Sweden. To put this characterization in a wider perspective, the Swedish water samples are also compared with four other semi-aquatic to aquatic Calliergonaceae moss species, with which *C. megalophyllum* has either been confused in the Netherlands, or ones that it sometimes grows together with in Sweden: the closely related *C. giganteum*, and the species *Scorpidium scorpioides* (Hedw.) Limpr., *Sarmentypnum trichophyllum* (Warnst.) Hedenäs, and *Sarmentypnum exannulatum* (Schimp.) Hedenäs. These species have partly similar, but also slightly different habitats. With this information, we will discuss the perspectives of survival of the species in countries such as the Netherlands, with high environmental pressure.

## Study sites and methods

*Calliergon megalophyllum* was found in October 2014 in a fen pool in National Park Weerribben-Wieden (Fig. 1), a wetland area known for its base-rich and transitional fens belonging to the Natura 2000 habitattype H7140 (van Wirdum 1991, Cusell 2014). The area is also a hotspot for characteristic rich-fen bryophytes (Kooijman 2012). The coordinates of the locality are: 52°42'54.1779"N; 6°7'5.4821"E, and a voucher is deposited in herbarium S (reg. no. B211008). The fen itself consists of a peat layer of approximately 60 cm thickness, formed on top

of cover sand from the last Ice age. The fen is mostly covered by *Sphagnum* spp. and *Polytrichum commune* Hedw. *Calliergon megalophyllum* was found in a pool of 10 cm depth in the central part of the fen, probably in remnants of isolated former lakes that terrestrialized only in the 1960s. In the National Park, fens are mainly fed by rain and surface water, as the wetland area is surrounded by lower lying agricultural polders, blocking the influence of groundwater seepage in the nature reserve itself (van Wirdum 1991). In this particular *C. megalophyllum* fen, the central parts are supplied with base-rich surface water by small ditches which are connected to larger ditches and channels.

After discovery of *C. megalophyllum*, all other plant species in the habitat were recorded. Vascular plant names are used according to van der Meijden (2005). Samples of surface water and soil pore water were collected for analysis of water chemistry. Soil samples and aboveground vascular plant biomass were collected for their nutrient composition. Depth profiles of the electrical conductivity (EC) were recorded until the sandy bottom was reached with a EC-prikstok, a sampling stick of 2 m which can measure EC and temperature at different depths (van Wirdum 1991). In addition, EC-values of the surface water were recorded with a field meter along a transect from the *C. megalophyllum* pool, through the small channels, to the ditch where the water originates from. This ditch is located relatively far away from the main channel to reduce nutrient input. EC in the main channel was also measured, and water samples for chemical analysis were collected in both ditch and channel.

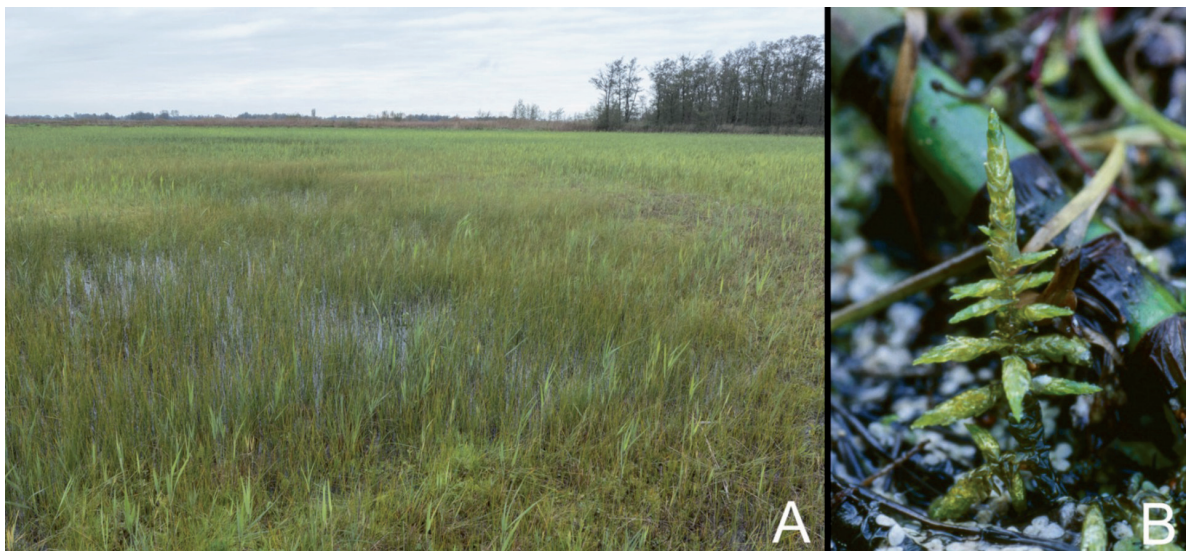


Figure 1. Habitat of *Calliergon megalophyllum* at the newly found locality in National Park Weerribben-Wieden, the Netherlands, with the species growing in the portions with open water (A), and close-up of *C. megalophyllum* in a locality in Medelpad, Sweden (B). Photographs: L. Hedenäs.

