SUPPLEMENTARY MATERIAL

Biochemical determinants of litter quality in 15 species of *Sphagnum*,

Plant and Soil

Fia Bengtsson*, Håkan Rydin, Tomáš Hájek

*Corresponding author: Uppsala University, fia.bengtsson@ebc.uu.se

Supplement 1: Methods for metabolite extractions

Some of the biochemical extractions that follow earlier work will be thoroughly described below.

Holocellulose

To obtain the fraction of structural polysaccharides dried litter was coarsely homogenised with a food processor and bleached, following (Ballance et al. 2007) as follows:

We put 0.9–1.4 g of litter and 150 mL deionised water into a 250 mL Erlenmeyer flask and placed in a water bath (75 °C). After temperature equilibration, we added 1.5 mL concentrated acetic acid followed by six 1-mL doses of 25% sodium chlorite solution every 10 min during the first hour. The flasks were loosely capped with inverted 25 mL Erlenmeyer flasks in order to keep liberated chlorine dioxide and shaken by hand every 10–15 minutes until the end of bleaching. After 3 h we repeated the additions of acetic acid and sodium chlorite and after another 3 h, the slurry was cooled and filtered through a 250 μm nylon mesh. The bleached litter was then thoroughly washed with 20 mM HCl and deionised water and dehydrated with acetone. Holocellulose in its acidic form was dried at 60 °C and weighed.

Sphagnan

Sphagnan extraction by acid autohydrolysis follows Ballance et al. (2007), and was performed as below:

We placed 0.5–0.7 g of the extracted HC into 100 mL glass flasks and filled the flasks with 40 mL of boiling (i.e. degassed) deionized water. Headspaces of the flasks were flushed with nitrogen in order to minimize oxygen concentration during the extractions. The flasks were heated at 98 °C for 72 h. The slurries were then transferred into 50 mL PP tubes and centrifuged at 2000 ×*g* for 20 minutes. The supernatants were collected and the pellets resuspended with deionized water and centrifuged again. Both supernatants were pooled and filtered through 1 μm glass microfiber filters and 0.45 μm membrane filters. All the 90 filtrates were then dialysed together using dialysis tubings (MWCO of 12 kDa) in a shaking barrel with 30 L of deionized water for 48 h (the water was exchanged after 24 h) and freeze dried.

Soluble phenolics

For extraction of soluble phenolics we followed Bärlocher and Graça (2005), the extraction was performed as follows:

We added 8 mL of acetone (70%) into 95–100 mg of ball-milled litter in 15 mL PP conical centrifuge tubes. After 70 min of shaking at 21 °C we centrifuged the suspension at 4777 ×*g* for 20 min. We collected 0.75 mL of the supernatant and diluted it with 0.25 mL deionised water; then 5 mL of 2% Na_2CO_3 dissolved in 0.1 M NaOH was added and the tube was shortly shaken. After 5 min we added 0.5 mL

Folin-Ciocalteu reagent and shook the tubes. After two hours the intensity of blue coloration was measured spectrophotometrically at 760 nm.

References

Ballance S, Børsheim KY, Inngjerdingen K, Paulsen B, Christensen B (2007) A re-examination and partial characterisation of polysaccharides released by mild acid hydrolysis from the chlorite-treated leaves of *Sphagnum papillosum*. Carbohydrate Polymers 67:104-115. doi: 10.1016/j.carbpol.2006.04.020

Bärlocher F, Graça MAS (2005) Total phenolics. In: Graça MAS, Bärlocher F, Gessner MO (eds) Methods to study litter decomposition: A practical guide Springer, Dordrecht, pp 45–48

SUPPLEMENTARY MATERIAL

Biochemical determinants of litter quality in 15 species of *Sphagnum,* Plant and Soil

Fia Bengtsson*, Håkan Rydin, Tomáš Hájek
*Corresponding author affiliation: Uppsala University, contact: fia.bengtsson@ebc.uu.se

Supplementary Table 1. Biochemical variables and mass loss in standard laboratory conditions of Sphagnum litters (means ± SE).

