Received: 15 October 2023 | Revised: 7 May 2024 | Accepted: 20 May 2024

DOI: 10.1111/ejss.13518

RESEARCH ARTICLE

European Journal of Soil Science WILEY

Peatland carbon chemistry, amino acids and protein preservation in biogeochemically distinct ecohydrologic layers

Anne Yalien Yusuf[1] | **Ewen Silvester**[2] | **Robert Brkljaca**[3] | **Christina Birnbaum**[1,4,5] | **James Chapman**[6] | **Samantha Grover**[1]

[1]Applied Chemistry and Environmental Science, RMIT University, Melbourne, Australia

[2]Research Centre for Applied Alpine Ecology (RCAAE), School of Agriculture, Biomedicine and Environment (SABE), La Trobe University, Wodonga, Australia

[3]Monash Biomedical Imaging, Monash University, Clayton, Australia

[4]School of Agriculture and Environmental Science, The University of Southern Queensland, Toowoomba, Australia

[5]Center for Crop Health, The University of Southern Queensland, Toowoomba, Australia

[6]School of Environment and Science, Griffith University, Nathan, Australia

Correspondence
Anne Yalien Yusuf, Applied Chemistry and Environmental Science, RMIT University, Melbourne, Australia.
Email: anne.yusuf@student.rmit.edu.au

Funding information
RMIT University

Abstract

Peatlands play a significant role in global carbon and nitrogen cycles due to their carbon storage capabilities. However, there are key knowledge gaps in our understanding of how peatland hydrology influences the biogeochemical properties that drive peatland functioning and health. This study examines peatland hydrology and biogeochemical dynamics by exploring the variations in carbon chemistry, total amino acid ('protein') content and amino acid composition in the ecohydrologic layers: acrotelm, mesotelm and catotelm. The dynamic movement of the water table recorded half-hourly over 4 years was used to assist in identifying the boundaries between these layers. Peat amino acids were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Carbon chemistry was analysed by solid state Cross Polarization Magic Angle Spinning (CPMAS) ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy, with the alkyl:O-alkyl ratio used to quantify the extent of decomposition. Our result revealed a strong positive correlation between the extent of decomposition and total protein content, indicating selective preservation of proteinaceous materials during peat decomposition. Each ecohydrologic layer displayed a distinct amino acid composition and carbon functional group composition. The acrotelm was relatively enriched in seven amino acids and two carbon functional groups. The mesotelm was relatively enriched in four amino acids, while the catotelm was relatively enriched in three amino acids and four carbon functional groups. The variations in amino acid composition reflect differences in microbial function and efficiency, while variations in carbon functional groups provide insights into long-term carbon sequestration in peatland. Collectively, these results provide more insights into nutrient cycling and changes in organic matter composition during peat decomposition. These findings demonstrate that peatland biogeochemistry is closely linked to ecohydrology and suggest that changes to water table dynamics could affect the ability of peatlands to sequester and store carbon in the future.

KEYWORDS
amino acids, carbon, decomposition, nitrogen, peat, protein, water table

1 | INTRODUCTION

Peatlands store large pools of terrestrial carbon (C) (Fekete et al., 2017; Parish et al., 2008) and nitrogen (N) as dead organic matter (Limpens et al., 2006). The ability of peatlands to store C and N is due to the slow rates of decomposition that result from permanent saturation, anaerobic and acidic conditions (Limpens et al., 2008). Carbon chemical composition also influences the stability and susceptibility of organic carbon pools to decomposition, with certain carbon functional groups representing recalcitrant or labile carbon (Grover & Baldock, 2012; Ji et al., 2020). Therefore, the process of peat decomposition is key to understanding carbon cycling and nutrient dynamics in these ecosystems. However, there are some key knowledge gaps in understanding the dynamics of organic matter transformation and how these changes through the peat profile.

The depth profile of peatlands is important for understanding peat formation, development and biogeochemical processes. The peat soil profile of peatlands is conceptualized in terms of three ecohydrologic layers: the acrotelm, mesotelm and catotelm (ordered from top to bottom; Clymo, 1984; Tfaily et al., 2014). The acrotelm is mostly oxic, characterized by a fluctuating water table, high hydraulic conductivity and dominated by living plants and their roots (Clymo, 1984; Ingram, 1978; Qiu et al., 2018; Tfaily et al., 2014). In contrast, the catotelm is the anoxic lower layer that consists of permanently saturated peat, where organic matter is predominantly stored (Clymo, 1984; Ingram, 1978; Tfaily et al., 2014). This layer is often called the 'carbon bank' of peatlands because decomposition is limited by the saturated, anoxic conditions (Tfaily et al., 2014). The mesotelm is the transition zone located between the upper acrotelm and the deeper catotelm (Clymo & Bryant, 2008). Like the catotelm, the mesotelm layer is often anoxic and only periodically oxic (Clymo & Bryant, 2008; Tfaily et al., 2014). The mesotelm exhibits relatively fast anoxic decomposition and rapid C turnover, compared to the other two layers (Lin et al., 2014; Tfaily et al., 2014). Although several studies have investigated the variations in the biogeochemical properties of peat across these ecohydrologic layers (Birnbaum et al., 2023; McAnallen et al., 2017; Thormann, 2011; Xiang et al., 2023; Yang et al., 2023; Younes & Grasset, 2017), very few studies (none found) have linked carbon chemistry and amino acid composition from ecohydrologic layers, despite their importance in soil C and N stabilization.

Highlights

- Water table dynamics reveal three ecohydrologic layers, acrotelm, mesotelm and catotelm, in an Alpine *Sphagnum* peatland.
- Each ecohydrologic layer had distinct biogeochemical properties.
- Peat decomposition correlated with total protein content, evidencing selective protein preservation.
- This study yielded a deeper understanding of decomposition and nutrient cycling in this endangered ecological community.

Amino acids are the building blocks of proteins, and certain amino acids are associated with specific soil microbial functional groups, such as bacteria or fungi (Glaser et al., 2004; Moe, 2013). For example, a higher proportion of amino acids associated with fungi may suggest a shift towards fungal-dominated decomposition (Glaser et al., 2004; Moe, 2013). Additionally, soil proteins have been proposed to play a significant role in organic C and N stabilization (Rillig et al., 2007). Protein contributes significantly to soil organic N and is a key indicator of the accumulation of different microbial residues and of soil health (Bastida et al., 2009; Bünemann et al., 2018; Hurisso et al., 2018; Rillig et al., 2007; Starke et al., 2019). Protein decomposition is considered a key driver of terrestrial N cycles because it has been suggested as the main process by which inaccessible chemically bonded N that is stored in litter or soil becomes accessible to microorganisms and/or plants (Knicker & Hatcher, 1997; Mooshammer et al., 2014; Schimel & Bennett, 2004; Wild et al., 2015). Therefore, to properly understand nitrogen cycling in peatlands the selective preservation of protein needs to be studied. Despite its relevance, the selective preservation of proteinaceous materials during organic matter decomposition is understudied. The preservation of protein in mineral soils has been reported previously (Henrichs, 1995; Knicker & Hatcher, 1997; Mayer, 1994; Rillig et al., 2007; Sollins et al., 1996), but little is known about this process in highly organic soils like peat. Consequently, it is important to better understand the role of peat proteins in C and N cycling dynamics in peat soils, considering the significant amounts of C stored in these ecosystems (Fekete et al., 2017; Limpens et al., 2006; Parish et al., 2008).

This study focuses on the preservation of organic N as protein during peat decomposition and examines the variations in carbon chemistry and amino acids content in the peatland ecohydrologic layers (acrotelm, mesotelm and catotelm). In doing so, our broader objective is to better understand the dynamics of carbon and nitrogen cycling and stabilization in peatlands. We hypothesize that: (i) the biogeochemical properties of peat will be distinctly different between the three ecohydrologic layers, and (ii) protein content will be positively correlated with extent of peat decomposition, indicating selective protein preservation. Although some of these peatland properties have been well studied individually, no study investigated how they are linked. Therefore, investigating these peatland properties will address this knowledge gap by contributing to better understanding of peatland processes and providing insights on the composition and stabilization of soil C and N, as well as nutrient cycling in this ecosystem.

2 | MATERIALS AND METHODS

2.1 | Site description

The peatland (Heathy Spur-1) investigated in this study is located within the Watchbed Creek catchment ($36^{o}53^{I}58^{II}$S, $147^{o}19^{I}38^{II}$E) on the Bogong High Plains, Victoria, Australia. This subalpine landscape is protected within the Alpine National Park and the 'Alpine *Sphagnum* bog and associated fen' ecosystem is listed as a threatened ecological community under the Australian state and federal legislation (EPBC Act, 1999). The Watchbed Creek catchment spans approximately 3.35 km^2 (Lawrence, 1995). The vegetation composition of the peatland is dominated by *Sphagnum* moss (*Sphagnum cristatum*), in association with candle heath (*Richea continentis*), alpine baeckea (*Baeckea gunniana*) and rope rush (*Empodisma minus*) (Wahren et al., 1999). The peat depth ranges between 0.3 m and greater than 1 m (Gunawardhana et al., 2021). Watchbed Creek peatland is fed by both rainfall and groundwater. The dominant water source is groundwater, with discharge ranging from minor seepages to spring flows (Silvester, 2009). The annual mean rainfall for the area is 2244 mm (BOM, 2021) and the mean annual maximum and minimum temperatures are 9.4 and 2.7°C, respectively (BOM, 2021). Stream flow and dissolved organic carbon (DOC) export at the study site varies seasonally, with higher DOC and lower stream flow in summer, with maximum stream flows occurring soon after spring snow melt (Silvester, 2009). The pH of the groundwater feed is between 5.25 and 5.40 and the electrical conductivity ranges from 6 to 10 μS/cm (Silvester, 2009).

