

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

Fia Bengtsson  · Håkan Rydin · Tomáš Hájek

Received: 25 August 2017 / Accepted: 22 January 2018 / Published online: 5 February 2018  
© The Author(s) 2018. This article is an open access publication

## Abstract

**Background and aims** *Sphagnum* mosses are ecosystem engineers that create and maintain boreal peatlands. With unique biochemistry, waterlogging and acidifying capacities, they build up meters-thick layers of peat, reducing competition and impeding decomposition. We quantify within-genus differences in biochemical composition to make inferences about decay rates, related to hummock–hollow and fen–bog gradients and to phylogeny.

**Methods** We sampled litter from 15 *Sphagnum* species, abundant over the whole northern hemisphere. We used regression and Principal Components Analysis (PCA) to evaluate general relationships between litter quality

parameters and decay rates measured under laboratory and field conditions.

**Results** Both concentrations of the polysaccharide sphagnan and the soluble phenolics were positively correlated with intrinsic decay resistance, however, so were the previously understudied lignin-like phenolics. More resistant litter had more of all the important metabolites; consequently, PC1 scores were related to lab mass loss ( $R^2 = 0.57$ ). There was no such relationship with field mass loss, which is also affected by the environment. PCA also revealed that metabolites clearly group *Sphagnum* sections (subgenera).

**Conclusions** We suggest that the commonly stated growth-decomposition trade-off is largely due to litter quality. We show a strong phylogenetic control on *Sphagnum* metabolites, but their effects on decay are affected by nutrient availability in the habitat.

---

Responsible Editor: Per Ambus.

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s11104-018-3579-8>) contains supplementary material, which is available to authorized users.

---

F. Bengtsson (✉) · H. Rydin  
Department of Plant Ecology and Evolution, Evolutionary  
Biology Centre, Uppsala University, Norbyvägen 18D, SE-752  
36 Uppsala, Sweden  
e-mail: fia.bengtsson@ebc.uu.se

T. Hájek  
Department of Experimental Plant Biology, Faculty of Science,  
University of South Bohemia, Branišovská 1760, CZ-370  
05 České Budějovice, Czech Republic

T. Hájek  
Department of Functional Ecology, Institute of Botany of the  
Czech Academy of Sciences, Dukelská 135, CZ-379 82 Třeboň,  
Czech Republic

**Keywords** Peatland · Decay resistance · Sphagnan · Phenolics · Lignin · Hummock–hollow

## Introduction

Northern peatlands store immense amounts of carbon, according to a recent estimate 436 Gt (Loisel et al. 2014), which is equivalent to >50% of the carbon stored as CO<sub>2</sub> in the atmosphere (IPCC 2013). The carbon is stored as peat, i.e. poorly decomposed organic matter. This is largely due to anoxia in the waterlogged habitat and the litter quality of the species that occupy these peatlands. Bryophytes, mainly from the genus

*Sphagnum*, make up around 45% of the peat in boreal peatlands (Turetsky 2003) and are the organisms creating and maintaining many of these ecosystems, particularly bogs (Rydin and Jeglum 2013). Bogs are composed of meters-thick layers of peat accumulated during thousands of years; the low hydraulic conductivity of peat facilitates the creation of waterlogged, anoxic habitats. Anoxia is particularly linked to the lower peat layers (the permanently water-logged catotelm), while the acrotelm is intermittently oxic (Morris et al. 2011; Rydin and Jeglum 2013). Decay resistance of dead plants differ for different growth forms (Dorrepaal 2005), but also between different species of *Sphagnum* (Turetsky et al. 2008; Hájek 2009; Bengtsson et al. 2016a). Consequently, knowledge about the litter quality of ecosystem engineers, such as *Sphagnum* spp., is crucial to the understanding of ecosystem functions in northern peatlands.

Several biochemical compounds produced by *Sphagnum* have been pointed out to be important in deterring decomposers or providing structural resistance to decay. Among these, soluble phenolics were earlier widely considered to be secondary metabolites with antimicrobial properties (Verhoeven and Liefveld 1997). However, the concentrations of soluble phenolics in *Sphagnum* have been considered too low to control decomposition (Painter 1991; Mellegård et al. 2009). On the other hand, Bragazza et al. (2006) attributed higher decay rates to less phenolics in litter (under oxic conditions). Freeman et al. (2001) provided a mechanism by which phenolics accumulate and retard decomposition due to impaired phenol oxidase activity in anoxic peat. In conclusion, while not directly antimicrobial, the soluble phenolics seem to hamper decomposition indirectly by inactivating enzymes.

The much more abundant compounds, here called “lignin-like phenolics”, are thought to contribute to structural integrity of the cell walls of *Sphagnum*. Bryophytes do not synthesize “true” lignin (Kremer et al. 2004; Maksimova et al. 2013), which strengthens the cell walls of vascular plants (tracheophytes) and shields polysaccharides within the walls against microbial degradation (Talbot and Treseder 2012; Berg 2014). Similar to true lignin, the lignin-like phenolics in *Sphagnum* are thought to shield the polysaccharides by forming an amorphous coat on the cell wall surface (Tsuneda et al. 2001). Removal of polymeric phenolics from the litter did not substantially increase the rate of *Sphagnum* decomposition in vitro (Hájek et al. 2011); however,

their concentration seems to correlate with *Sphagnum* decomposability in the field (Turetsky et al. 2008; Hájek et al. 2011).

Some studies found structural, i.e. cell-wall polysaccharides (Turetsky et al. 2008), especially the pectic cell-wall polysaccharide “sphagnan” (Painter 1991; Børsheim et al. 2001; Painter 2003; Hájek et al. 2011), to be the main compound influencing litter quality in *Sphagnum*. Although the antimicrobial properties of sphagnan were attributed to acidification (Ballance et al. 2007; Stalheim et al. 2009), sphagnan addition has been found to block nitrogen mineralisation of plant litters (Hájek et al. 2011). Hájek et al. (2011) interpreted such active inhibition of microbial decay as electrostatic inactivation of microbial extracellular enzymes by the negatively charged sphagnan polymer (Ballance et al. 2008). The negative charge is provided by unesterified carboxyl groups of uronic acid units (Ballance et al. 2007), which determine the cation exchange capacity (CEC) of *Sphagnum* shoots (Clymo 1963). CEC at neutral pH should thus be good proxy of sphagnan concentration (Richter and Dainty 1989). Moreover, the CEC also determines pH-buffering (acidifying) capacity of the species, contributing to the competitive advantage of sphagna, and possibly to their decay resistance (Clymo 1963).

As different authors have suggested either polysaccharides or soluble/polymeric phenolics to be the key to decay resistance of *Sphagnum* litter, it is of interest how much variation in decomposability these compounds explain, and whether there is a biochemical trade-off indicating specialization in production of either of the compounds. Such a trade-off could be linked to the niche differentiation in *Sphagnum* species along the hummock–hollow gradient (i.e., gradient of acrotelm thickness). A hollow has a thin acrotelm with near-surface anoxia, consequently, decay is to a large extent ruled by habitat, whereas a hummock has a thick acrotelm where the aerobic decay is more strongly determined by litter quality (Bengtsson et al. 2016a). Shoots of most species grow better in wet habitats, but only those able to avoid desiccation can survive high above the water table (summarized in Rydin et al. 2006). The desiccation avoidance of hummock sphagna (mediated by large, empty hyaline cells) and the structural persistence of *Sphagnum*-built hummocks are thus interlinked via resource allocation to structural, rather than photosynthesizing tissues (Rice et al. 2008; Hájek 2014). Thus, the compounds providing either structural

integrity or other protection against decomposers could plausibly be trade-offs for competitive growth (Turetsky et al. 2008; Hájek 2009; Laing et al. 2014; Bengtsson et al. 2016a); in contrast to the fast-growing hollow species, the hummock species often have greater decay resistance (Clymo 1965; Johnson and Damman 1991; Belyea 1996; Limpens and Berendse 2003). Consequently, we expect higher amounts of compounds that slow down decomposition in hummock species.

Here, we have the means to test which compounds are important to decay resistance. We collected litter from 15 *Sphagnum* species from different habitats along microtopographical (hummock–hollow) and nutritional (bog–fen–forest) gradients, and compared the biochemistry of individual species with their decay rate in standard conditions, i.e. with the intrinsic decay resistance (decomposability, litter quality). We aim to analyse:

- Metabolite composition as predictor of decay resistance: which compounds can best explain differences in decay resistance among species/samples?
- Relationships between metabolites: are there trade-offs between production of different compounds?
- Phylogeny versus habitat: are differences in biochemical composition among species explained by relatedness or by habitat affinity of the species?

