COMMISSIONED REPORT

Commissioned Report No. *HP801*

Consultancy Service: To establish soil indicators to assess the impact of atmospheric deposition on environmentally sensitive areas.

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COMMISSIONED REPORT

Summary

Consultancy Service: To establish soil indicators to assess the impact of atmospheric deposition on environmentally sensitive areas.

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Background

The overall purpose of this research project is to identify suitable soil indicators to assess the impact of atmospheric deposition from point sources on soil quality in habitats of conservation interest. Nitrogen is the primary pollutant of interest. This research follows on from work undertaken by the UK Soil Indicators Consortium (UKSIC) which aims to obtain "information from soil monitoring to determine how we are meeting national policy requirements and complying with international laws and agreements for protecting the environment". Additionally, monitoring information will also support the development and implementation of future soil and environmental policy by providing evidence on the state of soils.

Main findings

- Seven indicators were selected as the most suitable, at present, 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.
- These indicators are: soil carbon / nitrogen (C/N) ratio, fungal species fruiting bodies, bacterial to fungal ratio, base cation / aluminium (Al) ratio, soil pH, soil solution ammonium (NH_4) / nitrate (NO_3) and phosphomonoesterase.
- By providing information on a range of soil properties and processes, these indicators would inform on the maintenance and vulnerability of five soil functions which are recognised within the Scottish Soil Framework.
- There is a requirement to establish suitable baselines and reference values for habitats of conservation interest. Existing soils data could be used to develop these.
- The applicability of published thresholds (e.g. soil C/N, pH, base cation / Al ratio), should be tested in habitats of conservation interest.
- Further development is required to establish relevant thresholds for soil solution NH_4/NO_3, fungal species fruting bodies, bacterial to fungal ratio and phosphomonoesterase, with regards to habitats of conservation interest and atmospheric pollution.
- Further development of site-level monitoring of soils in habitats of conservation interest would benefit from a trial using one or more case-study sites to take the application of the selected indicators all the way from designing an appropriate sampling strategy to interpreting the resultant data. This would effectively assess the capacity to address the knowledge gaps (e.g. baselines, sampling designs, etc) and provide a realistic of evaluation of logistics and costs for habitats of interest.

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1 INTRODUCTION

1.1 Introduction to the report

The overall purpose of this research project is to identify suitable soil indicators to assess the impact of atmospheric deposition on soil quality in habitats of conservation interest. This research follows on from work undertaken by the UK Soil Indicators Consortium (UKSIC) which aims to obtain "information from soil monitoring to determine how we are meeting national policy requirements and complying with international laws and agreements for protecting the environment". Additionally, monitoring information will also support the development and implementation of future soil and environmental policy by providing evidence on the state of soils.

1.2 Background to the report

A three tier approach to soil monitoring has been adopted by UKSIC (Figure 1.1). A number of projects have identified indicators of soil quality for national soil monitoring (i.e. Tier 1). However these indicators may not be adequate for determining impacts of atmospheric pollution on soils, particularly in habitats of conservation interest and subsequent wider environmental impacts. Following the UKSIC tiered approach, site level monitoring considered by this project is Tier 2 monitoring since the objective is to determine if there are impacts on specific areas of risk from a specific threat which may be from a known source. In this context, soil monitoring is targeted towards protecting and improving habitat site condition rather than broad scale monitoring for national reporting. The soil information gained from this level of monitoring would be relevant to a range of regulatory and policy objectives aimed at protecting soil, water, air resources and, ultimately, human health and well being.

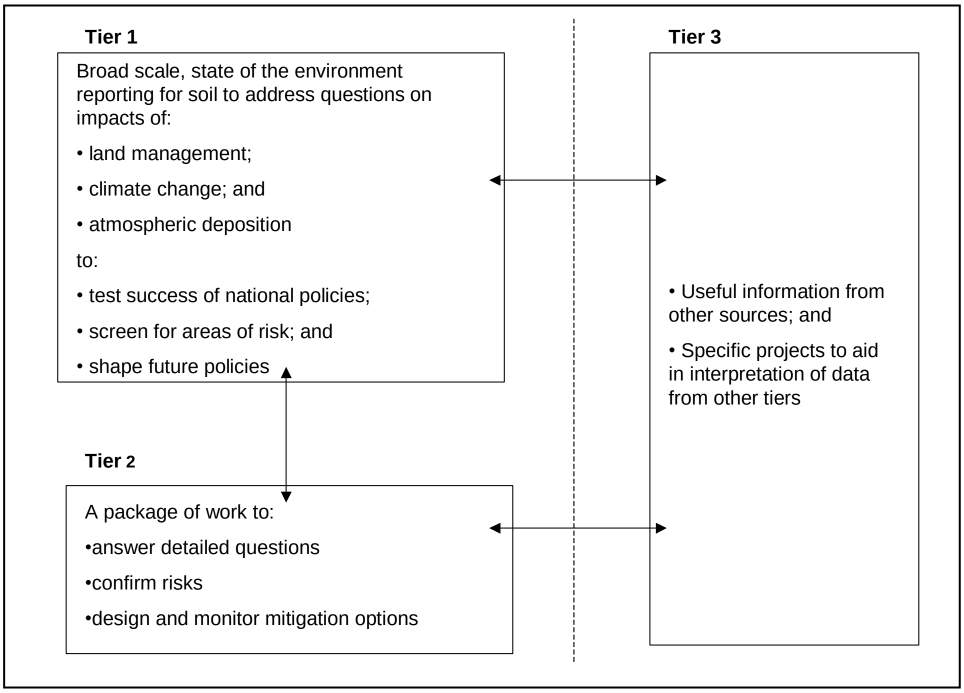


Figure 1.1 Three tier monitoring scheme proposed by UKSIC

The use of soil indicators is of prime importance to regulatory agencies in understanding how long semi-natural ecosystems may be able to retain nitrogen (N). For example, the concentration of soil N in acid and nutrient sensitive habitats can be diagnostic of the habitats vulnerability to atmospheric N deposition. If the capacity of an ecosystem to assimilate extra N (through plant and microbial nutritional demand) is exceeded, the system will eventually become N saturated and leaching to surface waters will commence (Agren and Bossatta, 1988, Aber et al., 1989; Aber et al., 1998).

Thus the continued deposition of sulphur (S) and N requires reliable soil indicators to monitor / assess the progression of, or recovery from, acidification and eutrophication in habitats of conservation interest. A key element of resolving reliable soil indicators will be characterising timescales for both responsiveness of indicators and ultimate consequences for the range of soil functions. In combination with above-ground assessments and following the principles of the EU Soil Thematic Strategy and Scottish Soil Framework, environmental protection measures are increasingly looking to maintain (or improve) soil functions, which include the role of soil in supporting habitats (e.g. supplying appropriate nutrient levels), biodiversity (including conservation status soil-associated species of fungi, ants and beetles), soil carbon stocks and water quality (including limiting dissolved organic carbon release).

1.3 Aims and objectives

The project objectives are listed below:

- Review literature to determine a range of suitable soil quality indicators which will inform on the impacts of atmospheric deposition on soil quality in habitats of conservation interest.
- Select indicators, taking into account physical, chemical, biological, and morphological parameters (preferably suitable for both mineral and organic soils) which:
    - reflect the status of the soil,
    - reflect any changes in processes occurring in the soil (e.g. nutrient cycling) as a result of atmospheric N and S deposition, or
    - enable an assessment to be made of the resilience of the soil to atmospheric N and S deposition.
- Evaluate the effectiveness of these indicators for detecting changes in soil as a result of atmospheric deposition, taking into account the sensitivity of indicators (e.g. number of measurements needed before they provide useful information), the accuracy of indicators (spatial and temporal variability), the timescale over which these indicators will provide meaningful information as well as costs and time associated with analysis. Evaluate whether indicators can distinguish between historic and (very) recent exposure.
- Link selected soil indicators to eventual impacts on habitat and species feature. If possible, identify threshold values for the indicators, taking into account not only changes in vegetation, but perhaps also more sensitive soil organisms (e.g. mycorrhizal fungi) as well as other soil functions (e.g. risk of nitrate (NO_3) leaching).
- Identify what other information would be required to allow meaningful interpretation of soil indicator results.
- Provide recommendations on how to test the indicators (including sampling strategies and analytical methodologies).

1.4 Background

1.4.1 Risk to habitats of conservation interest

Even with recent reductions in atmospheric pollution (c.f. NEGTAP, 2001), acidification and eutrophication from S and, increasingly, N remain significant threats to habitats of conservation interest, as demonstrated by the 2002-2004 critical load exceedance map for UK bog habitats (Figure 1.2). In 2005, the area of critical load exceedance of sensitive habitats was calculated at 58% and 59% of total habitat area for acidity and eutrophication, respectively (JNCC, www.jncc.gov.uk). Air concentrations of S have declined significantly in recent years with S now considered a relatively minor contributor to atmospheric pollution

(NEGTAP, 2001) although some upland areas of the UK may still be at risk from S due to continued long range transport (NEGTAP, 2001). By contrast, total N deposition has not declined significantly and continues to contribute to acidification and eutrophication of terrestrial habitats, soils and waters through both nitrogen oxides (NO_x) and ammonia (NH_3) inputs. Large areas of the country exceed the critical loads for nutrient nitrogen (Figure 1.2) and critical levels for ammonia, and are predicted to continue to do so in 2020 despite reductions in emissions of reactive nitrogen gases (NEGTAP, 2001).

Under the international agreements of the United Nations Economic Commission for Europe (UNECE) Convention on Long Range Transboundary Air Pollution (CLRTAP, e.g. Aarhus and subsequent Gothenburg Protocols such as the multi pollutant-multi-effects Gothenburg protocol), there has been a substantial reduction in S deposition over the past 20 years with emissions now dominated by a few large sources. There is now clear evidence from national surveys that UK soils, including those in Scotland, are recovering from acidification which has been attributed to reductions in S deposition (Haines-Young et al., 2000; NEGTAP, 2001, Carey et al, 2008). However model predictions indicate that N deposition could reinstate widespread acidification, within the next 20 or so years, if emission levels are not lowered below current Gothenburg targets (Helliwell et al., 2003, Curtis et al., 2005). In parallel, recent GB-wide changes in plant communities in semi-natural habitats, especially upland grassland and woodland, have been attributed to nutrient eutrophication from nitrogen deposition, stimulating plant growth and a demand for other plant nutrients (Smart et al., 2003; Kirby et al., 2005).

Atmospheric pollution from N is primarily composed of NH_3 and NO_x which can be deposited in wet (rainfall, snow, etc) and dry (volatiles) forms and can have acidifying and nutrient enrichment impacts on habitats and soils through a range of direct and indirect mechanisms. Recent emissions abatement strategies are reducing levels of N deposition (NEGTAP, 2001). Fossil fuel combustion is the major source of NO_x with increasing numbers of road vehicles making a greater contribution to this source. Agriculture, especially intensive livestock units e.g. poultry and pigs, is the primary source of NH_3, though vehicle catalytic converters are an increasing source (NEGTAP, 2001). In general, therefore, effects of NH_3 are more localised and reflect proximity to source. Recent research indicates that NH_3 may be more detrimental to semi-natural habitats, in particular lower plants / mosses with impact dependant upon both concentration and type of deposition (wet versus dry). Wet N deposition levels are often higher where there is low rainfall and correspondingly lower where high rainfall dilutes the pollutant. Nitrogen effects via acidification are associated with NO_3 which is not readily immobilised in the soil. Historical direct acidification effects on habitats have primarily been attributed to S deposition. In the main acidification impacts on habitats from NO_3 reflect feedback to plants via soil acidification.

Point sources may release other pollutants (e.g. heavy metals, persistent organic compounds) which can also pose a threat to habitats, biodiversity and soils. However there has been little consideration of how these pollutants may interact with NO_x or NH_3 to influence site level risks. These interactions are beyond the scope of this project but do warrant further consideration. Climate change (especially changes to rainfall) could also alter site-level critical loads exceedance and pollution deposition.

With regard to habitats of conservation interest, site relevant critical loads of acidification and eutrophication for SACs (Special Areas of Conservation) and SPAs (Special Protection areas) have been developed for current emissions and for 2010 scenarios, along with estimates of acidic and nutrient nitrogen deposition and their sources (e.g. industry, agriculture and transport). Figure 1.3 demonstrates this for a blanket bog SAC. This information is available from the UK Air Pollution Information website (www.apis.ac.uk) and could be used to develop a framework for determining how to deploy indicators of soil quality within these habitats.

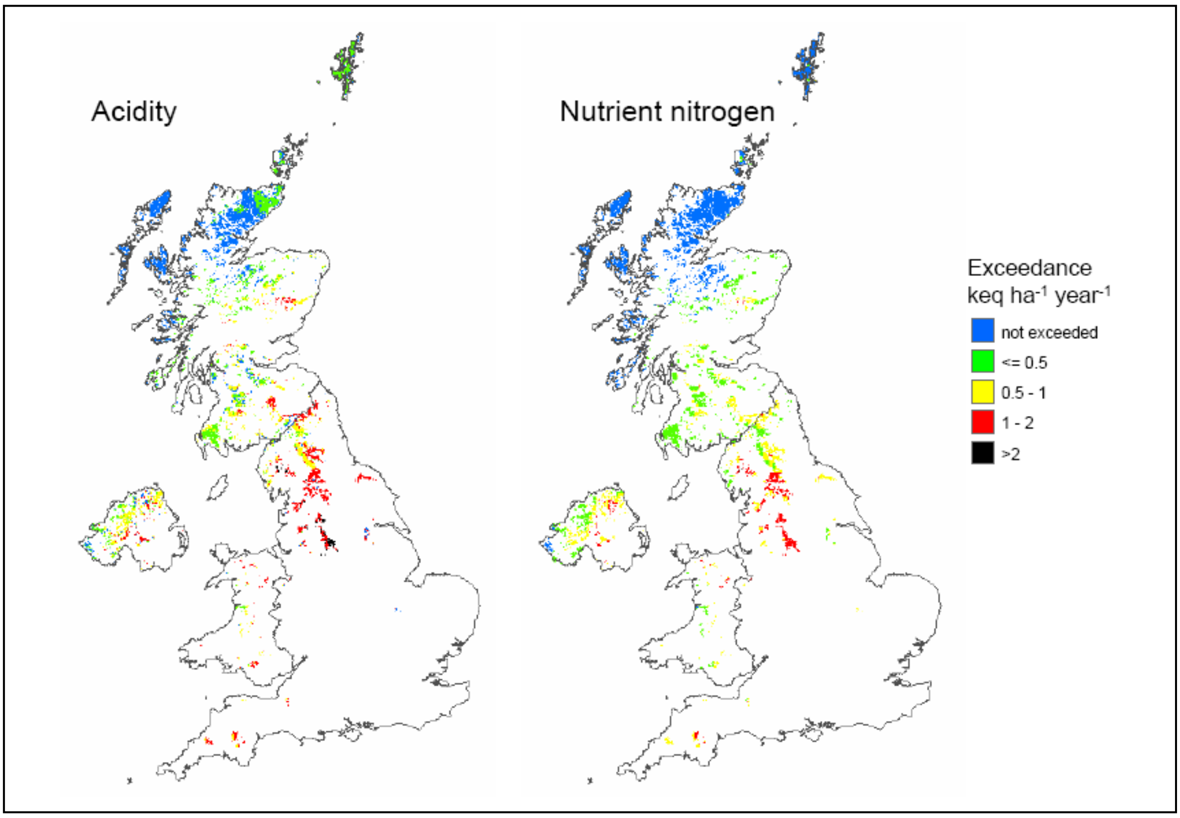


Figure 1.2 Exceedance of bog critical loads by deposition for 2002-04 Source: www.critloads.ceh.ac.uk

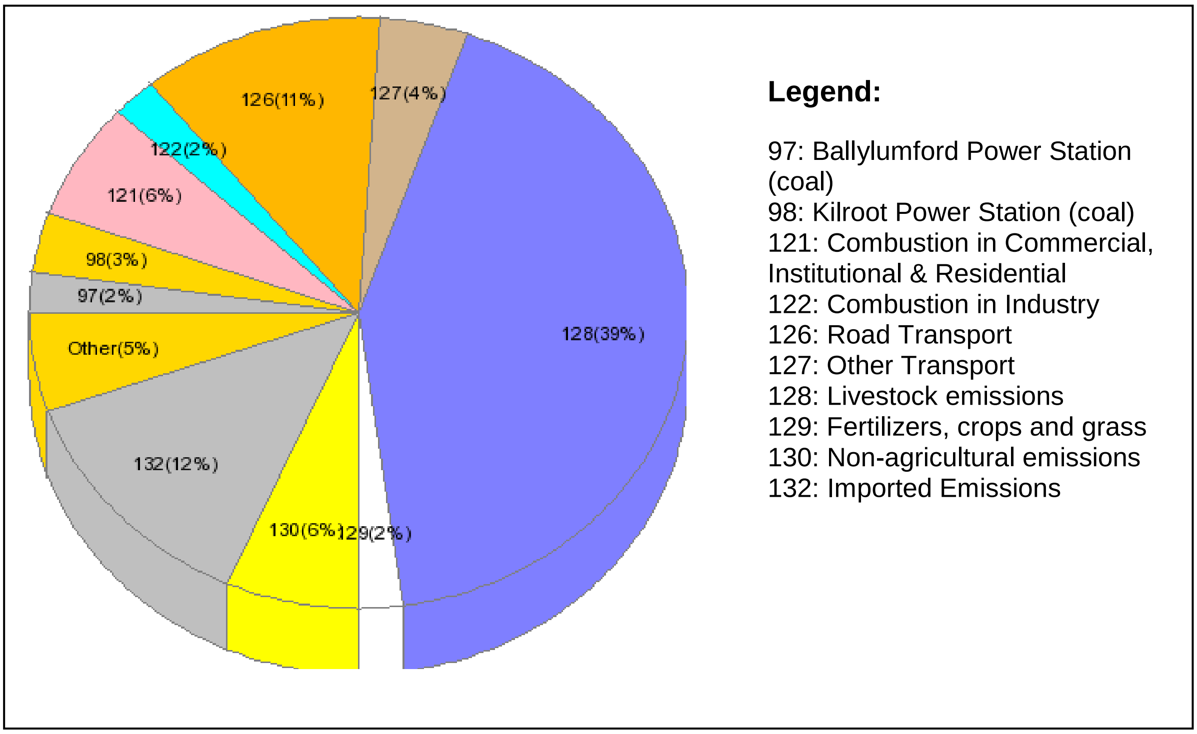


Figure 1.3 N pollution sources for Airds Moss, blanket bog SAC. Source: www.apis.ac.uk

1.4.2 Impacts of atmospheric pollution on soils

Impacts of atmospheric deposition on soils may be produced by either the direct or indirect actions of N and S on soil properties and processes. Direct actions are primarily due to the deposition of N or S onto the soil through rainfall (wet deposition) or dry deposition of volatile NH_3 compounds onto surface litter. Within the soil system, the pollutants induce a series of interacting reactions and cascades which are initially driven by changes to the structure and activity of the soil biological community and in the chemical composition of the soil. S, as an acidifying agent, directly influences the capacity of soil to retain and exchange cations (e.g. aluminium (Al), calcium (Ca), magnesium (Mg), potassium (K)) and the status of hydrogen ions (i.e. pH)) in soil. Changes to these characteristics have consequences for the reactivity of a wide range of soil chemical and biochemical processes and the structure and activity of the soil biological community since many soil organisms have physiological responses to the pH conditions in soil. These chemical and biological changes to soil can have consequences for plant establishment and growth through, for example, root toxicity and nutrient availability. An important consequence of acidification is an enhanced level of aluminium in the soil solution which can have significant effects on ecosystems (Bareham, 1996). High levels of soluble Al at low pH values disrupt cell wall structure in plant roots and inhibit nutrient uptake (Kennedy, 1992). Al can also kill earthworms at high concentrations and leach into water, affecting aquatic life (e.g. Battarbee, 1984).

