Impact of Spatial Variability on Soil Organic Matter in an Alpine System

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MSc by Research

University of York

Environment and Geography

June 2022

Abstract

Mountain soils are understudied carbon stores subject to accelerated elevation-dependent warming where topographic complexity amplifies localised microclimates and spatial variation. Better understanding of soil organic matter (SOM) distribution in alpine regions is vital for accurately modelling the impact of climate change, particularly focusing on labile fractions of water extractable organic matter (WEOM) which have been found in high proportions in alpine soils. This study explored the impact of aspect on belowground nutrient content and stochiometric relationships, environmental variables, and semivariance within the system in the Cairngorms National Park, Scotland. 128 topsoil samples were collected from the north and south facing slopes of three mountains at 750 m asl. Total carbon, nitrogen and phosphorus and water extractable carbon, nitrogen and phosphorus were measured along with carbon quality measures using FTIR and UV-Vis spectrometry. Huge variance in nutrient content was recorded, with total carbon content ranging from 3.24% to 46.42% with a mean of 17.60% (± 0.84). Aspect showed a limited effect on nutrient content with only mean WEON values showing variation between north (11.44 ± 1.20 μg per g dry soil) and south (12.58 ± 0.78 μg per g dry soil). Gravimetric moisture content, pH, slope angle and soil depth strongly correlated with SOM and WEOM. No spatial autocorrelation was found as high background variance suggests that to detect semivariance in these systems requires working at either much smaller or much greater scales than the 0.5 m to 50 m scale used in this study. Post-hoc power analyses highlighted the difficulty in detecting changes in total carbon concentration, showing that to detect a 4% and 0.4% change would require 119 and 11,757 samples respectively. This study shows that existing monitoring schemes do not currently operate at scales suitable to detect climate change induced belowground changes in this montane area and that future studies require thoughtful experimental design.

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Abbreviations

ANOVA	Analysis of Variance statistical test
AROCH	Aromatic waveband region 855-740 cm^{-1} measured using FTIR spectrometry
C	Carbon
CB	Carn Bheadhair
CB-N	Carn Bheadhair north site
CB-S	Carn Bheadhair south site
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
FTIR	Fourier Transform Infrared
GC	Geal Charn
GC-N	Geal Charn north site
GC-S	Geal Charn south site
ML	Meall an Lundain
ML-N	Meall an Lundain north site
ML-S	Meall an Lundain south site
N	Nitrogen
NMR	Nuclear magnetic resonance
NNR	National Nature Reserve
NP	National Park
P	Phosphorus
POLY	Polysaccharide waveband region 1,185-915 cm^{-1} measured using FTIR spectrometry

SAT	Saturated waveband region 2,985–2,820 cm^{-1} measured using FTIR spectrometry
SOC	Soil organic carbon
SOM	Soil organic matter
TC	Total carbon
TN	Total Nitrogen
TOC	Total organic carbon
TP	Total phosphorus
UNSAT	Unsaturated waveband region 1,800-1,525 cm^{-1} measured using FTIR spectrometry
UV-Vis	Ultraviolet-visible
WEOC	Water extractable organic carbon
WEOM	Water extractable organic matter
WEON	Water extractable organic nitrogen
WETP	Water extractable total phosphorus

Acknowledgements

For Lucy

I would like to thank my supervisor Dr Rob Mills for sharing his unending enthusiasm for learning, exploration and mountain adventures with me; Ash Taylor for his incredible support and positivity both in the hills and the labs; Dr Sylvia Toet for thoughtful feedback on my Thesis Advisory Panel; Professor Markus Egli and Professor Rob Marchant for their time and constructive comments; and the valuable technical advice from Rebecca Sutton, Deborah Sharp, and Dr Matt Pickering. I would like to extend my thanks to NatureScot, and the staff at RSPB Loch Garten and National Trust for Scotland Mar Lodge Estate for allowing me access to these beautiful parts of the world.

I am deeply grateful to Fergus for his unwavering belief in me throughout: you made this possible. Thanks to my friends especially Abi and Maria for always lifting my spirits; my parents for your endless encouragement and support; Jo for her wonderful words of wisdom; and Nessa for showing me the priorities in life.

Finally, thank you to Lucy: an inspiration and a lovely woman.

> *“On the summit, your soul becomes part of the mountain. It makes you feel alive.”*
>
> \- Nimsdai Purja MBE

Declaration

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References. Many of the ideas in this thesis were the product of discussions with my supervisor Dr Rob Mills (University of York) and colleague Ash Taylor (University of York).

1. Introduction

Mountain regions are topographically complex areas resilient to extremes in seasonal climatic variation, yet are facing accelerated rates of temperature change amplified at high altitude (Rangwala & Miller, 2012). Elevation-dependent warming threatens to change mountain ecosystem functions including belowground processes such as carbon sequestration, nutrient retention and hydrological regimes (Futter *et al*., 2009; Mountain Research Initiative EDW Working Group, 2015). Alpine soils store large amounts of labile organic C which is especially vulnerable to release into the atmosphere if environmental conditions are altered by climate change (Budge *et al*., 2011; Hagedorn *et al*., 2009; Leifeld *et al*., 2009). However, soil organic carbon (SOC) turnover and distribution in mountain regions has been difficult to quantify. SOC inventories are limited by the extent of existing regional data, our understanding of links between abiotic and biotic factors and SOC stocks, and high spatial variability in the study systems (Hoffmann *et al*., 2014). Mountain regions are difficult to access and characterised by complex topography, with elevational compression, surface roughness and precipitation gradients sustaining heterogenous belowground function (Immerzeel *et al*., 2020). Limited accessibility therefore means that the majority of historical data comes from mountain ranges such as the European Alps where pre-existing networks of summit infrastructure have supported scientific monitoring (Beniston *et al*., 1997; Rangwala & Miller, 2012). Vast altitudinal gradients condensed across small physical distances augment variation in microclimate, driving patterns in soil function, microbial processes and vegetation communities (Winkler *et al*., 2016). Mountains act as natural laboratories providing opportunities to study the mechanisms behind spatial ecological patterns across a condensed model landscape (Graham *et al*., 2014). Developing our understanding of biogeochemical processes in alpine soil will allow more accurate estimation of mountain carbon stocks and predictions of future impacts on soil organic matter (SOM) under a changing climate regime.

In alpine systems, belowground processes are largely influenced by topography which moderates solar radiation intensity and hydrological conditions. Large scale resolution studies encompass regional-sized features such as glacial valleys, whereas fine resolutions consider localised elements like snowbeds (Björk & Molau, 2007; Hoffmann *et al*., 2014; Trivedi *et al*., 2008). Incident solar radiation is redistributed by slope aspect, creating shading effects which, in the absence of trees, is a principal mechanism controlling soil temperature and moisture content (Winkler *et al*., 2016). Additionally, aspect determines wind exposure which controls soil temperature through direct evaporative cooling and redistribution of snow cover, which can insulate soil from frost, delay final snowmelt, and stabilise water availability into the growing season (Björk & Molau, 2007; Wundram *et al*., 2018). Aspect has been recognised as an explanatory factor for variation in SOC distribution (Garcia-Pausas *et al*., 2007; Zhu *et al*., 2017), total nitrogen (TN) stocks (Yimer *et al*., 2006) and microbial community structure (Wu *et al*., 2017) in mountain regions. SOM decomposition rates are temperature regulated, with colder temperatures inhibiting microbial metabolization, slowing enzymatic reaction rates and decreasing the speed of SOC turnover (Conant *et al*., 2011; Davidson & Janssens, 2006). However, net primary productivity is also subject to temperature limitation, with low temperatures at high altitudes restricting vegetation growth and low plant productivity regulating top-down carbon input into alpine soils (Garcia-Pausas *et al*., 2007; Hoffmann *et al*., 2014). Furthermore, the steep slope angles characteristic of mountainous landscapes influence SOM distribution, microbial and vegetation communities by restricting water storage capacity and altering soil erosion dynamics (Huang *et al*., 2013; Riebe *et al*., 2015).

As topography controls soil infiltration rate and localised microclimate, it has a direct relationship with moisture-dependent heterotrophic respiration which will be influenced by global changes in precipitation levels (Falloon *et al*., 2011). Coupled with the findings that SOM decomposition rates in cold regions are most vulnerable to increase with warming temperatures, mountain regions face additional pressure on their complex belowground systems over decadal timescales (Conant *et al*., 2011).

Developing a more robust understanding of how spatial variation impacts belowground systems is essential for accurately predicting the response of mountain regions to climate change (Chen *et al*., 2017; Hagedorn *et al*., 2009). The unique features of mountain environments such as condensed vertical gradients, topography-controlled microclimates and large seasonal amplitude result in uncertainties when predicting belowground function at altitude compared to low elevation habitats (Garcia-Pausas *et al*., 2007). As spatial uncertainty and low predictability of alpine SOC stocks limit our understanding of the role of mountain soils as sinks or sources of greenhouse gases, this study aims to answer questions about the local-scale variation in belowground function by investigating semivariance in SOM and attempting to detect spatial autocorrelation to better understand at what scale variation occurs in the system (Hoffmann *et al*., 2014). Particularly, I focus on dissolved organic matter (DOM), the fraction of SOM most readily available for microbial uptake and vulnerable to atmospheric loss found to be disproportionately present at high altitudes compared to low elevations (Budge *et al*., 2011; Hagedorn *et al*., 2009; Puissant *et al*., 2017). Limited studies of this nature have been conducted in an alpine environment, especially lacking in the Scottish Highlands, presenting an opportunity to utilise this area as a natural laboratory to investigate the relationship between topographic variables, climatic conditions and soil dynamics. Utilising both wet laboratory and spectroscopy analytical techniques to gain soil information at quantitative (precise nutrient content) and qualitative (soil structure and chemistry) levels will provide detailed insights into belowground processes alongside a broad dataset for wider comparison (Wadoux *et al.*, 2021). Developing our understanding of alpine soil biogeochemical processes in the Cairngorms National Park (NP) will improve modelling of existing SOC stocks and allow more accurate predictions of localised changes in a warming climate. This study aims to investigate how topographical variables describe the belowground function of mountain soils in the Cairngorms NP, by:

1. Determining the relative impact of aspect on topsoil nutrient content and stochiometric relationships
2. Quantifying the degree of semivariance in the system and its role in within-site variation
3. Identifying additional explanatory abiotic and biotic factors of residual variance in the system

2. Materials and Methods

2.1. Study Sites and Sampling Locations

The study sites were located in the Cairngorms NP in the Scottish Highlands. The NP is the most extensive mountainous region in the United Kingdom containing one-third of all land area above 600 metres altitude and four of the five highest summits. The geomorphology is characterised by glacially sculpted features including extensive plateaus and weathered bedrock (Gordon *et al*., 2002). Upland soil properties are relatively thin and stony with screes and rank soils less than 10cm deep derived from underlying solid geology, while podzols (50% of soil within the NP) and alpine podzols (18% of soils above 550 m) indicate relative stability in historical conditions (Cairngorms National Park Authority, 2006). The alpine zone experiences a subarctic-oceanic climate with cool, wet summers and cold winters with substantial snowfall (Watson *et al*., 2010). Average annual total precipitation approaches 1500 mm at mountain summits with over 30% falling as snow during the winter period (Soulsby *et al*., 2000; Tetzlaff *et al*., 2009). Mean annual temperature is 1.6 °C at Cairngorm Summit (1245 m asl) and 5.6 °C at the Cairngorm chairlift meteorological station (663 m asl) (Burt & Holden, 2010). High elevation annual snow coverage is recorded lasting between 157 and 260 days (Andrews *et al*., 2016). The potential treeline has remained stable at approximately 650 m for the last 1000 years reflecting limitation by wind exposure and grazing pressure, indicating a stable vegetation community in the montane environment (Nagy *et al*., 2013). Above 700 m, vegetation consists of alpine heath communities including dwarf shrubs (*Empetrum nigrum, Calluna vulgaris* and *Vaccinium vitis-idaea*), bryophytes (*Racomitrium lanuginosum*), rushes (*Juncus trifidus*), and lichen (*Cladonia* spp.). Grazing pressure from deer and sheep increased within the NP over the last 200 years, however extensive management including culling has reduced numbers significantly to a level considered more representative of historic levels, from 21.2 deer per km ^{2} in 1991 to 0.7 deer per km ^{2} within the Mar Lodge Estate (Rao, 2017).

Three mountains were selected as study sites based on the criteria of being over 700 m asl, having accessible north and south facing slopes, and lacking footpaths to avoid impacts of human disturbance. The mountains were also spread across the Cairngorms NP ensuring a good coverage of different regions in the area (Fig. 1).

Carn Bheadhair (CB; Fig. 1b) (804 m asl; latitude 57.186, longitude -3.565), is located within the Abernethy Nature Reserve owned and managed by the Royal Society for the Protection of Birds. It extends from 200 m asl to the summit of Cairn Gorm at 1309 m asl, covering 137 km ^{2} almost 20% of which is within the montane zone. Many Arctic plant species are found in the alpine heath and bryophyte-dominated snow beds (Beaumont *et al*., 2005). The site is part of the Nethybridge psammite formation with a quartzite bedrock (British Geological Survey, 2021).