2.2 | Water table monitoring

The dynamic movement of the water table was continuously monitored over 4 years, from December 2016 to December 2020, at five locations in the peatland (Figure 1). TruTrack water height loggers (WT-HR 500; TruTrack Ltd, Christchurch, New Zealand) were

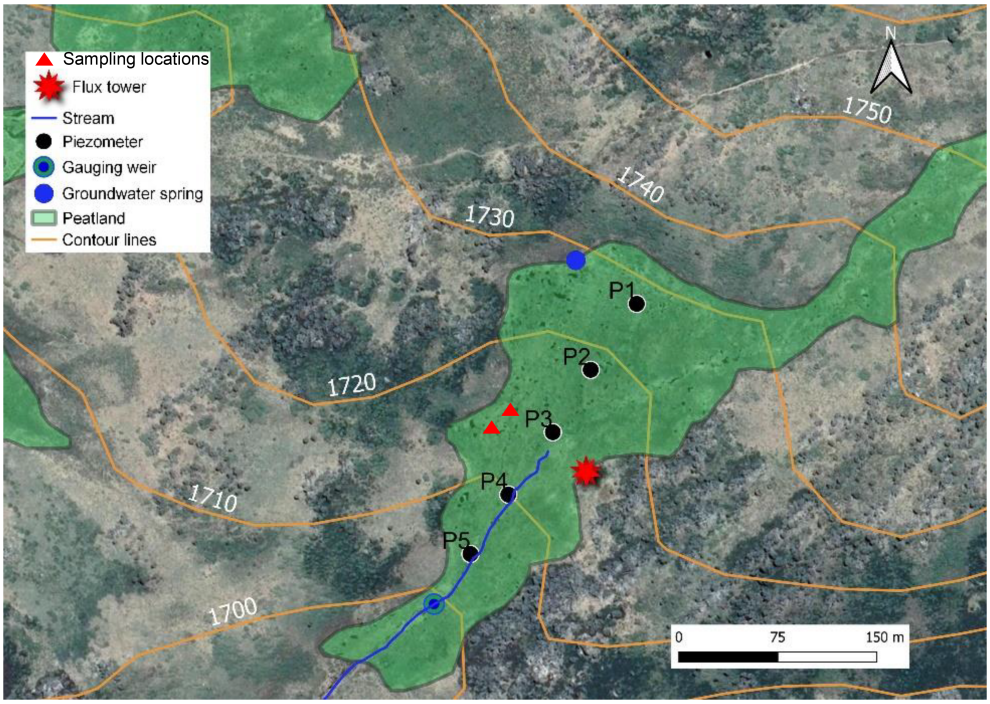

FIGURE 1 Study sites showing the peat core sampling locations and five piezometers (P1–P5) at Watchbed Creek peatland, Australia, adapted from Gunawardhana et al. (2021).

configured to record water height, water temperature and logger atmospheric temperature at 30 min intervals (Karis et al., 2016). The loggers were confined in a 32 mm piezometer tube inserted into the peat and anchored to the peatland substrate. Data were downloaded with Omni7 software (TruTrack Ltd, Christchurch, New Zealand).

2.3 | Field sampling

Peatlands in this region have a typical micro-topography of hummocks and hollows; hummocks are elevated mounds alternating with the lower and (typically) wetter hollows. Peat cores were collected with a Russian D peat corer in March 2019. A 60 cm core was collected from the hummock microtopography and a 45 cm core was collected from the hollow microtopography. The cores collected from the hummock and hollow microtopographies were subsectioned into 3-5 cm samples, based on the colour and texture of the peat, yielding 11 samples for each microtopography (Figure S1). In the following, depth profiles are plotted using the median depth of each core subsection. Replicate cores were not collected due to government research permit restrictions associated with working in this threatened ecosystem.

2.4 | Carbon and nitrogen content

Oven-dried and ground peat soil samples were analysed for total C and N using a LECO TruMac CHN analyser (LECO Corporation, USA) at the Environmental Analysis Laboratory, Southern Cross University. The C:N ratios of the samples were calculated from these results.

2.5 | Amino acids analysis

Amino acid concentrations were analysed for each of the peat samples following the methods adapted from Silvester et al. (2021). In summary, peat samples were frozen at −70°C and then freeze-dried at −50°C. The samples were then finely ground and homogenized. A 10 mg subsample was weighed and combined with 500 µL 6 N HCl containing 0.02% phenol. The solutions were hydrolysed by purging the samples with argon for approximately 1 min and then digested on a heating block at 110°C for 24 h. The hydrolysed samples were transferred to 1.5 mL Eppendorf tubes and the digestion tubes were rinsed with 300 µL Milli-Q water. The dilute HCl was removed using a rotating vacuum concentrator (RVC, Martin-Christ, Germany) for 7 h at 40°C. Dried samples were reconstituted in 10 mL of 0.1% formic acid and 1 mL of the sample was filtered through 0.45 µm cellulose acetate membranes. Approximately 20 µL of the filtered sample was derivatized with 60 µL borate buffer and 20 µL of 6-aminoquinolyl-N-hydroxysuccinimyidyl carbamate (AQC; 3 mg/mL in acetonitrile) reagent and heated to 55°C for 10 min for tyrosine conversion. Tagged samples were further diluted with 900 µL of 0.1% formic acid for analysis.

Tagged amino acid samples were analysed by liquid chromatography/tandem mass spectrometry (LC-MS/MS). The LC-MS/MS system comprised of a Shimadzu Nexera X2 UPLC attached to a Shimadzu 8030 triple quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan). Bovine insulin was used as a quality control standard and five blanks were included in each sample run. To minimize potential effects of variations between batch runs, duplicate or triplicate subsamples were run in separate batches and with samples from different depths and/or topographies.

Amino acids are referred to using their three-letter abbreviations: Histidine (HIS), Aspartic acid (ASP), Asparagine (ASN), Arginine (ARG), Serine (SER), Glutamine (GLN), Glutamic acid (GLU), Glycine (GLY), Cysteine (CYS), Threonine (THR), Alanine (ALA), Proline (PRO), Lysine (LYS), Tyrosine (TYR), Methionine (MET), Valine (VAL), Isoleucine (ILE), Leucine (LEU) and Phenylalanine (PHE). During the sample acid hydrolysis process, asparagine and glutamine are de-aminated to aspartic acid and glutamic acid, respectively. Therefore, these pairs of amino acids are referred to as ASX (= ASN + ASP) and GLX (= GLN + GLU) (Fountoulakis & Lahm, 1998). Total protein content is always underestimated by acid hydrolysis due to incomplete recovery of some amino acids (Knicker & Hatcher, 1997). Amino acid results were converted to amount of protein mg/g-soil and %-protein in N.

2.6 | ^{13}C cross polarization/magic angle spinning nuclear magnetic resonance analysis

The carbon chemistry of the peat samples was analysed by solid state Cross Polarization Magic Angle Spinning ^{13}C Nuclear Magnetic Resonance (^{13}C CP/MAS NMR) spectroscopy using an Agilent 500 MHz spectrometer (Agilent, USA) equipped with a 4 mm rotor triple resonance probe. To maximize the observability of this method, a variety of CP transfer times were evaluated, and the CP that displayed the best quality and resolution of C types across the entire spectrum was used for all experiments. The same CP transfer time was used across

all samples to evaluate how the C content changed across the different peat samples. Dried and ground peat samples were packed into a 4 mm zirconia cylindrical rotor and spun at 10000 ± 10 kHz with spectra collected at ambient temperature. A cross-polarization tancpx sequence was used with a pulse width of 4.5 μs, 1.5 s recycle delay, 1 ms transfer time, spectral width of 397.969 ppm, acquisition time of 20 ms, 1000 data points and 5000 scans. The chemical shifts were externally referenced to adamantane (δC 29.5). Line broadening (50 Hz), phasing, baseline correction and integration of the spectra were performed using the Advanced 1D NMR 12 Processor software (ACD, Version 12). Each spectrum was integrated over eight spectral regions. The signal intensities within these regions were combined to determine the contribution of alkyl (0–45 ppm), N-alkyl (45–60 ppm), O-alkyl (60–95 ppm), di-O-alkyl (95–110 ppm), aryl (110–145 ppm), O-aryl (145–165 ppm), carbonyl (165–190 ppm) and ketone (190–215 ppm) carbon functional groups (Figure S2). The phasing, baseline correction and integration of the spectra over these regions were performed using the Advanced 1D NMR 12 Processor software (ACD, Version 12).