Nitrogen pollution can be both a nutrient and acidifying agent with a wide range of direct and indirect actions on soil (see NEGTAP, 2001). Inputs of N to soil occur through deposition on litter, infiltration into the soil or through changes to the quality of plant litter and roots. These inputs will alter the activity and structure of the soil biological community as the soil microbial biomass captures, uses and transforms this fundamental nutrient, with consequences for the entire soil food web and in nutrient availability for plants. Changes to biologically driven nutrient cycling processes (e.g. mineralization and mobilisation of N) also alter the chemical form of N within the soil and produce an acidifying effect. Plant community structure in many habitats of conservation interest is dictated by the availability of soil N. Therefore, with increased availability of soil N, plant community structure changes as individual plant species grow and compete for this soil nutrient with increasing dominance of faster growing more competitive species. This “eutrophication” of plant communities has been detected in many habitats across Britain (Haines-Young et al., 2000, Smart et al., 2003 Kirkby et al, 2005). A feedback loop exists between soil and plants with eutrophication exacerbated as litter and soil C/N ratios lower through plant responses to N which in turn further alter soil N availability. Where soil N availability exceeds plant demand, this can lead to NO_3 leaching and pollution of water. In more acid soils and highly organic soils, a large proportion of NH_4 is immobilised by the soil microbial biomass and readily utilised by plant. In soils above pH 4, however, ammonium (NH_4) can be nitrified to NO_3 which also leads to acidification.

Detecting impacts of N or S on soils is highly dependant upon historical as well as current deposition inputs along with local site conditions (e.g. mineralogy, rainfall, soil organic matter content, phosphorus availability). The critical loads approach (c.f. Nilsson & Grennfelt, 1988) has been used to assess the likelihood that nutrient N and acidifying pollutants will cause damage to soils using empirical and mass balance modelling which take into consideration the inherent buffering capacity of different soils (see http://critloads.ceh.ac.uk/).

(i) ***a critical load*** a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge (source www.critloads.ceh.ac.uk).

(ii) ***a critical level*** refers to pollutant concentrations in the atmosphere which usually have direct effects on vegetation or human health (source www.critloads.ceh.ac.uk).

Critical loads thresholds and critical levels have been calculated at site level for habitats of known conservation status (www.apis.ac.uk) and can be used to determine whether the related soils are likely to show impacts from N and S deposition i.e. whether ther resilience of the ecosystem to these pollutants has been compromised. Figure 1.4 illustrates this for Airds Moss. For 2003, the S inputs are only slightly above the critical load threshold, therefore S impacts will be relatively low in this soil, although consideration should be given to recovery from past inputs. The N inputs are much greater than the critical load and indicate that acidification from N will be a continuing issue for this soil. Using the critical load projections for current deposition levels and future predicted deposition patterns can be used to assess whether individual sites are likely to experience recovery from acidification and whether the exceedance will be sufficient to be detectable in soil indicators.

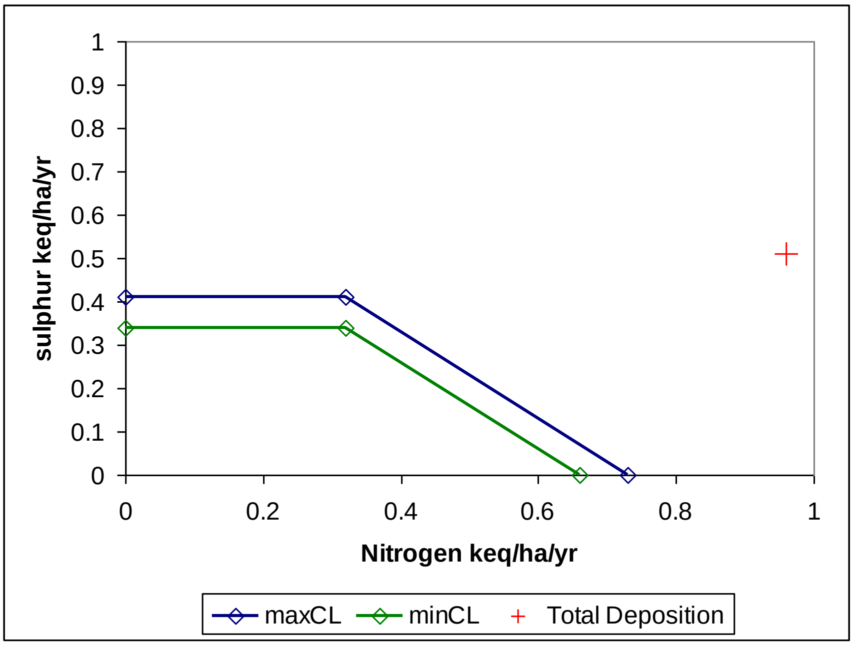


Figure 1.4 Critical load of acidity for Airds Moss blanket bog for 2003 with estimated total deposition showing that these loads are exceeded, with N being the dominant pressure. Source: www.apis.ac.uk

Attributing impacts of different pollution sources on soil properties and processes is primarily based on the relative contributions of these sources using mathematical calculations to account for a range of factors including pollutant forms and concentrations, average wind speed and direction etc (see www.apis.ac.uk). It may be possible to determine the relative contribution of a single source to overall deposition, or attribute the impacts of a single source to a site, if this has a characteristic stable isotope signal, e.g. 15N in ammonia, or trace metal isotope values (Skinner et al., 2006). This approach needs further evaluation to determine whether such a signal would be consistent outside an experimental setup and where multiple sources are involved.

It is worth noting that assessing status and change in soil indicators may require different sampling strategies (Black et al., 2008a). The requirements for sampling can only be fully assessed from prior knowledge of the local variability in soils both in space and time. This information is unlikely to be available at the outset of a site assessment and therefore some baseline sampling maybe required. Information from UK research and monitoring programmes, particularly forthcoming variability results from the National Soil Inventory for Scotland resampling (NSIS), could be used to inform on statistically robust sampling strategies.

Ultimately, assessing both status and change in soil indicators will be reliant upon adequate baseline and/or reference information. This can be used to determine whether soils are changing over time against a known point in time (baseline) or whether the indicator is deviating from what is considered acceptable for a habitat or site (reference and/or threshold). There are no soil baselines for individual sites for habitats of conservation interest or established reference conditions and only a few thresholds with respect to maintaining soil functions or impacts of N or S pollution. Current soils information (e.g. NSIS, National Soil Inventory (NSI), Environmental Change Network (ECN), Countryside Survey, experimental and site surveys) could be used to explore and assist in establishing suitable reference conditions / baselines for habitats of conservation interest. One objective should be to establish where sites of good and poor ecological status sit in relation to these conditions. It is possible that a lack of data for specific habitats (e.g. native pine woods) may limit this approach but this could be established from a review of the suitability of available data. Figure 1.5 illustrates the potential for this approach using GB wide data on soil carbon to nitrogen ratio (C/N) for a range of land uses.

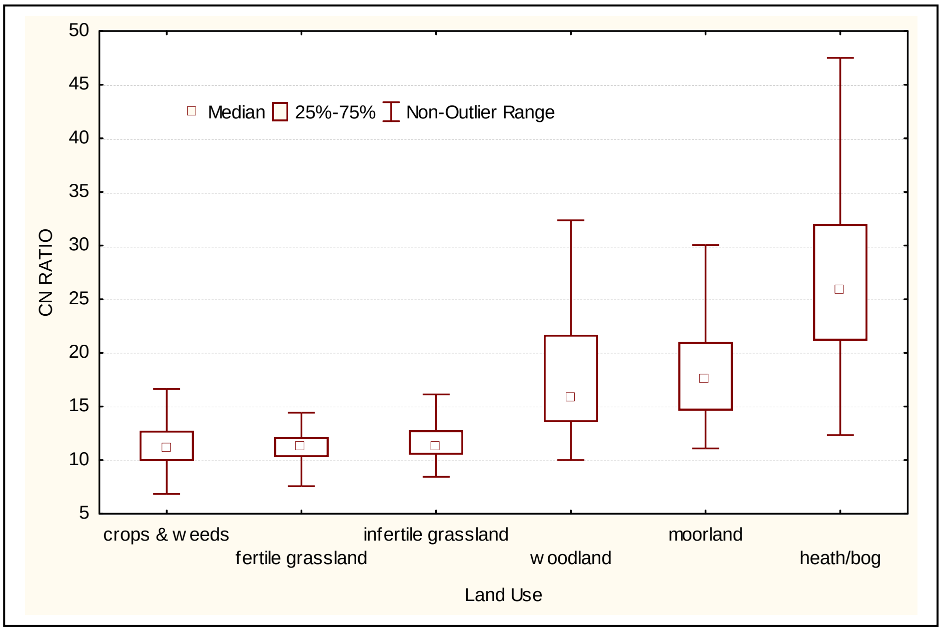


Figure 1.5 Box plot of soil C/N ratios for land uses across Britain showing medians, quartiles and non-outlier ranges. Source data: NERC Countryside Survey (http://www.countrysidesurvey.org.uk/)

1.4.3 Soil functions and habitats of conservation interest

A healthy soil, which is delivering a range of soil functions, requires a range of soil properties and processes to support these functions (c.f. Blum, 2005). The Scottish Soil Framework, in line with developments of the EU Soil Framework Directive, identifies seven soil functions which should be protected, maintained and enhanced. Of these, five are relevant to habitats of conservation interest and the impacts of atmospheric pollution. Table 1.1 lists these soil functions and illustrates which soil properties and processes are required to maintain these functions. Although the primary function of soil in habitats of conservation interest may be considered the support of biodiversity (plant and animal), these soils are also important in protecting water quality, releasing greenhouse gases, C storage, and protecting buried archaeological remains.

Ideally, indicators of soil quality will be selected to provide information on the range of soil properties and processes that are likely to be impacted by the relevant pressure or driver and necessary to support these diverse soil functions. It is important to note that, in addressing the range of soil functions, there may well be different thresholds or target values for the same indicator. For example, soil pH, as an indicator informing on the regulation of acidity, could have a different threshold for regulation of water quality compared to the storage of C or for supporting plant species in habitats of conservation interest.

All soil properties and processes in Table 1.1 could be impacted by extremely high inputs of N (e.g. excessive fertilisation at several 100's of kg N ha^{-1}). Under relatively low N inputs, e.g. from a point source, impacts will be detectable first in soil properties and processes which are directly related to increased availability of N or acidification as indicated by **(N)** against the soil process or properties in Table 1.1.

Table 1.1 Linking soil properties and processes to soil functions

	Soil properties and processes required for the provision of soil functions													
Soil functions, as defined in the Scottish Soil Framework	Aeration of soil	Breakdown and decomposition of organic matter **(N)**	Food source for above-ground food web **(N)**	Nutrient availability **(N)**	Mineralization / immobilisation of carbon **(N)**	Organic matter content **(N)**	Pest / pathogen biocontrol	Physical medium with structural integrity	Regulating acidity **(N)**	Regulating release of P and N **(N)**	Water infiltration / retention characteristics	Release of DOC to waters **(N)**	Release of GHGs to air **(N)**	Retention / degradation of pollutants (metals, POPs, etc) **(N)**
Controlling and regulating water flow and quality	X	X			X	X	X	X	X	X	X	X		X
Preserving cultural and archaeological heritage	X				X	X		X	X		X			X
Providing the basis for food, forestry and other biomass production	X			X	X	X	X	X	X	X	X			
Providing valued habitats & sustaining biodiversity	X	X	X	X	X	X	X	X	X	X	X			X
Storing carbon and maintaining the balance of gases in the air	X	X			X	X		X	X	X	X		X	

(N) – Soil property / processes known to be impacted by N deposition

2 SELECTION OF INDICATORS: OVERVIEW OF APPROACH AND RESULTS

2.1 Introduction

The following subsections outline the approach taken to meet the objectives summarised in section 1.3 and provide an overview of the results as they developed throughout the project. The following chapter highlights the main outcomes of the work.

A desk based literature review was undertaken to identify potential indicators of soil quality and soil process indicators focussing on the effects of S and N deposition, specifically acidification and eutrophication, on vulnerable sites with a focus on habitats of conservation interest and point sources. The first objective was to review the available literature to identify a range of indicators of soil quality which would be suitable to monitor for impacts of atmospheric deposition of N or S on the soils of Scottish habitats of conservation interest. This was carried out in stages using a combination of literature review and expert assessments. The first was a rapid coarse assessment of the large number of potential indicators of soil quality to eliminate, in a structured and consistent manner, indicators obviously unsuitable for the specific interests of this project. A total of 158 indicators were identified and assessed from the literature and previous reviews, of which 51 were carried forward for further evaluation. The second stage of the review was rapid relative assessment of the remaining (51) indicators within four groups which reflected their classification as biological, biochemical, chemical or physical indicators of soil quality. The physical indicators group also included morphological indicators. To simplify the review at this stage, it was assumed that all relevant functions of soil could be monitored by selecting a range of suitable indicators from these four classes. Within each group, the indicators were assessed to provide more detail on their responsiveness to atmospheric N deposition and whether one indicator could be more useful than another i.e. whether there was any surrogacy between indicators. The project was refocused on N deposition to reflect the increasing importance of continued N deposition in both acidification and eutrophication alongside the much reduced impact of S deposition in recent decades. The third stage of the review focussed on assessing the relevance of the short-list of selected indicators to Scottish habitats of conservation interest and for the protection of soil functions within these habitats with consideration of sensitivity, thresholds, variability (spatial and temporal variability), timescales for response and historic versus more recent exposure. The ultimate aim from this process was to identify, through a series of structured and increasingly detailed stages, a realistic range of soil quality indicators that could be considered for deployment in Scottish habitats to assess and / or monitor for impacts of atmospheric deposition of nitrogen from point sources.

2.2 Rapid assessment of potential indicators of soil quality

In the last 15 years, numerous research initiatives have addressed impacts of atmospheric N and S pollution on soils and, more recently, potential indicators of both impacts and recovery (e.g., for UK, Scottish Government Rural and Environment Research and Analysis Directorate (RERAD) Programme 3, Defra Terrestrial Umbrella, Loads and Dynamic Modelling, Freshwater Umbrella, Natural Environment Research Council (NERC), Global Nitrogen Enrichment (GANE) programme). Many of these have characterized habitat and/or site-specific impacts of atmospheric deposition of N and/or S. It was not possible to complete an in-depth and detailed review of the literature from first principles, therefore the information from these sources along with recent developments in soil quality indicators for UKSIC (e.g. Loveland and Thompson, 2002; Black et al., 2008a) and Scottish Government (Davidson and Wilson, 2006; Morecroft et al., 2006; Hough et al., 2007; Black et al., 2008b) were used as invaluable resources in identifying more habitat focused indicators (e.g. Tier 2 or 3). The focus of this task was on reviewing these projects and identifying any new indicators that have appeared in the last 12 to 24 months. Relevant and additional references were identified using the expert knowledge of those involved in the project of the literature, scientific literature databases (e.g. Web of Knowledge), general internet searches (e.g. Google) and specific searches of the main research commissioners websites in the UK and abroad (e.g. Scottish Government, Northern Ireland Executive, Defra, Scottish Environment Protection Agency (SEPA), Environment and Heritage Service (EHS), Environment Agency, Forestry Commission, European Commission and the United States Environmental Protection Agency (US EPA)).

Table 2.1 provides a summary of the distribution of the indicators identified between indicator types and grouping. Indicator type broadly classifies which soil parameters are being analysed for each indicator while the indicator group broadly illustrates the primary information that individual indicators provide about a soil. For a full list of indicators considered see Appendix A.

Table 2.1 Summary of potential soil indicators identified

Indicator Group	**Indicator Type**				**Total**
	Physical	**Chemical**	**Biological**	**Biochemical**	
Water availability	6				6
Carbon store/cycling	1	6	2	16	25
Soil stability	8				8
Toxicity		3	1	4	8
Regulation of multiple processes	6	6	1	12	25
Phosphorus cycling		2		3	5
Nitrogen cycling		5	3	14	22
Nutrient cycling		4			4
Sulphur release		1		2	3
Plants			3	1	4
Microbial			8	13	21
Invertebrates			10		10
Fungi			11	6	17
Total	21	27	39	71	**158**

To establish a robust, auditable and repeatable review process, a simplified SQID (Scoping Biological Indicators of soil Quality) approach was adopted. This approach has recently been reviewed and proposed as an international standard for selecting indicators and is designed to act as a decision support tool to assist in formulating a prioritized list of potential indicators, and not be an unequivocal and definitive list (Ritz et al., 2008). A simplified framework was completed for each indicator identified using the expertise of the project group, and their knowledge of published data and current experimental work. This rapid assessment was used to prioritise potentially useful indicators for more detailed evaluation, and to exclude indicators with little information or relevance to monitoring acidification or eutrophication. As a first cut a decision was made whether to pass the indicator to the next level of assessment based on:

a. Identification through the literature or expert opinion that the indicator had been used for acidification or eutrophication studies previously, and
b. An assessment of whether an indicator could provide relatively consistent responses to any or all of the following:
    - acidification from sulphur deposition,
    - acidification from nitrogen deposition,
    - deposition of NO_3 or NH_4 or both,
    - wet or dry deposition or both,
    - eutrophication from nitrogen disposition.