Geal Charn (GC; Fig. 1c) (920 m asl; latitude 57.090, longitude -3.843) is located in the Allt a' Mharcaidh catchment in the Invereshire and Inshriach National Nature Reserve (NNR). Managed by NatureScot, the catchment has been subject to long-term monitoring as part of the UK Environmental Change Network since 1998 (Andrews *et al*., 2016). Covering 10 km ^{2}, it ranges from 300 m to 1111 m asl with an underlying geology composed of biotite granite with superficial surface gravel deposits (British Geological Survey, 2021; Soulsby *et al*., 2000).

Meall an Lundain (ML; Fig. 1d) (777 m asl; latitude 57.035, longitude -3.547) is located within the Mar Lodge Estate, which at 290 km ^{2} is the largest NNR in the UK. Managed by the National Trust for Scotland since 1995, deer populations are controlled to conserve arctic tundra vegetation and allow natural regeneration of remnant Caledonian Pine forest at lower elevations (McDonald *et al*., 2008). The bedrock is part of the Gaick psammite formation (British Geological Survey, 2021).

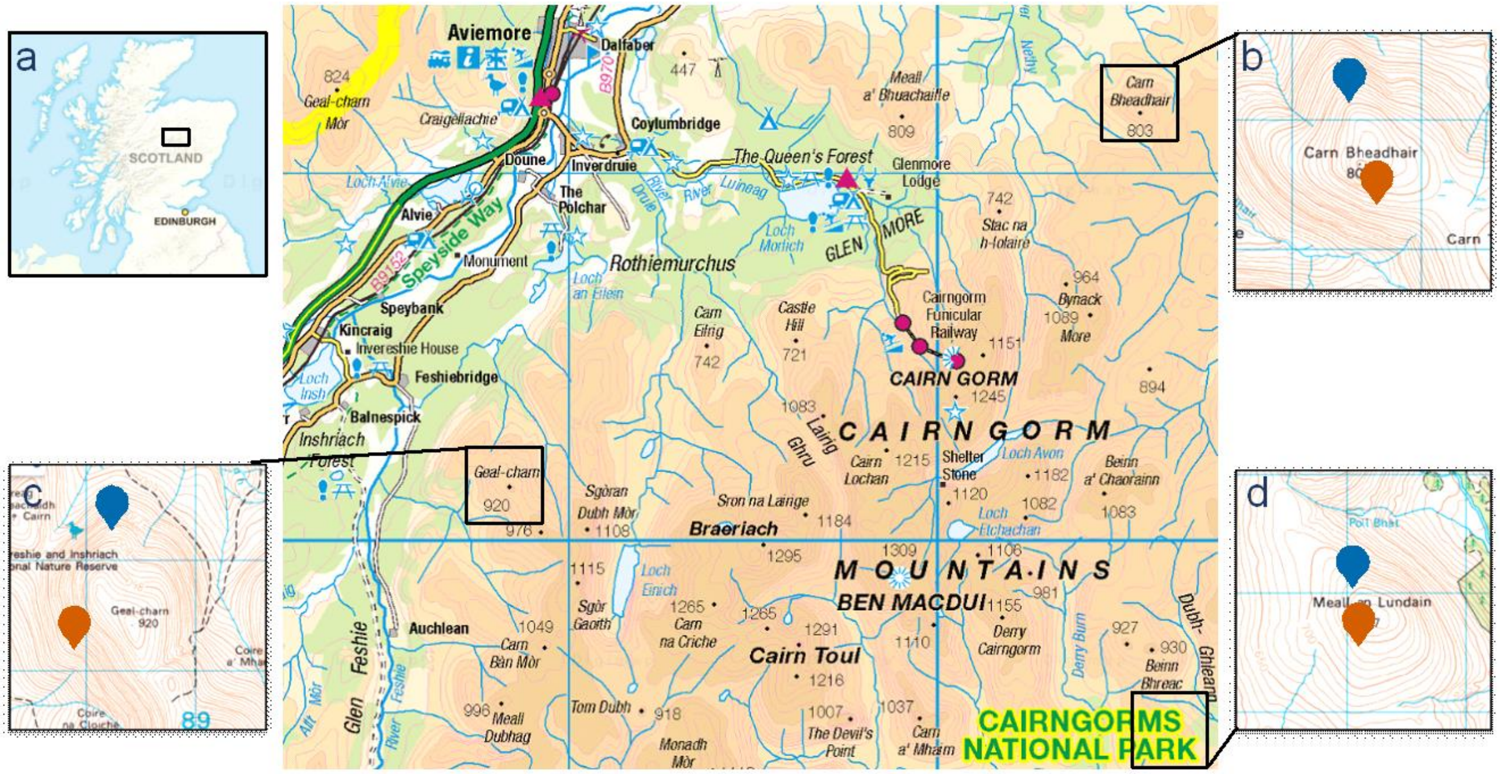

Figure 1: Map showing a) the position of the Cairngorms NP within Scotland, and the locations of b) Carn Bheadhair, c) Geal Charn, and d) Meall an Lundain, with north and south sampling locations marked in blue and red respectively.

2.2. Field Methods

I conducted a two-week field campaign in August 2021 with sampling design planned to facilitate accessing sites on foot and retrieving samples from remote locations. At each site I measured out a spatial survey grid on the north and south facing aspects. Survey grids were positioned by placing the bottom left corner at a GPS point along the 750 m contour line where the north aspect faced between 355° and 005° degrees and the south facing aspect between 175° and 185° degrees. Location was confirmed using a Garmin eTrex 22x and Ordnance Survey Locate iPhone application. Survey grids consisted of four 31.5 m transects parallel to the line of greatest slope and spaced 12 m apart (Fig. 2). Each transect had seven sampling points distributed at hierarchical intervals, totalling 28 sampling points per grid and 168 sampling points across the three sites.

At each sampling point a standard field protocol was followed. I positioned a 20 cm quadrat to the immediate right of the transect and measured soil depth with a metal rod at five points to calculate the mean depth (Fig. 3). I recorded GPS coordinates, slope steepness in degrees measured using a clinometer and slope aspect of each quadrat using a compass. Each quadrat was photographed for later calculation of functional plant type percentage ground coverage. After surface litter was removed, I collected a 10 cm deep soil core from as close to the centre of the quadrat as possible. Soil samples were collected using a trowel, brick bolster, mallet and ruler to measure depth due to constraints of shallow stony soils and did not retain a cylindrical structure when stored in labelled zip lock bags.

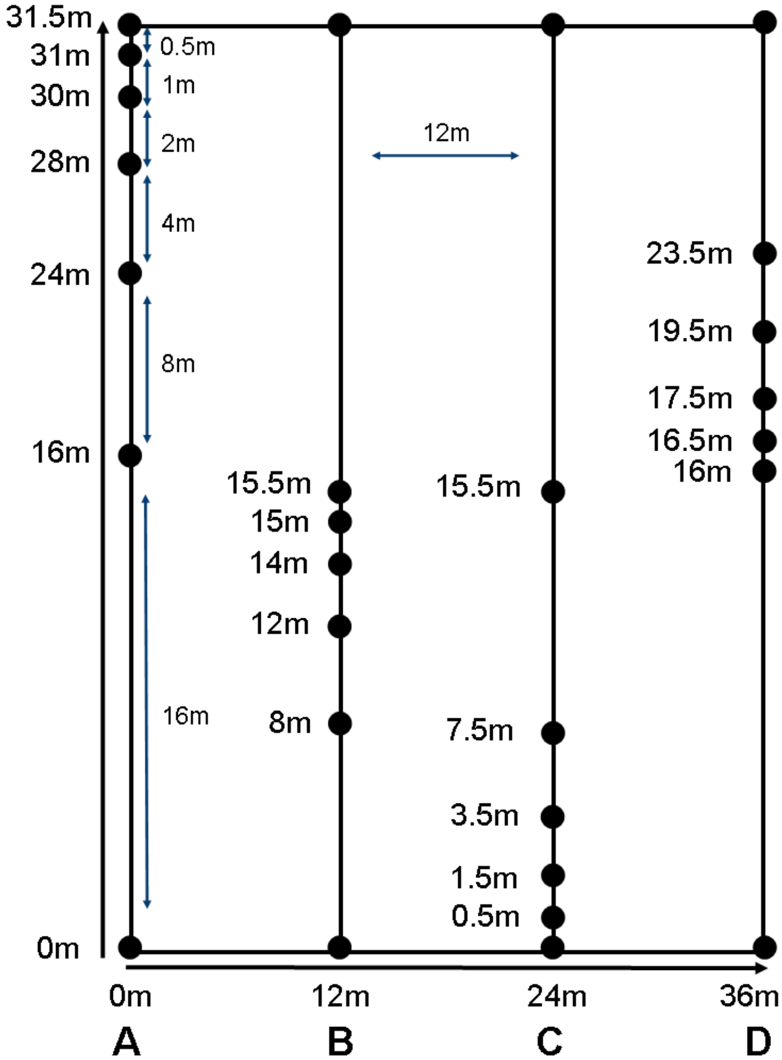

Figure 2: Layout of the spatial survey grids which were positioned on the north and south aspects of each of the three study sites. Sampling positions (n = 28) are shown as circles located at set distances along four transects (A to D).

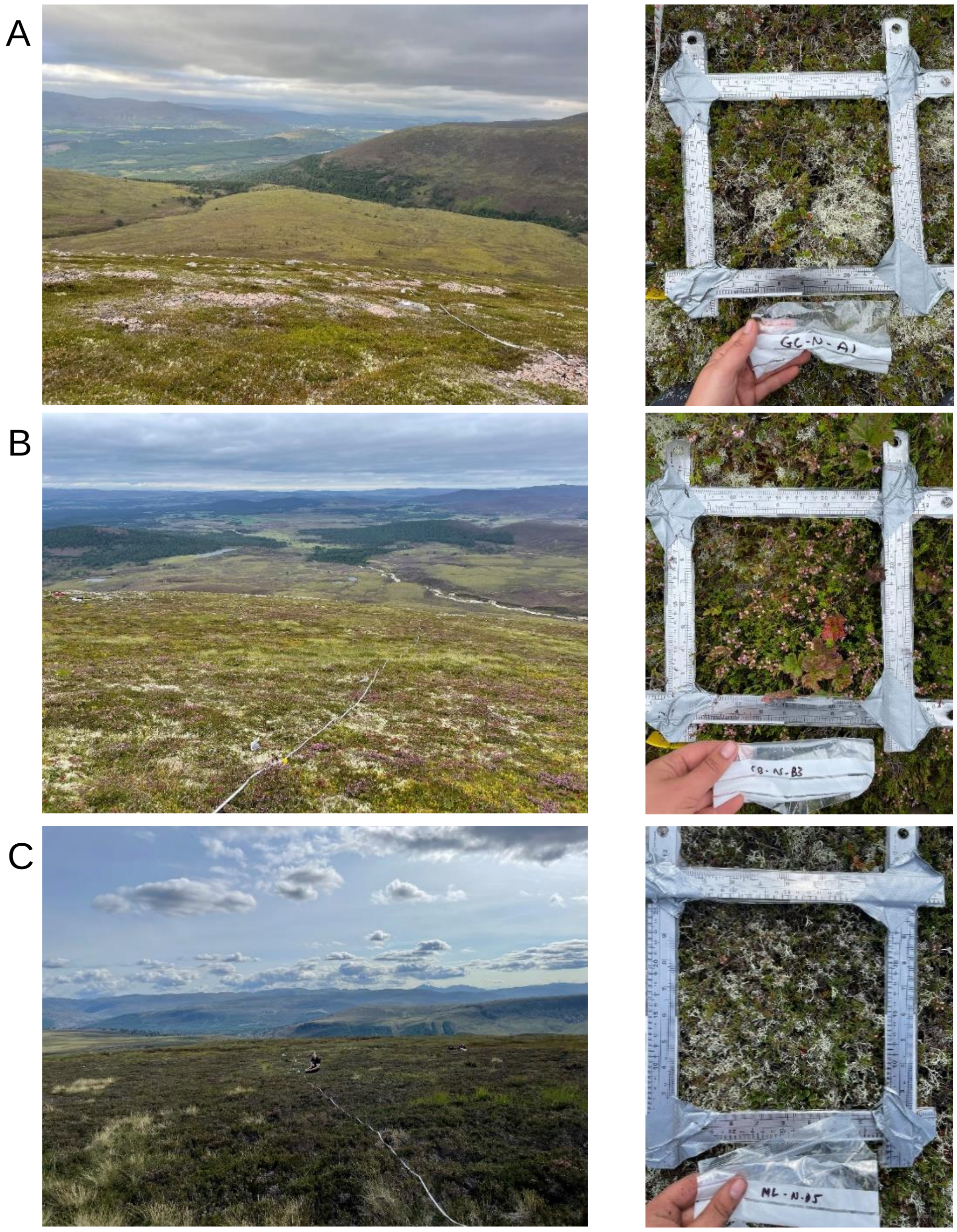

Figure 3: Photographs from field sampling in the Cairngorms National Park in August 2021, showing transects and quadrats *in situ* at a) Geal Charn north slope, b) Carn Bheadhair north slope, and c) Meall an Lundain north slope.

