



COMMISSIONED REPORT

Commissioned Report No. TT1508

APPLYING SOIL INDICATORS AT BIOMONITORING SITES

For further information on this report please contact

Claudia Erber
SEPA Corporate Office
Strathallan House
Castle Business Park
STIRLING
FK9 4TZ
Telephone: 01786-457700
E-mail: Claudia.Erber@sepa.org.uk

This report should be quoted as:

Mitchell, R.J., Beesley, L., Donald, C., Green, G. Hewison, R.L., Owen, I.J., Newman, G., Sturgeon, F., White, D., Williams, E, Black, H.I.J. (2016). Applying soil indicators at biomonitoring sites

Scottish Environment Protection Agency Commissioned Report No. TT1508.

This report, or any part of it, should not be reproduced without the permission of Scottish Environment Protection Agency. This permission will not be withheld unreasonably. The views expressed by the author(s) of this report should not be taken as the views and policies of Scottish Environment Protection Agency.



COMMISSIONED REPORT

Summary

APPLYING SOIL INDICATORS AT BIOMONITORING SITES

Commissioned Report No. TT1508

Contractor: Mitchell, R.J., Beesley, L., Donald, C., Green, G. Hewison, R.L., Owen, I.J., Newman, G., Sturgeon, F., White, D., Williams, E, Black, H.I.J.

Year of publication: [2016]

Background

UK nature conservation agencies are required to assess and report on the condition of designated features on protected sites, such as SSSIs and SACs. In Scotland, the Site Condition Monitoring (SCM) programme managed by Scottish Natural Heritage (SNH) monitors the status of designated features using the Common standards monitoring (CSM) guidance developed by the Joint Nature Conservation Committee and the UK conservation agencies. However these guidelines do not assess the impact of air pollution. SEPA is keen to improve their monitoring and assessment of air pollution impacts on the soil and this project is the third phase in a process of developing suitable soil indicators for assessing the impact of N deposition.

This project tested the three most promising soil indicators identified by previous work (soil pH measured in CaCl_2 , phosphomonoesterase and base cation/Al ratio) at 11 SEPA biomonitoring sites to establish links between the soil indicators, vegetation indicators (species richness, Shannon diversity index, cover weighted Ellenberg N and cover weighted Ellenberg R), climate (average maximum and minimum daily temperature and average annual rainfall) and present (total N, NH_3 , NO_x , SO_2) and cumulative (NO_x , NH_y , SO_x) pollution.

Main findings

- Soil pH measured in CaCl_2 was significantly affected by total N deposition, and NH_3 deposition. When climatic differences between the sites were taken into account only the relationship between soil pH and NH_3 remained significant. However this relationship was due to two sites with higher pH which may have been influenced by the underlying geology.
- Phosphomonoesterase was significantly affected by cumulative NH_y but once climatic differences between the sites were taken into account the relationship was no-longer significant.

- Phosphomonoesterase was significantly affected by average maximum daily temperature and soil pH was significantly affected by both average maximum and minimum daily temperatures.
- Cover weighted Ellenberg R was the only vegetation indicator that was significantly affected by N pollution: being correlated with current NO_x deposition levels. However this relationship was solely due to the results from one site (West Fannyside Moss).
- The soil and vegetation indices were related to each other: Species richness was negatively correlated with increasing phosphomonoesterase and base cation/Al ratio; however this relationship was driven by a few outlying data points and the relationship was not significant when these points were removed from the analysis. Increasing pH was positively correlated with cover weighted Ellenberg R scores, an expected relationship given that high Ellenberg R scores indicate species niche requirements for growing in less acidic conditions.
- When the three soil indicators were analysed together in a multivariate analysis (essentially treating them as one combined indicator) current NH₃ levels, cumulative SO_x and average maximum daily temperature were significant in explaining the variation in the indicators.
- Using vegetation composition data from the sites current NO_x, average maximum daily temperature and average rainfall were all significant in explaining the variation in the composition.
- Variation partitioning showed that most of the variation in the soil indicators was jointly explained by vegetation, climate and pollution, thus it is very hard to pick out a significant impact of N pollution alone and be sure it is due to N pollution and not due to differences in climate or vegetation.
- The soil indicators performed no worse than the vegetation indicators in terms of acting as assessment of N impacts. Of the four vegetation indicators studied, only one, cover weighted Ellenberg R, was significantly affected by N deposition (NO₂), and only one of the soil indicators, pH, was significant once climate had been taken into account.
- Possible reasons the soil indicators were not as successful in indicating N pollution as they had been in previous studies are:
 - The N gradient across the sites assessed was too short
 - Previous work was done at one site so variations in climate and geology were not relevant in this context.
 - Two sites had very deep sphagnum cover resulting in humified peat for sampling not being reached until below the water table. This resulted in the samples being taken from water logged, anaerobic, conditions which will have influenced biological measurements such as phosphomonoesterase.
 - Differences between sites in underlying geology were not taken into account but may have influenced the results.
 - The sites had previously had high historical pollution (sulphur and nitrogen), these systems will therefore have already changed making it hard to pick up differences due to current background pollution levels. Changes due to a point source of pollution can however be observed against background high historical pollution levels.
- These soil indicators were originally tested for the impact of point source pollution over one site. This study is the first time they have been tested against diffuse pollution over a range of sites and the results suggest that these soil indicators are not suitable for multi-site monitoring where there are large differences in climate and relatively small differences in N deposition combined with a significant background of historical atmospheric N and S deposition.

Acknowledgements

We thank the land owners for access to their land.

Table of Contents		Page
1	INTRODUCTION	1
1.1	Objectives	1
1.2	Questions to be answered	2
2	SITES	3
3	SOIL SAMPLING	7
3.1	Soil sample preparation	8
3.2	Soil analysis and quality control	9
3.3	Collation of environmental data	10
3.4	Vegetation data	13
3.5	Data analysis	13
3.5.1	Soil or vegetation indicators v pollution or climate data.	13
3.5.2	Multivariate analysis of soil indicators and vegetation % cover data	13
4	RESULTS	16
4.1	Variation between sites as noted by the soil surveyors	16
4.2	Variation in soil indicator results	16
4.3	Variation in vegetation indicators	19
4.4	Soil indicators v current pollution levels	22
4.5	Soil indicators v cumulative pollution levels	22
4.6	Soil indicators v climate	22
4.7	Multivariate analysis of soil indicators	26
4.8	Vegetation indicators v current pollution, cumulative pollution and climate	29
4.9	Multivariate analysis of the vegetation	33
4.10	Soil indicators v vegetation indicators	38
4.11	Explaining the variation in soil indicators	40
5	DISCUSSION	41
5.1	Do the soil indicators show a response to N-deposition?	41
5.2	Is the response to N-deposition in accordance with the findings from Whim Moss (see project phase II)? If not what might be the reasons/factors?	41
5.3	Is there a relationship between the vegetation indicators (e.g. species composition, cover-weighted Ellenberg N) and the soil indicators?	43
5.4	How can the soil indicators be used for the interpretation of site impacts from N deposition?	43
5.5	Could the soil indicators potentially be used as an “early warning system”?	43
5.6	What benefits would a repetition of the soil monitoring provide? What time scales would be reasonable?	43
5.7	Next steps	43
6	CONCLUSION	44
7	REFERENCES	45
8	APPENDIX 1: SOIL INDICATOR DATA	47
9	APPENDIX 2: GRID REFERENCES	52

Figure 1 Location of sampling sites. See Table 1 for sites codes	3
Figure 2 Position of soil samples relative to the quadrat	7
Figure 3 Picture of box corer used for sampling	8
Figure 4 Preparation of the soil samples prior to analysis	9
Figure 5 pH results. Each circle is the result from one quadrat with the mean shown by a cross.	17
Figure 6 Phosphomonoesterase results. Each circle indicates the results from one quadrat with the mean shown by a cross.	18
Figure 7 Base cation/Al ratio results. Each circle represents the results from one quadrat with the mean shown by a cross.	18
Figure 8 Species richness results. Each circle represents the results from one quadrat with the mean shown by a cross.	20
Figure 9 Shannon diversity results. Each circle represents the results from one quadrat with the mean shown by a cross.	20
Figure 10 Cover weighted Ellenberg N results. Each circle represents the results from one quadrat with the mean shown by a cross.	21
Figure 11 Cover weighted Ellenberg R results. Each circle represents the results from one quadrat with the mean shown by a cross.	21
Figure 12 Relationship between 3 soil indicators and current pollution levels. Graph titles in bold and underlined indicate a significant relationship	23
Figure 13 Relationship between 3 soil indicators and cumulative pollution levels. Graph titles in bold and underlined indicate a significant relationship.	24
Figure 14 Relationship between 3 soil indicators and climate. Graph titles in bold and underlined indicate a significant relationship.	25
Figure 15 Ordination diagram from PCA of soil indicators.....	26
Figure 16 Ordination diagram from PCA of soil indicators without samples CM4, AM2, AM5, SG1....	27
Figure 17 Correlation between pH and phosphomonoesterase	27
Figure 18 Ordination diagram from RDA of soil indicators with current and cumulative pollution variables as explanatory variables, only significant variables included.....	28
Figure 19 Ordination diagram from RDA of soil indicators with climatic variables as explanatory variables, only significant variables included. Note as only one climatic variable was significant the second axis is unconstrained (not correlated with any climatic variables)	29
Figure 20 Relationship between 4 vegetation indicators and current pollution levels. Graph titles in bold and underlined indicate a significant relationship.	30
Figure 21 Relationship between 4 vegetation indicators and cumulative pollution levels. None of the relationships were significant.	31
Figure 22 Relationship between 4 vegetation indicators and climate. None of the relationships were significant.	32
Figure 23 Ordination of samples from DCA of vegetation	33
Figure 24 Ordination of species from DCA of vegetation. (See Table 5 for species codes). Only those species with a weight of 10% or more are included in the diagram.	34
Figure 25 Ordination of samples from CCA of vegetation with current and cumulative levels of pollution as explanatory variables. Only those variables that were significant are included. Note as only one pollution variable was significant the second axis is unconstrained (not correlated with any climatic variables).....	36
Figure 26 Ordination of species from CCA of vegetation (See Table 5 for species codes). Only those species with a weight of 10% or more are included in the diagram.	36
Figure 27 Ordination of samples from CCA of vegetation with climate as explanatory variables. Only those variables that were significant are included.	37
Figure 28 Ordination of species from CCA of vegetation (See Table 5 for species codes). Only those species with a weight of 10% or more are included in the diagram.	38
Figure 29 Relationship between 4 vegetation indicators and 3 soil indicators. Graph titles in bold and underline indicate significant relationships, but note that some of these relationships were driven by a few quadrats – see text.	39
Figure 30 Partitioning the variation in soil indicators between three groups of variables: pollution, climate and vegetation. ns = not significant and * = significant.	40

List of Tables**Page**

Table 1 Easting and Northing of sampling sites	4
Table 2 Reproducibility of soil pH and base cation / aluminium ratio based on historical data from reference soil	10
Table 3 Environmental variables included in the analysis	11
Table 4 Current and cumulative pollution data for the sites and climate data. See Table 3 for data sources.	12
Table 5 Species codes used in ordination diagrams	35
Table 6 Codes used for soil data	47
Table 7 Soil data	49
Table 8 Details of column headings used for soil data	51
Table 9 Grid references of soil sampling points. The references is for the central soil core with four other soil cores taken to the N, E, S and W of this point each 0.5m away.....	52

1 INTRODUCTION

Protected areas are designated to meet the requirements of international directives and treaties and national legislation. They represent the very best of our national landscapes, plants and animals, rocks, fossils and landforms. The UK nature conservation agencies are required to assess and report on the condition of nature conservation features within these protected sites. In Scotland, the Site Condition Monitoring (SCM) programme managed by SNH monitors the status of designated features and assesses whether they are likely to be maintained under current management regimes and wider environmental drivers. This assessment uses a set of Common Standards developed at UK level. Atmospheric deposition is known to have an adverse impacts on terrestrial and freshwater natural features, SCM does not currently record evidence of pollution impacts on soil and vegetation. SEPA is keen to improve their monitoring and assessment of air pollution impacts on the soil and this project is the third phase in a process of developing suitable soil indicators for assessing the impact of N deposition.

Phase 1 of this work identified potential soil indicators through a literature review and expert evaluation in the SEPA project HP801 “To establish soil indicators to assess the impact of atmospheric deposition on environmentally sensitive areas” (Black et al. 2009). This project reviewed soil indicators to assess the impact of atmospheric deposition from point sources on soil quality in habitats of conservation interest, with nitrogen as the primary pollutant of interest. At that time, given the published literature available, seven soil indicators were selected as the most suitable to assess the status of soil quality in habitats of conservation interest in Scotland with respect to atmospheric pollution, with an emphasis on N deposition. By providing information on a range of soil properties and processes, these soil indicators could inform on the maintenance and vulnerability of five soil functions which are recognised within the Scottish Soil Framework.

The soil indicators identified by Phase 1 were:

- soil pH,
- soil carbon / nitrogen (C/N) ratio,
- base cation / aluminium (Al) ratio,
- solution ammonium (NH₄) / nitrate (NO₃),
- bacterial to fungal ratio (PLFA),
- fungal species fruiting bodies (an alternative DNA based approach was used in Phase 2),
- phosphomonoesterase.

Phase 2 of the work tested these seven soil indicators on an experimental site at Whim Moss, the results of which are reported in the SEPA report ‘*Testing soil quality indicators. Scottish Environment Protection Agency Commissioned Report No.HP1108.*’ The three soil indicators which showed the most promise as indicators of N deposition (i.e. a significant difference could be found in their values along a gradient of 8 to 64kg N ha⁻¹ yr⁻¹) were soil pH, base cation / aluminium ratio and phosphomonoesterase.

This current project (Phase 3) aims to use the three most promising soil indicators identified by the Phase 2 project at 11 SEPA biomonitoring sites to establish links between atmospheric deposition, impacts on soil and impacts on vegetation.

1.1 Objectives

The principal objectives of the project were:

Objective 1: Apply soil indicators at 11 sites where SEPA’s biomonitoring vegetation surveys have been conducted.

Objective 2: Explore whether the selected soil indicators relate to N-deposition rate and/or vegetation community measures and whether they can be used as an early warning system before habitat/vegetation changes have become apparent. Provide recommendations on which type of monitoring (soil and/or vegetation) is most effective, depending on the kind of information available for a given site.

These objectives contain four tasks:

- Task 1: Soil sampling
- Task 2: Soil sample analysis
- Task 3: Data analysis
- Task 4: Reporting of the results and findings

1.2 Questions to be answered

As a result of the project SEPA wish to answer the following questions:

1. Do the soil indicators show a response to N-deposition?
2. Is the response to N-deposition in accordance with the findings from Whim Moss (see project phase II)? If not what might be the reasons/factors?
3. Is there a relationship between the vegetation indicators (e.g. species composition, cover-weighted Ellenberg N) and the soil indicators?
4. How can the soil indicators be used for the interpretation of site impacts from N deposition?
5. Could the soil indicators potentially be used as an “early warning system”?
6. What benefits would a repetition of the soil monitoring provide? What time scales would be reasonable?

2 SITES

Eleven sites, all of which are SSSI's and SAC's that had previously been surveyed as part of SEPA's biomonitoring programming were sampled (Table 1, Figure 1). While a variety of habitats are present within each SSSI, in each case the habitat sampled was a bog habitat with a deep organic soil.

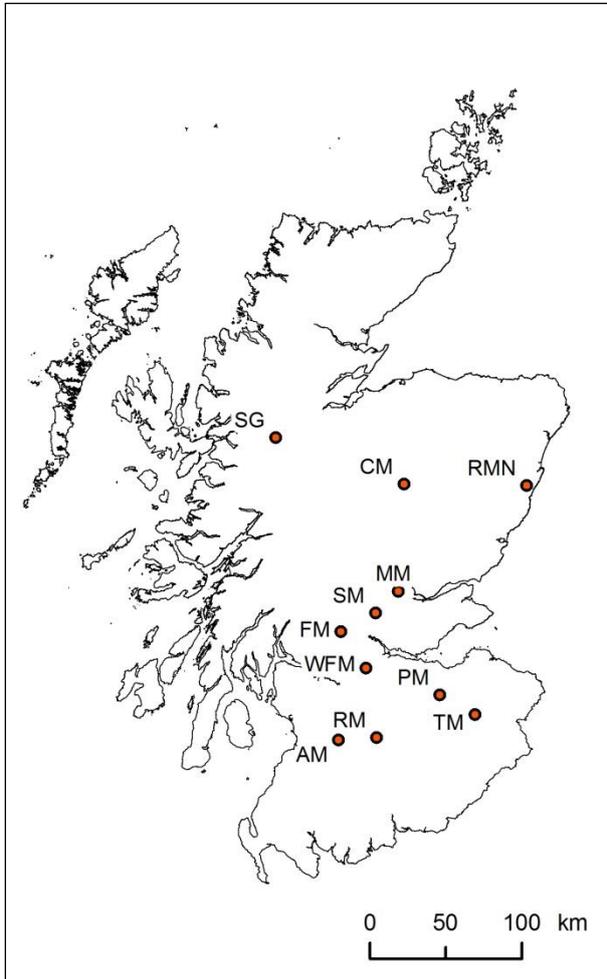


Figure 1 Location of sampling sites. See Table 1 for sites codes

Table 1 Easting and Northing of sampling sites

Site	Code	Easting	Northing	Alignment of quadrats
Airds Moss	AM	261988	625258	N to S
Cairngorms	CM	305066	794895	N to S
Flanders Moss	FM	263550	697007	N to S
Methven Moss	MM	301438	723898	N to S
Peeswit Moss	PM	328731	655108	N to S
Red Moss	RM	287154	626696	N to S
Red Moss of Netherley	RMN	385950	794042	NW-SE
Shelforkie Moss	SM	286210	709550	N to S
Strathglass	SG	220618	825702	N to S
Threepwood Moss	TM	351723	642227	N to S
West Fannyside Moss	WFM	279940	672998	Quadrats 1-3 aligned N-S, Quadrats 4 and 5 aligned NNW-SSE

Brief descriptions of the 11 sites, taken from or based on the Site of Special Scientific Interest (SSSI) citations (SiteLink SNH information gateway) are provided below.

The blanket bog of Airds Moss SSSI (AM) displays features typical of this habitat but is unusual in that these have developed at a relatively low altitude. The blanket bog has developed over a series of gently undulating ridges of glacial till. Although this landform is generally obscured by the development of the deep peat deposits across its surface, in places the mineral ridges rise above the peatland surface. Fen and acid grassland habitats are found around the periphery of the moss. Some of the surface features of Airds Moss, such as the development of a pool system at its eastern end, show affinities to the blanket bogs of north-west Scotland. In contrast, at its western end the deeper peats support vegetation communities more normally associated with lowland raised bogs. Here extensive lawns of the bog mosses *Sphagnum magellanicum* and *S. papillosum* dominate the vegetation, with cranberry *Vaccinium oxycoccos* and crowberry *Empetrum nigrum* frequent, and the nationally scarce bog rosemary *Andromeda polifolia* scattered over wide areas. Other species present, and indicative of undisturbed habitat, include white beaked sedge *Rhynchospora alba* and long-leaved sundew *Drosera anglica*.

The Cairngorms SSSI (CM) support extensive areas of blanket bog both on the lower slopes - it gives way to Northern Atlantic wet heath and European dry heaths as the gradient increases. Blanket bog is also found at a higher altitude than on any other SSSI in the UK, around 1000 m. The bogs at higher altitude are M19 *Calluna vulgaris* – *Eriophorum vaginatum* blanket mire and some of these are moderately extensive on the gently sloping plateaux below the mountain tops. Above about 850 m, heather *Calluna vulgaris* disappears from the blanket bog and is replaced by mountain crowberry *Empetrum nigrum* ssp. *hermaphroditum* and bog bilberry *Vaccinium uliginosum*. Dwarf birch *Betula nana* occurs locally in this higher-altitude bog. Lichens of the reindeer group (*Cladonia arbuscula* and *C. rangiferina*) are abundant, and the Cairngorms have some of the best examples of lichen-rich bogs.

Flanders Moss SSSI (FM) lies in the Carse of Stirling, 15 km west of Stirling. Flanders Moss holds one of the largest areas of near-natural raised bog in Britain and represents a significant proportion of the European resource. Degraded areas of raised bog on the site retain significant nature conservation value and are recovering. The site is the largest of a few isolated and protected remnants of the lowland raised mire system that once occurred more widely across the Carse of Stirling. This lowland raised mire system was one of the

most extensive of its type in Britain. Intact lowland examples of raised mire are becoming increasingly rare and declining in global terms. This makes Flanders Moss, with its biological and geomorphological features, of international importance.

Methven Moss SSSI (MM) lies on the watershed between the River Almond and the River Earn 9 km west of Perth. Methven Moss forms an important ecological link between the numerous small sites of the Central Belt and the scattered, drier sites of the Grampian Plain. Although the site has been damaged by past drainage activity it retains a significant area of intact surface and continues to support typical bog vegetation and species. The bog takes the form of an elongated active dome, the north and western end being intact, and the south and east end being “cut-over” but still regenerating. The bog surface is raised, and receives its water exclusively in the form of rain and snow fall. The bog is surrounded by wet woodland dominated by birch. The vegetation is dominated by cross-leaved heath *Erica tetralix*, cotton grass *Eriophorum vaginatum*, heather *Calluna vulgaris*, and typical bog mosses of raised bogs such as *Sphagnum magellanicum* and *Sphagnum cuspidatum*, the latter in small pools scattered throughout the site. The site is notable for the presence of white beaked sedge *Rhynchospora alba* in a lowland situation.

