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Source: Canadian Journal of Soil Science, 102(3) : 785-795

Published By: Canadian Science Publishing

URL: https://doi.org/10.1139/cjss-2021-0137

Canadian Journal of Soil Science

OPEN ACCESS | Article

Biochemical components of *Sphagnum* and persistence in peat soil

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Abstract

The amounts and arrangements of polysaccharides (cellulose and hemicellulose), proteins, phenolic lignin, and pectin that make up plant tissue, in part, determine its decay rate. Lignin-rich and/or nitrogen-poor tissue has been described as biochemically recalcitrant causing a slow decay rate. Although a controversial mechanism for organic matter storage in soils with mineral particles, biochemical recalcitrance is still poorly understood in organic peat soil (Histosols). To investigate the role of *Sphagnum* in formation of peat soil, we characterize biochemical components for 10 species and examine persistence of the components in soil to 150 cm depth in three peatland ecosystems. We hypothesize that species from hummock microforms have more biochemical structural components and cohesion than species from hollows. Relative proportions of biochemical components changed markedly between plant material and the top 10 cm of peat soil, suggesting that decomposition occurred at the peat soil surface, but thereafter relative proportions of biochemical components did not vary significantly to 150 cm deep. A few differences in biochemical components that distinguished hummock species from hollow species persisted to the deepest depth sampled. Although persistence of the lignin-like component was expected, persistence of soluble and ionically bound pectin compounds was surprising as these biopolymers are thought to be readily decomposable. Our findings indicate that structural components of *Sphagnum*, specifically polysaccharides and pectin in addition to oft-cited phenolic lignin-like components, persist in peat soil and should not be overlooked in trying to understand carbon dynamics in *Sphagnum*-dominated ecosystems.

Key words: bogs, decomposition, litter quality, northern peatland, soil organic matter

Résumé

La quantité de polysaccharides (cellulose, hémicellulose), de protéines, de lignine phénolique et de pectine qui forment en partie le tissu végétal, et la manière dont ces composés sont structurés, déterminent la rapidité avec laquelle celui-ci se décompose. On dit que les tissus riches en lignine ou pauvres en azote sont « biochimiquement récalcitrants », ce qui explique la lenteur de leur dégradation. Bien que cette méthode de stockage de la matière organique dans les sols renfermant des particules minérales soit controversée, la récalcitrance biochimique reste un mécanisme mal connu dans les sols tourbeux organiques (histosols). Pour préciser le rôle de la sphaigne dans la genèse des sols tourbeux, les auteurs ont déterminé la composition biochimique de dix espèces et mesuré la persistance des composés dans le sol de trois tourbières, à une profondeur de 150 cm. Selon eux, les espèces poussant sur les micro-tertres possèdent plus de composants structuraux et une meilleure cohésion biochimique que celles poussant dans les creux. Les proportions relatives des composants biochimiques varient nettement avec la matière végétale et dans les dix premiers centimètres du sol tourbeux pour ensuite demeurer relativement stables jusqu'à 150 cm de profondeur. Les quelques différences relevées entre les composés biochimiques des espèces qui poussent sur les tertres et e celles poussant dans les creux subsistent dans les échantillons prélevés à la plus grande profondeur. Même si la persistance des composés semblables à la lignine est prévisible, celle des composés de la pectine solubles et liés de façon ionique a de quoi surprendre, car on pensait que ces biopolymères se décomposaient aisément. Ces constatations révèlent que les composants structuraux de la sphaigne, plus précisément les polysaccharides et la pectine, persistent dans le sol tourbeux, en plus des composants, souvent mentionnés, de la lignine phénolique, et qu'il ne faudrait pas les négliger quand on tente d'élucider la dynamique du carbone dans les écosystèmes où domine la sphaigne. [Traduit par la Rédaction]

Mots-clés : tourbières, décomposition, qualité de la litière, sol tourbeux nordique, matière organique du sol

Introduction

The paradigm that explains transformation of plant material into persistent soil organic matter is changing. The older "biochemical recalcitrance" hypothesis asserts that certain components of plant material, such as lignin, resist microbial decomposers (recalcitrant) and partially decomposed products undergo secondary syntheses into new stable humic substances (refractory) forming the nucleus of soil organic matter (Kononova 2013). However, newer paradigms recognize that no components in plant material are truly recalcitrant (Liang et al. 2017) and secondary synthesis of humic substances is an outcome of extraction methods (Kleber and Lehmann 2019). The new paradigm attributes persistence of soil organic matter to physical interaction with mineral particles, thereby preventing further microbial decomposition (Kleber et al. 2021). Thus, the "biochemical recalcitrance" hypothesis for formation and persistence of soil organic matter has become passe.

But how well paradigms developed for soils with mineral particles fit organic peat soils, especially those derived from *Sphagnum*, is less straightforward. *Sphagnum*-dominated peatlands cover <3% of the global land area (Xu et al. 2018) yet hold about 30% of the soil carbon (Yu 2012). Peatlands provide many ecosystem services in addition to carbon storage, such as regulation of water flow, protection of biodiversity, food and fuel, and opportunities for recreation (Page and Baird 2016). In addition, layers of accumulated peat soil contain an archive of information that is valuable for deciphering past climate, vegetation, and human activity (Rydin et al. 2013). Thus, it is imperative to understand the transformation of plant material into *Sphagnum*-derived peat soil.

Any paradigm to explain formation and persistence of *Sphagnum*-derived peat soil must consider the unique role for *Sphagnum* plant material. One is that *Sphagnum* has biochemical components with functional properties that constrain microbial decomposition. These components include phenolic compounds, structural biopolymers that shield polysaccharides, and cell-wall pectin with abundant acidic functional groups, among others (Painter 1991; Lang et al. 2009; Mellegård et al. 2009; Hájek et al. 2011; Ballance et al. 2012). Second is evidence that decomposition of *Sphagnum* does produce humic substances (Zaccone et al. 2018; Fissore et al. 2019). Third is the very low amounts of mineral particles required to stabilize soil organic matter (Zaccone et al. 2013; Savichev et al. 2020). Fourth, and perhaps the ultimate reason, is that a very slow decay rate of plant material is central to the peat-forming process (Clymo 1984).