## Materials and methods

### Sampling sites and litter collection

The study was conducted at two localities: Kulflyten and Glon, which were also the study sites of our earlier study about *Sphagnum* species' growth and decomposition traits (Bengtsson et al. 2016a). The main site Kulflyten (59°54'N, 15°50'E) is a mire complex in central-southern Sweden (Västmanland province). The central area is a raised ombrotrophic bog with well-developed hummock–hollow patterning, bog pools, and wetter poor-fen soaks (minerotrophic seepage into the bog; Rydin and Jeglum 2013). The angiosperm vegetation of the bog is sparse, and typical of ombrotrophic bogs in this region (Sjörs 1948). Parts of the outer edge of the bog are covered by Scots pine (*Pinus sylvestris*) and can be described as a relatively shaded wooded marginal slope (pine bog). The bog is surrounded by a lagg fen, which is oligotrophic, but

richer in solutes than the ombrotrophic bog because it receives water from the surrounding mineral soil. In addition to the sampled peat mosses, it is characterized by sedges (*Carex* spp.). The mire is surrounded by young Norwegian spruce (*Picea abies*) forest on peaty soil. The bottom layer here is dominated by *Sphagnum girgensohnii* and common feather mosses. The mean temperature is 16.6 °C in July, and −2.6 °C in December, and the annual mean precipitation is 733 mm (1982–2013). Glon is a small rich fen in northern Uppland (60°31'N, 17°55'E), which has developed on lime-rich moraine. It is relatively open, but reed (*Phragmites australis*) is abundant and there are hummocks with Scots pine. Mean July temperature is 16.8 °C and −1.0 °C in December and the annual mean precipitation is 649 mm. Weather data were extracted from the SMHI online service LuftWebb (SMHI 2014). Nitrogen deposition is ca. 0.6 g m<sup>-2</sup> yr.<sup>-1</sup> at both localities (Granath et al. 2009).

We chose 15 *Sphagnum* species from habitats typical of the species so as to represent different habitats, both along the microtopographical gradient, the pH gradient, and four different sections (subgenera) of *Sphagnum* (Table 1, where rich fen samples are from Glon, the others from Kulflyten). The species represent open, ombrotrophic bog from hummocks to bog pools, rich fen, lagg fen, pine bog, and spruce forest. Sampling patches were chosen to be as uniform as possible. We had 5 replicates per species, in total 90 samples; these were roughly half the sampling points used in Bengtsson et al. (2016a, b) from where the decomposition data come. The same patches were used to sample litter for litter quality analyses and to measure decomposition. Two species (*S. fuscum* and *S. magellanicum*) were sampled in two and three different habitats, respectively. We sampled five replicate patches from each habitat. At each sampling patch, a perforated pipe was inserted into the peat so that the height above water table (HWT) could be measured as the distance between the water table and the top of the *Sphagnum* vegetation. HWT was measured in June 2012 (data in Bengtsson et al. 2016a, b).

In October 2013 we collected *Sphagnum* shoots from the patches for chemical analyses of litter quality. As usual for late autumn the sites were quite wet. One or two cores of 10 cm diameter were sampled at each patch, with the aim to collect 2 g of dry litter. The capitula were removed, and the 3 subsequent cm were kept and defined as litter. For *S. tenellum* and for some

**Table 1** Species, the habitats they were sampled from and number of replicates. Nomenclature according to Flatberg (2013)

<i>Sphagnum</i> species	Section	Author citation	Microtopographical habitat*	Vegetation type	n	Species code
<i>S. capillifolium</i>	Acutifolia	(Ehrh.) Hedw.	Hummock	Pine bog	5	CA
<i>S. fuscum</i>	Acutifolia	(Schimp.) H.Klinggr.	Hummock	1) Open bog 2) Rich fen	5 5	FU_1 FU_2
<i>S. girgensohnii</i>	Acutifolia	Russow	Hummock	Spruce forest	5	GI
<i>S. rubellum</i>	Acutifolia	Wilson	Low hummock	Open bog	5	RU
<i>S. warnstorfi</i>	Acutifolia	Russow	Low hummock	Rich fen	5	WA
<i>S. angustifolium</i>	Cuspidata	(Russow) C.E.O.Jensen	Low hummock	Bog edge	5	AN
<i>S. balticum</i>	Cuspidata	(Russow) C.E.O.Jensen	Lawn	Open bog	5	BA
<i>S. cuspidatum</i>	Cuspidata	Hoffin.	Carpet-pool	Open bog	5	CU
<i>S. fallax</i>	Cuspidata	(H.Klinggr.) H.Klinggr.	Lawn	Lagg fen	5	FA
<i>S. lindbergii</i>	Cuspidata	Lindb.	Carpet	Open bog	5	LI
<i>S. majus</i>	Cuspidata	(Russow) C.E.O.Jensen	Carpet	Open bog	5	MJ
<i>S. tenellum</i>	Cuspidata	(Brid.) Brid.	Lawn	Open bog	5	TE
<i>S. magellanicum</i>	Sphagnum	Brid.	Lawn-carpet Hummock Hummock	1) Open bog 2) Pine bog 3) Spruce forest	5 5 5	MG_1 MG_2 MG_3
<i>S. papillosum</i>	Sphagnum	Lindb.	Carpet	Fen soak	5	PA
<i>S. contortum</i>	Subsecunda	Schultz	Lawn	Rich fen	5	CO

\*Hummock – raised element with dwarf-shrubs; Lawn – firm moss mat with sedges; Carpet – soft moss mat with only little vascular plants (Rydin and Jeglum 2013)

replicates of *S. balticum* we used only 2 cm to exclude shoots with signs of structural decay. The litter was freed of alien species and debris, rinsed in deionized water and oven-dried at 60 °C until constant weight and stored in paper bags until further analysis.

### Biochemical analyses

Details about the extraction procedures for holocellulose, sphagnum and soluble phenolics are given in Supplement 1.

### Holocellulose

To obtain the fraction of structural polysaccharides (holocellulose, HC; Table 2) dried litter was coarsely homogenised with a food processor and bleached, following Ballance et al. (2007). The holocellulose in its acidic form was dried at 60 °C and weighed.

### Sphagnum

Sphagnum extraction by acid autohydrolysis follows Ballance et al. (2007). The sphagnum we yielded was a brown solid, expressed as mg g<sup>-1</sup> of HC and litter.

However, since these calculations were highly correlated ( $p < 0.0001$ ,  $r = 0.98$ ,  $df = 84$ ), we use only the litter calculation further on. Although not all sphagnum was liberated during the extraction, the yield of further extractions is expected to be small and difficult to separate (Ballance et al. 2007). This extraction can be considered to be a measure of sphagnum “releaseability”, i.e. the species’ potential to release high-molecular soluble polysaccharides.

Sphagnum concentration can also be estimated indirectly using cation exchange capacity (CEC,  $\mu\text{eq g}^{-1}$ ); at pH 7.0 the CEC is provided almost exclusively by entirely dissociated carboxyl group of uronic acids in cell-wall pectins, i.e. in bound sphagnum (Richter and Dainty 1989). We determined the CEC as the amount of exchangeable  $\text{NH}_4^+$  at pH 7.0 in litters saturated with  $\text{NH}_4^+$  (Spearing 1972). Around 50.0 mg of the litter was placed in polyester mesh bags (mesh size 150  $\mu\text{m}$ ). All 90 bags were washed together with 20 mM cold hydrochloric acid (5 °C) in order to elute possible cations associated with carboxyls. The acid was then removed with deionised water and the bags placed into a flask with 2 L of 1 M ammonium acetate solution to associate all cation-exchange sites with  $\text{NH}_4^+$ . The saturation took place during 10 min under continuous shaking. The acetate solution

**Table 2** Summary of the measured compounds, different terms used, units and the material used for extraction

Term used in our study	Method-based term	Also known as / denotes	Extracted from (measured on)	Unit used in our study
<i>Carbohydrates</i>				
Holocellulose (HC)	Chlorite-treated litter	Structural polysaccharides	Homogenised litter	mg g <sup>-1</sup>
Sphagnum	Hot-water hydrolysable carbohydrate	<i>Sphagnum</i> pectin	Holocellulose (expressed based on homogenised litter)	mg g <sup>-1</sup>
Cation exchange capacity (CEC)	NH <sub>4</sub> <sup>+</sup> exchange capacity at pH 7.0	A proxy for polygalacturonic acid or bound sphagnum	Homogenised litter	μeq g <sup>-1</sup>
<i>Phenolics</i>				
Soluble phenolics	Acetone-soluble phenolics; Folin–Ciocalteu phenols	Total (i.e. extractable) phenolics	Milled litter	mg g <sup>-1</sup>
Klason lignin (KL)	Acid-insoluble residuum	Lignin-like phenolics; Acid-insoluble lignin; Polymeric phenolics	Milled litter	mg g <sup>-1</sup>
Soluble Klason lignin	Acid-soluble fraction detected spectrophotometrically at 205 nm	Dissolved lignin-like phenolics; Acid-soluble lignin	Milled litter	mg g <sup>-1</sup> , % of total KL
Total Klason lignin	Sum of lignin-like and dissolved lignin-like phenolics	(Total) lignin-like phenolics	Milled litter	mg g <sup>-1</sup>

was exchanged after 4 min and the pH was increased to 7.0 using ammonia solution. Then we thoroughly washed the bags with deionised water (six times for 10 min) to remove free ammonium ions. A sample of washing collected after the 6th wash confirmed no detectable ammonium. Each bag was then eluted with 50 mL of 20 mM HCl. After 20 min of shaking the eluate was sampled, 10 times diluted, preserved with a drop of chloroform and analysed for ammonium colorimetrically using flow injection analysis (FIA; Foss Tecator AB, Sweden).