Species	Section	Holo-cellulose mg g^{-1}	Sphagnan mg g^{-1}	Soluble phenolics mg g^{-1}	Klason lignin mg g^{-1}	Soluble KL % of total KL	Total KL mg g^{-1}	CEC µeq g^{-1}	C mg g^{-1}	N mg g^{-1}	C:N ratio	PO_4 –P µg g^{-1}	Deviance from 100% (dev100)	205:280 nm absorbance ratio	Lab litter mass loss %
S. angustifolium	Cuspidata	674 ± 6.7	33.1 ± 2.2	5.9 ± 0.43	178 ± 30	20.8 ± 3.3	220 ± 28	640 ± 24	440 ± 0.6	6.3 ± 0.37	70.8 ± 4	488 ± 63	-10.6 ± 3.04	2.06 ± 0.02	62.5 ± 1.6
S. balticum	Cuspidata	720 ± 5	31.8 ± 3	5.7 ± 0.46	165 ± 21	25 ± 2.7	217 ± 21	621 ± 14	442 ± 1.1	4.5 ± 0.07	99.3 ± 1.5	160 ± 9	-6.3 ± 1.91	2.15 ± 0.03	43.8 ± 3.9
S. capillifolium	Acutifolia	700 ± 7	46.3 ± 3.6	7.8 ± 0.43	289 ± 32	13.8 ± 1.8	333 ± 30	869 ± 18	443 ± 1.3	5.1 ± 0.29	88.8 ± 5.2	261 ± 34	3.3 ± 3.57	2.22 ± 0.02	16.6 ± 3.7
S. contortum	Subsecunda	711 ± 6.6	48.1 ± 1.8	8.6 ± 0.44	251 ± 22	16.2 ± 1.3	298 ± 21	644 ± 29	442 ± 1.8	7.1 ± 0.51	64 ± 4.9	281 ± 9	0.9 ± 2.68	2.23 ± 0.04	26.6 ± 0.5
S. cuspidatum	Cuspidata	668 ± 16.4	25.3 ± 1.0	6.6 ± 0.36	206 ± 26	20.3 ± 2.8	255 ± 25	511 ± 9	443 ± 1.6	7.4 ± 1.02	63.6 ± 7.6	215 ± 17	-7.7 ± 3.49	2.15 ± 0.09	62.5 ± 1.6
S. fallax	Cuspidata	654 ± 9.9	34.4 ± 1.6	5.6 ± 0.32	138 ± 23	27.1 ± 3.2	186 ± 21	602 ± 9	440 ± 0.8	5.5 ± 0.24	80.7 ± 3.5	265 ± 25	-16 ± 2.58	1.94 ± 0.02	46.5 ± 3.3
S. fuscum - bog	Acutifolia	684 ± 3.8	46.7 ± 2.7	10.1 ± 0.41	352 ± 11	10.3 ± 0.5	392 ± 11	913 ± 4	451 ± 1.5	4 ± 0.17	114.3 ± 5	169 ± 15	7.6 ± 1.3	2.25 ± 0.01	8.4 ± 2.1
S. fuscum - fen	Acutifolia	678 ± 5.9	47.3 ± 3	15.3 ± 0.67	313 ± 12	11.9 ± 0.3	355 ± 12	850 ± 17	451 ± 1.4	5.2 ± 0.27	87.9 ± 5.1	286 ± 28	3.4 ± 1.06	2.28 ± 0.03	16.7 ± 1.2
