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Development of a nitrophobe/nitrophile classification for woodlands, grasslands and upland vegetation in Scotland.

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Executive Summary

- National surveys have shown that changes in vegetation have occurred that are consistent with increase in atmospheric N deposition. Decreases in frequency in plants of arable and horticultural habitats and calcareous grassland occurred throughout the UK. Decreases in plants of acid grassland, dwarf shrub heath, bogs and montane habitats, however, did not occur to the same degree in the Scottish Highlands. Changes in species composition have also occurred in vegetation adjacent to livestock farms in Scotland and England and have been shown through pollution monitoring and other bioassays to be related to ammonia concentration and deposition.
- The Ellenberg N Index, an indicator system for vascular plants of central Europe which describes the response of individual species to N, was used to assess both the regional and local scale changes and correlated well with measured N deposition. The classification of epiphytic lichens into nitrophytes and acidophytes was also used very successfully at sites in Scotland and England to detect ammonia emissions from poultry farms.
- First attempts to develop an "acidophyte/nitrophyte" classification for higher plants and bryophytes (using plants with an extreme Ellenberg response) indicated a higher sensitivity than the classical Ellenberg approach. A large body of data on woodland flora adjacent to livestock farms was already available, but there was clearly a need to improve the woodland database and to extend the approach to other habitats. The selected habitats include woodland, grasslands because of recorded decline in species richness in calcareous grasslands, and upland vegetation, because of the large areas occupied by acid grasslands, heaths and bogs in Scotland.
- An existing lichen acidophyte/nitrophyte index is based largely on the response of lichens to tree bark pH. However, for higher plants and bryophytes a division between N-loving plants and N-hating plants was sought and the terminology **nitrophobe/nitrophile** was thought to be more appropriate for these species groups.
- The nitrophobe/nitrophile classifications were developed using a 3 stage approach. In Stage 1, the National Vegetation Classification (NVC) was used to determine suitable communities within the broad vegetation types (woodland, heaths, grassland, etc.). NVC community maps were examined to select those communities found extensively in Scotland. These communities were then crosschecked with the Habitats Directive and the UK Biodiversity Action Plan (BAP). NVC lists of plants characteristic of a community provided the basis for the classification. Stage 2 involved an extensive search of the literature for habitat specific species shown to respond either positively or negatively to atmospheric N. In Stage 3, the Ellenberg N Index for individual species, published species responses and expert judgement were used to classify species as nitrophobes or nitrophiles, or those which do not have a clear preference.
- The resulting classifications for woodland are comprehensive and should prove useful. Those for grasslands and heaths are more limited due to the paucity of relevant published data and will undoubtedly need to be refined following field testing.
- This initialnitrophobe/nitrophile classification should be used in the field in combination with all available site information (NVC classification of the site, the pollution climate, soil moisture, management practices etc.) and complementary methods if necessary.
- Future work is necessary to refine the developed classification, develop protocols, provide staff training through workshops, field testing at key sites and the extension of the approach to other habitats.

Contents

1. Introduction

1.1. Changing flora of the UK in relation to Scotland

National changes in plant species composition were recorded in the Countryside Survey of Britain (Haines-Young *et al*. 2000; Smart *et al*. 2003, 2005), and the application of the Ellenberg N Index showed increased fertility associated with infertile grasslands, moorland, upland woodlands and heath/bog. The new Atlas of the British and Irish Flora (Preston *et al*. 2002a) also showed strong evidence of decline in species typical of nutrient-poor habitats and a corresponding increase in species of habitats where nutrient levels are higher. Decreases in frequency in plants of arable and horticultural habitats and calcareous grassland occurred throughout the UK. Decreases in plants of acid grassland, dwarf shrub heath, bogs and montane habitats however, did not occur to the same degree in the Scottish Highlands. Increasing eutrophication (NEGTAP 2001), together with habitat loss and changes in farming practice, were cited as probable causes. Ellenberg N Index was applied to the results of the two Atlas surveys (1950s and 1990s) and it was found that species with an index below 5 had declined and those above 5 had increased. The pattern did not occur in the Scottish Highlands, and was less marked in Eastern Scotland than elsewhere in the UK. Similar changes in vegetation have been recorded elsewhere in Europe (Diekmann and Dupre 1997; Hofmeister *et al*. 2002; Ejrnaes *et al*. 2003).

1.2. Ellenberg Nitrogen Index

Ellenberg devised a comprehensive indicator system for vascular plants of central Europe (Ellenberg 1979; Ellenberg *et al.* 1991) to describe the response of individual species to a range of ecological conditions (light, temperature, continentality, moisture, pH and nitrogen). The Ellenberg N Index (ENI) allocates an N score to each plant species, so that the overall community has a score on a scale of nutrient poor (1) to nutrient rich (10). While most studies have focused on higher plants, the approach has been extended to cover bryophytes using indicator values from (Siebel 1993) and lichens using indicator values from Wirth (1992). The Ellenberg N Index has been used on local and regional scales to detect the impact of enhanced N deposition on species composition (van Dobben 1993; Pitcairn *et al*. 2002, 2003) and a recent review of published studies on the Ellenberg N Index, (Sutton *et al*. 2004) concluded that in general, the index is a useful tool for detecting floristic shifts consistent with increased nutrient availability and ecosystem eutrophication.

1.2.1. Examples of local-scale application in Europe

The impact of N additions on the plant indicator score was examined by Van Dobben *et al.* (1999) in Swedish coniferous woodland experiments in which N or acidity had been added over a period of 15 years. Calculated mean indicator values were either **unweighted using presence/ absence** for each species or **weighted by using cover/abundance** of each species. The nitrogen fertilisation treatment had the strongest effect, an addition of around 60 kg N ha^{-1} y^{-1} causing a shift from ericaceous species with acrocarpous mosses and lichens to dense carpet of *Deschampsia flexuosa*, pleurocarpous mosses and ruderal species such as *Chamaenerion angustifolium* and *Rubus idaeus* after 15 years of treatment. Better relationships were found between measured environmental variables and indicator values based on presence/absence data, rather than on cover/abundance data. Similar conclusions were reached by Diekmann (1995), in a study of a Swedish deciduous forest.