This process is summarised for those indicators to be further evaluated for assessing impacts of acidification or eutrophication from point sources in tables 2.2 to 2.5. Appendix B summarises the process for those indicators not considered suitable for further evaluation as effective indicators of the impact of atmospheric deposition on soil quality.

Table 2.2 Physical indicators (including morphological indicators) for further evaluation as effective indicators of atmospheric deposition

Indicator	Indicator group	Identified response to atmospheric deposition (				Reasons why indicator identified as suitable for further evaluation as an indicator of atmospheric deposition	Reference
		Increase	Decrease	No response	Complex response		
Changes to the structure of the upper horizons of soil	Carbon store/ cycling	X	0	0	0	Relatively consistent responses to atmospheric deposition identified; increasing depth with additional inputs of litter or reduced decomposition but changes generally take a long time to become significant. Changes relate to soil or litter C/N ratios, which are generally respond faster	1

1) Complex response – potential for the indicator to increase, decrease or not to respond in response to atmospheric deposition. This general complex response may be explained by consideration of other variables e.g. source and type of deposition and soil type. This is considered within the comment in the table as well as later in the report.
2) Consistent response – indicator recorded in the literature as increasing or decreasing in response to atmospheric deposition. Failure to identify a complex response may relate to literature only considering one type of deposition or one soil type. This was considered further later in the report.

Table 2.3 Chemical indicators for further evaluation as effective indicators of atmospheric deposition

Indicator	Indicator group	Identified response to atmospheric deposition				Reasons why indicator identified as suitable for further evaluation as an indicator of atmospheric deposition	Reference
		Increase	Decrease	No response	Complex response		
Exchangeable S	Sulphur release	X	0	0	X	Consistent response identified under acidifying conditions in certain soils. Responses dependant upon mineral weathering of S from parent material	1-7, 117
Base cation (Ca+Mg+K)/Al	Regulation of multiple processes	0	X	0	X	In general there is a decline in base cations and an increase in Al mobility under acidification. Complex responses related to geology, soil and deposition type	1, 4, 5, 7-21, 37, 45, 86, 113-125, 129
Soil pH	Regulation of multiple processes	0	X	0	0	Consistent response identified, particularly in topsoil. Decline in pH with increased inputs of S and N. Acidification effect of N dependant on soil N status.	3, 7, 10, 17, 18, 20, 23-41, 82, 86, 94-96, 98, 102, 111, 115, 116, 118, 121, 122, 124
Base saturation	Regulation of multiple processes	0	X	0	X	Complex response identified is related to soil and deposition type	8, 18, 25, 26, 30, 32-34, 37, 42-47, 121, 126
Cation exchange capacity	Regulation of multiple processes	0	0	X	X	Complex response identified as release linked to soil and deposition type	3, 25, 26 28, 34, 37, 45, 46, 115,121,124
Available P	Phosphorus cycling	X	0	0	0	Consistent response identified	48-50, 124,125
Exchangeable Ca	Nutrient cycling	0	X	0	X	Consistent response identified	2, 7, 10, 11 13, 17, 19, 20, 24, 31, 34, 45, 46, 51-55, 82, 85, 92, 96, 114-19
Exchangeable Ca, Mg, Na, K	Nutrient cycling	0	X	0	0	Consistent response identified	2, 4, 7, 14, 17, 19, 25, 26, 39, 45, 56, 82, 85, 117
Extractable Mg	Nutrient cycling	0	X	0	0	Consistent response identified	2, 7, 10, 14, 20, 24, 31, 36, 45, 52, 54, 55, 57, 82, 85, 92, 117, 118, 125
NO_3 leaching	Nitrogen cycling	X	0	X	X	Complex response identified as release linked to soil and deposition type	6, 7, 15, 16, 19, 58-62, 81, 85, 86, 91, 92, 95, 100, 103, 105, 108, 110, 111, 117, 118, 121, 126, 127, 128
Soil solution NH_4/NO_3	Nitrogen cycling	X	0	X	X	Complex response identified as release linked to soil and deposition type	1, 7, 10, 11, 12, 14, 18, 31, 35, 61-63, 71, 81-88, 91, 92, 95, 108, 114, 117, 118, 122
Soil total N	Nitrogen cycling	X	0	0	0	Consistent response identified. Can take long time period to become significant	23, 26, 28, 64, 65
^{15}N content in soil	Nitrogen cycling	X	0	0	0	Changes, generally increases, maybe detected where there is a distinct isotopic signal in the source ^{15}N.	66, 67, 98
Dissolved organic N	Carbon store/ cycling	X	0	0	0	Consistent response identified	12, 62, 68, 69-71, 91, 93, 98
Soil C/N ratio	Carbon store/ cycling	0	X	X	0	Consistent response identified in specific habitats (woodlands). Less consistent other habitats (may reflect time period)	3, 26, 28, 30, 33, 59, 69, 72, 73, 94-111
Dissolved organic C	Carbon store/ cycling	X	0	0	X	Complex response identified related to soil and habitat	3, 14, 16, 38, 69, 70, 71, 74-78, 82, 89, 90, 100
Topsoil SOC %	Carbon store/ cycling	X	0	0	0	Consistent response identified. Can take long time period to become significant	24, 25, 28, 33, 79, 80, 100 104, 112
Soil C stock estimate	Carbon store/ cycling	X	0	0	0	Consistent response identified. Can take long time period to become significant	23, 65, 69, 76, 104, 107

1) Complex response – potential for the indicator to increase, decrease or not to respond in response to atmospheric deposition. This general complex response may be explained by consideration of other variables e.g. source and type of deposition and soil type. This is considered within the comment in the table as well as later in the report.
2) Consistent response – indicator recorded in the literature as increasing or decreasing in response to atmospheric deposition. Failure to identify a complex response may relate to literature only considering one type of deposition or one soil type. This was considered further later in the report.

Table 2.4 Biological indicators for further evaluation as effective indicators of atmospheric deposition

Indicator	Indicator group	Identified response to atmospheric deposition				Reasons why indicator identified as suitable for further evaluation as an indicator of atmospheric deposition	Reference
		Increase	Decrease	No response	Complex response		
Bacterial to fungal ratio	Microbial	X	X	0	X	Complex response identified which is related to habitat and soil types. Considerable evidence for response to N deposition	1, 2, 97-99
Bacterial counts (CFU)	Microbial	X	0	0	0	Consistent response identified.	3, 4
Protozoan	Microbial	0	X	0	0	Consistent response identified	5, 100
Enchytraeids	Invertebrates	X	X	0	X	Complex response identified is related to habitat and soil types. Considerable evidence for response to N deposition	6-9, 93, 100, 103-06
Nematodes	Invertebrates	0	0	0	X	Complex response identified is related to habitat and soil types. Considerable evidence for response to N deposition	1, 8, 10-13, 78, 100, 107
Microarthropod community structure	Invertebrates	0	0	0	X	Complex response identified is related to habitat and soil types.	1, 14, 15, 100
Earthworm content	Invertebrates	X	X	0	0	Complex response identified is related to habitat and soil types.	16-20, 70-84, 96, 101, 102, 107, 108
Ground-dwelling invertebrates (e.g. spiders and beetles)	Invertebrates	0	X	0	0	Consistent response identified.	17, 19-22, 74, 75, 78, 81, 84, 102, 107
On site visual recording – fauna (ants, earthworms casts etc.)	Invertebrates	0	0	0	0	Further investigation of literature required	23
Ants	Invertebrates	0	0	0	0	Further investigation of literature required	22
Ectomycorrhizal fungi	Fungi	0	X	0	X	Complex response identified is related to habitat and soil types	8, 24-38 , 58, 60, 63, 87, 89, 92, 101, 107-109
AM fungi colonisation	Fungi	0	X	0	0	Consistent response identified	32, 39-44, 58, 66
Fungi (CFU)	Fungi	X	X	0	0	Further investigation of literature required	3, 45
AM fungi (DNA based methods)	Fungi	0	0	0	0	Further investigation of literature required	39, 46, 86-89, 92
Saprotrophic fungi	Fungi	0	0	0	0	Further investigation of literature required	35, 57, 86, 107
Fungal species (DNA based ITS)	Fungi	0	X	0	X	Further investigation of literature required	56, 57, 59 ,61-63, 65, 85, 86, 90, 91
fungal species fruiting bodies	Fungi	0	X	0	0	Further investigation of literature required	56, 57, 59, 61-63, 65
Litter decomposition	Carbon store/ cycling	X	X	X	X	Complex response related to deposition type and habitat.	14, 47-56, 59, 64, 67

1) Complex response – potential for the indicator to increase, decrease or not to respond in response to atmospheric deposition. This general complex response may be explained by consideration of other variables e.g. source and type of deposition and soil type. This is considered within the comment in the table as well as later in the report.
2) Consistent response – indicator recorded in the literature as increasing or decreasing in response to atmospheric deposition. Failure to identify a complex response may relate to literature only considering one type of deposition or one soil type. This was considered further later in the report.

Table 2.5 Biochemical indicators for further evaluation as effective indicators of atmospheric deposition

Indicator	Indicator group	Identified response to atmospheric deposition				Reasons why indicator identified as suitable for further evaluation as an indicator of atmospheric deposition	Reference
		Increase	Decrease	No response	Complex response		
Sulphatase	Sulphur release	X	0	0	0	Consistent response to acidic atmospheric deposition identified.	1-3, 47
Microbial S - Chloroform fumigation	Sulphur release	X	0	0	0	Consistent response to acidic atmospheric deposition identified.	65, 66
Phosphatase	Phosphorus cycling	X	0	0	X	Consistent response to acidic atmospheric deposition identified in a few studies. However complex responses related to P and N status of soil.	1-5, 42, 44
Phosphomonoesterase	Phosphorus cycling	X	0	0	X	Consistent response to acidic atmospheric deposition identified in a few studies. However complex responses related to P and N status of soil.	2, 6-11, 41-47
N fixation	Nitrogen cycling	0	X	0	0	Consistent response to atmospheric deposition identified.	67
N mineralization	Nitrogen cycling	X	X	0	X	Complex response to atmospheric deposition related to deposition and soil type.	8, 9, 12-24, 48, 51, 52, 55, 61, 63, 64
Nitrification	Nitrogen cycling	0	X	0	0	Consistent response to atmospheric deposition identified.	10, 12-15, 7-21, 25-31, 49-52, 55, 61, 62
Microbial N (Chloroform fumigation)	Nitrogen cycling	0	X	0	0	Consistent response to atmospheric deposition identified.	1, 2, 17, 32, 33, 42, 52-54
Denitrification (short-term incubation)	Nitrogen cycling	0	0	X	0	Further investigation of literature required.	10, 34, 35, 50
Glomalin (AM fungi)	Fungi	0	0	X	0	Further investigation of literature required	68
Ergosterol	Fungi	X	0	X	0	Further investigation of literature required	36, 37
AM fungi (NLFA)	Fungi	0	X	0	0	Consistent response to atmospheric deposition identified	38
C mineralization	Carbon store/ cycling	X	X	0	X	Complex response to atmospheric deposition related to deposition and soil type	9, 22, 39, 64
Substrate induced respiration	Carbon store/ cycling	0	0	0	X	Further investigation of literature required	38, 40, 54, 56-60

1) Complex response – potential for the indicator to increase, decrease or not to respond in response to atmospheric deposition. This general complex response may be explained by consideration of other variables e.g. source and type of deposition and soil type. This is considered within the comment in the table as well as later in the report.
2) Consistent response – indicator recorded in the literature as increasing or decreasing in response to atmospheric deposition. Failure to identify a complex response may relate to literature only considering one type of deposition or one soil type. This was considered further later in the report.

From the comprehensive list of indicators (158) collated from the literature and other sources, 51 (32%) were identified as potential indicators of soil quality for impacts of either (or both) atmospheric deposition of N or S. At this stage, no distinction was made between the sources of N since the majority of current literature deals with impacts (or simulated impacts) from long range transboundary pollution. As illustrated in table 2.6, the majority of potential indicators would reflect changes in the status or cycling of N and, to a lesser extent, C. This reflects a historical research emphasis towards relating N impacts on soils to pollution of water sources and sensitivity of habitats and plant species to N (e.g. nitrate or mycorrhizal changes). In recent years, there has been an increasing interest in the wider consequences of N deposition in soil ecosystems with significant emphasis on impacts to the organisms regulating C and N cycling, in particular indicators of microbial community structure (e.g. bacterial to fungal ratio), activity (e.g. potential N mineralization) and function (e.g. nitrifiers / denitrifiers). Although many of these more functional indicators are giving useful results, there is currently insufficient information for habitats of conservation interest or in the transferability of results for them to be considered robust enough to be taken forward as soil indicators within this project.

There is a clear knowledge gap regarding both the impacts of N on soil physical and morphological conditions with only one indicator from the physical group (Changes to the structure of the upper horizons of soil (L, F& H)) considered to be sensitive to atmospheric N deposition. When considering ecosystem consequences (e.g. regulation of water flow or release of greenhouse gases), this area warrants further investigation since soil physical conditions, such as soil aeration and pore size, are influenced by other soil properties such as organic matter content and biological activity (e.g. annelid worms and fungi), which are known to be impacted by N to a lesser or greater degree.

Table 2.6 Summary of soil indicators for further evaluation as effective indicators of atmospheric deposition

Indicator Group	**Indicator Type**				**Total**
	Physical	**Chemical**	**Biological**	**Biochemical**	
Water availability					0
Carbon store/cycling	1	5	1	2	9
Soil stability					0
Toxicity					0
Regulation of multiple processes		4			4
Phosphorus cycling		1		2	3
Nitrogen cycling		4		5	9
Nutrient cycling		3			3
Sulphur release		1		2	3
Plants					0
Microbial			3		3
Invertebrates			7		7
Fungi			7	3	10
Total	1	18	18	14	**51**

2.3 Relative assessment of biochemical, biological, chemical and physical soil indicators for impacts of N deposition

An internal project team workshop was held to discuss and review the indicators that passed through the first level sieve described above in section 2.2. At this point in the project, through discussion with the project steering group, the focus of the project was further defined to aid the selection of indicators. The project steering group's primary interest in point sources of N and S, rather than diffuse sources, led to the agreement that N should be the pollutant of concern, with regard to eutrophication and acidification in Scottish soils, since, given current emission controls, point source S releases to air are low. As a consequence, S release related indicators were removed from further consideration.

Reviewing this smaller group of indicators allowed the indicator types to be viewed as whole and relative to one another and therefore the most relevant indicator for each type could be selected, where as previously they had been reviewed as individual indicators. This meant, for example, where a number of indicators were present representing the same function or element of soil a number could be removed from further analysis.

Table 2.7 summarises those indicators which through discussion were not selected for further consideration along with the reasons for this decision.

Table 2.7 Indicators not selected to be taken forward after internal project team workshop
a) Physical/morphological indicators and chemical indicators

Indicator	Indicator Group	Reason not selected for further consideration
Physical / morphological indicators		
Changes to the structure of the upper horizons of soil	Carbon store/ cycling	Responses differ between habitats. Limited published data available to support application of indicator.
Chemical indicators		
Exchangeable S	Sulphur release	Removed due to focus of study on N deposition
Base saturation	Regulation of multiple processes	More appropriate alternative indicators within this group. Related to geological weathering rate. Response considered too complex.
Cation exchange capacity	Regulation of multiple processes	Linked to organic status of soils, potentially a secondary indicator in relation to N deposition due to slow response. More appropriate alternative indicators within this group (Base cation (Ca+Mg+K)/Al)
Available P	Phosphorus cycling	Responsiveness inconsistent. Varies with historical deposition, current status and soil type (Williams and Anderson, 1999).
Exchangeable Ca, Mg, Na, K	Nutrient cycling	More appropriate alternative indicators (Base cation (Ca+Mg+K)/Al)
Extractable Mg	Nutrient cycling	More appropriate alternative indicators (Base cation (Ca+Mg+K)/Al)
Exchangeable Ca	Nutrient cycling	More appropriate alternative indicators (Base cation (Ca+Mg+K)/Al)
NO_3 leaching	Nitrogen cycling	More appropriate alternative indicators within this group (Soil solution NH_4/NO_3 taken forward as a precursor to the leaching of N).
Litter total N	Nitrogen cycling	Litter N highly variable with inconsistent response to deposition. More appropriate alternative indicators within this group (soil C/N ratio).
15N content in soil	Nitrogen cycling	Indicator of input rather than impact
Dissolved organic N	Nitrogen cycling	Responsiveness inconsistent. More appropriate alternative indicators within this group (Soil solution NH_4/NO_3)
Topsoil SOC %	Carbon store/ cycling	Takes too long to respond. More appropriate alternative indicators within this group (Soil C/N ratio)
Soil C stock estimate	Carbon store/ cycling	Takes too long to respond. More appropriate alternative indicators within this group (Soil C/N ratio)
Dissolved organic C (DOC)	Carbon store/ cycling	Responsiveness inconsistent, dependant on historical deposition, soil type and plant community. More appropriate alternative indicators within this group (Soil C/N ratio)

b) Biological and biochemical indicators

Indicator	Indicator Group	Reason not selected for further consideration
Biological indicators		
Bacterial counts (CFU)	Microbial	Difficult to link to soil function and outdated technique. More appropriate alternative indicators within this group (Bacterial to fungal ratio)
Protozoan (direct counts from soil extracts + microscopy)	Microbial	Difficult to link to soil function. Complex responses. More appropriate alternative indicators within this group (Bacterial to fungal ratio) which reflect changes in soil food web dynamics
Nematodes	Invertebrates	Response to atmospheric deposition complex and inconsistent. Further work required to assess responsiveness of key indices (e.g. Maturity Index)
On site visual recording - fauna	Invertebrates	Technique used to assess worms through casting and as a broad biodiversity assessment but utility regarding atmospheric deposition is undetermined
Ants - in situ visual recording	Invertebrates	May not be present on site. More appropriate alternative indicators within this functional group (enchytraeids and earthworms)
Microarthropod community structure	Invertebrates	Collembola and mite respond to atmospheric deposition but there is a lack of consistent or transferable results.
Enchytraeids	Invertebrates	Highly relevant to organic soils and function. Sensitive to N inputs but requires further work to establish responsiveness in habitats of conservation interest
Earthworm content	Invertebrates	Highly relevant to soil function. Sensitive to N inputs but requires further work to establish responsiveness in habitats of conservation interest
Ground dwelling invertebrates	Invertebrates	Complex responses relating to habitat, management. Further work needed on species of known conservation value.
Fungi (CFU / community structure / species)	Fungi	Difficult to link to soil function. CFU is a rather outdated technique while DNA methods require further development. Alternative indicators available (Bacterial to fungal ratio and fungal species fruiting bodies)
AM fungi (DNA based methods; colonisation & diversity)	Fungi	Inconsistent responses. Alternative indicators within this group (Bacterial to fungal ratio, fungal species fruiting bodies)
Ectomycorrhizal fungi	Fungi	Considerable evidence that mycorrhizal fungi are sensitive to N and S deposition. Specific to forest ecosystems with DNA methods offering potential. At present, more appropriate alternative indicators within this group (Bacterial to fungal ratio, fungal species fruiting bodies)
Saprotrophic fungi	Fungi	Although saprotrophs sensitive to atmospheric deposition, there is insufficient literature available to identify indicators for community structure. DNA methods offer potential. Alternative indicators within this group (Bacterial to fungal ratio, fungal species fruiting bodies).
Decomposition (from litter bags or bait sticks)	Carbon store/ cycling	Response too complex with variable responses over space and time. More appropriate alternative indicators within this group (soil C/N ratio)
Biochemical indicators		
Sulphatase	Sulphur release	Removed due to focus of study on N deposition
Microbial S	Sulphur release	Removed due to focus of study on N deposition
Denitrification (short-term incubation)	Nitrogen cycling	High temporal and spatial variability and may not be suited to monitoring. More appropriate alternative indicators within this group. Changes in indicator not significant in uplands.
Nitrification	Nitrogen cycling	As denitrification. More appropriate alternative indicator within this group
N fixation	Nitrogen cycling	More appropriate alternative indicator within this group
N mineralization	Nitrogen cycling	Clear link between N mineralization, N leaching, soils responses and plant community responses in experimental habitats (grasslands, moorlands, forests). High temporal and spatial variability and requires further work. At present, more appropriate alternative indicators within this group. Look to Countryside Survey and NSIS resampling for progress on this indicator
Glomalin (AM fungi)	Fungi	Further investigation of the literature showed no consistent response and difficult to relate to function
Ergosterol	Fungi	Further investigation of the literature showed no consistent response and difficult to relate to function
AM fungi (fatty acids)	Fungi	More appropriate alternative indicators within this group (fungal species fruiting bodies, bacterial to fungal ratio)
Long-term C mineralization	Carbon store/ cycling	More appropriate alternative indicators within this group (soil C/N ratio)
Substrate induced respiration	Carbon store/ cycling	High temporal and spatial variability and requires further work since few studies under N deposition. More appropriate alternative indicators within this group (soil C/N ratio).
Phosphatase	Phosphorus cycling	More appropriate alternative indicators within this group (Phosphomonoesterase)