2.3. Laboratory Methods

Soil samples were refrigerated at 4 °C in a cold room until analysis, before being weighed, coarsely sieved in a 5mm sieve, root-picked for 5 minutes per sample, homogenised and re-weighed. Soil pH in distilled water (pH_w) and 0.01 M $CaCl_2$ (pH_{Ca}) was measured using a Thermo Scientific Orion Star A111 benchtop pH meter calibrated with pH 4.01 and 7.00 commercial buffer solutions. pH_{Ca} minimises the impact of salt concentration in soils and provides a more accurate result for interannual comparisons. Aliquots of moist soil were weighed (*mwet*), oven dried for 48 hours at 105 °C and reweighed to determine dry weight (*mdry*). Gravimetric water content (*θg*) was calculated for each sample as:

$$\theta g = (mwet - mdry) \div mdry$$

I prepared soil for total carbon (TC), TN and total phosphorus (TP) analysis by airdrying 50 g of each soil sample at 40 °C for 72 hours, finely sieving to 0.5 mm and ball milling for 3 minutes at 25 rpm. TC and TN percentage were measured using a Elementar VarioMacro analyser with glutamic acid as a calibration standard. TP was determined using a colorimetric method outlined by Harwood, Steenderen and Kuhn (1969). 0.5 ml of 50% w/v $Mg(NO_3)_2$ was added to 0.1 g of sample and ashed at 550 °C in a muffle furnace for 2 hours. After cooling, 10 ml of 1 N HCl was added to samples which were then vortexed and shaken for 16 hours. Then 3 ml of each sample was measured into a 15 ml centrifuge tube and added 0.84 ml of a mixed reagent of equal parts 4 N H_2SO_4 and 0.96% w/v ammonium molybdate, and 0.33 ml of a mixed reagent of equal parts 10% w/v ascorbic acid and 0.27 % w/v K-antimony tartrate. Following the molybdenum blue reaction, the samples were measured with a Shimadzu UV-

1800 UV-Vis spectrophotometer at 890 nm and plotted against a calibration curve of known standards to obtain TP content.

To calculate key indicators of SOM quality, approximately 0.5 g of milled sample was analysed using a Thermo Scientific Nicolet iS20 Fourier Transform Infrared (FTIR) Spectrometer to produce a full spectral profile. Spectral data were processed using in RStudio version 1.4.1717 (RStudio Team, 2021) using the R package 'soilspec' (Wadoux *et al*., 2021) to characterize the following waveband regions: the saturated (SAT) region 2,985–2,820 cm^{-1}; the unsaturated (UNSAT) region 1,800–1,525 cm^{-1}; the polysaccharide (POLY) region 1,185–915 cm^{-1}; and the aromatic (AROCH) region 855–740 cm^{-1}, and corresponding C=O and C=C bonds, as outlined in Pengerud *et al.* (2013). These peak ratio comparisons were used to calculate aromaticity (C=C / TC), maturation (C=C / UNSAT), hydrophobicity (SAT / TC), condensation (AROCH / C=C) and polysaccharide (POLY / TC) indices.

I obtained water extractable organic matter (WEOM) by adding 40 mL of deionised water to 5 g aliquots of soil and shaking for 30 minutes at 120 rpm. Samples were allowed to stand for 30 minutes before collecting 20 mL of extractant using a syringe and spinning in a centrifuge for 10 minutes at 3000 rpm. Supernatant was then filtered using a syringe with a 0.45 μm Whatman GMF filter. Water extractable organic carbon (WEOC) and water extractable organic nitrogen (WEON) content was measured using a Elementar VarioCube analyser with five standards for calibration.

Water extractable total phosphorus (WETP) was measured using a colourimetry for waters method. I digested WEOM samples by adding 75 μL of H_2SO_4 and 600 μL of $K_2O_8S_2$ to 3 mL of sample in a centrifuge tube and boiling in a beaker of water on a hotplate for 30 minutes. Once cooled, to each 2.5 mL of digested sample I added 400 μL of a solution of 8 g ammonium molybdate and 0.2 g antimony potassium tartrate diluted to 1 L with distilled water, and 200 μL of a solution of 60 g ascorbic acid diluted to 1 L with distilled water. Samples were vortexed and following colour development were measured with an ultraviolet-visible (UV-Vis) spectrophotometer at 880 nm. WETP concentration was determined using a standard curve from five known standards prepared from stock phosphorus solution which were digested and prepared identically to the samples. WEOC, WEON and WETP content was corrected to mass of element per gram of dry soil.

Spectral profiles for WEOM were produced by pipetting 0.2 ml of sample with three replicates into a quartz microplate and measuring absorbance at 1nm intervals across 210 to 800 nm using a Jasco V560 Peltier UV-Vis spectrophotometer. This was used to calculate specific indicators of DOM quality. E2:E3 and E4:E6 values show the ratio of absorption coefficients at 250:365 nm and 465:665 nm respectively. $SUVA_{254}$ normalises the specific ultraviolet absorbance at 254 nm to TC content. $S_{220-465}$ describes the spectral slope between 220-465 nm calculated using a linear regression between the intervals.

2.4. Statistical Analysis

All statistical analyses were conducted in RStudio version 1.4.1717 (RStudio Team, 2021). Stochiometric values were non-normally distributed with no homogeneity of variance despite transformations, so nonparametric ANOVA equivalents were used. Comparisons between multiple sites were made using the Kruskall-Wallis test with Dunn-Bonferroni post hoc method using package ‘FSA’ (Ogle *et al*., 2022). Values at north and south aspects were compared using the Mann-Whitney-Wilcoxon test. Relationship strength between variables were measured using the Pearson correlation coefficient. Spatial autocorrelation was calculated using Moran’s I to identify similarity in response values based on sample distance, using package ‘ape’ (Paradis & Schliep, 2019).

Mean values for gravimetric moisture content, slope steepness, soil depth, pH_W and pH_{Ca} were calculated and reported with standard error (±). Vegetation percentage cover was measured using Adobe Photoshop 2021 version 22.4.2 to manually select functional plant types and calculate total pixel numbers within the quadrat.

Graphs were created in RStudio using package ‘ggplot2’ (Wickham, 2016). Boxplots show the median value as a central line with the box representing the interquartile range between the 25^{th} and 75^{th} percentile and whiskers showing the most distant data point that is not an outlier. All individual observations including outliers are shown to illustrate the distribution of values.

2.5 Power Analysis

Power analysis is a statistical tool with a well-established role in experimental design. It can be used to indicate whether lack of statistical significance is due to inadequate replication and estimate the number of samples needed to demonstrate differences between experimental treatments (Kravchenko & Robertson, 2011; Smith, 2004). To consider the reliability of modelling approaches and sampling techniques and their implications for future research, I imagined designing a warming experiment to detect 0.04 %, 0.4 % and 4 % changes in elemental concentrations based on the ‘4 per mille’ aspiration to increase global soil organic matter stocks by 0.4 % percent per year as a climate change mitigation solution (Minasny *et al*., 2017). I did a series of post-hoc power analysis using the whole dataset using the standard deviations to calculate the number of samples necessary in a hypothetical experiment to detect a change in soil carbon equivalents across aspect, site, and site x aspect in RStudio using the package ‘pwr’ (Champely *et al*., 2020).

3. Results

3.1 Environmental Variables

Most physical environmental variables showed consistency between aspects, with comparable mean values in gravimetric moisture content, slope steepness, soil depth and pH_w between north and south facing slopes (Table 1, Table 2). The exception was pH_{CaCl} measurements which were more acidic on south-facing slopes (Table 1, Table 2).

However, vegetation cover showed greater variation between spatial survey grids on north and south aspects (Table 2, Fig. S1). Although shrubs were the dominant vegetation type on both aspects, there was a higher percentage coverage on south-facing slopes in contrast to northern slopes where lichen and moss were more prominent ground cover components (Table 2, Fig. S1).

Correlations were found between environmental variables and belowground nutrient content. Soil moisture content was strongly positively correlated with SOC and SON content (Table 4). Mean slope angle was correlated with TN content, with shallower slopes containing higher concentrations particularly on south facing slopes (Table 4). Soil depth was a consistent predictor of SOM and WEOM nutrient concentrations, with increasing depth correlated with higher concentrations in all measurements excepting TP (Table 4). This pattern was also consistent with mean pH_{CaCl} which showed a positive correlation between increasing soil acidity and higher concentrations of TC, WEOC, TN, WEON and WETP (Table 4). This correlation was consistent on north facing slopes; however, it was limited to WEOC and WEON on south facing slopes with a generally weaker correlation shown. Of the vegetation types measured, only lichen cover showed a relationship with belowground nutrient content with a weak positive correlation with TC and TN content (Table 4).

3.2 Stochiometric Relations

There was no generalisable aspect effect in TC and TN concentration with contrasting patterns in distribution shown between sites (Fig. 4a-b). There was a tendency toward higher TP content on north-facing slopes driven by high values at CB-N, although TP values elsewhere were more consistent (Table 2, Fig. 4c). Both WEOC and WETP content showed no aspect effect and contrasting patterns between sites (Fig. 4c, 4e, Table 2). However, WEON content was higher on south-facing slopes while values at northern spatial survey grids were in general more condensed (Fig. 4d, Table 2). The exception to this was at CB-N where WEON content varied by an order of magnitude between the lowest and highest value (0.004 and 0.049 mg N per g dry soil respectively).

Stochiometric relationships of total nutrient concentrations were consistent between aspects, with TC:TN strongly positively correlated at all six spatial survey grids (Table 3, Fig. 5a). However, the relationship strength of TC:TP and TN:TP were weaker in particular at ML (Table 3, Fig. 5b-c). WEOC:WEON showed stronger positive correlation than the other water extractable stochiometric ratios (Table 3) with the strongest relationship on north-facing slopes (Table 2, Fig. 5d). WEOC:WETP showed no aspect effect and differed in relationship strength with site, with a weaker positive relationship at GC (Table 2, Table 3, Fig. 5e). WEON:WETP also showed a weaker relationship at GC, although overall there was an aspect effect with a stronger positive correlation on north-facing slopes (Table 3, Fig. 5f). Positive relationships were found between TC:WEOC, TN:WEON and TP:WETP (Table 3, Fig. 5g-i) with a stronger correlation found for N and P on north slopes (Table 2). However, TP:WETP also showed a distinct site effect with CB and GC showing a positive relationship whereas ML showed no correlation (Table 3).

Additional stochiometric relationships were explored as in Tipping *et al*. (2016; Fig. S2). TN:TC versus TP:TC showed contrasting patterns based on aspect with a strong positive relationship on north-facing slopes compared to no correlation on south-facing slopes, whereas WN:WC versus WP:WC showed no correlation (table 3). TC versus TN:TC showed a strong negative relationship on north slopes but no correlation on south slopes and TC versus TP:TC showed a negative relationship on both aspects although it was stronger on north-facing slopes (Table 3). WEOC (%) versus WEON:WEOC showed no overall correlation due to contrasting patterns between sites with CB showing a strong negative relationship compared to positive correlation at ML (Table 3). WEOC (%) versus WETP:WEOC showed a positive correlation consistent between aspects (Table 3).