Peeswit Moss SSSI (PM) is a small raised bog lying at 278 m on the edge of the Moorfoot Hills, 1.5 km north-west of Gladhouse Reservoir. It is one of the best examples of active raised bog in Midlothian. The bog vegetation is typical of a site where some drainage has occurred and slightly altered the plant communities present. Cross-leaved heath dominates the wetter areas of the bog surface along with hare’s tail cotton-grass and various species of bog moss. Heather-dominated hummocks are found on the drier areas. Much of the bog is surrounded by surface-water fed lagg fen or wetland areas, a typical feature of fully intact bogs. Such intact raised bogs are uncommon in the Central Lowlands and this example is of particular interest due to both the extent and relatively unmodified nature of the bog. Despite being altered by past management it still demonstrates the classic dome shape of a lowland raised bog and retains the species typical of such a bog.

Red Moss SSSI (RM) lies approximately 2 km north of Crawfordjohn. It comprises three raised bogs with associated fen situated along the broad valley of the Black Burn and its tributaries. The raised bog is one of the best examples in Lanarkshire. The raised bogs are dominated by deergrass *Scirpus cespitosus* and hare’s tail cottongrass *Eriophorum vaginatum* with heather *Calluna vulgaris*, bog asphodel *Narthecium ossifragum*, round-leaved sundew *Drosera rotundifolia* and cranberry *Vaccinium oxycoccos* all constant in the field layer. *Sphagnum* moss cover is generally extensive, especially on the northern raised bog, and is made up of *Sphagnum papillosum*, with *S.tenellum*, *S. cuspidatum*, *S. magellanicum* and *S. capillifolium*. The nationally scarce *S.austinii* is found in locally frequent tall hummocks on the northern raised bog.

The Red Moss of Netherley SSSI (RMN) is located 12 km north of Stonehaven. It comprises a raised bog, modified by peat cutting in the past. A central area of uncut deep peat is surrounded by re-vegetated peat-cuttings with a fairly extensive fringe of poor-fen, and birch and willow fen-woodland. It is the best example of a lowland raised bog in the Aberdeen area and one of the largest in the north-east. It has a good representation of bog vegetation associated with the eastern lowlands of Scotland, being dominated by ling heather *Calluna vulgaris* and hare’s-tail cotton grass *Eriophorum vaginatum*. Locally, towards the centre of the site, the bog is actively regenerating. Here, bog myrtle *Myrica gale* is frequent and major peat-building bog mosses, *Sphagnum papillosum* and most notably *S. magellanicum*, are abundant.

Shelforkie Moss SAC (SM) is part of Carsebreck and Rhynd Lochs SSSI and lies north of the A9 between Dunblane and Auchterarder in lowland Perthshire. Shelforkie Moss is a raised bog still growing (ie. accumulating peat). It is one of the largest in the Tayside region

and considered of international importance. Part of the bog has been modified, by drainage or peat-cutting, but still retains a thickness of peat to sustain bog communities. The flush and fen communities support a relatively high number of plant species, including several of restricted distribution.

Strathglass SAC (SG) is part of the Affric – Cannich Hill SSSI and is 6 km west of the village of Cannich and 40 km SW from Inverness. The majority of the site lies on the southern shores of Loch Beinn a' Mheadhoin and Loch Affric, with two small outliers at Cougie and Coille Ruigh na Cuileige. It is notified for its native pinewood habitats and associated lichen and bird assemblages. The bogs and lochans within the site support a very rich dragonfly assemblage.

Threepwood Moss SSSI (TM) is located in a depression in the high ground 6 km south of Lauder just to the south of Threepwood. It is the largest and most intact example of a raised mire and one of the few remaining in the Scottish Borders. The moss retains a typical raised dome with a bog vegetation of heather *Calluna vulgaris*, cottongrass *Eriophorum vaginatum* with localised cranberry *Vaccinium oxycoccus* and bog asphodel *Narthecium ossifragum*. The sloping margin (rand) of the dome has *Sphagnum* (bog-moss) pools and some actively growing hummocks (9 *Sphagnum* spp. recorded to date), and grades into a birch/willow carr along the wetter, peripheral lagg (depression) where peat digging has been less intense. In the north-eastern section is an area of typical mesotrophic (medium nutrient status) fen and fen-meadow vegetation which complements the bog habitat. The site contains national and regional plant rarities including small tussock sedge *Carex diandra*, early marsh orchid *Dactylorhiza incarnata*, globeflower *Trollius europaeus*, tea-leaved willow *Salix phylicifolia* and lesser twayblade orchid *Listera cordata*.

West Fannyside Moss SSSI (WFM), located approximately 2 km south-east of Cumbernauld, is nationally important for its extensive area of blanket bog supporting peat-forming vegetation. It displays features typical of this habitat but is unusual in that these have developed at a relatively low altitude. Intact bogs are uncommon in the central lowlands and this example is of importance as it is relatively undisturbed and one of the best examples of active blanket bog in Lanarkshire. West Fannyside Moss supports a range of bog communities, which includes a large area of intact wet heath and blanket bog, typified by a cross-leaved heath/bog moss community. In addition areas dominated by a heather/hare's-tail cotton-grass community are found around the peripheral areas of the site with areas of wet heath dominated by deer-grass/cross-leaved heath also occurring. Extensive lawns of the bog mosses *Sphagnum papillosum* and *S. recurvum* dominate the vegetation with crowberry *Empetrum nigrum* and blaeberry and a further six species of *Sphagnum* mosses. Other typical bog species are present including the locally rare cranberry *V. oxycoccus* as well as round-leaved sundew *Drosera rotundifolia* and bog asphodel *Narthecium ossifragum*, both of which are uncommon in Lanarkshire.

3 SOIL SAMPLING

The 11 sites were sampled between 30th September 2015 and 9th October 2015. At each of the 11 sites five quadrats (2 m x 2 m) had previously been established by SEPA in 2014. The locations of the quadrats had been recorded with a high accuracy GPS. Within in quadrat the species present and their percentage cover had been recorded.

At each site the soil surveyors relocated these quadrats using a Trimble mapping grade GPS device. Soil sampling was carried out 2 m north of the NW corner of the quadrat where five cores were collected (Fig. 2). The sampling followed that proposed by Mitchell et al. (2013). The central soil core was 2 m north of the NW corner of the quadrat (Fig. 2). Four additional cores were then taken each 0.5 m away from the central core in each of the four compass directions (N, E, W, S).

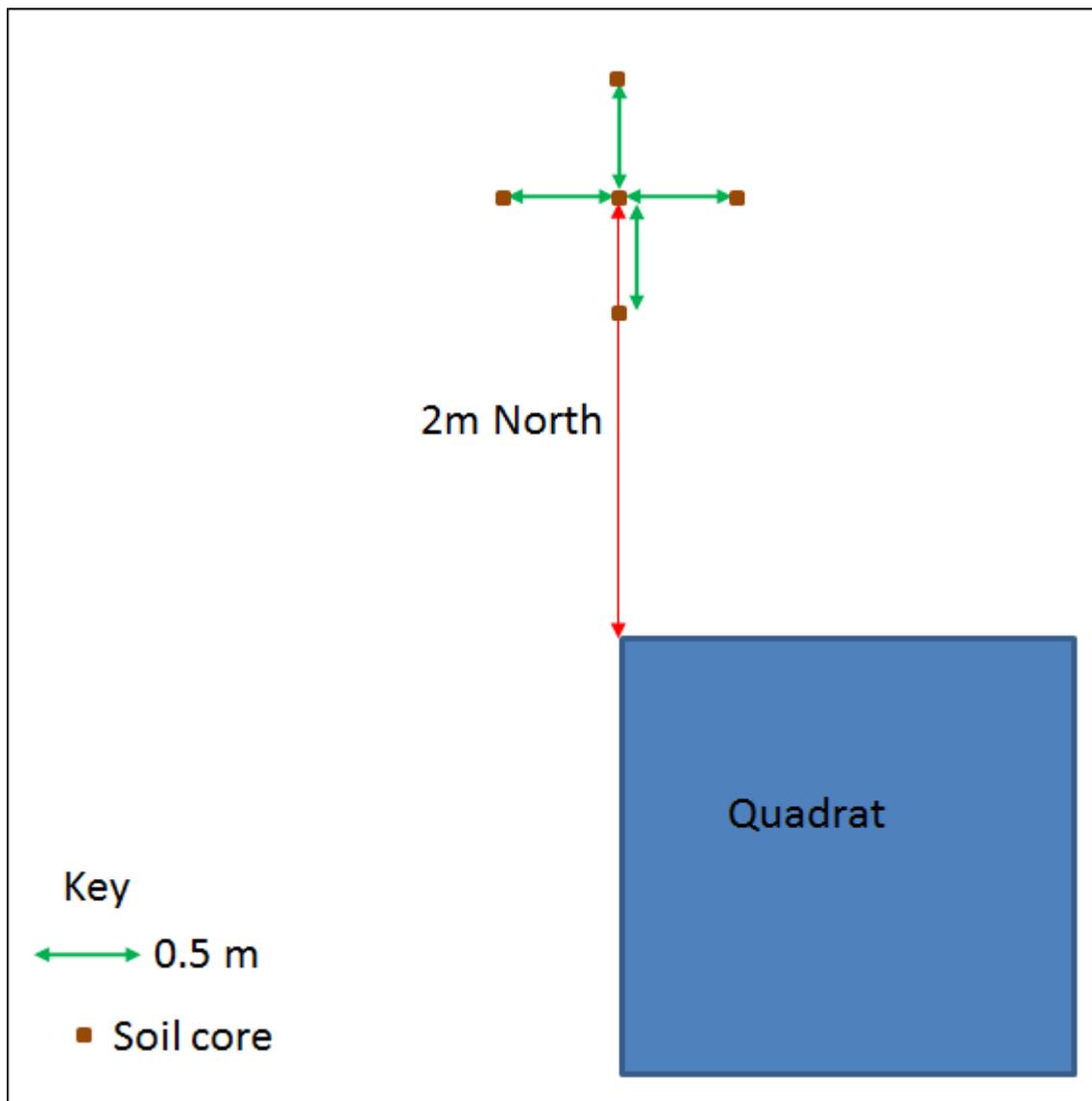


Figure 2 Position of soil samples relative to the quadrat

The majority of the soil cores were taken with a 40 cm long box corer (Fig. 3), but a longer, 1 meter version was required in certain circumstances where the surface was particularly saturated or there was a thick layer of live Sphagnum. Both corers were 5 cm by 5 cm and peat samples were taken to a depth of at least 15 cm excluding the litter layer and any living sphagnum which in some cases could be up to 40 cm in depth. The depth from the surface

at which the cores were taken thus varied quite significantly both between and within sites and even within plots depending on the depth of living sphagnum present. It is often difficult, particularly in active Sphagnum-rich bogs, to decide what constitutes a 'litter' layer and where the 'peat' horizon begins. We attempted to retrieve relatively humified peat (i.e. brown to black in colour) and not plant material that was alive and retaining its original colour. The cores were usually taken to a deeper depth than required and then trimmed to 15 cm in the lab before analysis. Soil cores were individually wrapped and labelled and kept cool until they were returned to the lab.

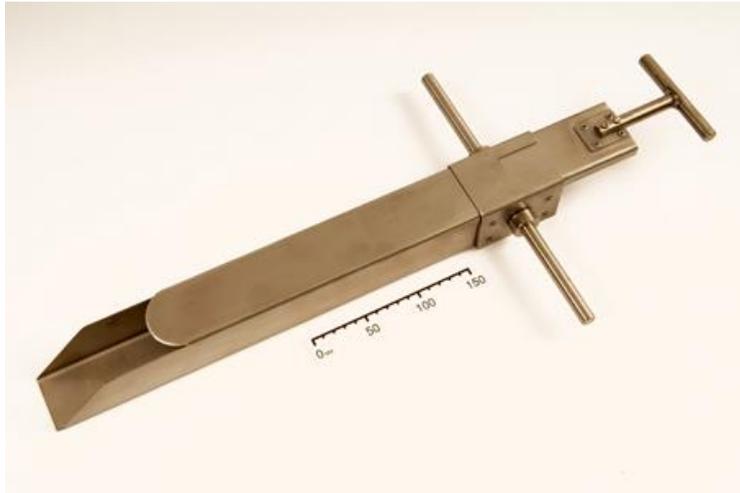


Figure 3 Picture of box corer used for sampling

3.1 Soil sample preparation

The sample preparation followed that suggested by Mitchell et al. (2013), except that the cores were split in half length ways not into quarters; as only three soil indicators were being assessed, not the seven done in the Phase 2 report, only two bulked soil samples were required not four (Fig. 4).

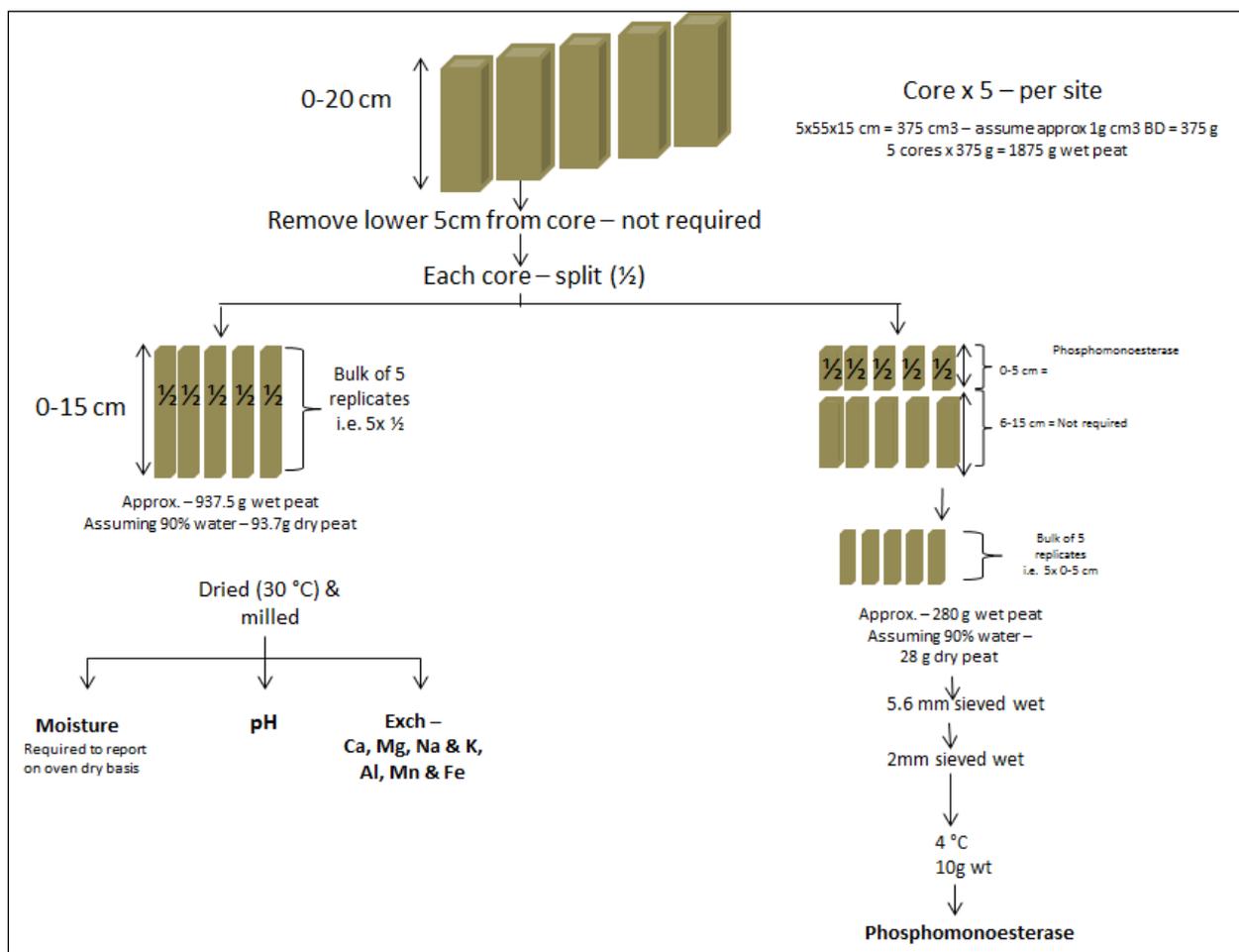


Figure 4 Preparation of the soil samples prior to analysis

3.2 Soil analysis and quality control

Soil analysis was carried out by the analytical department at the James Hutton Institute and followed the methods detailed in Appendix B of Mitchell et al. (2013).

Determination of pH and base cations (Ca, Mg, K & Na), followed appropriate United Kingdom Accreditation Service (UKAS) accredited methods. Aluminium followed the method used to determine cations however it is not accredited under the UKAS method.

Analytical results will vary over time due to slight changes in machines etc. In order to monitor this variability and to assess the repeatability of results three samples of a soil used as an internal reference soil are analysed with each batch of samples. Each determinant is assessed against historical data and where applicable the internal reference soil is referenced against certified reference materials. The reproducibility of results based on this internal reference material over time is shown in Table 2. In this project the small sample size (55 samples) ensured that all samples were analysed together in one batch at one time further reducing any variability between results.

Table 2 Reproducibility of soil pH and base cation / aluminium ratio based on historical data from reference soil

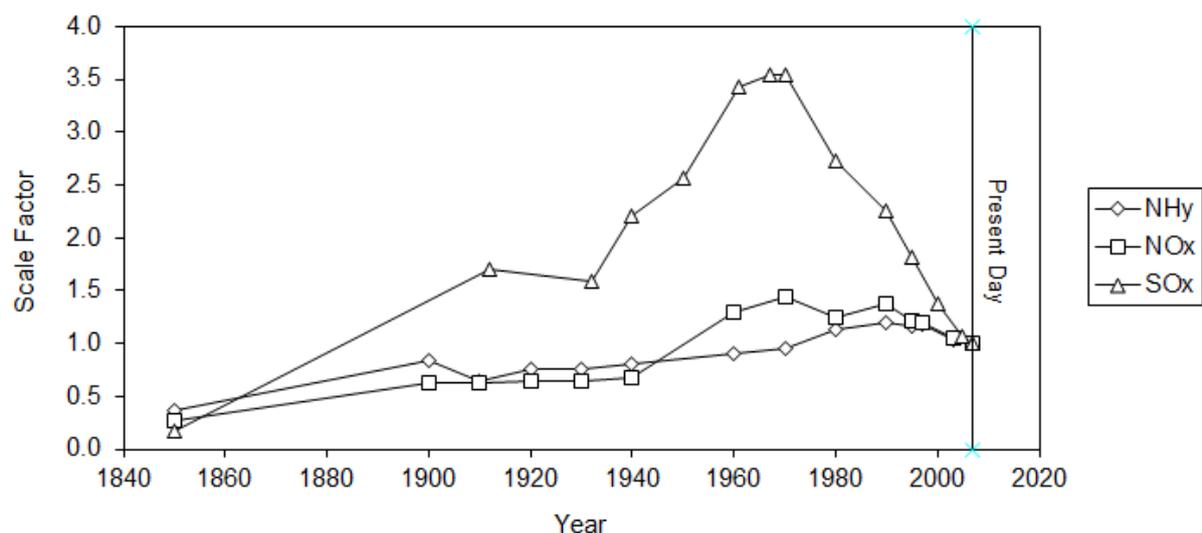
Determinant	Average	± (95% Confidence level)	Units
pH	5.40	0.16	pH in CaCl ₂
Ca	7.63	0.77	meq 100 g ⁻¹
Mg	0.32	0.05	meq 100 g ⁻¹
K	0.12	0.06	meq 100 g ⁻¹
Na	0.16	0.078	meq 100 g ⁻¹
Al	0.14	0.10	meq 100 g ⁻¹

3.3 Collation of environmental data

The soil indicators may be influenced by a range of other environmental factors including current and past (cumulative) pollution both of nitrogen (N) and sulphur (S), the form of the pollutant (NO_x or NH_y) and climate. Data on current pollution levels was taken from the UK Air Pollution Information System (APIS) <http://www.apis.ac.uk/> for the year range 2011-2013. Data on cumulative total nitrogen and sulphur deposition on a 5 km x 5 km grid for the period between 1850 and 2015 was obtained from the CBED-model (Smith et al. 2000) using historical scaling factors from the FRAME model (See Box 1 for further details). Table 2 lists the environmental data collated to include in the analysis and its source.

Box 1: Calculation of cumulative S, NO_x, NH_y from 1850

The calculation of cumulative deposition is based on the CBED model (described in Smith et al. 2000) but this project used the surface dataset from 2004 to 2006 (data from CEH). The Fine Resolution Atmospheric Exchange model (FRAME)(Fournier et al 2003; Fournier et al. 2004) and MAGIC models (developed by Crosby et al. 1985 but updated e.g. Helliwell et al. 2014) calculate S, NO_x, NH_y deposition in 1850, 1910, 1930, 1940, 1950, 1960, 1970, 1980, 1990, 1991-2005 (every year). These models provide a scaler factor by which to multiple the 2005 deposition to estimate the deposition in these 13 years (scaler factors obtained from CEH). We interpolated the scale factor between years to provide a scaler factor for every year based on the 2005 deposition data. The deposition in each year was then summed to provide a cumulative load for S, NO_x, NH_y back to 1850. This methodology is similar to that used by Fowler et al. (2004) but their data only goes back to 1900.



Data used from FRAME model to interpolate scale factor for NH_y, NO_x and SO_x for each year until 1850.