#### *Soluble phenolics*

For extraction of soluble phenolics we followed Bärlocher and Graça (2005). The concentration of acetone-soluble phenolics was expressed in mg g<sup>-1</sup>, calculated using 4-hydroxybenzoic acid as a standard, which is a representative of *Sphagnum*-abundant simple monophenolics (Rasmussen et al. 1995; Mellegård et al. 2009). This standard gave 1.37 times lower yields than the commonly used tannic acid standard; however, tannic acid represents more complex polyphenolics that are quantitatively insignificant in *Sphagnum*.

#### *Lignin-like phenolics*

The concentration of the polymeric, lignin-like phenolics (mg g<sup>-1</sup>) was assessed gravimetrically as sulphuric

acid-insoluble residuum known as Klason lignin (e.g., Templeton and Ehrman (1995); Wieder and Starr (1998)). This procedure may overestimate lignin fraction as sulphuric acid digestion leaves cutins and some condensed tannins and proteins (Templeton and Ehrman 1995; Preston et al. 1997); however, these compounds of non-structural character have low abundance in bryophytes. We modified the standard method in order to avoid autoclaving (to minimize lignin dissolving) and to allow batch processing of very small samples. We placed 95–100 mg of ball-milled litter into 50 mL PP tubes, added 1.25 mL of 72% (w/w; 12 M) sulphuric acid and thoroughly vortexed the tubes. After 1 h of incubation at 21 °C we added 35 mL of hot deionised water and kept the slurry in an oven at 100 °C for 150 min. Then we centrifuged the tubes at 3000×g for 15 min and collected the supernatants. The pellets representing Klason lignin were then resuspended with deionized water, centrifuged, dried and weighed. In the first supernatants, we measured dissolved phenolics, i.e. soluble Klason lignin (following Ehrman 1996) as the polymeric phenolics of *Sphagnum* are prone to dissolve by acid hydrolysis (Farmer and Morrison 1964; Straková et al. 2010). A sample of the supernatant was diluted 36 times with 2.48% sulphuric acid and its absorbance at 205 nm was measured spectrophotometrically. We prepared a specific standard solution based on the mean proportion of three prevailing phenolic

monomers in degradation products of the cell walls in 13 *Sphagnum* species (Williams et al. 1998): 4-hydroxybenzoic acid (18.4%), 4'-hydroxyacetophenone (60.6%) and 4-hydroxybenzaldehyde (20.9%) (synthesis grade; Merck Schuchardt OHG, Germany). The mass attenuation (extinction) coefficient of the standard was  $91.4 \text{ L g}^{-1} \text{ cm}^{-1}$  (cf.  $110 \text{ L g}^{-1} \text{ cm}^{-1}$  commonly used for soluble lignin in tracheophytes; Hatfield and Fukushima 2005).

#### Chemical stability parameters

As a proxy of carbohydrate concentration, we also measured the absorbance at 280 nm in the supernatant after hot-acid digestion and calculated the ratio of 205 nm and 280 nm absorbances. Phenolics absorb at 280 nm due to aromatic rings; however, also heterocyclic aldehydes, particularly furfural and hydroxymethylfurfural, absorb considerably at around 280 nm (but only little at 205 nm). These aldehydes are common products of hot acid degradation of pentoses and hexoses (Hatfield and Fukushima 2005; Chi et al. 2009). Hence, greater absorbance at 280 nm relative to 205 nm (i.e. lower 205:280 nm absorbance ratio) indicates a greater concentration of carbohydrates in the litter and/or its greater chemical degradability.

Holocellulose (HC) and total Klason lignin (KL) together represent almost 100% of dead plant litters as they are free of protoplasts and the other cell-wall fractions (proteins, lipids) are expected to be quantitatively less important. Deviations of the HC and total KL fraction sums from 100% can be then explained as artefacts of their extraction. Positive deviation (HC + total KL > 100%) denotes that total KL was not entirely extracted during HC isolation; lower deviation indicates that not all HC was recovered. Therefore we introduce the deviation as a parameter *dev100* evaluating the chemical resistance of the litters:

$$dev100 = \frac{HC + total\ KL}{10} - 100$$

HC and total KL are holocellulose and total Klason lignin in  $\text{mg g}^{-1}$  (HC + total KL is divided by 10 to make it into percentage); the more positive *dev100* the greater litter chemical resistance. Such parameter may represent a potential proxy of decay resistance. We are aware that the sum of other (non)extractables still represents several percent of the litter; therefore we do not interpret the absolute values of *dev100* but use the proxy

to compare species and sections and to correlate it with other parameters.

#### C, N, P

The carbon and nitrogen concentrations ( $\text{mg g}^{-1}$ ) in ball-milled litters were analysed with an ECS 4010 Elemental Analyzer (Costech Analytical Technologies, USA). Phosphorus ( $\text{mg g}^{-1}$ ) was analysed as phosphate using FIA after digestion of milled litter with perchloric acid.

#### Decomposition

To relate the biochemical composition to decomposability of the litter we used data from Bengtsson et al. (2016a, b). Briefly, we collected litter from the sampling patches in the beginning of the summer 2013, and incubated it in nylon mesh bags either at their patches of origin around 5 cm under the *Sphagnum* surface, or under controlled conditions in separate containers together with a peat water inoculum for 14 months, to obtain both “field mass loss” and “lab mass loss”. The bags in the lab were partly covered with plastic films to allow air to enter, while avoiding evaporation. Decay rates are given as the % mass loss.

#### Statistical analyses

The data were analysed in R version 3.3.0 (R Core Team 2016). We used correlation analyses to test relationships among chemical variables and decomposition (mass loss), i.e. work out which compounds can best explain differences in decay resistance among species/samples and to detect trade-offs between production of different compounds. These relationships were also illustrated by PCAs, using the Nipals algorithm (ade4 package in R; Dray and Dufour 2007) that can handle observations with missing data for some variables without removing the whole observation. We did one PCA including only the metabolites (holocellulose, sphagnum, soluble phenolics, total Klason lignin and CEC = cation exchange capacity) to see how well they group species, and one PCA, including also decomposition and C, N, P, which would be influenced by the environment, to see how species were grouped now to compare to the purely innate qualities of the litter.

To test if metabolite composition can be used as a predictor of decay resistance we used simple and

multiple linear regressions with PCA scores, or metabolites, as predictors of decay rates. After concluding that there were no trade-offs between production of different compounds we also analysed whether the sum of the centered and scaled metabolite concentrations of sphagnum, soluble phenolics and total KL (the “important” metabolite compounds) could be used as a predictor for decay rate in a simple regression.

To test for differences among sections for specific variables we used ANOVAs (residuals were checked for normality and homogeneity of variance and data were log-transformed when needed). When ANOVAs were significant post hoc Tukey HSD tests were used to identify which factor levels differed. Samples of the same species (*S. fuscum* and *S. magellanicum*) from different habitats were treated as different species in these analyses.

**Data availability** The data generated during the current study are available in the Dryad Digital Repository, doi: <https://doi.org/10.5061/dryad.4f8d2>.

## Results

The sections of the genus *Sphagnum* differed in concentrations of sphagnum, phenolics and Klason lignin, and in CEC (ANOVA:  $P < 0.0001$  for all; Fig. 1, detailed data in Supplementary Tables 1 and 2), with the differences generally due to high concentrations in sect. *Acutifolia* and lower in sect. *Cuspidata*. These four variables were positively correlated ( $0.5 < r < 0.57$ ; Table 3). Samples and species growing high above the water table also had a higher CEC ( $r = 0.64$ ,  $df = 84$ ,  $P < 0.0001$  and  $r = 0.72$ ,  $df = 16$ ,  $P = 0.0008$ , respectively).