To compare the Dutch *C. megalophyllum* habitat with more general habitat characteristics of the species in Sweden, we used a part of the dataset of water chemistry and plant species composition of 481 bryophyte localities in wetlands and rich-poor fens throughout Sweden (L. Hedenäs and A. M. Kooijman unpubl.). This survey was centered on species of the Calliergonaceae and took place in 1991–1997. Even though Sweden has a large population of *C. megalophyllum*, the species is also relatively uncommon, and only 13 localities could be included (Appendix 1). Thus, to put the data for *C. megalophyllum* in a wider perspective, the Swedish water samples were also compared with four other semi-aquatic to aquatic Calliergonaceae moss species. The first one is the closely related *C. giganteum* (n = 21), with which *C. megalophyllum* has been confused in the Netherlands. The other three are *Scorpidium scorpioides* (Hedw.) Limpr. (n = 26), *Sarmentypnum trichophyllum* (Warnst.) Hedenäs (n = 6), and *S. exannulatum* (Schimp.) Hedenäs (n = 24), which are sometimes growing together with *C. megalophyllum*, and have slightly different habitats.

The pH and EC of the water samples were measured immediately after collection. The Swedish water samples were divided in two bottles, one acidified with HNO<sub>3</sub> to prevent precipitation of Fe, and the other conserved with chloroform to prevent biological changes. In the Dutch sample, alkalinity (HCO<sub>3</sub><sup>-</sup>) was also measured directly after sampling, with titration to pH 4.2, using 0.01 M HCl. For all samples, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, Cl and SO<sub>4</sub><sup>2-</sup> concentrations were measured spectrophotometrically with a Skalar auto-analyzer (Westerman 1990). For the Swedish samples, concentrations of Na, K, Ca, Mg, Fe, Al and HCO<sub>3</sub><sup>-</sup> were also measured in this way. For the Dutch samples, Na, K, Ca, Mg, Fe and Al were measured with an ICP (Westerman 1990). For the Swedish samples, ionic ratio, a proxy for the relative contribution of groundwater versus rainwater was calculated according van Wirdum (1991), based on Ca- and Cl-concentrations. In the Dutch samples, concentrations of Cl were unfortunately unreliable, as they were 13–35 times lower than Na, while they are normally more or less the same in the Dutch rich fens (I. Mettrop unpubl.). To give at least some indication of the ionic ratio of the Dutch sample, this was based on Na rather than Cl. Soil material and aboveground vascular plant tissue were dried and ground for CNS-analysis (Westerman 1990). P- and K-contents were determined after microwave-digestion with HNO<sub>3</sub>. Nutrient concentrations were measured with ICP.

As there was only one Dutch locality with *C. megalophyllum*, statistical testing of the habitat conditions was not possible, but values generally fell within the Swedish range. Differences in habitat conditions between the Swedish localities of *C. megalophyllum*, *C. giganteum*, *S. scorpioides* and *S. trichophyllum* were tested with general linear models, and post-hoc lsmeans tests.

## Results

### Plant species composition

In the new Dutch locality of *Calliergon megalophyllum*, accompanying bryophytes were *S. scorpioides*, *Calliergonella cuspidata* (Hedw.) Loeske and *Sphagnum contortum* Schultz, which point to relatively base-rich conditions (Table 1). Vascular plant species included *Carex lasiocarpa* Ehrh., *Menyanthes trifoliata* L., *Pedicularis palustris* L., *Juncus subnodulosus* Schrank and *Ranunculus flammula* L., which are also species of relatively base-rich fens. Other vascular plant species of the Dutch locality also occurred in the Swedish localities, such as *Carex rostrata* Stokes, *Comarum palustris* (L.) Scop. and *Nymphaea alba* L., the latter pointing to the former presence of open water. More eutrophic species were also present, such as *Lycopus europeus* L. and *Lythrum salicaria* L. The Swedish localities ranged from backwaters of rivers to small (ox-bow) lakes and reed shores. Besides *C. megalophyllum*, the bryophyte *Sarmentypnum exannulatum* (Schimp.) Hedenäs was frequently found, which points to slightly lower buffer capacity than in the Netherlands. Among the vascular plants, *Equisetum fluviatile* L. and *Carex aquatilis* Wahlenb. frequently occurred. In many Swedish localities, at least some relatively eutrophic species were present, such as *Caltha palustris* L., *Lysimachia vulgaris* L., *Epilobium palustre* L., *Carex pseudocyperus* L., *Phalaris arundinacea* L., *Typha latifolia* L. and *Lemna minor* L. Typically northern (fen) species were generally absent, even in the north.