Table 3 Environmental variables included in the analysis

Variable	Units	Source
Current pollution levels		
Total N	kg N ha ⁻¹ yr ⁻¹	APIS accessed 12/01/2016
NH ₃	µg m ⁻³	APIS accessed 12/01/2016
NO _x	µg NO _x (as NO ₂) m ⁻³	APIS accessed 12/01/2016
SO ₂	µg m ⁻³	APIS accessed 12/01/2016
Cumulative pollution levels		
Cumulative SO _x	kg ha ⁻¹	Smith et al. 2000
Cumulative NH _y	kg ha ⁻¹	Smith et al. 2000
Cumulative NO _x	kg ha ⁻¹	Smith et al. 2000
Climate		
Average daily maximum temperature 1981-2010	°C	Annual average national gridded data (5km resolution) taken from http://www.environment.scotland.gov.uk/get-interactive/data/climate-trends/ accessed 15/01/2016
Average daily minimum temperature 1981-2010	°C	Annual average national gridded data (5km resolution) taken from http://www.environment.scotland.gov.uk/get-interactive/data/climate-trends/ accessed 15/01/2016
Average annual rainfall 1981-2010	mm	Annual average national gridded data (5km resolution) taken from http://www.environment.scotland.gov.uk/get-interactive/data/climate-trends/ accessed 15/01/2016

Table 4 Current and cumulative pollution data for the sites and climate data. See Table 3 for data sources.

Site name	Total N kg N ha ⁻¹ yr ⁻¹	NH ₃ µg m ⁻³	NO ₂ µg NO _x m ⁻³	SO ₂ µg m ⁻³	Cumulative SO _x kg ha ⁻¹	Cumulative NH _y kg ha ⁻¹	Cumulative NO _x kg ha ⁻¹	Max Temp °C	Min Temp °C	Rain mm
Airds Moss	12.74	0.81	3.41	0.69	1120.4	1113.9	585.5	11.71	4.44	1299.55
Cairngorms	7.98	0.13	2.19	0.44	1603.2	670.5	1002.9	8.76	1.76	1094.19
Flanders Moss	12.04	0.73	3.34	0.76	1712.5	921.4	770.6	12.98	5.09	1292.27
Methven Moss	11.48	0.96	4.38	0.87	1256.4	462.0	626.2	12.31	4.64	929.42
Peeswit Moss	16.38	1.34	5.11	1.13	1477.8	896.2	771.9	11.31	4.22	921.25
Red Moss	14.84	0.58	4.44	0.6	1701.9	1290.5	883.5	10.83	3.53	1243.31
Red Moss of Netherley	15.68	1.13	5.06	0.99	872.3	618.7	598.6	11.19	4.79	881.83
Shelforkie Moss	11.62	0.61	5.16	1.12	1693.9	776.7	815.2	11.58	4.07	1533.57
Strathglass	5.74	0.07	1.71	0.38	1643.2	418.2	678.7	7.65	1.87	1959.59
Threepwood Moss	16.24	1.27	4.31	0.92	1155.0	663.8	617.0	11.24	4.12	855.00
West Fannyside Moss	13.72	1.25	10.77	1.85	1261.7	942.6	659.0	12.04	4.78	1201.38

3.4 Vegetation data

Vegetation data was received from SEPA for the quadrats. This data contained the percentage cover of each species recorded in the quadrat and derived cover weighted Ellenberg N and R scores for each quadrat, species richness and Shannon diversity data for each quadrat. Ellenberg scores provide a measure of the ecological requirements of a plant species for Nitrogen (N) and acidic conditions (R) on a scale from 1 to 9. Scores for British plants are provided by Hill et al. (2004). The scores per quadrat can either be calculated as the average score of all the species present in the quadrat or the average score weighted by the cover of each species (cover weighted scores). In this project the cover weighted scores were used. Species richness is the number of species recorded in the quadrat and Shannon diversity provides a measure of the diversity and cover of the species in the quadrat.

3.5 Data analysis

All linear statistics were performed in SASv9.4 (SAS2015) and all multivariate statistics in CANOCO v 5 (Ter Braak & Smilauer 2012). There were two sets of indicators: soil indicators and vegetation indicators. The soil indicators consisted of soil pH measured in CaCl_2 (referred to as soil pH throughout), base cation to aluminium ratio calculated as $(\text{Ca}+\text{K}+\text{Mg})/\text{Al}$, (all data in $\text{meq } 100\text{g}^{-1}$) and phosphomonoesterase (mmoles pNP g^{-1}). There were four vegetation indicators: species richness, Shannon diversity index, and cover weighted Ellenberg N and R scores.

3.5.1 Soil or vegetation indicators v pollution or climate data.

Generalized Linear Mixed Models (GLMMs) were used to test if there was a relationship between the soil or vegetation indicators and current or cumulative pollution levels or climate (Box 2). For any indicator for which there was a significant relationship with one of the current pollution variables a second GLMM was run with climate and cumulative pollution variables included as co-variables. This analysis tested if the current pollution variables were still significant once differences between sites in cumulative pollution levels and climate were taken into account. In each case site was included as a random effect.

The relationship between soil and vegetation indicators was also assessed using GLMM. Once again site was included as a random effect.

Box 2: A brief introduction to Generalized Linear Mixed Models (GLMM)

Generalized Linear Mixed Models (GLMM) do not assume a linear relation between the soil indicators and the pollution and climate variables but test for non-linear (curved) relationships. GLMM also allows inclusion of random effects. In this study site is included as a random effect as this takes account of the fact that multiple samples were taken from each site all of which have the same climate and pollution levels. In statistical terms this is called taking account of the fact that samples from each site are not independent from each other.

We first of all used GLMMs to assess if there was a relationship between the soil/vegetation indicator and a single pollution/climate variable. We then tested to see if this relationship is still significant taking into account climatic and cumulative pollution levels at the sites.

In this report a software package called SAS is used to carry out GLMM using a procedure called ProcMixed.

3.5.2 Multivariate analysis of soil indicators and vegetation % cover data

The soil indicators and the vegetation data (% cover) were analysed using Principal Components Analysis (PCA) and Detrended Correspondence Analysis (DCA) respectively to

assess how they differed between sites (Box 3). The vegetation data percentage cover data was transformed ($\log_{10} + 1$).

To assess if the climate and pollution variables explain the variation in the soil indicators and vegetation data constrained ordination (Redundancy Analysis (RDA) – soil indicators, Canonical Correspondence Analysis (CCA) – vegetation data) was performed. Two separate analyses were carried out, the first with the current and cumulative pollution variables (Table 3) as explanatory variables and the second with climate as the explanatory variable. Once again the percentage cover vegetation data was $\log_{10} + 1$ transformed.

Variation partitioning was used to assess how much of the variation in the soil indicators was explained by each of three groups of variables: 1) current and cumulative pollution, 2) climate, 3) vegetation composition. Within each of these three groups only variables that explained a significant amount of the variation were included. For the vegetation composition it is not possible to include each species as a variable (as there would be too many variables), so the axis scores from the DCA of the vegetation were included as explanatory variables.

Box 3: A brief introduction to multivariate statistics

Univariate statistics only enables analysis of how one indicator or species is affected by one or many variables such as climate or pollution. GLMM is a type of univariate statistics. Multivariate statistics enables one to assess how many indicators or many species change together – e.g. how the community composition changes rather than just one species. Multivariate statistics summarizes the variation (differences) between samples/plots in their species composition in two dimensions (two axes of a graph called ordination diagrams). It is common to state how much of the variation in the composition of the plots/samples can be explained along each axis. Plots or samples that are plotted close to each other on the graph have a similar species composition, plots or samples that are at opposite ends of the axes have a very different species composition from each other. The results from multivariate statistics can be shown as graphs of samples or species and the two graphs can be related to each other; for example species found at the positive end of the first axis are more commonly found in plots/samples that are shown at the positive end of the first axis than in plots at the negative end of the first axis. Principal Components Analysis (PCA) and Detrended Correspondence Analysis (DCA) are two types of multivariate statistics that carry out the analysis described above. Both PCA and DCA carry out similar types of analysis but are suited for different types of data, so in this report DCA is used to analyse the vegetation data and PCA is used to analyse the soil indicators.

There is a second type of multivariate statistics called constrained ordination where environmental data such as pollution or climate variables is used to explain differences between plots/samples in their species composition. It is possible to test if the environmental variables explain a significant amount of the variation in the species composition. The results from this type of analysis are again shown as a graph or ordination diagram. The environmental variables are usually shown as arrows, plots/samples/species that occur near the tip of the arrow are associated with higher levels of that environmental variable than plots/samples/species that occur near the base of the arrow. The longer the arrow in the ordination diagram the more variation in the plots/samples/species it explains. Canonical Correspondence Analysis (CCA) and Redundancy Analysis (RDA) are both types of constrained ordination used in this report. CCA and RDA are most suited for different types of data but carry out a similar type of analysis which is why both types of analysis are used in this report: CCA to assess how the climate and pollution data explain variation in the vegetation data and RDA to assess how the climate and pollution data explain variation in the soil indicators.

4 RESULTS

4.1 Variation between sites as noted by the soil surveyors

The surveyors noted that Flanders Moss and Shelforkie Moss were the most active bog habitats of the sites visited. Both these sites often had 30-40cm of live sphagnum moss resulting in humified peat for sampling not being reached until below the water table. West Fannyside also had some deep sphagnum but not as deep as these two sites. The other sites had a much shallower live Sphagnum/litter layer.

Red Moss of Netherley was noted by the surveyors as being different from the other sites as it had been cut over for peat. Strathglass moss was slightly different from the other sites as it was an eroded blanket bog with active peat hags. Methven Moss was notable as having had a lot of young *Betula* sp. (Birch) tree removed from it fairly recently. Threepwood Moss was noted as being the most *Calluna* dominated moss and West Fannyside was noted as having very mixed vegetation.

Sites at Strathglass and Cairngorms were different from the other sites which were all lowland bogs in a hollow. These two sites were on a slight slope with water seeping through them.

The biggest difference of those noted by the surveyors was that of the deep sphagnum and sampling below the water table at Flanders Moss and Shelforkie Moss. As this may have impacted the results these two sites are plotted with crosses in all graphs in the report while the other sites are plotted as either open or closed symbols.

4.2 Variation in soil indicator results

Before assessing the relationship between the soil indicators and current and cumulative pollution and climate the variation in the soil indicators was assessed to see if particular results or sites might drive any of the relationships or if there were any outliers.

Mean soil pH ranged from 2.81 at Threepwood moss to 3.21 at Strathglass. Flanders Moss, Methven Moss, Peeswit moss, Shelforkie Moss and Threepwood Moss all had very similar mean pH values (2.8). Airds Moss, Red Moss, Red Moss of Netherley and West Fannyside all had a mean pH of 2.9 with the site at Cairngorm having a mean value of pH 3.09. The variability within each site was generally low (Fig. 5) with 0.18 pH units or less between the highest and lowest values within a site. At Cairngorm the variation in pH was much greater with 0.46 pH units between the highest and lowest values, however there were no outliers within the dataset.

Mean phosphomonoesterase ranged from 123 mmoles pNP g⁻¹ at Shelforkie Moss to 816 mmoles pNP g⁻¹ at Strathglass. At most sites there was large variation within the results. The greatest within site variation was at Airds Moss with a difference between the lowest and highest phosphomonoesterase values of 926 mmoles pNP g⁻¹. Strathglass had the lowest within site variation: a difference of only 63 mmoles pNP g⁻¹ between the lowest and highest values. Despite the large within site variation there were no obvious outliers within the dataset (Fig. 6).

Mean base cation/Al ratio ranged from 190 at West Fannyside Moss to 1691 at Airds Moss. The greatest within site variation was at the Cairngorms where the ratio ranged from 28 to 4117. This was due to one sample (Quadrat 3) which was an outlier with an extremely high value (Fig. 7). The ratio of the other four samples ranged from 28 to 291. Quadrat 1 from Strathglass also had a very high ratio (2317) compared to the other four quadrats which ranged from 123 to 331. There were also two samples from Airds Moss (Quadrats 2 and 5)

that had very high ratios, greater than 2000. All other samples had a ratio of less than 2000. Thus in interpretation of the data any relationship that might be influenced by these four potential outliers will be reanalysed without these data points.

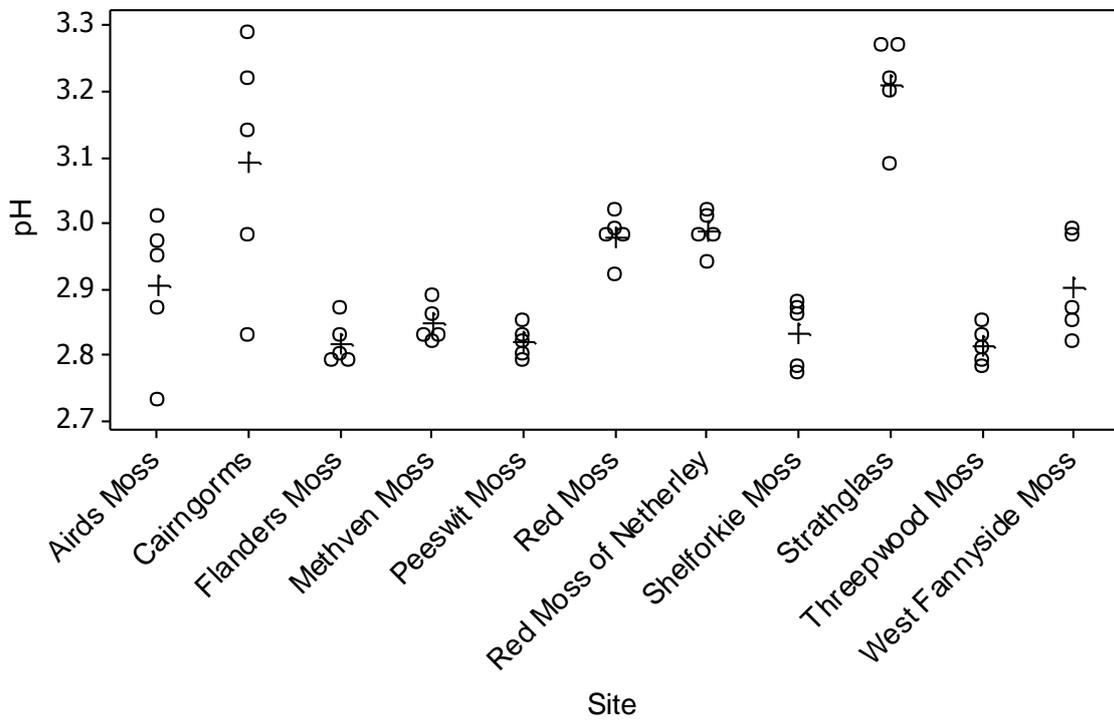


Figure 5 pH results. Each circle is the result from one quadrat with the mean shown by a cross.

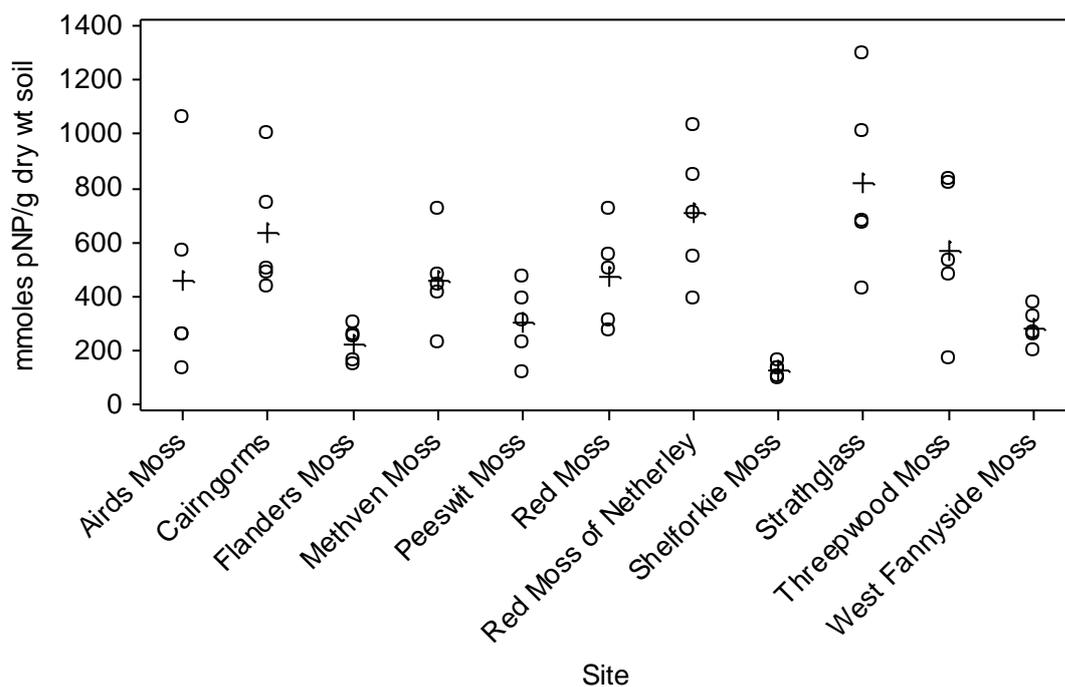


Figure 6 Phosphomonoesterase results. Each circle indicates the results from one quadrat with the mean shown by a cross.

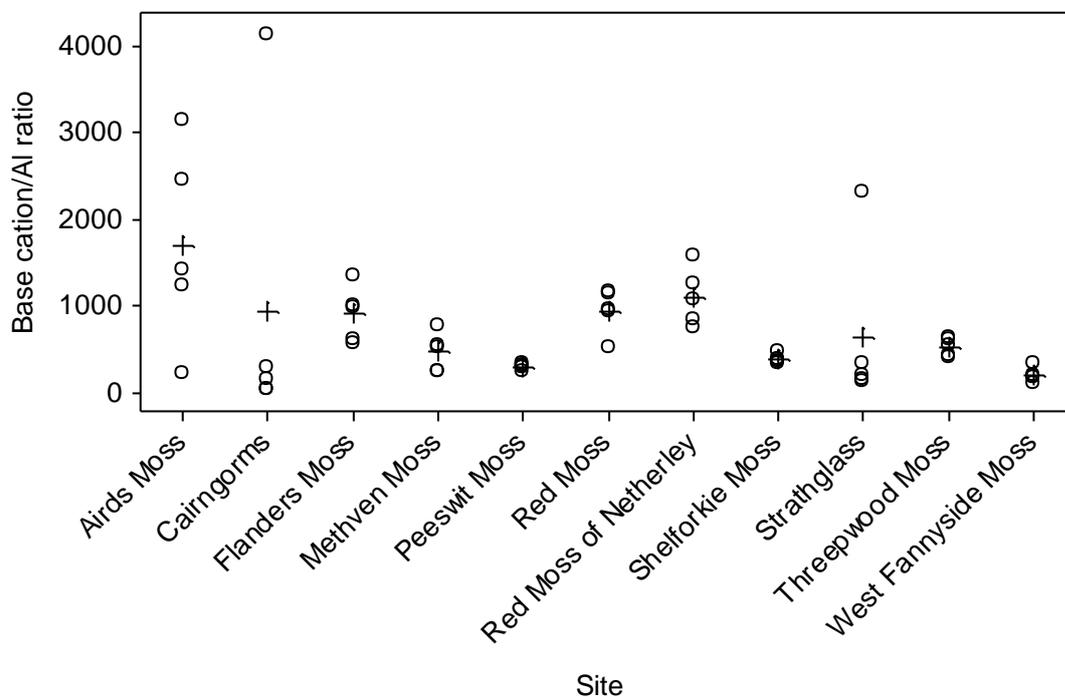


Figure 7 Base cation/Al ratio results. Each circle represents the results from one quadrat with the mean shown by a cross.

4.3 Variation in vegetation indicators

The variation in the four vegetation indicators was assessed in the same way as for the soil indicators. Mean species richness ranged from 9.8 at West Fannyside Moss to 21.4 at Airds Moss. The greatest range in species richness also occurred at Airds Moss with species richness ranging from 17 to 27. West Fannyside had the smallest range in species richness, ranging from 8 to 11 species. There were no outliers in terms of species richness (Fig. 8) and no indication that the outliers in terms of base cation/Al ratio had a lower or higher species richness than the other plots.

Mean Shannon Diversity Index was lowest at Shelforkie Moss (1.5) and greatest at Strathglass (2.3) (Fig. 9). Strathglass had the greatest variation in this diversity index (2.1-2.7) and Peeswit Moss the least variation (1.9-2.1). Quadrat 5 at Airds Moss was an outlier having an index of 1.77 compared to the other four quadrats that ranged from 2.1 to 2.6. This quadrat was also an outlier in terms of its base cation/Al ratio. There were also two quadrats (2 and 3) that were slight outliers at Cairngorm, having a lower index (1.3) than the other three quadrats that ranged from 1.6 to 1.9. However, it was a different quadrat at Cairngorm than gave an outlier result for base cation/Al ratio.

On average Cairngorm had the highest cover weighted Ellenberg N score (1.9) and Red Moss the lowest (1.1). Variation in this index was greatest at Airds Moss with scores ranging from 1.0 to 2.1 (Fig. 10), however this was due to one outlier (Quadrat 2) which had a much higher score than the other plots which ranged from 1.0 to 1.4. Quadrat 2 was one of the two quadrats at Airds Moss with a much higher base cation/Al ratio than the others, however, Quadrat 5, the other outlier in terms of base cation/Al ratio had the lowest cover weighted Ellenberg N score. Thus there was no clear link between outliers in terms of base cation/Al ratio and Shannon diversity index.

The site with the lowest average cover weighted Ellenberg R score was West Fannyside moss (1.6) with Strathglass having the highest score (2.2). The greatest variability in this indicator was at Airds Moss which ranged from 1.6 to 2.9 (Fig. 11). As with the Ellenberg N scores Quadrat 2 had a much higher score (2.9) than the other quadrats at this site which ranged from 1.6 to 2.0.

