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Effects of anthropogenic nitrogen inputs on dune grassland

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EFFECTS OF ANTHROPOGENIC NITROGEN INPUTS ON DUNE GRASSLAND

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by

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IN THE NAME OF ALLAH,

THE MOST GRACIOUS, THE MOST MERCIFUL

DEDICATION

"To the loving memory of my father, Mohd Said Sirin whose wish was my success, and to my mother Sarah, wife Wati and my two little daughters Amalina and Nadia."

SUMMARY

The effects of anthropogenic nitrogen inputs on dune grassland at Newborough National Nature Reserve, in Anglesey, North Wales were investigated using both field surveys and experimental approaches.

In a survey on the study area conducted at eight transect sites from coastal to inland sites, total N of vegetation and soil increased from the coastal towards the inland sites, it also differed significantly between sampling years. Effects of grass mowing treatments in a long-term experiment on total N of vegetation were examined and tissue N does not show any significant difference between mowing treatments. However, total N per unit area was significantly different, attributed to the higher biomass obtained from the unmown plots. Nine environmental variables were determined and discussed. Species composition was surveyed at each transect in three-year survey. An analysis using MATCH programme in VESPAN package indicated a vegetation sequence of mobile dune communities to mesotrophic grassland communities from coastal to inland sites. Separation between these two end communities was clearly shown by ordination using DECORANA. The relationships between species and their environmental variables were determined using Canonical Correspondence Analysis (CCA), which shows that the relationships between species are positive or negative depending on their demands on the environmental sources.

Survey of atmospheric nitrogen dioxide using diffusion tubes revealed a small amount of NO_2 deposited to the area. The NO_2 concentrations do not differ between sites but it shows a very highly significant difference between sampling periods, with greater concentrations during winter months. Annual mean concentration of 3.26 ppb indicates that atmospheric NO_2 is unlikely to contribute much N inputs at Newborough area.

The large poultry farm near Newborough NNR is a point sources of increased concentration of atmospheric ammonia in the area. The NH_3 concentrations were measured at six distances south-west of the farm across the dune, and at several other locations near the farm and at a sewage works. In all months, NH_3 concentration decreased significantly with distance from the farm. After 1000 m distance south-west from the farm, no measurable effect could be distinguished. NH_3 concentrations close to the farm are very large with annual mean concentration of 60.05 $\mu g\ m^{-3}$ compared to background of 0.94 $\mu g\ m^{-3}$. Tissue N content of ground cover species samples declined with distance from the farm, and a positive relationship was shown between tissue N and NH_3 concentrations along the dune transect.

In the pot experiments, the responses of models of dune plant communities to various N inputs revealed that most species were positively responsive to the gradient of N concentrations, by increasing in dry weight. The Relative Ecological Performance (REP) based on plant yields, enabled the seven species to be compared. Faster growing mesotrophic grassland species such as *Dactylis glomerata, Plantago lanceolata* and *Festuca rubra* had higher REPs than dune species such as *Galium verum, Leontodon hispidus, Achillea millefolium* and *Centaurea nigra*.

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CHAPTER 1

INTRODUCTION

1.0 GENERAL INTRODUCTION

As most of biodiversity of earth can be found in natural or semi-natural ecosystems, human activities may threaten the structure and functioning of these ecosystems which could affect the natural variety of plant and animal species. One of the major threats in recent years is the increase of air-borne nitrogen pollution (NO_x and NH_y), the concern of scientists has lead to a number of studies conducted on this matter (e.g. Wilson *et al,* 1995, Morecroft *et al.,* 1994 and Bobbink *et al.,* 1992). The concern about the increased nitrogen deposition is because nitrogen is the most limiting nutrient for plant growth (e.g. Chapin 1980) in which this factor along with other plant nutrients could affect the dynamics of species composition in terrestrial ecosystems. Since species diversity is an important parameter of a plant community and one of the major criteria for nature conservation, therefore any anthropogenic sources that contribute to the effects on the plant communities should be determined in order to control their emission.

In general, the structure of terrestrial ecosystems is too complex to allow for a detailed description of all functions of the whole system and of all changes occurred. One has to build and apply models using reduced sets of variables and correlations, which would reflect only rough structures of the real complexity. Due to this, I chose a manageable set of variables to determine

inputs of nitrogen on a terrestrial ecosystem, i.e. a dune grassland ecosystem, and furthermore I have tried to describe its development under the influence of increased nitrogen inputs in relation to physical climate as well as management factors.

This thesis is a study of the effects of anthropogenic nitrogen sources on dune grassland in Anglesey, North Wales. It involves surveys of atmospheric nitrogen inputs and also other environmental parameters. The idea of this study emerged mainly from large attention given by the public on nature reserves that under threat of excess nitrogen inputs. Although many related studies have been carried out at various places, data on the relative magnitudes of the different processes at specific sites are still needed to recognise the potential importance of nitrogen deposition. Hence, two objectives of the study have been raised as follows:

1. To determine evidence of effect of atmospheric nitrogen deposition at Newborough, and whether a chicken farm nearby is a measurable source.
2. To determine whether outcome of plant competition for nutrients changes across a nitrogen gradient.

In order to develop an understanding of those processes and relationships between plant communities with the variables, a review of literature was made in the subsequent sections to give an overview of the topic. Since studies of nitrogen are very broad topic, therefore the review could not cover all areas of nitrogen studies. In Chapter 2, a survey on the study area was conducted to describe the characteristics of the area. This chapter starts with monitoring the

area's weather using an automatic weather station, which include data on air temperature, wind and rainfall. The survey follows with measurement of nitrogen content of vegetation along a transect line, and also measures the N content of vegetation in mowing plots. The mowing plot measurements were done with a view to practice several basic methodologies in research. Subsequently, soil parameters were investigated, and vegetation was then surveyed. The vegetation survey was used to determine the sequence of vegetation to show that a successional sequence of vegetation has occurred at Newborough dune system. In addition, changes in soil nitrogen were also observed at each site along the line. Chapter 3 concentrates on atmospheric nitrogen dioxide measurement using passive diffusion tubes, which is the cheapest and most reliable method available. The changes of nitrogen dioxide concentration between months were noted and discussed. Chapter 4 reports atmospheric ammonia monitoring at the study area by means of badge samplers and diffusion tubes. A large poultry farm near the Reserve is believed to emit a significant amount of ammonia gas to the atmosphere, hence a survey to monitor this emission is quite vital in order to determine its effect on the natural vegetation. Species composition at each ammonia site was surveyed as well as their nitrogen content. This is to determine their relationships with the atmospheric ammonia concentration.

In Chapter 5, several pot experiments on effects of nitrogen deposition on dune plant communities were conducted. Seven dune plant species and mesotrophic grassland species were established in pots and exposed to different form of nitrogen deposition. The first experiment was a preliminary experiment using

Long Ashton nutrient solution (Hewitt, 1966) with different nitrogen concentrations in the form of nitrate solution, with a view to prove that the increased nitrogen deposition would contribute effects to the plant communities. In the second experiment, models of plant communities were exposed to nitrogen treatment in the form of ammonium nitrate mist in the misting units. The mist treatment would represent wet pollutant deposition, thus the way plant communities respond to this form of deposition could be investigated. Subsequently, the models of plant communities in pots were exposed to ammonia gas in several open-topped chambers. The ammonia gas was provided by allowing ammonia to evaporate from different concentrations of ammonia solutions. The ammonia concentration in the air in each chamber was also measured using ammonia diffusion tubes. Finally, the established plant communities in pots were exposed at five locations at Newborough site, at various distances from the chicken farm towards the dune. This is to investigate response of plant communities to atmospheric ammonia emitted by the poultry farm.

Each chapter is presented with its own detailed discussion where ecological interpretation is given. A final discussion and conclusion is provided in the final chapter (Chapter 6).

1.1 REVIEW

1.1.1 The Nitrogen Cycle

The nitrogen cycle is a process by which nitrogen circulates between the atmosphere and the biosphere or subsidiary cycles within the overall process, to

which nitrogen atom can move between gaseous, liquid and solid phase. This cycle has attracted scientific study for years, and its practical significance is beyond question. Main components of the nitrogen cycle can be seen in Figure 1.1, which shows the principal forms of N, and the pathways between them. There are five main components in a nitrogen cycle. They are: (1) nitrogen fixation (2) assimilation (3) ammonification (4) nitrification and (5) denitrification.

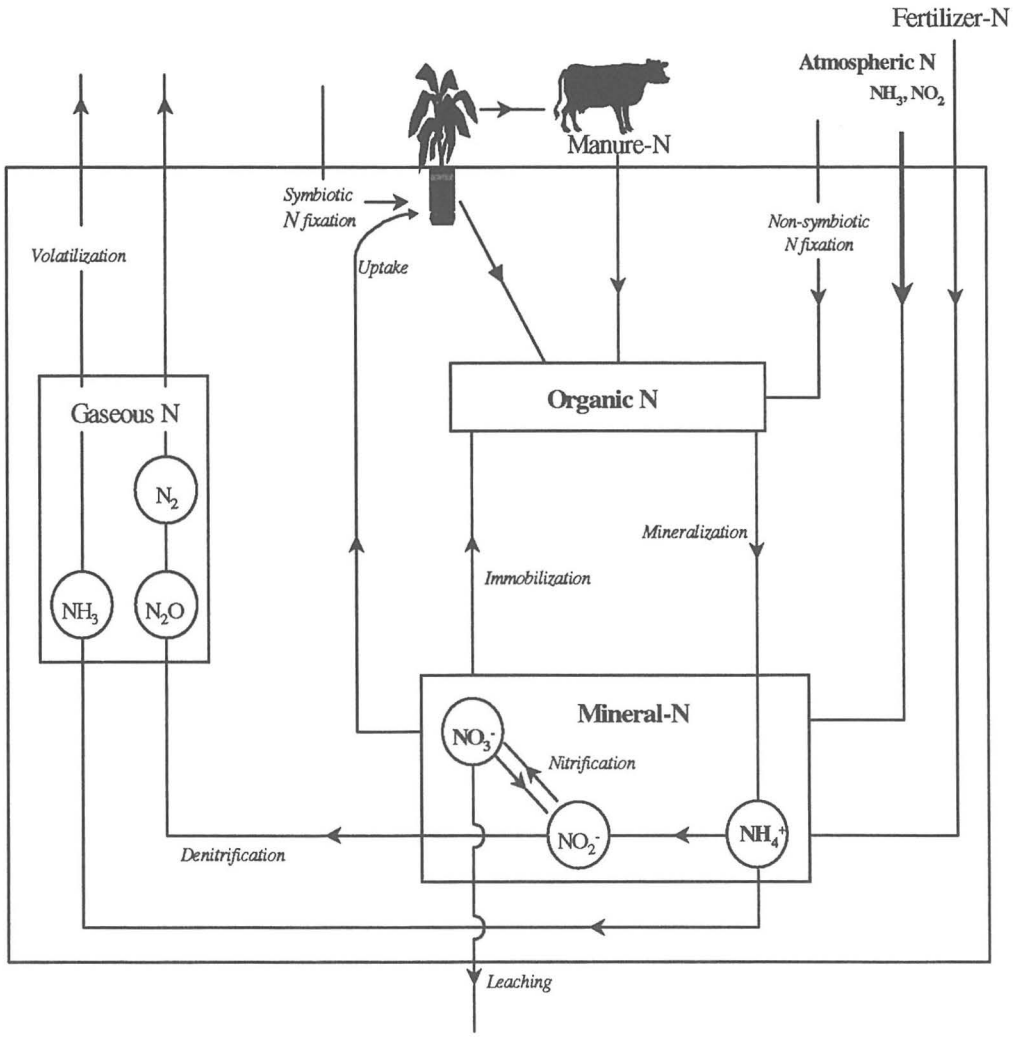


Figure 1.1 Schematic diagram of a nitrogen cycle (adapted from Rowel (1994))

It is well known that the large pool of nitrogen in the atmosphere is in the form of nitrogen gas (N_2) (Wellburn, 1994). Nitrogen from this pool is transferred into organisms via nitrogen fixation, which is the conversion of dinitrogen gas from the air to ammonium (NH_4^+). This process can be achieved biologically at normal ambient temperature and air pressures, carried out by small numbers of bacteria; or artificially by chemical processes requiring high temperatures and pressures. Nitrogen can also be converted from gaseous to liquid forms as a consequence of lightning, and by dry deposition of oxides of nitrogen to surfaces. A small number of bacteria capable of fixing nitrogen biologically can be divided into two groups; free-living bacteria, which carry out non-symbiotic N fixation and symbiotic bacteria which exist in a mutualistic partnership with plants.

The most important N fixers belong to the genus *Rhizobium*, and members of this genus live symbiotically in the roots of leguminous plants, where they form characteristic nodules. Enzymes produced by the bacterium accomplish the fixation of N, and the host plant provides energy and an environment suitable for the bacterium to carry out this fixation. As for non-symbiotic N fixation, certain members of blue-green algae (cyanobacteria) and a few actinomycetes are capable of fixing N from the atmosphere. Inputs of nitrogen to the soil from biological N_2-fixation vary widely from one ecosystem to another. For example, tropical legumes such as alfalfa have been shown, on occasion, to fix at rates as high as 400 kg N ha^{-1} y^{-1} (Tisdale *et al.,* 1985). On the other hand, in soils upland pastures in the UK, free-living (e.g. *Azotobacter*) and symbiotic (mainly clover) N_2-fixation may only account for an input of 5-30 kg N ha^{-1} y^{-1} (Batey,

1982). It is fixation by grain and forage legumes that make up by far the largest component of biologically fixed nitrogen in temperate agricultural soils. Despite the substantial contribution that this makes to the soil N-cycle of these temperate system, the input is relatively small compared with that added as fertiliser. In the UK, for example, about 0.4 x 10^6 t of nitrogen are fixed biologically, whereas fertilisers supply about 1.15 x 10^6 t (Royal Society, 1983). Globally, because of the many natural, unfertilised ecosystems, this picture is reversed, biological N_2-fixation supplies more nitrogen to the soil than fertilisers.

Nitrogen is essential for life as it is a necessary component of amino acids and proteins. Plants assimilate N in inorganic forms and incorporate these into organic N compounds. Inorganic N taken up by plants is mainly from the soil and in the form of ammonium (NH_4^+) and nitrate (NO_3^-) ions. Conversely, animals including humans obtain N by consuming plants and animals. Much of the N assimilated by plants and animals remains in plant and animal tissues until the death of the organisms, at which stage decomposition occurs by microorganisms and nitrogen is released. In the decomposition process, immobilized nitrogen (organic form) will be mineralized and ammonium (NH_4^+) and nitrate (NO_3^-) will again appear in soil solution. However, most of the immobilized nitrogen remains in the organic form. Part of the ammonium-N is assimilated by the microorganisms themselves and converted into microbial constituents, which will be released when the microbe dies. Moreover, there are groups of bacteria in the soil known as nitrifiers capable of converting ammonium ion to nitrate ion. The conversion process is important for these bacteria to provide their own energy for growth and reproduction, and also

nitrate produce from the process could be used for assimilation by certain plants that prefer nitrate to ammonium ions.

An important further component in the N cycle is the denitrification process which converts nitrate to N gases, such as nitrous oxide (N_2O) and nitric oxide (NO). This is also a biological process that is carried out by microorganisms, but only occurs under certain environmental conditions. The process uses nitrate as a substrate and requires anaerobic conditions and a source of energy for the organisms. Waterlogged or compacted soils result in an anaerobic condition in the soil and contribute N_2O into the atmosphere. A good drainage system and ploughing the soil may perhaps reduce the denitrification process. Recent trends in farming which uses artificial fertilizers widely, has disturbed the natural balance between the processes described and it also discourages microbial N_2 fixation. These excess fertilizers that have not drained away, are usually removed by denitrification. This also increases the N_2O in the atmosphere.

1.1.2 Grassland Nitrogen Cycle

The biogeochemistry of nitrogen in grassland is similar to that in other ecosystems in which no unique components or pathways exist. However, the distribution of many components and the rates of many processes in these grasslands are vastly different from those in ecosystems having large woody components. The most notable differences between grasslands and other natural ecosystems are the annual nature of most aboveground matter, the dominance of below ground biomass and its influence on pathways and transformations in nitrogen cycling, and the important role of the larger

herbivores. Table 1.1 shows data from Woodmansee *et al.* (1981) representing ranges for nitrogen contained in various components of grasslands. They concluded that more than 90% of the nitrogen is in soil organic matter, and in most grasslands, mineral nitrogen is less than 0.5% of the total nitrogen in the system. As an example, Woodmansee *et al.* (1978) studied distribution of nitrogen in semiarid grassland in which they reported that 99.5% nitrogen was in organic forms and the remaining as mineral nitrogen. A variable amount of nitrogen may be temporarily fixed as exchangeable NH_4^+ in clay minerals and can then be presumed to be in equilibrium with the NH_4^+ in the soil water solution.

Table 1.1 *Representative ranges for nitrogen contained in various components of grasslands. (From Woodmansee* et al., *1981).*

Component	Range (g N m^{-2})
Tops (live)	1-10
Tops (dead)	0-2
Litter	<1-9
Roots (live)	2-10
Roots (dead)	2-20
Microorganisms	2-15
Soil organic matter	90-1600
Invertebrates	<0.01-0.3
Large invertebrates	0-3

In dune ecosystems created from freshly blown sand, soil nitrogen is nearly zero during the formation of the dune. Most of nitrogen is fixed by the vegetation or by components of soil microflora from atmospheric nitrogen. In the slacks of a sand dune, early colonizing cyanobacteria may fix nitrogen, which is assimilated by higher plants, so helping to stabilize the slacks. In studies of a sand dune slack at Blakeney Point, Norfolk, UK, rich in *Nostoc*, it was shown by means of

^{15}N studies that the surface centimetre of soil (the region rich in cyanobacteria) became highly labelled (Stewart, 1967). Within three weeks, higher plants (*Agrostis capillaris*, *Glaux maritima* and *Suaeda maritima*) and the moss *Bryum pendulum* were found to be significantly labelled, indicating transfer of the biologically fixed nitrogen from the soil surface to the vegetation. Furthermore, the nitrogen-fixing bacterium *Rhizobium*, symbiotic with numerous forbs and shrubs including *Anthyllis vulneraria*, *Lotus corniculatus*, *Ononis repens*, *Trifolium* spp., *Ulex europaeus* and *Lupinus arboreus*, is also very important in enhancing the nitrogen status of the dune systems (Packham and Willis, 1997).