S. girgensohnii	Acutifolia	691 ± 13.1	40.3 ± 2.7	7.3 ± 0.49	269 ± 28	14.4 ± 1.7	313 ± 25	798 ± 28	439 ± 1.3	9.4 ± 1.15	49 ± 4.9	1049 ± 164	0.3 ± 3.25	2.38 ± 0.05	45.2 ± 3.5
S. lindbergii	Cuspidata	661 ± 8.8	35.4 ± 2.8	7.8 ± 0.37	227 ± 26	18.4 ± 2.3	275 ± 24	644 ± 39	450 ± 1	6.5 ± 0.59	71.1 ± 6	194 ± 10	-6.4 ± 1.95	2.15 ± 0.03	28.5 ± 2.3
S. magellanicum - open bog	Sphagnum	671 ± 7.7	51.2 ± 3.3	8.7 ± 0.3	278 ± 7	12.9 ± 0.3	319 ± 7	734 ± 60	445 ± 1.1	5.8 ± 0.21	77.3 ± 3	388 ± 24	-1.1 ± 1.1	2.28 ± 0.02	23.5 ± 2.5
S. magellanicum - pine bog	Sphagnum	671 ± 4.4	42.7 ± 2.1	8.1 ± 0.47	315 ± 20	11.5 ± 0.9	356 ± 19	744 ± 10	452 ± 1.7	7.1 ± 0.93	67.2 ± 6.9	150 ± 19	2.6 ± 1.75	2.3 ± 0.04	19.3 ± 3.7
S. magellanicum - spruce forest	Sphagnum	652 ± 6.3	40.3 ± 3.7	8.4 ± 0.7	262 ± 25	13.7 ± 1.5	303 ± 23	753 ± 20	443 ± 0.5	9.3 ± 0.47	48.3 ± 2.4	823 ± 88	-4.5 ± 2.56	2.31 ± 0.03	38.7 ± 6.6
S. majus	Cuspidata	679 ± 11.7	34.3 ± 1.5	7.4 ± 0.7	186 ± 29	22.7 ± 2.9	237 ± 27	562 ± 28	445 ± 0.5	6.3 ± 0.76	74 ± 7.5	215 ± 23	-8.5 ± 3.35	2.16 ± 0.03	47.5 ± 5.2
S. papillosum	Sphagnum	636 ± 8.7	32.7 ± 3	9.8 ± 0.71	294 ± 24	11.8 ± 1	333 ± 24	642 ± 11	453 ± 1	6.3 ± 0.54	73.8 ± 6.7	197 ± 27	-3.2 ± 2.74	2.23 ± 0.04	29.2 ± 3.3
S. rubellum	Acutifolia	702 ± 8	39.8 ± 1.7	7.5 ± 0.34	238 ± 11	17.7 ± 1.2	289 ± 10	868 ± 13	446 ± 1.9	4.6 ± 0.26	97.1 ± 4.8	181 ± 10	-1 ± 0.69	2.27 ± 0.02	38.1 ± 2.2
S. tenellum	Cuspidata	712 ± 3.1	36.3 ± 1.9	5.4 ± 0.17	142 ± 20	30.6 ± 3.8	200 ± 18	644 ± 7	445 ± 0.6	5.1 ± 0.31	89 ± 4.8	207 ± 36	-8.8 ± 1.9	2.26 ± 0.01	40.4 ± 3.3
S. warnstorfii	Acutifolia	728 ± 6	52.6 ± 3.7	10.8 ± 0.32	261 ± 17	16.3 ± 1.2	311 ± 16	869 ± 9	448 ± 0.9	6 ± 0.14	74.9 ± 1.8	247 ± 14	3.9 ± 1.49	2.33 ± 0.03	19.7 ± 2