### pH and buffer capacity

The Dutch locality of *C. megalophyllum* had a relatively high pH of 6.4 (Table 2). However, buffer capacity was relatively low. Electrical conductivity was only 138 µS cm<sup>-1</sup>, and Ca and HCO<sub>3</sub><sup>-</sup> concentration 289 µmol l<sup>-1</sup> (7.6 mg l<sup>-1</sup>) and 560 µmol l<sup>-1</sup> respectively. These values are 3–6 times lower than in the base-rich fens dominated by *Scorpidium* spp. in the area (Cusell et al. 2014). The pH in the Dutch locality was comparable to the Swedish ones. However, buffer capacity in the Dutch locality, albeit low for Dutch standards, was on the highest part of the Swedish range. In the Swedish localities, mean EC values were 51 (± 32) µS cm<sup>-1</sup>, and Ca-concentrations 136 (± 110) µmol l<sup>-1</sup>, which is approximately two times lower than in the Dutch locality.

In the Swedish localities, pH values for *C. megalophyllum* were also relatively high, at least compared to the other moss species (Table 3). The pH values were comparable to those of *C. giganteum*, but higher than for *S. scorpioides*, and especially *S. trichophyllum* and *S. exannulatum*. Despite high pH, however, buffer capacity for *C. megalophyllum* was relatively low. Electrical conductivity, ionic ratio,

Table 1. Plant species composition in the only *Calliergon megalophyllum* locality in the Netherlands (NL) and 12 localities throughout Sweden (samples 20–459). Nomenclature of bryophytes is according to van Tooren and Sparrius (2007), and of vascular plant species according to van der Meijden (2005), except for *Sarmentypnum tundrae* (Arnell) Hedenäs, *S. trichophyllum* (Warnst.) Hedenäs, and *Carex juncella* (Fr.) Th.Fr., which do not occur in the Netherlands.

	NL	20	175	165	195	252	167	459	461	194	150	24	147
<i>Calliergon megalophyllum</i>	*	*	*	*	*	*	*	*	*	*	*	*	*
<i>Sphagnum contortum</i>	*												
<i>Scorpidium scorpioides</i>	*	*											
<i>Calliergonella cuspidata</i>	*		*										
<i>Sarmentypnum exannulatum</i>		*	*	*	*	*	*	*	*				
<i>Drepanocladus aduncus</i>				*	*					*			
<i>Climacium dendroides</i>				*									
<i>Bryum pseudotriquetrum</i>				*									
<i>Calliergon giganteum</i>						*							
<i>Calliergon cordifolium</i>						*							
<i>Sarmentypnum tundrae</i>						*							
<i>Sphagnum squarrosum</i>						*							
<i>Sphagnum riparium</i>						*							
<i>Sarmentypnum trichophyllum</i>											*		
<i>Carex lasiocarpa</i>	*												
<i>Menyanthes trifoliata</i>	*												
<i>Hydrocotyle vulgaris</i>	*												
<i>Lythrum salicaria</i>	*												
<i>Lycopus europeus</i>	*												
<i>Pedicularis palustris</i>	*												
<i>Thelypteris palustris</i>	*												
<i>Juncus subnodulosus</i>	*												
<i>Mentha aquatica</i>	*												
<i>Ranunculus flammula</i>	*												
<i>Utricularia minor</i>	*												
<i>Carex rostrata</i>	*		*	*	*					*	*		
<i>Comarum palustris</i>	*		*	*	*	*		*	*				
<i>Galium palustre</i>	*			*	*				*	*			
<i>Cardamine pratense</i>	*			*				*	*				
<i>Nymphaea alba</i>	*				*								
<i>Peucedanum palustre</i>	*		*										
<i>Phragmites australis</i>	*		*			*							
<i>Utricularia intermedia</i>	*					*							
<i>Equisetum fluviatile</i>		*	*	*	*		*	*	*				
<i>Utricularia vulgaris</i>		*											
<i>Subularia aquatica</i>		*											
<i>Sparganium</i> sp.		*							*			*	
<i>Myrica gale</i>			*			*							
<i>Lysimachia vulgaris</i>			*			*							



Table 1. Continued.