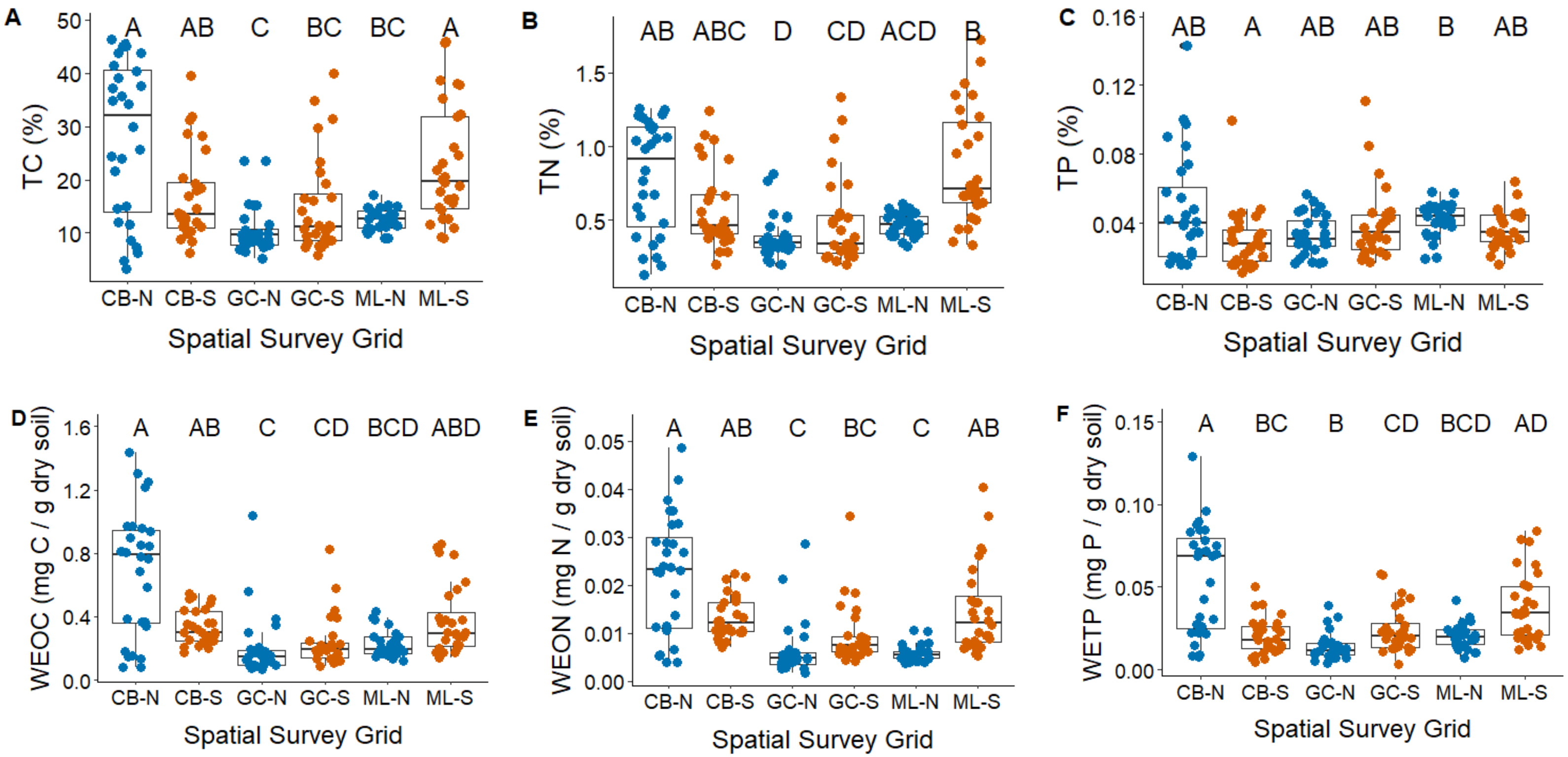

Figure 4: Stochiometric values showing a) TC (%), b) TN (%), c) TP (%), d) WEOC (mg C / g dry soil), e) WEON (mg N / g dry soil), and f) WETP (mg P / g dry soil) at the six spatial survey grids (n = 168). Variables with different letters are significantly different at the < 0.05 level (Table 1, Table 2). Colours show samples from north facing slopes (blue) and south facing slopes (red).

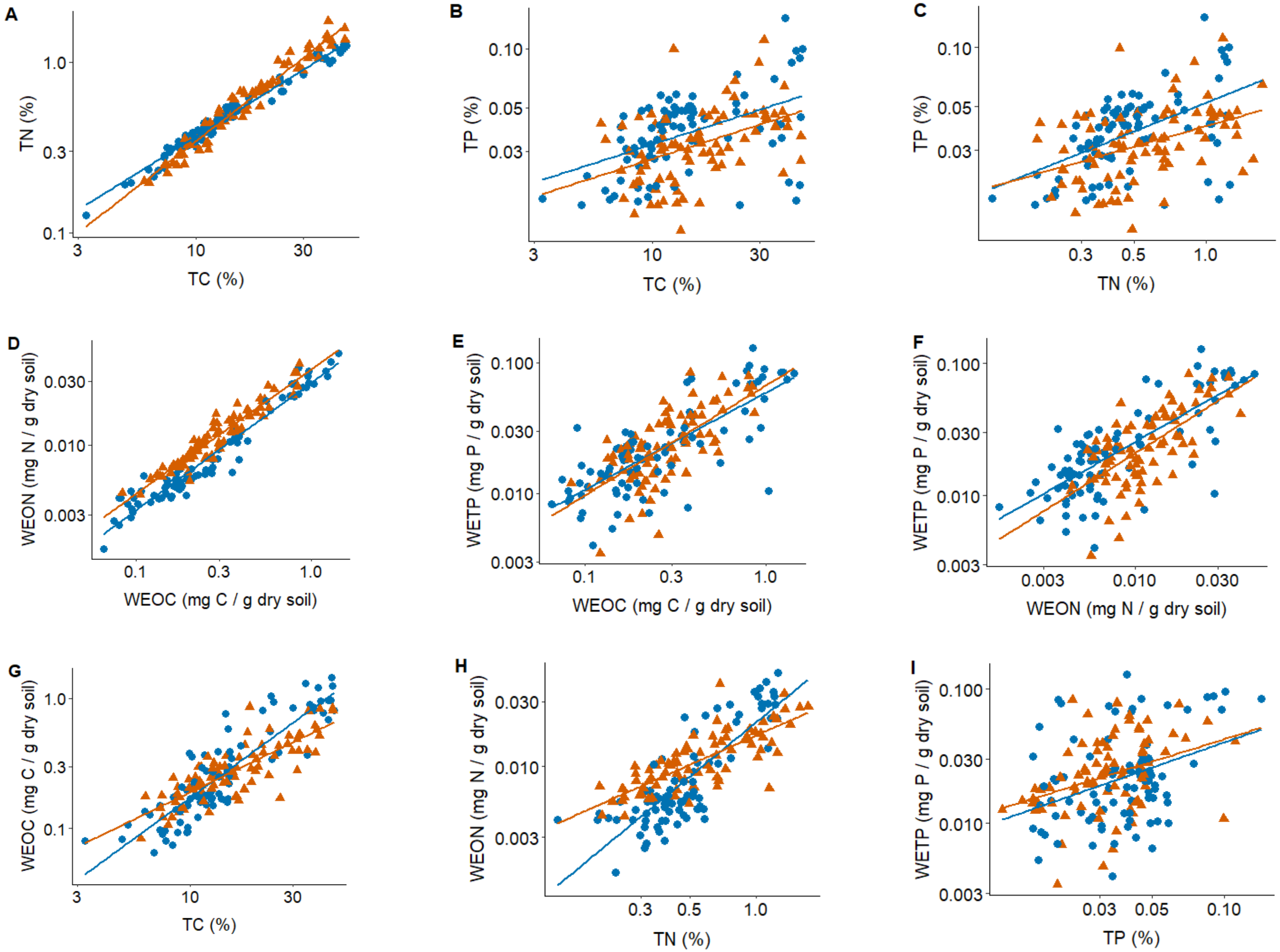

Figure 5: Stochiometric relationships showing a) TC:TN, b) TC:TP, c) TN:TP, d) WEOC:WEON, e) WEOC:WETP, f) WEON:WETP, g) TC:WEOC, h) TN:WEON, and i) TP:WETP (n = 168). Colours show samples from north facing slopes (blue) and south facing slopes (red).

Table 1: Mean (±SE) stochiometric and environmental values at the north and south spatial study grids at three study sites in the Cairngorms NP (n = 168).

	Carn Bheadhair		Geal Charn		Meall an Lundain	
	North	South	North	South	North	South
TC (%)	27.80 ± 2.79	16.81 ± 1.59	10.53 ± 0.85	15.00 ± 1.73	12.55 ± 0.39	22.89 ± 2.08
TN (%)	0.80 ± 0.07	0.58 ± 0.05	0.38 ± 0.03	0.48 ± 0.06	0.47 ± 0.01	0.86 ± 0.07
TP (%)	0.05 ± 0.006	0.03 ± 0.003	0.01 ± 0.002	0.04 ± 0.004	0.04 ± 0.002	0.04 ± 0.002
TC:TN ratio	33.41 ± 0.82	29.00 ± 0.32	27.43 ± 0.40	31.38 ± 0.50	26.80 ± 0.28	26.35 ± 0.50
TC:TP ratio	753.15 ± 118.28	606.75 ± 42.45	330.70 ± 20.13	397.43 ± 33.62	313.07 ± 18.24	659.80 ± 70.22
TN:TP ratio	21.70 ± 3.06	20.94 ± 1.48	11.96 ± 0.66	12.71 ± 1.09	11.67 ± 0.67	24.74 ± 2.42
WEOC (mg C per g dry soil)	0.68 ± 0.08	0.33 ± 0.02	0.20 ± 0.04	0.24 ± 0.03	0.23 ± 0.02	0.38 ± 0.04
WEON (mg N per g dry soil)	0.022 ± 0.002	0.013 ± <0.001	0.006 ± 0.001	0.010 ± 0.001	0.006 ± <0.001	0.015 ± 0.002
WETP (mg P per g dry soil)	0.055 ± 0.006	0.020 ± 0.002	0.014 ± 0.002	0.024 ± 0.003	0.020 ± 0.001	0.038 ± 0.004
WEOC:WEON ratio	30.38 ± 0.85	25.15 ± 0.56	30.04 ± 0.88	24.70 ± 0.63	38.32 ± 1.06	26.13 ± 1.03
WEOC:WETP ratio	13.99 ± 1.71	19.39 ± 1.61	16.51 ± 3.36	10.85 ± 1.06	12.26 ± 1.10	10.71 ± 0.86
WEON:WETP ratio	0.46 ± 0.05	0.77 ± 0.06	0.55 ± 0.10	0.45 ± 0.05	0.32 ± 0.03	0.43 ± 0.04
TC:WEOC ratio	45.74 ± 3.33	49.09 ± 2.19	68.04 ± 4.49	65.58 ± 3.59	61.29 ± 3.64	66.81 ± 4.50
TN:WEON ratio	41.33 ± 3.01	42.23 ± 1.77	74.66 ± 5.60	51.15 ± 2.65	86.16 ± 4.71	66.71 ± 5.20
TP:WETP ratio	1.18 ± 0.20	2.09 ± 0.38	2.97 ± 0.35	1.92 ± 0.20	2.42 ± 0.22	1.25 ± 0.13
Gravimetric moisture content (%)	73.34 ± 3.36	62.18 ± 1.55	46.25 ± 2.64	52.98 ± 2.87	53.36 ± 1.71	68.45 ± 2.25
Slope steepness (degrees)	17.86 ± 1.41	12.46 ± 0.76	25.43 ± 1.72	28.43 ± 1.24	13.57 ± 0.90	10.18 ± 1.10
Soil depth (cm)	14.59 ± 1.82	11.77 ± 0.94	16.42 ± 1.25	12.36 ± 1.29	12.86 ± 1.34	15.24 ± 2.13

pH (water)	4.22 ± 0.03	4.30 ± 0.04	4.72 ± 0.04	4.62 ± 0.05	4.60 ± 0.03	4.44 ± 0.03
pH (CaCl)	2.88 ± 0.03	2.90 ± 0.01	3.40 ± 0.03	3.17 ± 0.04	3.32 ± 0.04	3.13 ± 0.05
Shrub cover (%)	71.88 ± 3.89	91.91 ± 3.91	69.51 ± 3.68	83.70 ± 3.86	77.88 ± 3.54	88.95 ± 2.30
Moss cover (%)	10.09 ± 2.98	0.45 ± 0.45	1.70 ± 0.49	0.03 ± 0.03	1.11 ± 0.52	1.81 ± 1.41
Lichen cover (%)	16.58 ± 3.73	0.89 ± 0.47	28.10 ± 3.62	14.45 ± 3.81	20.61 ± 3.59	6.39 ± 1.73
Rush cover (%)	0.28 ± 0.20	6.74 ± 3.94	0.06 ± 0.06	0.80 ± 0.51	0.00 ± 0.00	0.50 ± 0.38
Grass cover (%)	0.30 ± 0.18	0.02 ± 0.02	0.39 ± 0.21	0.00 ± 0.00	0.00 ± 0.00	0.90 ± 0.47
Sedge cover (%)	0.88 ± 0.24	0.00 ± 0.00	0.25 ± 0.16	0.00 ± 0.00	0.00 ± 0.00	0.99 ± 0.57

Table 2: Outputs of statistical comparisons showing the effect of aspect, site and aspect-site interaction on response variables. Mann-Whitney-Wilcoxon test output (W), Kruskall-Wallis test output (H), degrees of freedom (df) and probabilities (P, in bold when significant) are presented.