1.2.2. Examples of local-scale application in Scotland

CEH Edinburgh has been monitoring atmospheric pollution level in Scotland and Northern England since 1980. The importance of ammonia emissions from livestock as a component of atmospheric N deposition was recognised in mid 1980s and expertise was developed in the difficult task of measuring ammonia concentrations and deposition to vegetation (Sutton *et al*. 1993, 1998). In 1995-1998, impacts of ammonia emissions from livestock farms on woodland flora, was investigate using a variety of techniques (tissue N content of mosses, herbs and trees, vegetation surveys and Ellenberg N Index). Tissue N of mosses proved to be an excellent indicator of N deposition but a reasonable relationship was also shown between Ellenberg N Index, (modified for British conditions by Hill *et al*. 1999) and atmospheric NH_3 concentrations for a gradient of ammonia concentration and N deposition downwind of 2 poultry farms, a pig farm and a dairy farm, where changes in species composition had been measured (Pitcairn *et al.* 1998, 2002).

Following this work, the suitability of the Ellenberg N Index (and other novel biomonitoring methods) for assessing the effects of N on condition and integrity of sites of conservation interest was investigated in 4 intensive study sites in 2004, which include 2 sites adjacent to a gaseous N pollution source (vehicular NO_x and agricultural NH_3) and 2 sites with similar habitats, but different inputs of wet deposited N (Leith *et al*. 2005). The Ellenberg Index correlated well with atmospheric N deposition at the 4 intensive sites, confirming the strength of the method in indicating enhanced N deposition. It also provided a useful assessment of the N status of a site, particularly along known gradients in N deposition. The index appeared to be a weaker predictor of the relative N status of the blanket bog sites dominated by wet deposited N. At such sites, the presence of only stress-tolerant, low N value species and the absence of propagules of high Ellenberg N Index plants may restrict changes in the mean Ellenberg N Index.

1.3. Acidophyte/Nitrophyte Index

Biomonitoring, using tree lichens to detect atmospheric N in agricultural areas, used first in the Netherlands (van Herk 1999) and later in the UK (Wolseley and James 2002), involved the development of indices of nitrophyte and acidophyte lichens. Acidophytes prefer naturally acidic bark while nitrophytes prefer enriched, more basic bark, resulting from enhanced NH_3 concentrations. This approach was used very successfully at Earlston poultry farm in southern Scotland (Sutton *et al*. 2004) and later at sites described in Leith *et al*. (2005). Subtraction of the nitrophyte score from the acidophyte score provided an index showing whether the flora was dominated by acidophytic or nitrophytic species.

A first attempt was made to apply this approach to the vascular plant and bryophyte species recorded at the 4 intensive sites described above, paying particular attention to Piddles Wood, Dorset (Leith *et al.* 2005). Species were described as acidophyte or nitrophyte mainly on the basis of their Ellenberg N Index scores – e.g. those with scores of 4 and below were described as acidophytes and those with 6 and above, as nitrophytes. In general, the acidophyte species tended to be vernal species, orchids, woodrushes and several bryophyte species typical of acid woodlands and moorland. Nitrophytes tended to be ‘weed species’ such as nettle, dock, hogweed, chervil, willow herbs and grasses typical of arable land together with a few bryophytes, which prefer N enriched habitats. Nitrophytic species were found to dominate the flora at NH_3 concentrations greater than 3 $\mu g\ m^{-3}$.

Overall, this first test of the Acidophyte/Nitrophyte approach for higher plants and bryophytes indicated a high sensitivity compared with the classical Ellenberg approach. Expansion of databases and refinement and validation of the method was recommended, and as a result a study was commissioned by SEPA to develop lists of acidophytes and nitrophytes for a range of important habitats.

This literature review aims to identify acidophytes and nitrophytes for a specific range of habitats. CEH research has already shown the impact of intensive livestock farms on woodland vegetation and now this database needs to be expanded to include additional published data. A decline in species richness in calcareous grasslands has been noted for Scotland (Preston *et al*. 2002b) and some general increase in Ellenberg N Index for Eastern Scotland. Although little decline in plants of acid grassland, dwarf shrub heath, bogs and montane habitats was found for the Scottish Highlands, these habitats occupy large areas of Scotland. Thus on this basis, the selected habitats include woodland, grasslands and upland vegetation.

1.4. Evolution of terminology: nitrophobe/nitrophile classification

The use of the terms acidophyte and nitrophyte is appropriate for lichens (van Herk 1999, Wolseley and James 2002, Sutton *et al*. 2004) particularly those found on tree bark. Acidophytes prefer naturally acidic bark while nitrophytes prefer enriched, more basic bark, resulting from enhanced NH_3 concentrations. Hence the terms acidophyte – acid plant and nitrophyte - nitrogen plant, describe the lichen response to N pollution. For higher plants the division is misleading. Ideally we wish to separate nitrogen loving plants from non- nitrogen loving plants. Thus the use of nitrophyte versus oligophyte (low nutrient plant) might be appropriate, although somewhat of a mouthful. Some workers correctly refer to nitrogen-loving plants as nitrophiles (Gordon *et al*. 2001) and it is proposed to use the terms nitrophile and nitrophobe to describe the response of plants to enhanced nitrogen deposition. This is more in line with the useage of calcicoles and calcifuges for calcium loving or hating plants. Although many species characteristic of more fertile sites such as fertile mixed deciduous woodlands may have quite high Ellenberg N indices and could not be said to be nitrogen – hating plants, they are sensitive to high levels of N deposition and hence can be referred to as nitrophobes.

2. Methods

2.1. Introduction

The species denoted as nitrophobes and nitrophiles need to be defined on a habitat specific basis ideally according to the results of experimentation on N deposition responses. Such experimentation has been confined to very few habitats (e.g. woodland, *Calluna* heath) and frequently focussed on impacts on one or a few species (*Racomitrium lanuginosum, Calluna* etc). Impacts of point sources of N such as intensive livestock farms has by necessity focussed on semi-natural vegetation adjacent to farms, most usually woodland /shelter belt.