Young dune soils are often low in mineral nutrients. Many sand-dune systems are limited by low levels of major nutrients, notably of nitrogen, phosphorus and potassium. The calcium content of shell fragments may range from very high to very low, leading to calcareous and acidic conditions respectively. The ability of dune soils to retain water and mineral nutrients rises as organic matter content increases. The classic study by Salisbury (1922) at Blakeney Point, Norfolk, demonstrated that organic matter content is greater in fixed than in mobile dunes, though calcium carbonate content and soil pH drop along transect running inland from the shore. At Blakeney, almost all the sands are acidic and low in nutrient content but concentrations of calcium, magnesium, sodium and nitrate are higher in the mobile dune sands than in those of the grey dunes; the opposite is true of soluble inorganic phosphate and potassium (Gorham, 1958).

1.1.3 Anthropogenic Nitrogen Inputs

1.1.3.1 Emission of Oxide Nitrogen

The major sources of deposited atmospheric nitrogen (N) are the gases nitric oxide (NO), nitrogen dioxide (NO_2), ammonia (NH_3) and nitrous oxide (N_2O). Many different sources are responsible for the emission on N gases. Emissions of NO and NO_2 (collectively known as NO_x) and N_2O primarily arise from fossil fuel combustion, biomass burning, lightning, and microbiological emissions from both natural and agricultural soils. Ammonia emissions arise from large number of sources, including volatilisation from animal waste and synthetic fertilisers, losses from soils under natural vegetation, agricultural crops, emissions from human excreta and waste and also from industrial processes (Lee *et al.,* 1997; Bouwman *et al.,* 1997).

Since 1970, total NO_x emissions have remained fairly constant, but some evidence of an increase in recent years showed that emissions from motor vehicles have risen rapidly and now account for just over half the total with power stations contributing a further 28% (Gilham *et al.*, 1992). The UK emitted an estimated 2.73 million tonnes of oxides of nitrogen (expressed as NO_x) in 1990 and is the third largest source of NO_x in Europe (UK-PORG, 1990). Globally, Olivier *et al.* (1998) estimated that anthropogenic emission of NO_x for 1990 is about 31 million ton N $year^{-1}$ and ammonia of about 54 million ton N $year^{-1}$. The emissions of both sources of nitrogen do not seem to decrease since we may still see localised increases in total N deposition in rural areas due to expansion of tourist traffic (Ashenden and Edge, 1995). It is hoped that introduction of catalyst technology will lead to a decrease in emissions from cars

over the next decade, with cold-start emissions becoming relatively more important.

Apart from industrial sources, NO_x is also emitted from soil through nitrification, the microbial oxidation of NH_4^+ to NO_3^-, and denitrification, the anaerobic reduction of NO_3^-. The emission inventories of oxidized N in industrial countries generally show that soils make a minor contribution to annual emissions. In the UK for example, total emissions of NO_x in 1992-4 averaged 740 kT N annually, and soil emissions contributed only 50 kT (7%) of the total (Fowler *et al.,* 1998a). Few measurements on rate of NO emission are available for British soils, and in one study, Skiba *et al.* (1992) displayed NO emission rates in the range of <0.1 to ~7 ng N m^{-2} s^{-1}. Emission increased with increasing soil temperature and was the largest shortly after the application of N fertiliser. This was shown by Bouwman & Sombroek (1990) who reported an average of annual emission rate for fertilised cultivated land in temperate climates of 15 ng N m^{-2} s^{-1}.

Another oxide of nitrogen is nitrous oxide (N_2O), which is present in the atmosphere in trace quantities. In 1990, its concentration was about 310 parts per billion by volume (ppbv), about 1000 times less than that of CO_2, and it is increasing at the rate of about 0.8 ppbv y^{-1} (Watson *et al.,* 1992). The sources and the causes for the increase in N_2O are not well known. It is generally accepted that the most important source is natural soils, seconded by emissions from the oceans (Seiler & Conrad, 1987) although there is significant uncertainty regarding the distribution and magnitude of the sources themselves. For some time it was thought that, like CO_2, the primary cause for the increasing

concentration was combustion of fossil fuels, in particular, coalburning power plants producing electricity (Hao *et al.,* 1987). However, identification of an artifact in the flask sampling procedure ruled out combustion (including biomass burning) as the major cause of the trend (Muzio and Kramlich, 1988). Minor sources so far include agricultural fields amended with nitrogenous fertilizers, animal manure, sewage, industry, automobiles, biomass burning, land clearing and trash incineration (Watson *et al.,* 1992; Khalil and Rasmussen, 1992). In soils and aquatic systems, N_2O and NO_x are produced by both denitrifiers (Firestone *et al.,* 1980) and nitrifiers (Lipschultz *et al.,* 1981). Estimates of annual N_2O release from natural soils have been variously estimated in several different studies. They are in the range of 7-16 Tg (Tg = 10^{12} g) (Bowden, 1986), 3-25 Tg (Banin, 1986), 3-9 Tg (Seiler and Conrad, 1987) and 2.8-7.7 Tg (Watson *et al.,* 1992).

1.1.3.2 Ammonia

Agriculture is the most important source for ammonia with NH_3 emissions mainly resulting from livestock management and to a minor part of them resulting from fertilizer application. Ammonia emissions from livestock farming derive mainly from the decomposition of urea in animal wastes and uric acid in poultry wastes. Emissions occur from housed animals, waste storage, land-spreading of wastes and from animals grazing in the field. At global scale, emission of ammonia in 1990 is estimated about 54 million ton NH_3-N y^{-1}, of which 43 million ton NH_3-N stems from anthropogenic sources (Olivier *et al.,* 1998). The major anthropogenic sources identified in this estimation are excreta of domestic animals (50%, of which non-dairy cattle 25%, dairy cattle 13%, and pigs 10% of

the global total), use of synthetic N fertilisers (25%), biomass burning (15%), crops (10%), and human population and pets (8%). Bouwman *et al.* (1997) estimated the total global NH_3 emission to be of the order of 50 Mt N yr-1 and the sources of emission are shown in Table 1.2. In the UK the total emission of ammonia is in the range of 260 to 400 kt N yr^{-1} (INDITE, 1994) of which the major contribution is from agricultural sources.

Table 1.2 *Source of global NH_3 emissions (Bouwman et al., (1997))*

Source	Emission (Mt N yr^{-1})
Domestic animals	21.7
Human excrements	2.6
Synthetic fertilizers	9.0
Agricultural crops	3.6
Biomas burning in agriculture and biofuel use	2.7
Fossil fuel combustion	0.1
Industry	0.2
Subtotal anthropogenic emissions	39.9
Wild animals	0.1
Undisturbed ecosystems	2.4
Biomass burning in natural ecosystems	3.2
Sea	8.2
Subtotal natural emissions	13.9
Total	53.8

Most ammonia is relatively quickly deposited near to sources of emission. The gas is highly reactive, forming solution products and aerosols, and it is estimated to have a short half-life of around seven hours in the atmosphere (Raven *et. al.* 1992). The growing awareness of ammonia as a further atmospheric toxin has shown that it can make up a substantial portion of total nitrogen deposition. This is true as ammonia/ammonium represents a major constituent of the total N deposition and frequently contributes over half of the total N (range 47-87%) (Pearson & Stewart, 1993). This range agrees well with the 70% average for Western Europe estimated by Dierderen and Duyzer (1988). In The United

Kingdom, measurements of the contribution of ammonia vary, whereby values can range from 40% to 80% of this total (RGAR, 1990). Further confirmation of this estimate comes from studies on heathland vegetation in The Netherlands where 75% of total nitrogen was deposited as ammonia/ammonium (Bobbbink *et. al.*, 1992). Therefore, in many instances ammonia can exceed oxides of nitrogen as a major nitrogenous pollutant. This topic will be discussed again in Chapter 4.

1.1.4 Effects of increased anthropogenic N input on plant community

Several studies on the impact of atmospheric NH_3 and NO_2 on plants have shown that both gases can contribute to the N required for growth (Rowland *et al.,* 1987; Raven 1988; Pearson & Stewart 1993; Wellburn 1990). These affect plant functioning via foliar and root uptake in which the impact can be both stimulation and inhibition of plant growth (Perez-Soba *et al.,* 1998). In higher plants, the foliar uptake takes place mainly via the stomata and uptake via the cuticle is negligible (Van Hove & Bossen, 1994).

At community level, various changes to natural and semi-natural vegetation have been associated with the increased N deposition. Deleterious effects on a range of ecosystems in Britain have been reported in several studies. Thompson and Baddeley (1991) showed that increased nitrogen deposition has affected the abundance of bryophyte species, such as *Racomitrium lanuginosum* that has become less abundant in some areas of Britain. Ecological modification and successional change by means of nitrogen deposition will be most obvious in systems poor in nitrogen, because species

adapted to nitrogen deficiency will soon be outcompeted by species with higher nitrogen demand (Tilman & Wedin, 1991). Most species found in natural and semi-natural communities have much lower nitrogen demands. For these species, an increased supply may be ecologically deleterious, even where the shoot growth of an individual is stimulated. If potentially aggressive competitors respond more vigorously, this may result in species with a more limited capacity for growth being out-competed. A study by Morecroft *et al.* (1994) on effects of ammonium sulphate addition on two semi-natural grasslands (calcareous and acidic grasslands) showed a decline in the moss *Rhytidiadelphus squarrosus* on the acid site, but no other change in vegetation composition was detected after 3 years of treatment.

Changes in species composition associated with increased nitrogen deposition have occurred over much of Europe. The range of affected communities is large and includes lowland heathlands, forest ground flora, calcareous grasslands, coastal dunes, wetlands and upland moorland (Sutton *et al.*, 1993). In The Netherlands there has been a change from *Calluna*-dominated heathland to grassland dominated by *Molinia caerulea* in wet sites and *Dechampsia flexuosa* and *Molinia caerulea* in drier sites (Aerts & Heil, 1993). It has been estimated that more than 35% of former Dutch heathland has changed into grassland (Bobbink *et al.*, 1993). This has been caused by very high nitrogen deposition rates of 40 kg N ha^{-1} y^{-1}, mostly in the form of ammonium.

As aforementioned, nitrogen is one of the major nutrients taken up by plants and consequently any lack of this nutrient will significantly affect their growth. Early studies by Willis (1963) on the effects of nutrients in dry and wet dune grasslands at Braunton Burrows, England proved that N is the most important nutrient in stimulating the growth of some grass species (*Festuca rubra* and *Poa pratensis*). This enhanced growth significantly reduced the abundance of many small plants, mosses and lichens. Moreover, sensitivity of plant species to atmospheric pollutants is an important aspect in determining species composition. This has been shown by Ashenden and Mansfield (1978) who conducted a study on the sensitivity of four grass species, *Dactylis glomerata, Lolium multiflorum, Phleum pratense* and *Poa pratensis*, when NO_2 and SO_2 were present in experimentally controlled atmospheres. They found that both single pollutants affected the yield of *D. glomerata* and *P. pratensis* whereas *P. pratense* was only affected by SO_2. *L. multiflorum* was unaffected by either gas. Therefore the varying sensitivity between species to pollutants may have serious effects on species composition in mixed grass species in the field.

Changes in the species composition of the plant community can be used to detect pollution and specific pollutants. Many species function as 'biological indicators' in which the presence of every plant is a measure of the conditions under which it is existing or existed previously. For example, the occurrence of stinging nettles (*Urtica dioica*) is an indication of possible high levels of nitrogen in the soil, the appearance of rosebay willow herb (*Chamaenerion angustifolium*) indicates disturbed soil or some kind perturbation (Spellerberg, 1991). In relation to the biological indicator, Ellenberg (1988) gave 'indicator value' for

nearly 2000 vascular plants with respect to soil moisture, acidity, available nitrogen and also the salt and the heavy metal content of the soil. The indicator value is the ecological behaviour in respect of any of the habitat factors, which is expressed as a number in a nine-point scale where '1' indicates a low value and '9' a very high value. This value may help plant ecologists to have a brief idea on characteristics of species studied.

1.1.5 Plant Succession

Changes of vegetation described in those examples are closely related to vegetation succession. Vegetation succession is a broad subject, which is unlikely that any single factor or process will ever explain all successions. For instance, each species in a plant community has physiological, morphological, and life history characteristics that are unique to it, and also, each habitat has a unique substrate, geomorphology, climate and past history (Tilman, 1988). The initial densities of colonists and the probabilities of colonization by various species are unique to each successional event. Tilman (1988) concluded that succession is unavoidably stochastic because colonization is necessarily a probabilistic event whereby successful colonists can have great potential for rapid growth in a previously vacant habitat. Nevertheless, succession is an often repeatable process locally that shares many features from habitat to habitat.

Plant succession occurs if a directional sequence of populations of plant species differs on a site between the end-point and the starting point (Burrows, 1990). It is often that quite long sequences are apparent, with more or less distinct

phases, characterized by the dominance of different species. As succession is a very broad topic, Grime (1979) classified it into two kinds, that is, successional change and cyclical change. He explained successional change as a progressive alteration in the structure and species composition of the vegetation, whereas cyclical change occurs when similar vegetation types recur in the same place at various interval times. In the case of succession, a further distinction may be drawn between the changes. These occur during the colonization of a new and skeletal habitat initially lacking in soil and vegetation (primary succession). There is more common circumstance in which succession is a process of recolonization of a disturbed habitat (secondary succession) whereby soil and at least some of the original plant populations are not completely destroyed.

Several studies recognize the importance of physical environmental factors in determining the response of plant communities to disturbance that caused succession (e.g. White 1979; White & Pickett, 1985). Current models on how plant communities respond to disturbance and develop through secondary succession are based on the assumption that resource availability changes as a result of disturbance and continues to change with time (Grime, 1977; Tilman, 1985; Vitousek, 1985). An understanding of how soil nutrient availability changes following disturbance and through successional time is needed to predict species change. This starts when nutrient availability increases for a short period immediately following disturbance because of increased decomposition and decreased biological demand, and subsequently decreases

through time (Vitousek & Reiners 1975; Bormann & Likens 1979), and this has been demonstrated in several studies of nitrogen (Vitousek *et al.*, 1989).

1.1.6 Nutrient Availability in Plant Succession

Many studies have suggested that the availability of such soil resources as nitrogen, water, and phosphorus may influence successional dynamics (e.g. Lloyd and Pigott 1967; Walker *et al.* 1981). Nutrient availability is known to be highly heterogeneous in terrestrial plant communities and is often associated with variation in plant species distributions (Grime 1979; Tilman 1988). Studies by Robertson & Vitousek (1981) and Zak *et al.* (1989) showed that variation in plant species composition and structure between sites in a successional sere is associated with differences in the rate of and variability in nutrient cycling. Even within a site, nutrient levels can vary by up to an order of magnitude over relatively small spatial scales (Robertson *et al.*, 1988).

In primary succession of a mesic habitat (e.g. after sand dune formation and glacial recession), the major soil factor limiting plant growth is nitrogen. Except for nitrogen, all of the mineral elements required by plants occur in the parent material in which most soils form (Jenny, 1980). Early plant communities should be capable of overcoming this nitrogen problem through their ability to fix nitrogen in the air. Early dominants in glacial succession in Alaska have been reported to have the ability to fix nitrogen on this type of habitat (Lawrence, 1979). In the case of foredunes, they may be colonised by sand-binding grasses (e.g. *Ammophila arenaria*) and shrubs because they are exposed to the full force of wind and weather. When these early dominant grasses lost leaves

or roots, and as they died, the nitrogen containing organic matter they produced became available to various decomposer species. These decomposers retained much of this nitrogen as well as nitrogen provided from atmospheric sources, causing total soil nitrogen levels to increase (Tilman, 1988). Furthermore, the decomposer species also excreted nitrogenous wastes, some of which were then available to vascular plants. Therefore, the early dominance by nitrogen-fixing organisms, as well as the accumulation of nitrogen provided from various atmospheric sources, led to gradual increase in total soil nitrogen levels.

Patterns of nitrogen availability in primary succession is different from secondary succession (disturbed habitats). In secondary succession, nitrogen supply is expected to increase greatly following disturbance, and then may continue at low levels for several years afterwards (Robertson & Vitousek 1981). This is true in inherently rich soil and in soils that receive heavy doses of fertiliser during cultivation, nitrogen availability can decline during the few years of succession. In contrast, on sandy nutrient-poor soil like dune ecosystem, soil nitrogen concentration generally increases with succession (Inouye *et al.,* 1987). An early study revealed that organic carbon, and with it total nitrogen, augments with increasing age and increasing density of plant cover on dunes. Salisbury (1925) found that organic matter augments slowly at first, but appreciably faster after about 200 years in the dunes especially in the higher rainfall climate at Southport, Lancashire. Such increases in total soil nitrogen, at least over this great a range, should correspond with increased rates of nitrogen supply, this may be measured as the nitrogen mineralization rate of the soil. Nitrogen mineralization rates are known to depend on many factors, such as soil pH, soil

oxygenation, moisture, temperature, organic matter quality, carbon to nitrogen ratios, and total soil nitrogen (Chichester *et al.,* 1975; Melillo *et al.,* 1982; Vitousek *et al.,* 1982). Robertson and Vitousek (1981) reported that the mineralization potential of the sand dune soils of southern Lake Michigan increased with their total nitrogen content, and thus with their age. This means that during the first 100 or 200 years of primary succession, a bare mineral substrate that is extremely nitrogen deficient becomes an increasingly nitrogen-rich organic soil with a higher rate of nitrogen supply.

Apart from nutrient availability in the soil, successions are also influenced by some other possible mechanisms, particularly the effects of grazing animals. A study by Gibson and Brown (1992) revealed that grazing deflected succession in species-rich calcicolous grassland, but was subordinate to an intrinsic rate of vegetation change and to local variations in its direction. Furthermore, they explained that succession only moved immediately towards the composition of the local ancient grasslands under the heaviest grazing treatment. The interactions of organisms that consume plants have always been included as one of the many factors influencing succession, however in this thesis, mechanisms of succession would be restricted to the interactions of plants with their physical environment or with other plants.

1.1.7 Plant Competition for Nutrient in Succession

Many studies show that species composition, diversity, and growth form of plant communities change in a general and predictable manner along productivity gradients (Whittaker, 1975; Grime, 1979; Tilman, 1988). Predictable patterns

are thought to arise because factors such as plant competition, physical stress, and herbivory vary in a predictable manner in either their intensity or quality along productivity gradients (Grime, 1979; Coley, 1987; Tilman, 1988).