2.4 Selected soil indicators and their relevance to the protection of soil functions in Scottish habitats of conservation interest

Table 2.8 lists the small number of indicators that were finally prioritised from the review process. These indicators were primarily selected as they have been shown to have relatively consistent responses to N deposition in habitats that are relevant to Scottish habitats of conservation interest. Although an overall complex response may have been identified initially in Table 2.2 to 2.5 for some of these indicators, the responses may be consistent when other factors such as deposition type and soil type are considered. The available literature is insufficient to enable detailed review at the level of specific Priority Habitats or equivalent and therefore the JNCC Broad Habitats were used as the basis of the review. Table 2.9 summarise responses of these indicators within JNCC Broad Habitats which include habitats of conservation interest, e.g. calcareous grassland includes the Machair Priority Habitat. The Priority Habitat of native pine wood (*Pinus sylvestris*) was included in the assessment (table 2.9) since sufficient information was available from various northern Europe and Scandinavian studies to support its specific inclusion.

Table 2.8 Selected indicators of soil quality

Indicator	Indicator Group
Soil C/N	Carbon store/ cycling
Fungal species fruiting bodies	Fungi
Bacterial to fungal ratio	Microbial
Base cation/Al	Regulation of multiple processes
Soil pH	Regulation of multiple processes
Soil solution NH_4/NO_3	Nitrogen cycling
Phosphomonoesterase	Phosphorus cycling

These seven indicators could provide a range of information on both the status and change in a range of soil properties and processes which support the five soil functions primarily relevant to habitats of conservation interest (also see table 2.10);

1) Controlling and regulating water flow and quality
2) Preserving cultural and archaeological heritage
3) Providing the basis for food, forestry and other biomass production
4) Providing valued habitats & sustaining biodiversity
5) Storing carbon and maintaining the balance of gases in the air

Table 2.9. Responsiveness of selected indicators to N inputs by JNCC Broad Habitats

	Indicator relevant to habitat and likely to be impacted by atmospheric N from a point source, i.e. indicator known to be impacted by N inputs within this habitat									**Evidence of impacts from N on this indicator in habitats (in order of priority Scotland (S), UK(U), Elsewhere(E)). Only recorded where impact noted in previous section**									
Indicator	Bogs	Calcareous Grassland	Native Pine Wood	Deciduous Woodland	Dwarf Shrub Heath	Montane habitats	Moorland Grass Mosaic	Acid Grassland	Coniferous woodland	Bogs	Calcareous Grassland (Machair)	Native Pine Wood	Deciduous Woodland	Dwarf Shrub Heath	Montane habitats	Moorland Grass Mosaic	Acid Grassland	Coniferous woodland	References (also see Table 2.3 and 2.4)
Soil C/N	↓			↓	↓	↓	↓	↓	↓	U			U	U	S	E	U	S	Pilkington et al. (2007a), Magill et al. (2004), Hyvonen et al. (2008)
Fungal species fruiting bodies			↓	↓			↓	↓	↓			E	E			E	E	E	Boujon (1997), Giovannetti & Panicucci (1995), Ruhling & Tyler (1991), Manning et al. (2008)
Bacterial to fungal ratio				↑			↑	↑	↑				E			U	U	E	Chung et al. (2007; 2009); Demoling et al. (2008); Cooke (2007); Bardgett et al. (2007)
Base cation (Ca+Mg+K) / Al	↑		↑	↑		↑	↑	↑	↑	S		S	S		S	U	U	S	Pilkington et al. (2005a); De Vries et al. (2003) Miller et al. (2001); Helliwell et al. (1998)
Soil pH	↓	↓	↓	↓	↓	↓	↓	↓	↓	S	U	S	S	U	S	U	U	E	Pilkington et al. (2005a), Emmett et al. (1998), Horswill et al. (2008), Prietzel et al. (2006), Blake et al. (1999)
Soil solution NH_4/NO_3	↑		↑	↑	↑	↑	↑	↑	↑	S		E	E	U	S	E	U	E	Pilkington et al. (2005a;b; 2007b); De Vries et al. (2003); Magill et al. (2000; 2004)
Phosphomonoesterase	↑	↑			↑	↑	↑	↑	↑	U	U			S	S	U	U	E	Pilkington et al. (2005c); Taniguchi et al. (2007); Johnson et al.(1998; 1999)

↓	Decrease
↑	Increase
	Response unknown

Table 2.10 Linking changes in an indicator to impacts on soil function through processes / properties, i.e. a change in an indicator would indicate that the following soil properties or processes will be impacted

	Soil properties and processes required for the provision of soil functions													
Indicator	Aeration of soil	Breakdown and decomposition of organic matter **(N)**	Food source for above-ground food web **(N)**	Nutrient availability **(N)**	Mineralization / immobilisation of carbon **(N)**	Organic matter content **(N)**	Pest / pathogen biocontrol	Physical medium with structural integrity	Regulating acidity **(N)**	Regulating release of P and N **(N)**	Water infiltration / retention characteristics	Release of DOC to waters **(N)**	Release of GHGs to air **(N)**	Retention / degradation of pollutants (metals, POPs, etc) **(N)**
Soil C/N		X		X	X	X				X				
Fungal species fruiting bodies			X	X										X
Bacterial to fungal ratio		X		X	X									
Base cation / Al				X	X	X	X	X	X	X				X
Soil pH		X		X	X	X			X	X			X	X
Soil solution NH_4/NO_3				X	X				X	X			X	
Phosphomonoesterase				X	X					X				

(N) - Soil properties / processes known to be impacted by N deposition

The selected indicators would all provide information on nutrient availability for plants which includes signs of nutrient enrichment which could be detrimental to plants with preferences for habitats with low nutrient availability and also issues of toxicity for plant growth due to excessive acidification. Both relate to the function of biomass production and providing valued habitats. With respect to the regulating functions of carbon and water flows, storage and quality, five indicators would provide information on the regulation of C, N and P to water (primarily N releases from N deposition), five indicators would inform on the turnover and status of soil organic matter content and two indicators would indirectly inform on the capacity for soils to release greenhouse gas emissions and decomposition. There were no indicators to inform on physical soil characteristics which would support plant growth and movement of air, water and nutrients within the soil. This reflects a lack of study of related indicators or physical methods in experimental N deposition studies. There appears to have been an assumption that soil physical characteristics will not respond or respond very slowly. However this may be an oversight since atmospheric N deposition is known to influence soil organisms related to these physical characteristics (e.g. fungi and annelid worms) and elevated N deposition and its impacts are likely to be manifest for several decades.

Although not comprehensive, this set of indicators could be used within habitats of conservation interest to establish the status of soil quality, the maintenance of the soil functions and status regarding acidification and eutrophication. The indicators would also establish a baseline to monitor for progressive acidification and eutrophication, or recover from these pollutants.

It should be noted that a number of other indicators show significant potential regarding acidification and eutrophication in soils, e.g. mineralization of N, base saturation, worms (enchytraeids and earthworms), substrate induced respiration, DNA-based assessments of fungal diversity and microarthropod community structure. However, these require further development to address issues of transferability, relevance to habitats of conservation interest, thresholds and/or variability in space and time. A few of these are currently under investigation within the Defra Terrestrial Umbrella and other research programmes, e.g. Countryside Survey 2007 and NSIS resampling.

The following section briefly describes the selected indicators with respect to:

- *what is the indicator*
- *what can it tell us about soil functions, including the maintenance of habitats and species of conservation interest*
- *how does it respond to N*
- *are ecological thresholds already established or suitable baselines to monitor against*
- *how variable is the indicator over space and time?*

2.4.1 Soil C/N

- *What is the indicator?*

Soil C/N is a measurement of the relative proportions of carbon and nitrogen in the soil. Changes to soil C/N under N deposition reflects the incorporation of N enriched litter into the soil organic matter layers through decomposition. Litter can become N enriched through increased plant uptake of N from the soil or from direct deposition of N on plant leaves.

- *What can it tell us about soil functions?*

With specific reference to N deposition and water quality issues, declines in the soil C/N ratio correspond to an increasing risk of NO_3 leaching from a range of ecosystems and therefore increased risk of water pollution. However, concentrations of NO_3 in surface waters cannot be explained solely by soil C/N ratios (Curtis & Simpson, 2007).

Decomposition and subsequent turnover of carbon and nitrogen in soil is influenced by the ratio of C/N in both soils and litter. High C/N values are indicative of soils where decomposition is a relatively slow process with the soil fungal community playing a significant role in breaking down complex organic compounds in relatively recalcitrant organic matter. High C/N ratios are therefore indicative of soils where carbon storage is important. Soil C/N ratios have also been used (in conjunction with other soil information) to predict the release of greenhouse gases. The ratio has also been used to determine the suitability of soil conditions for UK plant species (Smart et al., 2005)

- *How does it respond to N?*

N deposition decreases soil C/N in all habitats that have been assessed (table 2.9). Responses are primarily driven by increased availability of N, with responses brought about by different mechanisms. Elevated levels of available N in soil result in greater plant uptake which is translated into plant litter with higher N status. This litter is decomposed and enriches the N pool sufficiently to alter soil C/N. In addition, more readily available N can result in increased plant biomass with the consequence that more litter (with lower C/N) becomes available for decomposition processes. The time taken for responses to become significant may be several years as it is dependant upon the organic matter content of soil, inherent nutrient limitations and other factors. The indicator could provide useful information on changes to the soil microbial community structure and activity which can be related to the suitability of soil conditions for habitats and plant species (Smart et al., 2003).

- *Have ecological thresholds or suitable baselines already been established?*

A critical threshold of 25 has been proposed for soil C/N in moorland, grassland and forest habitats with several experimental and survey studies showing NO_3 leaching initiated at soil C/N ratios below this level (Emmett et al., 1998, Pilkington et al., 2007a, see table 2.2). There are uncertainties over the applicability of soil C/N in UK upland ecosystems (Curtis et al., 2005) and further information is needed to establish the broad scale use of this ratio in Scottish habitats.

Status of soil C/N has been determined within the National Soil Inventory for Scotland, the Countryside Survey and Environmental Change Network (ECN) with information on change in soil C/N also available from these surveys. More general soil surveys (e.g. Scottish Soils Knowledge and Information Base (SSKIB)) also contain information of soil C/N. These data sources could be used to explore suitable baselines for individual habitats, local relationships in soil C/N to N deposition levels and to identify where information gaps remain.

- *How variable is the indicator over space and time?*

Soil C/N has a relatively low spatial and temporal variability. Spatial variability in soil C/N will reflect local variability in soil types which can be addressed through adequate sub-sampling. Outwith changes from environmental pressures such as N deposition, temporal variability is low within and between years.

- *Site sampling requirements*

Soil C/N is generally measured from the upper soil horizons where responsiveness will be first detected. A recent report on biodiversity monitoring (Morecroft et al., 2006) recommends soil sampling at 0 to 5 cm and from 20 to 30 cm, which are used within the ECN sites, while many experimental studies have sampled organic horizons and soil surveys have sampled horizons and depths. The decision on sampling horizon or depth should take into consideration which data will be used to establish suitable baselines and acceptable ranges or thresholds.

- *How can it be measured (e.g. are there ISO or BSI standard methods)?*

There are BSI and ISO standard methods available for analyses of both carbon and nitrogen from soil samples (BS7755-3.8:1995 & BS7755-3.7:1995 respectively). Soil C/N can be derived from results of these analyses. However different analytical approaches have been used by various UK surveys and monitoring schemes to derive soil C/N (e.g. total C and N analysers, loss on ignition, etc). A key requirement would be to establish that methods applied for site assessment are entirely compatible with methods used to derive site sampling strategies, baselines or thresholds i.e. compatible with existing data.

2.4.2 Fungal species fruiting bodies

- *What is the indicator?*

The presence of fungal species fruiting bodies at the soil surface with a particular emphasis on species with a recognised conservation interest (e.g. BAP species such as *Royal bolete*). A fruiting body is a visual indicator of the presence and activity of fungi below-ground and has been widely used in recording schemes to assess the biodiversity of fungi within habitats of conservation interest. Visual assessmet is the only viable approach to monitor key vulnerable fungal species until molecular genetic approaches have been trialled appropriately.

- *What can it tell us about soil functions?*

Soil fungi are an important component of habitats, particulary those of conservation interest. Many plant species have symbiotic associations with mycorrhizal fungi to access nutrients (especially P) and assist in disease control. Fungi are also important food sources for higher animals. In addition, there are more than 50 fungal species on the UKBAP list. Monitoring fungal fruiting bodies would therefore inform on the vulnerability of important BAP species protected under existing legislation and on potential threats to plant survival and establishment through losses in symbiotic fungal partners.

- *How does it respond to N?*

In general, the abundance of most fungal fruiting bodies is significantly reduced under increased N deposition, with increased availability of N proposed as the primary mechanism for these reductions (see table 2.8). Since fungi are particularly sensitive to eutrophication, declines may become obvious within a few years of increased N inputs. Some wood decomposer fungi may increase in number if increased plant (tree) growth results in the greater availability of substrate materials over time.

- *Have ecological thresholds or suitable baselines already been established?*

There is no information on thresholds or baselines in the literature. Biological recording from individual sites may provide some baseline information. As many Scottish habitats have already experienced elevated inputs of N, it is possible that soil fungi have already declined, with corresponding reductions in above-ground fruiting bodies (see table 2.8). This needs to taken into account when establishing baseline data for this indicator.

- *How variable is the indicator over space and time?*

The occurrence of fruiting bodies above ground is highly variable in both space and time. This variability would need to be determined through initial site surveys. In many instances, future monitoring could build upon historical site recording which will have identified the most appropriate periods of time for monitoring. Advances in molecular genetic tools (i.e. fungal species specific primers) may offer a way of monitoring the presence / absence of fungal species of conservation interest from soil samples without being reliant upon fruiting bodies (e.g. Turrini et al., 2008) although this will require improved knowledge of below-ground fungal species occurrence and community structure.

- *How can it be measured (e.g. are there ISO or BSI standard methods)?*

There are no BSI or ISO standard methods for this indicator. Sampling should be complementary to approaches used to establish baselines etc and be compatible with relevant recording schemes.

2.4.3 Bacterial to fungal ratio

- *What is the indicator?*

This ratio provides information on the relative proportions of bacteria and fungi in the soil microbial community and therefore how the soil microbial community is regulating C and nutrient cycling.

- *What can it tell us about soil functions?*

There is increasing evidence that bacterial to fungal ratio reflect soil C and nutrient cycling pathways and turnover. A low ratio is characteristic of a soil food web where fungi are more prevalent. As a consequence, soil C and nutrient cycling is generally slower with the decomposition and turnover of organic compounds reflecting a diversity of biochemical complexity and mycorrhizal fungi playing a significant role in providing supply of nutrient such as phosphorus (Wardle, 2002). A recent study of grassland extensification from a large survey of grasslands in England and Wales (Bardgett et al, 2007; Smith et al, 2003) supports the theory that this ratio could be a reliable indicator of changes in soil quality as a consequence of nutrient enrichment and that a decrease in the ratio corresponds to conditions more suitable for the establishment and growth of plant species of conservation interest (see table 2.4).

- *How does it respond to N?*

As this ratio is a relatively new approach, there are only a few studies so far which have assessed changes to bacterial to fungal ratio (using PLFAs) within the specific context of atmospheric deposition. These suggest that bacterial to fungal ratio increase as N deposition increases, reflecting an increase in bacterial biomass and (often lesser) a decline in fungal biomass. This ratio increase however may be limited since increases in bacterial biomass can be limited by carbon availability and other nutrient supplies (see table 2.8). This is a relatively new approach with some uncertainty over responsiveness to N deposition in Scottish habitats since there is little relevant information from this area. A recent report on biodiversity monitoring (Morecroft et al., 2006) recommends the inclusion of PLFA within the proposed ECBN soil monitoring network as a biochemical marker for bacterial and fungal functional groups and microbial biomass.