The percentage of each carbon functional group was calculated by dividing the area of that group by the total spectrum area (Figure S2). The ratio of alkyl C-to-O-alkyl C (alkyl/O-alkyl ratio) is representative of the extent of decomposition of the organic material given a common vegetational origin (Baldock et al., 1997). This alkyl/O-alkyl ratio has been widely used to characterize peat soils (Grover & Baldock, 2010, 2012; Helfrich et al., 2006; Kang et al., 2021; Normand et al., 2017; Stricker et al., 2019). The alkyl/O-alkyl ratio was calculated by dividing the area of the alkyl region (0–45 ppm) by the area of the O-alkyl region (45–110 ppm). Carbonyl and N-alkyl carbon are a focus of this study; the carbonyl region includes the amide group of proteins while the N-alkyl region includes the C–N bond of proteins (Figure S2).

Although ^{13}C CP/MAS NMR experiments are more sensitive to carbon signals compared to direct polarization, this technique has some inherent biases. The ^{13}C CP/MAS NMR method slightly underestimates the proportion of aromatic C, whilst slightly overestimating the proportion of aliphatic C. However, this is more pronounced in polyaromatic rich material such as lignite and coal (Wright et al., 2011). As peat has approximately 5%–10% aromatic C of which very little is polyaromatic C, this will have very little effect on the data. Additionally, variations in cross-polarization rates for different carbon types may affect the signal intensity. When samples have para- and/or ferromagnetic centres, some carbons may be undetectable due to their proximity to those centres, leading to a strong reduction of the rotating-frame proton longitudinal relaxation time (Mikita, 2020). However, this has no effect on our results because our samples do not have para- and/or ferromagnetic centres.

2.7 | Statistical analyses

Data analysis was conducted using R (R core team, 2021), the figures were prepared using 'ggplot2' (Wickham, 2016). Pearson's correlation analysis was used to determine the relationship between total protein, percentage protein in N, carbon to nitrogen ratio and extent of decomposition using the function 'scatter' in ggplot2. The proportion of individual amino acids was calculated, and the similarities and dissimilarities between each amino acid were explored with a principal component analysis (PCA) using function 'biplot' in ggplot2. To assess differences in biogeochemical properties between the hummock and hollow micro-topographies, a permutational multivariate analysis of variance (Permanova) was used with 9999 permutations in R package 'vegan'. A Wilks' lambda pairwise test was performed to assess the differences in amino acid and carbon functional group between the acrotelm, mesotelm and catotelm.

3 | RESULTS

3.1 | Water table depth demarcates peatland ecohydrologic layers

A regular seasonal pattern in the water table depth (WTD) below the surface of the peatland was observed over 4 years and plotted as a density distribution (Figures 2 and S5). A sharp peak in the density plot indicates that the water table is quite stable at that depth in that season, while a broad peak indicates that the water table varies over a range of depths. The seasonal density plots (Figure 1) show seasonal peaks at -30 cm for Sites 1, 2 and 3. The density plot for Site 4 peaked at a shallower depth, around -20 cm, while at Site 5 the residence time density plot peaked at the deepest depth of around -40 cm (Figures 1 and S5). In general, all piezometer sites show a generally higher water table in winter-spring compared to summer-autumn. This difference was most pronounced at Site 4, with the highest water table levels observed in winter and spring (−23 cm) and the lowest water table levels occurring in summer (−36 cm). A broadly similar seasonal pattern was observed at Sites 1, 2 and 5. On average, across all five measured locations, 87% of all recorded WTDs were between −20 and −40 cm below the surface of the peatland (Figure 2).

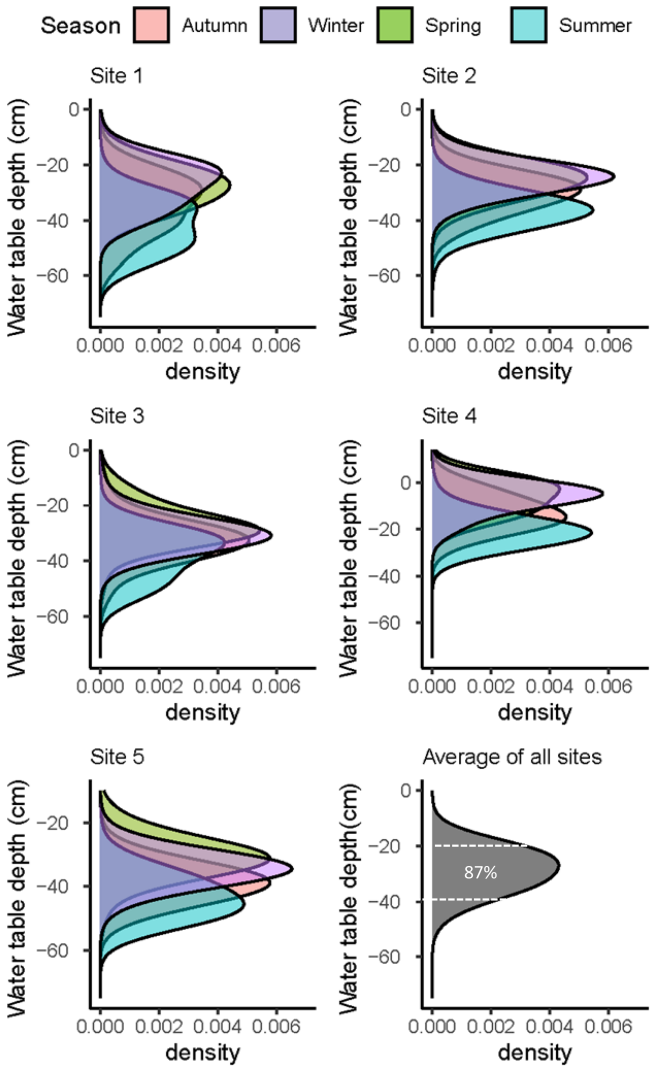

FIGURE 2 Density plots of the water table (below the surface of the peat) at five locations in the Heathy Spur-1 peatland over 4 years from December 2016 to December 2020.

The dynamic movement of the water table supports the presence of three distinct ecohydrologic layers, as conceptualized for peatlands globally. Although Morris et al. (2011) argued that the three-layered model of conceptualizing peatlands is rigid and inefficient, our results support the presence of these layers. As noted above, when averaged across all piezometer sites, 87% of the time the water table fluctuated between −20 and −40 cm and was rarely recorded above or below these depths. This suggests that the acrotelm is approximately between 0 and −20 cm, the mesotelm between −20 and −40 cm and the catotelm below −40 cm. The boundaries between the ecohydrologic layers likely differ between the hummock and hollow microtopographies due to the relative elevation of hummocks compared to hollows (Nungesser, 2003). As will be shown, the mesotelm depth range inferred from water table behaviour (i.e., −20 to −40 cm) corresponds closely with the depth range over which strong changes in peat soil properties were observed for the hummock microtopography (e.g., C:N ratio, alkyl:O-alkyl ratios). The boundaries for the hollow microtopography have been assigned based on the depth range over which similar changes in soil properties were observed; these being: acrotelm from −0 to −10 cm, mesotelm from −10 to −30 cm and catotelm from −30 to −50 cm (Figure S6). For reasons of practicality (a sampling point at −30 cm), the boundary between mesotelm and catotelm is shown at −32 cm.

3.2 | Peat soil biogeochemical properties

The biogeochemical properties of C:N ratio, protein content and percentage of protein in nitrogen did not vary significantly between the hummock and hollow microtopographies (PERMANOVA $p > 0.05$; Table S4) and thus these results are described here together. The C:N ratio for the hummock microtopography decreased with increasing depth from 85 at the surface of the peatland to 32 at 57.5 cm depth (Figure 3a). This decreasing C:N ratio trend with increasing depth was interrupted at 22.5 cm, where a maximum value of 104 was measured (Figure 3a). The protein content increased from 8.6 mg/g at the surface of the peatland to 26.3 mg/g at 57.5 cm depth (Figure 3b). The percentage of protein in total N varied with depth, with a maximum of 46% at 27.5 cm, decreasing both above and below this depth (Figure 3c).

The C:N ratio of the hollow microtopography generally decreased with increasing depth from 96 at the surface to 27 at 43.5 cm depth except at 12.5 cm where a maximum value of 112 was recorded (Figure 3d). The protein content in the depth profile of the peatland ranged from 7.2 to 32.4 mg/g and increased with increasing depth (Figure 3e). This trend of increasing protein content with increasing depth was interrupted at 30 and 33.5 cm depth where 10 and 13.8 mg/g of protein were measured, respectively (Figure 3e). The percentage protein in nitrogen varied between 21% and 36.5% and weakly increased with increasing depth (Figure 3f).