In vegetation succession process, competition for nutrient plays an important role in productive and unproductive habitats. Grime (1979) and Keddy (1989) have raised two ideas of plant competition along productivity gradients. They suggested that the intensity of interspecific competition increases along productivity gradients; competition may be most intense in productive habitats because such habitats support high growth rates and large amounts of biomass that result in pre-emption of space and light. Unproductive habitats support lower growth rates and less aboveground biomass, have less shading, and may have lower intensities of competition. Species with high competitive ability might dominate productive habitats, while species with low competitive ability may be displaced to less productive habitats where competition is less intense. This perspective suggests that a quantitative change exists in the intensity of competition along productivity gradients. In contrast, Newman (1983) and Tilman (1988) suggest that unproductive habitats should be characterized by intense competition for soil resources. Tilman's (1988) theory of resource competition predicts that there may be no quantitative change in the intensity of competition along a productivity gradient, but that there may be an important qualitative change. This theory explains that plants mainly competing for soil resources in unproductive habitats and competing for light in more productive areas. Furthermore, it suggests that each species is specialized for a particular ratio of soil resources and light, and that the species that characterize a

particular habitat are also the superior competitors for the particular resource ratio of that habitat. Therefore, competition may be important at all points along a productivity gradient, but its quality may vary.

Competition for nitrogen has often been studied to determine plant species that dominate a successional sequence of vegetation. For example, Tilman (1986) conducted a study on nine plant species grown in soil with different total soil nitrogen, spanning the range of total soil nitrogen observed in a chronosequence of old fields at Cedar Creek Natural History Area, Minnesota. This study revealed a highly significant tendency for early successional species to grow more rapidly at low nitrogen levels and to acquire more nitrogen per plant from nitrogen-poor soils than late successional species. However, late successional species did not grow more rapidly at high nitrogen levels than early successional species. The greater growth rate of early successional species agrees with Tilman's resource competition theory, which allow the species to acquire a larger proportion of available soil nitrogen, and thus inhibit the growth of later successional species.

1.2 THE STUDY AREA, NEWBOROUGH NNR

Newborough is situated in the south-western corner of Anglesey, at the western extremity of the Menai Straits (Figure 1.2). Wayback in fourteenth century, it was believed the area between Newborough village and Llanddwyn Island was an intensive agricultural land. However, the prosperity of this area disappeared when a storm on 6th December 1331, destroyed 186 acres of agricultural land with sand cover (Abdy and Mayhead, 1988).

Figure 1.2 Map of Newborough shows all study sites (From Ordnance Survey, 1985)

Notes:

1. **Site A-F**: Ammonia monitoring sites southwest of the poultry farm across the dune. **Rhuddgaer**: Ammonia monitoring site at southeast of the farm in an agricultural land. **x**, **y** and **z** are sites at 30 m, 100 m and 300 northeast of the poultry farm respectively (see Table 4.1)

2. **Site 1-8**: Sites for survey of atmospheric NO_2, vegetation and soils. At **Site Ø**, only vegetation survey was conducted.

The Newborough area includes the Newborough village, the Newborough Forest on the southwest, Newborough Warren on the south, and agriculture lands cover most of the area south east of the village. The altitude ranges from sea level to over 30 metres on the Pre-Cambrian rock ridge that runs through the forest. The highest dunes on Newborough Warren do not exceed 15 metres above mean sea level, although blown sand occurs on the rock ridges.

Newborough Forest that is part of the dune covers an area of 800 hectares. It is owned by Forestry Commission, which planted the pines in 1948. The Nature Conservancy Council (NCC) has designated the majority of the forest as a Site of Special Scientific Interest (SSSI). This site is important for its populations of nationally and locally rare plants, mainly found along the ridges and in unplanted slack areas; and also for its geomorphologically features, namely a central rock ridge running through the centre of the forest and the dune structures from Ynys Llanddwyn. The NCC originally declared Newborough National Nature Reserve (NNR) (National Grid Ref. SH425635) as National Nature Reserve on 30th June 1955. Initially it only covered 46 hectares included Llanddwyn Island but it was expanded to include the salt marsh on the eastern side of the Cefni salt marsh and the area around Malltraeth cob pool, this increased the total area of the Reserve to 633 hectares. The Reserve is the sixth largest dune system in Great Britain (Ratcliffe, 1977), rich with fauna and flora.

1.3 MEASUREMENT METHODOLOGIES

This project involves several methodologies to cover broad aspects of the study. Several sites along a transect across the dune were chosen randomly at the

beginning of the programme, and they were extended as the programme went on. All sites are listed in Table 1.3 and the locations are shown in Figure 1.2. At these sites, several environmental parameters were measured which include soil and vegetation surveys that are discussed in subsequent chapters.

Table 1.3 *Site numbers and list of surveys*

Site	Distance from the sea (m)	Surveys conducted		
		NO_2	Vegetation	soil
1	50	✓	✓	✓
2	100	✓	✓	✓
3	200	✓	✓	✓
4	400	✓	✓	✓
5	600	✓	✓	✓
6	800	✓	✓	✓
7	1500	✓	✓	✓
8	2100	✓	✓	✓

Six more sites were chosen for atmospheric ammonia monitoring with Site A at a large poultry farm that is believed as a point sources of atmospheric ammonia (Figure 1.2), and Site F in the dune system. Details of the ammonia sites are explained in Chapter 4.

Vegetation survey is conducted according to the National Vegetation Classification (Rodwell, 1992) to look at the species composition of the study area. Most of the soil surveys were done on soil of the dune system, which was assumed as natural soil of the dune grassland. The soil survey was not carried out at ammonia sites due to the nature of the soil as agricultural soils. Relationships between parameters surveyed are analysed statistically, using multivariate analysis available in several computer programmes.

CHAPTER 2: SURVEY OF NEWBOROUGH

CHAPTER 2

SURVEY OF NEWBOROUGH

2.0 INTRODUCTION

Many surveys have been conducted at Newborough, in particular on the Newborough National Nature Reserve, commonly known as Newborough Warren. The Warren and the forest have high floristic, faunal and ornithological value, and therefore the surveys covered various areas of scientific interest. These include a detailed series of studies by Ranwell in 1950s, Morton (1974a, 1974b), Gibson (1984) and Hewett (1985). Apart from scientists' attraction, Newborough Warren and the Forest are also popular as recreation areas. Llanddwyn Beach is the main tourist attraction whilst limited access is allowed on designated footpaths in the Forest and on the Warren.

In early 1950s, Ranwell conducted several studies at Newborough. He surveyed various aspects of Newborough dune system such as the flora of the dunes and slacks in relation to water table of the area. He also found that the change of structure and vegetation composition on the Warren were due to decreasing of rabbit population by myxomatosis. Growth and flowering of most grasses and sedges increased with immediate effect after the cessation of rabbit grazing. A subsequent study was carried out by Morton (1974b) which focused on the causal factors determining the structure of the dune grassland. He considered a few factors that affect the grassland structure, i.e. mineral nutrient status of the soil, mineral nutrient status of the vegetation and biotic factors. Hewett (1985) concentrated his studies on effects of grazing and mowing as management tools

on dune plant communities at Newborough and Gibson (1984) investigated relationship between plant patterns and soil heterogeneity at the Warren in which the study included the shading effect on the growth of dune species.

Grazing paddocks and mowing plots are situated in the hinterland to the north of the area (refer Figure 1.2) and a full description of the area was given by Ranwell (1959, 1960), who classified the area as "closed low dune and *Salix* associes". The grassland consisted of *Festuca-Agrostis* turf with *Agrostis stolonifera*, *A. capillaris* and *Festuca rubra* being the dominant species, with patches dominated by *Ammophila arenaria* on the dunes. However, the vegetation has changed considerably since that time (e.g. Hope-Jones 1965; Hewett 1985; Gibson 1984).

Chandapilla (1970) surveyed the area in 1964 in which he reported that the area had become less sparse and increased in species diversity since Ranwell's survey. A subsequent survey by Hewett (1982) revealed notable changes in the frequency of *Arrhenatherum elatius*, which was nearly doubled from 1964 to 1982, and a decrease by almost half of frequency of *Agrostis capillaris* and *A. stolonifera* (important dune slack species). Within the grassland area, Morton (1970) reported that main vegetation type was *Festuca-Poa-Carex* grassland type, and later he noted large-scale heterogeneity within the grassland area produced by the large vigorous species *Arrhenatherum elatius* and *Avenula pubescens*.

Ranwell (1955), Morton (1970), Chandapilla (1970) and Gibson (1984) also investigated soils at Newborough Warren. Within the fixed dune area there exists a compositional gradient from relatively nutrient-rich stable "grassland" to

impoverished dry "dune" facies (Chandapilla 1970). The data from these surveys suggest that there have been few large-scale changes in the soil in the past twenty years. Although the upper maximum value for organic matter have increased from 10% to 36% over this period, this would be in accord with the theoretical expectation of increasing organic matter levels with time (Ranwell 1972).

In this chapter, surveys of Newborough site were carried out with a view to look at the characteristics of the area, which is important to provide information on the subsequent chapters. This comprehensive survey will cover meteorological aspects, soil characteristics and vegetation composition.

2.1 NEWBOROUGH: CLIMATE AND GENERAL DESCRIPTION

In this section, weather monitoring was conducted to determine meteorological characteristics of this area. Meteorological data of this survey would provide information relevant to Chapter 3 and Chapter 4, in order to relate findings of these particular chapters.

2.1.1 Meteorological survey

An automatic weather station was installed at Newborough to gather meteorological data for the site. The station was located on private land (Figure 1.2) for security, facing in a south-westerly direction approximately 1000 m from the edge of the Reserve. Meteorological data were automatically logged using a Campbell 21-X data logger for air and soil temperature (at 10 and 30 cm depth), solar radiation, wind speed and direction, and rainfall. Data presented covered from July 1997 to July 1998.

Collected rainwater samples were analysed for pH, cations, anions and conductivity. pH and conductivity were checked with the Corning 220 pH meter and conductivity meter respectively. Before analysing the rainwater samples for cations and anions, the samples were filtered with 0.45 µm Whatman Cellulose Nitrate Membrane Filters to remove particulate and bacteria. The filtered samples were then analysed for sodium and potassium by flame atomic emission spectrophotometry, and calcium and magnesium by flame atomic absorption spectrophotometry using a Perkin-Elmer 280 Atomic Absorption Spectrophometer. Chloride (Cl^-), sulphate (SO_4^{2-}) and nitrate (NO_3^-), and ammonium ion (NH_4^+), were analysed by ion exchange chromatography using a

Dionex DX-120 Ion Chromatograph (brief procedure in Appendix 2.1). Data are presented on basis of collection date.

2.1.2 Results and Discussion

The general meteorological data of Newborough between July 1997 to July 1998 are given as Figures 2.1, 2.2 and 2.3, which represents data for air temperature, irradiance and rainfall respectively. Data for November 1997 and December 1997 could not be recorded due to a mechanical fault with the data logger. The graph lines have, however, been plotted as continuous.

The summary of meteorological data for each month (Table 2.1) indicates that the Newborough area does not suffer extreme weather conditions. The highest mean air temperature was in August 1997 of 17.3 °C in which maximum temperature recorded on that month was 28.3 °C. The mean air temperature in Figure 2.1 was a true mean of all data logged for every 20 second by the data logger. Minimum and maximum temperatures were the lowest and the highest readings of air temperature recorded within the 24 hours. Radiation received was quite high and the highest was in summer in May 1998 and lowest in winter (December 1997). Wind speed was quite moderate with an average of 3.39 m s^{-1} and most wind directions were of south-westerly winds except in April 98 and May 98 of south-easterly and north-easterly winds respectively (Table 2.1). Rainfall was quite low and the total of rainfall for this period of survey was 780.2 mm, however the total rainfall is an underestimate due to the exclusion of the data for November and December 1997.

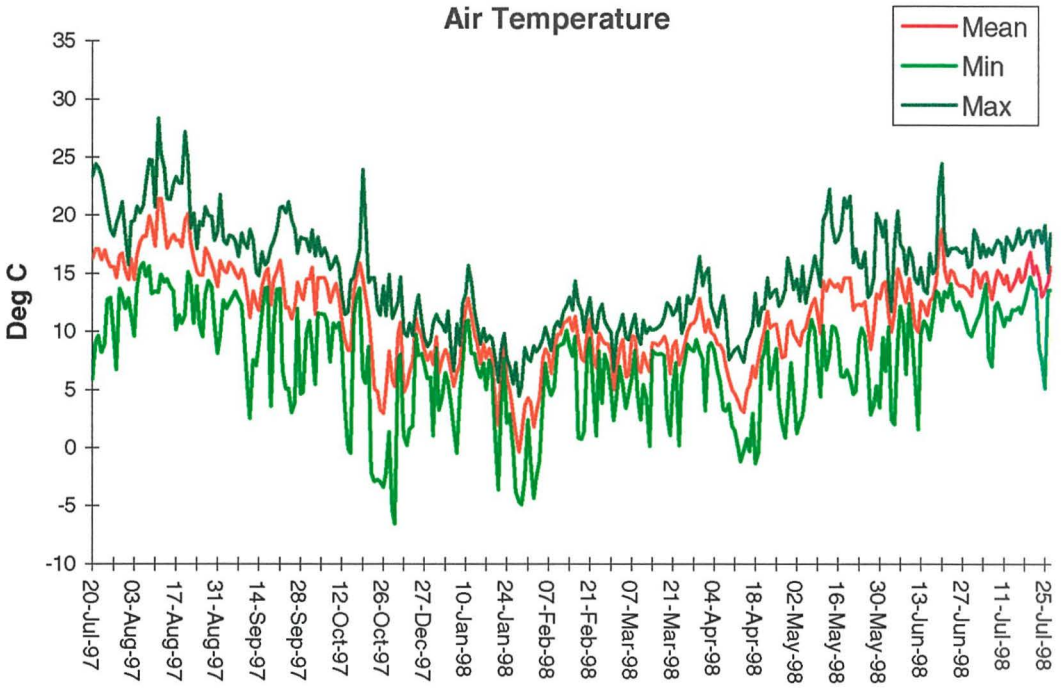


Figure 2.1 Mean, minimum and maximum daily air temperature at Newborough.

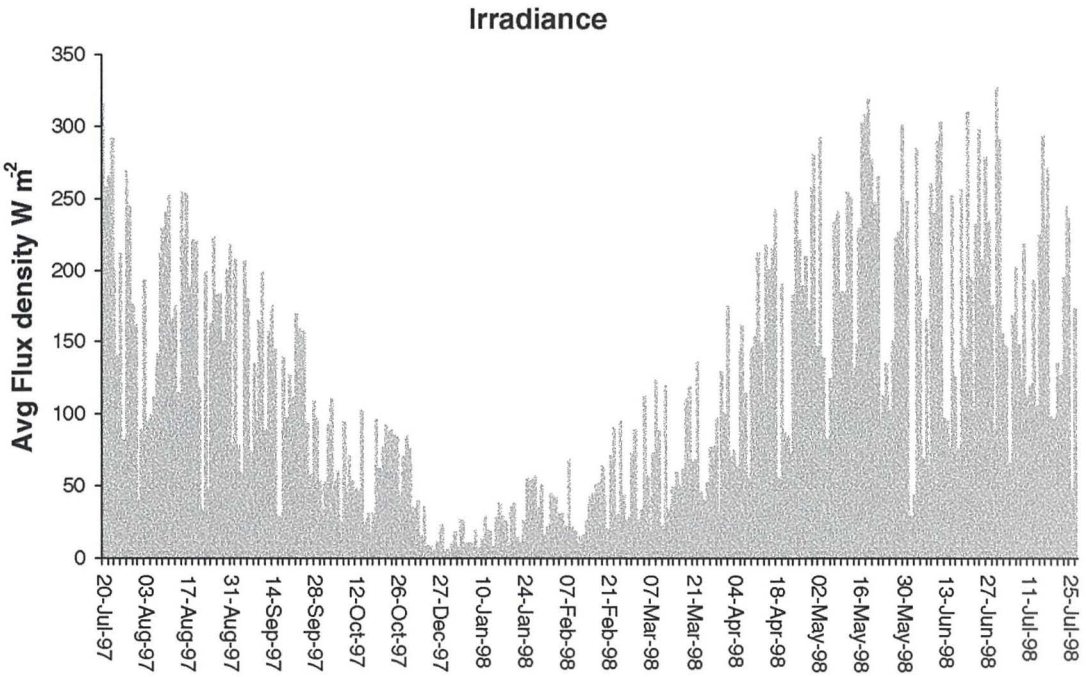


Figure 2.2 Average irradiance at Newborough

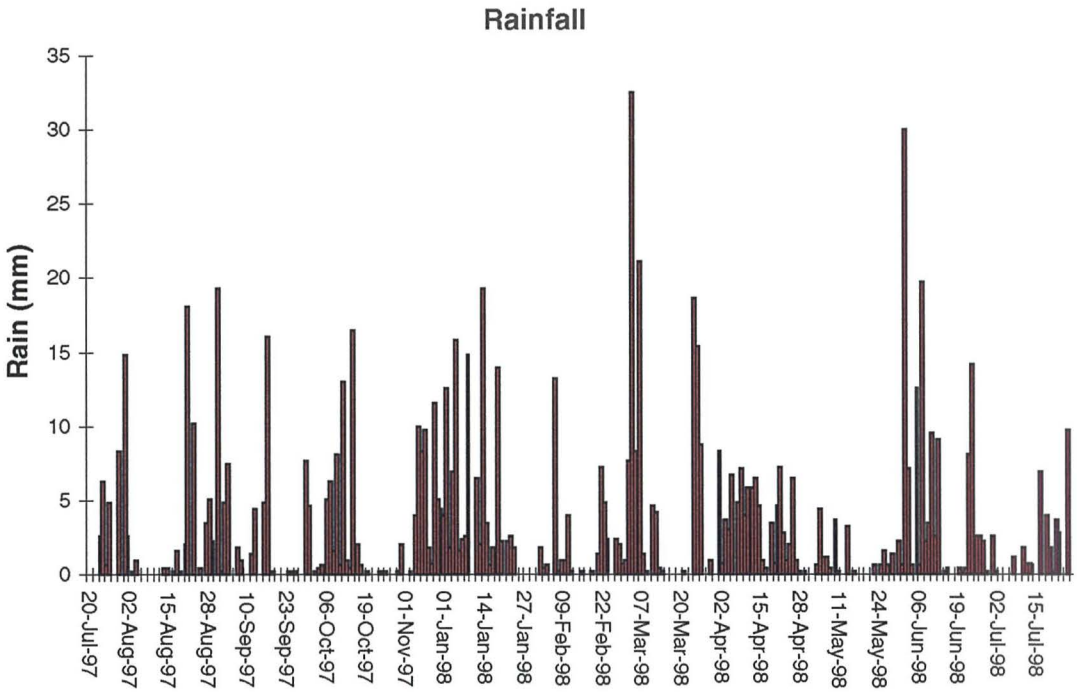


Figure 2.3 Daily rainfall at Newborough.