Response	Aspect		Site			Aspect x Site interaction		
	W	P	H	df	P	H	df	P
TC (%)	2988	0.087	26.57	2	**<0.001**	44.80	5	**<0.001**
TN (%)	3023	0.110	31.34	2	**<0.001**	47.45	5	**<0.001**
TP (%)	4176.5	**0.040**	7.32	2	**0.026**	17.75	5	**0.003**
TC:TN ratio	3336	0.544	49.30	2	**<0.001**	76.67	5	**<0.001**
TC:TP ratio	2241	**<0.001**	25.19	2	**<0.001**	52.99	5	**<0.001**
TN:TP ratio	2243	**<0.001**	24.61	2	**<0.001**	54.90	5	**<0.001**
WEOC (mg C per g dry soil)	3041	0.123	41.50	2	**<0.001**	52.35	5	**<0.001**
WEON (mg N per g dry soil)	2242	**<0.001**	41.16	2	**<0.001**	73.81	5	**<0.001**
WETP (mg P per g dry soil)	3227	0.341	17.92	2	**<0.001**	52.20	5	**<0.001**
WEOC:WEON ratio	6037	**<0.001**	14.84	2	**<0.001**	84.41	5	**<0.001**
WEOC:WETP ratio	3403	0.693	15.97	2	**<0.001**	31.16	5	**<0.001**
WEON:WETP ratio	2353	**<0.001**	24.04	2	**<0.001**	46.76	5	**<0.001**
TC:WEOC ratio	3289	0.449	31.54	2	**<0.001**	32.55	5	**<0.001**
TN:WEON ratio	4457	**0.003**	57.98	2	**<0.001**	72.41	5	**<0.001**
TP:WETP ratio	4294	**0.015**	21.20	2	**<0.001**	48.66	5	**<0.001**
TN:TC versus TP:TC ratio	2243	**<0.001**	24.61	2	**<0.001**	54.90	5	**<0.001**

TC (%) versus TN:TC ratio	2922	**0.045**	21.75	2	**<0.001**	43.30	5	**<0.001**
TC (%) versus TP:TC ratio	2512	**0.001**	27.71	2	**<0.001**	52.08	5	**<0.001**
WEON:WEOC versus WETP:WEOC ratio	2353	**<0.001**	24.02	2	**<0.001**	46.76	5	**<0.001**
WEOC (%) versus WEON:WEOC ratio	4003	0.132	22.56	2	**<0.001**	49.94	5	**<0.001**
WEOC (%) versus WETP:WEOC ratio	3041	0.123	41.50	2	**<0.001**	52.35	5	**<0.001**
Gravimetric moisture content (%)	3032	0.116	38.25	2	**<0.001**	57.88	5	**<0.001**
Slope steepness (degrees)	4082	0.079	84.89	2	**<0.001**	95.21	5	**<0.001**
Soil depth (cm)	3963	0.169	2.03	2	0.362	7.61	5	0.179
pH (water)	4002	0.133	70.47	2	**<0.001**	82.86	5	**<0.001**
pH (CaCl)	4476	**0.003**	78.22	2	**<0.001**	94.136	5	**<0.001**
Shrub cover (%)	1656	**<0.001**	6.01	2	0.050	48.03	5	**<0.001**
Moss cover (%)	4861	**<0.001**	7.20	2	**0.027**	50.15	5	**<0.001**
Lichen cover (%)	5336	**<0.001**	25.44	2	**<0.001**	61.48	5	**<0.001**

Table 3: Correlations of stochiometric response variables showing overall, aspect and site dependent relationships. Statistical outputs presented are Pearson correlation coefficient (r) and probabilities (P, in bold when significant).

Response	Overall		Aspect				Site					
			North		South		Carn Bheadhair		Geal Charn		Meall an Lundain	
	r	P	r	P	r	P	r	P	r	P	r	P
TC:TN ratio	0.961	**<0.001**	0.986	**<0.001**	0.972	**<0.001**	0.979	**<0.001**	0.984	**<0.001**	0.972	**<0.001**
TC:TP ratio	0.437	**<0.001**	0.494	**<0.001**	0.387	**<0.001**	0.513	**<0.001**	0.644	**<0.001**	0.049	0.722
TN:TP ratio	0.416	**<0.001**	0.511	**<0.001**	0.393	**<0.001**	0.479	**<0.001**	0.675	**<0.001**	0.106	0.438
WEOC:WEON ratio	0.956	**<0.001**	0.980	**<0.001**	0.949	**<0.001**	0.967	**<0.001**	0.959	**<0.001**	0.920	**<0.001**
WEOC:WETP ratio	0.769	**<0.001**	0.787	**<0.001**	0.725	**<0.001**	0.787	**<0.001**	0.524	**<0.001**	0.761	**<0.001**

WEON:WETP ratio	0.760	**<0.001**	0.784	**<0.001**	0.723	**<0.001**	0.746	**<0.001**	0.632	**<0.001**	0.782	**<0.001**
TC:WEOC ratio	0.822	**<0.001**	0.878	**<0.001**	0.801	**<0.001**	0.828	**<0.001**	0.773	**<0.001**	0.770	**<0.001**
TN:WEON ratio	0.773	**<0.001**	0.866	**<0.001**	0.739	**<0.001**	0.837	**<0.001**	0.837	**<0.001**	0.732	**<0.001**
TP:WETP ratio	0.403	**<0.001**	0.444	**<0.001**	0.332	**0.003**	0.481	**<0.001**	0.612	**<0.001**	-0.041	0.766
TN:TC versus TP:TC ratio	0.291	**<0.001**	0.685	**<0.001**	-0.103	0.349	0.292	**0.029**	0.389	**0.003**	0.139	0.307
TC (%) versus TN:TC ratio	-0.446	**<0.001**	-0.825	**<0.001**	0.060	0.586	-0.770	**<0.001**	-0.286	**0.032**	-0.198	0.114
TC (%) versus TP:TC ratio	-0.577	**<0.001**	-0.649	**<0.001**	-0.514	**<0.001**	-0.454	**<0.001**	-0.450	**0.001**	-0.724	**<0.001**
WEON:WEOC versus WETP:WEOC ratio	0.123	0.113	0.196	0.074	0.176	0.109	-0.109	0.423	0.218	0.106	0.305	**0.022**

WEOC (%) versus WEON:WEOC ratio	-0.056	0.471	-0.034	0.761	-0.024	0.832	-0.523	**<0.001**	0.110	0.419	0.408	**0.002**
WEOC (%) versus WETP:WEOC ratio	0.261	**0.001**	0.151	0.169	0.471	**<0.001**	0.468	**<0.001**	0.343	**0.010**	0.419	**0.001**

Table 4: Correlations of environmental measurements with stochiometric response variables showing overall, aspect and site dependent relationships. Statistical outputs presented are Pearson correlation coefficient (r) and probabilities (P, in bold when significant).

Response	Overall		Aspect				Site					
			North		South		Carn Bheadhair		Geal Charn		Meall an Lundain	
	r	P	r	P	r	P	r	P	r	P	r	P
Gravimetric moisture content versus TC	0.855	**<0.001**	0.869	**<0.001**	0.853	**<0.001**	0.897	**<0.001**	0.835	**<0.001**	0.815	**<0.001**
Gravimetric moisture content versus WEOC	0.826	**<0.001**	0.867	**<0.001**	0.833	**<0.001**	0.850	**<0.001**	0.801	**<0.001**	0.833	**<0.001**
Gravimetric moisture content versus TN	0.833	**<0.001**	0.855	**<0.001**	0.855	**<0.001**	0.882	**<0.001**	0.819	**<0.001**	0.789	**<0.001**
Gravimetric moisture content versus WEON	0.833	**<0.001**	0.850	**<0.001**	0.802	**<0.001**	0.847	**<0.001**	0.828	**<0.001**	0.813	**<0.001**
Gravimetric moisture content versus TP	0.348	**<0.001**	0.387	**<0.001**	0.341	**0.002**	0.393	**0.003**	0.614	**<0.001**	-0.084	0.539

Gravimetric moisture content versus WETP	0.765	**<0.001**	0.787	**<0.001**	0.763	**<0.001**	0.793	**<0.001**	0.707	**<0.001**	0.796	**<0.001**
Slope angle versus TC	-0.119	0.125	-0.037	0.740	-0.196	0.074	0.358	**0.007**	0.052	0.704	-0.238	0.077
Slope angle versus WEOC	-0.093	0.232	-0.034	0.761	-0.243	**0.026**	0.354	**0.007**	0.011	0.934	-0.170	0.211
Slope angle versus TN	-0.216	**0.005**	-0.086	0.435	-0.291	**0.007**	0.301	**0.024**	-0.018	0.894	-0.260	0.053
Slope angle versus WEON	-0.127	0.100	-0.031	0.776	-0.255	**0.019**	0.289	**0.030**	-0.021	0.878	-0.220	**0.103**
Slope angle versus TP	0.102	0.188	-0.001	0.999	0.190	0.084	0.428	**<0.001**	0.030	0.825	0.064	0.642
Slope angle versus WETP	-0.120	0.121	-0.109	0.324	-0.162	0.141	0.270	**0.044**	0.106	0.4371	-0.255	0.058
Soil depth versus TC	0.259	**0.001**	0.146	0.184	0.405	**<0.001**	0.400	**0.002**	-0.120	-0.380	0.462	**<0.001**

Soil depth versus WEOC	0.331	**<0.001**	0.245	**0.025**	0.524	**<0.001**	0.473	**<0.001**	-0.074	0.589	0.733	**<0.001**
Soil depth versus TN	0.247	**0.001**	0.106	0.338	0.391	**<0.001**	0.381	**0.004**	-0.076	**0.577**	0.392	**0.003**
Soil depth versus WEON	0.369	**<0.001**	0.237	**0.030**	0.611	**<0.001**	0.472	**<0.001**	-0.055	0.689	0.728	**<0.001**
Soil depth versus TP	-0.030	0.695	-0.229	**0.036**	0.178	0.105	-0.107	0.432	0.074	0.586	0.002	0.987
Soil depth versus WETP	0.323	**<0.001**	0.264	**0.015**	0.424	**<0.001**	0.517	**<0.001**	-0.122	0.372	0.483	**<0.001**
pH_{CaCl} versus TC	-0.543	**<0.001**	-0.759	**<0.001**	-0.213	0.052	-0.720	**<0.001**	-0.426	**0.001**	-0.427	**0.001**
pH_{CaCl} versus WEOC	-0.623	**<0.001**	-0.768	**<0.001**	-0.446	**<0.001**	-0.658	**<0.001**	-0.430	**0.001**	-0.619	**<0.001**
pH_{CaCl} versus TN	-0.425	**<0.001**	-0.712	**<0.001**	-0.094	0.393	-0.692	**<0.001**	-0.299	**0.025**	-0.313	**0.019**

pH_{CaCl} versus WEON	-0.635	**<0.001**	-0.775	**<0.001**	-0.365	**0.001**	-0.684	**<0.001**	-0.444	**0.001**	-0.554	**<0.001**
pH_{CaCl} versus TP	-0.085	0.273	-0.290	**0.008**	0.153	0.164	-0.322	**0.016**	-0.129	0.343	0.233	0.084
pH_{CaCl} versus WETP	-0.493	**<0.001**	-0.734	**<0.001**	-0.087	0.432	-0.565	**<0.001**	-0.456	**<0.001**	-0.426	**0.001**
Shrub cover % versus TC	-0.031	0.690	-0.048	0.665	-0.068	0.539	-0.289	**0.031**	0.032	0.817	0.219	0.105
Shrub cover % versus WEOC	-0.130	0.094	-0.148	0.179	-0.016	0.887	-0.322	**0.015**	-0.188	0.166	0.135	0.320
Shrub cover % versus TN	0.015	0.847	-0.046	0.675	-0.034	0.757	-0.271	**0.043**	0.023	0.864	0.240	0.075
Shrub cover % versus WEON	-0.057	0.461	-0.153	0.164	0.020	0.855	-0.284	**0.034**	-0.115	0.400	0.228	0.091
Shrub cover % versus TP	-0.022	0.779	0.064	0.562	-0.011	0.921	-0.134	0.327	0.092	0.501	0.060	0.661

Shrub cover % versus WETP	-0.027	0.732	-0.010	0.930	-0.002	0.988	-0.263	0.051	0.109	0.425	0.206	0.128
Lichen cover % versus TC	-0.166	**0.032**	-0.170	0.121	-0.134	0.226	0.098	0.473	-0.058	0.671	-0.323	**0.015**
Lichen cover % versus WEOC	-0.079	0.309	-0.102	0.357	-0.199	0.070	0.160	0.240	0.133	0.329	-0.240	0.075
Lichen cover % versus TN	-0.192	**0.013**	-0.157	0.155	-0.157	0.153	0.069	0.611	-0.048	0.724	-0.342	**0.010**
Lichen cover % versus WEON	-0.147	0.058	-0.100	0.364	-0.205	0.061	0.114	0.404	0.076	0.579	-0.334	**0.012**
Lichen cover % versus TP	0.021	0.792	-0.031	0.777	-0.043	0.696	0.176	0.194	-0.121	0.375	0.043	0.752
Lichen cover % versus WETP	-0.102	0.189	-0.190	0.084	-0.014	0.901	0.229	0.090	-0.127	0.352	-0.277	**0.039**

3.3 Soil Quality

Soil quality indicators derived from FTIR measurements were highly consistent between spatial study grids (Fig. S3). Aromaticity and Maturation showed a statistically significant aspect effect with higher values at northern sites, but values were at such a small scale that the effect was minimal (Table S1). UV-Vis indicators of WEOM quality also showed strong similarity across sites and aspects (Fig. S3, Table S1).

Aromaticity, Maturation and Polysaccharide were all negatively correlated with TC, with a stronger relationship between Aromaticity:TC and Maturation:TC on north-facing slopes (Table S1, S2). SUVA254 showed a negative correlation with WEOC, whereas Slope220-465 had a positive relationship with WEOC that was strongest on north-facing slopes (Table S1, S2).

3.4 Spatial Autocorrelation

Moran's I values of spatial autocorrelation for TC and TN were highly variable with no overall pattern and no aspect effect between north (r^2 = 0.01 and r^2 = 0.02, respectively) or south facing slopes (r^2 = 0.04 and r^2 = 0.01) for TC (Fig. 6a) or TN (Fig. 6b). TP concentration showed high variability and no aspect effect between north facing slopes (r^2 = 0.09) and south facing slopes (r^2 = <0.01; Fig. 6c).