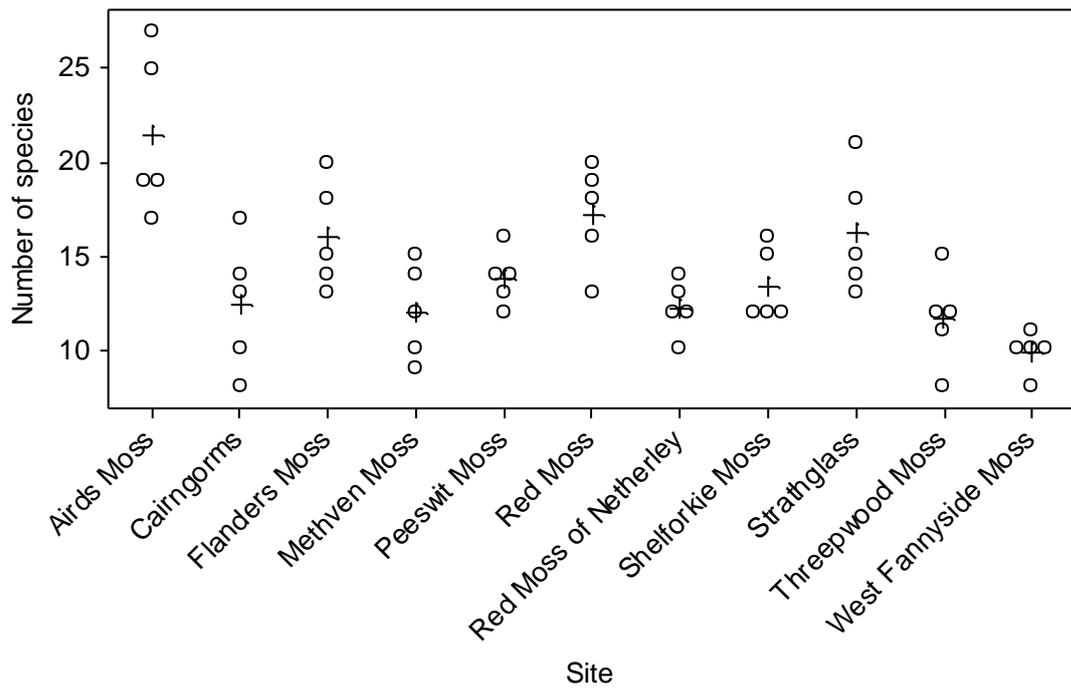


Figure 8 Species richness results. Each circle represents the results from one quadrat with the mean shown by a cross.

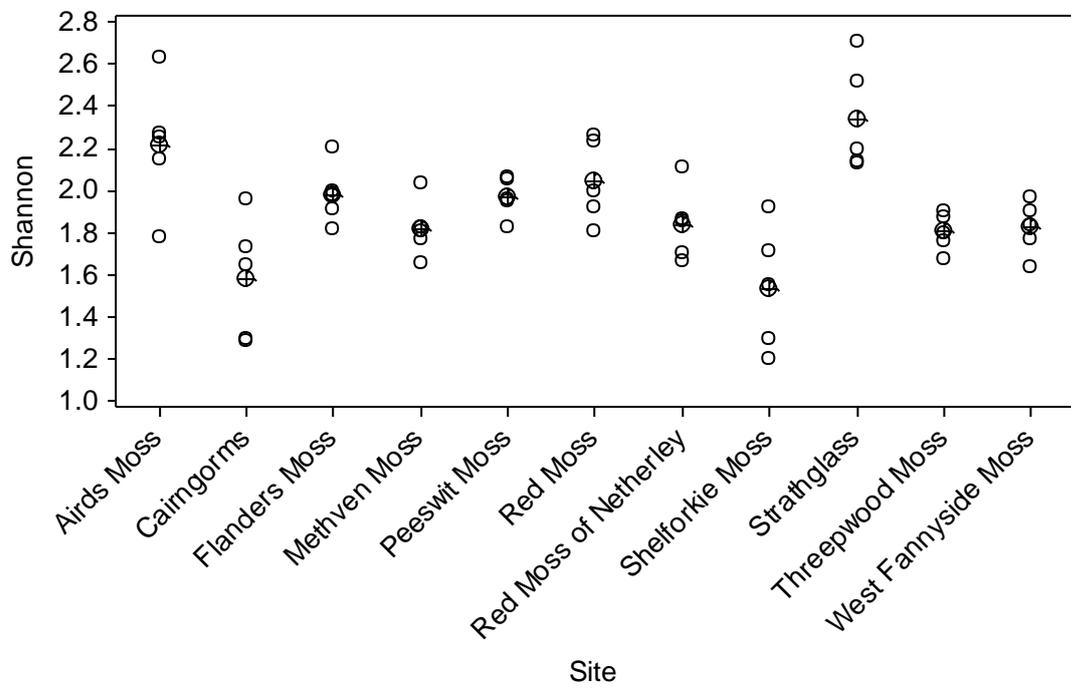


Figure 9 Shannon diversity results. Each circle represents the results from one quadrat with the mean shown by a cross.

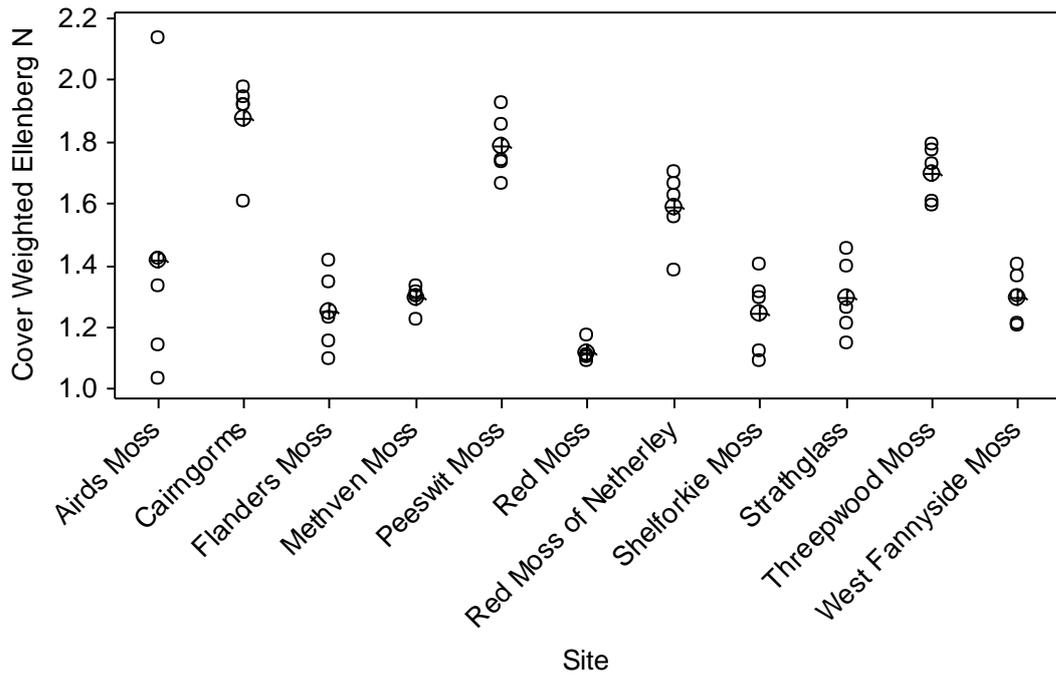


Figure 10 Cover weighted Ellenberg N results. Each circle represents the results from one quadrat with the mean shown by a cross.

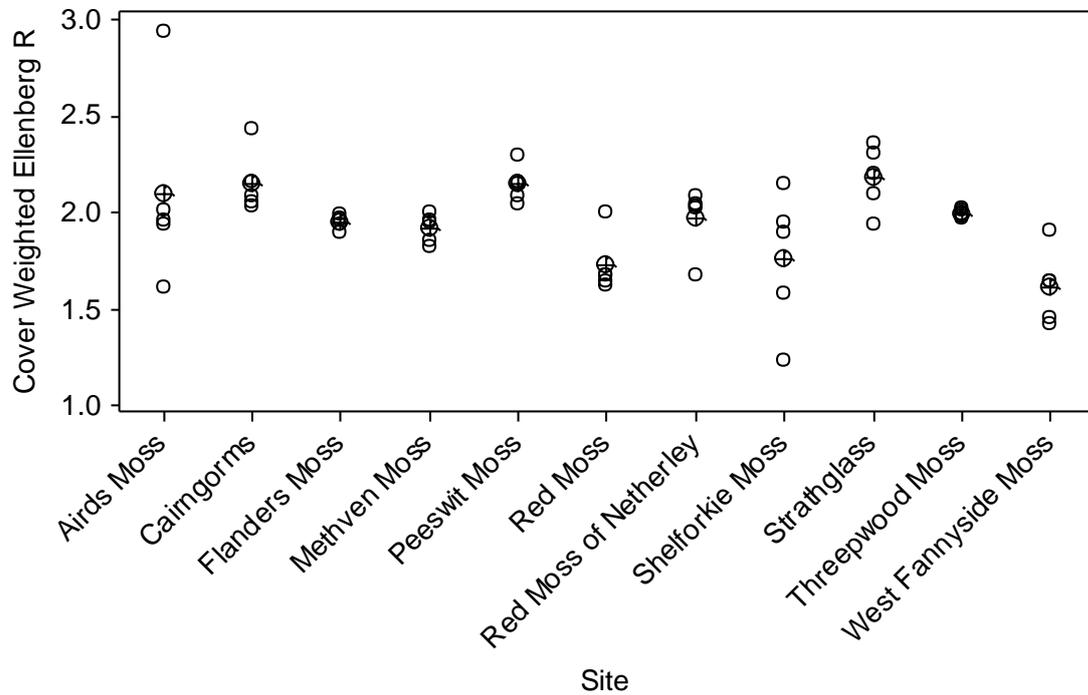


Figure 11 Cover weighted Ellenberg R results. Each circle represents the results from one quadrat with the mean shown by a cross.

4.4 Soil indicators v current pollution levels

Of the three soil indicators tested there were significant relationships between the soil indicators and current pollution levels for soil pH and total N deposition ($F_{1,9} = 8.23$; $P < 0.05$) and soil pH and ammonia concentration ($F_{1,9} = 10.98$; $P < 0.001$) (Fig. 12).

Current ammonia concentration levels were still found to have a significant effect on soil pH even once maximum and minimum average daily temperatures were taken into account ($F_{1,7} = 6.04$; $P < 0.05$). However once maximum and minimum average daily temperatures were taken into account total N deposition was found to no-longer be significantly correlated with soil pH.

The relationship between ammonia and soil pH was entirely due to two sites (Cairngorms and Strathglass). The higher pH at these sites may be due to their underlying geology and/or the water flow through the sites as observed by the surveyors (see section 5 Discussion). The robustness of soil pH as an indicator of the impact of ammonia concentration across multiple sites requires further investigation.

4.5 Soil indicators v cumulative pollution levels

The only significant relationship between any of the three soil indicators and cumulative pollution levels was a significant negative relationship between phosphomonoesterase and cumulative NH_y levels ($F_{1,18} = 4.40$, $P < 0.05$, Fig. 13). However once climate (average maximum and minimum daily temperature) was taken into account this relationship was no-longer significant.

4.6 Soil indicators v climate

Climate had a significant impact on phosphomonoesterase and soil pH but not on the base cation to aluminium ratio (Fig. 14). There was a significant negative relationship between phosphomonoesterase and average maximum daily temperature ($F_{1,9} = 10.47$, $P < 0.01$). The relationship between phosphomonoesterase and average minimum daily temperature was only significant at the $P < 0.1$ level ($F_{1,9} = 4.66$, $P = 0.059$). Soil pH was negatively correlated with average maximum daily temperature ($F_{1,9} = 37.21$, $P < 0.001$) and average minimum daily temperature ($F_{1,9} = 17.92$, $P < 0.05$). The relationship between pH and climate is the opposite to that expected, one would expect a lower pH in colder sites. The relationship between temperature and soil pH was entirely due to two sites (Cairngorms and Strathglass). The higher pH at these sites may be due to their underlying geology and/or the water flow through the sites resulting in the sites being slightly flushed enriched, as observed by the surveyors (see Discussion). Average total annual rainfall had no effect on any of the three soil indicators.

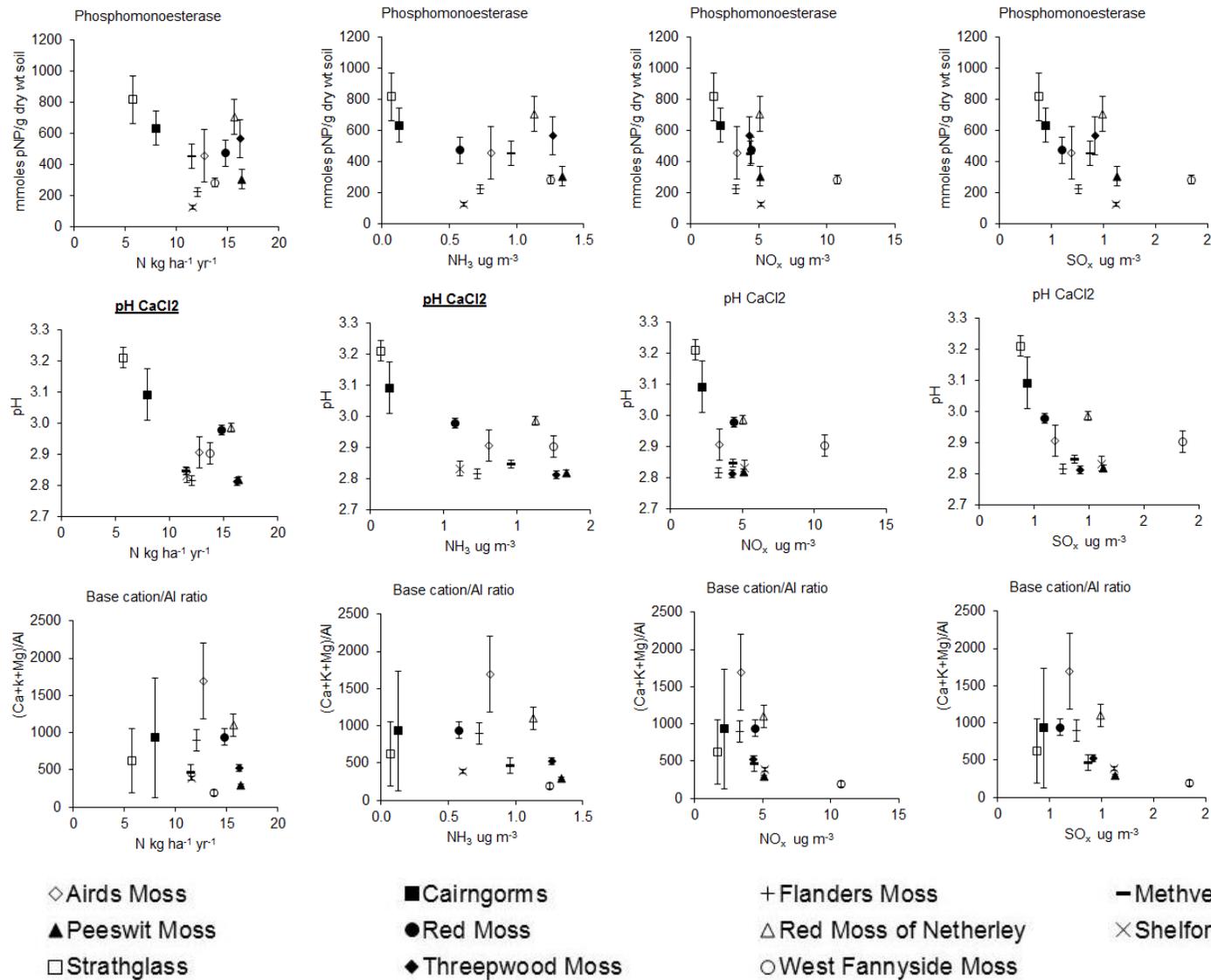


Figure 12 Relationship between 3 soil indicators and current pollution levels. Graph titles in bold and underlined indicate a significant relationship

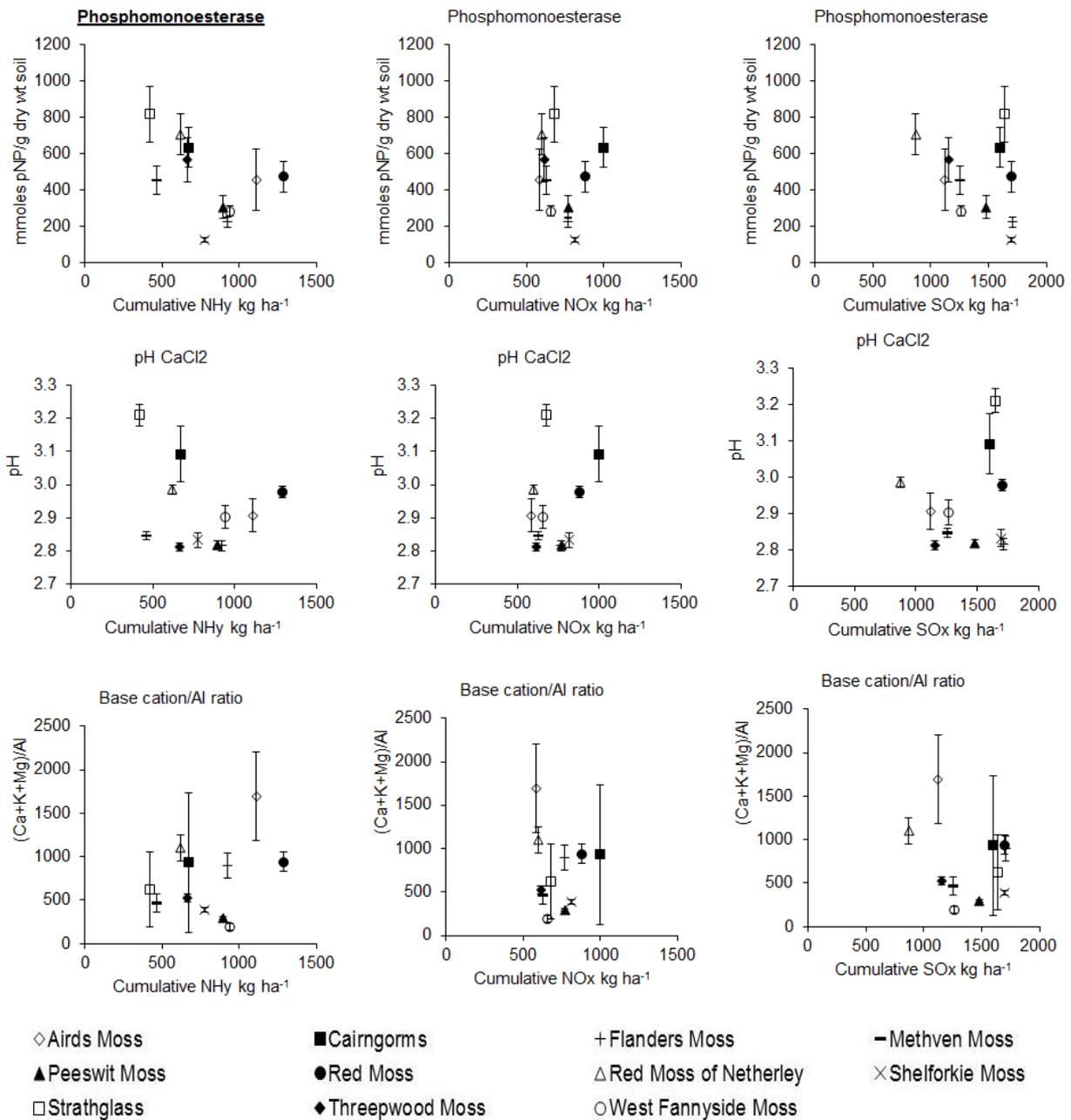


Figure 13 Relationship between 3 soil indicators and cumulative pollution levels. Graph titles in bold and underlined indicate a significant relationship.