	NL	20	175	165	195	252	167	459	461	194	150	24	147
<i>Carex aquatilis</i>			*				*	*	*	*			
<i>Carex vesicaria</i>			*					*	*				
<i>Hydrochaeris morsus-rana</i>			*										
<i>Myosotis scorpioides</i>			*										
<i>Caltha palustris</i>			*	*				*					
<i>Filipendula ulmaria</i>				*									
<i>Juncus filiformis</i>				*									
<i>Carex curta</i>				*									
<i>Calamagrostis canescens</i>				*									
<i>Carex juncella</i>				*									
<i>Potamogeton natans</i>					*		*				*		
<i>Epilobium palustre</i>					*						*		
<i>Equisetum palustre</i>					*								
<i>Calla palustris</i>					*								
<i>Cicuta virosa</i>						*							
<i>Lysimachia thyrsiflora</i>						*							
<i>Carex pseudocyperus</i>						*							
<i>Scirpus lacustris</i>						*							
<i>Carex elata</i>						*							
<i>Carex diandra</i>						*							
<i>Sparganium erectum</i>							*						
<i>Nymphaea candida</i>							*						
<i>Phalaris arundinacea</i>								*					
<i>Veronica scutellata</i>								*	*				
<i>Hippuris vulgaris</i>									*				
<i>Ranunculus repens</i>									*				
<i>Typha latifolia</i>									*	*			
<i>Myosotis laxa</i>										*			
<i>Rorippa palustris</i>										*			
<i>Alisma plantago-aquatica</i>										*			
<i>Sagittaria sagitifolius</i>										*			
<i>Poa palustris</i>										*			
<i>Lemna minor</i>										*			
<i>Glyceria fluitans</i>										*			
<i>Polygonum amphibium</i>										*			
<i>Alopecurus geniculatus</i>										*			
<i>Sparganium minimum</i>											*		
<i>Potamogeton</i> sp.												*	

Table 2. Chemical composition of the surface water in the only *Calliergon megalophyllum* locality in the Netherlands (NL) and 13 localities throughout Sweden (samples 20–459). \* = ionic ratio (IR) in the Netherlands was based on Ca and Na, instead of Ca and Cl, because Cl-concentration measurements were unreliable.

	NL	20	175	165	195	252	167	459	461	194	150	24	147	332
pH	6.4	6.5	6.7	6.2	6.9	6.6	6.4	6.8	6.9	6.7	6.7	6.6	6.9	7.0
EC ( $\mu\text{S cm}^{-1}$ )	138	44	37	52	23	98	28	43	75	54	33	14	37	130
IR	0.34*	0.71	0.56	0.64	0.22	0.72	0.38	0.67	0.78	0.56	0.58	0.47	0.84	0.60
water level (cm)	1	0	-4	4	-10	-10	3	-4	-4	-6	-20	0	0	6
Ca ( $\mu\text{mol l}^{-1}$ )	289	103	67	103	15	214	53	116	206	98	78	38	295	391
HCO <sub>3</sub> <sup>-</sup> ( $\mu\text{mol l}^{-1}$ )	560	344	394	393	196	412	98	301	572	377	230	49	246	659
Fe ( $\mu\text{mol l}^{-1}$ )	4	7	5	6	3	4	3	14	17	4	5	10	4	4
SO <sub>4</sub> <sup>2-</sup> ( $\mu\text{mol l}^{-1}$ )	115	31	57	52	41	121	31	49	53	56	31	31	10	94
NH <sub>4</sub> <sup>+</sup> ( $\mu\text{mol l}^{-1}$ )	0	0	12	16	36	0	4	14	14	0	18	2	10	4
NO <sub>3</sub> <sup>-</sup> ( $\mu\text{mol l}^{-1}$ )	0.0	0.0	0.5	0.3	0.6	1.5	0.3	0.0	0.0	2.3	1.3	0.3	1.3	0.0
PO <sub>4</sub> <sup>3-</sup> ( $\mu\text{mol l}^{-1}$ )	0.30	0.00	0.32	0.63	0.21	116	0.11	0.42	0.63	0.53	0.21	0.00	0.32	0.11
K ( $\mu\text{mol l}^{-1}$ )	72	23	14	23	72	7	54	24	21	36	18	3	10	18

Ca- and HCO<sub>3</sub><sup>-</sup>-concentrations were significantly higher for *C. giganteum* and *S. scorpioides* than for *C. megalophyllum*. Buffer capacity of the latter species was instead more comparable to *S. trichophyllum* and especially *S. exannulatum*. All three species had relatively low values for EC, IR, Ca and HCO<sub>3</sub><sup>-</sup>, although lowest values were reached for *S. trichophyllum*. These results support that the *C. megalophyllum* habitat has relatively high pH, but low buffer capacity in both Sweden and the Netherlands.