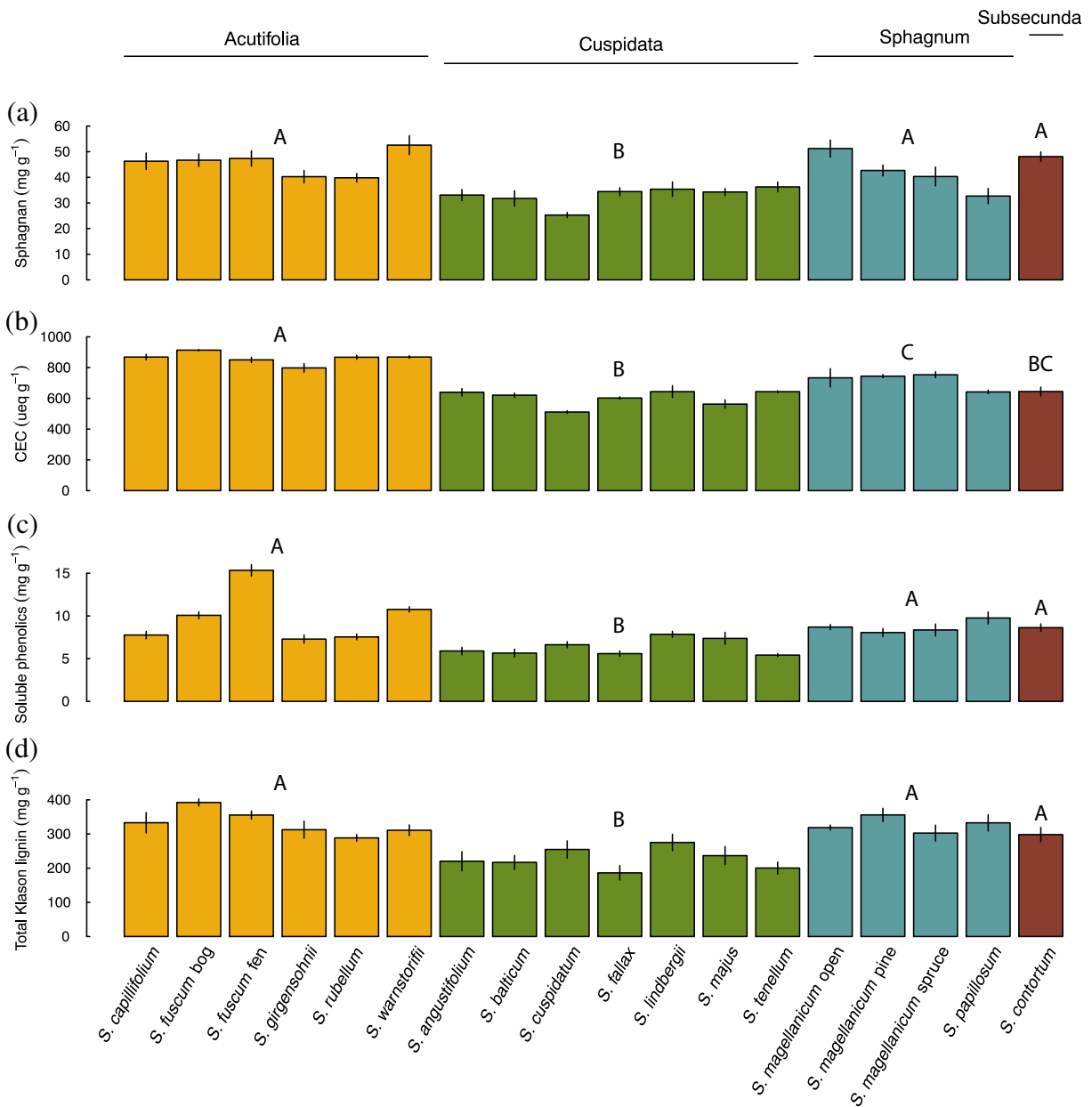
In a PCA based on concentrations of the six metabolites (Fig. 2) we see a strong clustering of species according to their taxonomic position at section level, indicating a strong phylogenetic signal. PC1 clearly separated the *Acutifolia* and *Cuspidata* species. It is notable that samples of *S. magellanicum* from three different habitats are very similar in their metabolite signals. The compounds with the strongest effects on axis 1 were total Klason lignin, soluble phenolics and sphagnum (also when expressed as CEC). The main influence on axis 2 was HC. The two first axes together explained 74% of the variation. Regressions analysing how lab mass loss can be predicted by the PC scores were significant only for PC1 ( $P < 0.0001$ ;  $R^2 = 0.57$ ;

$DF = 87$ ), while there was no significant relationship for PC2 (dominated by HC) and lab mass loss ( $P = 0.48$ ;  $R^2 = 0.006$ ;  $DF = 87$ ).

To illustrate relationships between all variables in the data and how the species relate to each other we performed a second PCA including all variables describing mass loss (in lab and field conditions), concentrations of metabolites and nutrients (Fig. 3). Here, the two first axes together explained 59% of the variation. As with Fig. 2, the first axis was controlled by the metabolites. However, the second axis was determined by mineral nutrients N and P (Fig. 3), as a consequence of the two outlying nutrient-rich samples collected in forested habitats, *S. girgensohnii* (sect. *Acutifolia*) and one set of *S. magellanicum* (sect. *Sphagnum*).

In support of the PCA, correlation analyses showed that lab mass loss was negatively correlated with sphagnum (and thus CEC), soluble phenolics and total KL, roughly at equal strength (Table 3). To test if they jointly could explain more of the variation in decomposition we performed a multiple regression with these three variables as predictors for lab mass loss. In the model all three were highly significant, and after removal of non-significant interactions the equation for litter mass loss in lab (%) as a function of the concentrations of sphagnum ( $x_1$ ,  $\text{mg g}^{-1}$ ), soluble phenolics ( $x_2$ ,  $\text{mg g}^{-1}$ ) and total Klason lignin ( $x_3$ ,  $\text{mg g}^{-1}$ ) was: lab mass loss =  $93 - 0.65 \times x_1 - 1.67 \times x_2 - 0.068 \times x_3$  ( $P < 0.0001$ ; Adj.  $R^2 = 0.53$ ;  $DF = 82$ ). Using the sum of centered and scaled concentrations of sphagnum, soluble phenolics and total KL as a predictor for mass loss yielded a similar result to the multiple regression (Fig. 4). Adding the nutrients (N or P) as predictors in the model including all three metabolites as separate predictors, slightly (but significantly) raised the variation explained (Adj.  $R^2 = 0.56$  with N; Adj.  $R^2 = 0.59$  with P). Other potential models (e.g. replacing sphagnum by CEC) also yielded similar results (Supplementary Table 3).

Since we had field decomposition data available for our samples, we also analysed the relationships between field mass loss and litter quality. The relationships were overall much weaker compared to the ones between lab mass loss and litter quality parameters. We found no relationships between field mass loss and the metabolite PCA axis 1 ( $R^2 = 0.005$ ,  $P = 0.5$ ), or between field loss and the scaled and summed sphagnum, soluble phenolics, and Klason lignin ( $R^2 = 0.003$ ,  $P = 0.6$ ). Field loss only correlated significantly ( $P < 0.05$ ) with carbon concentration, CN ratio and phosphorous concentration



**Fig. 1** Species means ( $\pm$ SE) of (a) sphagnum, (b) cation exchange capacity (CEC), (c) soluble phenolics and (d) total Klason lignin concentrations. Different letters above panels show significant

differences between the sections (based on Tukey tests, which was applied on log transformed values in the case of soluble phenolics)

( $r = -0.33, -0.28, 0.31$ , respectively). The correlation between field and lab loss variables was  $r = 0.3$  ( $P = 0.004$ ).

The parameter of litter chemical resistance represented by *dev100* (Supplementary Table 1) showed a much stronger correlation with total KL ( $r = 0.92, P < 0.0001$ ) than with HC ( $r = 0.39, P = 0.0001$ ), suggesting a failure in one of the two estimation methods. There were

positive correlations with most chemical parameters we measured, but no correlations with mineral nutrients. The correlation between *dev100* and lab mass loss was negative ( $r = -0.58, P < 0.0001$ ). The chemical resistance of fast-decomposing species in sect. Cuspidata and partly also in sect. Sphagnum was significantly lower compared to sect. Acutifolia species (ANOVA:  $F_{3,86} = 25.7, P < 0.0001$ ).

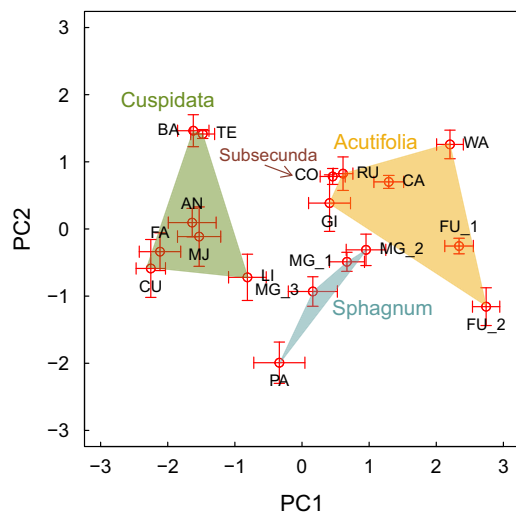
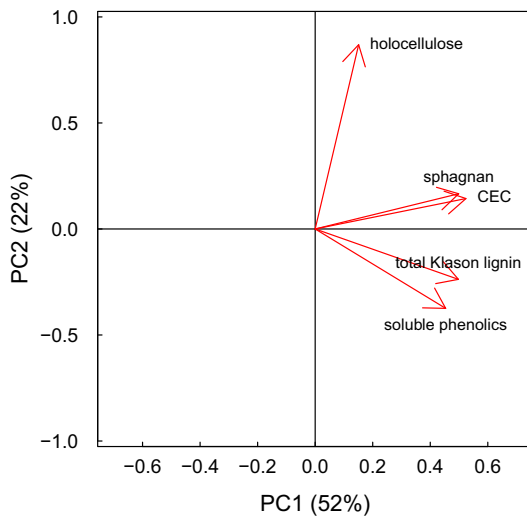


**Table 3** Correlations (showing  $r =$ ) among variables describing lab mass loss and concentrations of metabolites and nutrients; bold denotes significant correlation ( $P < 0.05$ )