The dynamics of plant litter decomposition are of central importance to paradigms of soil organic matter formation. The typical approach uses litterbags; plant samples of known mass are allowed to decompose for a known period before being retrieved and reweighed to quantify mass loss. For example, among the 20 or more *Sphagnum* species that can co-occur within a peatland (Piatkowski and Shaw 2019), decay-resistant compounds and slower decay rates are more pronounced for species that inhabit hummock microforms, which rise above the local water table, compared with species found in co-occurring hollows, which are closer to the local water table (Johnson and Damman 1991; Belyea 1996; Mäkilä et al. 2018). However, short-term studies of plant litter decay are only part of the "biochemical recalcitrance" hypothesis.

Accordingly, a better understanding of long-term plant litter decay in peat soil relies on other types of data. For example, an increase in the C:N ratio reflects more decomposition because microbial decomposers oxidize organic carbon to carbon dioxide or methane while retaining nitrogen for anabolism (Kuhry and Vitt 1996). Also, decomposition shifts stable isotope ratios of carbon and nitrogen to heavier values given that microbial decomposers preferentially oxidize the lighter isotopes (Drollinger et al. 2019). Likewise, the ratio of lignin-like compounds to cellulose and hemicellulose has shown the increases during decomposition (Herman et al. 2008). Similarly, the ratio of lignin-like compounds to nitrogen decreases during decomposition (Osono 2017). Another measure is lesser amounts of soluble organic compounds coming from more decomposed peat soil (Kalbitz and Geyer 2002). Since no single measure is perfect, (cf., Biester et al. 2014; Drzymulska 2016) we use several of these types of data.

Here we use two methods that fractionate plant material into basic components to better understand relationships between *Sphagnum* composition and peat soil. One is the forage fiber method, popularized by Van Soest et al. (1991), which distinguishes lignin-like compounds, cellulose and hemicellulose, from each other. A second method is taken from the plant physiology literature and uses solvents with different chemical properties to sequentially extract biochemical components based upon the way they held in the structural architecture of the cell wall (Selvendran and Ryden 1990). These components include hemicellulose, pectin, protein, and phenolic compounds. Thus, we evaluate structural cohesion of plant material rather following fates of individual molecules as typically done in diagenesis studies of organic matter (cf., Hedges and Prahl 1993).

We apply the analyses to 10 *Sphagnum* species and to samples of peat soil. Two hypotheses formed the basis for the study. First, structural components are weightier in *Sphagnum* species on hummocks than on hollows. Second, differences in biochemical components of between hummock species and hollow species persist in peat soils beneath the distinctive microforms. The second hypothesis examines the 'decomposition funnel' hypothesis (Swift et al. 1979; Fierer et al. 2009), which posits that during the decomposition process all plant material—regardless of differences in chemical composition—will converge toward a common chemistry as it becomes soil organic matter.

Materials and methods

Study sites

We used three *Sphagnum*-dominated peatlands located near Ithaca, NY, USA. Present-day climate is humid, continental with a mean annual temperature of 8.9 °C and with monthly mean temperatures ranging from –4.8 °C in January to 20.4 °C in July. Mean annual precipitation is 890 m, including 171 cm of snow. Regional vegetation belongs to Braun's (1950) hemlock, white pine, northern hardwoods formation. Bedrock of

Devonian age varies from limestone and calcareous shales and sandstones to acid shales. Soils formed in the glacial till, outwash, and lake sediment are mostly fine silt loams. Soil pH ranges from circumneutral with values of 6–7 to acidic with values of 3.5–4.5.

Sphagnum-dominated peatlands initiated in the kettle-kame landscape that formed following Wisconsin glaciation 2000–14 000 year B.P. (Gajewski et al. 2001). Most started out as ponds that transitioned into fens, dominated by graminoid plant species, after which they transitioned into bogs as peat soil accumulated and plants become further removed from nutrients in local ground water. Although no longer having a boreal or subarctic climate, the *Sphagnum*-dominated bogs have persisted and with similar plant species composition (Andrus 1986) and nutrient biogeochemistry (Dettling et al. 2006) compared with more northern counterparts (Wieder and Yavitt 1994). We collected plant material and a peat core from 0 to 150 cm depth in one hummock and one hollow per site.

Dryden Bog (42°26′51.5″N, 76°15′33.3″W) is a small bog approximately 150 m across (1.75 ha area). The maximum peat depth is 8 m. The surface consists of well-developed tall hummocks covered by the shrub *Chamaedaphne calyculata* (leatherleaf) with deep hollows. The water table is close to the surface of hollows following spring snowmelt and drops about 15 cm below the surface of the hollows by midsummer. We collected several shoots of *Sphagnum magellanicum* and *S. fuscum* from hummocks and shoots of *S. angustifolium* and *S. fallax* from hollows. In addition, we collected leaves from *C. calyculata* and the graminoid *Eriophorum vaginatum*. Further details of the site are in Yavitt et al. (2019).

McLean Bog (42°32′47.5″N, 76°15′59.4″W) is a kettle hole bog approximately 70 m across (0.4 ha area). Maximum peat depth is 8 m. The bog surface has low hummocks and larger hollows than in Dryden Bog. Evergreen shrubs include leatherleaf, *Rhododendron groenlandicum* (Labrador tea), and *Kalmia angustifolia* (sheep laurel). Cotton grass and *Dulichium arundinaceum* (three-way sedge) have moderate cover. We collected several shoots of *S. capillifolium* and *S. angustifolium* from hummocks and shoots of *S. recurvum* and *S. cuspidatum* from hollows. Further details of the site are in Brauer et al. (2004).