In the Dutch locality, the *C. megalophyllum* pool was located inside the fen, isolated from the surface water, except for the small ditches which presumably provide base-

rich water from the ditch. In the pool, EC-values were below 150  $\mu\text{S cm}^{-1}$  from the surface to a depth of 50 cm, and EC-values lower down were hardly higher (Fig. 2). In the small ditch system, EC values were around 231  $\mu\text{S cm}^{-1}$  in the ditch, and gradually decreased along the way to 138  $\mu\text{S cm}^{-1}$  (Fig. 3). In the ditch, EC values were approximately two times lower than in the main channel, which does not provide water to the fen and pool directly, due to the long pathway before the water reaches the supplying ditch. This was also true for Ca-concentrations, which were 989 and 559  $\mu\text{mol l}^{-1}$  in the main channel and supplying ditch, respectively.

Table 3. Chemical composition of surface water in localities throughout Sweden for *Calliergon megalophyllum* (n = 13), compared to three other (semi-)aquatic moss species *Calliergon giganteum* (n = 21), *Scorpidium scorpioides* (n = 26), *Sarmentypnum trichophyllum* (n = 6) and *Sarmentypnum exannulatum* (n = 24). Values given are mean values and standard deviations. \* = significant general effect ( $p < 0.05$ ); different letters indicate significant differences for a parameter between particular species.

	<i>C. megalophyllum</i>	<i>C. giganteum</i>	<i>S. scorpioides</i>	<i>S. trichophyllum</i>	<i>W. exannulatum</i>
pH*	6.7 (0.2) <sup>c</sup>	6.7 (0.6) <sup>c</sup>	6.3 (0.6) <sup>b</sup>	6.0 (0.7) <sup>ab</sup>	5.6 (0.6) <sup>a</sup>
EC ( $\mu\text{S cm}^{-1}$ )*	51 (32) <sup>a</sup>	142 (102) <sup>b</sup>	111 (107) <sup>b</sup>	42 (24) <sup>a</sup>	60 (65) <sup>a</sup>
Ionic ratio*	0.60 (0.17) <sup>a</sup>	0.76 (0.16) <sup>b</sup>	0.74 (0.15) <sup>b</sup>	0.46 (0.13) <sup>a</sup>	0.50 (0.16) <sup>a</sup>
Water level (cm)*	-3.4 (7.0) <sup>a</sup>	3.2 (1.5) <sup>b</sup>	1.5 (2.1) <sup>b</sup>	-6.8 (7.0) <sup>a</sup>	2.0 (3.9) <sup>b</sup>
Ca ( $\mu\text{mol l}^{-1}$ )*	136 (110) <sup>a</sup>	465 (447) <sup>b</sup>	400 (443) <sup>b</sup>	68 (28) <sup>a</sup>	141 (168) <sup>a</sup>
HCO <sub>3</sub> <sup>-</sup> ( $\mu\text{mol l}^{-1}$ )*	328 (171) <sup>a</sup>	1034 (880) <sup>b</sup>	861 (841) <sup>b</sup>	164 (31) <sup>a</sup>	266 (236) <sup>a</sup>
Fe ( $\mu\text{mol l}^{-1}$ )	7 (4) <sup>a</sup>	10 (9) <sup>a</sup>	18 (23) <sup>b</sup>	8 (5) <sup>a</sup>	23 (28) <sup>b</sup>
SO <sub>4</sub> <sup>2-</sup> ( $\mu\text{mol l}^{-1}$ )	51 (30) <sup>a</sup>	96 (67) <sup>b</sup>	80 (74) <sup>ab</sup>	82 (52) <sup>ab</sup>	72 (40) <sup>ab</sup>
NH <sub>4</sub> <sup>+</sup> ( $\mu\text{mol l}^{-1}$ )	10 (10) <sup>a</sup>	19 (35) <sup>a</sup>	11 (19) <sup>a</sup>	14 (17) <sup>a</sup>	5 (7) <sup>a</sup>
NO <sub>3</sub> <sup>-</sup> ( $\mu\text{mol l}^{-1}$ )*	0.6 (0.7) <sup>a</sup>	1.5 (1.4) <sup>a</sup>	1.0 (1.4) <sup>a</sup>	5.9 (11.0) <sup>b</sup>	0.8 (0.9)
PO <sub>4</sub> <sup>3-</sup> ( $\mu\text{mol l}^{-1}$ )*	0.36 (0.32) <sup>b</sup>	0.26 (0.27) <sup>ab</sup>	0.12 (0.16) <sup>a</sup>	0.14 (0.05) <sup>a</sup>	0.14 (0.15) <sup>a</sup>
K ( $\mu\text{mol l}^{-1}$ )*	25 (19) <sup>b</sup>	25 (40) <sup>b</sup>	7 (9) <sup>a</sup>	7 (8) <sup>a</sup>	10 (10) <sup>a</sup>