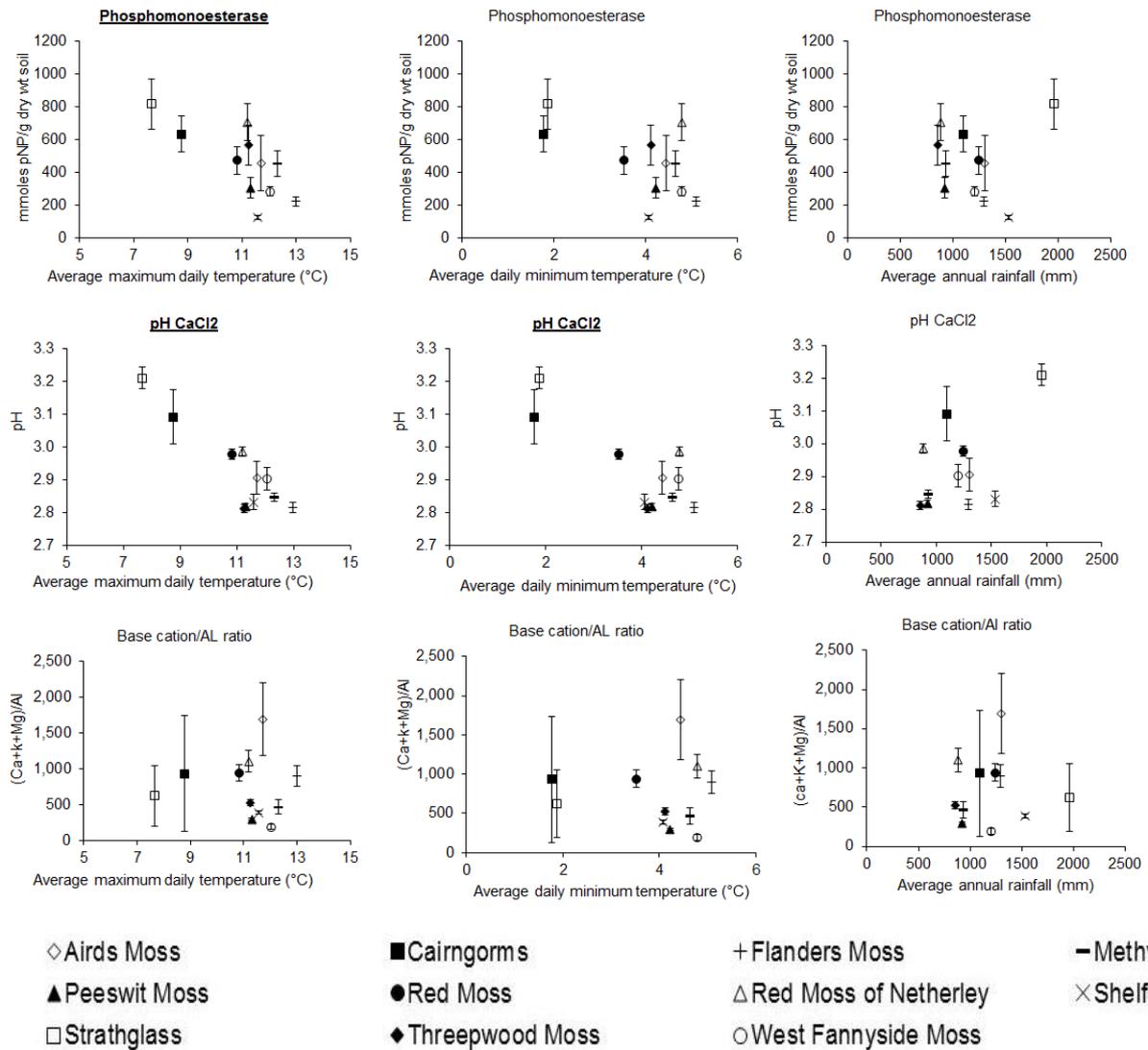


Figure 14 Relationship between 3 soil indicators and climate. Graph titles in bold and underlined indicate a significant relationship.

4.7 Multivariate analysis of soil indicators

PCA analysis of the soil indicators (Fig. 15) accounted for 54% of the variation in the soil indicators on the first axis. The first axis was correlated with increased soil pH and increased phosphomonoesterase. Generally samples from the Cairngorms and Strathglass had higher values of these soil indicators and hence occurred towards the positive end of the first axis. Some of the samples from Shelforkie Moss and Flanders Moss were at the negative end of the first axis and hence correlated with low levels of phosphomonoesterase and pH (see Section 5 for discussion on relationship between phosphomonoesterase and pH and water logged conditions). The second axis explained 32% of the variation and was correlated with an increase in the base cation/Al ratio. Individual samples from Airds Moss (samples AM1, AM5), Cairngorms (sample CM4) and Strathglass (sample SG1) were most closely correlated with high base cation/Al ratio. However when the analysis was repeated without these potential outliers a similar relationship was found (Fig. 16) with samples from Strathglass and the Cairngorms having high pH and phosphomonoesterase and samples from Shelforkie Moss and Flanders Moss having low values of these indicators. Red Moss and Red Moss of Netherley had higher base cation/Al ratio than the other sites. GLMM analysis of pH and phosphomonoesterase confirmed the significant correlation between these two indicators ($F_{1,25}=16.7$, $P<0.001$) (Fig. 17).

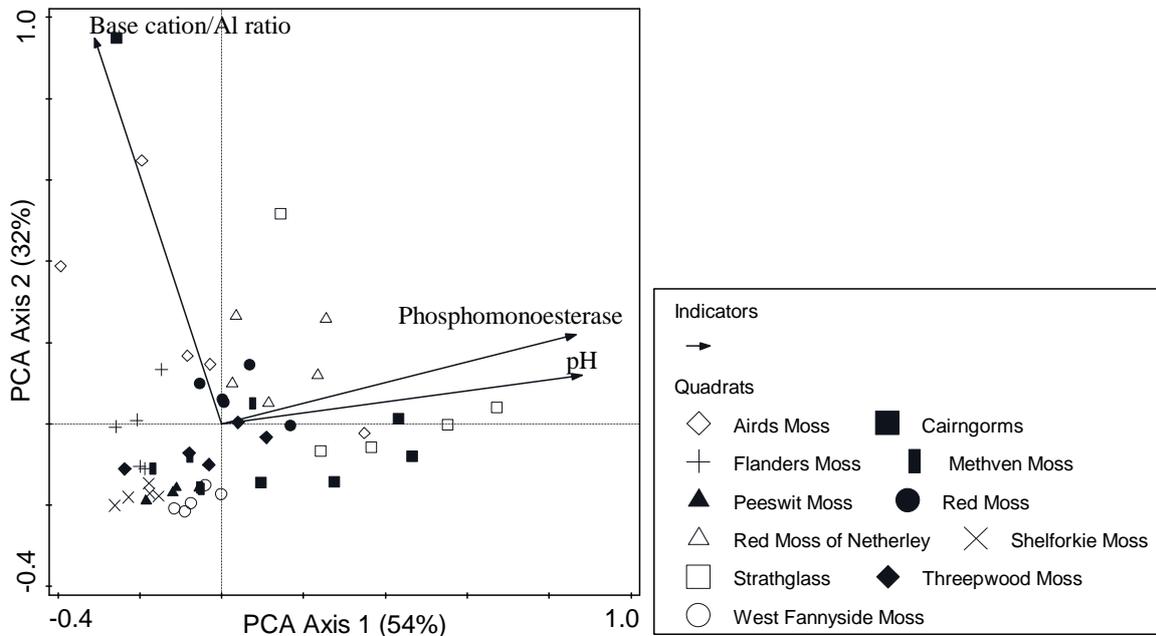


Figure 15 Ordination diagram from PCA of soil indicators

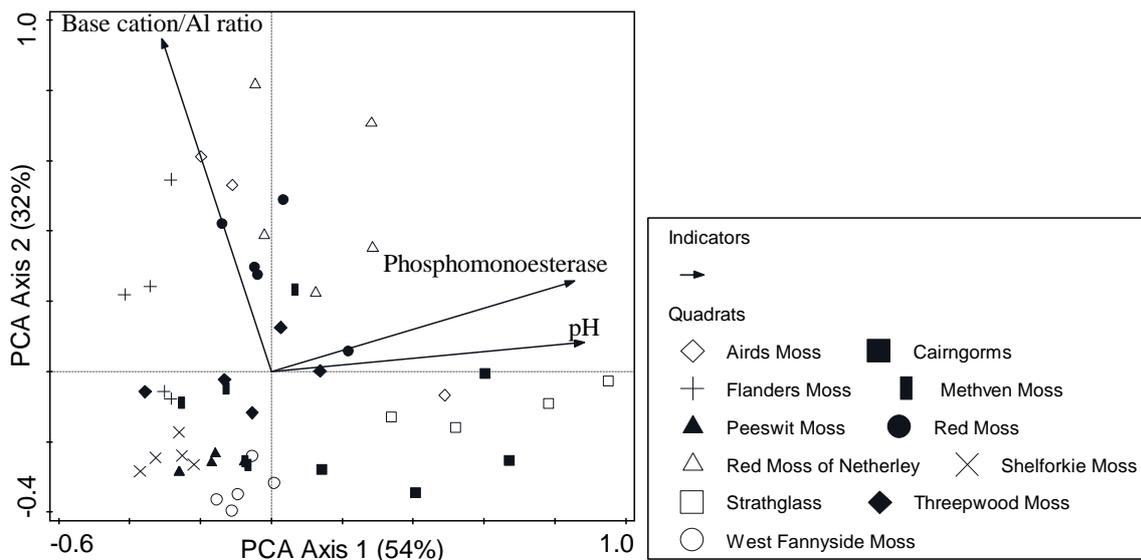


Figure 16 Ordination diagram from PCA of soil indicators without samples CM4, AM2, AM5, SG1.

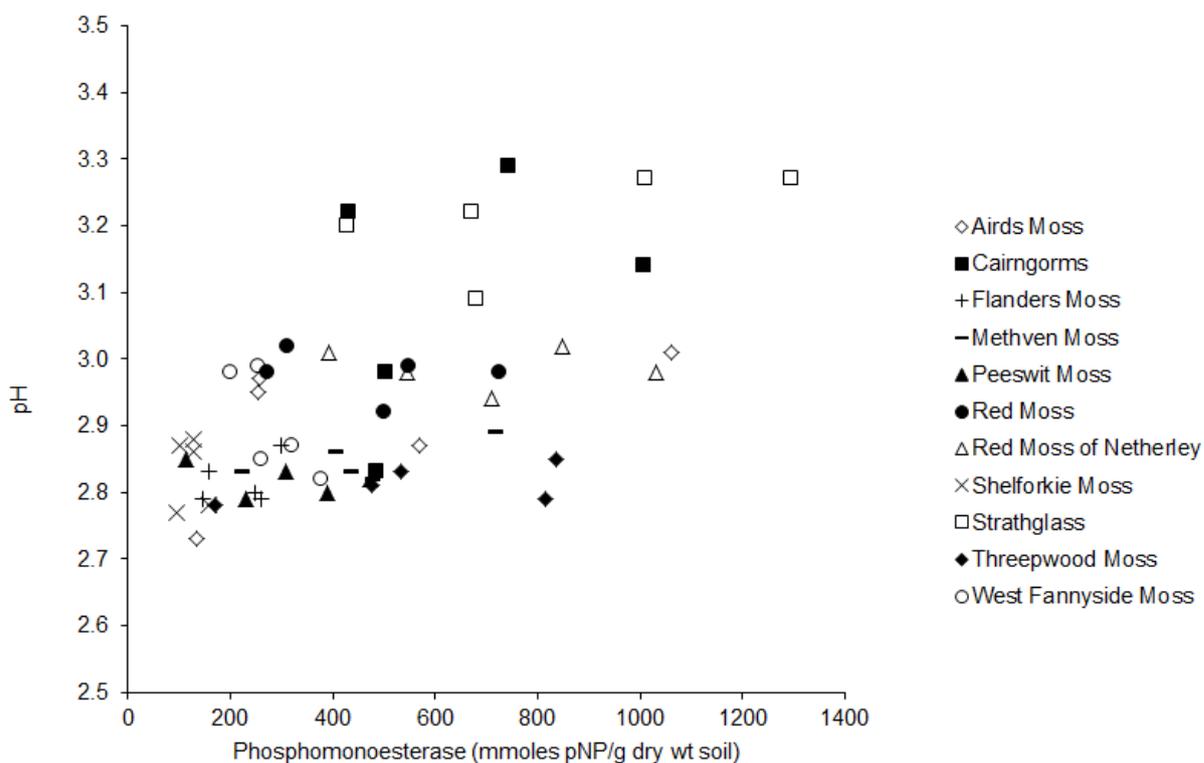


Figure 17 Correlation between pH and phosphomonoesterase

An RDA of the soil indicators with current and cumulative pollution variables included as explanatory variables explained 24% of the variation along the first axis and 4% along the second axis (Fig. 18). NH_y and cumulative SO_x were the only variables from this group that were significant in explaining the variation in the soil indicators. Samples from Strathglass and Cairngorms were negatively correlated with high levels of NH_y and had high soil pH. Samples from West Fannyside Moss and Threepwood Moss had high levels of NH_y and

lower values of pH. Samples from Red Moss, Flanders Moss and Shelforkie Moss had high levels of cumulative SO_x and low levels of phosphomonoesterase and base cation/Al ratio. Red Moss of Netherley, Airds Moss and Methven Moss had low levels of cumulative SO_x and higher levels of phosphomonoesterase.

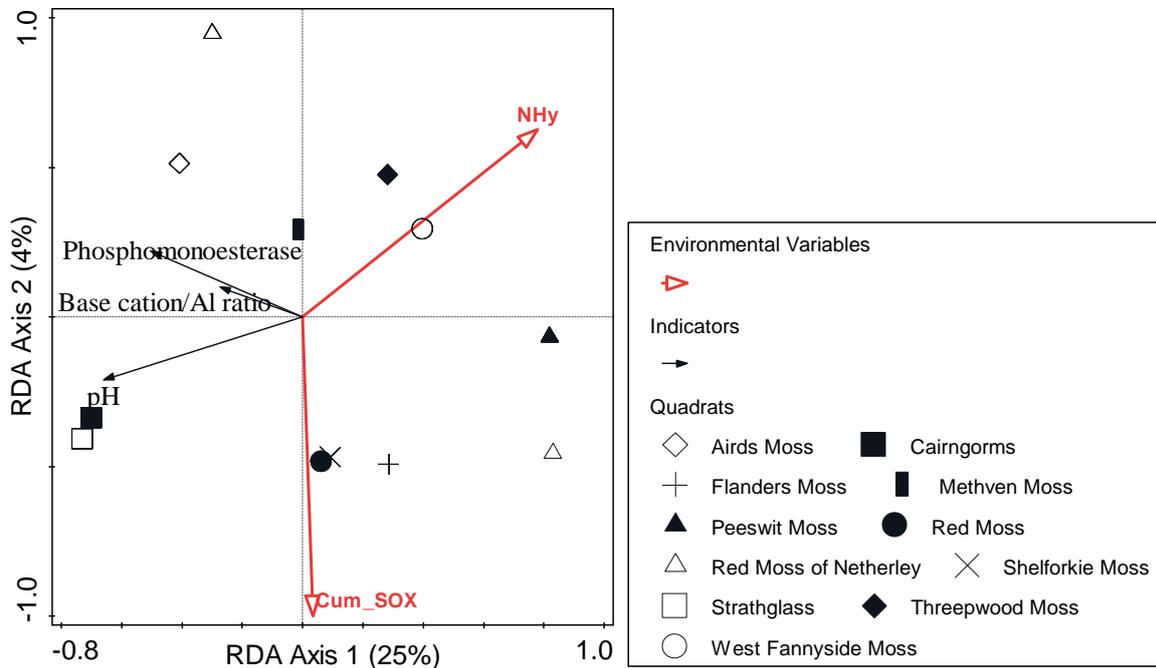


Figure 18 Ordination diagram from RDA of soil indicators with current and cumulative pollution variables as explanatory variables, only significant variables included

An RDA of the soil indicators with climate variables included as explanatory variables explained 29% of the variation along the first axis. Maximum average daily temperature was the only climate variable that was significant in explaining the variation in the soil indicators. Soil pH and phosphomonoesterase increased as maximum temperature declined (Fig. 19). Samples from Strathglass and the Cairngorms were correlated with low maximum temperatures and higher levels of phosphomonoesterase and pH. The second axis of the RDA separated out the 4 quadrats (Cairngorms Quadrat 4, Airds Moss Quadrats 2 and 5 and Strathglass Quadrat 1) known to have higher base cation/Al ratios than the other quadrats. As this second axis was not correlated with any climatic variables these 4 outliers are not affecting the relationship between maximum temperature and soil pH and phosphomonoesterase.

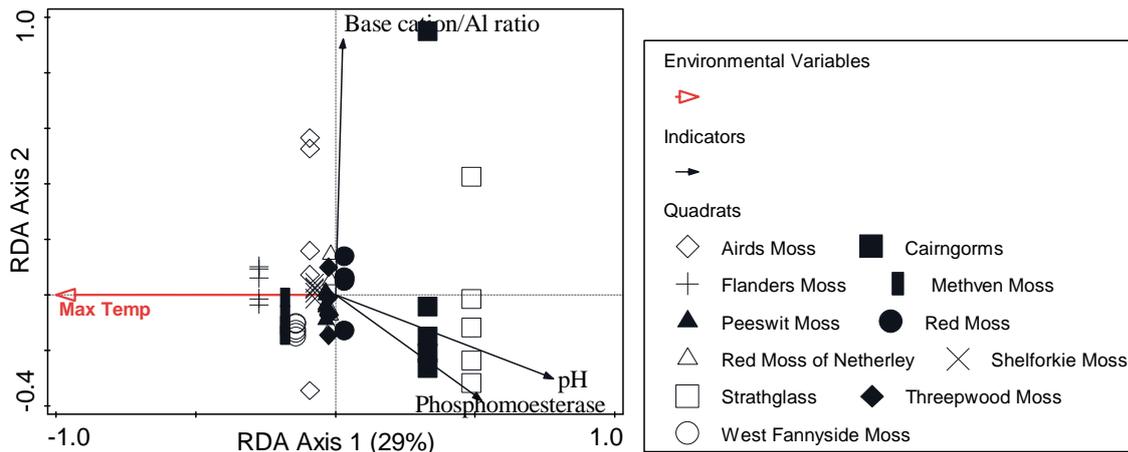


Figure 19 Ordination diagram from RDA of soil indicators with climatic variables as explanatory variables, only significant variables included. Note as only one climatic variable was significant the second axis is unconstrained (not correlated with any climatic variables)

4.8 Vegetation indicators v current pollution, cumulative pollution and climate

The four vegetation indicators (species richness, Shannon diversity index, cover weighted Ellenberg N and cover weighted Ellenberg R) were tested against each of the current pollution, cumulative pollution and climate variables in the same way as the soil indicators were (Figures 20, 21 & 22). The only significant relationships were between cover weighted Ellenberg R and the current pollution levels for NO_x ($F_{1,9} = 11.7$, $P < 0.05$) and SO_x ($F_{1,9} = 6.07$; $P < 0.05$). These relationships were strongly influenced by the results for West Fannyside Moss, without the data from this site there would have been no significant relationship.

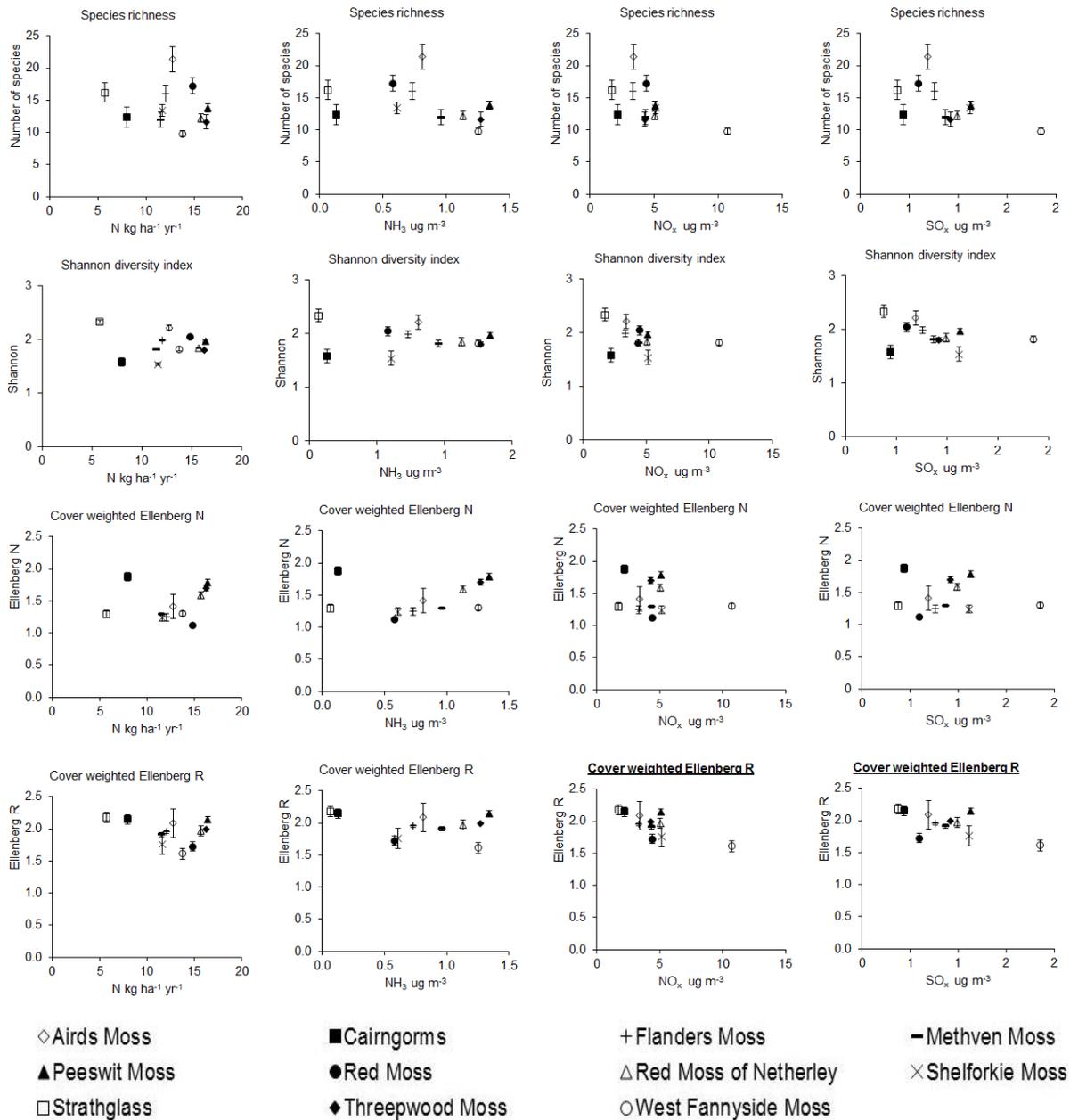


Figure 20 Relationship between 4 vegetation indicators and current pollution levels. Graph titles in bold and underlined indicate a significant relationship.