- *Have ecological thresholds or suitable baselines already been established?*

None available at present however information from recent UK projects may be used to inform developments of this indicator. None available at present however results from the Defra grasslands project (Smith et al, 2003), Defra SQID phase II and the NSIS resampling could be used to assess the potential to set thresholds and ranges.

- *How variable is the indicator over space and time?*

The ratio demonstrates seasonal variability which reflects annual patterns in microbial biomass size and activity as a consequence of plant growth and weather conditions, amongst other factors. Sampling therefore needs to account for inter-annual variability. Spatial variability does not limit the application of the indicator since adequate sub-sampling can be used to account for this variability.

- *How can it be measured (e.g. are there ISO or BSI standard methods?)?*

This indicator is normally determined in the laboratory through phospholipid fatty acid analysis (PLFA) of soils which is used to characterise the relative proportions of individual fatty acids attributable to bacterial organisms and different fungal groups, PLFA 18:2 omega 6,9) (Baath and Anderson, 2003). Fatty acids are extracted from soil and analysed using a GC-MS with an appropriate column. The resultant peaks from the GC-MS are associated with bacterial and fungal organisms through fatty acids considered characteristic with each group. Relative abundances of the PLFA groups are then used to determine the bacterial to fungal ratio. There is a range of PLFA extraction and GC-MS methods in common use which currently limits data comparisons.

There is a range of PLFA extraction and GC-MS methods in common use which currently limits data comparisons along with the lack of a common environmental standard. A standard method protocol has been developed to cope with soils of differing SOC content which is being trialled on soils in the NSIS resampling and Defra SQID project.

2.4.4 Base cation to aluminium ratio

- *What is the indicator?*

This indicator is derived from the molar ratio of soil base cations (Ca+Mg+K) to Al cations and provides information on the sensitivity of a soil (or habitat) to acidification. The ratio has been widely used to develop critical loads (e.g. Hornung et al., 1995; NEGTAP, 2001). The suitability of this ratio for following soil acidification is questionable in areas with elevated deposition rates of sea salts, in particular Mg. In organic soils (soil pH<4.5), protons from decomposition processes are the major determinants of soil acidity (NEGTAP, 2001) so the base cation/Al ratio will be less useful (c.f. Calver et al., 2004).

- *What can it tell us about soil functions?*

The molar ratio of base cation (Ca+Mg+K) to Al cations has been used as an indicator of potential reduction in plant growth from acidification (Sverdrup & Warfvinge, 1993). Although this index has received much criticism, few alternatives have been proposed and the ratio does provide information on when soil functioning is likely to be detrimentally altered. There is little in the literature to support acid buffering capacity per se as an indicator for monitoring (as opposed to acid neutralising capacity for water monitoring). There have been recent developments in the analytical methods for acid buffering but these are as yet untested for monitoring and have not achieved quality standards. To date, research has gone into applying understanding of acid buffering by soil type to establish critical loads. Therefore, in a sense, critical loads exceedance is a surrogate indicator of acid buffering. Exceedances do not directly monitor changes to the soil but assume that inherent soil characteristics are unchanged and it is the acid inputs which change and the soils capacity to buffer these. Various parameters are required to calculate critical loads for individual soils / sites and therefore base cation to aluminium ratio has been widely adopted to monitor for real changes to soil buffering capacity.

- *How does it respond to N?*

The proportions of base cations or protons can be altered through the deposition of N via a series of complex decomposition, mineralization and immobilisation interactions and also through accumulation of organic matter. Acidification occurs when Al species dominate the exchange complex with a corresponding loss of base cations through leaching. Although acidification is a natural process, atmospheric inputs of S and N impact on the balance of base cations to Al to increase the dominance of Al species.

- *Have ecological thresholds or suitable baselines already been established?*

Although Sverdrup and Warfvinge (1993) proposed a range of base cations to Al ratio values for different plant receptors, a critical soil threshold of 1 has been is widely adopted (e.g. Smith et al., 2004; van Scholl et al., 2004; Belyazid et al., 2005; Pannatier et al., 2005). Thresholds have not been established in Scottish habitats although results from long term surveys and model predictions suggest that critical thresholds are relevant and warrant further development (Helliwell et al., 1998; Miller et al., 2001).

- *How variable is the indicator over space and time?*

Spatial variability of individual cations is highly dependant upon local site conditions but adequate estimates of status can be obtained through appropriate sampling designs (e.g. Stutter et al., 2004). Temporal variability in cation concentrations can be high, reflecting soil water table fluctuations, rainfall patterns, litter decomposition rates, etc. Yanai et al. (2005) suggest that intensive sampling would be needed to detect a small change. Miller et al. (2001) propose a time interval of ~5 years would be sufficient to detect known changes in base cations using ECN methods i.e. through regular time-series monitoring as opposed to single inter-annaul surveys. Existing data could be used to explore how intensive sampling would need to be to detect changes at sites with varying spatial variability.

- *How can it be measured (e.g. are there ISO or BSI standard methods)?*

Standard methods and QC standards are available to support reproducibility of results. Cation concentrations can be determined by ICP-OES from ammonium nitrate soil extractions and this method is widely used in Scotland and UK (Sykes and Lane, 1996). This method can also be used to assess base saturation which would provide further information on the acidification status of the soil. A key requirement would be to establish that methods applied are compatible with methods used to derive site sampling strategies, baselines or thresholds.

2.4.5 Soil pH

- *What is the indicator?*

Soil pH is a measure of soil acidity or alkalinity (pH units) and is derived from the negative logarithm of the hydrogen ion concentration. The majority of soils in Scotland exhibit a soil pH<5.5 (pH in water) i.e. Scottish soils are predominately acidic.

- *What can it tell us about soil functions?*

Soil pH (as an indicator of H^+ concentrations) plays a major role in the regulation of many soil processes. For example, most cations become soluble and more available to plants at $pH_{H20}<5$ while phosphorus is immobilised in acidic soils. Soil biological community structure and activity is also regulated by pH. An extreme example is the rapid, changes in fungal and earthworm communities brought about by applying lime to acid soils. Many plant species have optimal soil pH ranges. This information can be used at a site level to establish potential risk to plant species and community structure from acidification (c.f. Smart et al., 2005).

- *How does it respond to N?*

Soil pH will lower under acidification from N deposition, which will be dependant on soil N status and soil type. Organic acid soils tend to be more resilient to acidification than other soils but significant changes can still occur. Although recovery in soil pH has been recorded for Scottish soils since the 1970's (Carey, 2008), with lowered S (acid rain) deposition, model predictions for future deposition scenarios predict acidification to recommence with increasing levels of ecosystem N (NEGTAP, 2001).

- *Have ecological thresholds or suitable baselines already been established?*

There are a variety of soil pH thresholds and optimal ranges for different soil processes and plant species which could be used to assess risk to soil functions and habitats of conservation interest based on the local requirements and conservation priorities of individual locations.

- *How variable is the indicator over space and time?*

Spatial variability in soil pH is highly dependant upon local site conditions (e.g. organic matter content, rooting depths, geology, etc) while there is seasonal variability in soil pH, especially when measured in H_2O. However, both can be relatively easily overcome to assess both status and change in soil pH through appropriate sampling designs (e.g. Black et al., 2008a). Available data (e.g. ECN, National Soil Inventory Scotland 2) could be used to establish reliable sampling schemes.

- *How can it be measured (e.g. are there ISO or BSI standard methods)?*

Two methods are in common use; soil pH generally is measured in water or weak salt solution ($CaCl_2$) from a soil slurry / paste. Both have BSI and ISO standard method protocols. There is no linear relationship between the measured values from the two methods although soil pH in water is generally higher than that taken using the $CaCl_2$ method, particularly in acid soils where the difference can be up to 1.5 pH units. $CaCl_2$ method is less responsive to seasonal variations in H^+. Both methods are widely applied and the selection of which method to use should consider what baseline information is available. One option, given the simplicity of both methods, would be to use both methods to allow comparisons with all available data. Standard methods and QC standards are available to support reproducibility of results for both approaches however a key requirement would be to establish that methods applied are compatible with methods used to derive site sampling strategies, baselines or thresholds.

2.4.6 Soil solution NH_4/NO_3

- *What is the indicator?*

The soil solution concentrations of both NH_4 and NO_3 (often listed as exchangeable NH_4 and NO_3) are increasingly being used as measures of the release of N from soils and therefore vulnerability of waters to acidification and eutrophication. NO_3 and NH_4 are used to determine both nitrogen status and change in nitrogen status of soils since changes in soil N content (%) is a small change in a large pool and measurements of soil solution chemistry are considered easier than bulk soil with determinations made on a filtered solution. Morecroft et al. (2006) comment that nitrogen mineralization and nitrification are better indicators of nitrogen supply to plants than spot measurements of NH_4 and NO_3, but they are substantially more expensive and require further development; both the NSIS2 and Countryside Survey 2007 will provide information on these.

- *What can it tell us about soil functions?*

Changes in the concentrations and relative proportions of extractable NH_4 and NO_3 inform on the balance between nitrogen supply and utilisation by plants and the soil microbial biomass, which is a major control of nitrate leaching. Therefore, NH_4 and NO_3 and their ratio can provide information on shifts in microbial functioning (e.g. nitrification / denitrification) and have been linked to shifts in plant species due to preferential usage of NH_4 or NO_3 by plants (Emmerton, 2001). Tietema et al. (1998) suggested that changes to NO_3 release and soil microbial populations are the first ecosystem compartments to be affected by N deposition.

- *How does it respond to N?*

Responses in soil solution NH_4/NO_3 are highly dependant upon the prior nutrient and acid status of the site. Where there are no limiting nutrients and acidification has not become significant, deposition of both NOy and NHx form will be readily utilised by plants, with consequences for community structure and diversity. In this way, plant uptake can mitigate acidification from N for a period of time. However progressive deposition of either NOx or NHy will result in the formation of NO_3, and increasing concentrations of NO_3, as other nutrients become limiting while plant uptake and microbial turnover are insufficient to mop up excess N within the soil system. Therefore in N limited systems, acidification from N deposition may only occur when the plant-soil system is no longer N limited which can take several years to decades. Where N is not limiting, NH_3 and NO_3 inputs will be equally acidifying with NH_4 is converted (nitrified) to NO_3. Recent model scenarios of declining deposition (N and S), indicate that recovery from acidification may also take several decades (Helliwell et al., 2003; Sverdrup et al., 2005; Reinds et al., 2009).

- *Have ecological thresholds or suitable baselines already been established?*

There are no recognised thresholds for Scottish soils and habitats. Rowe et al. (2006) propose upper soil thresholds for saturation of total inorganic N (NH_4 + NO_3) as 24 mol C mol^{-1} N in deciduous woodland and acid grassland and 51 mol C mol^{-1} N in coniferous woodland and heathland. The suitability of these limits requires testing in Scotland.

- *How variable is the indicator over space and time?*

The responsiveness of NH_4/NO_3 is related to the vegetation type with relatively fast responses in coniferous forests (Magill et al., 2000) and much slower, almost decadal, responses in deciduous woodlands (e.g. Magill et al., 2004). Past deposition history is also important as it plays a significant role in how close a habitat or soil is to N saturation. High temporal and spatial variability are significant factors and an appropriate sampling strategy would be required to overcome these issues, such as regular targeted within-year monitoring or an annual intensive sampling campaign.

- *How can it be measured (e.g. are there ISO or BSI standard methods)?*

Nitrate is often measured from in water or from a weak salt solution extraction while NH_4 is widely determined by KCl extraction (ISO14256-2:2005). A key requirement would be to establish that methods applied are compatible with methods used to derive site sampling strategies, baselines or thresholds.

2.4.7 Phosphomonoesterase

- *What is the indicator?*

Phosphomonoesterase (PME) belongs to the phosphatase group of soil enzymes which are produced by bacterial, fungal or plant root activity (Florkin & Stotz, 1964). These enzymes are important in the mineralization of organic phosphorus which is a significant source of this critical plant nutrient in many semi-natural habitats. PME activity is particularly important in soils with a low P status (e.g. acid soils with high organic matter content) where organic phosphorus is a dominant source of this nutrient for plant growth and health (Speirs & McGill, 1979). PME activity has been shown to be more responsive to N deposition than other measures of P availability (Pilkington et al., 2005c). PME activity can be measured from soil or root surface samples (e.g. Johnson et al., 1999). Further work is required to determine which approach would be more appropriate for site-level monitoring.

- *What can it tell us about soil functions?*

It is widely reported that N enrichment from atmospheric deposition has led to soil P availability limiting plant productivity in many semi natural systems, although there is recent debate over the significance of nutrient limitations under N deposition (Perring et al., 2008; Rowe et al., 2008). An increase in the activity of PME is considered an indicator of increasing plant demand for P and, correspondingly, an indicator of decreasing soil P availability. There are various implications of this increased activity for soil functioning. These include an increased risk of N leaching, as plants cannot utilise available N, and an increasing constraint on plant productivity and associated changes to plant community structure (Aerts & Berendse, 1988; Johnson et al., 1999; Emmett et al., 1995; Pilkington et al., 2005c). Therefore P limitations may act to suppress the influence of excess N availability and in determining the likelihood of plant community changes.

- *How does it respond to N?*

The response of PME to N deposition is closely tied up with the capacity for the above-ground vegetation to utilise the increase in available nitrogen, with a corresponding increase in demand for P uptake. Experimental work has demonstrated consistent relationships between increasing PME activity and increasing N inputs, both NO_3 and NH_4, with a great deal of this research having been carried out in the UK (e.g. Johnson et al.1999, Pilkington et al. 2005c, Phuyal et al., 2008).

- *Have ecological thresholds or suitable baselines already been established?*

No thresholds have yet been proposed for PME and there are no consistent data with which to establish baselines. Some progress may be made towards determining UK-relevant baselines by integrating available data from experimental work. Investigation of both thresholds and baselines is greatly hampered by a lack of consistency in published work due to a wide range in sampling approaches, analytical methods and activity units (c.f. Johnson et al., 2005). There could be some resolution of these inconsistencies by a comparative assessment of the different approaches in the short term while the adoption of a standard protocol would be beneficial in the longer term.

- *How variable is the indicator over space and time?*

Spatial variability in soil PME activity, and other phosphatase enzymes, has been shown to be relatively low e.g. low variability over 100 metres (Askn and Kzlkaya, 2006). Temporal variability is far more significant (Tscherko & Kandeler, 1999) since the enzyme responds to factors that show seasonal variability, e.g. soil moisture, temperature and plant growth. Suitable baseline sampling, along with consideration of available UK data, would aid in establishing the most appropriate sampling window.

- *How can it be measured (e.g. are there ISO or BSI standard methods)?*

There is no standard method for PME at present with modifications of different methods in wide use (c.f. Rejsek, 2007). A few of these have been applied to Scottish soils and the next stage would be to evaluate their efficiencies for use in habitats of conservation interest.

2.5 Identification of further indicator development requirements

As outlined above, there are generic issues that require development to bring these indicators to a deployable status for site-level monitoring. For example,

- An effective sampling design that can adequately address (site-level) temporal and spatial variability
- Standard protocols for all stages from sampling, through laboratory analyses to data analyses.
- Appropriate thresholds, baselines and or reference values for the indicators within individual habitats given local site conditions

3 HABITAT LEVEL ASSESSMENTS OF ACIDIFICATION AND EUTROPHICATION IMPACTS FROM ATMOSPHERIC DEPOSITION

3.1 Introduction

The review so far has concentrated on the generic utility of soil indicators to monitor for impacts of atmospheric deposition (primarily N). In this section, we consider in more detail the practical utility of the selected soil indicators for site-specific monitoring of point-source pollution in Scotland. Blanket bog, as one of the most extensive Scottish habitats of conservation interest, has been taken as a case study to illustrate how available information can be used to assist in the process of developing habitat- and location-specific monitoring strategies.

The selection of soil indicators to use for site-specific monitoring must consider the local context to ensure that the indicators chosen will be "fit-for-purpose". The selection will need to address which soil functions are important for this habitat, which indicators are most suitable given the local soil conditions and which indicators would be most appropriate given the current soil status and the site atmospheric deposition history. A simple framework for this process has been laid out in figure 3.1 as starting point for this selection process. The following sections discuss the various stages in further detail.