WEOC content showed a tendency toward positive spatial autocorrelation on south facing slopes (r^2 = 0.21) compared to the north facing slope (r^2 = 0.06; Fig. 6d). WETN and WETP content showed high variability and no aspect effect between north (r^2 = 0.07 and r^2 = <0.01, respectively) and south facing slopes (r^2 = 0.07 and r^2 = 0.04; Fig. 6e-f).

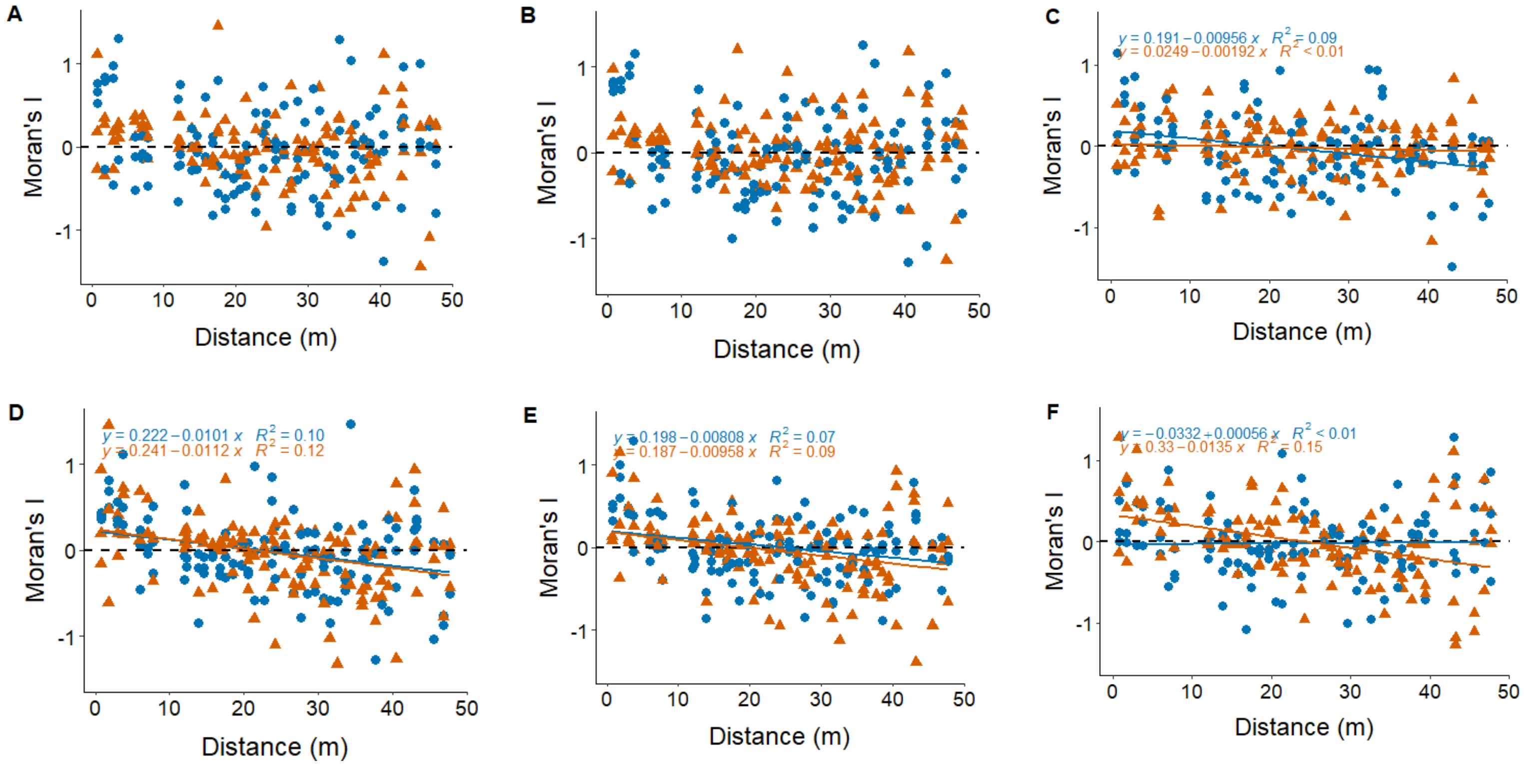

Figure 6: Moran's I value for spatial autocorrelation across distance between sampling points for a) TC (%), b) TN (%), c) TP (%), d) WEOC (mg C / g dry soil), e) WEON (mg N / g dry soil) and f) WETP (mg P / g dry soil). Samples from north- and south-facing slopes are shown in blue and red respectively.

3.5 Power Analysis

Given the huge variance that is seen in some elemental quantities and stochiometric relationships combined with the non-generalisability of aspect in a mountain system, it raises the question that ecological experiments look to measure changes in carbon accumulation may not take this background variance into account.

Using the standard deviation values from our entire data, you would need 11757 samples to detect a 0.4 % change in TC concentration. Standard deviation varied between survey grids, resulting in a variation of between 411 and 21366 samples needed to detect a 0.4 % change in TC showing that experimental design between study sites is highly varied and non-comparable. Our own design with n = 28 per spatial survey grid is consistently below the number of samples required to detect a 4 % change in TC effectively (Table 5). Detecting a 0.04% change in TC concentration requires between 41028 and 2136463 samples, an inordinate number which presents extensive challenges for experimental designs investigating SOC in alpine systems. Conversely, both TN and TP concentrations have lower standard deviation than TC, requiring fewer samples than collected in this study to accurately detect changes in elemental concentrations.

Table 5: Power analysis was conducted to calculate the number of samples (n) required to detect a change in mean elemental concentrations of 4%, 0.4% and 0.04% for Total Carbon, 0.4% and 0.04% for Total Nitrogen and 0.04% for Total Phosphorus with power = 0.8 and significance level = 0.05. Standard deviation (SD) was calculated from our data set and used to explore how differing levels of background variance alter experimental design requirements between aspects and sites.

	Total Carbon				Total Nitrogen			Total Phosphorus	
		n to detect:				n to detect:			n to detect:
	SD	4%	0.4%	0.04%	SD	0.4%	0.04%	SD	0.04%
All samples	10.95	119	11757	1175579	0.33	12	1085	0.02	5
North	11.79	137	13628	1362721	0.30	10	868	0.02	6
South	10.07	100	9947	994582	0.36	14	1273	0.02	4
CB	13.14	170	16928	1692753	0.35	13	1186	0.03	8
GC	7.48	56	5491	549013	0.24	7	571	0.02	4
ML	9.43	88	8731	873026	0.34	12	1136	0.01	3
CB-N	14.76	215	21366	2136463	0.38	15	1434	0.03	11
CB-S	8.43	71	6969	696841	0.27	8	738	0.02	4
GC-N	4.49	21	1978	197672	0.14	3	200	0.01	3
GC-S	9.14	83	8193	819207	0.30	10	911	0.02	5
ML-N	2.04	5	411	41028	0.08	2	58	0.01	2
ML-S	11.03	120	11936	1193528	0.39	16	1470	0.01	3

4. Discussion

Our project aimed to determine the impact of aspect on belowground function and stochiometric relationships in alpine soil in the Cairngorms NP, investigate the degree of semivariance in the system and identify additional abiotic and biotic factors explaining residual variance.

A key emergent factor from this study is that despite a general lack of microtopographic variation between and within the study sites, there was a massive amount of underlying variance in the system. This study's primary topographic focus was on aspect, yet the vastly different concentrations of SOM and WEOM measured were not explained by this dominant feature in the landscape. This is important as topographical approaches to estimating soil carbon stocks often categorise areas of spatial similarity into units presumed to have homogenous biogeochemical processes using factors such as relative height to surrounding features and slope gradient as terrain derivatives (Webster *et al*., 2011), or categorising environments based on dominant vegetation type (Morton *et al*., 2021). Previous studies in the Allt a'Mharcaidh categorised the environment based on plant community toposequences changing with altitude, yet recognised that within-group variation in range in carbon content in the alpine zone was considerably higher than the estimated global average soil C pools in arctic and alpine tundra (Britton *et al*., 2011).

Although this study found that key environmental measurements such as soil depth, pH and gravimetric water content were strongly correlated with specific SOM and WEOM concentrations, the high background variance in the system would make them unreliable predictors of carbon content if used in a modelling approach. In addition, selecting an appropriate unit cell size for estimating nutrient content within would be challenging as our spatial analysis has shown that between the scales of 0.5 m to 50 m there is no homogeneity in the system. Despite some studies recognising slope gradient as a control in SOC stock distribution (Conforti *et al*., 2016), and being regularly used as a topographic predictor in soil carbon content models (Obu *et al*., 2017; Tate *et al*., 2005), mean slope angle was only correlated with TN content with shallower slopes containing higher concentrations. Other studies have recognised slope curvature as an additional measurement of slope morphology that influences biogeochemical processes, although that was not measured in this study (Yoo *et al*., 2006). Recognising the huge underlying variance in soil nutrient content in areas of relatively homogeneous topography emphasises the importance of experimental design to accurately capture the variation in the belowground systems, as patterns may be overlooked due to inadequate sampling regimes.

Aspect showed a far less consistent effect on SOM and WEOM concentrations than other abiotic measurements. TC values were highly variable overall with range differing between sites and showing no evident aspect effect. This contrasts with previous studies which found aspect to be an important topographic factor affecting SOC distribution, attributed to aspect-induced variability in soil temperature and moisture (Garcia-Pausas *et al*., 2007; Zhu *et al*., 2017). However, this effect shows inconsistencies between studies and climatic regimes: Chen *et al* (2016) found that slope aspect alone explained 68.2% of SOC variation in an alpine environment, whereas Zhu *et al* (2018) found that slope aspect only accounted for 1.84% of SOC variability in a semi-arid alpine system with elevation and slope position having a much greater controlling effect on soil dynamics. Further research investigating how the strength of aspect effects on biogeochemical functioning in mountains is impacted by localised climate would be beneficial for understanding global patterns and potential impacts of climate change.

Whilst aspect failed to explain variance in TC, soil moisture was a dominant explanatory variable for all C data. Soil moisture has a key role in regulating SOC content by directly influencing soil respiration rates (Falloon *et al*., 2011), water availability for plant uptake, and plant-soil-microbial interactions (MatÍas *et al*., 2011), suggesting that detailed monitoring of localised precipitation levels and water retention quality of soil is an important factor when modelling SOC distribution. Soil moisture content was negatively correlated with slope angle. In addition, steeper slopes had lower TN content potentially indicating higher run off rates with increased N leaching compared to impeded water flow on shallower slopes (Weintraub *et al*., 2015). However, although slope angle offers an insight into some soil hydrological functioning at our sites it was not correlated with overall TC content. This could suggest that higher soil moisture content is a direct result of increased TC content rather than being a causative factor, due to SOM increasing the available water capacity of soil (Yost & Hartemink, 2019). This would imply that soil moisture content results from a combination of factors including SOM content and slope angle and is therefore not a useful proxy value for modelling TC content.

Stochiometric relationships showed that TP was a limiting factor at higher quantities of TC and TN. TP content of soil is strongly linked to underlying geology combined with atmospheric input of phosphorus through precipitation, local and long-distance dust deposition (Kopàček *et al*., 2011). In the Cairngorms NP the parent rock type is granite and schist, both of which contain intermediate amounts of phosphorus which may contribute to phosphorus-limited belowground systems (Britton & Fisher, 2007). Differences in underlying bedrock may account for some differences in P content between sites and contribute to high variability in TP and WETP content. Additionally, variation in TN:TP ratios may be due to differences in N deposition from atmospheric sources dependent on wind direction. TN:TC to TP:TC showed different patterns dependent on aspect. On the north slope there was a positive relationship consistent with the patterns identified by Tipping *et al*. (2016). However, the south slope showed high variability with no consistent pattern, showing a decoupling of TP content from TN availability.

Soil quality indicators showed that SOM quality was consistent between aspects with minimal variation, indicating that there was no considerable difference in the chemical nature or molecular accumulation of SOM (Mao et al., 2008). This is intriguing as despite varying stochiometric ratios between samples, FTIR analysis displays relatively similar SOM quality profiles. This is consistent with findings elsewhere (Egli *et al*., 2009) where soil quality remained the same between aspects whereas soil fractions and stochiometric relationships varied. However, the high amount on background variance at these sites raises the possibility that FTIR analysis is not sensitive enough to detect changes in SOM quality. Elsewhere, FTIR analysis has failed to observe changes in SOM humic fractions that were detected by complementary absorption spectroscopy analytical techniques such as nuclear magnetic resonance (NMR) spectroscopy (Mao *et al*., 2008). Whilst FTIR can quantitatively determine concentrations of molecules within a sample and identify presence and absence of functional groups, NMR can determine chemical structure of molecules potentially giving a greater insight into the organic structure (Gundlach *et al*., 2017). Where possible, utilising a range of spectroscopic techniques for investigating SOM quality would present a more complete picture of belowground function, benefiting from lower costs per sample, growing spectral libraries of soil analytical data, and robust comparisons between research projects (Nocita *et al*., 2015).