0 cm	138
10 cm	117
20 cm	112
30 cm	69
40 cm	120
50 cm	179
60 cm	183
sand bottom	

Figure 2. EC-depth profile ( $\mu\text{S cm}^{-1}$ ) in the Dutch locality with *Calliergon megalophyllum*, measured in october 2014.

### Nutrient availability

As already indicated, almost all Dutch and Swedish relevés contained at least some eutrophic species. Ammonium and nitrate seemed less important, but in both the Dutch and Swedish localities of *C. megalophyllum*, phosphate concentrations in the water were relatively high, with (mean) values of around  $0.3 \mu\text{mol l}^{-1}$ . In the Swedish localities, phosphate concentrations were also significantly higher for *C. megalophyllum* than for *S. scorpioides*, *S. trichophyllum* and *S. exannulatum*, with intermediate values for the closely related *C. giganteum*. More or less the same was true for K-concentrations, with higher values for the two *Calliergon* species. Because Fe and  $\text{SO}_4^{2-}$  may both affect P-availability, they were measured as well. However, concentrations were low in general, and did not generally differ between moss species. In the Dutch locality with *C. megalophyllum*, concentrations of N and K in vascular plant tissue showed values of  $16.5$  and  $15.3 \text{ mg g}^{-1}$  (Table 4), which are not elevated or very low. However, P-concentrations were relatively high, and showed values of  $1.2 \text{ mg g}^{-1}$ . Also, the N:P ratio of  $13.8$  was relatively low (Koerselman and Meuleman 1996).

## Discussion and conclusions

Based on the available information, the results suggest that the habitat conditions in the new Dutch locality of *Calliergon megalophyllum* fit well within the range described for Sweden. Also, comparison of Swedish localities for *C. megalophyllum* and the closely related *C. giganteum* makes clear that the two species have actually different habitats, especially with respect to buffer capacity. For *C. giganteum*, buffer capacity was approximately three

canal	364
ditch	231
10 m	240
20 m	172
30 m	176
40 m	156
50 m	181
60 m	181
70 m	152
80 m	195
90 m	142
100 m	146
pool	138

Figure 3. EC-surface profile ( $\mu\text{S cm}^{-1}$ ) in the dutch locality with *Calliergon megalophyllum*, from the ditch where the water comes from, through a series of small ditches inside the fen, to the pool where the species was found. EC-values were measured in October 2014. The mean EC-value in the main canal, which is not directly connected to fen and pool, is also given.

times higher than for *C. megalophyllum*. These habitat differences further supports that they are really different species (Jensen 1939, Tuomikoski 1940, Nyholm 1965, Tuomikoski and Koponen 1979, Hedenäs 1993a, b), in contrast to what was assumed by Rubers (1989).

### High pH and low buffer capacity

*Calliergon megalophyllum* is characteristic for habitats with a high pH of  $6.4\text{--}7.0$ , but nevertheless low buffer capacity. This is a difficult combination, as low buffer capacity may be advantageous to photosynthesis under water, but more difficult to combat acidification of the habitat. Low buffer capacity may to some extent help photosynthesis of submerged mosses. Under water,  $\text{CO}_2$  supply may be a problem due to low rates of diffusion, but is also chemically limited at high pH, when the main carbon form is  $\text{HCO}_3^-$ . Many aquatic macrophytes can use bicarbonate as alternative carbon source (Madsen and Sand-Jensen 1991), but there is no evidence that bryophytes can do this, except perhaps *Fontinalis antipyretica* Hedw. (Glime and Vitt 1984, Penuelas 1985). When buffer capacity is low at relatively high pH, submerged mosses may be able to capture at least some  $\text{CO}_2$  from the water. This may

Table 4. Nutrient concentrations in ( $\text{g kg}^{-1}$ ) and ratios in plant and soil in the Dutch locality with *Calliergon megalophyllum*.