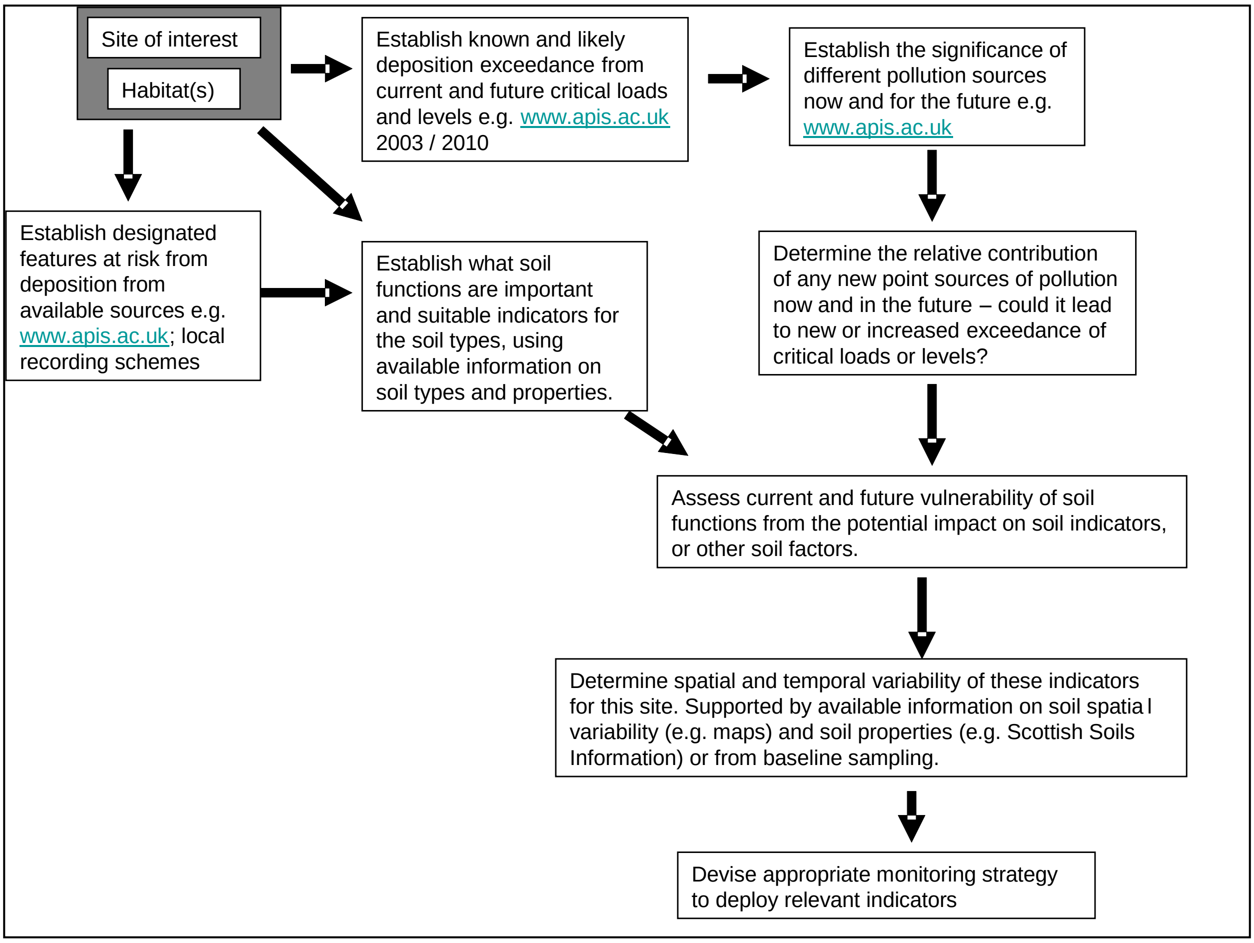


Figure 3.1 Simplified framework to assist in the selection of appropriate indicators of soil functioning for the monitoring of atmospheric deposition impacts in habitats of conservation interest

3.2 Description of a site to be monitored for impacts of atmospheric deposition

The details of a site can be captured from many sources. An example for a Blanket Bog site in SW Scotland (figure 3.2) is present in table 3.1 which summarises information from the APIS website and which could be expanded to include other relevant information. The site description will be essential in determining the scope and scale of the required monitoring by taking into consideration habitat size, environmental characteristics and variability, land use and management and conservation status of the habitat, its species and other features. For example, the blanket bog case study site has experienced various land uses and management practises while there are "areas of surface patterning" (table 3.1). All these could influence the choice of soil indicators and the production of robust baselines for monitoring. These factors need consideration in the design of a sampling strategy, which may include a range of options. The design might ensure that the sampling area is relatively homogeneous in terms of soils, habitat type, management and/or use. It could also ensure that there is sufficient sampling of site heterogeneity, such as vegetation patterns, to reduce the influence of spatial variability on the indicators. Temporal variability may also be considered from local weather information to review interannual seasonal patterns.

Table 3.1 Description of Airds Moss site

Airds Moss. Data derived from www.apis.ac.uk	
Country	Scotland
Unitary Authority	East Ayrshire
Centroid	NS613252
Latitude	55 30 10 N
Longitude	04 11 50 W
SAC EU code	UK0030218
Status	Designated Special Area of Conservation (SAC)
Area (ha)	1359.33
Annex I habitats that are a primary reason for selection of this site. 7130 Blanket bogs Priority feature. Airds Moss represents one of the few remaining areas of relatively low-altitude blanket bog in south-west Scotland, where agricultural conversion and forestry have reduced the original extent. The vegetation over some parts of the site is modified by past drainage and mineral extraction, and dominated by purple moor-grass *Molinia caerulea*. Elsewhere, areas of surface patterning occur, and more typical bog vegetation dominated by heather *Calluna vulgaris*, deergrass Trichophorum cespitosum and cross-leaved heath *Erica tetralix* is extensive, with locally abundant white beak-sedge *Rhynchospora alba*, cranberry *Vaccinium oxycoccos* and carpets of the bog-moss *Sphagnum magellanicum*.	

Figure 3.2 Location of Airds Moss SAC/SCI/cSAC (www.apis.ac.uk)

3.3 Site-specific atmospheric deposition sources, inputs and thresholds for acidification and eutrophication

Critical loads and levels for acidification and eutrophication have been calculated for all habitats of SPA and SAC status within the UK based (Wadsworth and Hall, 2007). At present, this information is available for 2003 and 2010 (see www.apis.ac.uk).

Critical Loads are thresholds for the deposition of a pollutant below which harmful indirect effects on a habitat or species do not occur, according to current knowledge. Pollutants that are associated with critical loads in APIS are N (kg N ha^{-1} $year^{-1}$) and acid deposition (keq ha^{-1} yr^{-1}). Additional deposition above the Critical Load is termed Critical Load Exceedance.

Critical Levels are thresholds for the atmospheric concentration of a pollutant above which direct adverse effects may occur on a habitat or species, according to current knowledge. Pollutant air concentrations above the Critical Level are termed Critical Level Exceedances. In the context of acidification and eutrophication, pollutants that are associated with critical levels in the APIS database are NH_4 (µg m^{-3}), sulphur dioxide (µg m^{-3}) and nitrogen oxides (µg m^{-3}).

Both critical loads and critical levels can be used to inform on the likely status of soils within individual habitats of conservation interest and the applicability of soil indicators to assess status of or future change in soils and soil functions. It should be borne in mind that, as these thresholds were developed for habitats and species, exceedance of critical loads or levels do not indicate that a threshold has been exceeded for any particular soil function. Suitable soil function thresholds still require development although certain soil property / process thresholds (e.g. soil C/N and potential mineralization) are under development within the Defra Terrestrial Umbrella and other initiatives. These are currently being reviewed under the ROTAP reporting process (Review of Transboundary Air Pollution, due for publication in 2010 as an update to NEGTAP).

The information available for a site-level assessment of current atmospheric inputs is illustrated in table 3.2 for Airds Moss, a blanket bog habitat of known conservation interest (see also figure 1.4). This information establishes whether the soils at the site of interest will have experienced significant pollutant inputs, whether these have already exceeded known thresholds and what the direction of future impacts may be (by comparing changes in the loads, levels, inputs and thresholds from 2003 to 2010). The attribution of pollution sources could be useful in establishing the potential impact of any new point sources and / or identifying the opportunities for reducing pollutant inputs. The relative significance of a new source may also reflect the choice of indicators for monitioring.

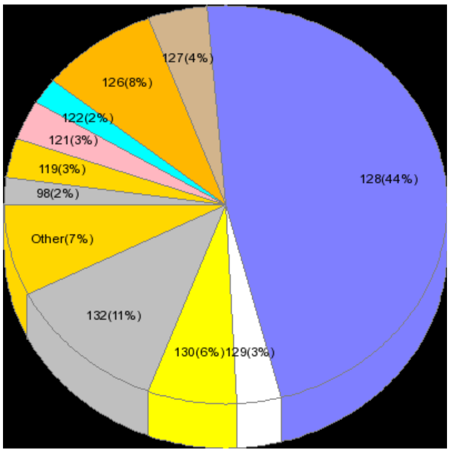
Monitoring impacts of, or recovery from, pollution from specific point sources in soils will need to consider the likely direction of future non-points source atmospheric N deposition. Even if the Gothenburg protocols are met, and atmospheric N deposition does decline significantly to the recommended levels, current soil modeling projections indicate that N deposition could re-initiate soil acidification within a few decades (NEGTAP, 2001). The significance of future atmospheric deposition against changes to point source inputs and / or site level management could be assessed through projections of critical loads or levels at a site-level, along with consideration of how the known status of the soils at a given site are likely to change as a consequence. It should be acknowledged however that recovery of soils, and habitats, that have experienced considerable historical atmospheric deposition may take several years to respond significantly to reductions in inputs.

Table 3.2 Site-level assessment of current atmospheric inputs to Airds Moss, a blanket bog habitat of known conservation interest. Data derived from www.apis.ac.uk

Pollutant	**Acid Deposition**	**N Deposition**	**Nitrogen Oxides**	**Ammonia**
Critical Level / Load	0.26 keq ha^{-1} $year^{-1}$	5-10 kg N ha^{-1} $year^{-1}$	30 µg NOx (as NO2) m^{-3}	1 - 3 µg m^{-3} (where lichens and bryophytes are integral to habitat use 1 µg NH_3 m^{-3})
Concentration / Deposition	1.3 keq ha^{-1} $year^{-1}$	12.7 N ha^{-1} $year^{-1}$	4.2 µg NOx (as NO_2) m^{-3}	0.8 µg m^{-3}
Exceedance	0.87 keq ha^{-1} $year^{-1}$	[7.7] to [2.7] kg N ha^{-1} $year^{-1}$	-25.8 µg NO_x (as NO_2) m^{-3}	[-0.2] to [-2.2] µg m^{-3}

Note: The Simple Site-Based Assessment should be used only to assist the user in obtaining a broad indication of the likely pollutant impact at a specific location. Where this method suggests likely significant pollutant impact, a detailed site-based assessment should be conducted.

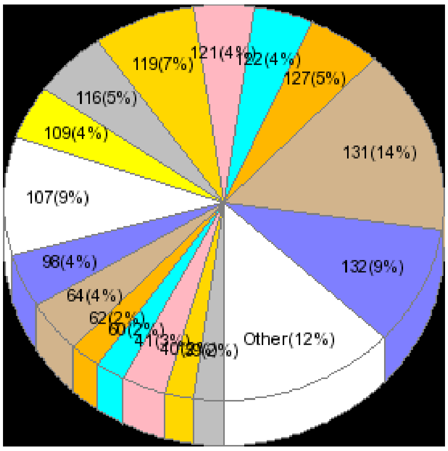
Source Attribution for 2010 for Airds Moss



N deposition (by Source ID and %)

Legend:

128: Livestock emissions
132: Imported Emissions
130: Non-agricultural emissions
126: Road Transport
127: Other Transport
129: Fertilizers, crops and grass
119: Other Point Sources
121: Combustion in Commercial, Institutional & Residential
122: Combustion in Industry
98: Kilroot Power Station (coal)



S deposition (by Source ID and %)

Legend:

131: SO2 emissions from shipping
132: Imported Emissions
39: Drax Power Station (coal)
40: Eggborough Power Station (coal)
41: Ferrybridge Power Station (coal)
60: Lynemouth Power Station
62: James Cropper
64: Fiddlers Ferry (coal)
98: Kilroot Power Station (coal)
107: BP Grangemouth Refinery
109: UPM, Caledonian Paper Mill
116: Longannet Power Station (coal)
121: Combustion in Commercial, Institutional & Residential
122: Combustion in Industry
127: Other Transport
119: Other Point Sources

3.4. Vulnerability of soil functions to atmospheric deposition in habitats of conservation interest

There has been considerable research activity on the impacts of atmospheric deposition on semi-natural habitats and their associated soils in recent decades with a significant contribution from the UK research community through initiatives such as the Defra Terrestrial Umbrella (http://www.bangor.ceh.ac.uk/terrestrial-umbrella/), the Critical Loads programme (http://critloads.ceh.ac.uk/), NERC GANE Thematic Programme (http://gane.ceh.ac.uk) and EU R&D projects (e.g. Nitroeurope www.nitroeurope.eu). Much of this information has been brought together through the APIS website which can be used as a source of information on the vulnerability of soils in specific habitats. Table 3.3 illustrates the level of information available on the vulnerability of soils to levels and forms of atmospheric deposition within peatland habitats. Although not comprehensive, this is a useful basis to start identifying the functions of soils in specific habitats and the relative importance of atmospheric deposition. The range of soil functions in blanket bog habitats is summarised in table 3.4 with a description of related soil properties and processes that could be monitored to assess changes to function. The selection of suitable indicators for monitoring of a specific habitat site would therefore be based on a combination of what is required for the soil functions relevant to this habitat and what indicators are currently considered suitable for monitoring impacts of atmospheric deposition. The final stage would be to review the application of the chosen indicators with respect to the site-level information on atmospheric deposition forms and thresholds. Table 3.5 illustrates this for the Airds Moss blanket bog case study site using the seven indicators selected in the previous section, with consideration of other indicator options. Since the selected indicators will have different responses and thresholds based on the habitat type and sitel-level conditions, this approach can be used to identify the suitability of individual indicators based on the pollutants of interest, current critical loads / exceedances and the suitability of the indicator to the local soil types. Table 3.5 illustrates that both S and N deposition exceed current critical loads for this Blanket Bog habitat. For acid deposition, soil pH, soil nitrate/ammonium ratios and base cations/Al (depending on depth of organic horizon) will be expected to have responded while for N deposition, fungal fruiting bodies, bacterial to fungal ratios, soil C/N, and phosphatase (PME) will be showing signs of changed status. Information is provided on the likely directions of change in status. This information can be used to determine whether baseline soil status of these indicators reflects what is already known about site-level pollutant inputs and to establish the likelihood of decting further changes to soil indicators. If site-level monitoring is to be used to provide insight on a range of issues then it will need to include a range of selected indicators and be reliant upon one or two indicators. For example, acidification can be detected using just soil pH or base cations/Al but each provides different information on consequences for the habitat; base cations/Al reflects changes to soil buffering capacity and an indication of increased risk to soil waters while soil pH can be related to changes in habitat suitability for plant species, dynamics of soil processes and changes to retention of toxins e.g. metals.

Table 3.3 Impacts of atmospheric pollutants (total N, NOx, NHy and S) on the soils of peatland ecosystems. Adapted from information available from APIS

	Total N Deposition	**NOx deposition**	**NH_3 deposition**	**S deposition**
Key Concerns	Rain-fed bogs are supplied with all nutrients and water from the atmosphere and are adapted to conditions of low N availability, making them extremely vulnerable to the effects of atmospheric pollutants (Thompson and Bottrell, 1998; Berendse et al., 2001). *Sphagnum* shows reduced growth, a change in morphology and increased tissue N content which can lead to loss of water holding capacity and hummock integrity. Threat of reduced carbon sequestration (Aerts et al., 1990). There are also changes in competitive ability: *Sphagnum* is overgrown and shaded out by *Calluna* and hypnaceous mosses (Sheppard et al., 2008). Algal growth increases. N accumulation in the peat with the potential to increase soil mineralization and decomposition rates.	NO_X may lead to ground flora changes related to eutrophication. Impacts on functioning of the peat ecosystem, e.g. decomposition, sulphate reduction, nitrate uptake, organic acid production (Wilson et al., 1994). Changes in vegetation composition, e.g. loss plant diversity and increase in *Eriophorum* (cotton grass).	Direct exposure to high concentrations of ammonia can lead to: Breakdown of Sphagnum hummocks and increase in bare peat which can increase the likelihood of erosion and surface oxidation. Increase in algal growth over *Sphagnum*. Suppression of root uptake of cations (Ca, Mg and K) leading to nutrient imbalances. Changes in the composition of groundflora, bryophyte and lichen communities.	Impacts observed on decomposition, sulphate reduction, nitrate uptake, organic acid production (Wilson et al., 1995), together with a decline in peat pH and drainage waters (e.g. ~ 0.5 pH units in Scottish peats). Peats with highest acidity and lowest base saturation tend to occur in areas where atmospheric deposition is highest (Skiba et al., 1989; Cresser et al., 1993). Changes to peat profiles in historically highly polluted Pennines over the last 200 years (Ferguson et al., 1978; Ferguson and Lee, 1983). Changes may also have occurred in bogs of upland areas in Dumfries and Galloway (largest acid deposition loads in Scotland). Establishing the role of S versus acidity as causes of detrimental change is difficult.
Additional Comments:	The height of the watertable exerts a significant effect on the ability of *Sphagnum* species to use N (Williams et al. 1999). Pollutant effects on bog vegetation and the underlying peat are closely related and should always be considered together. Plant species shift from bog mosses (*Sphagnum*) to grasses with the bog becoming a net emitter of CO_2; grasses increase organic matter decomposition, leading to gradual loss of the accumulated peat (Lawton et al., 1998) and drying out of peat, as grass species tend to have higher evapotranspiration rates. N impact is influenced by P limitation which will limit growth responses and 'grass' encroachment. Historical changes reflect responses to both N and S deposition. The highest deposition loads in upland Scotland are estimated to apply in Dumfries and Galloway, although N deposition to bogs is also an issue where these sensitive habitats occur near intensive agricultural activities in lowland areas (e.g. central Scotland poultry farms, Aberdeenshire pig farms, and dairy farms in Ayrshire and N. Ireland).	It is difficult to distinguish the effects of acidification due to oxidised N deposition from the direct effects due to NO_X.	Mainly a problem close to local sources, though some plant species sensitive to low NH_x concentrations downwind. Increased risk from fungal pathogens (e.g. *Phythopthora infestans)* in the summer when the water table is high and shoots have a high N content (Sheppard et al., 2008). Most evidence of damage to semi-natural ecosystems has largely come from the Netherlands. The contribution by NH_3 to total N deposition of N to unfertilised and semi-natural vegetation is likely to be more significant than direct exposure to NH_3. In most cases the contribution of NH_3 to N deposition leads to critical load exceedance, at much lower concentrations than the ammonia critical level.	Peats are naturally acidic and act as major sinks for NH_4^+ and, to a lesser extent, SO_4^{2-} (Bareham, 1996). Bogs are naturally low in base cations especially potassium and acid inputs will further restrict their availability, by displacing them from exchange sites, to be leached down the profile on mobile anions. Since the existing H^+ pool in peats is so large, it may take years for sustained acid inputs to influence acidity, making assessment difficult. There is some debate within scientific literature (see Wilson et al., 1994) as to the nature and extent of anthropogenic impact relative to the natural acidity of these ecosystems.
Critical Load :	5-10 kg N ha^{-1} $year^{-1}$.	30 µg NO_X (as NO_2) m^{-3} annual mean; 75 µg NO_X (as NO_2) m^{-3} 24-hour mean	1 or 3 µg NH_3 m^{-3} annual mean (where lichens and bryophytes are an integral part of the habitat use 1 µg NH_3 m^{-3})	0.1-1.0 keq^{-1} ha^{-1} yr^{-1}
Status	Valid for raised and blanket bogs [D1]. Description: change in species composition, N saturation of Sphagnum. Uncertainty: reliable.	Valid for all vegetation categories. Description: concentration units referenced as if all NO_X were NO_2. NO_x level should only be applied were SO_2 and O_3 are close to their critical levels. Uncertainty: quite reliable	Valid for higher plants (uncertainty ~2-4 µg m^{-3}). Description: direct visible injury; species composition changes. Ecosystems where sensitive lichens and bryophytes are an important part of the ecosystem integrity is set at 1 µg NH_3 m^{-3}. Uncertainty: expert judgement, only limited or no data available for this type of receptor	Raised bog and blanket bog, quite reliable, i.e. results of some studies comparable, the value within the range depends on the plant species composition and acidification of drainage water (UNECE, 1996).