Many studies employ the additions of a single large treatment dose or large concentrations of N in liquid additions. Consequently some species may give different responses to added N and published papers provide contradictory evidence.

Because of considerable experience in conducting experiments on the impacts of N on vegetation in chambers and field experiments and in botanical surveys in the field under different N deposition levels, expert judgement will be an essential element in assembling lists of nitrophobes and nitrophiles for the selected habitats.

The first attempts to described species as nitrophobes or nitrophiles for the 4 intensive study sites (Leith *et al*. 2005) were made mainly on the basis of their Ellenberg scores – e.g. those with scores of 4 and below were described as nitrophobes and those with 6 and above, as nitrophiles. Species with an index of 5 (intermediate N plants) were excluded together with those species, which are not so easy to identify and those which can be readily overlooked in the field, such as liverworts. Species with a low Ellenberg N Index, but known to respond to added N from studies in the UK, Netherlands and Sweden (Pitcairn *et al.* 1998) were described as nitrophiles because of their potential to out-compete small herbs in eutrophicated environments. These include the grasses *Deschampsia flexuosa*, *Festuca ovina* and *Molinia caerulea*. Although these species naturally occur as low N index plants in many habitats their presence and perhaps more importantly, a significant cover of such species should be carefully noted.

2.2. Stage 1: Identification of plant communities based on the NVC

To develop a list of nitrophobes and nitrophiles on a habitat specific basis, it is necessary to obtain a list of species typical or characteristic of that habitat, *i.e*. those species which would be expected and/or desired to be present. The National Vegetation Classification provides the standard classification used to describe vegetation in Britain (Rodwell 1992). This classification divides the broad vegetation types (woodland and scrub, heaths, grassland, etc.) into smaller communities and sub-communities designated by a number and name (*eg*. W9 *Fraxinus excelsior-Sorbus aucuparia-Mercurialis perennis* woodland).

All NVC community maps were examined to select those communities found extensively in Scotland. These communities were then crosschecked with the Habitats Directive and the UK Biodiversity Action Plan (BAP) (Averis *et al*. 2004). On this basis the following NVC communities were selected:

Woodlands:

W8 *Fraxinus excelsior-Acer campestre -Mercurialis perennis* woodland

W9 *Fraxinus excelsior-Sorbus aucuparia-Mercurialis perennis* woodland

W11 *Quercus petraea-Betula pubescens-Oxalis acetosella* woodland

W17 *Quercus petraea- Betula pubescens-Dicranum majus* woodland

W18 *Pinus sylvestris-Hylcomium splendens* woodland

Calcicolous grasslands:

CG10 *Festuca ovina-Agrostis capillaris-Thymus praecox* grassland

CG11 *Festuca ovina-Agrostis capillaris-Alchemilla alpina* grass-heath

CG12 *Festuca ovina--Alchemilla alpina-Silene acaulis* dwarf-herb community

CG13 *Dryas octopetala-Carex flacca* heath

CG14 *Dryas octopetala-Silene acaulis* ledge community

Calcifugous grasslands and montane communities:

U4 *Festuca ovina-Agrostis capillaries-Galium saxatile* grassland

U5 *Nardus stricta-Galium saxatile* grassland

U7 *Nardus stricta-Carex bigelowii* grass-heath

U10 *Carex bigelowii-Racomitrium lanuginosum* moss-heath

Mires:

M15 *Scirpus cespitosus-Erica tetralix* wet heath

M16 *Erica tetralix-Sphagnum compactum* wet heath

M17 *Scirpus cespitosus-Eriophorum vaginatum* blanket mire

M18 *Erica tetralix-Sphagnum papillosum* raised and blanket mire

M19 *Calluna vulgaris-Eriophorum vaginatum* blanket mire

M20 *Eriophorum vaginatum* blanket raised mire

Heaths:

H10 *Calluna vulgaris-Erica cinerea* heath

H12 *Calluna vulgaris-Vaccinium myrtilis* heath

H16 *Calluna vulgaris-Arctostaphylos uva-ursi* heath

H18 *Vaccinium myrtilis-Deschampsia flexuosa* heath

H20 *Vaccinium myrtilis-Racomitrium lanuginosum* heath

The NVC floristic tables produced for each community (Rodwell 1992) include all vascular plants, bryophytes and lichens that occur with a frequency of 5% or more in any one of the sub-communities. Species are recorded with their frequency and abundance. Frequency refers to presence of a species and is listed using Roman numerals on a scale of I to V. Species of frequency classes IV and V in a particular community are referred to as its constants. Those of class III are called common and those of II and I, occasional and scarce respectively. Abundance is used to describe how much of a plant is present in a sample (e.g. dominant, abundant, plentiful, sparse).

In this study, the constant species were listed together with most common and a few occasional species and Ellenberg and Siebel N index numbers were allocated to vascular plants and bryophytes respectively. In the first instance these species form the basis of the nitrophobes, i.e. the status quo, recognising that some may be potential nitrophiles, despite their Ellenberg N Index.

Because of their usually low frequency, many lichens particularly epiphytic lichens are not recorded in the NVC tables. Consequently the use of epiphytic lichens as biomonitors has been addressed separately in Section 2.5.

2.3. Stage 2: Literature Search

Databases have already been compiled for CEH woodland habitats. The next stage was to make as thorough a search of the literature in the available time, to determine species that had been lost or had declined from areas of high N deposition in the field, and those species which responded positively or negatively to added N. This latter group included results of fertiliser (or N addition) trials in the field (both short and long-term) and N addition experiments in chambers, microcosms etc. While some important publications included intensive vegetation surveys made before and after N additions, most involved impacts of N on single species or 2 or 3 species in competition. Some publications investigated effects of N and other environmental factors and management practices. Publications included records from many countries and continents. However, studies from Scandinavia, France, Germany, Switzerland and the Netherlands provided data more relevant to the UK climate and vegetation. The search provided some nitrophiles but a much larger list of nitrophobes.