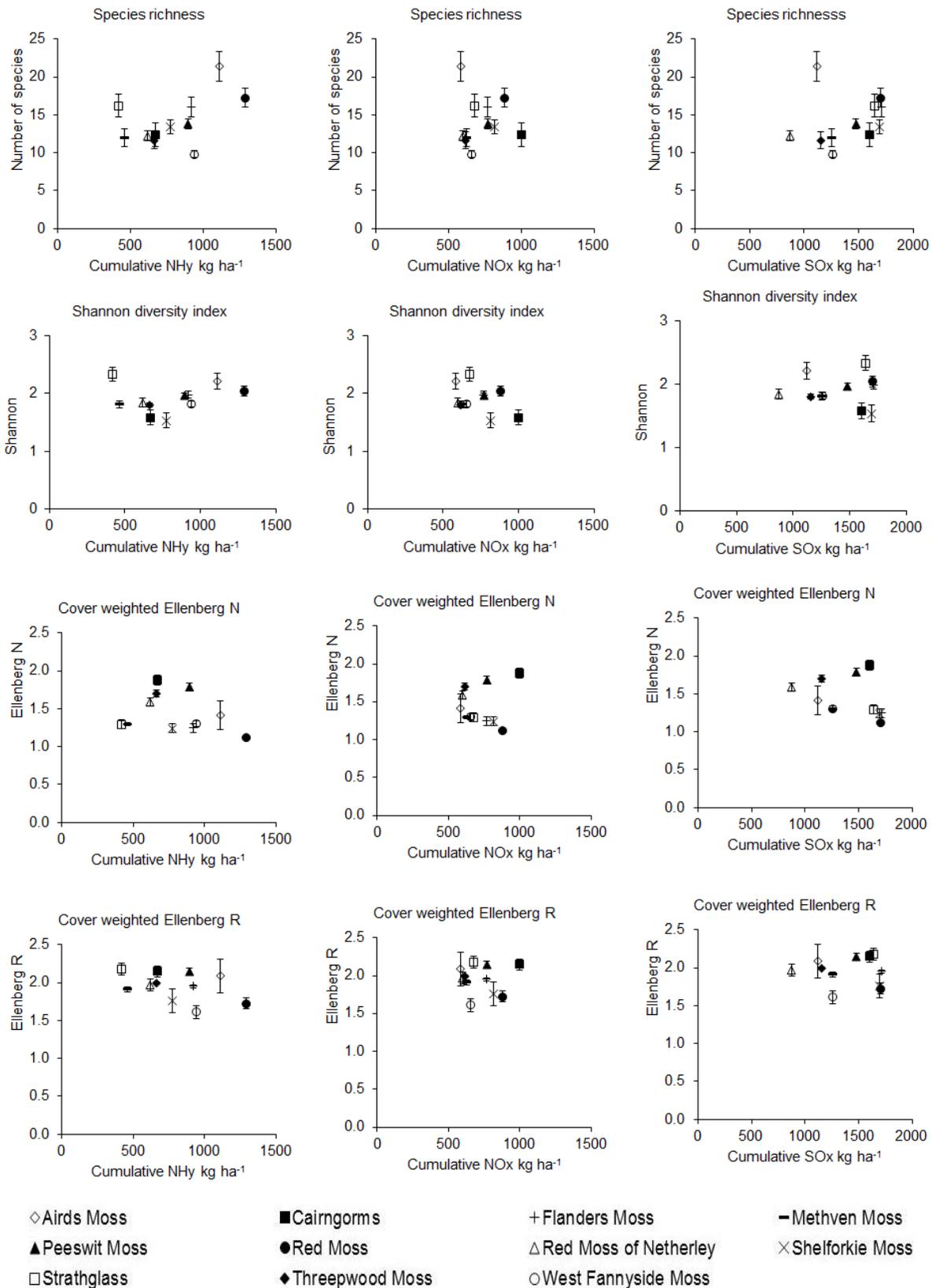


Figure 21 Relationship between 4 vegetation indicators and cumulative pollution levels. None of the relationships were significant.

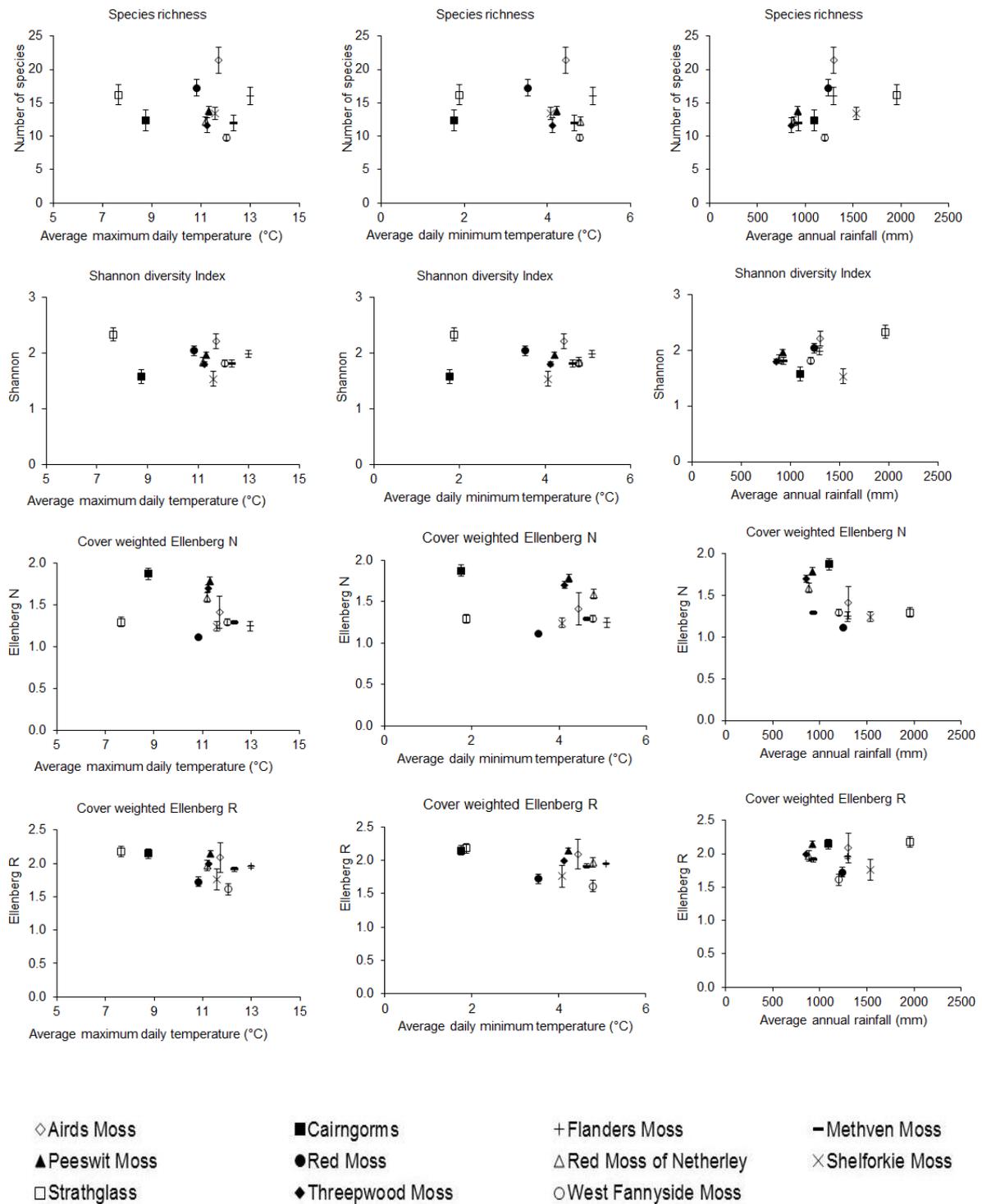


Figure 22 Relationship between 4 vegetation indicators and climate. None of the relationships were significant.

4.9 Multivariate analysis of the vegetation

DCA of the vegetation explained 14% of the variation along the first axis and 8% along the second axis. Samples clustered together by sites with samples from Strathglass being most dissimilar from the other sites, separated out from the other sites at the far positive end of the first axis (Fig. 23) and associated with *Eriophorum angustifolium*, *Cladonia portentosa* and *Trichophorum cespitosum* (Fig. 24). Threepwood Moss and Peeswit Moss had very similar vegetation with their samples clustering together (the two sites are close together geographically too). Shelforkie Moss and Methven Moss were also similar to each other in vegetation species composition with their plots clustering together in the ordination diagram and the two sites were close together geographically.

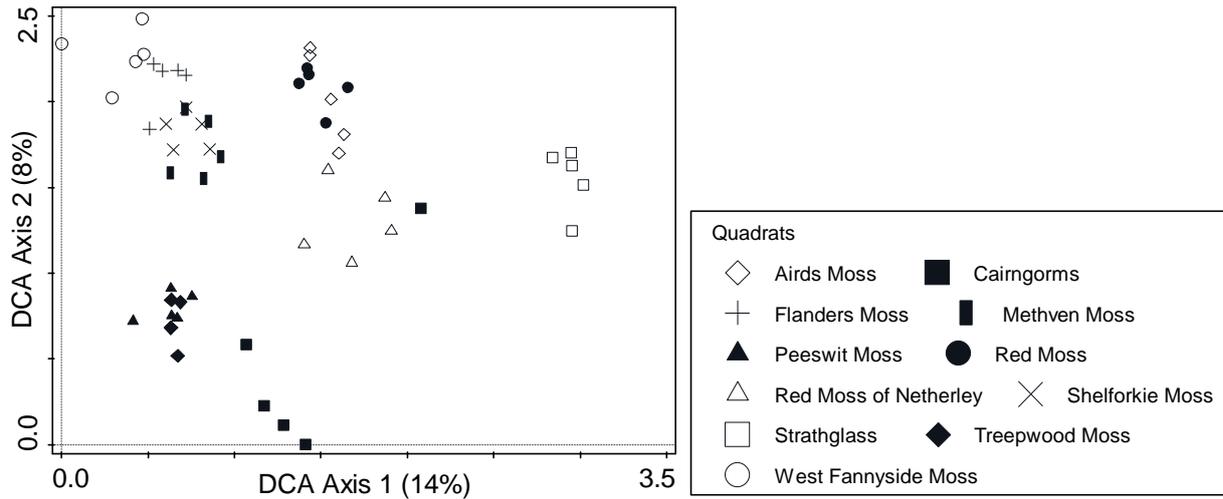


Figure 23 Ordination of samples from DCA of vegetation

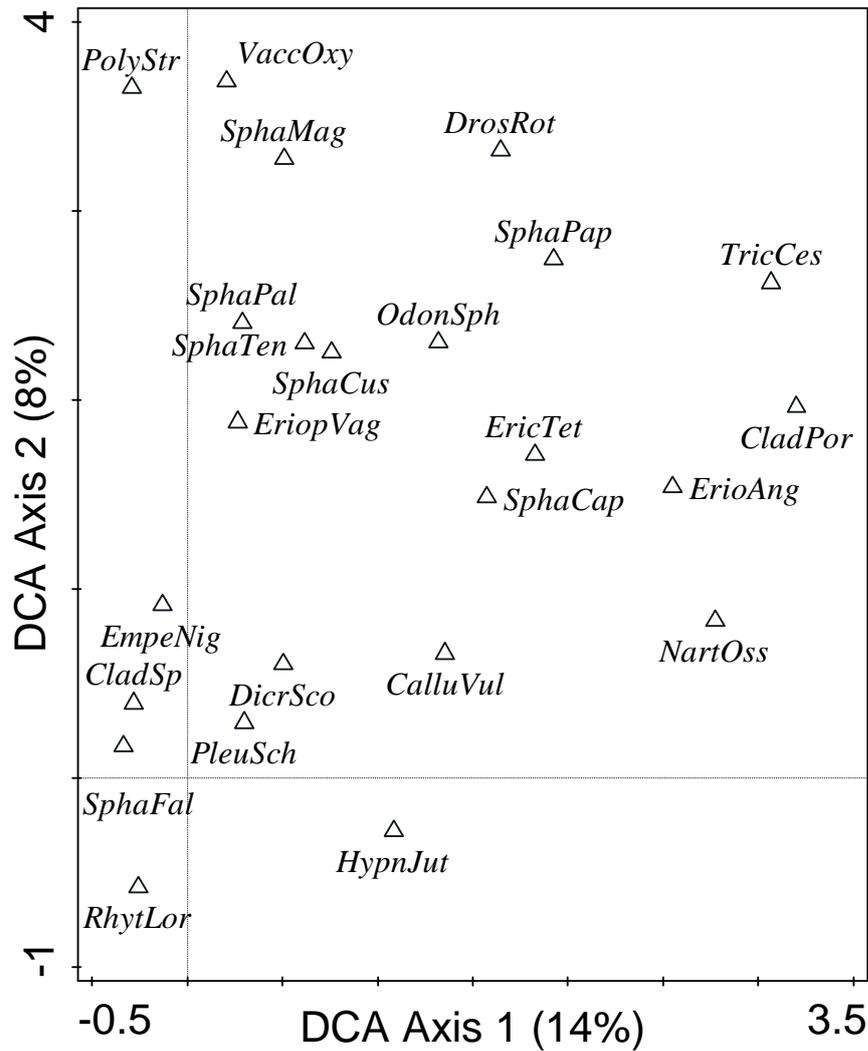


Figure 24 Ordination of species from DCA of vegetation. (See Table 5 for species codes). Only those species with a weight of 10% or more are included in the diagram.

Table 5 Species codes used in ordination diagrams

Species name	Species code used in ordination diagrams
<i>Calluna vulgaris</i>	CalluVul
<i>Cladonia portentosa</i>	CladPor
<i>Cladonia sp.</i>	CladSp.
<i>Dicranum scoparium</i>	DicrSco
<i>Drosera rotundifolia</i>	DrosRot
<i>Empetrum nigrum</i>	EmpeNig
<i>Erica tetralix</i>	EricTet
<i>Eriophorum angustifolium</i>	ErioAng
<i>Eriophorum vaginatum</i>	EriopVag
<i>Hypnum jutlandicum</i>	HypnJut
<i>Narthecium ossifragum</i>	NartOss
<i>Odontoschisma sphagni</i>	OdonSph
<i>Pleurozium schreberi</i>	PleuSch
<i>Polytrichum strictum</i>	PolyStr
<i>Rhytidiadelphus loreus</i>	RhytLor
<i>Sphagnum capillifolium</i>	SphaCap
<i>Sphagnum cuspidatum</i>	SphaCus
<i>Sphagnum fallax</i>	SphaFal
<i>Sphagnum magellanicum</i>	SphaMag
<i>Sphagnum palustre</i>	SphaPal
<i>Sphagnum papillosum</i>	SphaPap
<i>Sphagnum tenellum</i>	SphaTen
<i>Trichophorum cespitosum</i>	TricCes
<i>Vaccinium oxycoccos</i>	VaccOxy

In a CCA analysis of the vegetation with current and cumulative pollution as explanatory variables NO_x was the only variable that explained a significant amount of the variation in the vegetation composition (Fig. 23). West Fannyside Moss was shown as being very different to the other sites with a much higher level of NO_x. Cairngorms had the lowest levels of NO_x. Most species were associated with lower levels of NO_x but *Sphagnum tenellum*, *Sphagnum magellanicum* and *Sphagnum papillosum* were associated with higher levels of NO_x than most other species (Fig. 24).

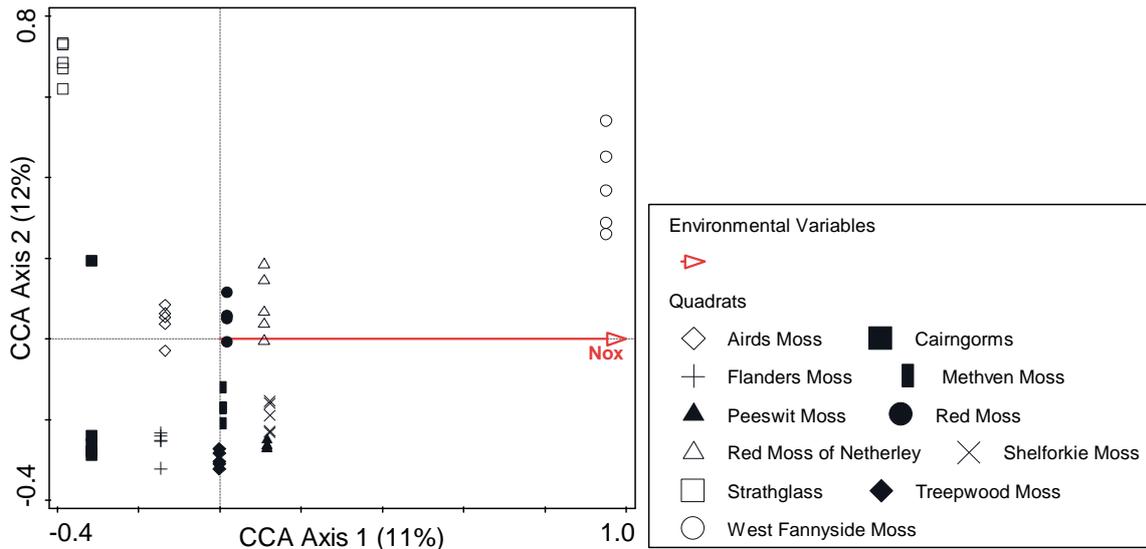


Figure 25 Ordination of samples from CCA of vegetation with current and cumulative levels of pollution as explanatory variables. Only those variables that were significant are included. Note as only one pollution variable was significant the second axis is unconstrained (not correlated with any climatic variables)

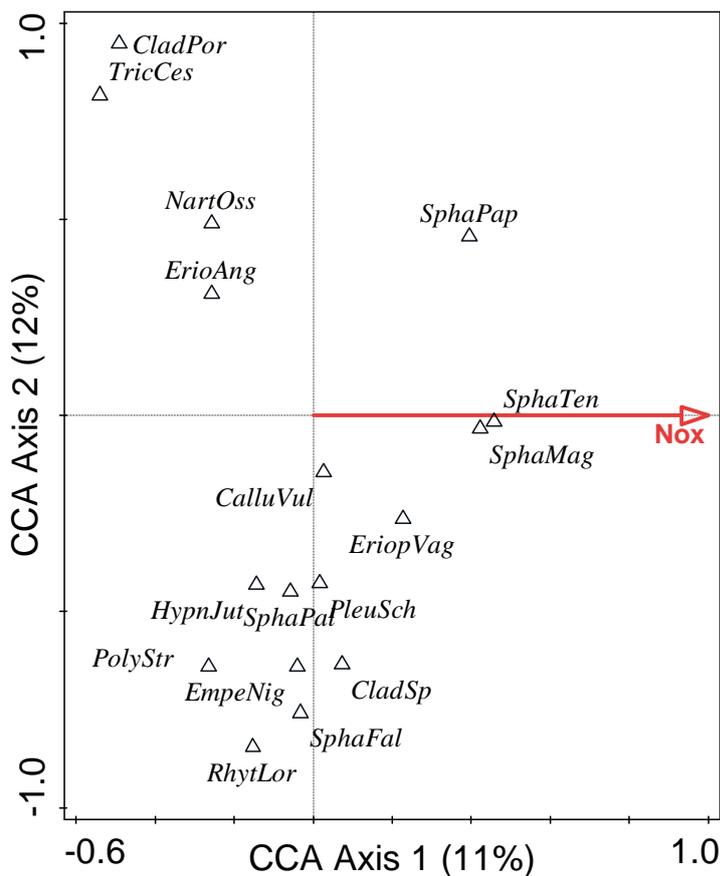


Figure 26 Ordination of species from CCA of vegetation (See Table 5 for species codes). Only those species with a weight of 10% or more are included in the diagram.

In a CCA analysis of the vegetation composition with climate as explanatory variables average maximum daily temperature and average annual rainfall were both significant in explaining the variation in vegetation composition (Figs. 27 and 28). This analysis differs from that in 4.7 where the vegetation indicators were analysed against climate. This analysis is based on species percentage cover data and shows that while indicators such as species richness, Shannon and Ellenberg R and N were not related to climate the actual species composition is related to climate. *Eriophorum angustifolium*, *Narthecium ossifragum* and *Trichophorum cespitosum* were more abundant at sites with higher annual rainfall. *Polytrichum strictum*, *Sphagnum cuspidatum* *Sphagnum tenellum* and *Vaccinium oxycoccos* were more abundant at sites with a higher average daily maximum temperature.

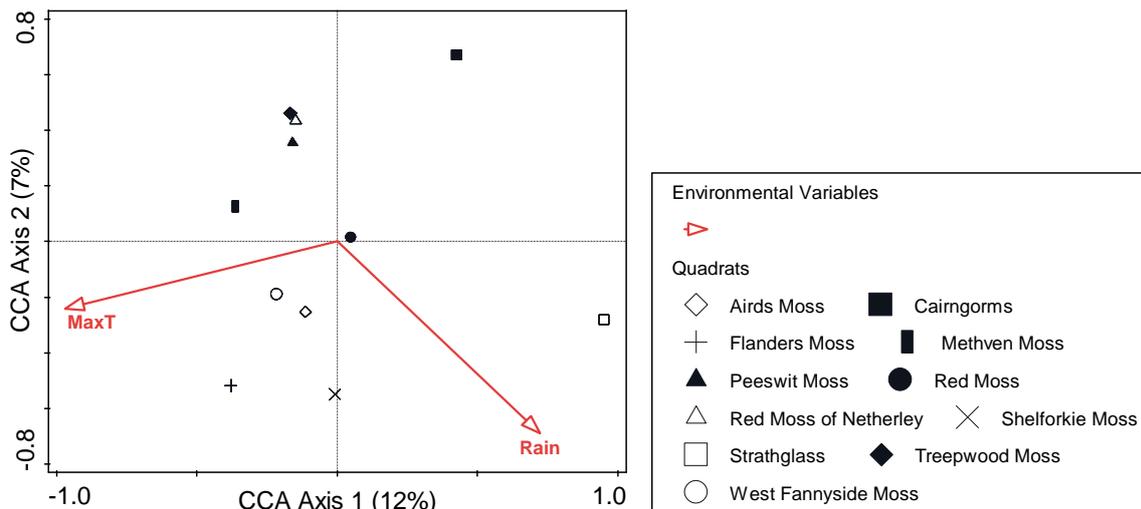


Figure 27 Ordination of samples from CCA of vegetation with climate as explanatory variables. Only those variables that were significant are included.