Labrador Hollow (42°46′52.3″N, 76°02′33.6″W) is a swamp forest dominated by *Acer rubrum* (red maple) and several conifer trees: *Pinus strobus* (white pine), *Tsuga canadensis* (eastern hemlock), and *Larix laricina* (larch). The understory is dominated by ferns, *Vaccinium corymbosum* (highbush blueberry), and *Toxicodendron radicans* (poison ivy). *Sphagnum* mosses cover about 75% of the ground area. There are fewer low hummocks than at the other two sites. We collected several shoots of *S. palustre* and *S. girgenshnii* from hummocks and shoots of *S. squarrosum* and from hollows. Further details of the site are available in Corteselli et al. (2017).

We collected a core of peat soil from a hummock and a hollow at each site using a Russian style peat core (Aquatic Research Instruments, Inc., Hope, ID, USA) that had a 5 cm diameter barrel. The cores extended from the surface of the peat deposit to a depth of 150 cm. We saved peat soil from five depth intervals: 0–10 cm, 10–20 cm, 30–40 cm, 100–110 cm, and 140–150 cm. Peat age at the 150 cm varied among sites: 2880 (2820 to 2940) year B.P. in Dryden Bog; 3080 (2950 to 3210) year B.P. in McLean Bog; and 2350 (2330 to 2470) year B.P. in Labrador Hollow (J.B. Yavitt, unpublished data). The fen-to-bog transition occurred about 4100 year B.P. in the region (McNamara et al. 1992) and is now at a depth of 2–4 m in the peat soil, depending on site.

Plant tissue

The samples were sorted by hand and any foreign matter was removed. Any damaged or abnormal-looking plant tissue was also removed. After rinsing under a light stream of water, the samples were dried to a constant mass at 35 °C before being finely ground using a mortar and pestle.

For the forage fiber method, portions of plant tissue were analyzed sequentially for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) on an ANKOM fiber analyzer (ANKOM Technology, Macedon, NY, USA). The analyses are used to approximate hemicellulose (NDF–ADF), cellulose (ADF–ADL), and lignin (ADL). Protocols are available at https://www.ankom.com/analytical-methods-support/fiber-analyzer-a2000. Note that, hereafter, we refer to the ADL fraction as lignin-like compounds (cf., Bengtsson et al. 2018), given that *Sphagnum* does not have lignin per se but rather the ADL fraction contains polyphenolic compounds that resemble lignin.

For the sequential extraction method, we used the procedure described in McLeod et al. (2007). Briefly, tissue (0.2 g) was extracted with 10% formic acid to release extracellular polysaccharides (fraction 1); with a phosphate buffer (200 mM NaH_2PO_4, 0.5% w/v chlorobutanol, 10 mM $Na_2S_2O_3$, adjusted to pH 7) to release weakly bound polysaccharides and proteins (Jamet et al. 2006) (fraction 2); with cyclohexanediamine tetra acetic acid (CDTA at pH 7.5) to remove calcium, which releases pectin held by ionic bonds (Jarvis 1982) (fraction 3); with urea to break hydrogen bonds, which mostly releases phenols and proteins (Loomis and Battaile 1966; Scalbert 1992) (fraction 4); with dilute sodium carbonate (200 mM Na_2CO_3 at 5 °C), which releases covalently ester-bound pectin within the cell wall (Gawkowska et al. 2018) (fraction 5); with strong sodium hydroxide (6 M NaOH, 1% w/v $NaBH_4$ at 37 °C) to release hemicellulose (Carpita 1984) (fraction 6); and finally with 5% formic acid to remove all remaining nonstructural polysaccharides (fraction 7). Each extraction took place in a 50 mL centrifuge tube with 6 mL of solvent added and incubated for 24 h. Each solvent was removed by centrifugation and saved for colorimetric analysis. The final residue, consisting of cellulose and lignin-like material, was weighed to compare with the analysis of the forage fiber method.

Total polysaccharides in the solvents were quantified using an o-phenanthroline colorimetric assay (Prado et al. 1998). Before the colorimetric assay, the extract was diluted with deionized water and de-salted through a 10 mL plastic syringe containing around 1 mL of microfiber glass wool and 9 mL exchange resin (Mixed Bed IONAC NM-60 H^+/OH^- Form, Type I, Beads 16–50 Mesh). De-salting was repeated until the remaining salt concentration was below 50 mS m^{-1}. The colorimetric assay was done by mixing 1 mL solution with 50 μL 0.1 M K_3 $Fe(CN)_6$ solution, 100 μL alkaline reagent, and 1 mL

color reagent (o-phenanthroline solution), and quantified by 505 nm wavelength.

The two methods produced independent estimates of hemicellulose. For plant tissue, the two estimates agreed with each other (Pearson correlation $r = 0.65$). However, for peat soil samples, the chemical extraction using sodium hydroxide consistently gave larger values than that from the forage fiber method. We assumed that the former included humic substances, as they are typically extracted from soil with sodium hydroxide (Olk et al. 2019), even though this has been questioned (Kleber and Lehmann 2019). Nevertheless, for the peat soil samples, we report hemicellulose from the forage fiber method and assume that humic substances are the excess values in the sodium hydroxide extract, i.e., raw value minus the amount of hemicellulose from the forage fiber method. But rather than further muddying the debate whether humic substances occur in soil organic matter or not (Lehmann and Kleber 2015), hereafter, we refer to the humic substances as alkali-extract excess.

Additional portions of plant material and peat soil were analyzed for concentrations of carbon and nitrogen and for ratios of stable isotopes of carbon and nitrogen using a continuous flow isotope ratio mass spectrometer (Thermo Finnigan Environmental Delta V) coupled to an elemental analyzer (Thermo Finnigan Carlo Erba NC2500) at the Cornell Stable Isotope Laboratory (COIL). Stable isotope ratios are expressed as delta values (per mil), which is the ratio of heavy to light isotopes of the sample relative to the international standards for carbon (Vienna-Pee-Dee Belemnite) and nitrogen (atmospheric N_2).