	Sphagnum mg g <sup>-1</sup>	Soluble phenolics mg g <sup>-1</sup>	Klason lignin mg g <sup>-1</sup>	Soluble KL% of total KL	Total Klason lignin mg g <sup>-1</sup>	CEC μeq g <sup>-1</sup>	C mg g <sup>-1</sup>	N mg g <sup>-1</sup>	C:N ratio	PO <sub>4</sub> -P μg g <sup>-1</sup>	Lab litter mass loss %
Holocellulose mg g <sup>-1</sup>	<b>0.268</b>	-0.071	-0.027	0.113	-0.004	<b>0.268</b>	-0.103	<b>-0.330</b>	<b>0.309</b>	-0.160	-0.093
Sphagnum mg g <sup>-1</sup>		<b>0.448</b>	<b>0.459</b>	<b>-0.400</b>	<b>0.471</b>	<b>0.568</b>	0.152	-0.088	0.059	0.085	<b>-0.618</b>
Soluble phenolics mg g <sup>-1</sup>			<b>0.518</b>	<b>-0.489</b>	<b>0.524</b>	<b>0.457</b>	<b>0.490</b>	-0.050	0.082	-0.048	<b>-0.565</b>
Klason lignin mg g <sup>-1</sup>				<b>-0.940</b>	<b>0.998</b>	<b>0.573</b>	<b>0.468</b>	0.012	0.025	0.046	<b>-0.583</b>
Soluble KL% of total KL					<b>-0.927</b>	<b>-0.504</b>	<b>-0.389</b>	-0.108	0.091	-0.140	<b>0.474</b>
Total KL mg g <sup>-1</sup>						<b>0.586</b>	<b>0.480</b>	-0.003	0.044	0.026	<b>-0.598</b>
CEC μeq g <sup>-1</sup>							<b>0.279</b>	<b>-0.229</b>	<b>0.319</b>	0.093	<b>-0.631</b>
C mg g <sup>-1</sup>								<b>-0.233</b>	<b>0.265</b>	<b>-0.429</b>	<b>-0.548</b>
N mg g <sup>-1</sup>									<b>-0.937</b>	<b>0.699</b>	<b>0.255</b>
C:N ratio										<b>-0.609</b>	<b>-0.315</b>
PO <sub>4</sub> -P μg g <sup>-1</sup>											<b>0.231</b>

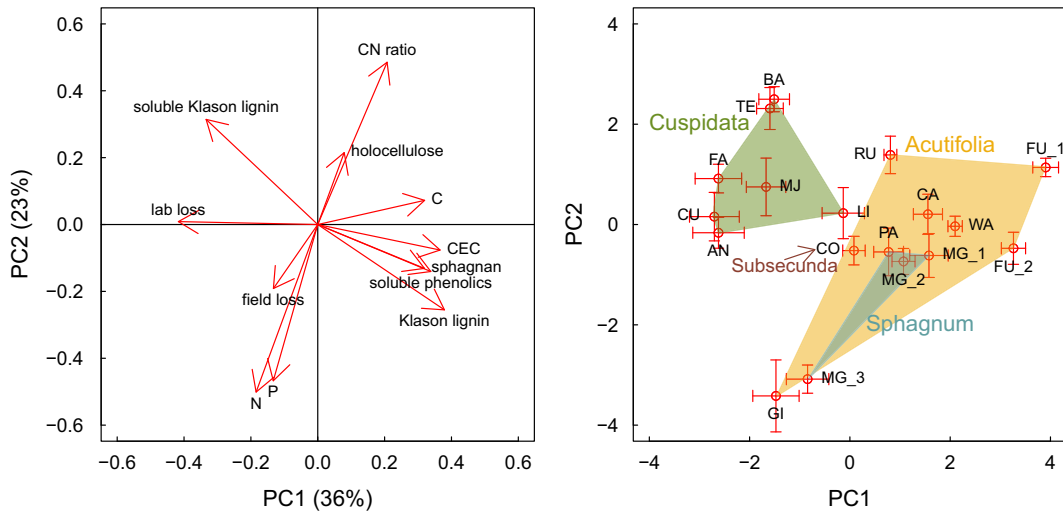
Soluble KL (as % of total KL; Supplementary Table 1), which characterises chemical stability of KL, was highly, but negatively correlated with total KL ( $r = -0.93$ ) and therefore the correlation

with lab mass loss was positive ( $r = 0.47$ ) (Table 3). This means that the abundant KL of decay-resistant *Acutifolia* is resistant to chemical acid-degradation.



**Fig. 2** PCA including only the measured metabolites. Left: The metabolites include holocellulose, sphagnum, soluble phenolics, total Klason lignin and CEC = cation exchange capacity. Right: The distribution of the species in the trait space (with x and y standard error bars), and envelopes around species from the same section. *Sphagnum* species codes: AN = *S. angustifolium*, BA = *S. balticum*, CA = *S. capillifolium*, CO = *S. contortum*, CU =

*S. cuspidatum*, FA = *S. fallax*, FU\_1 = *S. fuscum* (open bog), FU\_2 = *S. fuscum* (rich fen), GI = *S. girgensohnii*, LI = *S. lindbergii*, MG\_1 = *S. magellanicum* (open bog), MG\_2 = *S. magellanicum* (pine bog), MG\_3 = *S. magellanicum* (spruce forest), MJ = *S. majus*, PA = *S. papillosum*, RU = *S. rubellum*, TE = *S. tenellum*, WA = *S. warnstorfi*



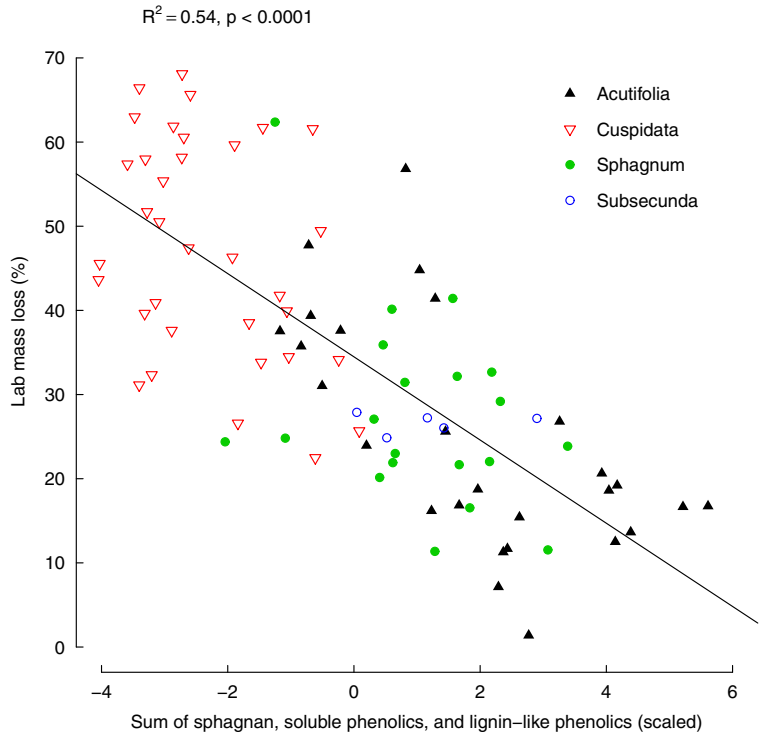
**Fig. 3** PCA including the measured metabolites, nutrients and litter mass loss in field and lab. Left: Variables included are holocellulose; sphagnum; soluble phenolics; Klason lignin; CEC = cation exchange capacity; soluble Klason lignin (% of total lignin); N = nitrogen concentration; C = carbon concentration; CN ratio; P = PO<sub>4</sub>-P concentration; lab loss (mass loss from litter bags after 14 months in laboratory conditions); field loss (mass loss from litter bags in the place of the litters' origins over two seasons). Right: The distribution of the species in the trait space (with x and

y standard error bars), and envelopes around species from the same section. *Sphagnum* species codes: AN = *S. angustifolium*, BA = *S. balticum*, CA = *S. capillifolium*, CO = *S. contortum*, CU = *S. cuspidatum*, FA = *S. fallax*, FU\_1 = *S. fuscum* (open bog), FU\_2 = *S. fuscum* (rich fen), GI = *S. girgensohnii*, LI = *S. lindbergii*, MG\_1 = *S. magellanicum* (open bog), MG\_2 = *S. magellanicum* (spruce forest), MJ = *S. majus*, PA = *S. papillosum*, RU = *S. rubellum*, TE = *S. tenellum*, WA = *S. warnstorffii*

A proxy of carbohydrate concentration (and/or chemical degradability), the 205:280 nm absorbance ratio of

the supernatant after hot-acid digestion of the litter (Supplementary Table 1), was also correlated with most

**Fig. 4** The lab mass loss (%) as a function of the summed metabolite concentrations of sphagnum, soluble phenolics and lignin-like phenolics (centered and scaled variables; DF = 84)



biochemical parameters, notably total KL, sphagnum, CEC and soluble phenolics ( $r=0.45$ ,  $0.40$ ,  $0.45$ , and  $0.36$ ,  $P<0.001$ ). Also height above water table (HWT) and lab mass loss were correlated with the absorbance ratio ( $r=0.33$ ,  $P=0.002$  and  $r=-0.38$ ,  $P=0.0002$ ). Hence, the 205:280 ratio was lower in Cuspidata than in sections Acutifolia and Sphagnum (ANOVA:  $F_{3,86}=17.57$ ,  $P<0.0001$ ).