2.4. Stage 3: Matching records

Stage 3 involved matching literature records and results from the extensive field studies carried out by CEH, with selected habitats. The limitations of the available data made it necessary to combine some of the NVC classes in order to avoid tables for habitats where no reliable data are available. Nitrophobes are nearly always species typical of the selected communities with an Ellenberg N Index of 4 or below. Nitrophiles can be species typical of the community with a high Ellenberg N Index (>6) and species (from the community or related communities) known to respond to increased N either from published records or through expert judgement irrespective of their Ellenberg N Index.

2.5. Lichens

The application of epiphytic lichen indicator species to detect increasing atmospheric ammonia associated with intensive livestock farming was developed in the Netherlands by analysing data from lichen communities on trunks of oak trees to determine nitrophyte and acidophyte indicator species (van Herk 1999, 2002). This technique was assessed in the UK, and a sampling protocol was devised for lichen communities of twigs (Wolseley and Pryor 1999) and combined with van Herk (1999, 2002) indicator species. The method has been successfully applied in Scotland (Sutton *et al*. 2004; Leith *et al*. 2005) and lists of acidophyte and nitrophyte species have been produced which can be applied to sites in the UK. A range of studies on different tree type largely confirm the major acidophyte and nitrophyte species (Lambley and Wolseley 2003). The results of all surveys have shown that lichens on twigs are better correlated with atmospheric conditions than lichens on trunks. Lichens are often long lived and nitrogen is rarely lethal so that communities on trunks may be relics and often contain species that are indicators of ecological continuity. This is important in sites of conservation importance but results show that lichens on twigs are able to provide an early warning system of changes that may take some time on older substrates.

Application of epiphytic lichens as indicators of NH_3 concentrations is appropriate where acid-barked tree species of the same species are present and where there is some habitat homogeneity. Clearly this approach is ideal for assessing woodlands but can also be used wherever sufficient trees of the same species are present. As described in Leith *et al*. (2005), trees adjacent to grasslands or heaths can be examined to give an indication of the pollution climate in the area. Consequently, a table of lichen acidophytes and nitrophytes has been included separately (Section 3.4) that is applicable to all habitats where trees may be found close by.

3. Results

3.1. Woodlands

In Europe, woodland may be conveniently divided into deciduous and coniferous woodland. In the UK most of the native woodland is mixed deciduous with naturally occurring coniferous woodland confined to a few pockets in Scotland. However, large tracts of coniferous plantation exist usually planted on infertile areas such as acid sands of East Anglia and Scotland and wet acid moorland of Scotland. In addition small patches may be planted as shelter belts and within existing woodland types. In a similar fashion, beech while outside its native range in Scotland occurs frequently planted in country estates and woodlands.

For the purposes of this exercise it may seem simpler to follow the division into mixed deciduous and coniferous. However, the basis of woodland classification is both climatic and soil type, and species which may be regarded as nitrophiles for less fertile woodland, may in fact be the constant species in more fertile woodland. Publications from Europe may refer to oak woodland, boreal forest etc. or pine plantation and it is important to use the data correctly.

Mixed deciduous and oak-birch woodlands in the cool and wet North Western sub-montane zone of the UK vary in line with soil differences. Rendzinas and brown earth calcareous soils support W9 *Fraxinus-Sorbus-Mercurialis* woodland. (In the south, such soils support W8 *Fraxinus-Acer-Mercurialis* woodland, and a few examples are found in Scotland). Brown earths of low base status support W11 *Quercus-Betula-Oxalis* woodland, while acid rankers, brown podzolic soils and podzols support W17 *Quercus-Betula-Dicranum* woodland. Floristic response to these soil variations depend on a range of additional factors not least sylvicultural treatments and moisture levels.

Planted *Pinus sylvestris* may be a replacement for other tree species in mixed deciduous and oak-birch woodlands in the UK. In Scotland, within its believed natural distribution, it forms the W18 *Pinus-Hylocomium* woodland, and is found on similar base poor soils as occupied by W17, often with a similar ground flora.

For the purposes of this study, species lists are provided for fertile mixed deciduous woodlands (W8, W9), infertile oak-birch woodlands (W11, W17) and pine woodlands (W18).

Tables 1-3 include columns of species designated as nitrophobes (although non-nitrophile may be more appropriate in some cases) and nitrophiles, both known and potential. In some cases the potential nitrophiles may be constant or common species in that community and research has shown the potential for these species to respond to increased N. Other cited nitrophiles may not be typical of the community but may be able to colonise from adjacent agricultural land or be brought in by grazing animals, walkers and forest workers and machinery. As some listed nitrophiles should not be counted as such unless present in abundance, it is important to also record a rough cover of all listed nitrophiles.

Table 1: Classification of species for mixed deciduous woodlands (W8, W9) into nitrophobes and nitrophiles using NVC, published reports and expert judgement. Ellenberg N Index (ENI).

Species	**ENI**	**Caveats and comments**
Nitrophobes		
Anemone nemorosa	4	
Anthoxanthum odoratum	3	
Blechnum spicant	3	
Conopodium majus	5	
Luzula sylvatica	4	
Oxalis acetosella	4	Constant species
Potentilla erecta	2	
Primula vulgaris	4	Ancient woodland indicator
Pteridium aquilinum	3	
Succisa pratensis	2	
Viola riviniana	4	Constant species. Ancient woodland indicator
Dicranum scoparium	2	
Hylocomium splendens	3	
Isothecium myosuroides	4	
Nitrophiles		
Agrostis stolonifera	6	Can invade rapidly.
Anthriscus sylvestris	7	
Arrhenatherum elatior	7	
Chaerophyllum temulentum	7	
Chamaenerion angustifolium	5	Increase may also be in response to sylviculture.
Dactylis glomerata	6	May respond to increased N.
Deschampsia flexuosa	3	Known to respond to increased N. Note abundance.
Festuca gigantean	5	May respond to increased N.
Galium aparine	8	
Geranium robertianum	6	Known to respond to increased N.
Geum urbanum	7	
Glechoma hederacea	7	Note size and abundance.