For carbon isotopes, it was necessary to adjust values of modern plant material to account for the global decrease in the delta ^{13}C value of atmospheric carbon dioxide because of fossil fuel burning over the past 150 years, i.e., the Suess Effect. Based on ice-core records (Indermuhle et al. 1999), we increased the amount of ^{13}C in modern plant material by 2.0 per mil (Dombrosky 2020). The correction makes a more acceptable comparison between modern plant material and plant residues in peat soil, deposited before fossil fuel burning (cf., Chamberlain et al. 2005).

Data analysis

We applied the lignocellulose index (LCI) to the data, which is defined as the amount of lignin divided by the sum of the amounts of lignin + cellulose + hemicellulose. We also applied the lignin nitrogen index (LNI) to the data, which is defined as the amount of lignin divided by the amount of nitrogen (Osono 2017).

We used repeated-measures analysis of variance (ANOVA) to test for overall differences in relative proportions of biochemical components among hummock plants and hollow plants, among hummock soils and hollow soils, and across depths in the peat soils. A repeated measures analysis is necessary because the individual biochemical components are not independent of each other in a plant tissue or a soil sample, nor are soil samples from a specific depth interval independent form another depth interval in the same core, both of which violate independence in linear model. Percentage values for biochemical components were logit transformed before analyses. To disentangle significant interaction effects in the analyses, we performed post hoc comparisons using *t* tests with a Bonferroni correction (Holm 1979).

Results

Plant material

The relative proportions of biochemical components in plant tissue (Table 1) differed significantly between *Sphagnum* species on hummocks versus hollows ($F_{[1,198]} = 17.09$, $P < 0.0001$). Hummock species had 54% more lignin-like components, whereas hollow species had 2.2 times more biochemical components in the formic acid extracts and 56% to 74% more biochemical components in the phosphate buffer and CDTA extracts. The significant difference for cellulose was 7% more in hollow species than in hummock species.

Compared with the *Sphagnum* species (Table 1), leaf material from shrubs had 76% more lignin-like components, 61% more hemicellulose, and 1.9 times more biochemical components in the urea extract. On the other hand, *Sphagnum* had 2.6 times more cellulose and 3.7 times more biochemical components in the CDTA extract compared with shrub leaf material. The shrub leaf material also had greater concentrations of nitrogen and lighter values for delta ^{15}N compared with *Sphagnum*. In contrast to *Sphagnum*, graminoid leaf material had 51% more hemicellulose, but *Sphagnum* had 4.0 times more biochemical components in the CDTA extract. Graminoid leaf litter also had heavier delta ^{15}N values compared with *Sphagnum* material.

Peat soil

Relative proportions of biochemical components were sharply different between plant material and peat soil from 0 to 10 cm depth interval (Fig. 1). The ANOVA revealed a significant difference for the hummock microform ($F_{[1,40]} = 7.02$, $P = 0.0115$). Although the main effect was not significant for the hollow microform ($F_{[1,40]} = 0.08$, $P = 0.7728$), the interaction term was significant ($F_{[9,40]} = 15.36$, $P = <0.0001$). In both microforms, relative proportions of cellulose and hemicellulose decreased as proportions of lignin-like components and the alkali-extract excess increased between plant material and the peat soil. Furthermore, in the hummock microform, relative proportions of biochemical components extracted by phosphate buffer and by CDTA increased by about 60% in the peat soil, whereas proportions for these extractable fractions decreased between plant material and peat soil in the hollow setting.

The ANOVAs revealed that relative proportions of the biochemical components did not vary significantly among the sampled depth intervals in the peat soils in either the hummock setting ($F_{[4,100]} = 0.44$, $P = 0.7818$) or the hollow setting ($F_{[4,100]} = 0.87$, $P = 0.4813$). On the other hand, a comparison of values between hummocks and hollows for the two deepest depth intervals indicated a marginally significant difference ($F_{[1,100]} = 3.42$, $P = 0.0675$) between microforms (Table 2). Hollow peat had 22% more of the lignin-like components, whereas hummock peat had 2.1 times more bio-

Table 1. Biochemical composition of *Sphagnum* species on hummocks and hollows and leaf material from co-occurring graminoids and shrubs.

Component	Hummock	Hollow	Graminoid	Shrub
Carbon (%)	42.3 ± 0.8	40.9 ± 0.4	42.0 ± 0.4	43.0 ± 0.3
Delta ^{13}C (per mil)	–27.9 ± 0.3	–27.0 ± 0.7	–27.8 ± 0.5	–27.5 ± 0.6
Nitrogen (%)	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	1.1 ± 0.2
Delta ^{15}N (per mill)	–4.9 ± 0.3	–4.7 ± 0.3	–2.0 ± 0.2	–6.0 ± 0.9
Forage fiber				
Lignin like (g kg^{-1})	**149 ± 27**	**97 ± 12**	103 ± 46	216 ± 38
Cellulose (g kg^{-1})	**373 ± 18**	**400 ± 15**	369 ± 1	151 ± 10
Hemicellulose (g kg^{-1})	140 ± 27	104 ± 12	222 ± 17	237 ± 73
Extractant				
Formic acid (1) (g kg^{-1})	**29 ± 16**	**94 ± 18**	39 ± 24	130 ± 37
Phosphate buffer (g kg^{-1})	**45 ± 8**	**77 ± 11**	27 ± 10	60 ± 16
CDTA (g kg^{-1})	**58 ± 14**	**91 ± 21**	15 ± 2	20 ± 4
Urea (g kg^{-1})	57 ± 20	55 ±± 13	67 ± 14	160 ± 8
Sodium carbonate (g kg^{-1})	111 ± 19	104 ± 12	114 ± 12	131 ± 36
Formic acid (2) (g kg^{-1})	**53 ± 14**	**84 ± 15**	4 ± 1	14 ± 13

Note: Values are mean ± 1 standard error (n = 11 for *Sphagnum* species; n = 3 for graminoids and shrubs). The delta ^{13}C values have been corrected by the addition of 2.0 per mill to account for the Suess Effect. Bold indicates significant difference between hummock species and hollow species ($P < 0.05$).

chemical components in the formic acid extract, 53% more in the phosphate buffer extract, and 71% more in the sodium carbonate extract.