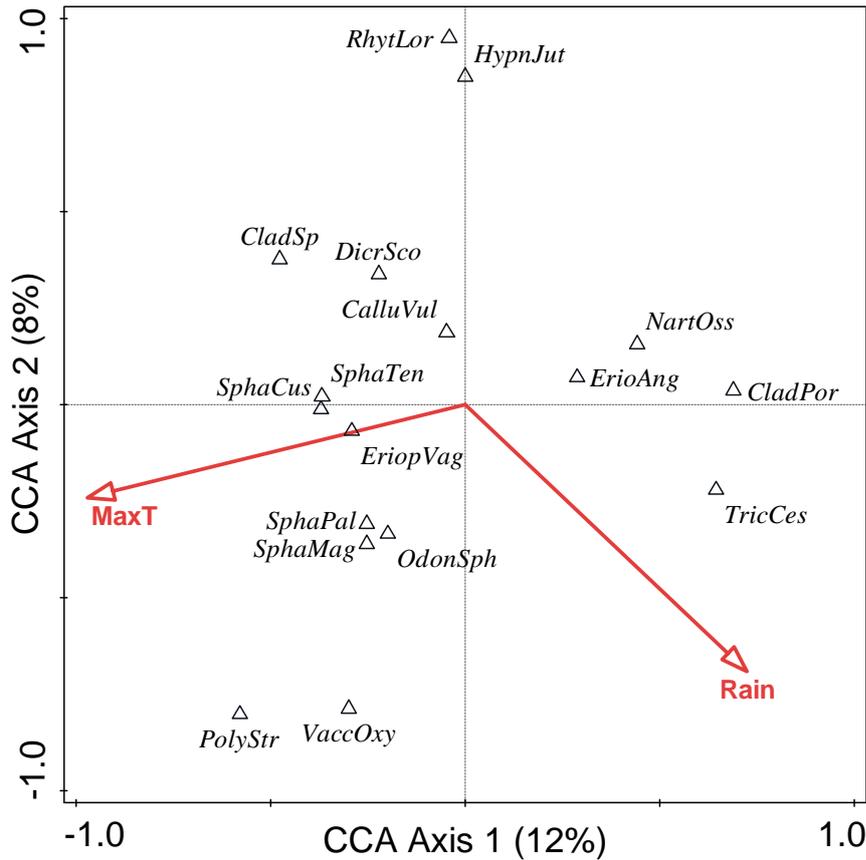


Figure 28 Ordination of species from CCA of vegetation (See Table 5 for species codes). Only those species with a weight of 10% or more are included in the diagram.

4.10 Soil indicators v vegetation indicators

The two types of indicators (vegetation and soil) were analysed together to assess if there was a relationship between them (Fig. 29). There was a significant relationship between vegetation species richness and phosphomonoesterase ($F_{1,53} = 5.46$; $P < 0.05$) and base cation/Al ratio ($F_{1,53} = 8.4$; $P < 0.01$). In each case, species richness declined as phosphomonoesterase or base cation/Al ratio increased. However these relationships were driven by a few quadrats. When Quadrats 1 and 2 from Airs Moss, which had high species richness and low phosphomonoesterase activity and Quadrat 2 from Strathglass with had medium species richness and high phosphomonoesterase activity were removed from the dataset this relationship was no-longer significant. The relationship between species richness and base cation ratio was entirely due to the outlier, Quadrat 4 from the Cairngorms. When this quadrat was removed from the dataset this relationship was no-longer significant. It is therefore unlikely that these two relationships are valid.

There was also a significant relationship between cover weighted Ellenberg R score and soil pH ($F_{1,46} = 7.42$; $P < 0.01$) with the Ellenberg R score increasing with soil pH. This is expected as higher Ellenberg R scores indicate a preference for higher soil pH.

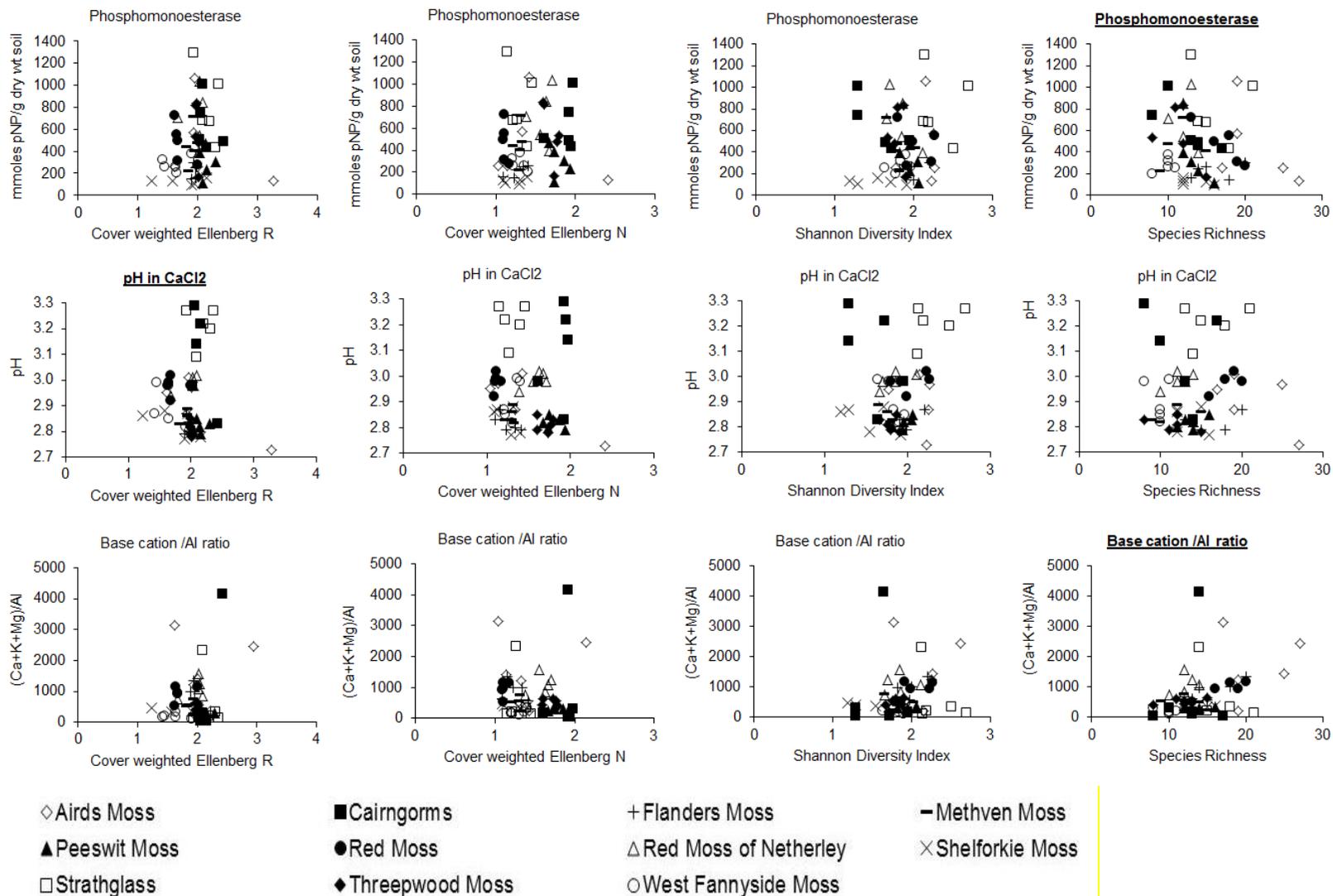


Figure 29 Relationship between 4 vegetation indicators and 3 soil indicators. Graph titles in bold and underline indicate significant relationships, but note that some of these relationships were driven by a few quadrats – see text.

4.11 Explaining the variation in soil indicators

Plant species composition, climate, current and cumulative pollution will influence the variation in the soil indicators. Variation partitioning using multivariate statistics was used to assess how much of the variation in the soil indicators was explained by these three groups of variables. The pollution variables NH_y and cumulative SO_x explained a significant amount (5%) of the variation in the soil indicators independent of the vegetation or the climate. Average maximum daily temperature also explained a significant amount of the variation (6%) independent of the vegetation and pollution variables. Vegetation did not explain a significant unique amount of variation in addition to pollution or climate variables. By far the greatest degree of variation in the soil indicators (18%) was jointly explained by all three groups of variables: pollution, vegetation and climate.

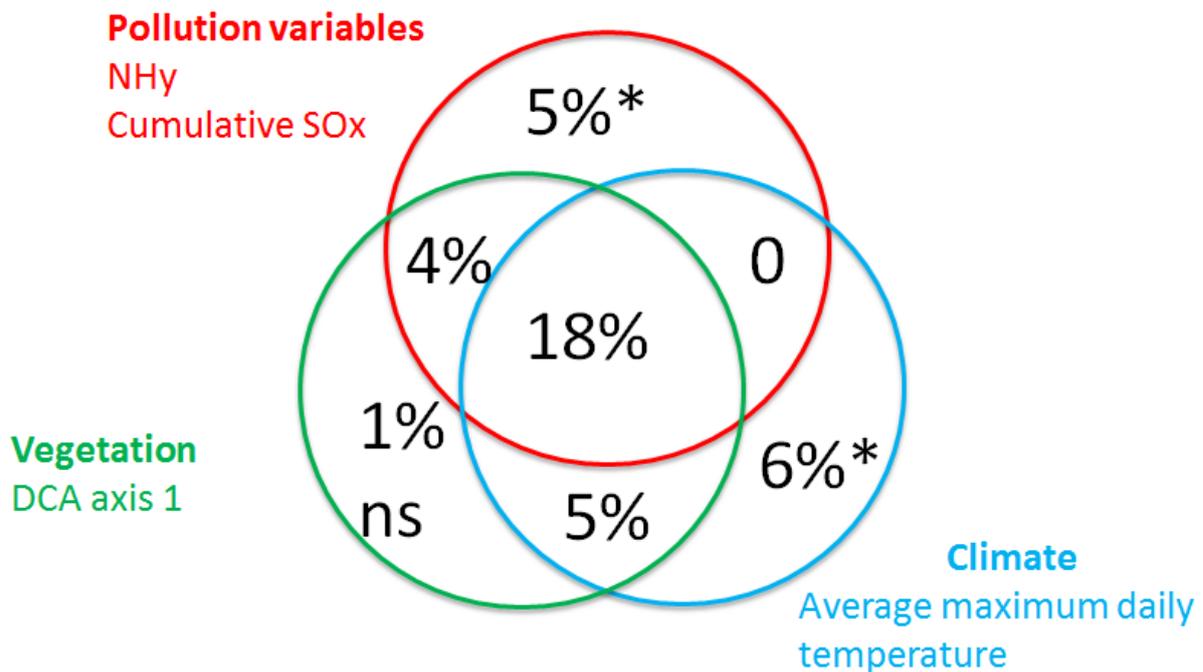


Figure 30 Partitioning the variation in soil indicators between three groups of variables: pollution, climate and vegetation. ns = not significant and * = significant.

5 DISCUSSION

5.1 Do the soil indicators show a response to N-deposition?

Of the three soil indicators tested only soil pH shows any consistent relationship to current pollution levels (both total N and NH₃). However when climate was taken into account only NH₃ showed any correlation with soil pH. However this relationship was solely due to two sites (Cairngorms and Strathglass) and differences in climate and underlying geology may be influencing the soil pH (see below). Further work would be needed to confirm if soil pH is a robust indicator of ammonia impacts across multiple sites with different geology. Phosphomonoesterase was correlated with cumulative NH₃ but once climate was taken into account this relationship was no longer significant. Thus the soil indicators were found to only have a very limited response to N deposition across these sites.

5.2 Is the response to N-deposition in accordance with the findings from Whim Moss (see project phase II)? If not what might be the reasons/factors?

The response of the indicators to N-deposition is far weaker than that expressed from the Whim Moss project. This is likely to be for several reasons.

- The N gradient across the sites assessed here was far shorter than that at the Whim Moss experiment. This work covered the range 5.7-16.2 kg N ha⁻¹ yr⁻¹ while the Whim Moss experiment ranged from 8 to 64 kg N ha⁻¹ yr⁻¹. In the final methodology recommended by Phase 2 Point 5 of Annex A has the following statement “Indicators are unlikely to show a response to N deposition if the difference between the high and low N deposition is less than 15-40 kg N ha⁻¹ yr⁻¹.” This work concurs with the Phase 2 report in that as expected the N gradient along which the soil samples were taken was too small to show a statistically significant effect.
- The Phase 2 samples were all taken from one site so there was no variation in climate to take into account.
- The Phase 2 samples were all taken from one site so there was no variation in geology to take into account. The British Geological Society (Geology Viewer of Britain <http://mapapps.bgs.ac.uk/geologyofbritain/home.html>) indicates that the sites are on the following deposits and bedrock types:
 - Airds Moss: peat over limestone
 - Cairngorm: peat over Glen Spean Subgroup which is dominantly siliceous, feldspathic and micaceous psammite with some quartzite.
 - Flanders moss peat over sandstone,
 - Methven moss: alluvium clay, silt and sand over sandstone with subordinate conglomerate, siltstone and mudstone
 - Peeswit moss: till diamicton over Scottish coal measures group - mudstone, siltstone, sandstone, coal, ironstone and ferricrete
 - Red moss: peat over Lanark group - sandstone and conglomerate
 - Red moss of Netherley: on boundary between peat and till diamicton over Argyll group composed of psammite, semipelite and pelite
 - Shelforkie Moss: Sand and gravel over Sandstone with subordinate conglomerate, Siltstone And Mudstone.
 - Strathglass: on the boundary between superficial peat /till - diamicton with the bedrock belongs to the Glenfinnan group - pelite
 - Threepwood Moss: peat over Gala Group – Wacke (a sedimentary bedrock)
 - West Fannyside Moss: peat over Clackmannan Group (a sedimentary bedrock).

These differences in geology will influence the soil pH. They may explain the unexpected relationship between climate and soil pH and suggest that further investigations with respect to the robustness of the relationship between soil pH and ammonia deposition is required.

- Differences in hydrology between the sites may result in differences in pH. The surveyors noted that Cairngorms and Strathglass had a flow of water through them compared to the other sites which were in hollows. This seepage of water may result in flushed areas and hence slightly higher pH.
- These soil indicators were originally developed to test for the impact of point source pollution over one site, this study is the first time they have been tested against diffuse pollution over a range of sites and the results suggest that these soil indicators are not suitable for assessment where there are large differences in climate and relatively small differences in N deposition.
- The multivariate analysis of the soil indicators showed that pH and phosphomonoesterase were correlated. Several other studies have shown similar relationships between phosphomonoesterase activity and soil pH, for example in permanent meadow (Traser-Cepeda & Gil-Sotres, 1987) and in ancient/modern woodland/forest systems with more ancient soil having a higher activity than a modern post-agricultural system (Orczewska et al., 2012). Activities of other soil enzymes have been shown to be correlated (positively & negatively) with soil pH (Sinsabaugh et al., 2008). Generally enzyme activity is inhibited by high acidity. Given the range of pH present at the different sites there may have been an interaction between these two indicators which could further mask N impacts.
- It was noted by the soil surveyors that Flanders Moss and Shelforkie Moss often had very deep layers (>30cm) of live sphagnum moss; the depth at which relatively humified peat was reached to take soil samples was often below the water level. Thus these samples were taken from water logged and therefore anaerobic conditions. Anaerobic conditions are likely to have affected biological measurements: the phosphomonoesterase. Most phosphomonoesterase activity is recorded in the first few centimetres of a soil profile (e.g. Juma & Tabatabai, 1978; Pang & Kolenko, 1986) decreasing sharply with depth. This may be influenced by the nature of the mineral content/underlying geology, plant type (Harrison, 1983), Al^{3+} (Trasar-Cepeda & Gil-Sotres, 1987) and organic C content (which may be correlated with organic P in acidic soils) (Juma & Tabatabai, 1978; Margesin & Schinner, 1994; Orczewska et al., 2012) and P (Margesin & Schinner, 1994). Most phosphomonoesterase activity seems to be found within the litter layer and is thought to be from fungal and plant sources rather than bacterial. The restriction of activity to the litter layer is not so surprising where root activity is limited at depth. A waterlogged (anoxic) system where there is limited fungal and/or plant-root activity but more bacterial activity would therefore be expected to have low levels of phosphomonoesterase activity. Figure 6 shows that phosphomonoesterase levels were lowest at Flanders Moss and Shelforkie Moss and this is the likely cause. At Whim Moss (for the Phase 2 work) there was only a thin layer of litter and sphagnum to remove before relatively humified peat was reached.
- All the sites had a history of previously high historical pollution (both nitrogen and sulphur), these systems will therefore have already changed making it hard to pick up differences due to current background pollution levels. Changes due to a point source pollution can however be observed against background high historical pollution levels, as seen in Phase 2.
- The variation partitioning results (Fig. 30) highlights the biggest problem in using these soil indicators: that most of the variation in them is jointly explained by vegetation, climate and pollution, thus it is very hard to pick out a significant signal of an impact of N pollution alone and be sure it is due to N pollution and not due to differences in climate or vegetation.

5.3 Is there a relationship between the vegetation indicators (e.g. species composition, cover-weighted Ellenberg N) and the soil indicators?

There was a significant relationship between vegetation species richness and phosphomonoesterase and base cation/Al ratio and between cover weighted Ellenberg R score and soil pH. The relationship between soil pH and Ellenberg R is to be expected as Ellenberg R is a measure of which plants can grow in acidic conditions. The relationships between species richness and phosphomonoesterase and base cation/Al ratio were entirely due to a few outlying data points driving the results and at this stage should not be treated as significant relationships, unless proved by further research.

5.4 How can the soil indicators be used for the interpretation of site impacts from N deposition?

Given the huge overlap between vegetation, climate and N pollution in explaining the variation in soil indicators these results suggest that it is difficult to use these soil indicators to interpret the impact of N deposition at the sites.

5.5 Could the soil indicators potentially be used as an “early warning system”?

The soil indicators showed little response to N deposition with only pH responding to NH_3 having the potential to be an early warning system to measure the impact of N pollution. However the soil indicators performed no worse than the vegetation indicators in terms of acting as assessment of N impacts. Of the four vegetation indicators studied only one, cover weighted Ellenberg R was significantly affected by N deposition (NO_x).

5.6 What benefits would a repetition of the soil monitoring provide? What time scales would be reasonable?

The data collected from this study cannot be used to give a statistical analysis of the frequency with which such monitoring would be required to detect change. However given the limited relationship found in this study between the soil indicators and N pollution it is suggested that this monitoring is not worth repeating at these sites and hence the question of benefits and timescales for repeated sampling does not arise.

5.7 Next steps

While acknowledging that it is disappointing that the soil indicators did not show a clear relationship to N pollutions at these sites this was always a possibility (see above) as the indicators had not originally been suggested for monitoring of diffuse pollution. This work suggests that these soil indicators are indeed not suitable for monitoring impacts of diffuse pollution although earlier work (phase 2) suggests they may be useful in monitoring impacts of point sources of pollution. It is suggested that any future work in developing these indicators should be targeted at monitoring the impact of point source pollution. In addition future methodology should be modified to record the depth of litter/sphagnum removed in the field before the consolidated peat is reached as this may help explain variation between samples/sites.

6 CONCLUSION

- Soil pH measured in CaCl_2 has the potential to be an indicator of current NH_3 deposition even given variation between sites in climate, but the robustness of this as an indicator across multiple sites requires further investigation particularly in relation to the impact of the underlying geology.
- Cover weighted Ellenberg R has the potential to be an indicator of current NO_x deposition levels.
- There is the potential to use the three soil indicators as one combined indicator to assess impacts over time of current NH_3 levels and cumulative SO_x using multivariate statistics. If the soil was resampled and data from both time points included in the ordination the relative position of the points in the ordination could be compared to assess changes over time. Such a method should be tested over a greater N gradient before being tested at diffuse pollution sites such as these.
- Vegetation species composition data was not strongly influenced by N pollution but was impacted by current NO_x levels.
- The variation in the soil indicators is jointly explained by vegetation, climate and pollution, thus it is very hard to pick out a significant impact of N pollution alone and be sure it is due to N pollution and not due to differences in climate or vegetation.
- There are many other factors attributing to the variation in the soil indicators that were not taken into account in this study. Only 39% of the variation in the soil indicators was explained by the vegetation, climate and pollution.
- The soil indicators performed no worse than the vegetation indicators in terms of acting as assessment of N impacts. Of the four vegetation indicators studied only one, cover weighted Ellenberg R, was significant affected by N deposition (NO_x), and only one of the soil indicators, pH, was significant once climate had been taken into account.
- Future work should measure the depth of litter/sphagnum removed before consolidated peat is reached and take this into account in the statistical analysis.
- Future work should investigate the impact of depth of litter/sphagnum removed before consolidated peat is reached and depth of water table on indicators, particularly in relation to phosphomonoesterase.
- These soil indicators were originally developed to test for the impact of point source pollution over one site, this study is the first time they have been tested against diffuse pollution over a range of sites and the results suggest that these soil indicators are not suitable for this type of work where there are large differences in climate and only small differences in N deposition.
- This study concurs with suggestions from previous studies that these soil indicators are only suitable to assess N pollution impacts along a large N gradient (difference between the high and low N deposition is at least $15\text{-}40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$).