## Discussion

Since both carbohydrates (Turetsky et al. 2008), notably sphagnum (Painter 1991; Børsheim et al. 2001; Painter 2003; Hájek et al. 2011), as well as insoluble phenolics (Tsuneda et al. 2001) and soluble phenolics (Freeman et al. 2001; Bragazza et al. 2006) have been suggested as determinants for decay resistance of *Sphagnum*, it would be possible to envisage that species allocate carbon to production of either primary or secondary metabolites, i.e. carbohydrates or phenolics. However, we found that there are relatively close positive correlations among all these metabolites as well as between them and litter decay resistance. These parameters were correlated also with several others, including those describing litter chemical resistance. As a result, we found no signs of trade-offs among species in production of metabolites related to decay resistance, but rather that the combination of high concentrations of these metabolites results in slower decay (Fig. 4). This does not contradict the trade-off in resource partitioning between metabolic and structural carbohydrates (Turetsky et al. 2008), which describes the partitioning between dead hyaline cells (cell walls) composed of decay-resistant polymers and living chlorophyllous cells that constitute an easily decomposable carbohydrate storage. Linked to this, our results support the suggested trade-off between growth and decay (Turetsky et al. 2008; Hájek 2009; Laing et al. 2014; Bengtsson et al. 2016a); fast growing species decompose fast, while slow growing species invest in metabolites which retard their decomposition.

### Metabolite composition as predictor of decay resistance

In our field decomposition experiment each species was exposed to its own environment, where the microhabitats greatly impact decomposition. Hummocks are well aerated, which means good decomposition potential, but hummock species generally have a high intrinsic

decomposition resistance (Bengtsson et al. 2016a). Hollows, on the other hand, are wet and have a poorer decomposition potential, but hollow species are less resistant to decomposition. This is illustrated in our results by the strong relationships between mass loss in standard conditions, which would represent intrinsic decay resistance, and litter quality, in contrast to the absent relationships between field mass loss and metabolites.

As an individual predictor of litter quality, sphagnum (and CEC) was marginally better than the other two (see also PCA in Fig. 2). Nevertheless, in the multiple-regression model, sphagnum, soluble phenolics and/or Klason lignin together predicted lab mass loss better than each one individually, indicating that they have complimentary functions. For example, plant soluble phenolics are known to delay litter microbial decomposition and this effect can be magnified in field conditions due to accumulation of phenolics under anoxic conditions in the catotelm (Freeman et al. 2001; however, cf. Mellegård et al. 2009). Conversely, carbohydrate polymers (Turetsky et al. 2008; Maksimova et al. 2013), specifically cellulose microfibrils of secondary cell walls (Kremer et al. 2004), confer structural integrity to the walls of hyaline cells.

The mechanism by which the lignin-like phenolics contribute to structural integrity of the cell-walls of *Sphagnum* has not been fully elaborated. To avoid collapse of the large but empty hyaline cells, their secondary walls are strengthened by rib-like fibrils. The fibrils exhibit intensive autofluorescence emitted by lignin-like phenolics (see Bragina et al. 2012 for photographs) proposing a structural function of the phenolic polymer. The lignin-like phenolics (together with hemicelluloses (Scheller and Ulvskov 2010)) provide the hyaline cells with rigidity preventing their collapse, e.g. during dehydration (see Fig. 8.22 in Clymo and Hayward 1982), but also during litter decomposition as the lignin-like phenolics seem to shield the polysaccharides against decomposers, particularly fungi (Tsuneda et al. 2001). All these properties are homologous with the roles of lignin in tracheophytes. In addition, the fibrils are strongly stained by ruthenium red stain, which indicates high density of sphagnum (Kremer et al. 2004; Ballance et al. 2012) that may serve as further protection against enzymatic degradation. Phenolic polymers seem to be responsible also for the rigidity of *Sphagnum* stems and branches as we observed considerable shoot softening after the delignification (holocellulose preparation).

We postulate that the previously overlooked but quantitatively dominant (compared to soluble phenolics; Fig. 1) lignin-like phenolics play an important role in *Sphagnum* decay resistance. Traditionally, as described in the introduction, decay resistance has been attributed to the small concentration of soluble phenolics. However, the soluble phenolics may in fact be derived from the lignin-like phenolics, seeing that their oxidation products (Williams et al. 1998) are to a large extent identical to phenolic monomers soluble in acetone or water, or even to those excreted into the environment (Rasmussen et al. 1995).

Although the metabolites that increase decay resistance determine the trade-off between growth and decay and thereby represent a cost, they also have positive effects on growth, mostly by alleviating the abiotic stress in hummock environments: (1) The large water retention capacity provide the hummock sphagna with a desiccation avoidance strategy that allows them to maintain photosynthetic C assimilation and growth during drought, while hollow sphagna or other bryophytes have to rely on their ability to tolerate desiccation (Hájek and Vicherová 2013). The desiccation avoidance strategy is possible only when the cell walls are strong enough to prevent collapse of the large capillary spaces as discussed above. (2) Low nutrient availability in rain-fed hummocks can be partly compensated by the greater CEC of hummock-forming sphagna provided by sphagnum (consistent with Clymo (1963); Painter (2003); Hájek and Adamec (2009)) as the cationic nutrients from atmospheric deposition can be retained on the cation-exchange sites before assimilation. Although CEC can be considered a measure of sphagnum, their correlation was not as high as expected, obviously due to the non-exhaustive sphagnum isolation. (3) Calcium and bicarbonate ions in fen water strongly suppress growth of most sphagna, and they can be overgrown by brown mosses (cf. Granath et al. 2010; Vicherová et al. 2015). Sphagna can establish and survive thanks to their high water retention capacity; the pH and ion concentration of the water can be reduced by the buffering action of the cell-wall carboxyls (here enumerated as CEC; Vicherová et al. (2017)). Furthermore, the typical bog hummock species *S. fuscum* can expand as its decay-resistant litter gives an ability to form high hummocks, thereby avoiding the influence of the fen water ions. Litter quality parameters were highly consistent between the two sets of *S. fuscum* collected in a bog and a rich fen (Fig. 3).

## Parameters related to chemical stability

All three parameters related to chemical stability of the litter components (*dev100*, soluble KL and 205:280 absorbance ratio) were correlated with the biological degradability. Lower chemical stability of KL after acidolysis in species of sect. *Cuspidata* was reported by Straková et al. (2010). We introduced *dev100* because our results revealed no trade-off in production between the two main components of *Sphagnum* cell walls, i.e. holocellulose and lignin-like phenolics. However, we identified such a trade-off in litters of the 8 *Sphagnum* species presented by Straková et al. (2010). Since we expected the summed weight of HC and total KL to give almost 100% of the initial litter, our results (ranging between -16 and +4%) raised a question of reliability of the two gravimetric analytical methods for bryophytes.

The positive values of *dev100* in sect. *Acutifolia* species (+3%) indicates a greater resistance of lignin-like phenolics to the sodium chlorite bleaching, resulting in an overestimation of holocellulose concentration (Reeves 1993). On the other hand, the lowest *dev100* in species of sect. *Cuspidata* (-9%) may be the result of greater degradation of HC, perhaps of the easily hydrolysable hemicelluloses (Kumar et al. 2013) rather than the highly resistant cellulose microfibrils or sphagnum (sphagnum was not depleted in *Cuspidata* during holocellulose extraction as shown by a constant ratio of litter-derived CEC and holocellulose-derived sphagnum). Litters in the study of Straková et al. (2010) had *dev100* between -13 and -17% in sections *Sphagnum+Cuspidata* and *Acutifolia*, respectively. Here, the difference between sections could be explained by hot-water extractives (4–8%). By contrast, Turetsky et al. (2008) reported *dev100* between -1% (*S. fuscum*) to -24% (mean in sect. *Cuspidata*) in assumedly living shoot segments, but this wide range could not be explained by extractives. Both studies used similar (but not identical) protocols for HC and KL isolations. The proposed greater degradability of holocellulose fractions through chlorite treatment is in agreement with the lower 205:280 absorbance ratio, which indicates a greater proportion of carbohydrates in *Cuspidata* litters. Moreover, Farmer and Morrison (1964) observed that material of *Sphagnum* sp. (but not of the vascular plant *Phragmites australis*), when subjected to mild acid hydrolysis, readily released fragments of phenolic polymers attached to oligosaccharides containing xylose and rhamnose. These sugars

are typical building blocks of hemicelluloses and are thus likely the reason to why the chemical degradation of lignin-like phenolics also removes some hemicelluloses. In conclusion, the chlorite extraction procedure may underestimate holocellulose concentration in litter of the easily decomposable *Sphagnum* species. As these species have considerably lower proportion of non-carbohydrate polymers, their holocellulose concentration is, in fact, most probably higher than in other species suggesting that chlorite extraction should not be used for holocellulose quantification in *Sphagnum*.

### Phylogeny vs habitat

The PCA based on the metabolites only (Fig. 2) indicate a strong phylogenetic signal, while when including nutrients and carbon (Fig. 3), the clustering is more similar to the results based on growth and decay traits (Bengtsson et al. 2016a). Mosses sampled in spruce forest (*S. girgensohnii* and one set of *S. magellanicum*) are now outliers, because of a greater nutrient availability and not because of their biochemistry. This reflects the additional effect of habitat, and specifically nutrient availability on decomposition. C/N ratio is commonly suggested to be a main factor determining decomposition rate in terrestrial plant litter (Bardgett 2005). Positive correlation between litter mass loss and N has also been reported for *Sphagnum* (Freeman et al. 2001; Limpens and Berendse 2003; Bragazza et al. 2006; Bragazza and Freeman 2007; Hájek 2009). However, we must stress that in our analysis, N and P had lower correlations with lab mass loss than any of the studied metabolites (see also Turetsky et al. 2008).