The indices for organic matter decomposition showed the largest change between plant material and peat soil in the 0–10 cm depth interval (Fig. 2). All changes were statistically significant (all $P < 0.05$), except the LNI in the hollow setting. The C:N ratio decreased, delta ^{13}C and delta ^{15}N showed heavier values, and the LCI increased between plant material and peat soil. In contrast, the ANOVAs revealed that values for the indices did not vary significantly among the sampled depth intervals in the peat soil for the C:N ratio ($F_{[4,20]} = 1.00$, $P = 0.4347$), delta ^{15}N values ($F_{[4,20]} = 1.28$, $P = 0.3177$), the LCI ($F_{[4,20]} = 1.29$, $P = 0.3067$), and the LNI ($F_{[4,20]} = 0.59$, $P = 0.6716$). However, delta ^{13}C values showed a distinctive pattern with the heaviest values in the 10–20 cm depth interval and marginally significant ($F_{[4,20]} = 2.73$, $P = 0.0579$) lighter values in near surface peat and in the deeper sampled depth intervals.

Discussion

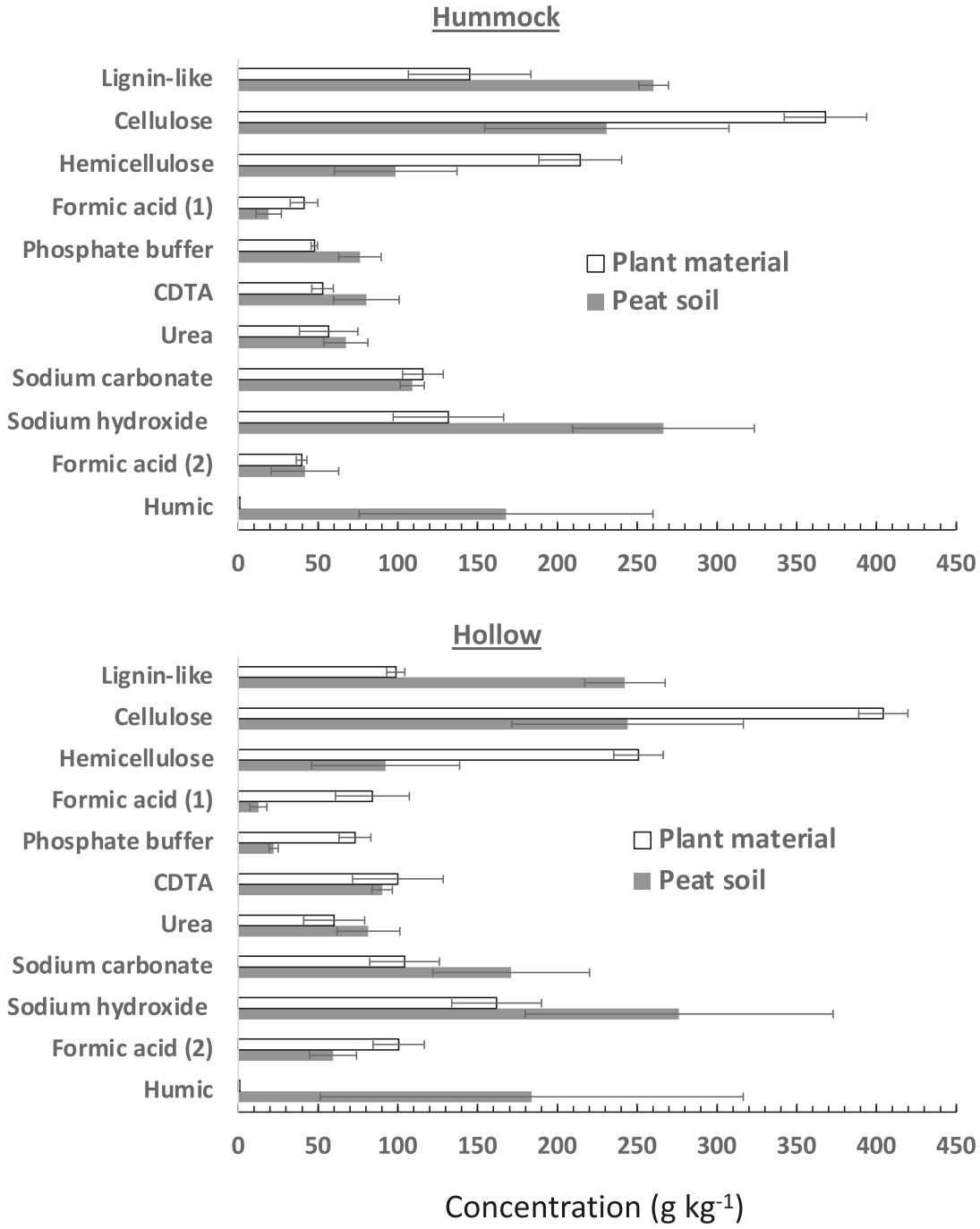
Plant material

Our first aim was to examine differences in the composition of *Sphagnum* species on hummocks versus on hollows. Prior studies for *Sphagnum* found more lignin-like components in hummock species than in hollow species (Bengtsson et al. 2018; Piatkowski and Shaw 2019), and Limpens et al. (2017) found that hummock species invest more in pectin, whereas hollow species invest more in hemicellulose. Also, hollow species have shown to have greater amounts of soluble phenolic compounds (Chiapusio et al. 2018).