In terms of mobile organic matter, WEON was the only measured water extractable element showing an aspect effect between our sites. South facing slopes had higher WEON content as well as greater variability in amount present, compared to lower values with less variation on north facing slopes. DOM is the fraction thought to be most readily available for microbial uptake (Marschner & Kalbitz, 2003) as well as being a potential product of microbial functions. The formation of dissolved organic nitrogen (DON) within soil is particularly complex with many potential sources, including as a waste product from microbial degradation of SOM and as the result of enzymatic release by microbes to make N sources bioavailable (McDowell, 2003). Further investigation of soil microbial communities would give us an insight into whether there is an aspect-associated difference in microbial function impacting belowground processes at these montane sites. Aspect is recognised as having a greater impact on bacterial community composition than other topographical features such as elevation (Wu *et al*., 2017). Lower microorganism content and reduced microbiological enzymatic activity have been observed in alpine soil with colder aspect-induced microclimates (Sidari *et al*., 2008). This limited microbial biomass could account for lower levels of WEON as a consequence of suppressed microbiological activity. Alternatively, the suppressed decomposition rate of SOM in colder soils could lead to limited N availability. To cope in N-limited soil environments, microbial decomposer communities can regulate their nitrogen use efficiency and retain the immobilised organic N (Mooshammer *et al*., 2014) leading to reduced amounts of bioavailable WEON in north facing slope soils.

Furthermore, WEON content was negatively correlated with pH with a pronounced relationship on north facing slopes. This corresponded strongly with aromaticity measurements which indicated higher phenolic concentrations in TC-limited soil where samples also had low WEON content. Higher phenolic concentrations decrease N mineralization and enhance N limitation to microorganisms (Min *et al*., 2015). Additionally, the acidic conditions associated with high phenol content suppresses microbial growth. Although samples from southern facing slopes were overall slightly more acidic, the stronger negative relationship between WEON content and increased acidity on north facing slopes could be due to the additional stress of low pH on microbial communities already subject to stress from colder temperatures.

N is the major limiting nutrient for plant growth, and as DON is both a large potential N source for plants and highly susceptible to leaching from soil, this could influence the different vegetation communities found between aspects at our sites (Jones & Willett, 2006). South-facing slopes were dominated by shrub vegetation, compared to higher levels of cryptogam coverage on north-facing slopes. Shrub communities were predominantly composed of Ericaceae including *C. vulgaris, E. nigrum* and *V. vitis-idaea*. *V. vitis-idaea* has been found to increase the acidity of alpine lichen heath associated soils in mountain-meadow systems as leaf litter from ericaceous shrubs has a high phenol content (Adamczyk *et al*., 2016; Makarov *et al*., 2019). Ericaceae are characterised by their symbiosis with ericoid mycorrhizal fungi which through targeted enzymatic activity are capable of degrading highly organic recalcitrant matter, providing access to organic N and allowing ericaceous shrubs to dominate in acidic soil conditions (Adamczyk *et al*., 2016; Cairney & Burke, 1998; Tybirk *et al*., 2000). This could lead to increased decomposition rates of SOM on south facing slopes compared to northern slopes where cryptogam species dominate, potentially sustaining the pattern of lower WEON availability and perpetuating aspect-associated patterns in belowground function and above-ground vegetation communities. Additionally, soil moisture is an important driver of vegetation community structure, yet as there was no difference in gravimetric moisture content between north and south aspects this does not account for the lower levels of vascular vegetation on north facing slopes. However, differences in general levels of insolation with higher incoming solar irradiance on south facing slopes may be a key contributor to increased shrub abundance driven by longer growing seasons, although further measurements of annual soil temperatures would be needed to confirm this (Bürli *et al*., 2021).

Spatial autocorrelation showed high variability with a non-generalisable distribution of values. When looking for patterns of semivariance there might generally be seen a cluster of similar values closest together subsequently increasing in variance with expanding distance. The lack of pattern and huge variation in values found at our sites for all stochiometric measurements suggests that the scale this study was working at was either not fine enough to capture the micro-scale patterns in distribution, or not on a large enough scale to show semivariance across the functional landscape. This study worked on a scale ranging between 0.5 m to 50 m distance between samples illustrating a macroscale distribution of values. Future work investigating whether patterns of semivariance are present at smaller values less than 0.5 m or greater than 50 m would help contribute to our understand of belowground function in complex alpine environments. Our post-hoc power analysis showed that the huge background variance in our systems would require a vast number of sample replicates to definitively demonstrate meaningful differences between experimental treatments. Although the figure of 11,757 samples required to detect 0.4% change in SOC concentration was presented as a hypothetical thought experiment which would be unfeasible to actively sample, it presents interesting implications for the design of future studies. To avoid overlooking significant differences by committing Type II errors based on insufficient replication, considered experimental design is needed to accurately reflect the values present in a sampling regime which combines realistic sampling effort with a representative number of samples. This is of particular importance for climate change studies which frequently look to detect small changes, and in studies which sample across subsurface horizons across which variability in C content is likely to vary (Kravchenko & Robertson, 2011). This study has shown huge variation in nutrient content within the same soil type and depth, indicating that introducing further variation in sampling location, for example between different mountain regions, would require sampling effort on an even greater magnitude to detect meaningful results.

The high variability in our measured TC content is not represented by existing land cover maps. One widely used map, the Topsoil Organic Carbon map of Scotland (Lilly *et al*., 2012), consistently underestimates C content at these study sites. For example, at ML-S, it estimates a TOC content of 8.27% compared to our mean of 22.89% with a maximum measured value of 45.80%. Errors in modelling upland C content are compounded by maps such as the UKCEH Land Cover Map 2020 (Morton *et al*., 2021) which categorises the majority of the Cairngorms NP as inland rock and heather, overlooking the important cryptogam communities and varied topography which contributes to the highly complex belowground function. It is important to accurately represent the SOC content and biodiversity of mountain environments to ensure they are recognised for their carbon storage potential and receive adequate protection in management regimes.

Further investigation of abiotic and biotic factors at montane sites would improve the accuracy of SOC stocks modelling in Scotland. Details of soil temperature differences between aspects at our sites would help us to understand how mean differences and fluctuation ranges vary between north and south. As our sites were at 700 m asl compared to the higher altitudes in other studies (e.g. 1270 m asl (Sidari *et al*., 2008), 2147 to 4606 m asl (Zhu *et al*., 2018)), differences in temperature extremes may be possible. However, although the altitude of summits in the Cairngorms NP is lower, the combination of latitude and proximity to the ocean results in an oceanic climate with severe wind exposure, low temperatures and persistent snow coverage in winter months (Fryday, 2009) resulting in the most southerly outpost of arctic habitat in Europe (Crabtree & Bayfield, 1998). Incoming wind direction can regulate soil temperature and moisture, with wind stress capable of desiccating soil, limiting plant recruitment and eroding substrate (Momberg *et al*., 2021). Wind speed loggers are difficult to utilise in mountain regions where speeds can reach upwards of 100 mph, but better understanding of near-ground conditions across microhabitats would improve modelling accuracy. As wind speed controls vegetation communities through direct (e.g. destruction of canopy) and indirect (e.g. redistribution of snow cover) methods, it has a key role in maintaining the distinctive vegetation communities found at these study sites including areas of ground-layer lichen cover, wind-clipped heath and vascular plant communities (Crabtree & Ellis, 2010). Contrasting wind exposure between north and south sites could potentially accentuate differences in vegetation community structure between aspects. An additional useful metric for understanding biotic variation between sites would be the abundance of herbivore populations monitored through line transect faecal surveys or camera trapping campaigns, as the seasonal habitat selection shown by alpine populations of red deer (*Cervus elaphus*) can influence succession in vegetation communities (Schütz *et al*., 2006; Zweifel-Schielly *et al*., 2009). Grazing pressure at our study sites is sustained at a level of low impact through active deer management, indicated by successful pine regeneration (Bayfield & Nolan, 2008; Rao, 2017). The limited historical record available means that the legacy of Anthropogenic land use within the Cairngorms NP on patterns of soil variability cannot precisely be calculated. Previous studies have recognised the potential impact of large herbivore presence on soil nutrient cycling (Mohr *et al*., 2005; Nakayama *et al*., 2021) However, as *C. elaphus* have been shown to preferentially use pine forest over open habitat, the impact of herbivores at our study sites is unlikely to be a controlling factor in belowground variation (Palmer *et al*., 2007).

The Cairngorms NP is important for its biodiversity, ecosystem services, and recreational value for people (Dick *et al*., 2016). The growing abundance of ecological restoration projects within the NP, such as the Cairngorms Connect project, present an opportunity to gather valuable evidence on landscape-scale processes, increase carbon storage potential, and monitor the impact of environmental factors over the long-term (Jones & Comfort, 2020). Collaboration with land managers, rewilding organisations and restoration projects could allow us to improve our knowledge of belowground function across the region, implement land management policies designed to maximise soil health, and ensure future resilience in a changing climate.

5. Conclusion

This study found high background variance in nutrient content in alpine soils in the Cairngorms NP. Aspect had a limited effect with south facing slopes containing higher WEON concentrations. Alternative abiotic and biotic factors including gravimetric moisture content, pH and soil depth correlated strongly with measured SOM and WEOM content. TN was correlated with slope angle, with highest concentrations on shallower slopes. Vegetation communities differed with aspect with ground-layer lichen more present on northern slopes and ericaceous shrubs dominating on southern slopes. SOM and WEOM quality were similar between sites and further analytical techniques such as NMR spectroscopy may be more effective for detecting variation in future studies. Controls over SOM accumulation remain relatively cryptic here and further research into soil-plant interactions and the legacy effect of antecedent conditions is needed to better understand driving factors in these montane environments. Further investigation of the role of wind exposure on soil and vegetation dynamics would increase our understanding of driving factors behind spatial variation. Lack of spatial autocorrelation and high variability have important implications for future research with post-hoc power analysis showing that a huge number of samples is required to accurately capture changes in elemental concentrations in alpine soils. The main recommendation is that monitoring schemes should operate at scales suitable to detect the high variation shown in this study, which existing natural resource studies like the Countryside Survey currently does not (Emmett *et al*., 2010). Better understanding of the belowground functions in the Cairngorms NP will allow more accurate carbon stock maps to be developed to accurately reflect the diversity and carbon storage potential in this area and develop accurate predictions for future impacts in the face of a changing climate. With the increase of ecological restoration projects within the NP committed to a vision of enhancing natural processes, there are opportunities for collaborative research to increase our knowledge of belowground function, projected future changes, and implement measures to improve the SOC quantity and quality within the NP. This presents a valuable chance to build landscape-scale resilience within an alpine environment in a changing climate.

6. References

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7. Supplementary Material

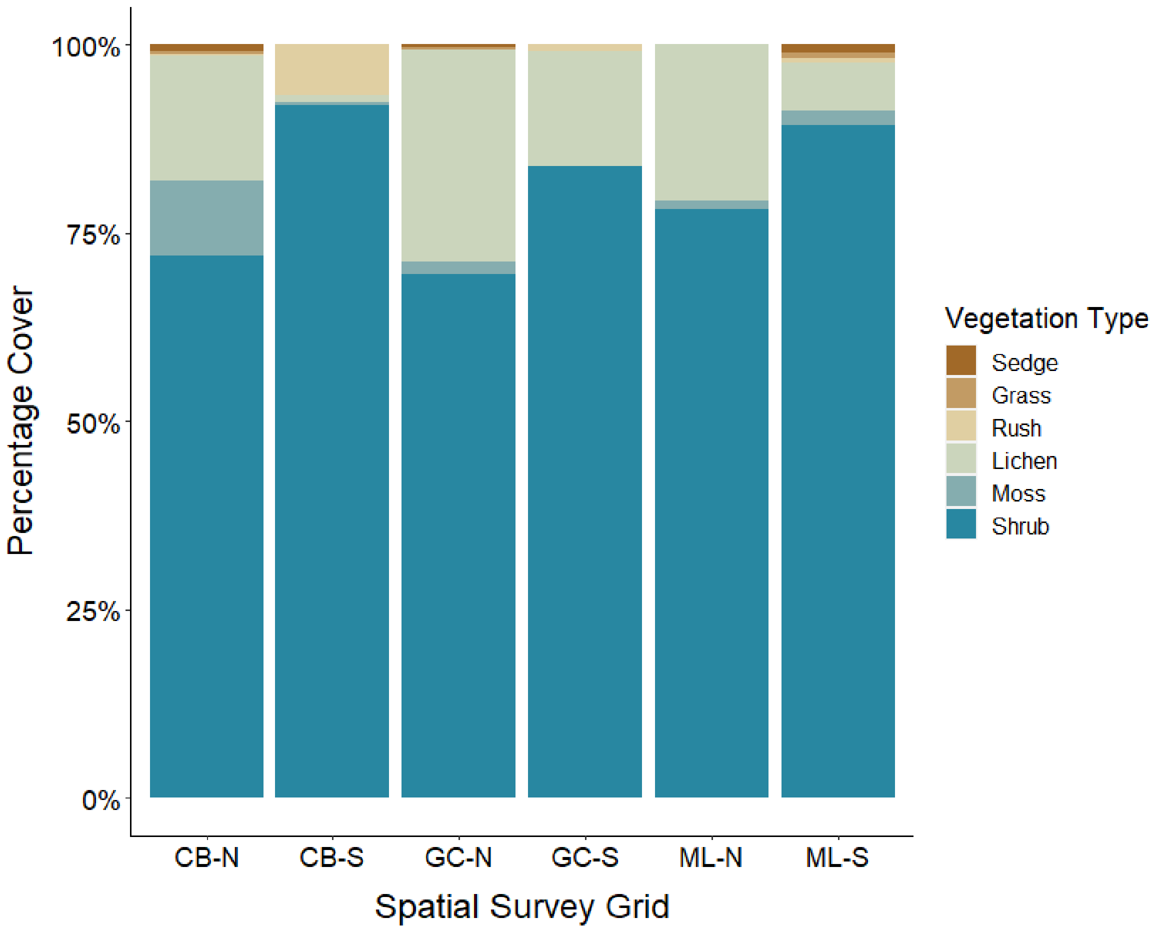

Figure S1: Vegetation coverage in percentage at each study grid (n = 168), showing functional plant types of sedge, grass, rush, lichen, moss and shrub.