	N	P	K	C:N ratio	N:P ratio	N:K ratio
Plant biomass	16.5	1.2	15.3	28.7	13.8	1.1
Soil material	14.8	0.6	1.2	34.4	24.7	12.1



be a reason why *S. trichophyllum*, which has lower pH and slightly lower buffer capacity than *C. megalophyllum*, is growing completely submerged, while the latter was found at or above the water surface in 50% of the cases. When buffer capacity becomes too high, such as for, e.g. *C. giganteum* and *S. scorpioides*, growing above the water may be a better option.

While low buffer capacity may be an advantage for photosynthesis in aquatic bryophytes, it is a problem to maintain a relatively high pH, especially in areas with high atmospheric deposition. In the Netherlands, weakly buffered habitats with *S. scorpioides* in the Pleistocene, sandy part of the country were still present in the 1960s (Kooijman and Westhoff 1985, Arts 1990). However, the species has disappeared from this region, probably due to high atmospheric deposition. In the Netherlands, approximately 1.5 times higher amounts of buffer components are needed to maintain the pH in the habitat around 6.5 compared to less polluted areas in e.g. Sweden (Kooijman 2012). It is not unlikely that *C. megalophyllum* has disappeared from part of the former Dutch localities by acidification in a similar way.

In the new Dutch locality, it is not clear whether *C. megalophyllum* is really new to the area, or whether it has been there for a long time. In any case, the buffer capacity is relatively high compared to Sweden, albeit low compared to real rich-fen habitats in the National Park dominated by *Scorpidium* spp. (Cusell et al. 2014). Buffer capacity is presumably maintained by input of relatively base-rich ditch water into the small channel system during high water levels in the National Park. In other parts of the National Park, similar channels, with higher buffer capacity, have led to an increase of rich-fen bryophytes such as *S. scorpioides*, *S. cossoni* (Schimp.) Hedenäs and *Hamatocaulis vernicosus* (Mitt.) Hedenäs (A. M. Kooijman, L. Hedenäs, I. Mettrop and C. Cusell unpubl.). More systematic research is needed, but it is very likely that occasional flooding with base-rich, relatively nutrient-poor water can mitigate at least some of the adverse effects of high atmospheric deposition.

## Nutrient availability

*Calliergon megalophyllum* seems to prefer intermediate to nutrient-rich habitats. In both Sweden and the Netherlands, phosphate concentrations in the water were relatively high, and eutrophic plant species occurred in almost all relevés. In at least some of the old Dutch localities, where *C. megalophyllum* has disappeared, eutrophic species were also present, such as *Glyceria maxima* (Hartm.) Holmb. and *Lycopus europeus* in the Belversven in 1947 (Appendix 1). Also, plant N:P ratios in the present locality suggest that P is not a limiting factor. Other *Calliergon* species are also growing in relatively nutrient-rich habitats, such as *C. cordifolium* and to some extent *C. giganteum*.

This may be an inherent habitat trait of the genus, inherited from ancestors during the evolution of the Calliergonaceae (Hedenäs and Kooijman 1996).

Growing under relatively nutrient-rich conditions may be an advantage in the Netherlands, where eutrophication in wetlands is a real problem (Lamers et al. 2014). In the National Park, approximately 79% of the annual P-input comes from adjacent agricultural polders, especially in winter (Cusell 2014). Nevertheless, values around  $0.3 \mu\text{mol P l}^{-1}$  in the water are not really high, and *C. megalophyllum* would probably not survive hypertrophic conditions. In some of the old Dutch localities, *C. megalophyllum* may have disappeared due to eutrophication of the water, such as in former ditches. In the National Park Weerribben-Wieden, its present locality, however, nutrient levels have decreased in the National Park over the past decades. Although total P did not decrease, phosphate levels in the main channels of the National Park have clearly dropped to values around  $0.1 \mu\text{mol l}^{-1}$  (Cusell 2014). In one of the best rich-fen complexes in the area, eutrophic bryophytes such as *C. cuspidata* have been replaced along the shoreline by characteristic species such as *S. scorpioides*, while aboveground vascular biomass decreased from approximately 1000 to  $250 \text{ g m}^{-2}$ . A further decrease in P-input is much more difficult to achieve, but there is no reason to say that the present conditions are not suitable for *C. megalophyllum*, especially when buffer capacity in the habitat is maintained the way it is.