Our results confirm the microform difference for lignin-like components and extend differences to cellulose and biochemical components extracted with formic acid, phosphate buffer, and CDTA. The formic acid extractant removes extracellular polysaccharides; formic acid limits the oxidation of these compounds (Parsons and Tinsley 1960). Studies using other plants suggest this fraction is mostly soluble pectin (Robichaux and Morse 1990), which likely applies to *Sphagnum* given its large amount of pectin. Therefore, hollow species have a greater magnitude of soluble pectin than hummock species. The fraction extracted with phosphate buffer includes loosely bound proteins, many of which have a structural role not only binding neighboring cells to each other (Cassab 1998), but also functioning in the expansion of tissue when needed (Cosgrove 2015). Therefore, greater magnitude of this fraction in hollow species could play a role in a faster rate of decomposition in a similar way that loosely bound proteins function in the softening of fruit when ripe (Vicente et al. 2007). CDTA removes calcium, which releases pectin held by ionic bonds (Jarvis 1982). This pectin fraction is mostly in the middle lamella where it functions to help to bind adjacent cells to each other (Bou Daher and Braybrook 2015). It is important to distinguish pectin in the CDTA-extractable fraction from pectin in the sodium carbonate-extractable fraction, as the latter releases components covalently bound to each other mostly in the cell wall. Although we did not find hummock species investing more in pectin, as reported by Limpens et al. (2017), our results do suggest that hummock species have more of their pectin in the cell wall compared with equal amounts in the middle lamella and cell wall for hollow species. Accordingly, cell wall pectin, particularly in *Sphagnum* is thought to be persistent and responsible, in part, for preservation (Ballance et al. 2012).

The extraction using urea is thought to release soluble phenolic compounds held in plant tissue by hydrogen bonds (Loomis and Battaile 1966; Scalbert 1992). Although we did not measure phenolics per se, our results show no difference

Fig. 1. Mean concentrations (±1 standard error) of biochemical components in *Sphagnum* plant material and in peat soil from 0 to 10 cm depth interval for hummock and hollow microforms in peatlands in New York State.



between hummock species and hollow species. In contrast, Chiapusio et al. (2018) found that hollow species had greater investment in free phenolic compounds. It is worth noting, however, that Fudyma et al. (2019) examined specific compounds in *S. fallax* and found only two phenols, which is much less than others had predicted. Thus, the role of phenolics compounds in decay resistance of *Sphagnum* is still open to debate (cf., Urbanova and Hajek 2021).

Sphagnum angustifolium was the only species that occurred on both hummocks and hollows. Although *Sphagnum* species are overwhelmingly microform specialists (Piatkowski and Shaw 2019) and *S. angustifolium* is known as a hollow-forming species, it can inhabit low hummocks (Andrus 1986). Notably, we did not detect differences in relative proportions of biochemical components among entities from the two microforms. However, the lack of statistically significant differences is, in part, related to the small sample size. Consequently, it would be interesting to use a larger sample size to assess the extent to which proportions of biochemical components vary with microform occupancy for *S. angustifolium* as

Table 2. Biochemical composition of peat soil from two depth intervals beneath hummocks and hollows.

	10–40 cm depth interval		100–150 cm depth interval	
Component	Hummock	Hollow	Hummock	Hollow
Carbon (%)	44.8 ± 1.1	44.7 ± 1.2	44.4 ± 4.0	47.4 ± 1.4
Delta ^{13}C (per mil)	–26.9 ± 0.3	–27.3 ± 0.2	–27.6 ± 0.3	–27.7 ± 0.2
Nitrogen (%)	1.5 ± 0.2	1.6 ± 0.1	1.5 ± 0.1	1.4 ± 0.1
Delta ^{15}N (per mill)	–0.17 ± 0.29	0.05 ± 0.26	–0.07 ± 0.12	0.14 ± 0.21
Forage fiber				
Lignin like (g kg^{-1})	244 ± 35	288 ± 27	**271 ± 23**	**314 ± 16**
Cellulose (g kg^{-1})	**196 ± 46**	**146 ± 27**	148 ± 21	161 ± 27
Hemicellulose (g kg^{-1})	101 ± 32	82 ± 23	71 ± 21	57 ± 18
Extractant				
Formic acid (1) (g kg^{-1})	33 ± 8	22 ± 7	**27 ± 4**	**11 ± 4**
Phosphate buffer (g kg^{-1})	49 ± 6	36 ± 8	**36 ± 10**	**17 ± 3**
CDTA (g kg^{-1})	65 ± 14	77 ± 14	80 ± 13	64 ± 16
Urea (g kg^{-1})	57 ± 10	64 ± 7	80 ± 7	74 ± 5
Sodium carbonate (g kg^{-1})	113 ± 15	99 ± 14	**112 ± 17**	**76 ± 17**
Formic acid (2) (g kg^{-1})	36 ± 8	28 ± 7	49 ± 11	59 ± 9
Alkali excess (g kg^{-1})	216 ± 56	215 ± 54	207 ± 80	235 ± 50

Note: Values are mean ± 1 standard error ($n = 6$). Bold indicates statistically significant difference between hummock and hollow per depth interval.

well as for *S. magellanicum*, another species that can populate both microforms (Oke et al. 2020).