7 REFERENCES

- Black, H.I.J., Britton, A.J., Helliwell, R.C., Langan, S.J., Taylor, A.F.S. & Booth P.D., (2009). *To establish soil indicators to assess the impact of atmospheric deposition on environmentally sensitive areas*. Consultancy Service, SEPA.
- Cosby, B.J., Hornberger, G.M., Galloway, J.N. & Wright, R.F. (1985) Modelling the effects of acid deposition; Assessment of a lumped parameter model of soil water and stream water chemistry. *Water Research*, 21, 51-63.
- Fournier, N., Dore, A.J., Vieno, M., Weston, K.J., Dragosits, U. & Sutton, M.A. (2004) Modelling the deposition of atmospheric oxidised nitrogen and sulphur to the United Kingdom using a multi-layer long-range transport model. *Atmospheric Environment*, 38, 683-694.
- Fournier, N., Pais, V.A., Sutton, M.A., Weston K.J., Dragosits U., Tang Y.S. & Aherne, J. (2003). Parallelization and application of a multi-layer atmospheric transport model to quantify dispersion and deposition of ammonia over the British Isles. *Environmental Pollution*, 116, 95-107.
- Fowler, D., O'Donoghue, M., Muller, J., Smith, R., Dragosits, U., Skiba, U., Sutton, M. & Brimblecombe, P., (2004). A chronology of nitrogen deposition in the UK between 1900 and 2000. *Water, Air, & Soil Pollution: Focus*, 4, 9–23.
- Harrison, A.F., (1983) Relationship between intensity of phosphatase activity and physico-chemical properties in woodland soils. *Soil Biology and Biochemistry*, 15, 93-99.
- Helliwell, R.C., Wright, R.F., Jackson-Blake, L.A., Ferrier, R.C., Aherne, J., Cosby, B.J.L., Evans, C.D., Forsius, M., Hruska, J., Jenkins, A., Kram, P., Kopacek, J., Majer, V., Moldan, F., Posch, M., Potts, J.M., Rogora, M. & Schopp, W. (2014) Assessing recovery from acidification of European surface waters in the year 2010: evaluation of projections made with the MAGIC model in 1995., *Environmental Science and Technology*, 48, 13280-13288.
- Hill, M.O., Preston, C.D. & Roy, D.B., (2004). *PLANTATT*. Attributes of British and Irish Plants: Status, Size, Life History, Geography and Habitats. Biological Records Centre, Centre for Ecology and Hydrology, Monks Wood, Cambridgeshire, UK.
- Juma, N.G. & Tabatabai, MA (1978) Distribution of phosphomonoesterases in soils. *Soil Science*, 126, 101-108.
- Margesin, R. & Schinner, F. (1994). Phosphomonoesterase, phosphodiesterase, phosphotriesterase, and inorganic pyrophosphate activities in forest soils in an alpine area: effect of pH on enzyme activity and extractability. *Biology and Fertility of Soils*, 18, 320-326.
- Mitchell, R.J., Beesley, L., Briggs, R., Cuthbert, A., Dawson, J.J.C., Helliwell, R.C., Hewison, R.L., Kerr, C., Leith, I.D., Owen, I.J., Potts, J.M., Newman, G., Smith, D., Shephard, L.J., Sturgeon, F., Taylor, A.F.S., White, D., Williams, A., Williams, E. & Black, H.I.J., (2013). *Testing soil quality indicators*. Scottish Environment Protection Agency Commissioned Report No.HP1108.
- Orczewska A., Piotrowska A. & Lemanowicz J. (2012) Soil acid phosphomonoesterase activity and phosphorous form in ancient and post-agricultural black alder [*Alnus glutinosa* (L.) Gaertn.] woodlands. *Acta Societatis Botanicorum Poloniae*, 81, 81-86 DOI:10.5586/asbp.2012.013
- Pang, P.C.K. & Kolenko, H. (1986) Phosphomonoesterase activity in forest soils. *Soil Biology and Biochemistry* 18, 35-40.
- Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N. Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S, Gallo, M. E., Gartner, T. B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H. (2008) Stoichiometry of soil enzyme activity at global scale. *Ecology Letters*, 11, 1252-1264. Doi: 10.1111/j.1461-0248.2008.01245.x

- Smith, R., Fowler, D., Sutton, M., Flechard, C. & Coyle, M., (2000). Regional estimation of pollutant gas dry deposition in the UK: Model description, sensitivity analyses and outputs. *Atmospheric Environment*, 34, 3757–3777.
- SNH site link: <http://gateway.snh.gov.uk/sitelink/index.jsp> (accessed January 2016)
- Ter Braak, C.J.F. & Smilauer, P. (2012) CANOCO reference manual and user's guide: software for ordination (version 5.0). . Microcomputer Power, Ithaca, NY, USA
- Trasar-Cepeda, M.C. & Gil-Sotres, F. (1987) Phosphatase activity in acid high organic matter soils in Galicia (NW Spain). *Soil Biology and Biochemistry*, 19, 281-287.

8 APPENDIX 1: SOIL INDICATOR DATA

Table 6 Codes used for soil data

Code	site	Quadrat	Sample ID
AM1	Airds_Moss	1	1221204
AM2	Airds_Moss	2	1221205
AM3	Airds_Moss	3	1221206
AM4	Airds_Moss	4	1221207
AM5	Airds_Moss	5	1221208
CM1	Cairngorms_Blanket_Bog	1	1221209
CM2	Cairngorms_Blanket_Bog	2	1221210
CM4	Cairngorms_Blanket_Bog	3	1221211
CM3	Cairngorms_Blanket_Bog	4	1221212
CM5	Cairngorms_Blanket_Bog	5	1221213
FM1	Flanders_Moss	1	1221214
FM2	Flanders_Moss	2	1221215
FM3	Flanders_Moss	3	1221216
FM4	Flanders_Moss	4	1221217
FM5	Flanders_Moss	5	1221218
MM1	Methven_Moss	1	1221219
MM2	Methven_Moss	2	1221220
MM3	Methven_Moss	3	1221221
MM4	Methven_Moss	4	1221222
MM5	Methven_Moss	5	1221223
PM1	Peeswit_Moss	1	1221224
PM2	Peeswit_Moss	2	1221225
PM3	Peeswit_Moss	3	1221226
PM4	Peeswit_Moss	4	1221227
PM5	Peeswit_Moss	5	1221228
RM1	Red_Moss	1	1221229
RM2	Red_Moss	2	1221230
RM3	Red_Moss	3	1221231
RM4	Red_Moss	4	1221232
RM5	Red_Moss	5	1221233
RN1	Red_Moss_of_Netherley	1	1221234
RN2	Red_Moss_of_Netherley	2	1221235
RN3	Red_Moss_of_Netherley	3	1221236
RN4	Red_Moss_of_Netherley	4	1221237
RN5	Red_Moss_of_Netherley	5	1221238
SM1	Shelforkie_Moss	1	1221239
SM2	Shelforkie_Moss	2	1221240
SM3	Shelforkie_Moss	3	1221241
SM4	Shelforkie_Moss	4	1221242
SM5	Shelforkie_Moss	5	1221243
SG1	Strathglass	1	1221244
SG2	Strathglass	2	1221245

Code	site	Quadrat	Sample ID
SG3	Strathglass	3	1221246
SG4	Strathglass	4	1221247
SG5	Strathglass	5	1221248
TM1	Threepwood_Moss	1	1221249
TM2	Threepwood_Moss	2	1221250
TM3	Threepwood_Moss	3	1221251
TM4	Threepwood_Moss	4	1221252
TM5	Threepwood_Moss	5	1221253
WF1	West_Fannyside_Moss	1	1221254
WF2	West_Fannyside_Moss	2	1221255
WF3	West_Fannyside_Moss	3	1221256
WF4	West_Fannyside_Moss	4	1221257
WF5	West_Fannyside_Moss	5	1221258

Table 7 Soil data

Code	pH(H ₂ O)	pH(CaCl ₂)	Moisture	LOI_450	LOI_900	Al	Ca	Fe	K	Mg	Mn	Na	Cation_ratio	PME_drywt	PME_sec
AM1	3.78	2.97	9.67	96.86	97.40	0.008	3.370	0.011	0.141	7.794	0.01381	1.007	1424.337	257.1223	0.142846
AM2	4.07	2.73	8.68	96.75	97.29	0.005	3.240	0.004	0.099	8.135	<0.00004	1.058	2445.420	134.18256	0.074546
AM3	4.20	3.01	8.91	95.66	96.31	0.051	2.861	0.025	0.931	7.625	0.00691	1.175	223.798	1060.2726	0.58904
AM4	4.23	2.87	8.44	96.34	96.86	0.009	3.019	0.008	0.426	7.515	0.00051	1.050	1225.671	568.69025	0.315939
AM5	4.07	2.95	9.02	97.04	97.54	0.004	3.189	0.003	0.070	8.127	<0.00004	1.061	3140.290	255.81277	0.142118
CM1	4.19	2.98	9.65	89.41	89.93	0.055	3.032	0.015	1.184	4.602	0.01129	0.750	161.718	502.18182	0.27899
CM2	4.22	3.14	9.63	94.37	94.91	0.041	5.712	0.013	0.992	5.243	0.02114	0.806	291.452	1005.9135	0.558841
CM4	4.39	3.29	10.39	90.50	91.36	0.202	2.636	0.033	0.763	2.401	0.01808	0.630	28.707	743.53174	0.413073
CM3	4.10	2.83	8.59	97.09	97.48	0.004	5.748	<0.001	1.039	8.249	0.01123	0.971	4145.997	485.64279	0.269802
CM5	4.42	3.22	9.07	91.97	92.58	0.159	2.984	0.011	0.876	3.065	0.03170	0.540	43.602	431.37165	0.239651
FM1	3.96	2.80	8.93	95.68	96.17	0.010	2.861	0.003	0.157	6.627	0.00404	1.010	984.161	248.34877	0.137972
FM2	3.87	2.79	8.96	95.48	96.11	0.010	2.946	0.005	0.145	6.563	0.00454	1.041	994.053	145.85453	0.08103
FM3	4.08	2.87	8.48	95.84	96.41	0.007	2.866	0.002	0.243	6.551	0.01746	0.990	1346.199	298.47219	0.165818
FM4	3.92	2.79	10.01	96.15	96.62	0.020	3.830	0.004	0.111	7.589	0.00644	1.077	562.463	259.59446	0.144219
FM5	3.82	2.83	9.20	95.65	96.10	0.016	3.450	0.001	0.069	6.663	<0.00004	0.953	617.755	157.64606	0.087581
MM1	4.02	2.89	9.76	96.32	96.83	0.019	4.984	0.003	0.307	9.468	0.00186	0.888	773.472	718.7101	0.399283
MM2	4.15	2.86	9.54	95.68	96.16	0.045	3.112	0.007	0.816	6.871	0.00204	0.781	240.966	408.79099	0.227106
MM3	3.99	2.83	9.65	96.40	96.90	0.022	4.217	0.003	0.607	7.254	0.03038	0.797	550.069	224.24197	0.124579
MM4	4.02	2.82	9.16	96.22	96.62	0.048	3.406	0.008	0.802	7.127	0.00738	0.865	238.128	479.14672	0.266193
MM5	3.86	2.83	9.14	96.33	96.83	0.024	4.244	0.004	0.401	8.175	0.00919	0.825	523.500	438.24202	0.243468
PM1	3.97	2.80	8.07	95.49	96.02	0.030	3.158	0.008	0.568	5.463	0.00097	1.009	307.355	390.77678	0.217098
PM2	4.17	2.85	7.64	95.22	95.72	0.028	3.208	0.008	0.492	5.446	0.00450	0.910	328.183	114.0912	0.063384
PM3	4.14	2.79	8.34	94.92	95.45	0.034	3.588	0.007	0.626	5.842	0.00185	1.001	300.150	230.56679	0.128093
PM4	4.00	2.83	7.58	94.62	95.16	0.031	3.843	0.006	0.561	4.898	0.00066	0.861	297.895	307.23546	0.170686
PM5	3.92	2.82	7.98	94.56	95.08	0.038	3.942	0.009	0.517	4.676	0.00100	0.857	240.357	472.63249	0.262574
RM1	3.91	2.98	8.86	97.36	97.93	0.026	4.598	0.014	0.649	8.237	0.00384	1.342	518.952	725.11558	0.402842
RM2	4.03	2.92	8.85	96.63	97.21	0.014	4.954	0.001	0.139	8.000	<0.00004	1.089	930.427	499.36909	0.277427

Code	pH(H ₂ O)	pH(CaCl ₂)	Moisture	LOI_450	LOI_900	Al	Ca	Fe	K	Mg	Mn	Na	Cation_ratio	PME_drywt	PME_sec
RM3	4.10	2.99	8.95	96.78	97.31	0.013	6.787	0.003	0.280	7.837	0.00113	1.102	1148.852	549.19663	0.305109
RM4	4.07	3.02	8.33	96.28	96.72	0.012	5.555	0.003	0.156	5.848	0.00013	0.970	950.015	310.25363	0.172363
RM5	4.05	2.98	9.26	97.04	97.54	0.011	5.228	0.001	0.101	7.864	<0.00004	1.044	1164.332	273.81441	0.152119
RN1	4.13	3.01	8.52	95.27	95.86	0.015	4.727	0.004	0.510	11.15	0.01321	1.397	1070.451	392.65935	0.218144
RN2	4.09	2.98	9.08	96.52	97.03	0.011	5.161	0.001	0.532	12.23	0.02656	1.481	1584.012	544.94743	0.302749
RN3	4.09	3.02	9.03	95.45	95.98	0.019	5.138	0.004	0.659	10.68	0.02085	1.427	850.352	847.80302	0.471002
RN4	3.92	2.98	8.87	95.71	96.33	0.012	4.566	0.002	0.351	9.859	0.01526	1.365	1256.736	1031.7939	0.573219
RN5	4.02	2.94	8.18	95.41	96.03	0.020	5.182	0.003	0.734	9.416	0.02533	1.408	753.767	708.93776	0.393854
SM1	3.72	2.78	8.48	94.95	95.61	0.023	3.284	0.006	0.213	5.241	0.00486	0.921	382.834	159.35779	0.088532
SM2	3.76	2.77	8.27	95.22	95.86	0.026	3.687	0.008	0.243	5.471	0.00806	0.953	357.932	96.510002	0.053617
SM3	3.91	2.88	8.09	94.89	95.70	0.025	3.270	0.007	0.165	5.199	0.00664	1.021	345.966	127.95982	0.071089
SM4	3.75	2.87	8.26	94.64	95.43	0.023	3.340	0.004	0.152	5.326	0.00477	0.895	391.125	102.23313	0.056796
SM5	3.70	2.86	8.74	96.29	96.98	0.023	4.085	0.013	0.193	6.377	0.01788	1.150	468.970	129.35855	0.071866
SG1	4.22	3.09	7.46	96.91	97.18	0.007	4.623	0.002	0.607	10.88	0.01603	1.188	2317.448	679.2635	0.377369
SG2	4.37	3.22	7.72	94.45	94.93	0.051	3.047	0.006	0.681	6.305	0.01414	0.986	196.879	670.22189	0.372345
SG3	4.24	3.20	7.96	96.42	96.87	0.030	2.553	0.008	0.594	6.809	0.00097	1.009	331.411	427.63173	0.237573
SG4	4.27	3.27	6.89	86.61	87.05	0.072	2.141	0.030	1.077	5.632	0.00775	0.933	123.682	1295.9348	0.719964
SG5	4.24	3.27	7.93	92.43	92.90	0.071	2.809	0.039	0.989	6.622	0.00343	1.023	147.775	1009.5911	0.560884
TM1	3.91	2.83	7.51	96.01	96.45	0.025	3.175	0.003	0.744	5.903	0.03826	0.937	399.054	533.37673	0.29632
TM2	3.88	2.81	8.33	96.50	96.96	0.019	3.383	0.001	0.653	6.285	0.03908	0.968	547.685	477.79861	0.265444
TM3	3.97	2.85	8.03	96.13	96.58	0.025	3.698	0.003	0.816	6.263	0.06799	1.017	437.178	835.94137	0.464412
TM4	3.94	2.79	8.07	96.06	96.52	0.017	3.766	0.004	0.605	6.002	0.02283	1.024	620.281	816.59925	0.453666
TM5	3.86	2.78	8.03	96.09	96.50	0.016	3.488	0.002	0.528	5.770	0.01863	0.969	625.216	169.73455	0.094297
WF1	3.75	2.87	8.22	93.78	94.65	0.051	3.779	0.019	0.383	4.179	0.00579	0.790	164.698	319.66406	0.177591
WF2	3.78	2.82	8.38	93.82	94.80	0.062	2.078	0.039	0.468	3.286	0.00014	0.964	93.475	376.59594	0.20922
WF3	3.64	2.85	8.38	94.43	95.35	0.039	3.113	0.011	0.223	3.336	0.00758	0.780	171.573	260.48898	0.144716
WF4	3.72	2.99	8.67	93.85	94.96	0.045	4.327	0.013	0.134	4.438	0.01736	0.732	199.387	255.64432	0.142025
WF5	3.66	2.98	8.62	94.67	95.47	0.030	4.640	0.009	0.103	4.947	0.01116	0.630	323.555	199.84198	0.111023

Table 8 Details of column headings used for soil data

Column heading	units	definition
site		Location of sample
Quadrat		There were 5 quadrats at each site
Sample ID		unique analytical number
pH(H ₂ O)	pH	pH in water
pH(CaCl ₂)	pH	pH in calcium chloride
Moisture	%	% moisture after sample air dried at room temperature - used for standardizing all data to results per oven dried weight
LOI_450	%	% Loss on ignition at 450 degrees
LOI_900	%	% Loss on ignition at 900 degrees
Al	meq/100g	Aluminium
Ca	meq/100g	Calcium
Fe	meq/100g	Iron
K	meq/100g	Potassium
Mg	meq/100g	Magnesium
Mn	meq/100g	Manganese
Na	meq/100g	Sodium
Cation_ratio	Ratio	Data presented as meq/100g. Ratio calculated as (Ca+Mg+K)/Al
PME_drywt	mmoles pNP/g dry wt soil	Phosphomonoesterase
PME_sec	mmoles pNP/g dry wt soil/sec	Phosphomonoesterase

9 APPENDIX 2: GRID REFERENCES

Table 9 Grid references of soil sampling points. The references is for the central soil core with four other soil cores taken to the N, E, S and W of this point each 0.5m away.

Site	Quadrat	Easting	Northing
Airds_Moss	1	261988.191000	625261.982000
Airds_Moss	2	261973.076000	625245.539000
Airds_Moss	3	261951.190000	625256.837000
Airds_Moss	4	261933.469000	625241.289000
Airds_Moss	5	261909.952000	625230.226000
Cairngorms_Blanket_Bog	1	305066.979000	794898.999000
Cairngorms_Blanket_Bog	2	305064.189000	794879.246000
Cairngorms_Blanket_Bog	3	305061.039000	794857.301000
Cairngorms_Blanket_Bog	4	305057.631000	794836.077000
Cairngorms_Blanket_Bog	5	305054.447000	794816.264000
Flanders_Moss	1	263549.932000	697011.631000
Flanders_Moss	2	263519.106000	697031.961000
Flanders_Moss	3	263490.241000	697048.681000
Flanders_Moss	4	263473.482000	697080.757000
Flanders_Moss	5	263455.723000	697108.845000
Methven_Moss	1	301438.323000	723902.001000
Methven_Moss	2	301408.455000	723883.884000
Methven_Moss	3	301378.359000	723866.154000
Methven_Moss	4	301348.571000	723848.066000
Methven_Moss	5	301318.516000	723830.020000
Peeswit_Moss	1	328731.097000	655112.347000
Peeswit_Moss	2	328711.218000	655110.337000
Peeswit_Moss	3	328691.426000	655109.280000
Peeswit_Moss	4	328689.714000	655129.380000
Peeswit_Moss	5	328667.459000	655125.047000
Red_Moss	1	287154.611000	626700.060000
Red_Moss	2	287134.387000	626703.829000
Red_Moss	3	287115.139000	626706.852000
Red_Moss	4	287094.768000	626706.487000
Red_Moss	5	287074.988000	626706.760000
Red_Moss_of_Netherley	1	385949.108000	794046.368000
Red_Moss_of_Netherley	2	385938.407000	794062.666000
Red_Moss_of_Netherley	3	385927.252000	794079.409000
Red_Moss_of_Netherley	4	385916.386000	794095.980000
Red_Moss_of_Netherley	5	385906.458000	794110.712000
Strathglass	1	220618.353000	825705.573000
Strathglass	2	220597.045000	825696.022000
Strathglass	3	220558.387000	825721.531000
Strathglass	4	220541.058000	825734.616000
Strathglass	5	220525.002000	825728.304000
Shelforkie_Moss	1	286209.911000	709554.415000
Shelforkie_Moss	2	286197.718000	709521.818000

Site	Quadrat	Easting	Northing
Shelforkie_Moss	3	286185.023000	709489.410000
Shelforkie_Moss	4	286172.276000	709456.928000
Shelforkie_Moss	5	286158.819000	709424.450000
Threepwood_Moss	1	351722.867000	642231.586000
Threepwood_Moss	2	351720.325000	642251.410000
Threepwood_Moss	3	351718.465000	642271.827000
Threepwood_Moss	4	351717.517000	642290.867000
Threepwood_Moss	5	351720.676000	642310.489000
West_Fannyside_Moss	1	279940.515000	673003.085000
West_Fannyside_Moss	2	279972.515000	673029.272000
West_Fannyside_Moss	3	280004.052000	673049.207000
West_Fannyside_Moss	4	280041.811000	673059.375000
West_Fannyside_Moss	5	280096.524000	673075.968000