Table 3.4 The vulnerability of soil functions within blanket bog to atmospheric N and S pollution through impacts on soil properties and processes.

Soil Function	Potential impacts of atmospheric N and S pollution
Storing carbon and maintaining the balance of gases in the air.	Organic soils are important stores of terrestrial carbon and protecting this C store may become an obligation under the Scottish Climate Change Bill. There is ongoing scientific debate over the impacts of combined acid and nitrogen deposition on the overall store of soil C since few studies or assessments have tackled the C stock issue directly and experimental studies are still contradictory regarding the role of N deposition on soil C turnover and accumulation. Although increasing soil pH can lead to accumulation of organic matter through slowing down decomposition processes, high historical acid deposition is considered a significant driver in the current degraded state of habitats and peatland soils in the Pennine area of Northern England (Holden et al., 2007). These organic soils are also important greenhouse gas emitters. There is some indication that atmospheric N may increase the release of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (c.f. de Vries et al., 2007). The significance of these impacts would need to be assessed against the influence of other factors, such as site drainage, and grazing, which can have a far more significant and immediate impact on GHG emissions.
Controlling and regulating water flow and quality.	Organic soils have a significant role to play in capturing and filtering pollutants within rain before releasing water to streams, rivers and groundwater. With regard to acidification and eutrophication, transfer of N (inorganic and organic) and acidity to waters is likely to be an issue where critical loads have been exceeded although the risk to local water provision and flow would need to be assessed at a site level.
Preserving cultural and archaeological heritage	Organic soils are a recognized reserve for archaeological remains and records of past climates. As noted by Davidson and Watson (2006), atmospheric deposition is unlikely to be a significant threat to this function. However, as soil pH and organic carbon are important to preserving remains, the significance of changes to either, in particular soil pH, should be assessed against the importance of a site for this function i.e. where there are important remains.
Providing the basis for food, forestry and other biomass production.	Atmospheric N and S deposition can influence the capacity for soils to maintain biomass production, including organic soils, with soil, and other, indicators deployed in trans-european level II forest monitoring (see http://www.forestresearch.gov.uk/fr/INFD-67MEVC).
Providing valued habitats & sustaining biodiversity.	The physical, chemical, and biological compositions of organic soils have a major role in dictating the suitability of an environment for plants, and therefore habitat establishment and maintenance. The biology (e.g. invertebrates and fungi) are also a major source of food for the above-ground food chain. Atmospheric N deposition, in particular, shifts low soil nutrient status, increasing availability of N in soil systems which has knock-on consequences for plant growth, competition and establishment with resultant eutrophication of plant community structure e.g. increasing predominance of grasses over mosses, which are particularly sensitive to direct impacts of N. A soil based constraint to this eutrophication is the limited availability of phosphorus which is supplied, in part, by fungal activity; many fungi are particularly sensitive to N. It has been difficult to quantify N impacts on fungi. Responses of associated phosphatase enzymes (e.g. phosphomonoesterase) are being increasingly used to assess P limitation.

Table 3.5 Monitoring to determine status and change in soils of Airds Moss blanket bog.

Simple Site-Based Assessment		**Grid Reference: NS613252**	**Habitat: Raised bog and blanket bog**	
Pollutant	**Acid Deposition**	**N Deposition**	**Nitrogen Oxides**	**Ammonia**
Exceedance	YES: 0.87 keq ha^{-1} year^{-1}	YES: [7.7] to [2.7] kg N ha^{-1} year^{-1}	None: -25.8 µg NO_x (as NO_2) m^{-3}	None: [-0.2] to [-2.2] µg/m^3
Monitoring to determine current status	Soils likely to have lowered pH from historical acid inputs, but now may be showing signs of pH increase under reduced acid inputs (following national trends).	Soils likely to be showing signs of excess N inputs (with corresponding impacts on plants).	Deposition is well below critical levels therefore would not expect any obvious impacts on soils beyond those from total N exceedance.	Deposition below critical level. Would not expect any obvious soil impacts. As close to critical level, there may be impacts on the plant/bryophyte community.
Monitoring for future status and change	It will be difficult to detect further inputs to this system unless they are significantly above current loads / levels.		Increased inputs in either NOx or NHy maybe detected in soil water since N exceedance has already been reached. It may be possible to use stable isotopes of 15N to distinguish N inputs from a distinctive source.	
Indicators of soil quality to determine the status of soils within this site given exceedance of acid and N deposition				
Soil C/N	Although acid inputs could increase soil C/N (by favouring acidophile plant species with more recalcitrant litter and associated fungi), the high inputs of N are likely to have been more pervasive.	Increased N within soil system may have lowered the soil C/N ratio to lower than typical C/N ratio of surface horizon organic matter. Threshold ratios for soil N leaching under investigation.	Critical levels are low and therefore indicators may not detect specific responses in soils from either NO_x or NHy inputs.	
Fungal species fruiting bodies	Although acid inputs could be favourable to fungal species, the high inputs of N are likely to have been more pervasive.	Sensitive species are likely to have declined but this will be difficult to assess unless historical records exist for this site.		
Bacterial to fungal ratio	Acid impacts unknown; although acid conditions may favour fungal dominance, N excess likely to override this influence.	Increased N within soil system is likely to increase dominance of bacteria in soil microbial community.		
Base cations to Al ratio	Less relevant in deep organic soils - increased mobility of Al maybe observed in organic soils with shallow surface horizon.	n/a		
Soil pH	pH of soil and soil water are likely to be highly acidic and at the lower end of Scottish and UK national data. Increases in pH can be observed under reduced inputs but will be limited to natural acidic conditions.	pH likely to re-acidify over time through maintained N inputs, though this maybe decadal.		
Soil solution NH_4^+/NO_3^-	Predominance of NO_3 to be expected in soil water given acidic conditions.	Signs of mobility of N in soil water, with predominace of NO_3		
PME	n/a	Signs of P limitation due to excess N with increased PME activity		
Other: potential N ineralization		Potential N mineralization could be used to assess potential for soil to release N as NO_3.		
Other: morphological	There may have been notable changes to the peat profile from decadal pollutant inputs, along with changes to plant community structure. However these may be difficult to determine if there are no suitable reference / historical comparative descriptions.			

4 SUMMARY OF PROJECT FINDINGS

4.1 Introduction

This chapter provides a summary of the main findings of the project.

4.2 Review literature to determine a range of suitable soil quality indicators

A total of 160 potential indicators of soil quality were reviewed through expert discussion and review of scientific literature and the reported outcomes of UK and other research programmes regarding acidification and eutrophication from atmospheric deposition. Seven indicators of soil quality were selected as the indicators which are currently most suitable to assess the status of soils in habitats of conservation interest in Scotland with respect to soil functions and atmospheric deposition, with an emphasis on N deposition, as agreed an early steering group meeting. These indicators are: soil C/N ratio, fungal species fruiting bodies, bacterial to fungal ratio, base cations to Al ratio, soil pH, soil solution NH_4/NO_3 and phosphomonoesterase. It is important to consider that if site-level monitoring will be providing information on a range of issues then it will need to include a range of selected indicators and not be reliant upon one or two indicators. For example, acidification can be detected using just soil pH or base cations/Al but each can provide different information on consequences for the habitat.

4.3 Select indicators suitable for both mineral and organic soils

By providing information on a range of soil chemical, physical and biological properties and processes, these indicators would inform on the maintenance and vulnerability of five soil functions which are recognised within the Scottish Soil Framework (controlling and regulating water flow and quality; preserving cultural and archaeological heritage; providing the basis for food, forestry and other biomass production; providing valued habitats & sustaining biodiversity; storing carbon and maintaining the balance of gases in the air). The selection therefore reflects a range of relatively reliable and informative indicators for each of these functions rather than the current most reliable indicators for assessing impacts of atmospheric deposition on soils. This distinction is important as it has focussed the selection of the indicators for the purpose of protecting functions within habitats of conservation interest as opposed to assessing impacts with no consideration of the significance of these impacts.

The 160 potential indicators of soil quality which were reviewed were assigned to an indicator group to reflect the primary information that each indicator would provide (e.g. regulation of multiple soil processes, phosphorus cycling, invertebrates). The majority of potential indicators would reflect changes in the status or cycling of N and, to a lesser extent, C. This reflects a historical research emphasis towards relating N impacts on soils to pollution of water sources and sensitivity of habitats and plant species to N (e.g. nitrate or mycorrhizal changes). There is a clear knowledge gap regarding both the impacts of N on soil physical and morphological conditions with only one indicator from the physical group.

In recent years, there has been an increasing interest in the wider consequences of N deposition in soil ecosystems with significant emphasis on impacts to the organisms regulating C and N cycling, in particular indicators of microbial community structure (e.g. bacterial to fungal ratio), activity (e.g. potential N mineralization) and function (e.g. nitrifiers / denitrifiers). There is potential to assess the transferability of recent results and to develop functional indicators for habitats of conservation interest to better reflect the status and dynamics of N availability and release.

This information was expanded for the final selection to ensure that coverage of most soil properties and processes which are known to be sensitive to atmosperhic deposition and relevant to habitats of conservation interest:

- Soil C/N ratio (carbon storage / cycling - plus N status);
- Fungal species fruiting bodies (fungi -vulnerability of sensitive species, in particular)
- Bacterial to fungal ratio (microbial - community structure changes reflecting changes to nutrient availability)
- Base cations/Al ratio (regulation of multiple processes – reflecting changes in soil buffering capacity)
- Soil pH (regulation of multiple processes – reflecting changes in soil buffering capacity)
- Soil solution NH_4/NO_3 (nitrogen cycling – reflecting changes in N availability and increasing risk of transfers to water)
- Phosphomonoesterase (phosphorus cycling – reflecting changes in P availability).

At present, the resilience of soil systems to atmospheric deposition of N and S is determined by the critical loads approach and, in part, through the development of thresholds for indicators reflecting risks to water quality and habitats. Further work is required to establish thresholds for a full suite of soil indicators to reflect limits to unacceptable changes in soils and the wider environment.

4.4 Evaluate the effectiveness of these indicators for detecting changes in soil

The indicators selected are all suitable for monitoring both the status in, and changes to, key soil properties and processes as a consequence of N deposition, primarily. Most of these indicators are included in broader-scale monitoring / survey schemes (soil C/N ratio, fungal species fruiting bodies, bacterial to fungal ratio, base cations to Al ratio, soil pH, soil solution NH_4/NO_3). Therefore spatial information is available to assist in the design of site-level sampling schemes to detect significant differences in status over time. There are far fewer temporal data from monitoring of changes in soil properties and processes. These currently include soil pH, C/N, and soil solution NH_4/NO_3. Design of site-level monitoring would have to take into consideration the uncertainities that a current lack of temporal information will introduce (see Black et al., 2008a). In this context, endusers need to determine whether monitoring both status and change are required for site-level monitoring or whether monitoring of change could be incorporated once sufficient site-level information had been gathered over more than one time period. Timescales for monitoring and detecting changes to soil indicators would be predicted from baseline site conditions and levels of pollutant inputs. Assessment of current site conditions would indicate whether exceedances had already been met through historical deposition and to what extent indicators would be expected to respond further to current or new pollutant inputs.

4.5 Link selected soil indicators to eventual impacts on habitat and species features

The relationships between the seven selected indicators and impacts on soil properties and processes are summarised in table 2.10. Gaps currently include water retention / release and release of DOC to waters. The significance of changes in each selected indicator to habitats and species features are discussed in general and thresholds identified where available. It was not feasible to cover the linkages between indicators and all habitats and species within this report. A structure for identifying the key issues of interest for site level assessments was proposed and demonstrated using Airds Moss as a blanket bog example. This approach could be replicated as the basis for individual site-level assessments to capture the information available from a variety of sources, in particular the site-specific critical loads assessments available from the APIS website (www.apis.ac.uk).

4.6 Identify what other information would be required to allow meaningful interpretation of soil indicator results

The site-level assessment framework and information on individual indicators sets out the additional information required to implement and interpret the selected indicators. The following summaries the main information required:

- site-level habitat characteristics and risk feastures (species and community-level)
- baseline soil conditions, including spatial variability of soils through sampling and / or soil maps
- soil functions relevant to soil types and habitats
- spatial and temporal information on selected indicators from other schemes which is relevant to the habitat under investigation
- site level critical loads / levels and exceedances for acid and nitrogen deposition, including NOx and NH_3
- deposition levels / concentrations (historical, current and future)
- source attribution of pollutant inputs

4.6 Provide recommendations on how to test the indicators

The main recommendation to establish site-level assessments using the selected indicators is thee-fold:

i. To develop and trial a site-level assessment in key habitats of interest in Scotland. A range of options are available and the choice should reflect known and future risks. For example, one option could be to trial across a minimum of three sites of the same habitat (e.g. blanket bog habitats on organic soils) with site selection reflecting low to high pollutant inputs. The first stage would review available information to determine how far soils have been stressed and the likely current status of selected indicators. The second stage would carry out baseline sampling for each indicator (based in part on information from ii below) and associated soil and site information. The third stage would analyse and interpret the information to establish current status of indicators, acceptable levels of future change and design of future monitoring, including spatial intensity and temporal frequency.

ii. To analyse existing spatial and temporal information for the selected indicators (in parallel to i). The information would be used to aid the design of sampling for site-level assessments and set thresholds / acceptable ranges for key habitats of interest, where possible. This would entail analyses of survey data e.g. NSIS2, Countryside Survey, NSI and RSSS and review of results from the Defra Terrestrial Umbrella and the forthcoming ROTAP report.

iii. To develop fully operational sampling and analytical methodologies with appropriate quality control measures. These should be compatible with the design requirements and with the methods used to establish thresholds or acceptable limits. This would involve reviewing current methods and devising either a single or combined method suitable to Scottish soils. The methods should be trialled during the baseline sampling of the trial site-level assessment.

4.7 Knowledge gaps and priorities

- The seven selected indicators are dominated by chemical and biochemical properties and processes which reflect the historical emphasis of soils research with respect to atmospheric deposition. The range of available information for these (surveys, experiments, model outputs, etc) can be used to inform development of suitable monitoring for habitats of conservation interest.
- Despite extensive literature on the negative impacts of N deposition on soil fungi, it is difficult, at present, to relate this to specific fungal indicators for Scottish habitats since we know little about fungal community structure <u>in the soil</u> of Scottish habitats,

as opposed to the presence/absence of fruiting bodies. Forthcoming molecular analysis of soil fungal communities will help to address this gap (RERAD Research Programme).

- No consideration has been given to the potential for a SOILPACS approach to monitoring soil quality (c.f. Weeks, 1998) which follows the principles of RIVPACS (Wright et al., 2000) whereby the quality and sensitivity of an ecosystem can be determined from the structure of the soil biological community. Given the relative importance of soil biodiversity in supporting habitats of conservation interest this approach warrants further exploration to determine the utility of taxonomic level indicators within the soil invertebrate community using available experimental and survey data.
- There is a lack of information regarding the broad applicability of potential soil indicators since information is primarily available from experiments across a limited number of habitats. There is a gap in geographical assessments to determine whether the indicators will provide consistent results in different locations and habitats. Statistical analysis of existing soil survey and other monitoring data could be used to start filling this gap for soil pH, soil C/N, base cations/Al and possibly fungal species fruiting bodies.
- There is a lack of baseline information or reference values for soil properties and processes in habitats of conservation interest. Statistical analysis of existing data (as above), along with habitat condition data if available, could be used to start filling this gap.
- Site level assessments and monitoring will require consideration of local spatial and temporal variability to design an optimal sampling strategy for these indicators. Statistical analysis of available soils data, along with habitat condition data, could be used to start filling this gap.
- Further development is required to establish relevant and robust thresholds for four of the selected indicators with regards to habitats of conservation interest and atmospheric deposition (soil solution NH_4/NO_3, fungal species fruting bodies, bacterial to fungal ratio, phosphomonoesterase).
- The applicability of proposed thresholds (e.g. soil C/N, pH, base cations to Al ratio) should be tested in habitats of conservation interest.
- Standard protocols should be developed to cover all aspects of monitoring at a site-specific level since there are currently a range of suitable approaches which have been adopted in broader-scale monitoring. They will need to cover sampling design, sampling procedures, analytical methods, data management, data analyses, etc. In most instances this will require resolution of the most appropriate analytical method for an indicator.
- A blanket bog case study demonstrated that soil monitoring in habitats of conservation interest should consider the local context of each site to assess whether indicators will be able to detect changes to soil functioning given local factors such as site heterogeneity, soil status, pollution history and future trajectories, relative source contributions.
- Further development of site-level monitoring of soils in habitats of conservation interest would benefit from a trial using one or more case-study sites to take the application of the selected indicators all the way from designing an appropriate sampling strategy to interpreting the resultant data. This would effectively assess the capacity to address the knowledge gaps (e.g. baselines / reference values, sampling designs, etc) and provide a realistic evaluation of logistics and associated costs for specific habitats of interest.