Table 2.1 *Summary of meteorological data at Newborough. Data are the average for each month except for rainfall.*

Month	Air Temperature (°C)	Irradiance (Wm^{-2})	Total Rainfall (mm)	Wind Speed ($m\ s^{-1}$)	Wind Direction (°)	Soil Temperature at 10 cm depth (°C)	Soil Temperature at 30 cm depth (°C)
August 97	17.3	16.8.8	62.9	2.5	206.8 (South-west)	15.5	16.0
September 97	13.9	121.0	70.0	3.0	226.9 (South-west)	13.8	14.8
October 97	10.7	63.8	65.4	2.7	198.4 (South-west)	11.6	12.9
November 97	N/A	N/A	N/A	N/A	N/A	N/A	N/A
December 97	N/A	N/A	N/A	N/A	N/A	N/A	N/A
January 98	6.6	25.8	121.2	4.4	197.1 (South-west)	6.7	7.9
February 98	8.2	39.8	43.4	4.2	220.7 (South-west)	7.2	8.0
March 98	8.6	70.7	132.8	3.7	235.2 (South-west)	8.0	8.9
April 98	8.0	153.0	93.4	3.7	167.8 (South-east)	8.9	9.4
May 98	12.2	205.1	22.5	2.8	57.8 (North-east)	12.4	12.2
June 98	13.5	181.3	131.1	3.5	230.4 (South-west)	14.1	14.1
July 98	14.6	176.0	37.3	3.4	255.1 (South-west)	15.4	15.5
August 98 §	-	-	-	3.2	247.9 (South-west)	-	-
September 98 §	-	-	-	3.1	222.5 (South-west)	-	-

N/A = Not available due to mechanical fault with data logger

§ - Wind data only (for Section 4.3.1)

From the data, we can conclude that Newborough's climate is remarkably dry and sunny compared with the rest of North Wales. Previous meteorological data collected from 1930 from the nearest station at RAF Valley near Holyhead, showed that between January 1931 and December 1986 the average monthly maximum temperature recorded was 23.6 °C in July and August and the average monthly minimum is -3.0 °C in January (Abdy and Mayhead, 1988). Ranwell (1960) reported the average of rainfall from 1950-1958 was 957 mm/annum, being highest in midwinter and lowest in the spring; and average monthly mean temperature ranges from 5.6 °C in February to 15 °C in July (Ranwell, 1959). Rainfall data recorded by the Forestry Commission at Parc Mawr between 1984-1988 which on average was 1000 mm per annum (see Abdy and Mayhead, 1988). The wettest months were September, October and December and the driest were February through May. Reynolds *et al* (1990) who conducted a study on bulk precipitation across the mountains also collected rainfall data at Newborough and compared it to other sites. For the period of December 1984 to April 1986, the rainfall was 1307 mm and for 30 year annual average was 900 mm/annum.

Chemical analysis of rain water revealed that rainfall at Newborough was consistently acidic with pH range between 4.18 to 5.68 (Table 2.2). Overall the rainfall has a low conductance, usually less than 100 μS cm^{-1}. However, rainwater sampled at the end of December 1997 (29/12/97) showed an extremely high conductance of 294 μS cm^{-1} compared with the rest of the samples. The highest conductance at this sampling date seems also related to the highest concentration of base cations and anions (Cl^- and SO_4^{2-}) in the particular sample. Analysis for base cations shows that concentration of sodium (Na) is relatively

higher compared to other cations for each particular sampling date. The similar pattern is also demonstrated by chloride ion (Cl^-), which is relatively higher than other anions, sulphate (SO_4^{2-}) and nitrate (NO_3^-). The higher concentration of sodium and chloride, as well as sulphate in the rainwater relative to other ions is caused by the influence of marine aerosols on the atmospheric inputs, because no other major sources of these ions are known in this area. Precipitation and throughfall are generally influenced by sea salt at sites within approximately 100 km from the coastline (Parker, 1983). The rainwater sampling site (at the weather station) is approximately 4.5 km inland, which suggests that a strong marine influence on atmospheric deposition can be expected. The sea is also a source of sulphur gases such as dimethyl sulphide (Barnard *et al.,* 1982; Turner and Liss, 1985), and sulphate arising from these emissions is perhaps contributed to the estimation of sulphate concentration in the Newborough rainwater.

Table 2.2 *Chemical rain water analysis*

Collection date	pH	Na	K	Ca	Mg	NH_4^+	Cl	NO_3-N	SO_4	Conductance ($\mu S\ cm^{-1}$)
		mg/L								
22-Aug-97	4.8	-	-	-	-	0.4	-	0.35	-	22
26-Sep-97	5.17	-	-	-	-	0.02	-	<0.01	-	44
19-Dec-97	4.52	4.3	0.14	0.3	0.52	0.17	7.74	0.31	2.27	-
29-Dec-97	5.14	43.7	1.66	1.84	5.14	0.13	31.3	0.1	12.16	294
15-Jan-98	5.21	12.9	0.48	0.6	1.58	0.16	23.5	0.15	3.8	96
27-Jan-98	4.69	5.9	0.22	0.34	0.76	0.32	11.58	0.36	2.62	55
10-Feb-98	4.65	9.1	0.33	0.52	1.12	0.94	16.58	0.31	4.11	72
28-Feb-98	5.56	9.4	0.37	0.84	1.14	0.6	17.84	0.44	3.96	77
14-Mar-98	5.31	5.1	0.16	0.28	0.58	0.1	9.43	0.08	1.66	41
31-Mar-98	4.84	2.4	0.06	0.16	0.28	0.26	3.43	0.18	1.55	24
15-Apr-98	4.66	9.4	0.25	0.42	1.06	0.33	16.6	0.33	3.59	74
1-May-98	4.74	3.9	0.21	0.26	0.46	0.27	7.02	0.23	2.24	36
31-May-98	4.18	5.9	0.4	1.64	0.86	0.5	11.01	1.1	6.73	86
15-Jun-98	4.46	2	0.04	0.2	0.22	0	3.25	0.21	1.75	26
30-Jun-98	4.91	1.8	0.14	0.24	0.22	0.18	3.31	0.16	1.36	18
31-Jul-98	5.64	4.5	0.89	0.5	0.54	0.12	8.14	0.05	2.12	45
31-Aug-98	5.68	2.4	0.17	0.28	0.26	0.75	-	-	-	24

Concentration of ammonium and nitrate ions in the Newborough rainwater is fairly low (Table 2.2 & Figure 2.4). Figure 2.4 also indicates that concentration of NH_4^+ in the rainwater is apparently greater in February 1998 sampling (collected on 10/2/98) and NO_3^- in May 1998 sampling (collected on 31/5/98).

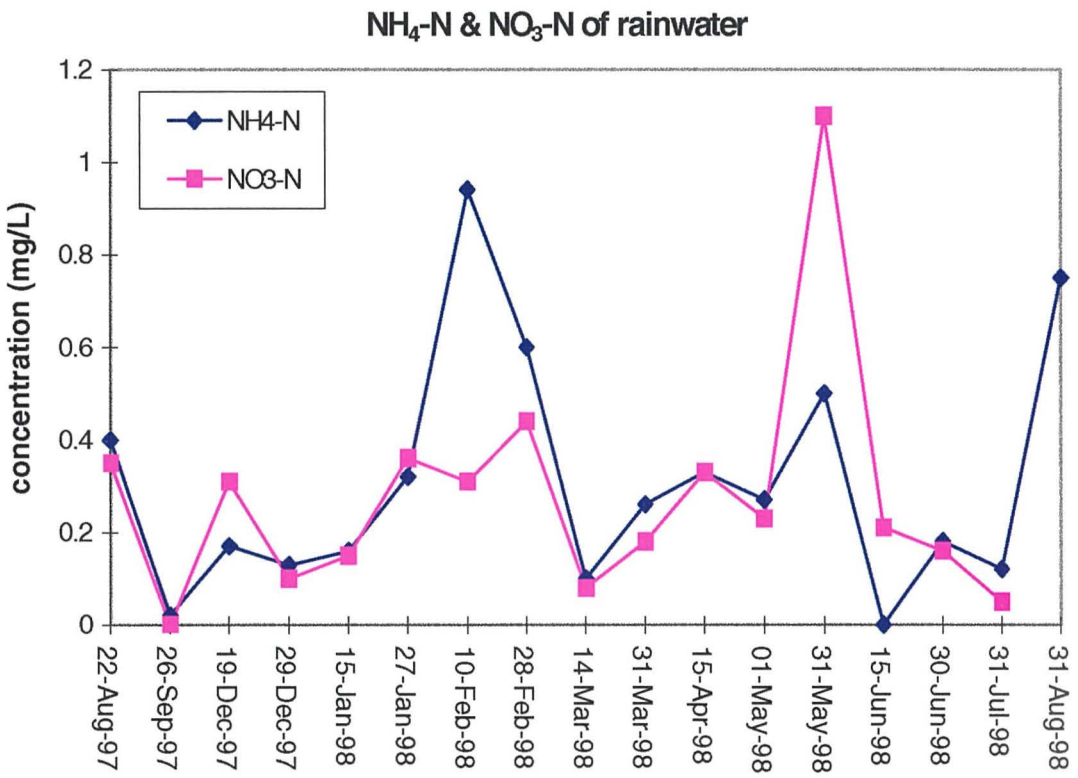


Figure 2.4 Concentration of ammonium-N and nitrate-N in Newborough rainwater

The acidity of precipitation and the concentration of pollutant-related ions seemingly correlated. The most acid rainfall occurred in samples collected on 31/5/98 and 10/2/98, and coincided with some of the highest concentrations of NO_3, NH_4 as well as SO_4. As this area supports intensive agriculture where fields periodically receive fertilizer additions and occasional applications of sewage sludge, these contribute to the concentration of the ammonium ions in the

rainwater. Moreover, the existence of large poultry farm in this area which is believed to be a point source of atmospheric ammonia, is also contributing to the higher ammonium concentration in the precipitation (survey of ammonia concentration in the air will be discussed in Chapter 4). In addition, the increased of NH_4^+ concentration in February 1998 samples may be partly contributed to marine aerosols aged by contact with land-based emissions. Aged marine aerosols are known to have higher concentrations of secondary ammonium sulphate and nitrate (Chow *et al.*, 1996).

2.2 VEGETATION NITROGEN CONTENT AT TRANSECT SITES

This survey was conducted to determine N content of wild vegetation on the Newborough dune system at each transect site from Site 1 to Site 8 (see Table 1.2). Data from this survey would provide a general idea of the N content of vegetation from the coastal area towards the inland area.

2.2.1 Material and Methods

Aboveground vegetation of the dune system was sampled along a transect line from Site 1 to Site 8. A 10 cm x 10 cm quadrat was placed randomly at each site, and vegetation in the quadrat was cut to ground level using a pair of scissors. Three replicate samples were taken; and samples collected were then kept in polythene bags, labelled and brought back to laboratory. Samplings were carried out three times in October 1996, August 1997 and April 1998.

All samples were dried in the oven at 70°C for more than 72 hours prior to analysis. Dried samples were weighed to determine biomass per quadrat area. Subsequently, samples were then milled using a bench milling machine and stored in small airtight polythene bags. Analysis of total nitrogen content of the samples was conducted by means of the Kjeldahl method outlined in Allen *et al.* (1989).

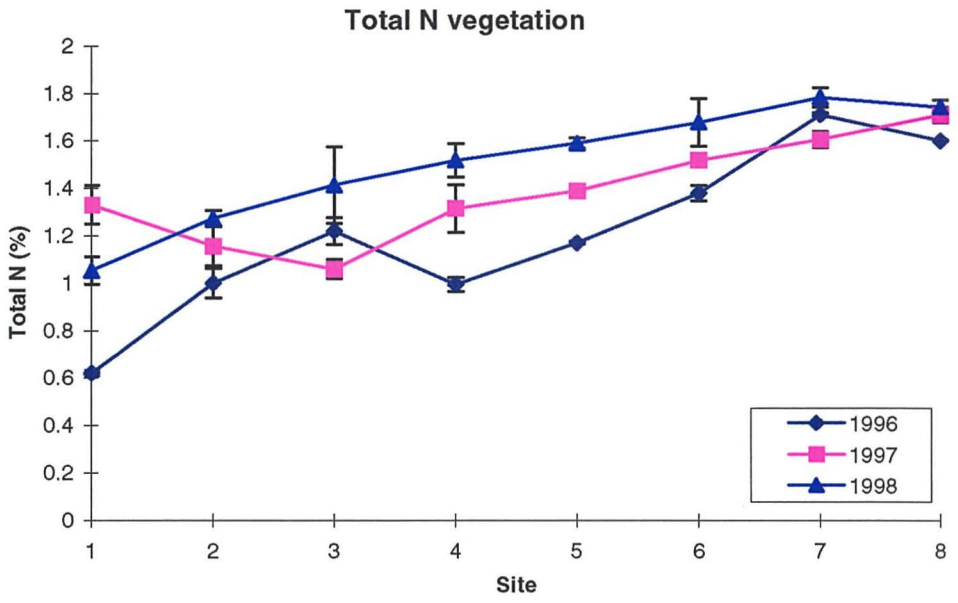
0.200 g milled samples were weighed accurately in duplicates into Kjeldahl tubes. Half a tablet of Kjeltabs TCT catalyst was put into each tube to increase digestion rate. Each tablet of Kjeltabs TCT contains K_2SO_4, $CuSO_4.5H_2O$ and TiO_2, in which K_2SO_4 is used to raise the digestion temperature and Cu and Ti are used to promote oxidation of organic matter (Bremner & Mulvaney 1982).

Concentrated sulphuric acid (5 ml) was added to each tube, and all tubes were then put in the preheated Kjeldahl digester at 420°C to digest the plant materials for 30-45 minutes until solutions became greenish in colour. After digestion, the solutions were left to cool at room temperature, and 10 ml of distilled water was added to the tubes to dilute the solutions. Each tube was run on a Kjeltec Auto 1030 Analyzer to determine nitrogen content in the samples, which is given as percentage of N in the sample. Then, total nitrogen content of vegetation at each site was calculated and expressed as kg N per hectare.

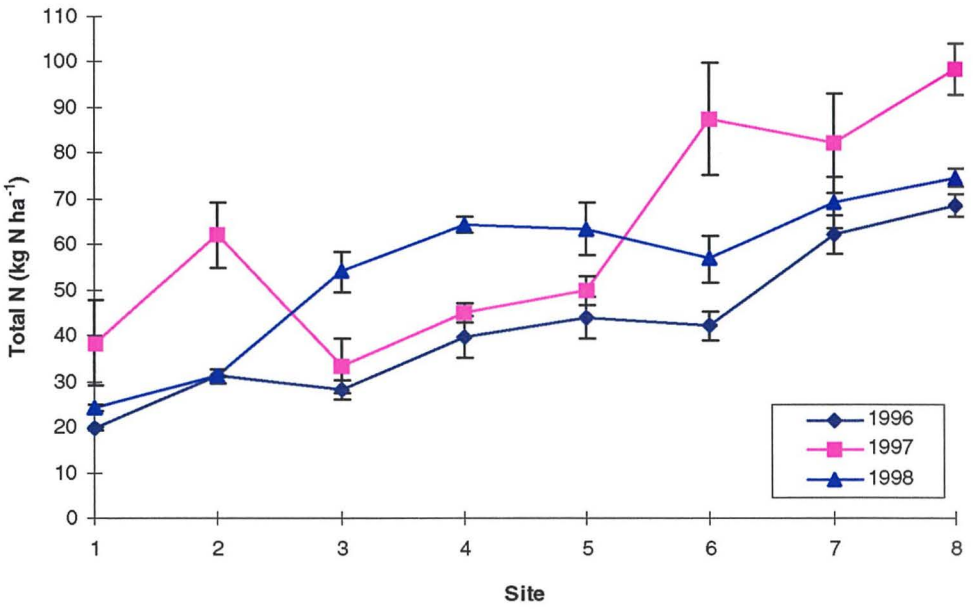
2.2.2 Results and Discussion

Results of annual measurements of total nitrogen content of wild vegetation at each site are shown as Figure 2.5. It is apparent that total N in the vegetation different significantly between sites ($p<0.001$) and increased in inland (Figure 2.5a). When total N was expressed as weight per unit area, inland sites have a much greater N content compared to coastal sites (Figure 2.5b). Though as shown in Figure 2.5a that nitrogen concentration of vegetation is higher at inland sites, the pattern is more obvious when expressed per unit area due to the higher vegetation biomass sampled from the inland sites. This is because total nitrogen content per unit area is a product of percentage of nitrogen content multiplied with biomass per unit area. Furthermore, there is also a highly significant difference in total N between sampling period ($p<0.001$), whereby nitrogen concentration is apparently the highest in the 1998-sampling (Figure 2.5a). However, a slightly different trend is shown when total N was expressed per unit area for each year. This shows that the 1997-sampling has a higher total N compare with the other two samplings. This is due to higher biomass obtained in

the sampling that was done in the summer compared to those in autumn and late winter.



(a)



(b)

Figure 2.5 Annual measurements of total N content of vegetation at each site expressed as (a) percentage of total N per unit weight (b) total N per unit area of vegetation. Bars show standard error of mean.

2.3 MOWING EXPERIMENT - VEGETATION N CONTENT

The mowing experiments at Newborough Warren NNR were set up in the early 1970's by the then Nature Conservancy Council (now the Countryside Council for Wales) with the initial objective to look for a management system for the dune vegetation, by comparing data of the grazing and mowing plots. As these plots are well established, they provide an opportunity to determine whether management of mowing on the system could affect nitrogen content of the vegetation. It is known that plants take mineral nitrogen as their nutrient mostly available from the soil. Nevertheless, atmospheric nitrogen is also another important source of N that should not be neglected, and this source of N is taken up by leaves via the stomata. Thus, grazing by animals may perhaps affect the uptake of nitrogen from the atmosphere due to the foliar grazing.

2.3.1 Aim

To investigate mowing as a means of determining whether grazing in the dune system could affect total nitrogen accumulation in vegetation.

2.3.2 Methods

A mowing experiment has been laid out at Newborough Warren on fixed dune communities near Llyn Rhos-Ddu. The experimental plots are based on a Latin Square design and consists of 25 plots each of 25 square metres. A one-metre wide pathway separates each plot. The plots were first mown in 1971 and have been mown every year since then. Details of these experimental plots can be found in Hewett (1985). Five cutting regimes were originally employed and they are:

A - Not mown

B - Mown in May

C - Mown in May, July

D - Mown in May, September

E - Mown in May, July, September

The experimental layout is as Figure 2.6 below. From 1992 onwards, the treatment 'E' was reduced to a maximum of 2 cuts per year, 'early' and 'late' cut treatments (Figure 2.7).