3.3 | Carbon chemistry of peat

The ^{13}C CP/MAS NMR spectra changed with depth in the peat profile, however, O-alkyl, alkyl, N-alkyl and di-O-alkyl C were the dominant carbon functional groups across all samples in the hollow and hummock microtopographies (Figure S8, Figure S3 and Figure S4). The signal intensity of alkyl and N-alkyl carbon increased with increasing depth for both micro-topographies while

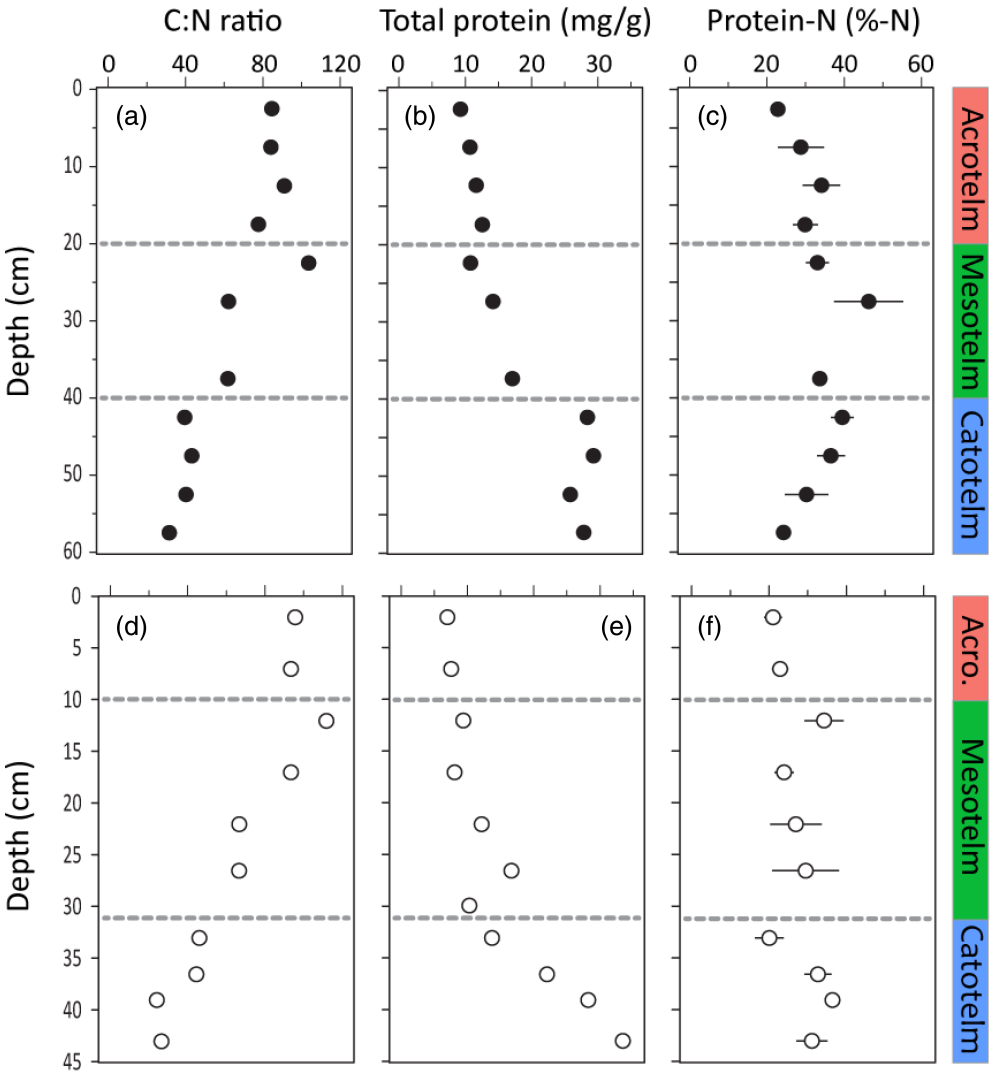

FIGURE 3 Peat soil biogeochemical properties with depth in the hummock (a–c) and hollow (d–f) microtopographies of the Heathy Spur-1 peatland. Figure 3a,d show the C:N ratio, Figure 3b,e show total protein (mg-protein/g-soil) and Figure 3c,f show the percentage of protein in total N. Error bars represent ±2 S.E. from duplicate or triplicate laboratory measurements. The errors from the total protein measurement are small, and in many cases not visible at this scale. The C:N ratio and percentage protein data from the hollow microtopography at 30 cm depth are missing due to insufficient sample.

O-alkyl and di-O-alkyl C decreased in the hollow microtopography (Figure S8).

The alkyl/O-alkyl ratio as well as the carbonyl and N-alkyl functional group contributions did not vary between the hummock and hollow microtopographies (PERMANOVA $p > 0.05$; Table S4). The alkyl/O-alkyl ratio of the hummock microtopography ranged from 0.17 to 0.49. No significant difference was observed between 0 cm and 27.5 cm depth, however, from 27.5 to 57.5 cm, the alkyl/O-alkyl ratio increased from 0.19 to 0.49 (Figure 4a). The signal intensity of the carbonyl functional group varied between 1.7% and 3.3%, with the minimum value occurring at 17.5 cm (1.65%; Figure 4b). Below this depth the signal intensity increased to a maximum value of 3.3% at 42.5 cm (Figure 4b) and then decreased to 2.11% at 57.5 cm (Figure 4b). The signal intensity of the N-alkyl functional group varied with depth, displaying a decreasing trend from 7.5 to 22.5 cm where the lowest value was recorded (Figure 4c). Below 22.5 cm the signal intensity of the N-alkyl functional group increased from 7.2% to 11.06% (Figure 4c). The alkyl/O-alkyl ratio of the hollow microtopography increased from 0.14 at the surface of the peatland to 0.58 at the bottom of the profile (Figure 4d). The signal intensity of the carbonyl group varied, with values ranging from 2.14% to 4.05% and the maximum intensity at 39.5 cm (Figure 4e). The signal intensity of N-alkyl ranged from 6.15% to 11.15%, increasing from the surface to a maximum at 37.5 cm and then decreasing below this depth (Figure 4f).

3.4 | Amino acid and carbon composition of the peatland ecohydrologic layers

On the average, the most dominant amino acids for the hummock and hollow microtopographies were GLY, ASX, ALA, GLX and SER, while MET, CYS and TYR were least abundant (Tables S1 and S2; Figure S7).

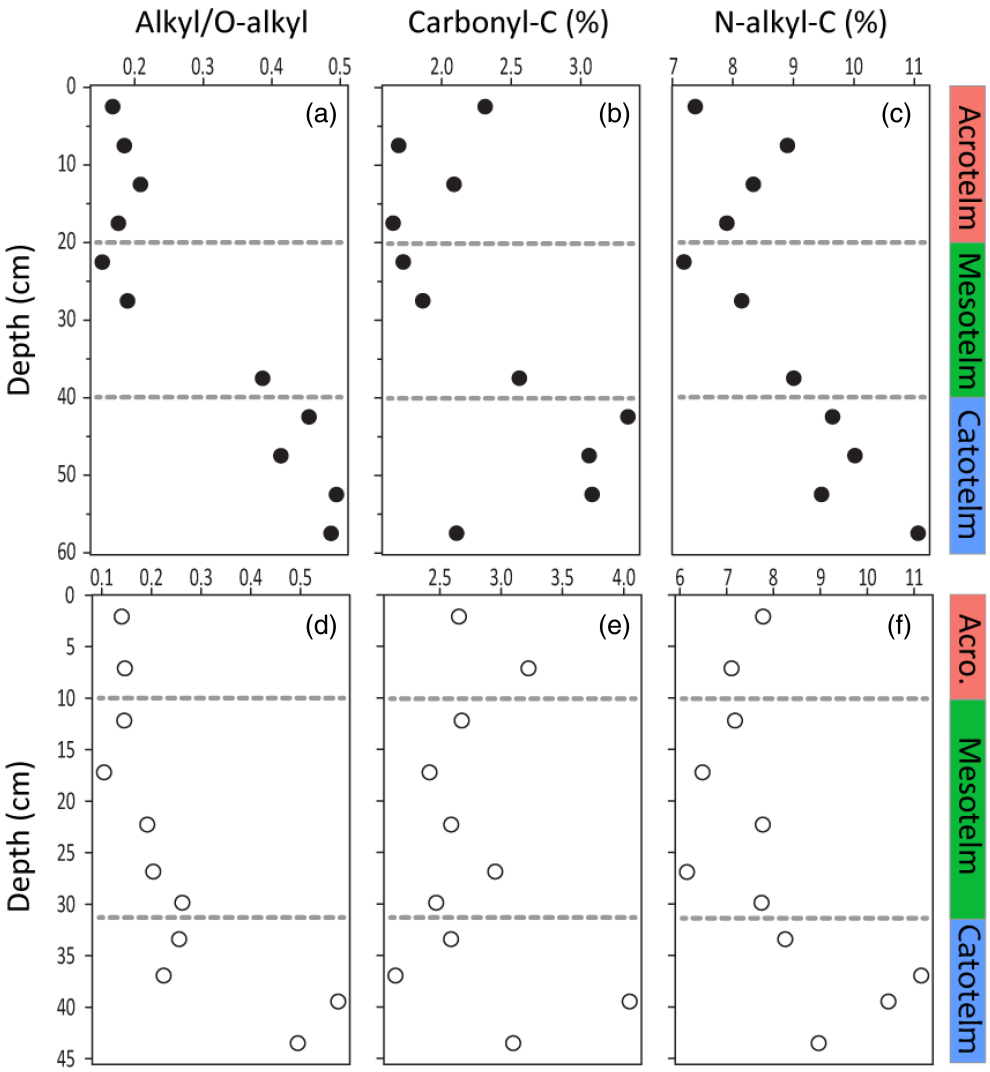

FIGURE 4 Carbon chemistry (from ^{13}C CP/MAS NMR) of the hummock (a–c) and hollow (d–f) micro-topographies of the Heathy Spur-1 peatland. Shown are: (a) and (d) alkyl/O-alkyl ratio, (b) and (e) signal intensity (%) of the carbonyl functional group and (c) and (f) signal intensity (%) of the N-alkyl functional group.