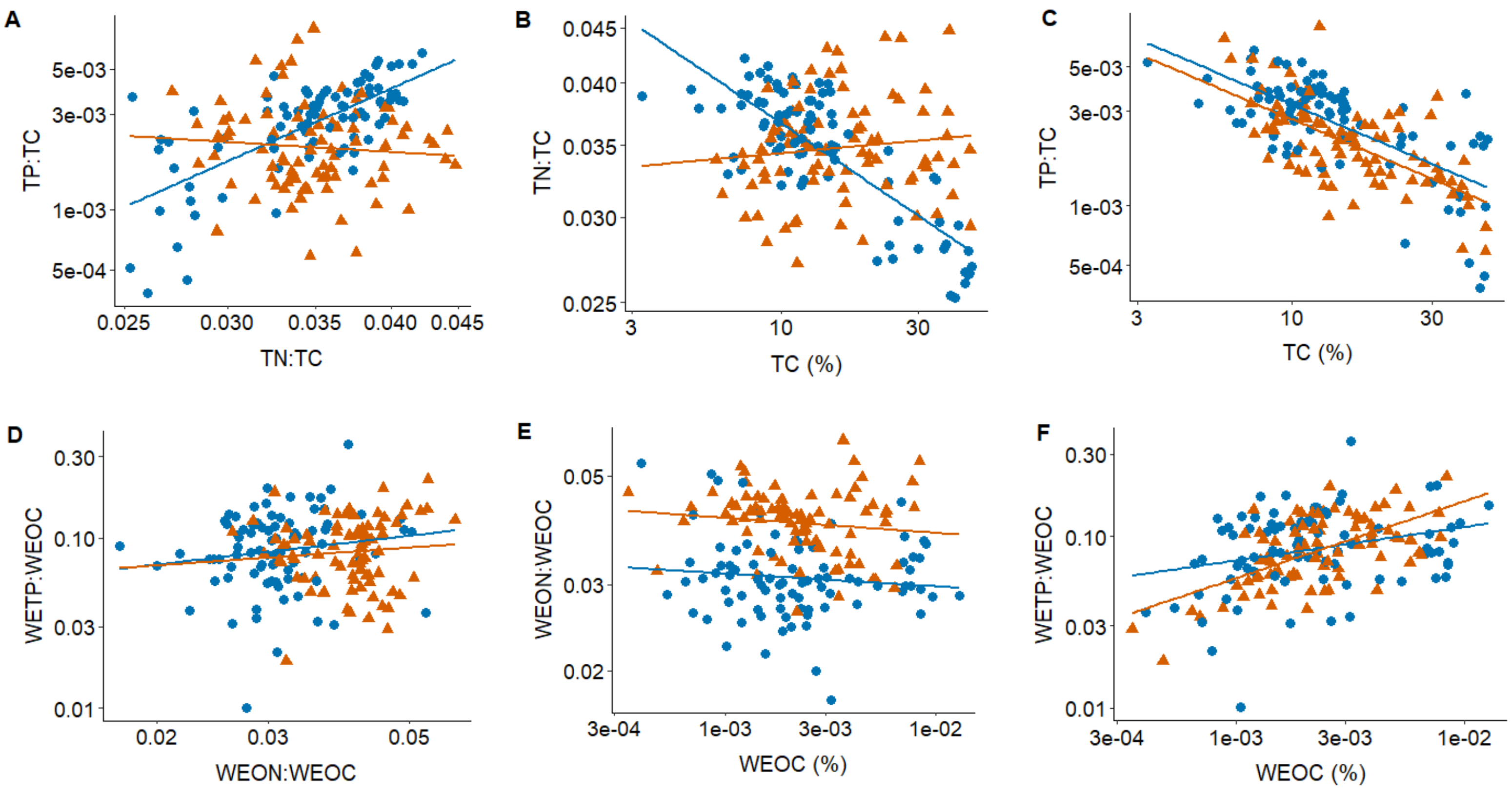

Figure S2: Stochiometric relationships showing a) TN:TC to TP:TC ratio, b) TC to TN:TC, c) TC to TP:TC, d) WEON:WEOC to WETP:WEOC ratio, e) WEOC to WEON:WEOC ratio, and f) WEOC to WETP:WEOC ratio for all samples (n = 168). Samples from north- and south-facing slopes are shown in blue and red respectively.

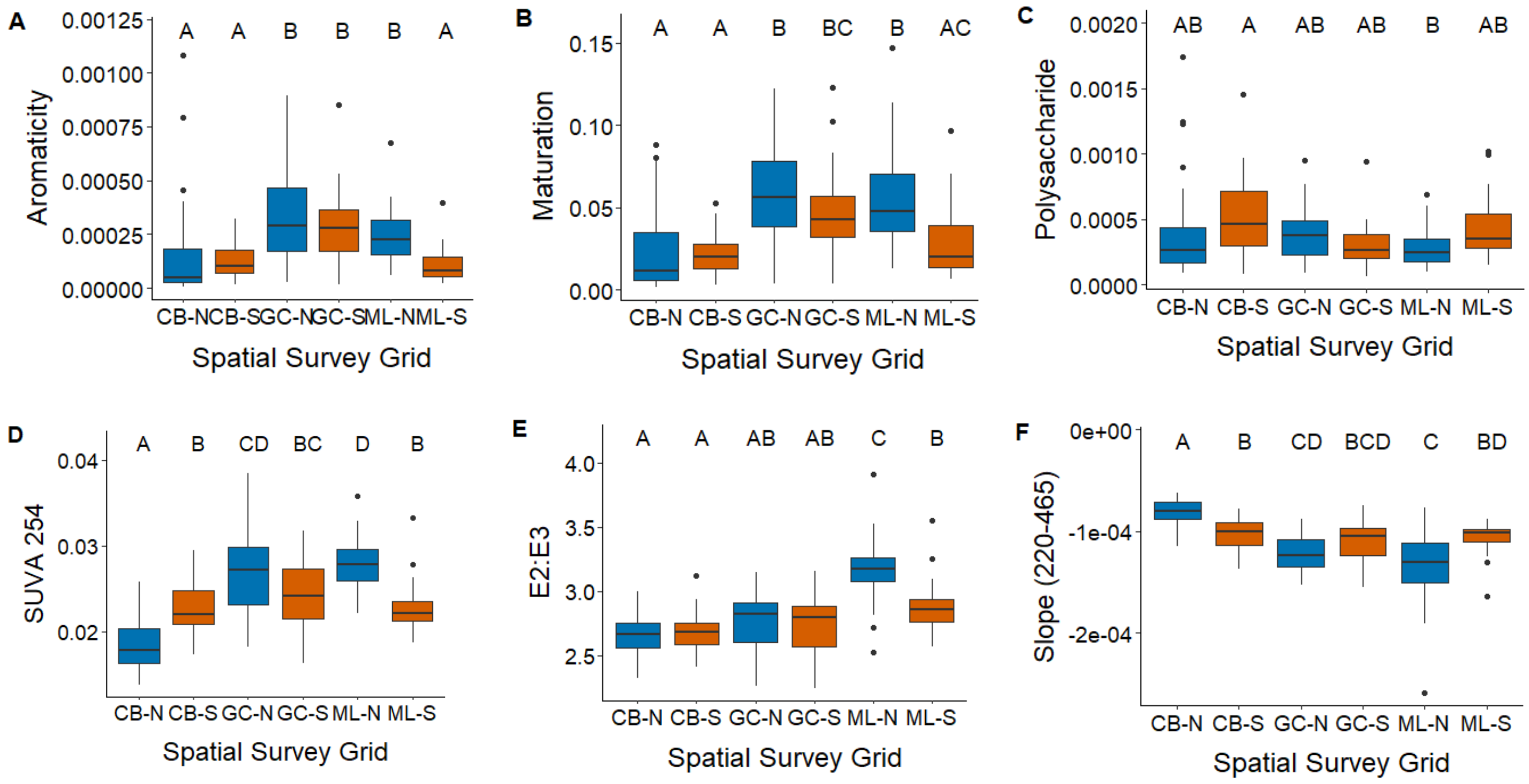

Figure S3: Soil quality measurements showing A) Aromaticity, B) Maturation, C) Polysaccharide, D) SUVA 254, E) E2/E3 ratio, and F) Slope (220-465) for all samples (n = 168). Variables with different letters are significantly different at the < 0.05 level (Table S1). Samples from north- and south-facing slopes are shown in blue and red respectively.

Table S1: Outputs of statistical comparisons of the effect of aspect, site and aspect-site interaction on soil quality indicators showing Mann-Whitney-Wilcoxon test output (W), Kruskall-Wallis test output (H), degrees of freedom (df) and probabilities (P, in bold when significant).

Response	Aspect		Site			Aspect x Site interaction		
	W	P	H	df	P	H	df	P
Aromaticity	4168	**0.042**	35.60	2	**<0.001**	51.29	5	**<0.001**
Maturation	4327	**0.011**	38.01	2	**<0.001**	52.50	5	**<0.001**
Hydrophobicity	3730	0.523	7.90	2	**0.019**	16.76	5	**0.005**
Condensation	3820	0.355	13.77	2	**0.001**	19.58	5	**0.002**
Polysaccharide	2861	**0.034**	1.29	2	0.524	16.36	5	**0.006**
SUVA254	4000	0.135	41.09	2	**<0.001**	77.80	5	**<0.001**
E2:E3 ratio	4083	0.079	56.36	2	**<0.001**	65.61	5	**<0.001**
E4:E6 ratio	3377	0.633	11.25	2	**0.004**	19.34	5	**0.002**
Slope (220-465)	3151	0.232	40.06	2	**<0.001**	69.16	5	**<0.001**
Aromaticity : TC	4184	**0.038**	33.07	2	**<0.001**	51.52	5	**<0.001**
Maturation : TC	4342	**0.010**	35.11	2	**<0.001**	53.44	5	**<0.001**
Polysaccharide : TC	3381	0.642	4.63	2	0.099	15.43	5	**0.009**
SUVA254 : WEOC	4105	0.068	49.78	2	**<0.001**	67.84	5	**<0.001**
E2:E3 : WEOC	4176	**0.040**	45.78	2	**<0.001**	60.24	5	**<0.001**
E2:E3 : Aromaticity	3006	0.098	35.95	2	**<0.001**	48.77	5	**<0.001**

Table S2: Stochiometric - quality relations showing correlations of response variables overall and between aspects and sites. Statistical outputs shown are Pearson correlation coefficient (r) and probabilities (P, in bold when significant).

Response	Overall		Aspect				Site					
			North		South		Carn Bheadhair		Geal Charn		Meall an Lundain	
	r	P	r	P	r	P	r	P	r	P	r	P
Aromaticity : TC	-0.560	**<0.001**	-0.629	**<0.001**	-0.564	**<0.001**	-0.602	**<0.001**	-0.520	**<0.001**	-0.548	**<0.001**
Maturation : TC	-0.558	**<0.001**	-0.658	**<0.001**	-0.408	**<0.001**	-0.661	**<0.001**	-0.351	**0.008**	-0.490	**<0.001**
Polysaccharide : TC	-0.481	**<0.001**	-0.454	**<0.001**	-0.539	**<0.001**	-0.749	**<0.001**	-0.545	**<0.001**	-0.275	0.040
SUVA254 : WEOC	-0.549	**<0.001**	-0.628	**<0.001**	-0.377	**<0.001**	-0.357	**0.007**	-0.574	**<0.001**	-0.524	**<0.001**
E2:E3 : WEOC	-0.011	0.885	-0.099	**0.368**	0.174	0.112	0.275	**0.040**	0.265	**0.048**	-0.067	0.625
Slope(220-465) : WEOC	0.377	**<0.001**	0.427	**<0.001**	0.277	**0.011**	0.262	0.051	0.498	**<0.001**	0.172	0.205
E2:E3 : Aromaticity	-0.057	0.465	-0.125	0.259	-0.025	0.825	-0.381	**0.004**	-0.002	0.987	0.228	0.091