A [1]	B [2]	C [3]	D [4]	E [5]
B [6]	E [7]	D [8]	A [9]	C [10]
C [11]	A [12]	B [13]	E [14]	D [15]
D [16]	C [17]	E [18]	B [19]	A [20]
E [21]	D [22]	A [23]	C [24]	B [25]

Figure 2.6 Experimental layout of mowing plots

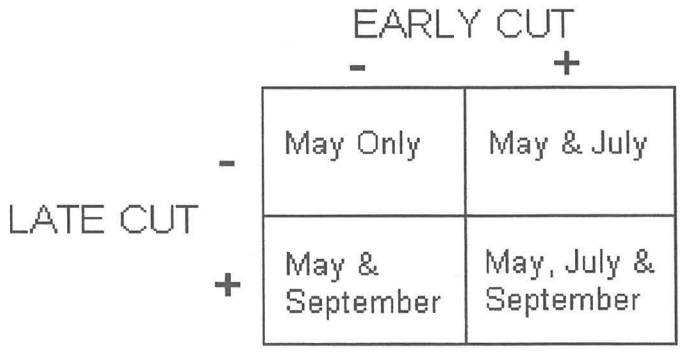


Figure 2.7 Summary of mowing treatments after reducing cutting treatments to 'early' and 'late' cut.

The clippings were spread on the plot with a view to cycle the nutrient back to the system. Aboveground vegetation was sampled three times, i.e. on 13/1/96, 24/7/96 and 29/10/96, representing winter, summer and autumn data respectively. Sampling methods and vegetation analyses were as described in Section 2.2.1.

In addition, several plant species in the mowing enclosure were collected to determine tissue N content. Species collected were chosen randomly, with most of them found to grow abundantly in the enclosure. Sampling was done only once in summer (July 1996), and the species were *Trifolium pratense, Rubus fruticosus, Galium verum, Festuca rubra, Briza media, Salix repens* and *Taraxacum spp.*

2.3.3 Results and Discussion

Using the MINITAB program, the experimental factors such as treatments, rows, columns, were separated in different columns and each analytical and derived variable were then added to the data sheet. Results of tissue N content and biomass of aboveground vegetation of winter, summer and autumn samplings are shown as in Table 2.3. The calculated total N per unit area was also displayed (Table 2.3).

Since the experimental design was a Latin Square, percentage of nitrogen content was tested for significance against treatment effects, row and column factors using ANOVA in the General Linear Model (GLM) in MINITAB. No significant difference was found on nitrogen concentration against these factors. However, when ANOVA was conducted on total nitrogen per unit area (kg N ha^{-1}),

there is a highly significant difference between the treatments ($p<0.001$), and sampling times differed significantly ($p<0.05$). Again, there was no significant difference between row and column factors (Table 2.4).

Table 2.3 *Tissue N content (%), biomass and total N (kg N ha^{-1}) of aboveground vegetation in mowing plots of three sampling times*

Treatment	Winter			Summer			autumn		
	N%	Biomass (g)	Total N (kg N ha^{-1})	N%	biomass (g)	Total N (kg N ha^{-1})	N%	biomass (g)	Total N (kg N ha^{-1})
A	1.38	6.67	92.05	1.43	5.61	80.22	1.51	5.02	75.80
B	1.12	4.76	53.31	1.44	4.14	59.62	1.29	3.87	49.92
C	1.25	4.30	53.75	1.28	4.99	63.87	1.37	3.21	43.98
D	1.15	3.61	41.52	1.23	4.56	56.09	1.11	3.48	38.63
E	1.20	3.81	45.72	1.18	4.40	51.92	1.20	3.41	40.92
Overall mean	1.22	4.63	57.27	1.31	4.74	62.34	1.30	3.80	49.85

Table 2.4 *ANOVA of total nitrogen per unit area (kg N ha^{-1})*

Source	DF	SS	Adj SS	Adj MS	F	P
Treatment	4	13279.8	13279.8	3319.9	10.84	0.000 ***
Row	4	1334.9	1334.9	333.7	1.09	0.370
Column	4	548.7	548.7	137.2	0.45	0.774
Sampling time	2	2065.6	2065.6	1032.8	3.37	0.041
Error	60	18380.7	18380.7	306.3		
Total	74	35609.6				

The significant difference shown by total nitrogen content when expressed per unit area (kg N ha^{-1}) was due to high biomass obtained from the uncut treatment (treatment A) compared to cut treatments (B, C, D & E). Percentages of variance of all factors are shown in Table 2.5. The ANOVA indicates that 37.29% of the variance is because of the treatment, when Sum of Squares (SS) is expressed as percentage of total variance. Most of the variances are due to error factor that represents 51.62% of the total variance.

Table 2.5 *Percentages of variance of all factors.*

Factor	Percentage of variance (%)
Treatment	37.29
Row	3.75
Column	1.54
Sampling Time	5.80
Error	51.62
TOTAL	100

Further ANOVA was conducted between the four cutting treatments to determine significance difference between them. Originally all four treatments had cutting in May, July and September. Different combinations of cuttings differentiate the treatments. Recently the cutting regimes became less regular and were reduced from strict monthly cuttings to an 'early' cut and a 'late' cut. July cutting represents early cut treatment whereas September cutting as a late cut treatment. Summarisation of the treatments is as Figure 2.7. ANOVA in the General Linear Model (Table 2.6) shows that there is a significant difference of total tissue nitrogen content (%) and subsequently total nitrogen per unit area (kg N ha^{-1}) between early and late cut treatments ($p<0.01$).

Table 2.6 *ANOVA on tissue N content (%) of early and late cutting treatments.*

Source	DF	SS	MS	F	P
Early cut	1	0.00894	0.00894	0.49	0.489
Late cut	1	0.19648	0.19648	10.68	0.002
Early*late	1	0.00083	0.00083	0.05	0.832
Error	56	1.02998	0.01839		
Total	59	1.23624			

Analysis of tissue N content of several species collected in the mowing enclosure shows that legume of *Trifolium pratense* has the highest N content and *Briza media* has the lowest compared with other species (Figure 2.8). The higher N

content in the legume species is expected because of the ability of the species to fix nitrogen from the atmosphere.

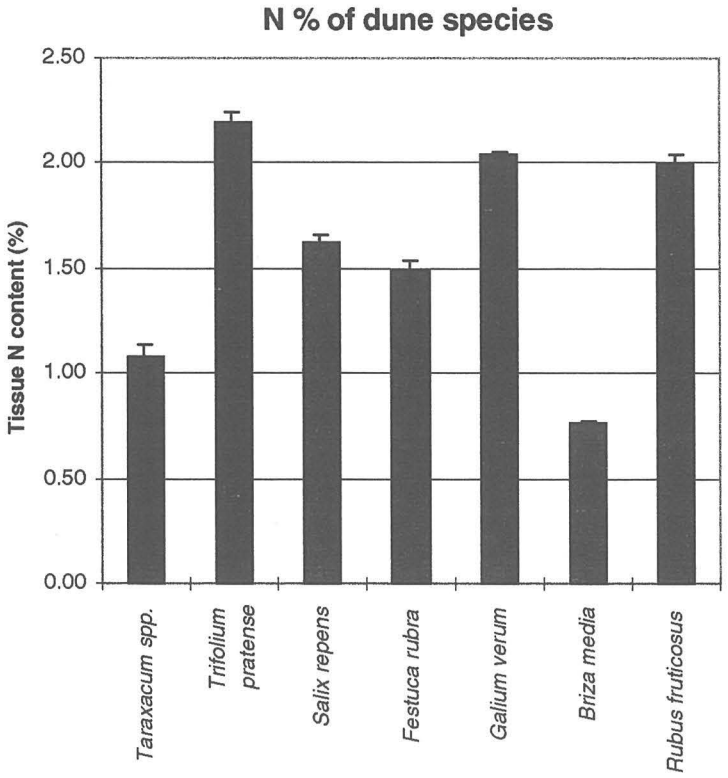


Figure 2.8 Tissue N content of several dune plant species at Newborough

The survey on the mowing plots indicates that N content of the aboveground vegetation though statistically different, is not greatly affected by management regime such as mowing. This type of management would affect biomass differences between treatments. Moreover, the treatments also affected species composition as described by Hewett (1985). In relation to the mowing regimes employed, there is some dispute regarding the effects on nutrient levels of returning clippings. Green (1972) has argued that returning clippings leads to

eutrophication, encourages nitrophilous species and promotes seral change. However, Wells (1980) found no differences in floristic composition by returning clippings and that removal of cuttings only caused a significant decrease in extractable phosphate and exchangeable magnesium, other nutrients were replaced either in rainfall, by soil mineralisation or by fixation by soil-micro-organisms.

2.4 SOIL SURVEY

2.4.1 General Material and Methods

Soil at each site was sampled three times in October 1996, August 1997 and August 1998, at 0-10 cm depth. Sampling was carried out using a small hand corer and samples were labelled and kept in polythene bags. Three replicate samples were taken, and samples were brought back to the laboratory, stored in a refrigerator prior to analysis. Replicate samples were not bulked together, but they were analysed individually. The following parameters were determined on all samples.

2.4.1.1 Soil pH

Soil pH was measured on a suspension of fresh field-moist soil in deionised water with the ratio of soil to water of 1:2.5 by weight. The method described here is based upon that employed by the Soil Survey of England and Wales (Avery & Bascomb 1974).

10 g of fresh field-moist soil were weighed into a 50 ml plastic pH beaker. 25 ml deionised water was added to each beaker from 2 x 12.5 ml squirts of the Oxford dispenser. The suspensions were thoroughly stirred with a glass rod and they were allowed to stand for 30 minutes during which they were occasionally stirred. Soil pH was measured electrometrically using the Corning 220 pH meter, calibrated beforehand.

2.4.1.2 Soil Moisture and Organic Matter Content

Empty porcelain crucibles were weighed accurately and the crucible identification was noted. Subsequently, the crucibles were filled up to three-quarters full with

field-moist soil, and they were reweighed. The soils were then placed in an oven set at 105 °C, and left overnight to drive off moisture. All crucibles were removed from the oven, cooled in a desiccator, and reweighed to obtain the moisture content. The crucibles were then placed in the muffle furnace set at 375 °C, and left overnight (16 hours) to burn off organic matter. Finally, crucibles were reweighed after cooling in the desiccator.

Soil moisture was determined as the difference between the weight of fresh, field-moist soil at ambient temperature and after 16 hours drying at 105 °C. Organic matter of the soil was determined as the "loss-on-ignition" from the soil after placing in a furnace overnight at 375 °C. Moisture content was expressed as a percentage of 105 °C dry-weight soil whereas organic matter content was expressed as a percentage of the 375 °C dry-weight soil.

2.4.1.3 Exchangeable Cations

Exchangeable cations were determined by extraction of field moist soil in 0.5 M ammonium chloride solution. 15 g of each sample of moist soil was weighed into a 250 ml conical flask, and 150 ml of 0.5 M NH_4Cl solution (3 x 50 ml squirts from Zippette) was added. Three blank extracts containing no soil were also included to test for contamination from glassware, filter papers, etc. All conical flasks were covered with cling film and were then shaken for 1 hour on the flatbed shaker. While the flasks were on the shaker, Whatman No. 542 filter papers were prepared and pre-washed by pouring through 50 ml of the NH_4Cl solution into labelled plastic bottles. The solution was discarded before filtering the soil suspension. Then, all soil suspensions were filtered and filtrates were retained

for analysis for Na, K, Ca and Mg. Na and K were analysed by flame atomic emission spectrophotometry whereas Ca and Mg were analysed by flame atomic absorption spectrophotometry, using the Perkin-Elmer 280 A.A.S. As for Ca and Mg analyses, extracts were diluted with lanthanum chloride solution ($LaCl_3$) by 1:1 for Mg, and 1:5 for Ca due to higher concentration of the latter ion.

2.4.1.4 Available Nitrate (NO_3^- -N) and Ammonium (NH_4^+ -N)

15 g of field moist soil of each sample was weighed into a 250 ml conical flask and to which was added 150 ml of 1 M Potassium Chloride (KCl) solution (3 x 50 ml squirts from Zippette). Three blank extracts containing no soil were also included to test for contamination from glassware, filter papers, etc. All conical flasks were covered with cling film and were then shaken for 1 hour on the flatbed shaker. While waiting for the flasks on the shaker, Whatman No. 44 filter papers were prepared and pre-washed by pouring through 50 ml of the KCl solution into labelled plastic bottles. The solution was discarded before filtering the soil suspension. Filtrates were retained in plastic bottles, and then analysed for ammonium-N and nitrate-N using a Skalar Continuous Flow AutoAnalyser SA-40. Brief procedure of running the samples on this machine is in Appendix 2.

2.4.1.5 Total Soil N Content

2 g air-dried soil were weighed into Kjeldahl tubes and soils were digested as procedure employed in section 2.2.1. The volume of sulphuric acid used for the digestion, 15 ml was greater than vegetation analysis. Also, two Kjeltabs tablets were used instead of half a tablet and soil was digested for two hours. Solutions were then run on the Kjeltec AutoAnalyzer to determine total nitrogen content of the soil.

In addition to the soil analyses, surface water was sampled once in 1998 at five sites, i.e. Site 4, Site 5, Site 6, Site 7 and Site 8. All water samples were analysed for ammonium-N and nitrate-N using a similar method to that for the rainwater analysis in Section 2.1.1.

2.4.2 Results and Discussion

Newborough Warren's soil is sandy soil, which is typical for soil of dune grassland. Soil characteristics vary between each survey site, which is closely related to the type of dune areas. Mean soil pH of Site 1 to Site 8 for the three surveys are within the range of 5.34 to 7.70 (Table 2.7).

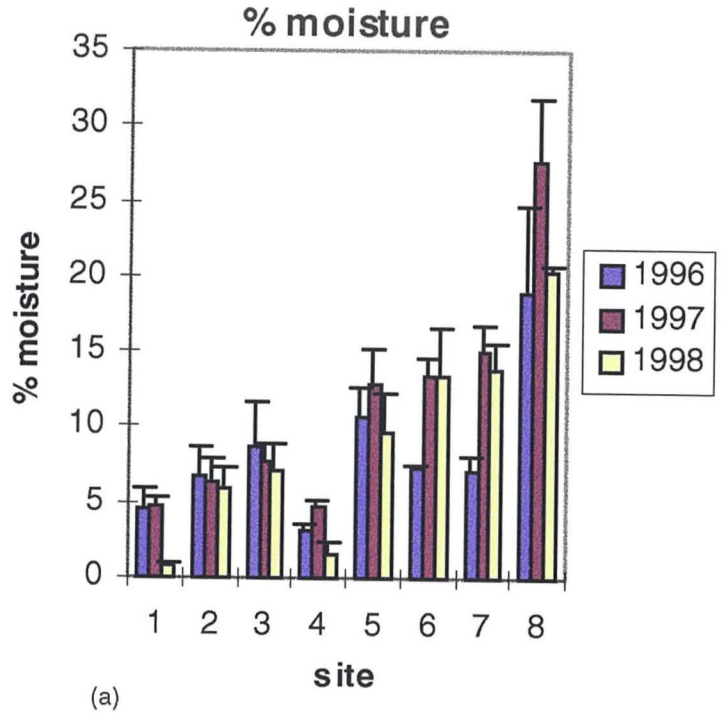
Table 2.7 *Soil pH of all sites from coastal towards inland for each sampling year.*

Site	1996	1997	1998	Mean
1	7.66	7.75	7.68	7.70
2	7.47	7.40	7.44	7.44
3	7.13	7.13	7.17	7.14
4	7.57	7.53	7.45	7.52
5	6.93	5.80	6.90	6.55
6	5.61	5.52	5.53	5.55
7	5.36	5.27	5.40	5.34
8	5.63	5.44	5.47	5.51

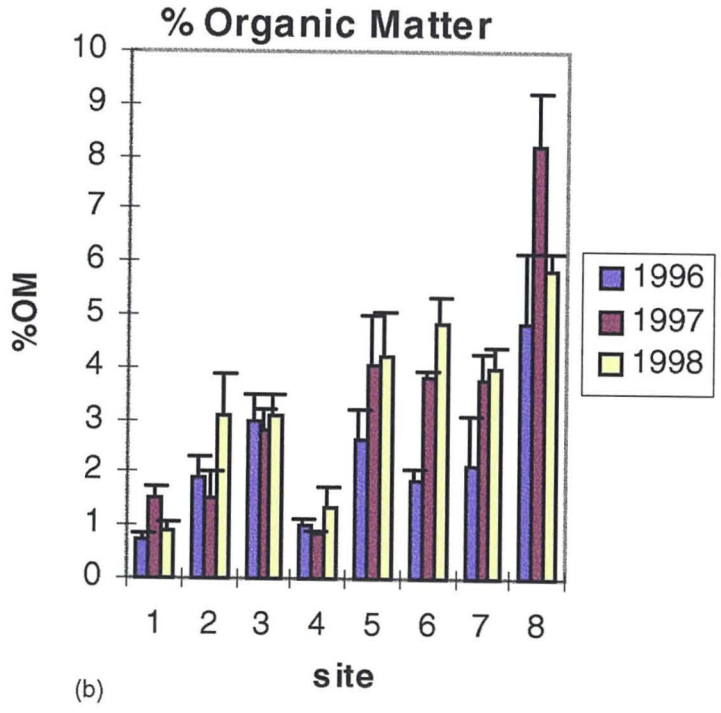
No significant changes in soil pH could be observed between sampling years. There is a clear trend that soils at coastal sites have significantly higher pH compared to inland sites (ANOVA, $p<0.001$). Higher pH at the coastal sites was probably due to higher content of shell fragments in the soil from very high at the coast to very low inland, leading to calcareous and acidic conditions respectively. Salisbury (1922) (cited in Packham and Willis, 1997) demonstrated long ago at dune system at Blakeney Point, Norfolk that soil pH declines along transects

running inland from the shore. However, Willis *et al.* (1959) reported a consistent high pH (alkaline) of soil at Braunton Burrows, even in the oldest parts of the dune system.