It is worth commenting on a few of the differences noted between *Sphagnum* and the vascular plant material. For example, *Sphagnum* was distinguished from shrub leaf litter by a much greater amount of cellulose but a lesser amount of hemicellulose. This finding might seem puzzling, given that cellulose is thought to decompose readily, except when bundled to and protected by hemicellulose (Silveira et al. 2013). The hemicellulose binds to cellulose, and in some cell walls to lignin (Scheller and Ulvskov 2010) providing strength. On the one hand, greater proportions of hemicellulose in vascular plant material than in *Sphagnum* is consistent with tougher leaf tissue for shrubs and graminoids compared with the flimsy *Sphagnum* shoot. However, decay of cellulose in *Sphagnum* material is more than a function of concentration or protection by hemicellulose but depends on nature of cellulose, including crystallinity, porosity, and degree of polymerization (Mansfield et al. 1999). Furthermore, cellulose decay depends on matching its structure with the type of microbial cellulase enzymes present, of which there are several (Wilson 2011). Hence, persistence of cellulose in *Sphagnum* is more plausible than expected by concentration alone (Santelmann 1992).

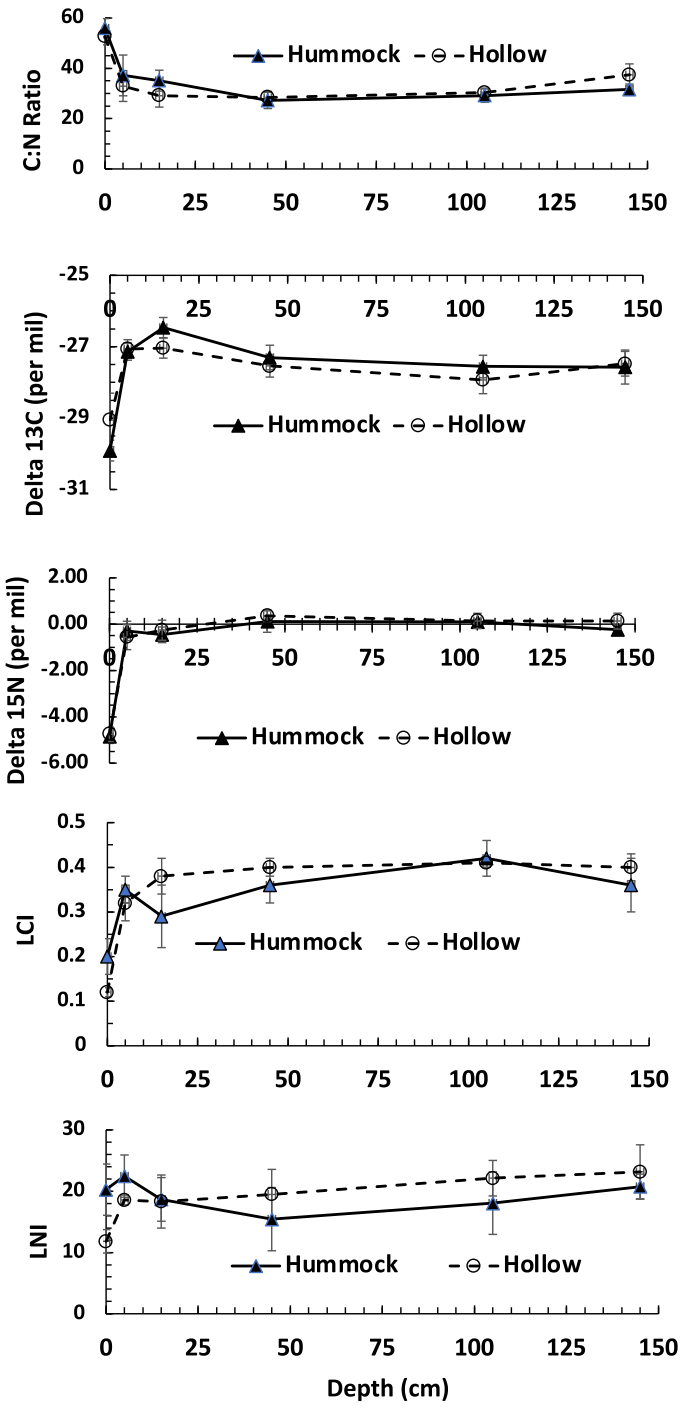
Another distinctive difference in our findings was larger investment in the CDTA-extractable fraction by *Sphagnum* than by vascular plant material. This fraction is principally pectin in the middle lamella. We can only speculate whether this pectin has the large amount of acidic functional groups that confer acidity to *Sphagnum*. On the other hand, the sodium carbonate-extractable fraction is pectin in the cell wall where it makes a strong barrier for transport of nutrient elements contained within the cell. Therefore, this prevalence of this fraction in both *Sphagnum* and in vascular plants is consistent with nutrient conservation by peatland plants (Aerts et al. 1999).

Although nitrogen concentrations in plant tissue did not differ among plant types, the delta ^{15}N values were relatively ^{15}N depleted. Asada et al. (2005) found ^{15}N depleted values for hummock species, whereas ^{15}N values were generally greater than −2.00 per mill for hollow species. However, our results are more like values for peatlands in Europe (Bragazza et al. 2005) that have been impacted by atmospheric deposition of pollutant nitrogen, resulting in no difference among microforms. *Sphagnum* mosses rely on several sources of nitrogen, and thus the concentration of nitrogen and the ^{15}N value reflects wet and dry deposition from the atmosphere, atmospheric nitrogen fixed by symbiotic organisms, and even organic nitrogen that cycled through plants. The relatively small amount of variation in delta ^{15}N values among the species suggests a similar source for all the species, and very little internal transport of nitrogen, consisting of a relatively drier habitat (cf., Aldous 2002).

Peat soil

According to peat formation theory (Clymo 1984), decomposition of plant material is essentially restricted to the upper portion of the peat soil, and decay rates decrease markedly as the residue becomes buried deeper in soil. One reason being that deeper peat soil is further removed from atmospheric oxygen that is essential to fuel aerobic microbial decomposers (McCarter et al. 2020 and references cited therein). The findings here are consistent with the theory by showing that the largest shifts in relative proportions of biochemical components occurred between plant material and peat soil in the 0–10 cm depth interval.