The three ecohydrologic layers showed distinct amino acids and carbon chemical composition, with statistically significant variations ($F = 9.8$, $p < 0.001$; Figure 5). Among these layers, the difference between the acrotelm and catotelm was more significant ($p = 0.0002$), followed by the difference between the catotelm and mesotelm ($p = 0.0004$). The acrotelm and mesotelm demonstrated the least significantly difference in biogeochemical properties ($p = 0.019$). The acrotelm layer was largely characterized by relatively higher proportions amino acids (LYS, PHE, MET, GLX, ASX, CYS and LEU; Table 1 and Figure 5) compared to the mesotelm and catotelm. Di-O-alkyl C and O-alkyl C were the dominant carbon functional groups in the acrotelm (Figure 5). The mesotelm was characterized by a strong transition between characteristics of the acrotelm and the catotelm. As a result, there were no carbon functional groups characteristic of this zone. In terms of amino acid (AA) composition, the mesotelm differed from the acrotelm with relatively higher levels of ALA, GLY, SER and PRO. The (relatively) most abundant amino acids in the catotelm were: ARG, TYR and VAL (Figure 5 and Table 1), while alkyl, N-alkyl, carbonyl, O-aryl were the dominant carbon functions groups in this layer.

3.5 | Relationships between extent of decomposition and protein content, total C, total N, C:N and % protein N

There was a significant strong positive correlation between the extent of decomposition of peat, as quantified by the alkyl/O-alkyl ratio, and total protein content ($R = 0.91$, $p = 6.5e{-}09$; Figure 6a) as well as total N ($R = 0.84$, $p = 1.2e{-}06$; Figure 6d). By contrast, a negative correlation was observed between the extent of decomposition and C:N ratio ($R = -0.87$, $p = 1.9e{-}07$; Figure 6e). No significant correlation was found between the extent of decomposition of peat, and total C ($R = -0.22$, $p = 0.33$; Figure 6c) or the percentage of protein in N ($R = 0.3$, $p = 0.19$; Figure 6b).

4 | DISCUSSION

This study aimed to investigate the preservation of organic N as protein during peat decomposition and examine the variations in carbon chemistry and amino acid content in the peatland ecohydrologic layers (acrotelm, mesotelm and catotelm). Our results suggest that the water table demarcates the peatland's ecohydrologic layers and that the biogeochemical properties of the peat soil show distinct trends in these ecohydrologic layers, as described below. These trends in amino acid and carbon composition suggest distinct microbial activity and decomposition dynamics in these layers. Our results also provide evidence of the selective preservation of protein during peat decomposition process.

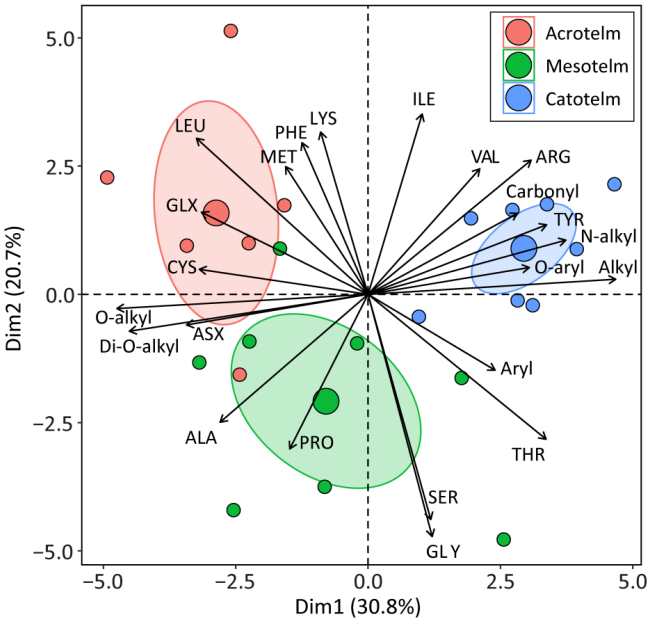

FIGURE 5 Principal component analysis (PCA; 52% explained) plot of the proportion of 17 amino acids and carbon chemistry (from ^{13}C CP/MAS NMR) measured in peat sampled from the three ecohydrologic layers of the Watchbed Creek peatland at a confidence interval of 95%. Data from hummock and hollow microtopographies were combined because there was no significant difference between the two microtopographies ($p = 0.261$, $R^2 = 0.05$).

4.1 | Peatland ecohydrologic layers demonstrate distinct biogeochemical properties

We hypothesized that the biogeochemical properties of peat would be distinctly different between the three ecohydrologic layers. Our results demonstrate that the acrotelm, mesotelm and catotelm have distinct biogeochemical properties, which supports our hypothesis. The acrotelm layer was largely characterized by relatively higher proportions of particular amino acids compared to the mesotelm and catotelm. Seven amino acids were enriched in this layer, indicating active decomposition of recently deposited organic matter by microorganisms in this layer. Amino acids are organic compounds that are released during the decomposition of organic matter (Senwo & Tabatabai, 1988). The richer amino acid composition in the acrotelm may suggest more organic nitrogen availability which is beneficial for plant growth and nutrient cycling (Limpens et al., 2006). The higher proportions of LYS, PHE, MET, GLX, ASX and CYS

TABLE 1 Trends in the biogeochemical properties of peat soils in the acrotelm, mesotelm and catotelm of the hollow and hummock micro-topography.

Ecohydrologic layer	Dominant amino acids	Protein content	% protein in total nitrogen	Dominant carbon functional groups	C:N ratio	Alkyl/O-alkyl ratio	Carbonyl C	N-alkyl C
Acrotelm	LYS, PHE, MET, GLX, ASX, CYS, LEU	Low	Increasing	Di-O-alkyl, O-alkyl	High	Low	Low	Low
Mesotelm	ALA, PRO, SER, GLY	Transition from Low to High	Increasing	Nil	Tran-sition from High to Low	Tran-sition from low to High	Tran-sition from Low to High	Tran-sition from Low to High
Catotelm	ARG, VAL, TYR	High	Increasing	Alkyl, N-alkyl, Carbonyl, O-aryl	Low	High	High	High