Percentages of moisture and organic matter content are illustrated as Figure 2.9, which shows a positive increase towards the inland sites. There are highly significant differences ($p<0.001$) in moisture and organic matter contents between sites. However, soil moisture does not show any significant difference between sampling time; conversely organic matter content does at $p=0.001$. Site 4 apparently has the lowest moisture and organic matter content because of the location of the site, which is situated on top of a dune. The soil of this site is consistently exposed to direct sunlight and strong wind, and vegetation cover was very sparse. Due to its location, it was quite often that this site received fresh mobile sand from the beach brought by the strong wind. Organic matter content is greater in the soils covered by dense vegetation due to the production of litter and dead roots (Berendse, 1990). The role of soil organic matter as a nutrient reservoir is especially important in sandy soils that are inherently low in fertility. Other studies have also highlighted the importance of soil organic matter in nutrient cycling and other ecosystem processes (e.g. Covington 1981; Gosz *et al.*, 1976).



(a)



(b)

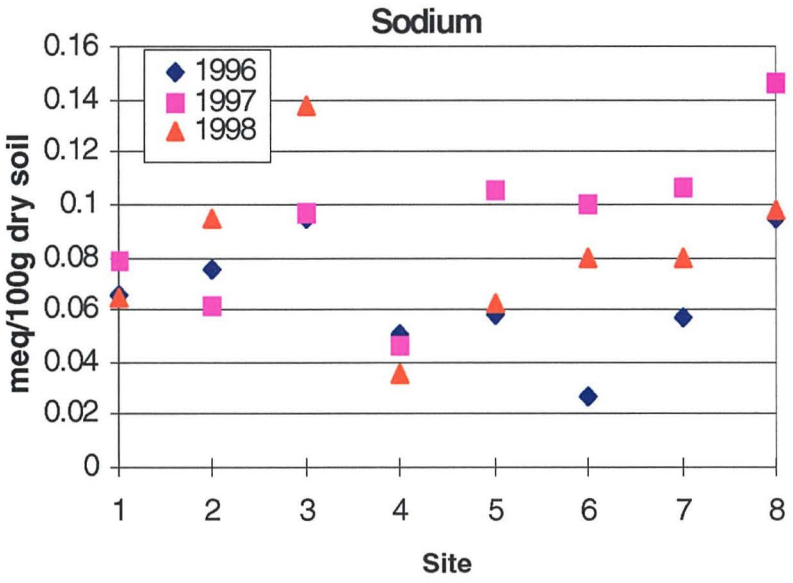
Figure 2.9 Percentages of (a) soil moisture and (b) organic matter content of Newborough soil

Exchangeable base cations show no clear pattern of sodium, potassium and magnesium between sites (Figure 2.10). Although there is no pattern shown, concentration of Na, K and Mg are different significantly ($p<0.001$, $p<0.05$ and $p<0.05$ respectively) between sites and sampling times. Furthermore, calcium concentration indicates a significant negative relationship with the site, whereby coastal sites show greater concentration compared to inland sites, with regression analysis shows R^2 of 44.2%. The higher calcium content in the soil of coastal sites is due to high content of shell fragments available in the soil blown in from the beach by storms.

Unpublished data by Ernst (cited in Ernst *et al.,* 1996) mentioned that shell of various bivalves contained between 10 and 11 mmol Ca g^{-1}, and this is a significant input of Ca to soils. Some Ca input into Newborough's soils is derived from spray deposition from the Irish Sea and the Menai Strait. Leaching is perhaps one of the reasons for the somewhat lower values of Ca in the older, inland parts, but even so these remain distinctly calcareous.

The concentrations of ammonium-N and nitrate-N show high variability of the data and no clear trend could be seen between survey sites. Ammonium-N is not different significantly between sites and sampling times, but nitrate-N differed significantly between site ($p<0.05$) and sampling times ($p<0.01$). The difference is primarily contributed by data of Site 5 and Site 6 of August 1997 surveys (Figure 2.11) that show very high concentration compared to the rest of the data. Total available inorganic N (the sum of available ammonium plus nitrate) is illustrated as Figure 2.12. Similar to Figure 2.10, no consistent trend could be seen from this figure.

Figure 2.10 Concentration of base cations of Newborough NNR soil at different sites with different sampling times. Data are mean of three samples for each sampling year.



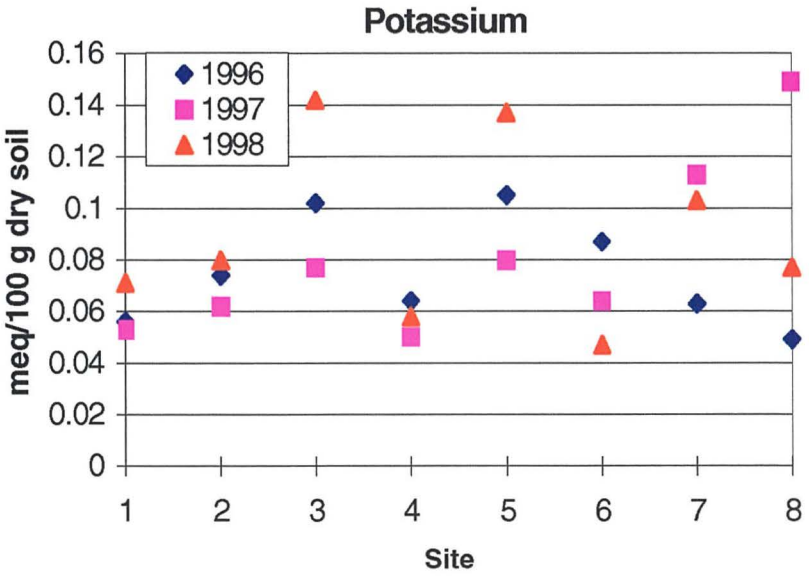
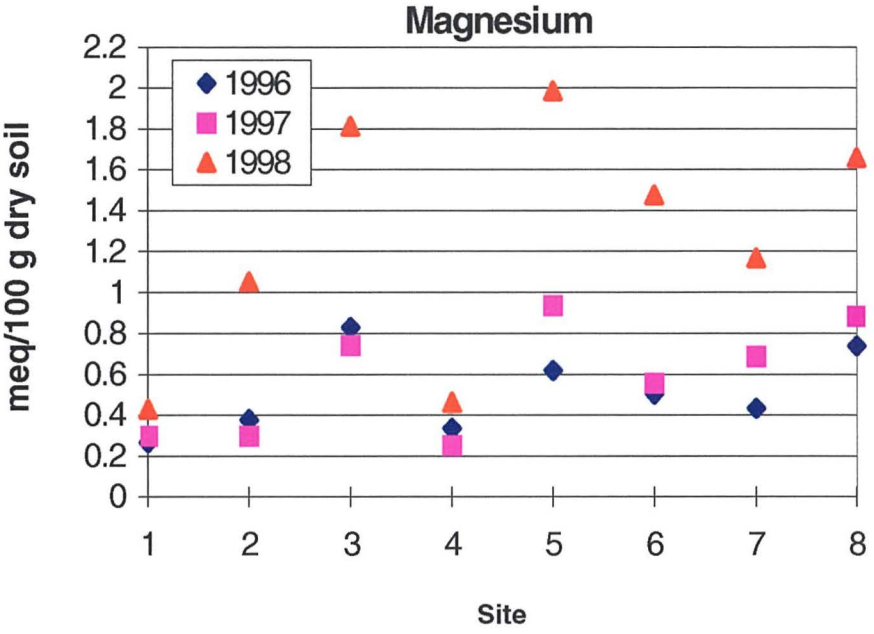
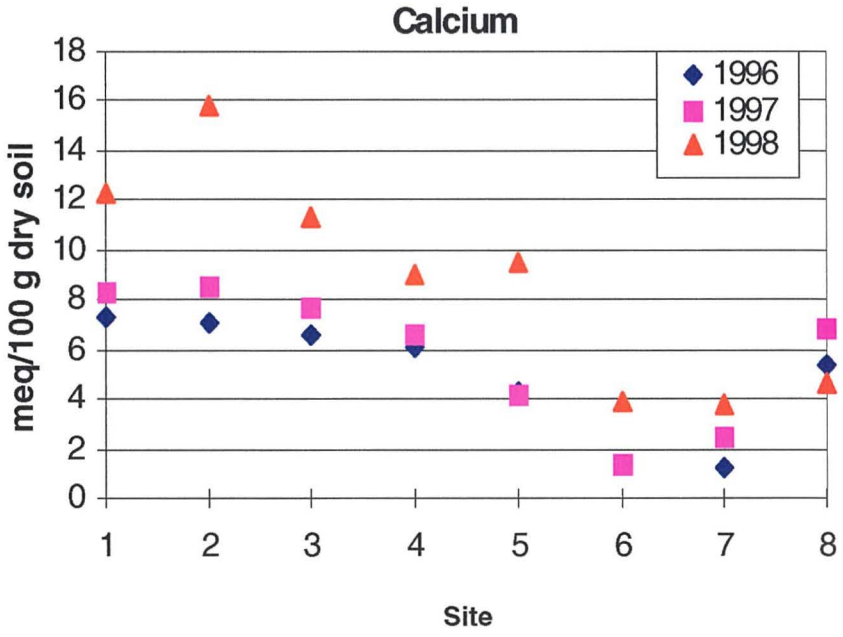


Figure 2.10 continued.





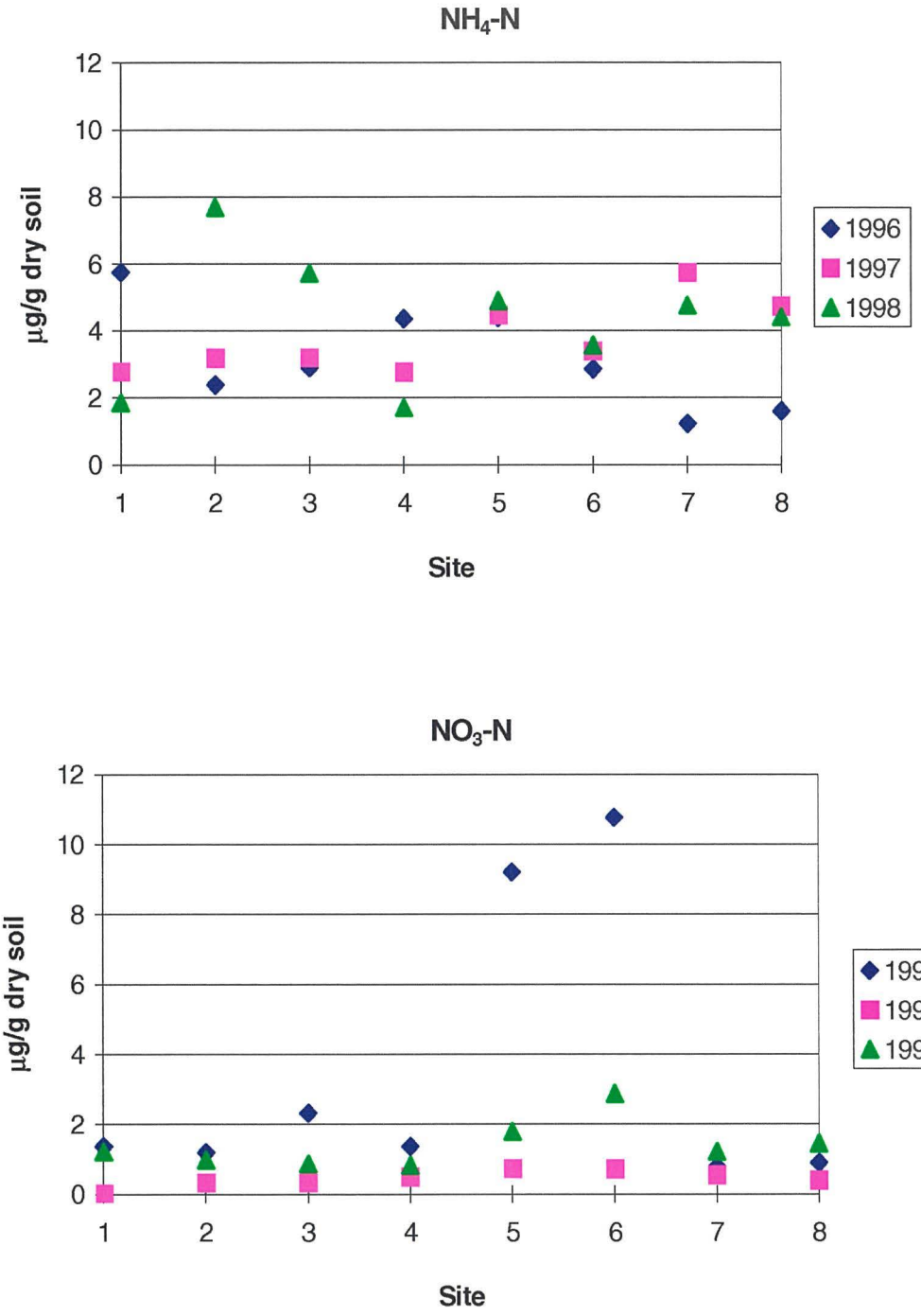


Figure 2.11 Concentration of NH_4-N and NO_3-N of Newborough soil at different sites and different sampling times. Data are mean of three samples.

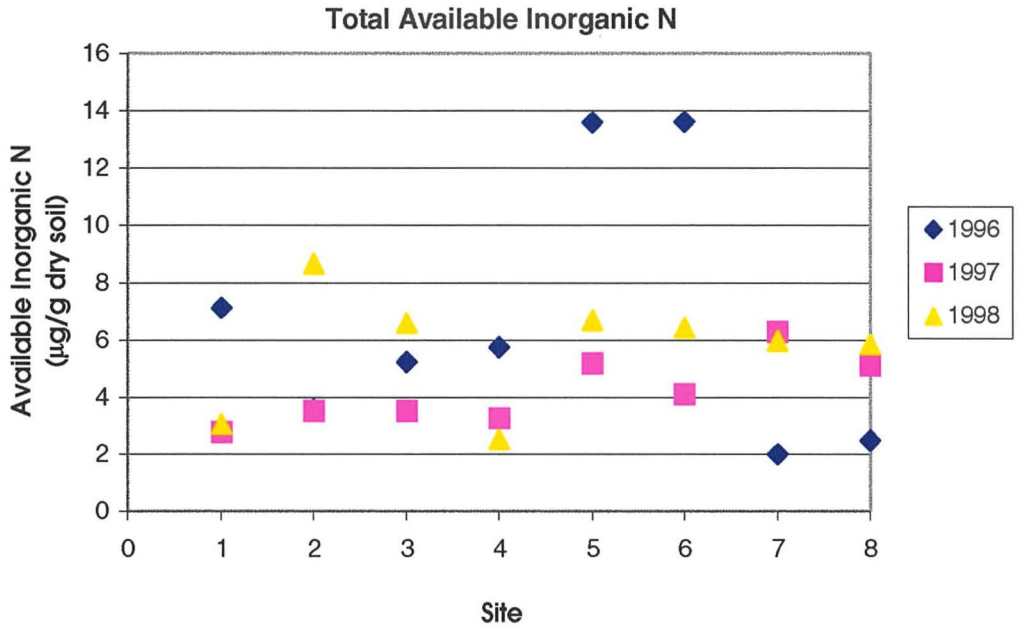
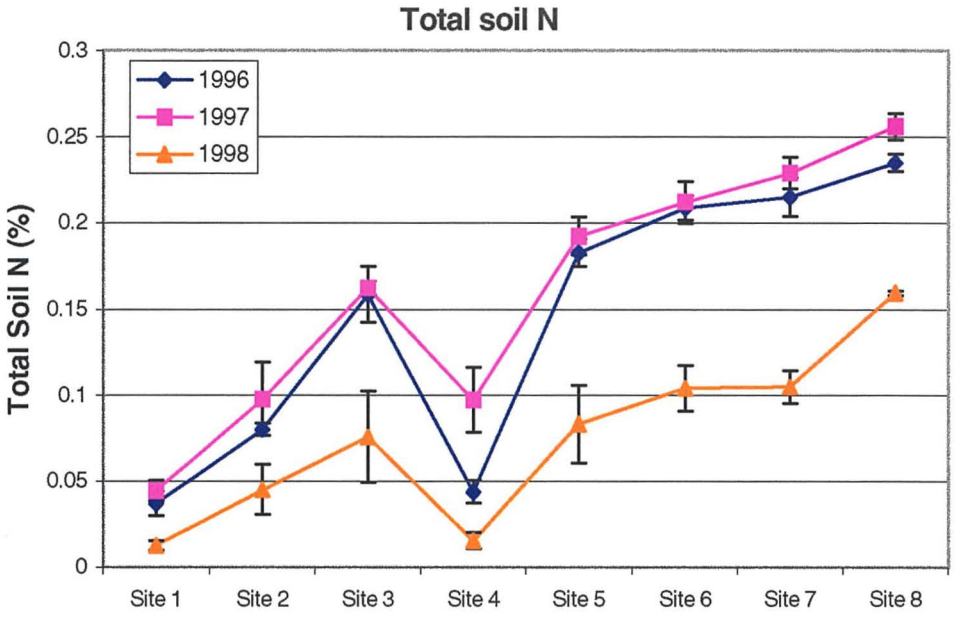


Figure 2.12 Total available inorganic N of Newborough soil at different sites and different sampling year.

A clear pattern of total N between sites can be seen in which total N increases from the coastal sites (mobile dune) towards the inland sites (dune slack) (Figure 2.13a). Again, an exception should be considered for Site 4, which total N is lower due to its soil type of fresh mobile sand (site located on top of a dune). Statistical analysis conducted on all of the data revealed that total N differed significantly between sites and sampling times (ANOVA, $p<0.001$). Between sampling years, total nitrogen content of the soil indicates an increase from 1996 sampling to 1997 sampling. However, a surprise result was obtained in which total N content of 1998 survey seems declining (Figure 2.13a).

(a)



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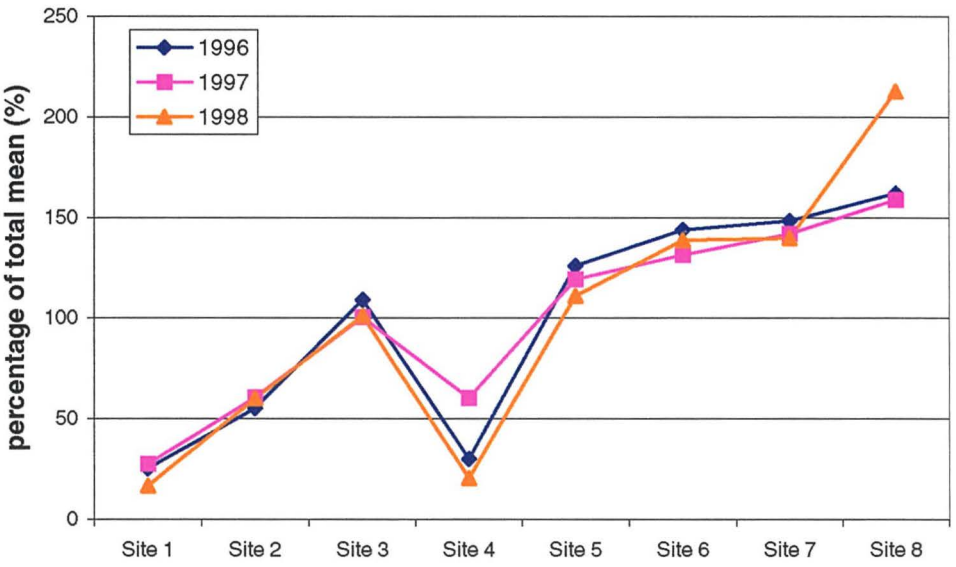


Figure 2.13 (a) Total soil N (%) of Newborough NNR soil at eight sites for each sampling year, and (b) total N expressed as percentage of total mean.