Fig. 2. Mean values (±1 standard error) for the C:N ratio, delta ^{15}N, delta ^{13}C, LCI, and the LNI in *Sphagnum* plant material (0 cm depth) and in peat soil to a depth of 150 cm in hummock and hollow microforms in peatlands in New York State. [Colour online.]



Decomposition of plant materials coincided with an increase in the proportion of lignin-like components relative to proportions for cellulose and hemicelluloses. This is consistent with cellulose decomposing more quickly in the top portion of the peat soil but much less so in deeper peat as shown by Santelmann (1992). It is important to stress that our results do not mean that lignin-like components resist microbial decomposition, but rather they decompose at a slower rate than other biochemical compounds. The finding that the phosphate- and CDTA-extractable fractions had increasing proportion in hummocks versus decreasing proportions in hollows provides insight into how microform influences decomposition processes. Litterbag studies with *Sphagnum* material have shown hummock species retain physical structure longer than hollow species (Johnson et al. 1990). Both phosphate- and CDTA-extractable fractions include biochemical compounds that help bind adjacent cells to each other in plant material. Therefore, the persistence of structural integrity of plant material in hummocks than in hollows correlates with slower decay rates in the hummock setting.

The occurrence of alkali-extract excess in the top 10 cm of the peat soil suggests that humic substances formed quickly. However, given the controversy whether alkali extracts humic substances from soil or not (Olk et al. 2019), the findings here should be taken as circumstantial evidence. But that said, humic substances are well-known components of brown coal and lignite, both of which are derived from peat soil (Francioso et al. 2003). Although we used a stronger alkali solution than typically used to extract humic substances, but as required for extraction of hemicellulose (Lawther et al. 1996), strong alkali did not overestimate the amounts of humic substances in lignite (Henning et al. 1997). Lastly, many studies show that concentrations of humic substances increase with depth in peat soil, but our findings did not. However, the pattern with depth might reflect selective preservation, as the formation of humic substances has been shown to correlate with quicker decay rates in surface peat soil (Zaccone et al. 2008).

The indices for organic matter decomposition provide additional evidence that the decay rate markedly slowed below 10 cm in the peat soil. For example, the depth profiles for delta ^{13}C values fit a profile that Kruger et al. (2014) specifically described for hummocks. Heavier values between plant material and the top of the peat soil suggests mostly aerobic decomposition with preferential loss of ^{12}C compared with ^{13}C. Below this is the "turning point" where the delta ^{13}C value decreases with depth as anaerobic decomposition causes an enrichment of slowly decomposing substances depleted in ^{13}C (Benner et al. 1987). Here the pattern was more distinctive for hummock peat than hollow peat, as predicted. The LCI reached a maximum value of 0.4, whereas it typically reaches a value of 0.7 for nearly complete decomposition of leaf litter in forests established on well-drained soil (Herman et al. 2008). Although the LNI was relatively insensitive to organic matter decomposition, it does so when the residue accumulates nitrogen, such as decomposing leaf litter in forest ecosystems (Osono 2017). Typically, highly decomposed peat typically does not produce large concentrations of dissolved organic compounds (Kalbitz and Geyer 2002). Although we did not specifically measure dissolved organic compounds in peat soil, the continued production of the formic acid fraction at all depths is notable since this is the source of dissolved organic compounds.

The slow rate of organic matter decomposition in peat soil, as our findings show, contrasts studies in peatlands in Canada (Moore and Bubier 2020), Minnesota (Hobbie et al. 2017), Finland (Esmeijer-Liu et al. 2012), and in Austria (Drollinger et al. 2019) where the indices changed gradually across the top 50 cm of peat. Regardless of the reason, slow rates of organic matter decomposition in our sites account for persistent differences in relative proportions of biochemical components between hummock peat and hollow peat even at 150 cm depth. The "biochemical recalcitrance" hypothesis attributes slow decay rates to sizeable concentrations of lignin, small concentrations of nitrogen, and/or production of humic substances in the decomposing material (Swift et al. 1979). We did not try to evaluate the hypothesis in these terms, since it pertains to litterbag studies. Nevertheless, a slow decay rate in peat soil is an obvious prediction from our findings, and the cause is not protection afforded by organic–mineral interactions (Kleber et al. 2021). Rather, as our findings suggest, the amounts of pectin compounds and their location within and between cells in *Sphagnum* tissue likely play a crucial role in the hypothesis. The search for a pectin signal in other peatland soils should be a high priority.

Furthermore, some studies in soils with mineral particles have shown that the biochemical composition of soil organic matter converges to one common composition, regardless of variation in biochemical composition of the plant material from which it formed, called the "decomposition funnel" hypothesis (Swift et al. 1979; Fierer et al. 2009). A few studies have tested this hypothesis, with mixed results, and mostly using just the initial stages of litter decomposition (Wickings et al. 2012; Liu et al. 2016). However, the "decomposition funnel" hypothesis seems unlikely in peat soil because litter decay rates slow too much.

Acknowledgements

We thank Molly Huber for help with the analyses in the laboratory, Brian Piatkowski and Blanka Aguero (Duke University, Durham, NC) for identifying the *Sphagnum* species, and two anonymous reviewers for insight comments and suggestions that improved the clarity of the presentation.

Article information

History dates

Received: 20 September 2021
Accepted: 27 March 2022
Accepted manuscript online: 14 April 2022
Version of record online: 25 August 2022

Copyright

Data availability

The entire data set is available to others by sending a request to the corresponding author.

Author information

Author notes

Joseph B. Yavitt currently serves as an Associate Editor; peer review and editorial decisions regarding this manuscript were handled by Fereidoun Rezanezhad.

Author contribution

GTP and JBY conceived and designed the study, carried out the field work, conducted the analyses, and analyzed the data. JBY initiated the manuscript and GTP assisted with the presentation.

Competing interests

The authors declare there are no competing interests.

Funding information

The authors declare no specific funding for this work.

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