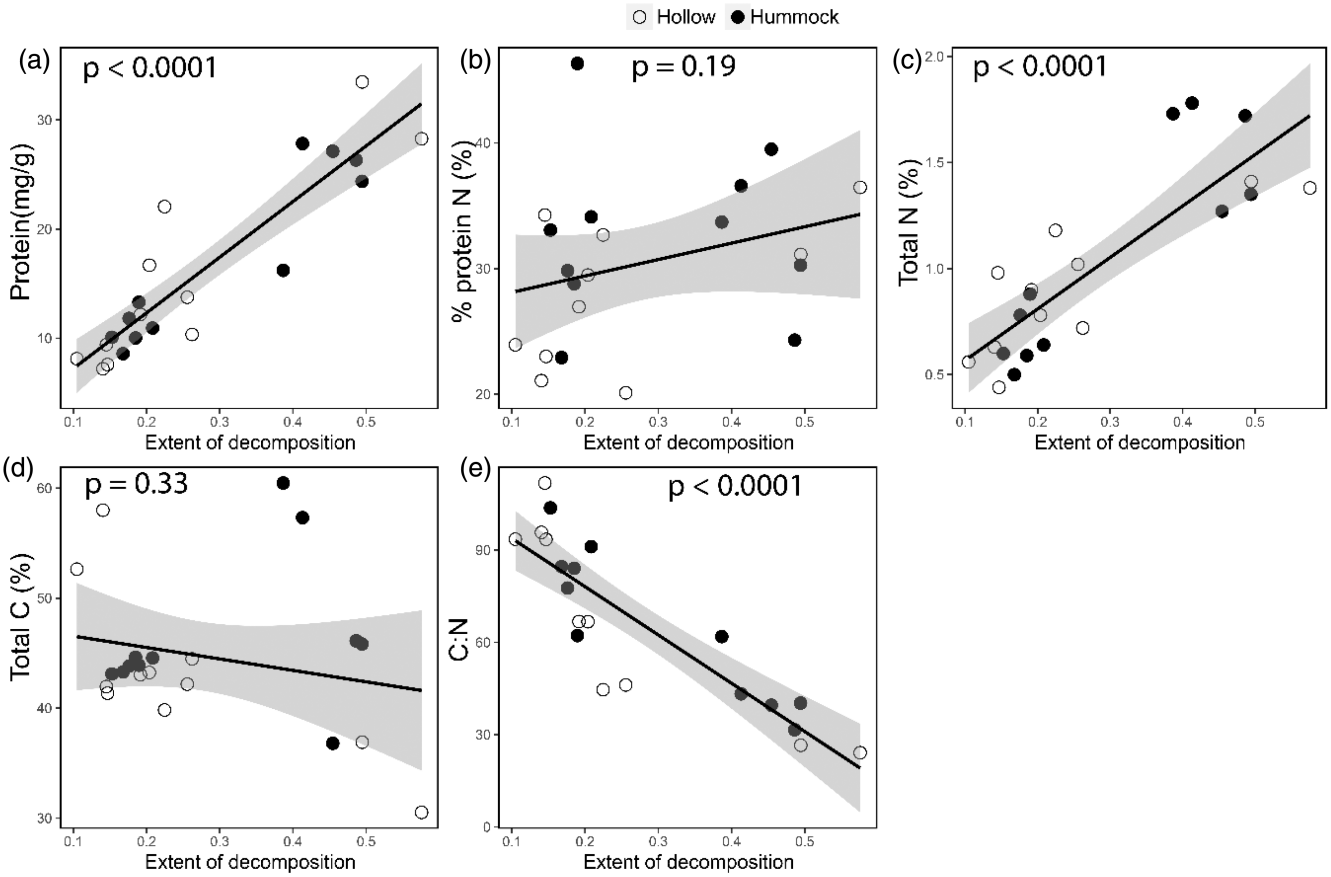

FIGURE 6 Relationships between the extent of peat decomposition (alkyl/O-alkyl ratio) and (a) total protein concentration (mg-protein/g-soil), (b) percentage of protein in total N, (c) total N, (d) total C and (e) C:N ratio. Grey envelopes represent the 95% confidence intervals for linear correlation.

observed in the acrotelm is somewhat similar to work by Kunnas and Eronen (1994), who found elevated levels of free ALA, GLY, GLX and ASX in near-surface peat relative to deeper layers. The higher abundance of ALA and ASX may be due to the presence of microbes that have high concentrations of these amino acids in their cell walls (Friedel & Scheller, 2002; Wagner & Mutatkar, 1968). We also observed that Di-O-alkyl C and O-alkyl C were dominant forms of carbon in the acrotelm, indicating elevated levels of complex carbon molecules in this aerobic layer, linked to plant-derived materials in the form of complex carbohydrates (Kögel-Knabner, 2000; Leifeld et al., 2012). The dominance of Di-O-alkyl C and O-alkyl C in the acrotelm may also be related to hydrolysis/oxidization processes taking place in this zone (Ono et al., 2015), particularly in summer and spring when water table levels fall (Figure 2; Ingram, 1978; Mezbahuddin et al., 2014). Our results demonstrate that the C:N of peat from the acrotelm were higher than those for the mesotelm and catotelm. This compares to the high C:N ratio for live *Sphagnum* moss of 60 ± 10 (Silvester et al., 2021) and are consistent with the low N content of *Sphagnum* compared to other vascular plants (Tfaily et al., 2014), resulting from the dominance of fresh and poorly decomposed *Sphagnum* in this layer.

In general, the mesotelm was characterized by strong transitions between acrotelm and catotelm characteristics likely because the mesotelm is a zone with high C turnover (Tfaily et al., 2014). As a result, no carbon functional groups were dominant in this zone. Our results demonstrate that the mesotelm differed from the acrotelm with relatively higher proportions of ALA, GLY, SER and PRO. The simple and stable structure of GLY may be responsible for high GLY in this layer, as GLY is produced during the breakdown of other amino acids (Kunnas & Eronen, 1994). The transitioning of the C:N ratio and protein content in the mesotelm can be explained by the enhanced peat decomposition in this layer (Tfaily et al., 2014). This fast rate of peat decomposition in the mesotelm may also be responsible for the peak in percent protein-N at 25–30 cm, as nitrogen is broken down very quickly in this layer.

The (relatively) most abundant amino acids in the catotelm were: ARG, HIS, TYR, THR and VAL (Figure 5 and Table 1). While the mechanistic reasons for the

relative enrichment of these AAs are not known, it appears likely that there is an association between the AA composition of the stored peat and the formation of recalcitrant protein. As noted previously, this zone was also characterized by relatively higher proportions of carbon functional groups, namely, alkyl-C, N-alkyl-C, carbonyl-C and O-aryl-C as well as low C:N ratios and higher protein content. This suggests a complex combination of recalcitrant and labile organic matter in this layer. The higher alkyl/O-alkyl ratio in the catotelm of the peat profile (representing more decomposed peat) is also consistent with the results of Stricker et al. (2019) and may reflect the accumulation of lipids and other recalcitrant byproducts of carbohydrate degradation (Baldock & Preston, 1995; Hopkins et al., 1997). Additionally, the observed higher protein content in the catotelm is consistent with that observed in an earlier study of woody Canadian peat where increasing protein content with depth was observed (Sowden et al., 1978). The stable C:N ratios and alkyl/O-alkyl ratio in the catotelm suggest very slow decomposition rates at that depth (Kuhry & Vitt, 1996; Tfaily et al., 2014; Vardy et al., 2000). Our study found increasing signal intensity of the N-alkyl functional group with increasing depth. This may reflect the formation of N-alkyl compounds as metabolic products of decomposer organisms (Tfaily et al., 2014). N-alkyl groups are also present in peptides, amino acids and proteins, therefore, the increase in N-alkyl may be linked to the increase in protein content (Figure 3). The high proportion of carbonyl functional group in the catotelm suggests the accumulation of recalcitrant organic matter in this anaerobic layer, formed via oxidative degradation in the upper layers (Baldock & Preston, 1995), but may also be linked to the increased levels of amide bonds associated with higher protein content. Additionally, the low C:N ratios in the catotelm suggest very slow decomposition at that depth (Kuhry & Vitt, 1996; Tfaily et al., 2014; Vardy et al., 2000). This low C:N ratio in the catotelm results from the high N content in this layer (Figure 3).

4.2 | Protein is preserved during peat decomposition

Our results demonstrate a strong positive correlation between peat protein content and extent of peat decomposition, which supports our second hypothesis that protein content will be positively correlated with extent of peat decomposition, indicating the selective preservation of protein during peat decomposition. The increase in protein content with increasing depth demonstrated by our result may also be due to the selective preservation of particular amino acids. This observed increasing protein content with increasing depth is consistent with that reported in an earlier study of woody Canadian peats where amino acid N increased with depth up to 40–60 cm (Sowden et al., 1978). This result also concurs with the findings from mangrove sediments that protein is preserved during decomposition, and it is stabilized in soils (Knicker & Hatcher, 1997). The selective preservation of protein during peat decomposition likely explains the strong positive correlation between the extent of decomposition and increasing N content of peat.

In peatlands, newly added, less decomposed plant materials are found at the top of the profile, and older, more decomposed peat is usually found at the bottom. The depth profile therefore also represents an age profile. Previous studies on the origin of peat from a number of peatlands across the Australian Alps suggest that peat began to form in the region within the last 3000 years (Dodson, 1987; Grover et al., 2012; Hope, 2002). Therefore, these results reveal that recalcitrant proteins are accumulated over many years in the lower layers of the peatlands and serve as long-term N storage. The fate of this recalcitrant stored protein under drier climatic conditions remains uncertain.

A number of studies have attempted to assess the mechanism by which protein is preserved in soils (Henrichs, 1995; Knicker & Skjemstad, 2000; Mayer, 1994; Rillig et al., 2007). For example, protein could be preserved due to inherent chemical recalcitrance (Henrichs, 1995; Rillig et al., 2007), inaccessibility due to physical protection (Knicker & Skjemstad, 2000; Mayer, 1994), or interactions with other soil molecules (Rillig et al., 2007). However, these studies were conducted on mineral soils, in which the primary interactions between proteins are with mineral soil components. Protein in organic soils has been explored by Knicker and Hatcher (1997), who measured protein in highly organic sediments from a mangrove lake and suggested that the preservation of protein may be due to the encapsulation of labile protein into refractory organic matter or its interaction with other refractory organic material. Previous studies have also suggested the role of microorganisms N preservation during organic matter decomposition (Manzoni et al., 2021; Melillo et al., 1984). Considering how little is known about the mechanism of protein preservation in organic-rich sediments, further research in this area is needed.

5 | CONCLUSION

This study demonstrates significant variations in carbon chemistry, protein content and amino acids across the three ecohydrologic layers at the Heathy Spur-1 peatland at Watchbed Creek. The acrotelm displayed a wide range

of amino acids, suggesting the dynamic nature of microbial activities in this layer and high turnover of organic matter. In the mesotelm, the protein content transitioned from low to high and C:N transitioned from high to low, suggesting the transitional nature of this layer. The abundance of four recalcitrant carbon functional groups in the catotelm indicates an accumulation of recalcitrant organic matter. Furthermore, the strong positive correlation between the extent of decomposition (described by the alkyl:O-alkyl ratio) and protein content indicates a selective preservation of protein during peat decomposition.