The decline of total soil N in the 1998 sampling was probably due to the method of analysis used for this particular sampling. For 1996 and 1997 samplings, the acid digestion method of the Kjeldahl AutoAnalyzer was used to analyse the samples. However, this machine was replaced in 1998 by a CHN AutoAnalyzer. One might expect some differences of readings between these two machines, and in fact we know from analysis of a standard soil that the CHN Analyser does not recover all the soil C and N even in quite organic-rich soil (Reynolds, unpublished data). Hence, to make the data more realistic, they were presented as percentage of total N over total mean and illustrated as Figure 2.13b.

From this soil survey, the increase of the organic matter and the accumulation of soil nitrogen are characteristic processes during vegetation succession. A study by Olff *et al.* (1993) on three dune microsites, a plain, a slope and a dune on the Waddenisland of Schiermonnikoog in The Netherlands revealed an increase in the thickness of the humus layer from 0.5-1.2 cm after 12 years of vegetation development to 1.8-6.8 cm after 28 years. At the same time the nitrogen amount per m^2 increased in the plain from 7.5 g N m^{-2} to 45 g N m^{-2}.

All soil data were analysed for correlation, and a full correlation matrix is shown as Table 2.8.

Correlations (Pearson)

	Na	K	Ca	Mg	NH4-N	NO3-N	%moist	%OM	pH
K	0.419								
Ca	0.045	0.033							
Mg	0.335	0.583	0.112						
NH4-N	0.281	0.509	0.171	0.483					
NO3-N	-0.447	0.130	-0.338	-0.009	-0.035				
%moist	0.541	0.509	-0.155	0.477	0.612	-0.044			
%OM	0.549	0.630	-0.254	0.636	0.442	-0.063	0.897		
pH	-0.289	-0.386	0.699	-0.411	-0.261	-0.204	-0.696	-0.770	
tot%N%	0.237	0.450	-0.582	0.549	0.424	0.290	0.662	0.727	-0.788

Table 2.8 *Correlation matrix of soil data from three replicate samples collected in each sampling year on a transect line across Newborough NNR for 1996-1998.*

It is apparent that organic matter content is highly positive correlated with soil moisture (r=0.897). This is entirely expected because higher organic matter enables the soil to hold a higher moisture content. Total N content of the soil is also positively correlated with organic matter (r=0.727), which indicates that nitrogen in the soil is mostly in the form of organic nitrogen. In contrast, soil pH is negatively correlated with organic matter content as well as total N. Higher organic matter (humus etc.) in the soil contributes to higher soil acidity due to the release of hydrogen ions from humus colloids (humic acids) when water is mixed with the soil. Humic acids that are important sources of acidity are formed by the partial decomposition of soil organic matter. Thus in the soil:

$$\underset{\text{Humus colloid}}{R\text{-}H} \overset{H_2O}{\rightleftharpoons} R^- + H^+ \text{ (the } H^+ \text{ gives acid soil pH measurement)}$$

Furthermore, the release of carbon dioxide by respiration of soil organisms to form H_2CO_3 acid with soil moisture will also contribute acidity to the soil solution, but this will only be a minor factor in determining soil pH. Such changes in soil

features can be expected to have an important impact upon the establishment and competitive ability of plant species and ultimately upon the dynamics of the species composition of the plant community.

Analysis of surface water sampled at six sites generally shows low concentration of ammonium-N and nitrate-N; the highest was at Site 7 with ammonium-N concentration of 0.26 mg/L (Table 2.9).

Table 2.9 *Concentration of NH_4 and NO_3 in surface water at several chosen sites on the transect lines*

	NH_4-N (mg/L)	NO_3-N (mg/L)
Site 4	0.09	0.01
Site 5	0.08	<0.01
Site 6	<0.01	0.01
Site 7	0.26	0.01
Site 8	0.04	0.01

2.5 DUNE TRANSECT VEGETATION SURVEY

Vegetation cover at Site 1 to Site 8 was surveyed for three times in summer 1996, 1997 and 1998. The survey used the National Vegetation Classification (NVC) methodology, which requires an ability to identify the British vascular flora and major bryophytes etc. Bryophytes were not surveyed in this study.

2.5.1 Methods

At each site, a 2 m x 2 m quadrat was used for the survey, and species occurrence within the quadrat were identified and noted. Three replicate quadrats were used at each site. The cover abundance of the plant species in the quadrat was then estimated using DOMIN scale as set out in Table 2.10. Plants were identified following Stace (1991). All surveys were as far as possible done at similar locations each year, and they were also independent of each other. The independence of the survey was assured by not referring to the previous survey data during the field work.

Table 2.10 *DOMIN cover abundance scale*

Cover/abundance		Domin score
91 - 100%		10
76 - 90%		9
51 - 75%		8
34 - 50%		7
26 - 33%		6
11 - 25%		5
5 - 10%		4
	} frequent	3
< 5%	} occasional	2
	} rare	1

2.5.2 Data analysis

Vegetation data were analysed using VESPAN package available at the University of Wales Bangor networking system. Analysis by MATCH programme in the VESPAN package was conducted to classify group of the sample points,

these were subsequently arranged in a successional sequence at Newborough. Furthermore, sequence of the transect sites and floristic gradients were investigated with Detrended Correspondence Analysis (DCA) (Hill & Gauch, 1980) using DECORANA computer programme. Also, Canonical Correspondence Analysis (CCA) (ter Braak, 1986, 1987) was conducted on the vegetation and environmental data using CANOCO programme to determine relationships between the species and the environmental variables. For all analyses, the species cover scale values were used. For the CCA, the soil data were used as the environmental variables. A brief explanation on DECORANA and CANOCO are given in subsequent sections to give overview on these two analyses.

2.5.2.1 DECORANA

Ordination of community data allows complex relationships between large samples and the often large abundances of their elements (i.e. species or families) to be represented in a more easily assimilated form; normally on a 2-dimensional graph where similar entities are placed closest together. Gauch (1982) provides a thorough review of the different ordination techniques available. Ordination is most effective where environmental factors that may structure communities are not readily apparent, and complement classification techniques such as TWINSPAN by indicating the possible role of environmental factors in producing the classification.

DECORANA (DEtrended CORrespondence ANAlysis) is a computer program developed by Hill (1979) which produces an ordination of community data by detrended correspondence analysis, a method derived from reciprocal averaging.

Reciprocal averaging is known has two main faults. Firstly, there is tendency for the second and higher axes to be related to the first axis. This is known as the arch effect (Gauch *et al*, 1977) or the horseshoe effect (Kendall 1971). This problem is not restricted to reciprocal averaging but also affects other ordination techniques such as principal component analysis. The problem is worse for the second axis since this typically possesses a high eigenvalue and is thus best able to show a gradient in normally distributed data. However the arch effect may lead to this gradient being displaced to higher, less obviously relevant axes where identification of true from false axes may prove extremely difficult (Gauch 1982). The second problem is the compression of the ends of the first axis such that the separation between elements or samples in the ordination cannot be interpreted as representative of their differences. However, DECORANA avoids these problems by ensuring - through a mathematical treatment detailed in Hill (1979), that there is no correlation of any kind between the first and higher axes.

2.5.2.2 CANOCO

A common problem in community ecology is to discover how a multitude of species respond to external factors such as environmental variables, pollutants and management regime. CANOCO, an acronym of **CANO**nical **C**ommunity **O**rdination is designed for data analysis in community ecology using the Canonical Correspondence Analysis (CCA). This program is a good technique to study community response with their environment whereby the analysis detects unimodal relationships between the species and the external variables (ter Braak 1986, 1987). CANOCO is an extension of DECORANA, which includes the indirect techniques of Principal Component Analysis (PCA), detrended correspondence analysis and principal coordinate analysis and also the direct

techniques of weighted averaging, canonical correspondence analysis, canonical variate analysis and redundancy analysis (ter Braak 1988). Also, CANOCO is particular efficient for ordination of 'sparse' data sets (data containing many zero values compared to the number of nonzero values).

2.5.3 Results and Discussion

In addition to the chosen sites, another site only 25 m from the beach, labelled as Site Ø, was also surveyed with a view to look at vegetation sequence on mobile dune built from newly blown sea sand. Table 2.11 shows full species list found at each site. No 1996-survey was done on Site 7 and Site 8 due to technical factor. The three-year vegetation survey is briefly summarised as follows:

- Site Ø: This site is a mobile dune area by the seashore. *Ammophila arenaria* (marram grass), as a dune builder grow abundantly with percentage cover of nearly 80-90%. In the second year and third year, *Rubus fruticosus* started to grow with cover of 10-15%.
- Site 1: *A. arenaria* was still dominating with cover of 76-90%, but semi-fixed dune communities such as *Festuca rubra*, started to present. Also, legume species of *Ononis repens* existed.
- Site 2: *A. arenaria* cover was reduced to 60-75%, and other semi-fixed dune communities present with cover range from 5-33%. These included *F. rubra, Leontodon autumnalis, R. fruticosus, Holcus lanatus* and *Salix repens*.
- Site 3: *A. arenaria* and *F. rubra* seemed to have similar percentage cover between 35-40%. Other species of *S. repens*, *O. repens*, *R.fruticosus* were growing abundantly with percentage cover increased between 10-35%.
- Site 4: *Festuca rubra* started to show dominance with cover of 51-75%. Covers of *A. arenaria* reduced and other fixed dune communities were

present. It was found that legume species *Anthyllis vulneraria* present abundantly in the third year survey with cover of nearly 65-75%.

- Site 5: Fixed dune communities present abundantly with *F. rubra*'s cover of more than 50%. The cover of *A. arenaria* declined to 5-10%. Other species occurred were *Holcus lanatus, L. autumnalis, R. fruticosus,* etc.
- Site 6: Diversity of species increased with the highest number of species present, 18 species in 1996 (Table 2.11). Nitrogen-loving species such as *Holcus lanatus* present significantly; also several species such as *Achillea millefolium, Agrostis capillaris* were noted to occur with a significant cover.
- Site 7: Mesotrophic grassland species were present. *Arrhenatherum elatius* grew abundantly with cover of nearly 90%. Grasses *Dactylis glomerata, F. rubra* also occurred with cover of 25-35%.
- Site 8: *F. rubra* was the dominant species in this mesotrophic grassland community. Diversity of the species was also high whereby there were 16 species present in both surveys. Other mesotrophic grassland species present were *Plantago lanceolata*, *D. glomerata* and *H. lanatus*.

Vegetation data were then matched to their community using MATCH programme in the VESPAN package, to determine their community and sequences according to the National Vegetation Classification. Output of the analysis is summarised as a vegetation sequence and is shown as Figure 2.14.

Table 2.11 Newborough Transect Floristic data for site surveys in 1996,1997,and 1998

Spp.		Site 0			Site 1			Site 2			Site 3			Site 4			Site 5			Site 6			Site 7		Site 8	
NVC	Species Name	1996	1997	1998	1996	1997	1998	1996	1997	1998	1996	1997	1998	1996	1997	1998	1996	1997	1998	1996	1997	1998	1997	1988	1997	1998
576	Festuca rubra				7	6	7	6	6	6	7	5	6	8	6	4	7	7	7	5	5	5	5	5	8	8
159	Ammophila arenaria	9	9	9	9	9	9	9	8	8	7	7	7	7	7	6	4	4	4							
768	Leontodon autumnalis				4	2		4	3	4	4	3	3	4	3	1	4	2	2	4		2			2	3
1179	Salix repens agg.							4	0	3	4	2	3			3	2	3	3	1	3	3	3	2	5	2
1136	Rubus fruticosus agg.		5	5	5	7	6	4	6	6	5	7	7	1	3		4	5	6							
680	Holcus lanatus							1	1	2				3	3	1	4	4	4	4	4	3		4	4	5
914	Ononis repens				4	3	1	4		3	4	3	2	1	3	2										
1422	Viola canina							1						1	1	1	1	1	1	2	1				3	3
104	Achillea millefolium																2	3	3	3	5	4	2	3	4	5
304	Carex arenaria				1	0					1		1	3	3					5	4	4				
197	Arrhenatherum elatius																			4	5	5	9	9	5	7
415	Cirsium arvense																1	1	2		1	2	1	1		
123	Agrostis capillaris																5	2	2	5	4	4				
465	Dactylis glomerata																	4	3				6	6	4	6
613	Galium verum																	3	2		5	4			5	4
1239	Senecio jacobaea																3	2	2	3	3	2				
1432	Viola tricolor								1			2	2					2	1					1		
1095	Ranunculus repens																4	2	1						1	2
1396	Veronica chamaedrys																4	2	2	3	3					
383	Cerastium glomeratum													1			4	2		2						
445	Crataegus monogyna (s)							1									4		2	1						
973	Plantago lanceolata													1						1		1				4
2982	Taraxacum seedling/sp																	4			4	1			1	
174	Anthyllis vulneraria													3	3	8										
807	Luzula campestris													2						2		2				
965	Hieracium pilosella group													3	1					1						
251	Briza media																								5	5
323	Carex flacca																			1		1				
362	Carlina vulgaris										2			1												
371	Centaurea nigra																								2	2
674	Hieracium vulgatum group										3		1													
718	Jasione montana											3			4											
788	Listera ovata																								2	2
800	Lotus corniculatus											2	2													
1043	Potentilla anserina																								2	2
1139	Rumex acetosa																						3	3		
1271	Sonchus arvensis														2	2										
1333	Thymus praecox arcticus													3	2											
1349	Trifolium pratense																								4	4
105	Achillea ptarmica																						3			
298	Carduus acanthoides														2											
460	Cynosurus cristatus																			4						
565	Euphorbia paralias				1																					
924	Ornithogalum umbellatum								3																	
1081	Ranunculus acris															2										
1227	Sedum anglicum													2												
1429	Viola riviniana																						3			
	Number of species per sample	1	2	2	7	6	4	9	8	7	9	9	10	16	14	10	15	18	17	18	13	15	9	9	16	16

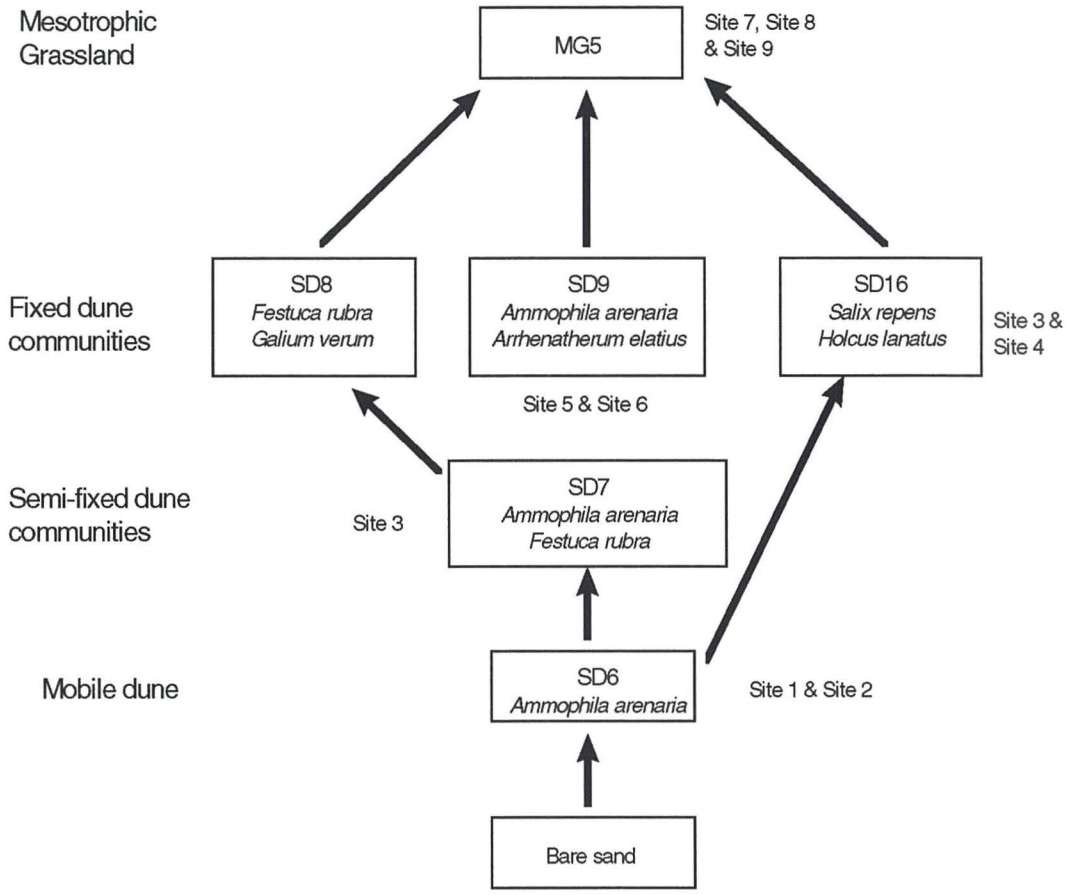


Figure 2.14 Vegetation sequence at Newborough NNR after analysing the species scores using MATCH programme in the VESPAN package.

Analysis of ordination technique using DECORANA on Newborough transect sites is shown as Figure 2.15 and Figure 2.16. In this analysis, the presence or absence data were not transformed before entering the analysis, but rare species were downweighted. This has been observed by Gauch (1982) that ordinations by DECORANA are quite sensitive to the effects of rare species. The first four DCA-axes were extracted and both the sites and species scores were plotted in two-dimensional graphs to allow manageable representation of the ordination.