As expected, the three metabolites we found to be the best predictors of litter quality were lowest in section Cuspidata. This section mainly includes wet-growing species, and their higher decomposability prevents litter accumulation that could otherwise shift the habitat towards a drier one (hummock), which the species are not adapted to. In contrast, Acutifolia species had the highest concentrations of metabolites related to decay resistance, allowing them to maintain the microhabitat they are well adapted to, and to avoid competition with the fast-growing species of wet habitats. The biochemistry controls decay resistance also within section Acutifolia as shown by the greater lab mass loss in the wettest-growing species *Sphagnum rubellum* and the forest outlier *S. girgensohnii* (Bengtsson et al. 2016a), although the latter species is rather under habitat control

(higher nitrogen concentration). These results were clearly reflected also in the PCA, but the effects of habitat versus phylogeny are hard to fully separate. Limpens et al. (2017) concluded that the composition of the dominant primary metabolites (carbohydrates) in *Sphagnum* is phylogenetically conserved, while the secondary metabolite composition and nutrient concentration are rather controlled by the environment. Instead, we found a strong phylogenetic control on both primary and secondary metabolites, and environmental control only on the tissue nutrient concentration. This discrepancy may result from different analytical approaches; while this study employed more direct and quantitative measures of phenolics quantification, Limpens et al. (2017) applied an indirect and less quantitative method of pyrolysate composition analysis.

### Conclusions

We conclude that the most important metabolites possibly affecting litter quality are sphagnum and soluble phenolics, both which have been independently acknowledged before, but we add the lignin-like phenolics to the discussion as a major contributor. Alternatively, the soluble phenolics could be negatively correlated with decay in lab because they may originate as oxidation products of the overlooked lignin-like phenolics.

The high intrinsic decay resistance of hummock sphagna, which is interlocked with their anatomical stress-avoiding adaptations, can be seen as a trade-off for fast competitive growth. Higher concentrations of the litter metabolites is necessary for success in drier habitats, while fast growth is facilitated by water availability in wetter habitats.

We found that the quantity of several metabolites produced by sphagna vary dependant on species and habitat. We conclude that metabolites are under strong phylogenetic control, but their effects on decay are modified by nutrient concentrations in the litter, which is under habitat control.

Our results call for reviewing the mechanisms of how and to which extent biochemistry affects the litter quality of *Sphagnum*, particularly because the significance of the soluble and lignin-like phenolics has previously been questioned. As the structural carbohydrates represent a majority of *Sphagnum* litter, the decay properties of holocellulose constituents of *Sphagnum* (including the interactions with other compounds) deserves detailed investigation.

The variation of metabolite concentration within species is small compared to between species, which indicates that comparisons over larger scale based on few samples of each species or literature data can be made.

**Acknowledgements** Financial support was obtained from the Swedish Research Council (to HR) and by scholarships from Kungliga Vetenskapsakademien, Svenska Växtgeografiska Sällskapet and Extensus (to FB). TH was supported by a long-term research development project of the Institute of Botany, Czech Academy of Sciences (RVO 67985939). Müge Apaydin, Sandra Maciarzová, Elisa Pedrotti, Päivi Turunen and Aaron Wan assisted in field and lab. Nils Cronberg and Håkan Hytteborn commented on the manuscript.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

## 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*. *Carbohydr Polym* 67:104–115. <https://doi.org/10.1016/j.carbpol.2006.04.020>
- Ballance S, Kristiansen KA, Holt J, Christensen BE (2008) Interactions of polysaccharides extracted by mild acid hydrolysis from the leaves of *Sphagnum papillosum* with either phenylhydrazine, *o*-phenylenediamine and its oxidation products or collagen. *Carbohydr Polym* 71:550–558. <https://doi.org/10.1016/j.carbpol.2007.06.025>
- Ballance S, Kristiansen KA, Skogaker NT, Tvedt KE, Christensen BE (2012) The localisation of pectin in *Sphagnum* moss leaves and its role in preservation. *Carbohydr Polym* 87:1326–1332. <https://doi.org/10.1016/j.carbpol.2011.09.020>
- Bardgett R (2005) *The biology of soil*. Oxford University Press, Oxford
- Bärlocher F, Graça MAS (2005) Total phenolics. In: MAS G, Bärlocher F, Gessner MO (eds) *Methods to study litter decomposition: a practical guide*. Springer, Dordrecht, pp 45–48
- Belyea LR (1996) Separating the effects of litter quality and microenvironment on decomposition rates in a patterned peatland. *Oikos* 77:529–539
- Bengtsson F, Granath G, Rydin H (2016a) Photosynthesis, growth, and decay traits in *Sphagnum* - a multispecies comparison. *Ecology and Evolution* 6(10):3325–3341. <https://doi.org/10.1002/ece3.2119>
- Bengtsson F, Granath G, Rydin H (2016b) Data from: photosynthesis, growth, and decay traits in *Sphagnum* – a multispecies comparison. Dryad Digital Repository. <https://doi.org/10.5061/dryad.62054>
- Berg B (2014) Foliar litter decomposition: a conceptual model with focus on pine (*Pinus*) litter — a genus with global distribution. *ISRN Forestry* 2014:22. <https://doi.org/10.1155/2014/838169>
- Børsheim KY, Christensen BE, Painter TJ (2001) Preservation of fish by embedment in *Sphagnum* moss, peat or holocellulose: experimental proof of the oxopolysaccharidic nature of the preservative substance and of its antimicrobial and tanning action. *Innovative Food Sci Emerg Technol* 2:63–74
- Bragazza L, Freeman C (2007) High nitrogen availability reduces polyphenol content in *Sphagnum* peat. *Sci Total Environ* 377:439–443. <https://doi.org/10.1016/j.scitotenv.2007.02.016>
- Bragazza L, Freeman C, Jones T, Rydin H, Limpens J, Fenner N, Ellis T, Gerdol R, Hájek M, Hájek T, Iacumin P, Kutnar L, Tahvanainen T, Toberman H (2006) Atmospheric nitrogen deposition promotes carbon loss from peat bogs. *Proc Natl Acad Sci USA* 103:19386–19389. <https://doi.org/10.1073/pnas.0606629104>
- Bragina A, Maier S, Berg C, Müller H, Chobot V, Hadacek F, Berg G (2012) Similar diversity of Alphaproteobacteria and nitrogenase gene amplicons on two related *Sphagnum* mosses. *Front Microbiol* 2:275. <https://doi.org/10.3389/fmicb.2011.00275>
- Chi C, Zhang Z, Chang H-m, Jameel H (2009) Determination of furfural and hydroxymethylfurfural formed from biomass under acidic conditions. *J Wood Chem Technol* 29:265–276. <https://doi.org/10.1080/02773810903096025>
- Clymo RS (1963) Ion exchange in *Sphagnum* and its relation to bog ecology. *Ann Bot* 27:309–324
- Clymo RS (1965) Experiments on breakdown of *Sphagnum* in two bogs. *J Ecol* 53:747–758
- Clymo RS, Hayward PM (1982) The ecology of *Sphagnum*. In: Smith AJE (ed) *Bryophyte ecology*. Chapman and Hall, London, pp 229–289
- Dorrepal E (2005) Are growth forms consistent predictors of leaf litter quality and decomposability across peatlands along a latitudinal gradient? Plant growth forms and litter quality. *J Ecol* 93:817–828. <https://doi.org/10.1111/j.1365-2745.2005.01024.x>
- Dray S, Dufour AB (2007) The ade4 package: implementing the duality diagram for ecologists. *J Stat Softw* 22:1–20
- Ehrman T (1996) Determination of acid-soluble lignin in biomass. Laboratory Analytical Procedure No.004. National Renewable Energy Laboratory, Golden, p 1–7
- Farmer VC, Morrison RI (1964) Lignin in *Sphagnum* and *Phragmites* and in peats derived from these plants. *Geochim Cosmochim Acta* 28:1537–1546
- Flatberg KI (2013) *Norges torvmoser*. Akademika, Oslo
- Freeman C, Ostle N, Kang H (2001) An enzymic ‘latch’ on a global carbon store. *Nature* 409:149
- Granath G, Strengbom J, Breeuwer A, Heijmans MMPD, Berendse F, Rydin H (2009) Photosynthetic performance in *Sphagnum* transplanted along a latitudinal nitrogen deposition gradient. *Oecologia* 159:705–715
- Granath G, Strengbom J, Rydin H (2010) Rapid ecosystem shifts in peatlands: linking plant physiology and succession. *Ecology* 91:3047–3056