Species	ENI	Caveats and comments
Holcus lanatus	5	Has potential to increase. Note abundance.
Hyacinthoides non-scripta	6	May respond to increased N in moist soils.
Lamiastrum galeobdolon	6	Note size and abundance.
Mercurialis perennis	7	Although a Constant, known to respond to increased N.
Milium effusum	5	Known to respond to increased N. Note abundance.
Rubus fructicosus	6	Known to respond to increased N. Note abundance.
Rubus idaeus	6	Known to respond to increased N. Note abundance.
Rumex obtusifolia	9	
Silene vulgaris	5	Known to respond to increased N.
Stachys sylvatica	8	
Stellaria media	7	
Urtica dioica	8	
Eurynchium praelongum	6	Constant species. Note abundance.
Brachythecium rutabulum	8	
Plagiothecium undulatum	3	Known to respond to increased N.
Thamnium alopecurum	7	

Table 2: Classification of species for Oak-birch woodlands (W11, W17) into nitrophobes and nitrophiles, based on NVC, published reports and expert judgement. Ellenberg N Index (ENI).

Species	ENI	Caveats and comments
Nitrophobes		
Agrostis capillaris	3	
A. canina	3	
Anemone nemorosa	4	
Anthoxanthum odoratum	3	
Blechnum spicant	3	
Calluna vulgaris	3	
Galium saxatile	3	
Luzula pilosa	4	
Oxalis acetosella	4	
Potentilla erecta	2	
Primula vulgaris	4	Ancient woodland indicator.
Pteridium aquilinum	3	
Vaccinium myrtilis	2	
Viola riviniana	4	Ancient woodland indicator.
Dicranum spp.	2	*D. scoparium*, *D. majus*
Frullania tamarisci	1	Indicator of low N. Easy to identify.
Hylocomium splendens	3	
Isothecium myosuroides	4	
Pleurozium schreberi	2	
Polytrichum formosum	4	
Rhytidiadelphus loreus	3	
R. triquetrus	2	
Thuidium tamariscinum	4	
Nitrophiles		
Aegopodium podograria	7	
Chamaenerion angustifolium	5	Known to respond to increased N. Note abundance.
Deschampsia flexuosa	3	Known to respond to increased N. Note abundance.
Galium aparine	8	
Geranium robertianum	6	
Hedera helix	7	

Species	ENI	Caveats and comments
Holcus spp.	5	*H. mollis, H. lanatus*
Rubus fructicosus	6	Known to respond to increased N. Note abundance.
Rubus idaeus	6	Known to respond to increased N. Note abundance.
Rumex obtusifolia	9	
Sambucus nigra	7	
Poa trivialis	6	Known to respond to increased N. Note abundance.
Stellaria media	7	
Urtica dioica	8	Not found in deep shade.
Eurynchium praelongum	6	Known to respond to increased N. Note abundance.
Brachythecium rutabulum	8	
Plagiothecium undulatum	3	Known to respond to increased N. Note abundance.

Table 3: Classification of species for *Pinus sylvestris- Hylocomium splendens* woodland (W18) into nitrophobes and nitrophiles, based on NVC, published reports and expert judgement. Ellenberg N Index (ENI).

Species	ENI	Caveats and comments
Nitrophobes		
Anthoxanthum odoratum	3	
Blechnum spicant	3	
Calluna vulgaris	3	
Empetrum nigrum	2	
Erica spp.	2	
Luzula pilosa	4	
Melampyrum pratense	3	
Oxalis acetosella	4	
Vaccinium myrtilis	2	
Dicranum spp	2	
Hylocomium splendens	3	
Hypnum jutlandicum	2	
Pleurozium schreberi	2	
Ptilium crista-castrensis	2	
Rhytidiadelphus spp.	2	*R. loreus, R. triquetrus*
Sphagnum capillifolium	2	
Cladonia spp		
Nitrophiles		
Chamaenerion angustifolium	5	
Deschampsia flexuosa	3	
Dryopteris dilatata	5	
Galium aparine	8	
Poa trivialis	6	
Rubus fructicosus	6	
Rubus idaeus	6	
Urtica dioica	8	
Brachythecium rutabulum	8	
Eurynchium praelongum	6	
Plagiothecium undulatum	3	

3.2. Grasslands

3.2.1. Calcareous grasslands

Within Scotland, the most common calcicolous grasslands are the northern montane and mire calcicoles CG10 *Festuca ovina-Agrostis capillaries-Thymus praecox*; the artic-alpine calcicolous grasslands – CG11 *Festuca ovina-Agrostis capillaries-Alchemilla alpina* grass-heath, CG12 *Festuca ovina-Alchemilla alpina-Silene acaulis* dwarf-herb community and CG14 *Dryas octopetala-Silene acaulis* ledge community; and the lowland Dryas heath CG13 *Dryas octopetala-Carex flacca* heath.

In CG10, depending on soil moisture and lime-saturation levels, both mesophytic and calcifugous species may be present causing some overlap with calcifugous grasslands. At higher altitudes increased rainfall means increased leaching and lower base richness and lower species diversity. The species richness of CG10, 11, 12, (to a lesser degree) and 14 grasslands is maintained by sheep grazing with some deer, rabbits and hares. CG13 is typical of ungrazed high, rocky ledges.

A single table (Table 4) has been produced for calcareous grasslands. The range of nitrophobe species is large and should be used in parallel with species lists of the vegetation classification. Published data for N effects on calcareous grasslands typical of Scotland is negligible and data from more southern grasslands has been included with caveats.

Table 4: Classification of species for calcareous grasslands (CG10, 11, 12, 13, 14) into nitrophobes and nitrophiles, based on NVC, published reports and expert judgement. Ellenberg N Index (ENI).