SUPPLEMENTARY MATERIAL

Biochemical determinants of litter quality in 15 species of *Sphagnum,* Plant and Soil

Fia Bengtsson*, Håkan Rydin, Tomáš Hájek

*Corresponding author: Uppsala University, fia.bengtsson@ebc.uu.se

Supplementary Table 2. Tests of differences among sections, section pairs with significant differences (P<0.05) in bold.

Sphagnan						
ANOVA				Tukey test	diff	P
Source	df	F	P	**Acutifolia vs Cuspidata**	**12.73**	**< 0.0001**
Section	3	19.69	< 0.0001	Acutifolia vs Sphagnum	3.88	0.25
Error	82			Acutifolia vs Subsecunda	-2.48	0.89
				Cuspidata vs Sphagnum	**-8.84**	**0.0002**
				Cuspidata vs Subsecunda	**-15.21**	**0.0001**
				Sphagnum vs. Subsecunda	-6.37	0.28
CEC						
ANOVA				Tukey test	diff	P
Source	df	F	P	**Acutifolia vs Cuspidata**	**257.8**	**< 0.0001**
Section	3	90.05	< 0.0001	**Acutifolia vs Sphagnum**	**142.9**	**< 0.0001**
Error	86			**Acutifolia vs Subsecunda**	**216.6**	**< 0.0001**
				Cuspidata vs Sphagnum	**-115.0**	**< 0.0001**
				Cuspidata vs Subsecunda	-41.2	0.53
				Sphagnum vs. Subsecunda	73.8	0.10
Soluble phenolics (log transformed)						
ANOVA				Tukey test	diff	P
Source	df	F	P	**Acutifolia vs Cuspidata**	**0.41**	**< 0.0001**
Section	3	20.9	< 0.0001	Acutifolia vs Sphagnum	0.09	0.53
Error	86			Acutifolia vs Subsecunda	0.09	0.83
				Cuspidata vs Sphagnum	**-0.32**	**< 0.0001**
				Cuspidata vs Subsecunda	**-0.32**	**0.016**
				Sphagnum vs. Subsecunda	0.003	1.0
Klason lignin						
ANOVA				Tukey test	diff	P
Source	df	F	P	**Acutifolia vs Cuspidata**	**109.76**	**< 0.0001**
Section	3	27.08	< 0.0001	Acutifolia vs Sphagnum	-0.389	1.0
Error	86			Acutifolia vs Subsecunda	36.22	0.53
				Cuspidata vs Sphagnum	**-110.15**	**< 0.0001**
				Cuspidata vs Subsecunda	**-73.54**	**0.03**
				Sphagnum vs. Subsecunda	36.61	0.55

SUPPLEMENTARY MATERIAL
Biochemical determinants of litter quality in 15 species of *Sphagnum,* Plant and Soil

Fia Bengtsson*, Håkan Rydin, Tomáš Hájek
*Corresponding author: Uppsala University, fia.bengtsson@ebc.uu.se

Supplementary Table 3. Models predicting decomposition of *Sphagnum* litter (mass loss (%) after 14 months incubation in lab).

Predictors*	Model (intercept+predictor coefficients)	R^2	P	DF
sphagnan	78.5-1.11x	$R^2 = 0.38$	<0.0001	1, 84
soluble phenolics	64.7-3.76x	$R^2 = 0.32$	<0.0001	1, 87
total Klason lignin	74.4-0.14x	$R^2 = 0.36$	<0.0001	1, 87
sphagnan+soluble phenolics+total KL	$93-0.65x_1-1.67x_2-0.068x_3$	$R^2_{adj} = 0.53$	<0.0001	3, 82
sphagnan+soluble phenolics	$86.2-0.81x_1-2.39x_2$	$R^2_{adj} = 0.48$	<0.0001	2,83
sphagnan+total KL	$91.3-0.77x_1-0.09x_2$	$R^2_{adj} = 0.49$	<0.0001	2, 83
sphagnan+soluble phenolics+total KL+N	$82.8-0.61x_1-1.62x_2-0.07x_3+1.58x_4$	$R^2_{adj} = 0.56$	<0.0001	4, 81
sphagnan+soluble phenolics+total KL+P	$89.6-0.7x_1-1.4x_2-0.079x_3+15.5x_4$	$R^2_{adj} = 0.59$	<0.0001	4, 81
CEC	93-82.3x	$R^2 = 0.4$	<0.0001	1, 87
CEC+soluble phenolics+total KL	$99.8-0.048x_1-1.83x_2-0.06x_3$	$R^2_{adj} = 0.52$	<0.0001	3, 85
CEC+soluble phenolics	$97.5-0.062x_1-2.35x_2$	$R^2_{adj} = 0.49$	<0.0001	2, 86
CEC+total KL	$97.8-0.056x_1-0.082x_2$	$R^2_{adj} = 0.47$	<0.0001	2, 86
CEC+soluble phenolics+total KL+N	$87.6-0.040x_1-1.83x_2-0.064x_3+1.45x_4$	$R^2_{adj} = 0.54$	<0.0001	4, 84
CEC+soluble phenolics+total KL+P	$96.2-0.052x_1-1.63x_2-0.057x_3+16.46x_4$	$R^2_{adj} = 0.58$	<0.0001	4, 84
Sum of sphagnan, soluble phenolics and total KL (each scaled and centered and used as one predictor)	34.5-4.9x	$R^2 = 0.54$	<0.0001	1, 84

*Predictor units: CEC in μeq g^{-1}, all other predictors in mg g^{-1}