The distinct biogeochemical properties of the peatland's ecohydrologic layers and their demarcation by the water table suggests that any future change in peatland water table levels will likely subsequently affect the biogeochemical properties of peat. Future research should explore the mechanisms driving these observed variations in carbon chemistry, protein content and amino acid composition and manipulative water table change experiments could yield further insights. Changes in the biogeochemical properties of peat may affect the ability of these peatlands to continue to store C and N and to provide other vital ecosystem services such as water filtration and biodiversity conservation.

AUTHOR CONTRIBUTIONS

Anne Yalien Yusuf: Conceptualization; investigation; formal analysis; visualization; writing – original draft; writing – review and editing; methodology; software; data curation; validation; funding acquisition; project administration. **Ewen Silvester:** Conceptualization; investigation; methodology; supervision; visualization; writing – review and editing; data curation; formal analysis; validation. **Robert Brkljaca:** Methodology; writing – review and editing; software. **Christina Birnbaum:** Writing – review and editing; supervision; formal analysis. **James Chapman:** Writing – review and editing; supervision. **Samantha Grover:** Funding acquisition; supervision; writing – review and editing; conceptualization; methodology; visualization; resources; investigation; validation.

ACKNOWLEDGEMENTS

We would like to acknowledge Lynne McDonald for providing advice on NMR spectral regions. The installation of piezometers was carried out in the Alpine National Park under the National Parks research permit No. 10005653 and peat soils were collected under research permit No. 10008607. Open access publishing facilitated by RMIT University, as part of the Wiley - RMIT University agreement via the Council of Australian University Librarians.

FUNDING INFORMATION

This research was financially supported by an Australian Government Research Training Program (RTP) Scholarship awarded to AY through RMIT University. Open access publishing facilitated by RMIT University, as part of the Wiley – RMIT University agreement via the Council of Australian University Librarians.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Anne Yalien Yusuf https://orcid.org/0000-0002-4104-8487

REFERENCES

Baldock, J., Oades, J., Nelson, P., Skene, T., Golchin, A., & Clarke, P. (1997). Assessing the extent of decomposition of natural organic materials using solid-state 13C NMR spectroscopy. *Soil Research*, *35*, 1061–1084.

Baldock, J., & Preston, C. (1995). Chemistry of carbon decomposition processes in forests as revealed by solid-state carbon-13 nuclear magnetic resonance. In W. William & J. M. K. McFee (Eds.), *Carbon forms functions in forest soils* (pp. 89–117). Wiley.

Bastida, F., Moreno, J. L., Nicolás, C., Hernández, T., & García, C. (2009). Soil metaproteomics: A review of an emerging environmental science. Significance, methodology and perspectives. *European Journal of Soil Science*, *60*, 845–859.

Birnbaum, C., Wood, J., Lilleskov, E., Lamit, L. J., Shannon, J., Brewer, M., & Grover, S. (2023). Degradation reduces microbial richness and alters microbial functions in an Australian Peatla'd. *Microbial Ecology*, *85*(3), 875–891.

BOM. (2021). Climate statistics for Australian sites. http://www.bom.gov.au/climate/averages/tables/ca_vic_names.shtml

Bünemann, E. K., Bongiorno, G., Bai, Z., Creamer, R. E., de Deyn, G., de Goede, R., Fleskens, L., Geissen, V., Kuyper, T. W., & Mäder, P. (2018). Soil quality–a critical review. *Soil Biology and Biochemistry*, *120*, 105–125.

Clymo, R. (1984). The limits to peat bog growth. *Philosophical transactions of the Royal Society of London. B, Biological Sciences*, *303*, 605–654.

Clymo, R., & Bryant, C. (2008). Diffusion and mass flow of dissolved carbon dioxide, methane, and dissolved organic carbon in a 7-m deep raised peat bog. *Geochimica et Cosmochimica Acta*, *72*, 2048–2066.

Dodson, J. (1987). Mire development and environmental change, Barrington tops, New South Wales, Australia. *Quaternary Research*, *27*, 73–81.

EPBC Act. (1999). Environment protection and biodiversity conservation act 1999 (Cth).

Fekete, I., Lajtha, K., Kotroczó, Z., Várbíró, G., Varga, C., Tóth, J. A., Demeter, I., Veperdi, G., & Berki, I. (2017). Long-term effects of climate change on carbon storage and tree species composition in a dry deciduous forest. *Global Change Biology*, *23*, 3154–3168.

Fountoulakis, M., & Lahm, H.-W. (1998). Hydrolysis and amino acid composition analysis of proteins. *Journal of Chromatography*, *826*, 109–134.

Friedel, J. K., & Scheller, E. (2002). Composition of hydrolysable amino acids in soil organic matter and soil microbial biomass. *Soil Biology & Biochemistry*, *34*, 315–325.

Glaser, B., Turrión, M. B., & Alef, K. (2004). Amino sugars and muramic acid—Biomarkers for soil microbial community structure analysis. *Soil Biology and Biochemistry*, *36*(3), 399–407.

Grover, S., & Baldock, J. (2010). Carbon decomposition processes in a peat from the Australian Alps. *European Journal of Soil Science*, *61*, 217–230.

Grover, S., & Baldock, J. (2012). Carbon chemistry and mineralization of peat soils from the Australian Alps. *European Journal of Soil Science*, *63*, 129–140.

Grover, S. P., Baldock, J. A., & Jacobsen, G. E. (2012). Accumulation and attrition of peat soils in the Australian Alps: Isotopic dating evidence. *Austral Ecology*, *37*, 510–517.

Gunawardhana, M., Silvester, E., Jones, O. A., & Grover, S. (2021). Evapotranspiration and biogeochemical regulation in a mountain peatland: Insights from eddy covariance and ionic balance measurements. *Journal of Hydrology: Regional Studies*, *36*, 100851.

Helfrich, M., Ludwig, B., Buurman, P., & Flessa, H. (2006). Effect of land use on the composition of soil organic matter in density and aggregate fractions as revealed by solid-state 13C NMR spectroscopy. *Geoderma*, *136*, 331–341.

Henrichs, S. M. (1995). Sedimentary organic matter preservation: An assessment and speculative synthesis—A comment. *Marine Chemistry*, *49*, 127–136.

Hope, G. S. (2002). The mountain mires of southern New South Wales and the Australian Capital Territory: Their history and future, Celebrating Mountains – An International Year of Mountains Conference, NSW.

Hopkins, D., Chudek, J., Webster, E., & Barraclough, D. (1997). Following the decomposition of ryegrass labelled with 13C and 15N in soil by solid-state nuclear magnetic resonance spectroscopy. *European Journal of Soil Science*, *48*, 623–631.

Hurisso, T. T., Moebius-Clune, D. J., Culman, S. W., Moebius-Clune, B. N., Thies, J. E., & van Es, H. M. (2018). Soil protein as a rapid soil health indicator of potentially available organic nitrogen. *Agricultural & Environmental Letters*, *3*(1), 1–5.

Ingram, H. (1978). Soil layers in mires: Function and terminology. *Journal of Soil Science*, *29*, 224–227.

Ji, H., Han, J., Xue, J., Hatten, J. A., Wang, M., Guo, Y., & Li, P. (2020). Soil organic carbon pool and chemical composition under different types of land use in wetland: Implication for carbon sequestration in wetlands. *Science of the Total Environment*, *716*, 136996.

Kang, H., Yu, W., Dutta, S., & Gao, H. (2021). Soil microbial community composition and function are closely associated with soil organic matter chemistry along a latitudinal gradient. *Geoderma*, *383*, 114744.

Karis, T., Silvester, E., & Rees, G. J. (2016). Chemical regulation of alpine headwater streams during a storm event (Bogong High Plains, Victoria, Australia). *Journal of Hydrology*, *542*, 317–329.

Knicker, H., & Hatcher, P. G. (1997). Survival of protein in an organic-rich sediment: Possible protection by encapsulation in organic matter. *Naturwissenschaften*, *84*, 231–234.

Knicker, H., & Skjemstad, J. (2000). Nature of organic carbon and nitrogen in physically protected organic matter of some Australian soils as revealed by solid-state 13C and 15N NMR spectroscopy. *Soil Research*, *38*, 113–128.

Kögel-Knabner, I. (2000). Analytical approaches for characterizing soil organic matter. *Organic Geochemistry*, *31*, 609–625.

Kuhry, P., & Vitt, D. H. (1996). Fossil carbon/nitrogen ratios as a measure of peat decomposition. *Ecology*, *77*, 271–275.

Kunnas, A. V., & Eronen, M. (1994). Identification of free amino acids in peat by gas chromatography and mass spectrometry. *European Journal of Soil Science*, *45*, 387–392.

Lawrence, R. (1995). The effects of grazing activity on the hydrology of the Bogong High Plains, Australia. *The Rangeland Journal*, *17*, 138–153.

Leifeld, J., Steffens, M., & Galego-Sala, A. (2012). Sensitivity of peatland carbon loss to organic matter quality. *Geophysical Research Letters*, *39*(14), 1–6.

Limpens, J., Berendse, F., Blodau, C., Canadell, J., Freeman, C., Holden, J., Roulet, N., Rydin, H., & Schaepman-Strub, G. (2008). Peatlands and the carbon cycle: From local processes to global implications–a synthesis. *Biogeosciences*, *5*, 1475–1491.