Species	**ENI**	**Caveats and comments**
Nitrophobes		
Agrostis capillaris	2	
Antennaria dioica	2	
Anthoxanthum odoratum	3	
Alchemilla alpina	3	
Asplenium viride	3	
Briza media	3	
Calluna vulgaris	2	
Campanula rotundifolia	2	Indicator of low N.
Carex flacca	2	Reduced by added N.
Carex spp.	2-3	
Dryas octopetala	2	In CG13 and 14
Euphrasia officinalis	3	
Galium spp.	3	*G. saxatile, G. boreale*
Helianthemum nummularis	2	“
Hieracium pilosella		Reduced by added N.
Hypochaeris radicata	3	“
Koeleria macrantha	2	“
Leontodon autumnalis	4	“
Linum catharticum	2	
Lotus corniculatus	2	
Luzula spicata	2	
Pinguicula vulgaris	2	
Plantago lanceloata	4	Reduced by added N.
Potentilla erecta	2	
Saxifraga spp.	2	
Selaginella selaginoides	2	
Sibbaldia procumbens	3	
Silene acaulis	1	
Succisa pratensis	2	
Thalictrum alpinum	3	
Thymus praecox	2	
Viola riviniana	4	
Ctenidium moluscum	2	
Dicranum scoparium	2	

Species	ENI	Caveats and comments
Ditricnum flexicaule	2	
Hylocomium splendens	3	
Racomitrium lanuginosum	1	
Rhytidiadelphus squarrosus	5	
Rhytidiadelphus spp.	2-3	*R. loreus, R. triquetrus*
Tortula tortuosa	3	
Nitrophiles		
Festuca rubra	5	
Festuca ovina	2	Known to respond to N in some situations
Nardus stricta	2	"
Vaccinium myrtilis	2	"

3.2.2. Calcifugous grasslands and montane communities

U4 *Festuca ovina-Agrostis capillaris-Galium saxatile grassland* is one of the most important pastures on acidic, well drained soils in western Scotland. U5 *Nardus stricta-Galium saxatile* grassland is more typical of moist, peaty, infertile mineral soils and provides poorer quality upland grazing. U7 *Nardus stricta-Carex bigelowii* grass-heath is found in the low-alpine zone of the Scottish Highlands, where floristic variation depends on precipitation and snow fall. Large stands of U10 *Carex bigelowii-Racomitrium lanuginosum* moss-heath are found over the windy ridges and summits of the Highlands. U4 and U5 are not of great conservation value for their flora but are important for supporting birds, insects etc. U7 and U10 are of interest because of their bryophyte and lichen flora.

A single table (Table 5) has been produced for the selected calcifugous grasslands. Published data for N effects tend to relate to grazing and fertiliser interactions, or to impacts on bryophyte and lichen species.

Table 5: Classification of species for calcifugous grasslands (U4, U5, U7, U10) into nitrophobes and nitrophiles, based on NVC, published reports and expert judgement. Ellenberg N Index (ENI).

Species	ENI	Caveats and comments
Nitrophobes		
Agrostis capillaris	2	
Alchemilla alpina	3	
Anthoxanthum odoratum	3	
Galium saxatile	3	
Luzula spp.	3	
Potentilla erecta	2	
Dicranum fuscesens	2	
Dicranum scoparium	2	
Hylocomium splendens	3	
Hypnum cupressiforme	3	
Pleurozium schreberi	2	
Racomitrium lanuginosum	1	
Sphagnum spp.	1-2	
Rhytidiadelphus loreus	5	
Cetraria islandica		
Cladonia spp.		*C. uncialis, C. arbuscula*
Nitrophiles		
Agrostis stolonifera	6	May invade from more fertile grasslands when N increases.
Carex bigelowii		Increased by added N.
Deschampsia flexuosa	3	Responds to increased N.
Festuca ovina	2	May respond to increased N. Note abundance.

Species	ENI	Caveats and comments
Poa trivialis	6	May invade from more fertile grasslands when N increases.
Cerastium fontanum	4	May invade from more fertile grasslands when N increases.
Nardus stricta	2	Increased by added N on heaths.
Plantago major	2	May invade from more fertile grasslands when N increases.

3.3. Mires and heaths

To represent the mires of Scotland, the following communities were selected: M15 *Scirpus cespitosus-Erica tetralix* wet heath, M16 *Erica tetralix-Sphagnum compactum* wet heath, M17 *Scirpus cespitosus-Eriophorum vaginatum* blanket mire, M18 *Erica tetralix-Sphagnum papillosum* raised and blanket mire, M19 *Calluna vulgaris-Eriophorum vaginatum* blanket mire, M20 *Eriophorum vaginatum* blanket raised mire.

The wet heaths M15 and M16 are characteristic of moist acidic and oligotrophic peats and peaty soils, whereas M16 is found mainly in the north-east of Scotland. M17 is the typical blanket bog of oceanic areas whereas M19 is typical of cold, wet high-level plateaux. M20 with its absence of dwarf shrubs, often arises as a result of widespread burning and grazing. M18 is similar to M17 but includes a more extensive *Sphagnum* carpet (Table 6).

Table 6: Classification of species for upland vegetation: wet heath and blanket bog, (M15, M16, M17, M18, M19) into nitrophobes and nitrophiles, based on NVC, published reports and expert judgement.
Ellenberg N Index (ENI).

Species	ENI	Caveats and comments
Nitrophobes		
Calluna vulgaris	2	
Drosera rotundifolia	3	
Empetrum nigrum	1	
Erica tetralix	1	
Eriophorum angustifolium	1	
Galium saxatile	3	
Myrica gale	2	
Narthecium ossifragum	1	
Polygala serpyllifolia	2	
Potentilla erecta	2	
Rubus chamaemorus	1	
Scirpus cespitosus	1	
Aulocomium palustre	2	
Dicranum scoparium	2	
Hypnum cupressiforme	3	
Hypnum jutlandicum	3	
Pleurozium schreberi	2	
Ptilidium ciliare	2	
Racomitrium lanuginosum	1	
Rhytidiadelphus loreus	2	
Sphagnum spp.	1-2	*S. fuscum, S warnstofii, S. papillosum, S magellanicum, S. compactum, S. tenellum, S.capillifolium, S. subnitens*
Cladonia spp.		*C. portentosa, C. uncialis*
Nitrophiles		
Agrostis stolonifera	6	Invading from fertile upland grasslands.
Deschampsia flexuosa	3	Responded to N additions in Dutch heaths.
Eriophorum vaginatum	1	Increased following N additions.
Festuca rubra	5	Invading from fertile upland grasslands.
Holcus lanatus	5	Invading from fertile upland grasslands.