- Hájek T (2009) Habitat and species controls on *Sphagnum* production and decomposition in a mountain raised bog. *Boreal Environ Res* 14:947–958
- Hájek T (2014) Physiological ecology of peatland bryophytes. In: Hanson DT, Rice SK (eds) *Photosynthesis in early land plants*. Springer, Dordrecht, pp 233–252
- Hájek T, Adamec L (2009) Mineral nutrient economy in competing species of *Sphagnum* mosses. *Ecol Res* 24:291–302. <https://doi.org/10.1007/s11284-008-0506-0>
- Hájek T, Vicherová E (2013) Desiccation tolerance of *Sphagnum* revisited: a puzzle resolved. *Plant Biol* 16:765–773
- Hájek T, Ballance S, Limpens J, Zijlstra M, Verhoeven JTA (2011) Cell-wall polysaccharides play an important role in decay resistance of *Sphagnum* and actively depressed decomposition in vitro. *Biogeochemistry* 103:45–57. <https://doi.org/10.1007/s10533-010-9444-3>
- Hatfield R, Fukushima RS (2005) Can lignin be accurately measured? This paper was originally presented at the lignin and forage digestibility symposium, 2003 CSSA annual meeting, Denver, CO. *Crop Sci* 45:832–839. <https://doi.org/10.2135/cropsci2004.0238>
- IPCC (2013) *Climate change 2013: the physical science basis*. In: Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) *Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, and New York, pp 1535. <https://doi.org/10.1017/CBO9781107415324>
- Johnson LC, Damman AWH (1991) Species-controlled *Sphagnum* decay on a south Swedish raised bog. *Oikos* 61: 234–242. <https://doi.org/10.2307/3545341>
- Kremer C, Pettolino F, Bacic A, Drinnan A (2004) Distribution of cell wall components in *Sphagnum* hyaline cells and in liverwort and hornwort elaters. *Planta* 219:1023–1035. <https://doi.org/10.1007/s00425-004-1308-4>
- Kumar R, Hu F, Hubbell CA, Ragauskas AJ, Wyman CE (2013) Comparison of laboratory delignification methods, their selectivity, and impacts on physicochemical characteristics of cellulosic biomass. *Bioresour Technol* 130:372–381. <https://doi.org/10.1016/j.biortech.2012.12.028>
- Laing CG, Granath G, Belyea LR, Allton KE, Rydin H (2014) Tradeoffs and scaling of functional traits in *Sphagnum* as drivers of carbon cycling in peatlands. *Oikos* 123:817–828
- Limpens J, Berendse F (2003) How litter quality affects mass loss and N loss from decomposing *Sphagnum*. *Oikos* 103:537–547
- Limpens J, Bohlin E, Nilsson MB (2017) Phylogenetic or environmental control on the elemental and organo-chemical composition of *Sphagnum* mosses? *Plant Soil*:1–17. <https://doi.org/10.1007/s11104-017-3239-4>
- Loisel J et al (2014) A database and synthesis of northern peatland soil properties and Holocene carbon and nitrogen accumulation. *The Holocene* 24:1028–1042. <https://doi.org/10.1177/0959683614538073>
- Maksimova V, Klavina L, Bikovens O, Zicmanis A, Purmalis O (2013) Structural characterization and chemical classification of some bryophytes found in Latvia. *Chem Biodivers* 10: 1284–1294. <https://doi.org/10.1002/cbdv.201300014>
- Mellegård H, Stalheim T, Hormazabal V, Granum PE, Hardy SP (2009) Antibacterial activity of sphagnum acid and other phenolic compounds found in *Sphagnum papillosum* against food-borne bacteria. *Lett Appl Microbiol* 49:85–90. <https://doi.org/10.1111/j.1472-765X.2009.02622.x>
- Morris PJ, Waddington JM, Benscoter BW, Turetsky MR (2011) Conceptual frameworks in peatland ecohydrology: looking beyond the two-layered (acrotelm–catotelm) model. *Ecohydrology* 4:1–11
- Painter TJ (1991) Lindow man, Tollund man, and other peat-bog bodies: the preservative and antimicrobial action of sphagnum, a reactive glycuronoglycan with tanning and sequestering properties. *Carbohydr Polym* 15:123–142
- Painter TJ (2003) Concerning the wound-healing properties of *Sphagnum* holocellulose: the Maillard reaction in pharmacology. *J Ethnopharmacol* 88:145–148. [https://doi.org/10.1016/S0378-8741\(03\)00189-2](https://doi.org/10.1016/S0378-8741(03)00189-2)
- Preston CM, Trofymow JA, Niu J, Sayer BG (1997) <sup>13</sup>C nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning investigation of the proximate-analysis fractions used to assess litter quality in decomposition studies. *Can J Bot* 75:1601–1613. <https://doi.org/10.1139/b97-872>
- Rasmussen S, Wolff C, Rudolph H (1995) Compartmentalization of phenolic constituents in *Sphagnum*. *Phytochemistry* 38: 35–39. [https://doi.org/10.1016/0031-9422\(94\)00650-I](https://doi.org/10.1016/0031-9422(94)00650-I)
- R Core Team (2016) *R: a language and environment for statistical computing*. Foundation for Statistical Computing, Vienna
- Reeves JB (1993) Chemical studies on the composition of fiber fractions and lignin determination residues. *J Dairy Sci* 76: 120–128. [https://doi.org/10.3168/jds.S0022-0302\(93\)77331-2](https://doi.org/10.3168/jds.S0022-0302(93)77331-2)
- Rice SK, Aclander L, Hanson DT (2008) Do bryophyte shoot systems function like vascular plant leaves or canopies? Functional trait relationships in *Sphagnum* mosses (Sphagnaceae). *Am J Bot* 95:1366–1374. <https://doi.org/10.3732/ajb.0800019>
- Richter C, Dainty J (1989) Ion behavior in plant cell walls. I. Characterization of the *Sphagnum russowii* cell wall ion exchanger. *Can J Bot* 67:451–459
- Rydin H, Jeglum JK (2013) *The biology of peatlands*, 2 ed. Oxford University Press, Oxford, p 432
- Rydin H, Gunnarsson U, Sundberg S (2006) The role of *Sphagnum* in peatland development and persistence. In: Wieder RK, Vitt DH (eds) *Boreal peatland ecosystems*, vol 188. Springer, Berlin Heidelberg, pp 47–65
- Scheller HV, Ulvskov P (2010) Hemicelluloses. *Annu Rev Plant Biol* 61:263–289. <https://doi.org/10.1146/annurev-arplant-042809-112315>
- Sjörs H (1948) Myrvegetation i Bergslagen. *Acta phytogeogr Suec* 21:1–299
- SMHI (2014) LuftWebb.Swedish Meteorological and Hydrological Institute <http://luftwebb.smhi.se/>
- Spearing AM (1972) Cation-exchange capacity and galacturonic acid content of several species of *Sphagnum* in sandy ridge bog, Central New York state. *Bryologist* 75:154–158
- Stalheim T, Ballance S, Christensen BE, Granum PE (2009) Sphagnum – a pectin-like polymer isolated from *Sphagnum* moss can inhibit the growth of some typical food spoilage and food poisoning bacteria by lowering the pH. *J Appl Microbiol* 106:967–976. <https://doi.org/10.1111/j.1365-2672.2008.04057.x>
- Straková P, Anttila J, Spetz P, Kitunen V, Tapanila T, Laiho R (2010) Litter quality and its response to water level draw-down in boreal peatlands at plant species and community

- level. *Plant Soil* 335:501–520. <https://doi.org/10.1007/s11104-010-0447-6>
- Talbot JM, Treseder KK (2012) Interactions among lignin, cellulose, and nitrogen drive litter chemistry–decay relationships. *Ecology* 93:345–354. <https://doi.org/10.1890/11-0843.1>
- Templeton D, Ehrman T (1995) Determination of acid-insoluble lignin in biomass. Laboratory Analytical Procedure No.003. National Renewable Energy Laboratory, Golden, p 1–13
- Tsuneda A, Thormann MN, Currah RS (2001) Modes of cell-wall degradation of *Sphagnum fuscum* by *Acremonium* cf. *curvulum* and *Oidiodendron maius*. *Can J Bot* 79:93–100
- Turetsky MR (2003) The role of bryophytes in carbon and nitrogen cycling. *Bryologist* 106:395–409. <https://doi.org/10.1639/05>
- Turetsky MR, Crow SE, Evans RJ, Vitt DH, Wieder RK (2008) Trade-offs in resource allocation among moss species control decomposition in boreal peatlands. *J Ecol* 96:1297–1305. <https://doi.org/10.1111/j.1365-2745.2008.01438.x>
- Verhoeven JTA, Liefveld WM (1997) The ecological significance of organochemical compounds in *Sphagnum*. *Acta Bot Neerlandica* 46:117–130
- Vicherová E, Hájek M, Hájek T (2015) Calcium intolerance of fen mosses: physiological evidence, effects of nutrient availability and successional drivers. *Perspect Plant Ecol Evol Syst* 17:347–359. <https://doi.org/10.1016/j.ppees.2015.06.005>
- Vicherová E, Hájek M, Šmilauer P, Hájek T (2017) *Sphagnum* establishment in alkaline fens: importance of weather and water chemistry. *Sci Total Environ* 580:1429–1438. <https://doi.org/10.1016/j.scitotenv.2016.12.109>
- Wieder RK, Starr ST (1998) Quantitative determination of organic fractions in highly organic, *Sphagnum* peat soils. *Commun Soil Sci Plant Anal* 29:847–857
- Williams CJ, Yavitt JB, Wieder RK, Cleavitt NL (1998) Cupric oxide oxidation products of northern peat and peat-forming plants. *Can J Bot* 76:51–62