## Concluding remarks

It is unknown why *C. megalophyllum* has disappeared from the Netherlands in the past. However, the particular habitat conditions of high pH, but low buffer capacity suggest that acidification has played at least some role, similar to the disappearance of weakly buffered habitats with *S. scorpioides* (Kooijman and Westhoff 1995). In addition, pollution of surface waters in the 1960s may have led to local extinction, although *C. megalophyllum* is a relatively eutrophic species and can at least stand slightly elevated nutrient levels, such as in the new Dutch locality. Whether it will survive there probably mainly depends on the buffer capacity, which is maintained by input of base-rich surface water through small channels. Whether the species can expand in the Netherlands is more difficult to say, since weakly buffered habitats with relatively high pH have become very rare.

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Appendix 1. Site description of *Calliergon megalophyllum* localities in NL (present and extinct) and Sweden (only localities sampled in this paper). The general position of the extinct Dutch localities is indicated on the map in van Tooren and Sparrius (2006).

Sample	Year	Site description
<u>The Netherlands</u>		
NL	2014	small pool in fen, National Park Weerribben-Wieden, 52°42'54.17"N; 6°70'54.82"E
NL	1864	Haren, Groningen; among <i>Fontinalis antipyretica</i> , together with <i>C. giganteum</i>
NL	1898	ditch, den Bosch, Noord-Brabant
NL	1898	ditch, Nieuwkuijk, Noord-Brabant
NL	1935	mill canal, Harendermolen, Groningen
NL	1943	in river Rosep, Oisterwijk, Noord-Brabant
NL	1947	shallow lake, Belversven, Noord-Brabant; with <i>Phragmites australis</i> , <i>Schoenoplectis lacustris</i> , <i>Glyceria maxima</i> , <i>Nymphaea alba</i> , <i>Menyanthes trifoliata</i> , <i>Carex rostrata</i> , <i>Comarum palustris</i> , <i>Peucedanum palustre</i> , <i>Mentha aquatica</i> , <i>Galium palustre</i> , <i>Carex curta</i> , <i>Lysimachia vulgaris</i> , <i>Lysimachia thyrsiflora</i> , <i>Lycopus europeus</i> , <i>Cicuta virosa</i> , <i>Scutellaria galericulatum</i> , <i>Rumex hydrolapathum</i>
NL	1948	shallow lake. Belversven, Noord-Brabant
NL	1948	shallow lake, Belversven, Noord-Brabant
NL	1948	shallow lake with <i>Phragmitetum</i> , Belversven, Noord-Brabant
NL	1965	partly terrestrialized shallow lake, Moerputten Vlijmen, Noord-Brabant; with <i>Comarum palustris</i>
<u>Sweden</u>		
S20	1991	backwater (ava) Stenselestryckan in River Vindelälven, Björksele, Lycksele Lappmark, 65°01'04.62"N, 18°45'47.25"E
S24	1991	backwater, Vindelgransele, Björksele, Lycksele Lappmark, 65°10'32.64"N, 18°29'55.83,,E
S147	1991	backwaters of River Umeälven, Kattisavan, Lycksele, Lycksele Lappmark, 64°75'75.45"N, 18°16'29.71"E
S150	1991	backwaters of River Umeälven, Kattisavan, Lycksele, Lycksele Lappmark, 64°75'75.45"N, 18°16'29.71"E
S165	1991	old backwater (ava), 2 km ESE of Strandåker, Vindeln, Västerbotten, 64°27'81.26"N, 19°24'57.76"E
S167	1991	old backwater (ava), W shore of Vindelälven opposite Renfors, Vindeln, Västerbotten, 64°21'77.69"N, 19°70'13.40"E
S175	1993	shore of the dammed River Dalälven, the island Kvarnön, W side, Söderfors, Uppland, 60°41'35.33"N, 17°20'46.70"E
S194	1993	small pond near Filadelfia church, Djurås, Gagnef, Dalarna, 60°55'59.58"N, 15°12'01.53"E
S195	1993	Lake Gallsjön, Överbacka, Gagnef, Dalarna, 60°56'49.46"N, 15°10'94.24"E
S252	1993	reed belt at S shore of Lake Mindalssjön, Turinge, Södermanland, 59°19'76.38"N, 17°49'33.76"E
S332	1996	reed belt at S shore of Lake Hacksjön, Botkyrka, Södermanland, 59°17'76.70"N, 17°94'59.05"E
S459	1997	ox-bow lakes W of the River Dammån, ca 1 km S of Lillstavallen, Mattmar, Jämtland, 63°23'50.51"N, 13°88'19.91"E
S461	1997	ox-bow lakes W of the River Dammån, ca 1.25 km S of Lillstavallen, Mattmar, Jämtland, 63°23'30.60"N, 13°88'68.05"E