Species	ENI	Caveats and comments
Molinia caerulea	2	Constant, but responded to N additions in Dutch heaths.
Festucal ovina	2	Responded to N additions in Dutch heaths.
Nardus stricta	2	Competes with Calluna following N additions.
Plagiothecium undulatum	3	
Rumex acetosa	4	Invading from fertile upland grasslands.
Vaccinium spp.	2	N additions increased biomass of *V. oxycoccus*, and occasionally. *V. myrtilis*. Note abundance.
Polytrichum strictum		
Sphagnum angustifolium		
Sphagnum fallax	4	

Typical Scottish heaths included H10 *Calluna vulgaris-Erica cinerea* heath, H12 *Calluna vulgaris-Vaccinium myrtilis* heath, H16 *Calluna vulgaris-Arctostaphylos uva-ursi* heath, H18 *Vaccinium myrtilis-Deschampsia flexuosa* heath, H20 *Vaccinium myrtilis-Racomitrium lanuginosum heath*. H10 is frequently called the 'Atlantic heather moor' being found largely on the west of the country. On cooler higher ground H10 is replaced by H12 which can be regarded as the boreal heather moor of Britain. H16 and H18 are both centred on the east-central Highlands where the climate is drier and temperature variations are more extreme. H20 has been included because it represents montane heath with lichens and mosses and because there are published data relevant to some of its species (Table 7).

Table 7: Classification of species for upland vegetation: Heath, (H10, H12, H16, H18, H21) into nitrophobes and nitrophiles, based on NVC, published reports and expert judgement. Ellenberg N Index (ENI).

Species	ENI	Caveats and comments
Nitrophobes		
Anthoxanthum odoratum	3	
Arctostaphylos uva-ursi	2	
Blechum spicant	3	
Calluna vulgaris	2	
Erica cinerea	1	
Erica tetralix	1	
Empetrum nigrum	1	
Galium saxatile	3	
Listera cordata	2	
Potentilla erecta	2	
Vaccinium spp	2	*V. myrtilis, V. vitis-idaea*
Dicranum spp	2	*D. majus, D. scoparium*
Hylocomium splendens	3	
Hypnum cupressiforme	3	
Hypnum jutlandicum	3	
Pleurozium schreberi	2	
Ptilidium ciliare	2	
Racomintrium lanuginosum	1	
Rhytidiadelphus loreus	2	
Sphagnum spp.	1-2	
Cetraria spp.		*C. ericetorum, C. nivalis*
Cladonia spp.		*C. mitis, C.stellaris, C. portentosa, C. arbuscula*
Nitrophiles		
Agrostis stolonifera	6	
Andromeda polifolia	1	Note abundance
Carex bigelowii	2	
Deschampsia flexuosa	3	Constant but responds to added N. Note abundance
Festuca ovina	2	Invading from fertile upland grasslands
Festuca rubra	5	

Species	ENI	Caveats and comments
Holcus lanatus	5	
Molinia caerulea	2	
Nardus stricta	2	
Rumex acetosa	4	
Polytrichum spp.	5	*P. commune, P. juniperinum.* Note abundance.

3.4. Lichens

In addition to the specific habitat lists, the lichen acidophyte and nitrophyte classification (Table 8) should be used where possible (See Leith *et al*. 2005; Section 7 and 11 for details of lichen frequency surveys at four intensive sites and at the UK scale).

Table 8: Acidophyte and nitrophyte lichens for use in and around all habitats.

Acidophytes	Nitrophytes
Bryoria fuscescens	*Candelariella reflexa*
Cladonia spp.	*Hyperphyscia adglutinata*
Evernia prunastri	*Phaeophyscia orbicularis*
Hypogymnia physodes	*Physcia adscendens*
Hypogymnia tubulosa	*P. tenella*
Lecanora conizaeoides	*Xanthoria candelaria*
L. pulicaris	*X. parietina*
Lepraria spp.	*X. polycarpa*
Parmelia saxatilis	
Platismatia glauca	
Pseudevernia furfuracea	
Usnea spp.	

The key to lichens on twigs produced by Wolseley *et al*. (2002) may be downloaded as an additional aid.
See Wolseley *et al.* (2002) http:/www.nhm.ac.uk/botany/lichen/twig

3.5. Field application of nitrophobe/nitrophile classification

It must be emphasized that the above classification should be used where possible in combination with knowledge of the NVC classification of the site. NVC lists should be studied before hand to obtain an idea of the potential species richness of the site. Any prior knowledge of the site such as management practices, soil moisture levels, exposure should be considered, as should any potential point sources of N (farms, sewage works, slurry dumps, animal shelter spots). In addition maps of N deposition (NEGTAP 2001) and the UK Air Pollution Information database (APIS 2004) should be consulted to be aware of pollution levels in the area.

Where a possible gradient of pollution is suspected records should be taken along a transect to match the gradient. Where no obvious pollution source is detected, a thorough examination of the entire site should be made. Because many nitrophobes are vernal species a site should ideally be visited twice, once in spring and once in early summer (late summer may make identification of species more difficult). Lichen and bryophyte surveys could be carried out at any time although species are easier to identify in cool moist periods.

Although a presence or absence record is valuable and probably sufficient for nitrophobes, the abundance of many nitrophiles should be noted. The presence of constant species of intermediated Ellenberg N Index, or those species with a low Ellenberg N Index but an ability to respond to N, may not be indicative of enhanced N. However a large cover/abundance of such species should be of concern. Species which need to be monitored for abundance are indicated in the lists. Where monitoring is taking place along transects, cover can be more easily estimated using quadrats (size determined by vegetation mosaic), but for more general quick surveys, rough estimates of cover should suffice.

4. Discussion

4.1. How robust are the classifications?

4.1.1. Limitations of available data

Excellent field data and additional data from the literature search were available for constructing the woodland lists. The 3 tables give a good range of nitrophobe and nitrophile species and together with use of the lichen acidophyte/nitrophyte list, should provide a very good aid to determining the N status of a site.