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LIST OF ABBREVIATIONS

Al	Aluminium
AM fungi	Arbuscular mycorrhiza fungi
ATP	Adenosine Tri-Phosphate
BAP	Biodiveristy Action Plan
Bc/Al	Base cation (Ca+Mg+K) to Al cation ratio
BSI	British Standards Institute
C	Carbon
Ca	Calcium
$CaCl_2$	Calcium chloride
CFU	Colony forming units
CLPP	Community Level Physiological Profile
CLRTAP	Convention on Long Range Transboundary Air Pollution
CO_2	Carbon dioxide
Cu	Copper
DNA	Deoxyribonucleic acid
EA	Environment Agency
ECBN	Environmental Change Biodiversity Network
ECN	Environmental Change Network
EHS	Environment and Heritage Service
F Horizon	Partially decomposed or comminuted litter, remaining from earlier years, in which some of the original plant structures are visible to the naked eye
GANE	Global Nitrogen Enrichment
GC-MS	Gas chromatography-mass spectrometry
H_20	Water
H horizon	Well decomposed litter, often mixed with mineral matter, in which the original plant structures cannot be seen
ISO	International Organization for Standardization
ITS	Internal transcriped spacers (in conjunction with gentic mehtods)
K	Potassium
L horizon	Fresh litter deposited during the previous annual cucle. It is normally loose and the original plant structures are little altered
Mg	Magnesium
MPN	Most probable number
MSIR	Multiple substrate induced respiration
N	Nitrogen
N/A	Not applicable
Na	Sodium
NEGTAP	National Expert Group on Transboundary Air Pollution
NERC	Natural Environment Research Council
NH_3	Ammonia
NH_4	Ammonium
Ni	Nickel
NLFA	Neutral lipid Fatty Acid
NO_3^-	Nitrate
NOx	Nitrogen Oxide
NSI	National Soil Inventory
NSIS	National Soil Inventory *of* Scotland
P	Phosphorus
PLFA	Phospholipid Fatty Acid
PME	Phosphomonoesterase
QC	Quality control

RERAD	Scottish Government Rural and Environment Research and Analysis Directorate
RNA	Ribonucleic acid
RoTAP	Review of Transboundary Pollution
S	Sulphur
SEPA	Scottish Environment Protection Agency
SIR-GLUC	Substrate induced respiration, glucose used as a substrate
SSKIB	Scottish Soils Knowledge and Information Base
SOC	Soil organic carbon
SQID	Scoping Biological Indicators of Soil Quality
UKSIC	UK Soil Indicators Consortium
UNECE	United Nations Economic Commission for Europe
Zn	Zinc

APPENDIX

APPENDIX A – Initial Indicator lists

Physical Indicators	
Water Availability	**Carbon store/cycling**
Least limiting water range	Changes to the structure of the upper horizons of soil
Soil moisture at 1m depth	***Soil Stability***
Subsoil permeability	Atterberg limits
Water content of soil	Erosion and sediment distribution
Water retention characteristics of soil	Estimated soil loss
Water-table depth and variability	Packing density
Regulation of multiple processes	Particle density
Bulk density	Surface crust
Depth of soil/topsoil/rooting	Top-soil aggregate stability
Porosity	Visual soil structure
Soil texture	
Temperature	
Visual evidence of eluviation (EH layer)	

Chemical Indicators	
Toxicity	***Nitrogen cycling***
Available Ni	15N content in soil
Available Zn	Litter total N
Available Cu	NO_3 leaching
Regulation of multiple processes	Soil solution NH_4/NO_3
Base cation (Ca+Mg+K)/Al	***Nutrient cycling***
Cation exchange capacity	Extractable K
Electrical conductivity	Exchangeable Ca
Base saturation	Exchangeable Ca, Mg, Na, K
Soil pH	Extractable Mg
Soil redox	***Carbon store/cycling***
Phosphorus cycling	Soil C/N ratio
Available P	Dissolved organic C (soil solution)
Olsen P	Dissolved organic N
Sulphur release	Particulate organic matter (soil solution)
Exchangeable S	Soil C stock estimate
	Topsoil SOC %

Biological indicators	
Toxicity	***Invertebrates***
Pinus sylvestris roots (biomass, density)	Ants
Regulation of multiple processes	Collembola
Fungal/bacteria biomass ratios (Microscopic determination)	Earthworm content
Plants	Earthworm reproduction
Plant seed bank - counts	Enchytraeids
Plant seed bank - germination	Ground-dwelling invertebrates (e.g. spiders and beetles)
Roots - size frequency & depth	microarthropod community structure
Nitrogen cycling	Nematodes
Ammonia oxidisers/denitrifiers (DNA based methods)	On site visual recording – fauna
Rhizobium (Most probable number (MPN))	Springtail reproduction
Rhizobium spp.	***Fungi***
Total N	Actinomycetes (DNA based methods)
Microbial	AM fungi (DNA based methods)
Archaea	AM fungi colonisation
Bacterial counts (CFU)	AM Fungi infectivity bioassay
Bacterial community structure (DNA based methods)	fungal species fruiting bodies
Bacterial to fungal ratio	Ectomycorrhizal fungi
Cyanobacterial counts (MPN)	Fungal community structure (DNA based methods)
Genetic profiling of keystone species	Fungal hyphal length/biovolume by microscopy
Microbial community size	Fungal species (DNA based ITS)
Protozoan	Fungi (CFU)
Carbon store/cycling	Saprotrophic fungi
Decomposition from litter bags or bait sticks	
Methanogens/ methanotrophs (DNA based methods)	

Biochemical Indicators	
Toxicity	***Carbon store/cycling***
Microtox	Acetylene inhibition of CH_4 oxidation
Pinus sylvestris fine root tissue Ca/Al molar ratio	Anoxic incubation for $C2H_4$ production
Stress proteins - a/body detection	Cellulase
Xenobiotics (catabolic potential selection assays)	Chitinase
Regulation of multiple processes	Dehydrogenase
Basal respiration	Galactosidase
Community level Physiological Profiles (Biolog)	Glucosidase
Fluorescein diacetate hydrolysis	Glucuronidase
Metabolic quotient	Invertase
Metabolomics	C mineralisation
Microbial quotient	Methane fluxes
Microplate fluorometric assay - multi-enzyme	Microbial C (fumigation)
Muramic acid (bacterial)	Substrate induced respiration
Phenol oxidase	N-acetyl-beta-glucosaminidase
Phylogenetic gene arrays	SIR-GLUC
Proteomics	Xylosidase
Radiorespirometry specific substrates	***Nitorgen cycling***
Microbial	Amidase
Adenylate energy charge	Aminopeptidase
Archaea	Denitrification (short-term incubation)
Bacteria + Biolog/API Identification (CFU)	Microbial N (Chloroform fumigation)
Bacterial biovolume by microscopy	N fixation
Bacterial DNA synthesis 3H-thymidine	N fixers (direct isolation)
Bacterial protein synthesis 14C-leucine	
Biosensor bacteria/fungi/algae	N mineralization
Chlorophyll a content, acetone extraction procedure (algae)	Nitrate reductase
Cytochrome (p450)	Nitrification
Functional gene arrays	Nitrite reductase
Microbial community activity (ATP)	Nitrous oxide fluxes
Phospholipid Fatty Acid (PLFA) profiles	Protease
Total PLFA	Urease
Total RNA	***Fungi***
Sulphur release	AM fungi (NLFA)
Ssulphatase	Ergosterol
Microbial S - Chloroform fumigation	Fungal growth (14C-Acetate)
	Fungilog (CLPP)
Phosphorus cycling	Glomalin (AM fungi)
Microbial P (Chloroform fumigation)	Glucosamine (fungal)
Phosphatase	***Plants***
Phosphomonoesterase	P32 Root uptake bioassay

APPENDIX B – Indicators not considered suitable for further evaluation as effective indicators of atmospheric deposition.

Table B.1 Physical indicators not considered suitable for further evaluation as effective indicators of atmospheric deposition

Indicator	Indicator group	Identified response to atmospheric deposition				Reasons why indicator identified as not suitable for further evaluation as an indicator of atmospheric deposition	Reference
		Increase	Decrease	No response	Complex response		
Water content of soil	Water availability	0	0	X	0	No significant responses identified	1, 2
Water retention characteristics of soil	Water availability	0	0	X	0	No significant responses identified	1
Water-table depth and variability	Water availability	0	0	X	0	No significant responses identified	1
Least limiting water range	Water availability	0	0	0	0	Insufficient information identified	
Soil moisture at 1m depth	Water availability	0	0	0	0	Insufficient information identified	
Subsoil permeability	Water availability	0	0	0	0	Insufficient information identified	
Erosion and sediment distribution	Soil stability	0	0	X	0	No significant responses identified	
Visual soil structure	Soil stability	0	0	X	0	No significant responses identified	
Top-soil aggregate stability	Soil stability	0	0	X	0	No significant responses identified	3
Estimated soil loss	Soil stability	0	0	X	0	No significant responses identified	
Atterberg limits	Soil stability	0	0	0	0	Insufficient information identified	
Packing density	Soil stability	0	0	0	0	Insufficient information identified	
Particle density	Soil stability	0	0	0	0	Insufficient information identified	
Surface crust	Soil stability	0	0	0	0	Insufficient information identified	
Bulk density	Regulation of multiple processes	0	0	X	0	No significant responses identified	
Depth of soil/topsoil/rooting	Regulation of multiple processes	0	0	X	0	No significant responses identified	
Soil texture	Regulation of multiple processes	0	0	X	0	No significant responses identified	
Visual evidence of eluviation (EH layer)	Regulation of multiple processes	0	0	X	0	No significant responses identified	
Porosity	Regulation of multiple processes	0	0	0	0	Insufficient information identified	
Temperature	Regulation of multiple processes	0	0	0	0	Insufficient information identified	

1) Complex response – potential for the indicator to both increase, decrease or not to respond to atmospheric deposition. This complex response may be explained by consideration of other variables e.g. N source and type of deposition and soil type.

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Table B.2 Chemical indicators not considered suitable for further evaluation as effective indicators of atmospheric deposition

Indicator	**Indicator group**	**Identified response to atmospheric deposition** (number of assessors commented)				**Reasons why indicator identified as not suitable for further evaluation as an indicator of atmospheric deposition**	**Reference**
		Increase	**Decrease**	**No response**	**Complex response**		
Available Ni	Toxicity	0	X	0	0	Increase in mobility related to decline in soil pH. Likely to be influenced by metal deposition	1
Available Zn	Toxicity	0	0	0	0	Insufficient information identified. Increase in mobility related to decline in soil pH. Likely to be influenced by metal deposition	
Available Cu	Toxicity	0	0	0	0	Insufficient information identified. Increase in mobility related to decline in soil pH likely under certain soil conditions. Likely to be influenced by metal deposition	
Soil redox	Regulation of multiple processes	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
Electrical conductivity	Regulation of multiple processes	X	0	0	0	Only one study relating to atmospheric deposition identified. Closely related to pH	2, 3
Olsen P	Phosphorus cycling	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
Extractable K	Nutrient cycling	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
Total N	Nitrogen cycling	0	0	X	0	No significant responses to atmospheric deposition considered	
Particulate organic matter (soil solution)	Carbon store/ cycling	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	

1) Complex response – potential for the indicator to both increase, decrease or not to respond to atmospheric deposition. This complex response may be explained by consideration of other variables e.g. N source and type of deposition and soil type.

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Table B.3 Biological indicators not considered suitable for further evaluation as effective indicators of atmospheric deposition

Indicator	**Indicator group**	**Identified response to atmospheric deposition** (number of assessors commented)				**Reasons why indicator identified as not suitable for further evaluation as an indicator of atmospheric deposition**	**Reference**
		Increase	**Decrease**	**No response**	**Complex response**		
Pinus sylvestris roots (biomass, density)	Toxicity	0	0	0	X	Response to atmospheric deposition highly variable	1, 10
Fungal/bacteria biomass ratios (Microscopic determination)	Regulation of multiple processes	0	X	0	0	Method of measurement has been superseded (PLFA method)	2
Roots - size frequency & depth	Plants	0	0	X	X	Response to atmospheric deposition highly variable	3, 4, 5, 6, 7
Plant seed bank - germination	Plants	X	0	0	0	Insufficient information identified in relation to atmospheric deposition	8
Plant seed bank - counts	Plants	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
Ammonia oxidisers/denitrifiers (DNA based methods)	Nitrogen cycling	0	0	0	X	Response to atmospheric deposition highly variable	10
Rhizobium (most probable number)	Nitrogen cycling	0	0	0	0	Insufficient information identified in relation to atmospheric deposition for non agricultural systems	
Cyanobacterial counts (MPN)	Microbial	0	0	0	0	Insufficient information identified in relation to atmospheric deposition.	
Genetic profiling of keystone species	Microbial	0	0	0	0	Insufficient information identified in relation to atmospheric deposition.	
Microbial community size (automated direct observation)	Microbial	0	0	0	0	Insufficient information identified in relation to atmospheric deposition.	
Bacterial community structure (DNA based methods)	Microbial	0	0	0	0	Insufficient information identified in relation to atmospheric deposition.	
Archaea	Microbial	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
Collembola	Invertebrates	X	0	0	X	Response to atmospheric deposition highly variable	9, 12
Springtail reproduction	Invertebrates	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
Earthworm reproduction	Invertebrates	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
Actinomycetes (DNA based methods)	Fungi	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
AM Fungi infectivity bioassay	Fungi	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
Fungal community structure (DNA based methods)	Fungi	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	11
Fungal hyphal length/biovolume by microscopy	Fungi	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
Methanogens/ methanotrophs (DNA based methods)	Carbon store/cycling	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	

1) Complex response – potential for the indicator to both increase, decrease or not to respond to atmospheric deposition. This complex response may be explained by consideration of other variables e.g. N source and type of deposition and soil type.

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Table B.4 Biochemical indicators not considered suitable for further evaluation as effective indicators of atmospheric deposition

Indicator	Indicator group	Identified response to atmospheric deposition				Reasons why indicator identified as not suitable for further evaluation as an indicator of <u>atmospheric deposition</u>	Reference
		Increase	Decrease	No response	Complex response		
Pinus sylvestris fine root tissue Ca/Al molar ratio	Toxicity	0	0	0	X	Response to atmospheric deposition highly variable	1
Xenobiotics (catabolic potential selection assays)	Toxicity	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
Microtox	Toxicity	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
Stress proteins - a/body detection	Toxicity	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
Microplate fluorometric assay - multi-enzyme	Regulation of multiple processes	0	0	0	X	Response to atmospheric deposition highly variable	2
Basal respiration	Regulation of multiple processes	0	X	0	0	No response at low levels of deposition	3-9
Community level Physiological Profiles (Biolog)	Regulation of multiple processes	0	0	0	X	Response to atmospheric deposition highly variable.	10-13
Phenol oxidase	Regulation of multiple processes	0	0	X	X	Response to atmospheric deposition highly variable	12, 14-20,48
Metabolic quotient	Regulation of multiple processes	X	0	0	X	Response to atmospheric deposition highly variable	21-25
Microbial quotient	Regulation of multiple processes	0	0	0	X	Response to atmospheric deposition highly variable	
Fluorescein diacetate hydrolysis	Regulation of multiple processes	0	0	0	X	Single enzyme response to atmospheric deposition highly variable	
Muramic acid (bacterial)	Regulation of multiple processes	0	0	X	0	Insufficient information identified in relation to atmospheric deposition	
Metabolomics	Regulation of multiple processes	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
Phylogenetic gene arrays	Regulation of multiple processes	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
Proteomics	Regulation of multiple processes	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
Radiorespirometry specific substrates	Regulation of multiple processes	0	0	0	0	Insufficient information identified in relation to atmospheric deposition	
P32 Root uptake bioassay	Plants	X	X	0	X	Response highly variable.	13
Microbial P (Chloroform fumigation)	Phosphorus cycling	0	0	0	X	Response highly variable	

Indicator	Indicator group	Identified response to atmospheric deposition				Reasons why indicator identified as not suitable for further evaluation as an indicator of atmospheric deposition	Reference
Nitrate reductase	Nitrogen cycling	X	0	X	0	Single enzyme response highly variable	
Nitrous oxide fluxes	Nitrogen cycling	0	0	0	0	Response highly variable	
Amidase	Nitrogen cycling	0	0	0	X	Single enzyme response highly variable	
Aminopeptidase	Nitrogen cycling	0	0	0	X	Single enzyme response highly variable	
Nitrite reductase	Nitrogen cycling	0	0	0	X	Single enzyme response highly variable	26
Protease	Nitrogen cycling	0	0	0	X	Single enzyme response highly variable	27
Urease	Nitrogen cycling	0	0	0	X	Single enzyme response highly variable	28
N fixers direct isolation	Nitrogen cycling	0	0	0	0	Insufficient information identified for non agricultural systems	N/A
Phospholipid Fatty Acid (PLFA) profiles	Microbial	0	0	0	X	Response highly variable	17, 18, 29-32, 54
Total PLFA	Microbial	0	X	0	X	Response highly variable. Indicator difficult to interpret without additional information.	16, 29, 31, 32, 54, 55
Adenylate energy charge	Microbial	0	0	0	0	Insufficient information identified	
Bacterial DNA synthesis 3H-thymidine	Microbial	0	0	0	0	Insufficient information identified	
Bacterial protein synthesis 14C-leucine	Microbial	0	0	0	0	Insufficient information identified	
Biosensor bacteria/fungi/algae	Microbial	0	0	0	0	Insufficient information identified	
Bacteria + Biolog/API Identification (CFU)	Microbial	0	0	0	0	Insufficient information identified . Method of measurement has been superseded	
Chlorophyll a content, acetone extraction procedure (algae)	Microbial	0	0	0	0	Insufficient information identified	
Cytochrome (p450)	Microbial	0	0	0	0	Insufficient information identified	
Functional gene arrays	Microbial	0	0	0	0	Insufficient information identified	
Total RNA	Microbial	0	0	0	0	Insufficient information identified	
Microbial community activity (ATP)	Microbial	0	0	0	0	Insufficient information identified	
Bacterial biovolume by microscopy	Microbial	0	0	0	0	Insufficient information identified	
Fungal growth (14C-Acetate)	Fungi	0	0	0	0	Insufficient information identified	
Fungilog (CLPP)	Fungi	0	0	0	0	Insufficient information identified	
Glucosamine (fungal)	Fungi	0	0	0	0	Insufficient information identified	
Methane fluxes	Carbon store/ cycling	0	X	0	X	Response highly variable	33

Indicator	Indicator group	Identified response to atmospheric deposition				Reasons why indicator identified as not suitable for further evaluation as an indicator of <u>atmospheric deposition</u>	Reference
Glucosidase	Carbon store/ cycling	0	0	X	X	Single enzyme response highly variable	12, 14, 17, 35, 36
Cellulase	Carbon store/ cycling	0	0	X	X	Single enzyme response highly variable	16, 34, 37
SIR-GLUC	Carbon store/ cycling	0	0	0	X	Response highly variable	12
Microbial C (fumigation)	Carbon store/ cycling	0	0	0	X	Response highly variable	4, 5, 23, 25, 18, 38-42, 49-53
Xylosidase	Carbon store/ cycling	0	0	X	X	Response highly variable	14, 43
Anoxic incubation for C2H4 production	Carbon store/ cycling	0	0	0	X	Response highly variable	
Dehydrogenase	Carbon store/ cycling	0	0	0	X	Single enzyme response highly variable	28, 44, 45, 47
Galactosidase	Carbon store/ cycling	0	0	0	X	Single enzyme response highly variable	
Glucuronidase	Carbon store/ cycling	0	0	0	X	Single enzyme response highly variable	12, 14
Invertase	Carbon store/ cycling	0	0	0	X	Single enzyme response highly variable	
Chitinase	Carbon store/ cycling	0	0	0	X	Single enzyme response highly variable	
Acetylene inhibition of CH4 oxidation	Carbon store/ cycling	0	0	0	X	Response highly variable	46
N-acetyl-beta-glucosaminidase (Nag)	Carbon store/ cycling	0	0	0	0	Insufficient information identified	

1) Complex response – potential for the indicator to both increase, decrease or not to respond to atmospheric deposition. This complex response may be explained by consideration of other variables e.g. N source and type of deposition and soil type.

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