Limpens, J., Heijmans, M. M., & Berendse, F. (2006). The nitrogen cycle in boreal peatlands. In *Boreal peatland ecosystems* (pp. 195–230). Springer Berlin Heidelberg.

Lin, X., Tfaily, M. M., Green, S. J., Steinweg, J. M., Chanton, P., Imvittaya, A., Chanton, J. P., Cooper, W., Schadt, C., & Kostka, J. E. (2014). Microbial metabolic potential for carbon degradation and nutrient (nitrogen and phosphorus) acquisition in an ombrotrophic peatland. *Applied and Environmental Microbiology*, *80*, 3531–3540.

Manzoni, S., Chakrawal, A., Spohn, M., & Lindahl, B. D. (2021). Modeling microbial adaptations to nutrient limitation during litter decomposition. *Frontiers in Forests and Global Change*, *4*(64), 1–26. https://doi.org/10.3389/ffgc.2021.686945

Mayer, L. M. (1994). Relationships between mineral surfaces and organic carbon concentrations in soils and sediments. *Chemical Geology*, *114*, 347–363.

McAnallen, L., Doherty, R., & Ogle, N. (2017). Organic composition and multiphase stable isotope analysis of active, degrading and restored blanket bog. *Science of the Total Environment*, *599*, 1779–1790.

Melillo, J. M., Naiman, R. J., Aber, J. D., & Linkins, A. E. (1984). Factors controlling mass loss and nitrogen dynamics of plant litter decaying in northern streams. *Bulletin of Marine Science*, *35*(3), 341–356.

Mezbahuddin, M., Grant, R., & Hirano, T. (2014). Modelling effects of seasonal variation in water table depth on net ecosystem CO_2 exchange of a tropical peatland. *Biogeosciences*, *11*, 577–599.

Mikita, M.A., 2020. *NMR of humic substances and coal: Techniques, problems and solutions*. CRC Press.

Moe, L. A. (2013). Amino acids in the rhizosphere: From plants to microbes. *American Journal of Botany*, *100*(9), 1692–1705.

Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K. M., Zechmeister-Boltenstern, S., & Richter, A. (2014). Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling. *Nature Communications*, *5*, 3694. https://doi.org/10.1038/ncomms4694

Morris, P. J., Waddington, J. M., Benscoter, B. W., & Turetsky, M. R. (2011). Conceptual frameworks in peatland ecohydrology: Looking beyond the two-layered (acrotelm–catotelm) model. *Ecohydrology*, *4*(1), 1–11.

Normand, A. E., Smith, A. N., Clark, M. W., Long, J. R., & Reddy, K. R. (2017). Chemical composition of soil organic matter in a subarctic peatland: Influence of shifting vegetation communities. *Soil Science Society of America Journal*, *81*, 41–49.

Nungesser, M. K. (2003). Modelling microtopography in boreal peatlands: Hummocks and hollows. *Ecological Modelling*, *165*, 175–207.

Ono, K., Hiradate, S., Morita, S., Hiraide, M., Hirata, Y., Fujimoto, K., Tabuchi, R., & Lihpai, S. (2015). Assessing the carbon compositions and sources of mangrove peat in a tropical mangrove forest on Pohnpei Island, Federated States of Micronesia. *Geoderma*, *245*, 11–20.

Parish, F., Sirin, A., Charman, D., Joosten, H., Minaeva, T. Y., & Silvius, M. (2008). *Assessment on peatlands, biodiversity and climate change*. Global Environment Centre, Kuala Lumpur and Wetlands International, Wageningen.

Qiu, C., Zhu, D., Ciais, P., Guenet, B., Krinner, G., Peng, S., Aurela, M., Bernhofer, C., Brümmer, C., Bret-Harte, S., Chu, H., Chen, J., Desai, A. R., Dušek, J., Euskirchen, S. E., Fortuniak, K., Flanagan, L. B., Friborg, T., Grygoruk, M., … Ziemblinska, K. (2018). ORCHIDEE-PEAT (revision 4596), a model for northern peatland CO_2, water, and energy fluxes on daily to annual scales. *Geoscientific Model Development*, *11*, 497–519.

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing Vienna, Austria.

Rillig, M. C., Caldwell, B. A., Wösten, H. A., & Sollins, P. (2007). Role of proteins in soil carbon and nitrogen storage: Controls on persistence. *Biogeochemistry*, *85*, 25–44.

Schimel, J. P., & Bennett, J. (2004). Nitrogen mineralization: Challenges of a changing paradigm. *Ecology*, *85*, 591–602.

Senwo, Z. N., & Tabatabai, M. A. (1988). Amino acid composition of soil organic matter. *Biology and Fertility of soils*, *26*(3), 235–242.

Silvester, E. (2009). Ionic regulation in an alpine peatland in the Bogong High Plains, Victoria, Australia. *Environmental Chemistry*, *6*, 424–431.

Silvester, E., Karis, T., Yusuf, A., Pengelly, J., Grover, S., & Rees, G. N. (2021). Organic carbon and nitrogen dynamics during a peatland storm event: How dissolved combined amino acids reveal the spatial and temporal separation of organic molecules. *Journal of Hydrology*, *597*, 126191.

Sollins, P., Homann, P., & Caldwell, B. A. (1996). Stabilization and destabilization of soil organic matter: Mechanisms and controls. *Geoderma*, *74*, 65–105.

Sowden, F., Morita, H., & Levesque, M. (1978). Organic nitrogen distribution in selected peats and peat fractions. *Canadian Journal of Soil Science*, *58*, 237–249.

Starke, R., Jehmlich, N., & Bastida, F. (2019). Using proteins to study how microbes contribute to soil ecosystem services: The current state and future perspectives of soil metaproteomics. *Journal of Proteomics*, *198*, 50–58.

Stricker, C. A., Drexler, J. Z., Thorn, K., Duberstein, J. A., & Rossman, S. (2019). Carbon chemistry of intact versus chronically drained peatlands in the southeastern USA. *Journal of Geophysical Research – Biogeosciences*, *124*, 2751–2767.

Tfaily, M. M., Cooper, W. T., Kostka, J. E., Chanton, P. R., Schadt, C. W., Hanson, P. J., Iversen, C. M., & Chanton, J. P. (2014). Organic matter transformation in the peat column at Marcell experimental Forest: Humification and vertical stratification. *Journal of Geophysical Research – Biogeosciences*, *119*, 661–675.

Thormann, M. N. (2011). In vitro decomposition of sphagnum-derived acrotelm and mesotelm peat by indigenous and alien basidiomycetous fungi. *Mires & Peat*, *8*(3), 1–12.

Vardy, S., Warner, B., Turunen, J., & Aravena, R. (2000). Carbon accumulation in permafrost peatlands in the Northwest Territories and Nunavut, Canada. *The Holocene*, *10*, 273–280.

Wagner, G. H., & Mutatkar, V. K. (1968). Amino components of soil organic matter formed during Humification of 14C glucose. *Soil Science Society of America Journal*, *32*, 683–686.

Wahren, C.-H., Williams, R., & Papst, W. (1999). Alpine and subalpine wetland vegetation on the Bogong High Plains, southeastern Australia. *Australian Journal of Botany*, *47*, 165–188.

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag.

Wild, B., Schnecker, J., Knoltsch, A., Takriti, M., Mooshammer, M., Gentsch, N., Mikutta, R., Alves, R. J. E., Gittel, A., Lashchinskiy, N., & Richter, A. (2015). Microbial nitrogen dynamics in organic and mineral soil horizons along a latitudinal transect in western Siberia. *Global Biogeochemical Cycles*, *29*, 567–582.

Wright, E. L., Black, C. R., Cheesman, A. W., Drage, T., Large, D., Turner, B. L. & Sjoegersten, S., (2011). Contribution of subsurface peat to CO2 and CH4 fluuxes in a neotropical peatland. *Global Change Biology*, *17*(9), 2867–2881.

Xiang, X., Wang, H., Man, B., Xu, Y., Gong, L., Tian, W., & Yang, H. (2023). Diverse Bathyarchaeotal lineages dominate archaeal communities in the acidic Dajiuhu peatland, Central China. *Microbial Ecology*, *85*(2), 557–571.

Yang, Q., Liu, Z., & Bai, E. (2023). Comparison of carbon and nitrogen accumulation rate between bog and fen phases in a pristine peatland with the fen-bog transition. *Global Change Biology*, *29*(22), 6350–6366.

Younes, K., & Grasset, L. (2017). Analysis of molecular proxies of a peat core by thermally assisted hydrolysis and methylation-gas chromatography combined with multivariate analysis. *Journal of Analytical and Applied Pyrolysis*, *124*, 726–732.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Yusuf, A. Y., Silvester, E., Brkljaca, R., Birnbaum, C., Chapman, J., & Grover, S. (2024). Peatland carbon chemistry, amino acids and protein preservation in biogeochemically distinct ecohydrologic layers. *European Journal of Soil Science*, *75*(3), e13518. https://doi.org/10.1111/ejss.13518