The literature did not yield many data for grasslands that were relevant to Scottish habitats. For example, one of the most cited indicators of N enrichment in calcareous grassland in Europe is the grass *Brachypodium pinnatum*. Although there is potential for this species to be a problem in certain English calcareous grasslands, the species scarcely occurs in Scotland. While there are many species which can be considered nitrophobes in calcareous grasslands, there is little of no evidence of nitrophiles in this habitat. The grass species *Festuca ovina* and *Nardus stricta* have been shown to respond to added N in some heathland experiments and for that reason, a note of their abundance should be made. Any influx of tall vegetation in general should be noted as many nitrophobes are low growing rosette species sensitive to shading. In the presence of gazing, tall species and grass are kept low and any effects of N deposition may be obscured.

There were also few records of nitrophiles for calcifugous grasslands, although the Countryside Survey (Smart *et al*. 2003, 2005) recorded the occurrence of a number of species of fertile grasslands in normally less fertile acid grasslands. Such species should be carefully noted as they have the potential to respond rapidly to enhanced N inputs.

Many of the records relating to heather moorland or heath come from the lowland heaths of the Netherlands, Denmark and southern England. They, together with the few records from uplands heather moor record the decline in *Calluna vulgaris* in response to N additions. In the lowland heaths and bogs, grass species such as *Deshampsia flexuosa*, *Festuca ovina* and *Molinea caerulea* tend to invade the C*alluna*. In upland sites, *Nardus stricta* may outcompete *Calluna* especially where gazing is high, but there is little evidence for nitrophilic species. In the recent study of 4 intensive sites, application of the Ellenberg N Index to the 2 blanket bog sites gave little indication of the N status of the sites. However the application of the Acidophyte/nitrophyte Index using largely low Ellenberg N grasses as potential nitrophytes, showed the grassy area of the upland blanket bog to be enriched or potentially enriched.

Such sites may lack propagules of fast growing species which respond to N. However as stated above, some species typical of more fertile grasslands are becoming more common in heaths and bogs and these species should be noted. Bog habitats have received much attention throughout Europe especially because of their important *Sphagnum* spp. But few of these studies are field based or include species common in Scotland. Nevertheless the list for heaths and bogs is fairly extensive and should prove useful.

4.1.2. Sensitivity to other factors

As the indices are initially based on the Ellenberg N Index, factors which may potentially influence spatial and temporal change in mean Ellenberg values should be noted. Differences in Ellenberg N values can result from disturbance such as mowing or grazing, as competitive, taller species tend to be those with high N values. Weather can also affect means. For example, Dunnett *et al*. (1998) analysed a 38 year record of changes in plant species performance related to weather and hypothesised that more competitive plants (largely high N value) should be favoured by cooler, wetter summers while warm and dry spring and summers should favour ruderal and stress tolerant species (low N value).

The response of species to soil N levels will depend in part on other conditions including light, water and nutrient availability. Increased mineral nutrition may allow plants to grow in deeper shade on alkaline, neutral and weakly acidic soil than on strongly acidic soils (Peace and Grubb, 1982; Ellenberg *et al*. 1991). Thus the presence of a species of low N requirements but high light requirements in deep shade, may not be indicative of low N soil levels but may be a response of that species to shade conditions.

The response of low N demanding species such as grasses to enhanced N deposition can confound the index to some extent. These species are not typical of N rich ecosystems and have fairly low Ellenberg numbers. However they are able to respond to an increase in available N and can dominate total cover in high N deposition areas. Because some species typical of low N ecosystems are able to respond positively to increased N availability, Ellenberg Index may underestimate eutrophication and obscure species composition change in some cases.

4.2. How important is it to record all species?

Research has shown that when examining a site by allocating Ellenberg N Index to all species present, incomplete lists do not greatly affect the overall N Index. The method relies on the dominance pattern of relatively few species and the deletion of low-abundance species affects overall results only weakly (Ewald 2003).

The lists produced here for the nitrophobe/nitrophile species combine more than one NVC classified habitat so obviously not all species listed will be found at any one site. Even constant species of the NVC are not found at every site. However, because this method focuses on only the polar ends of the scale (nitrophobes and nitrophiles), every effort should be made to record as many nitrophobe/nitrophile species as possible. By contrast there is no need to record non nitrophobe/nitrphyte species.

4.3. Complementary Methods

The ability of the nitrophobe/nitrophile classification to detect N deposition may be enhanced by using additional methods (Leith *et al*. 2005). For example, sampling key mosses for tissue N would provide a useful standard to compare with existing data. Similarly, the use of standardised grass transplants can give a rapid indication of atmospheric N levels. Where application of the nitrophobe/nitrophile classification to an important site is inconclusive additional monitoring of N deposition by physical methods may be employed e.g. passive gas samplers, bulk deposition collectors.

5. Recommendations for future work

5.1. Validation

Clearly the ability of these habitat based classification to predict or identify N impacts on a specific site and the potential for vegetation change must be fully tested in the field. Certain species may prove to be incorrectly allocated on the basis of a few studies which are often conducted under artificial conditions. The classifications using nitrophobe/nitrophile species require further field testing and should be refined following habitat specific field exposure systems.

5.2. Extension of habitats

Based on the results of this initial study with selected habitats the nitrophobe/nitrophile classification should be applied to more habitats. Decline in species of agricultural and horticultural habits recorded by Preston *et al.* (2000b) may make these habitats candidates for future study.

5.3. Training of staff

Training in use of the classification and identification of key species particularly, bryophytes and lichens could be provided to SEPA staff. This could take the form of a workshop in the laboratory with samples of key species or at a selected site. Protocols on application of the nitrophobe/nitrophile classification should be developed, tailored to the needs of SEPA staff and the wider user.

6. Conclusions

- Sufficient data were obtained to construct 3 comprehensive lists for woodland habitat, mixed deciduous, oak-birch, and pine woodland.
- Calcareous grassland lists were extensive but little data directly applied to Scotland.
- The data for calcifugous grasslands classification were limited. There was little evidence for nitrophiles in this habitat.
- Reasonable evidence was available for heath and bogs giving 2 workable classifications.
- Lichen acidophyte/nitrophyte classification should provide key additional information.
- Where the application of nitrophobe/nitrophile classification is inconclusive, complementary methods should be used.

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