Figure 2.15 shows a clear sequence of the sites from Site Ø to Site 8. It is obvious that the vegetation of Site Ø is separated far away from Site 8. Between sampling years, species do not vary obviously, however species in the first survey (1996) of Site Ø and Site 4 (point 1 and 21 respectively) are quite far apart from the two other surveys (1997 and 1998), indicate the presence of semi-fixed dune communities from mobile dune communities. There is a general trend of increasing values of the site scores along this axis from the coastal sites towards the inland sites. The low scores for the sites situated near the shore indicate a low diversity of species found on the mobile dune area, whereas the highest scores are found in the damp dune slack, which has higher diversity of fixed dune communities. Furthermore, the species scores of mobile and semi-fixed dune communities were represented on the left side of Figure 2.16. These include *Ammophila arenaria* (Point 4), *Ornithogalum umbellatum* (Point 29), *Euphorbia paralias* (Point 18) and *Lotus corniculatus* (Point 26). On the right side of Figure 2.16, groups of fixed dune communities and mesotrophic grassland species are present and they are listed in Table 2.13.

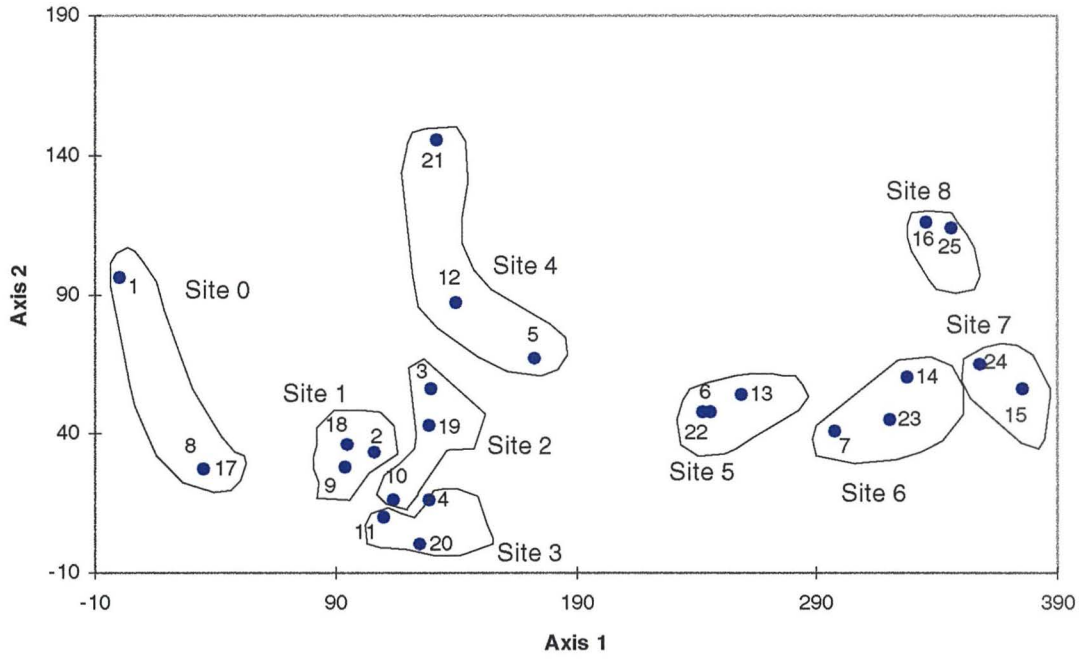


Figure 2.15 DECORANA on Newborough Transect Sites. Numbers indicate year of survey listed in Table 2.12.

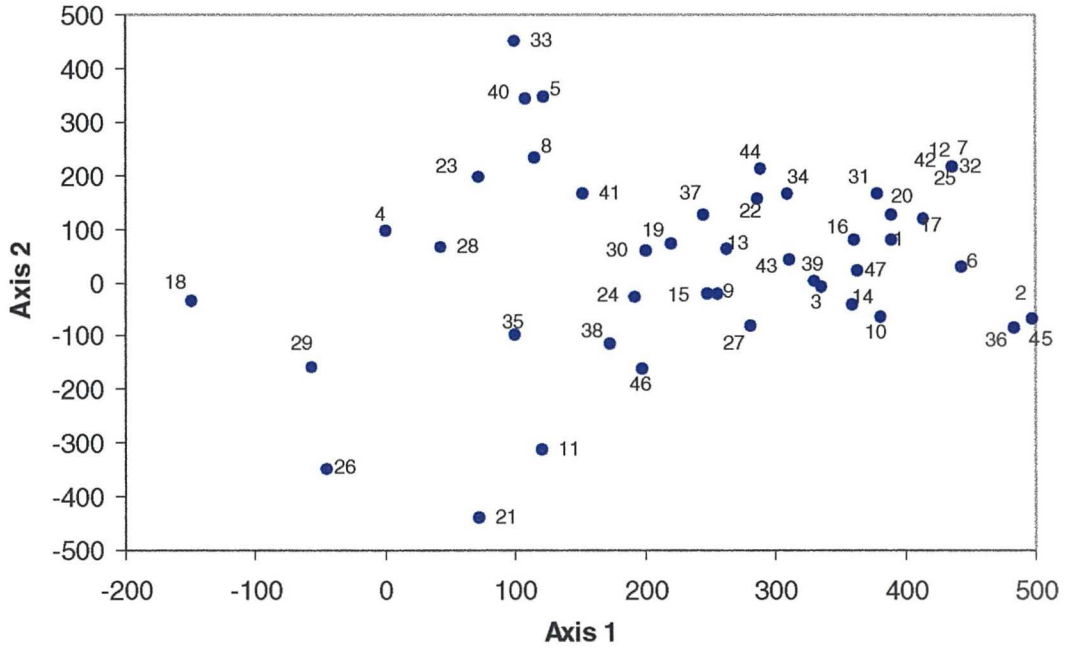


Figure 2.16 DECORANA on species of Newborough Transect Sites. Numbers represent species listed in Table 2.13.

Using CANOCO programme for Canonical Correspondence Analysis, the species-environment biplot is presented (Figure 2.17). The points represent individual species in which some of the species points are overlapped with each other, therefore it is difficult to visualise all the species points. An arrow representing each environmental variable is plotted pointing in the direction of maximum change of the environmental variable across the diagram. The length of the arrow is proportional to the magnitude of change in that direction; and for interpretation purposes, each arrow can also be extended backwards through the central origin. Those environmental factors that have long arrows are more closely correlated in the ordination than those with short arrows and are much more important in influencing community variation. A point corresponding to an individual species can be related to each arrow representing an environmental factor by drawing a perpendicular from the line of the arrow up to the point representing the species. The order in which the points project on to the arrow from the tip of the arrow downwards through the origin is an indication of the position of the species in relation to the environmental factor. Species with their perpendicular projections near to or beyond the tip of the arrow will be strongly positively correlated with, and influenced by the arrow. Those at the opposite end will be less strongly affected (ter Braak, 1987).

The species-environment biplot for the Newborough data (Figure 2.17) shows the species and community distribution very clearly, with various groupings of species emerging. Species of mobile dune and semi-fixed dune communities (*Ammophila arenaria, Rubus fruticosus, Festuca rubra, Hieracium vulgatum, Ononis repens,* etc.) are shown to the right of the plot. Fixed dune communities and mesotrophic grassland communities are shown to the left of the plot. These

Table 2.12 *Number of points in Figure 2.15 represent the sites and the survey year.*

No.	Site	Survey year		No.	Site	Survey year
1	Ø	1996		5	4	1996
8	Ø	1997		12	4	1997
17	Ø	1998		21	4	1998
2	1	1996		6	5	1996
9	1	1997		13	5	1997
18	1	1998		22	5	1998
3	2	1996		7	6	1996
10	2	1997		14	6	1997
19	2	1998		23	6	1998
4	3	1996		15	7	1997
11	3	1997		24	7	1998
20	3	1998		16	8	1997
				25	8	1998

Table 2.13 *List of species recorded on 9 sites at Newborough dune grassland in three-year survey. Numbers represent points in Figures 2.16, 2.17 and 2.19.*

1 *Achillea millefolium*
2 *Achillea ptarmica*
3 *Agrostis capillaris*
4 *Ammophila arenaria*
5 *Anthyllis vulneraria*
6 *Arrhenatherum elatius*
7 *Briza media*
8 *Carduus acanthoides*
9 *Carex arenaria*
10 *Carex flacca*
11 *Carlina vulgaris*
12 *Centaurea nigra*
13 *Cerastium glomeratum*
14 *Cirsium arvense*
15 *Crataegus monogyna*
16 *Cynosurus cristatus*
17 *Dactylis glomerata*
18 *Euphorbia paralias*
19 *Festuca rubra*
20 *Galium verum*
21 *Hieracium vulgatum*
22 *Holcus lanatus*
23 *Jasione montana*
24 *Leontodon autumnalis*
25 *Listera ovata*
26 *Lotus corniculatus*
27 *Luzula campestris*
28 *Ononis repens*
29 *Ornithogalum umbellatum*
30 *Hieracium pilosella*
31 *Plantago lanceolata*
32 *Potentilla anserina*
33 *Ranunculus acris*
34 *Ranunculus repens*
35 *Rubus fruticosus*
36 *Rumex acetosa*
37 *Salix repens*
38 *Sedum anglicum*
39 *Senecio jacobea*
40 *Sonchus arvensis*
41 *Thymus praecox arcticus*
42 *Trifolium pratense*
43 *Veronica chamaedrys*
44 *Viola canina*
45 *Viola riviniana*
46 *Viola tricolor*
47 *Taraxacum spp.*

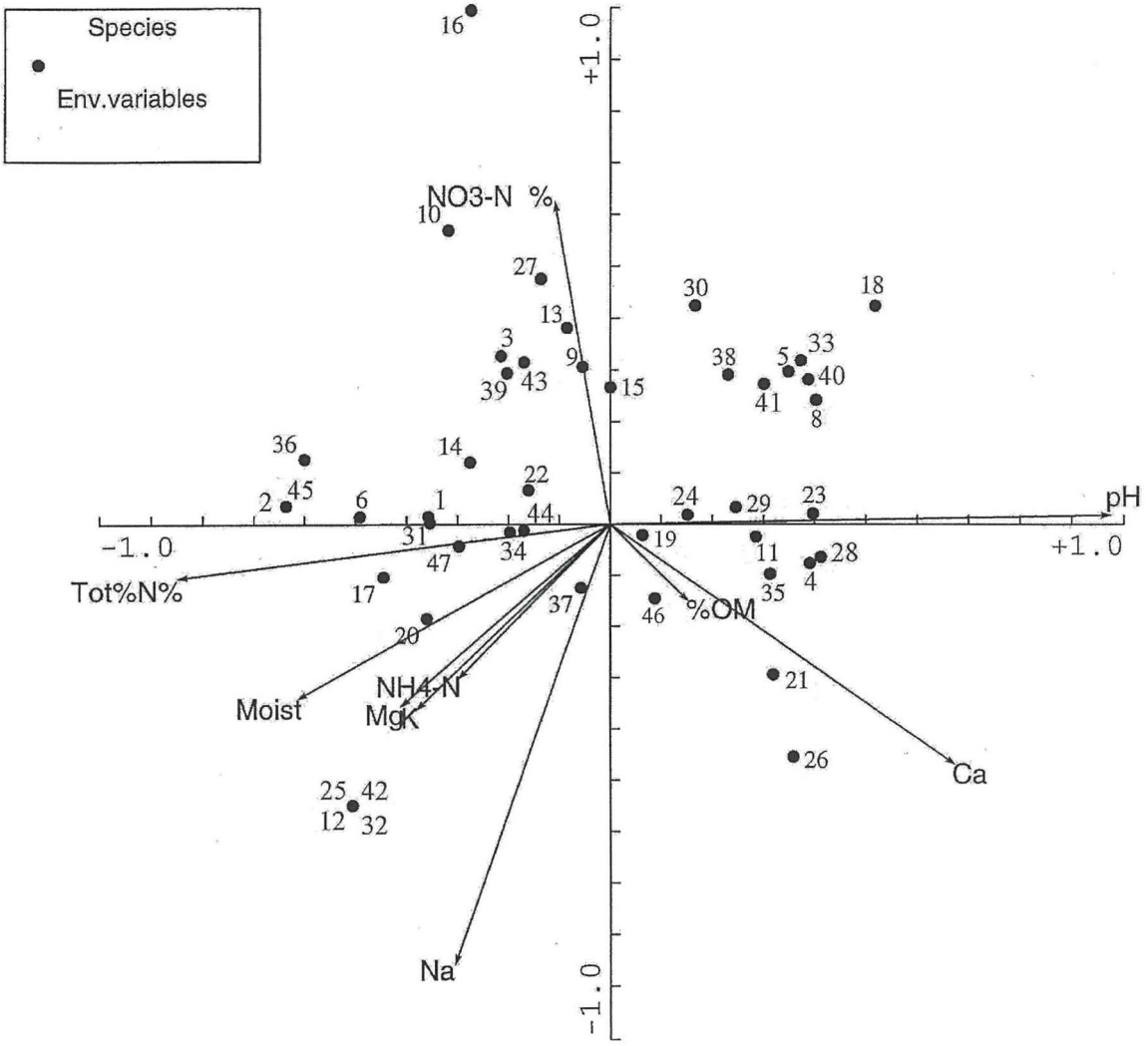


Figure 2.17 Canonical correspondence ordination of dune vegetation at Newborough Warren, Anglesey, North Wales, showing correlation between species and environmental variables. Each point represents the weighted average of each species listed in Table 2.13

include *Arrhenatherum elatius, Holcus lanatus, Achillea millefolium, Plantago lanceolata, Dactylis glomerata,* etc. The groupings and the individual species are clearly shown in relation to the arrows representing environmental factors and gradients. The mobile and semi-fixed dune species are shown to occur at soil with higher pH and calcium. The horizontal axis is most strongly influenced by total N on the left and soil pH on the right. The species *Festuca rubra, Leontodon autumnalis, Ornithogalum umbellatum, Carlina vulgaris* and *Jasione montana* are very closely related to soil pH by which they were present along the pH axis. The effects of total soil N was illustrated with existence of *Dactylis glomerata, Taraxacum* spp., *Ranunculus repens, Viola canina, Holcus lanatus, Achillea millefolium, Achillea ptarmica, Viola riviniana* and *Rumex acetosa.* The NO_3-N axis is in the opposite direction to the NH_4-N axis perhaps indicating that several species are closely related to the former axis. These include *Luzula campestris, Cerastium glomeratum, Carex arenaria Crataegus monogyna, Agrostis capillaris, Senecio jacobea* and *Veronica chamaedrys.* As a whole, a large number of species occur at the arrows of total N (Tot%N%) and NO_3-N, and these are related with the nitrogen availability in the soil. It is also found that *Galium verum* (Point 20) is very influenced by moisture content of the soil. Other environmental variables that are strongly correlated to other species are sodium and calcium. *Salix repens* are seen closely related to sodium whereas *Hieracium vulgatum* and *Festuca rubra* are related to calcium.

In addition, another biplots of the sites and environmental variables (Figure 2.18), and sites and species (Figure 2.19) were also plotted. Figure 2.18 describes the relationships of each site in each sampling year with their environmental variables. It is obvious that the coastal sites are very closely related to the higher

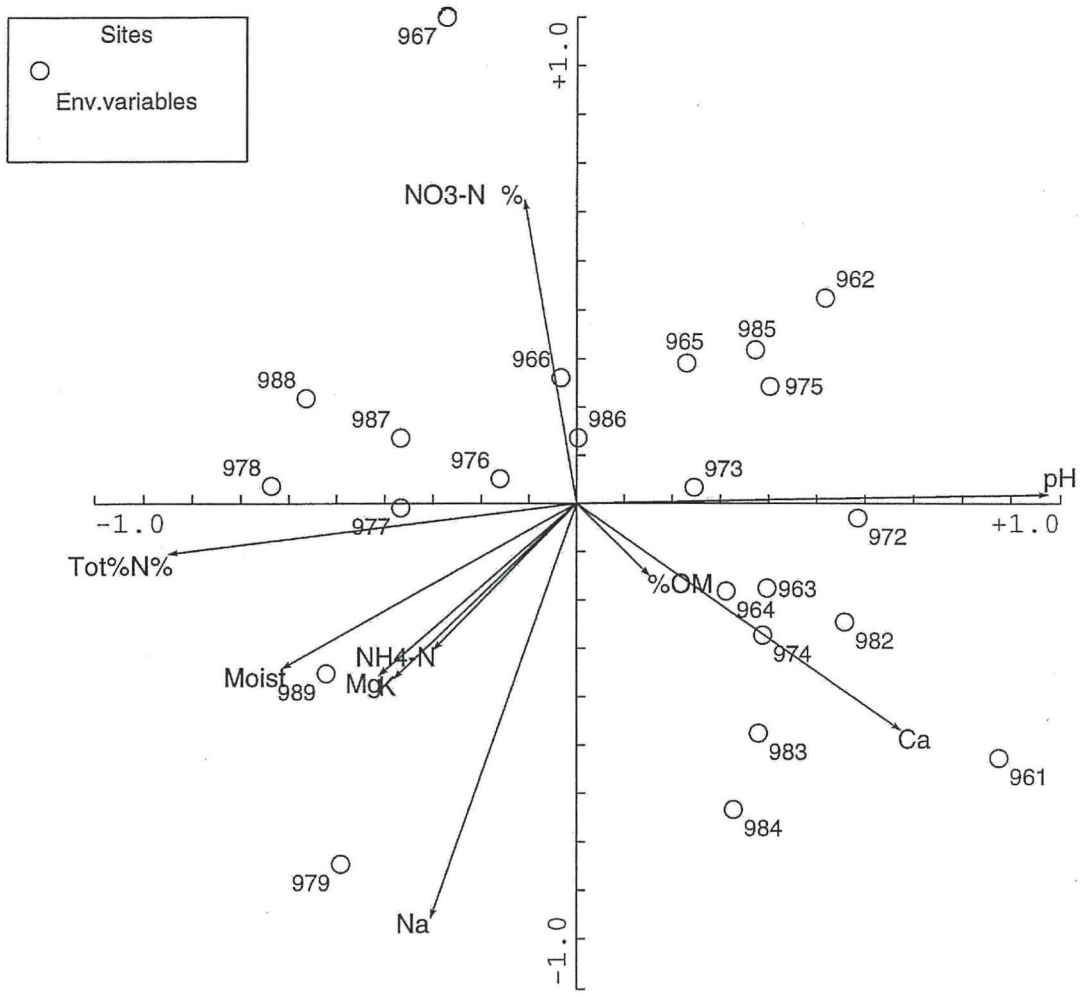


Figure 2.18 Canonical correspondence ordination of all survey sites of each sampling year, showing correlation with environmental variables. First two numbers of labels represent sampling years and the last number represents site number.

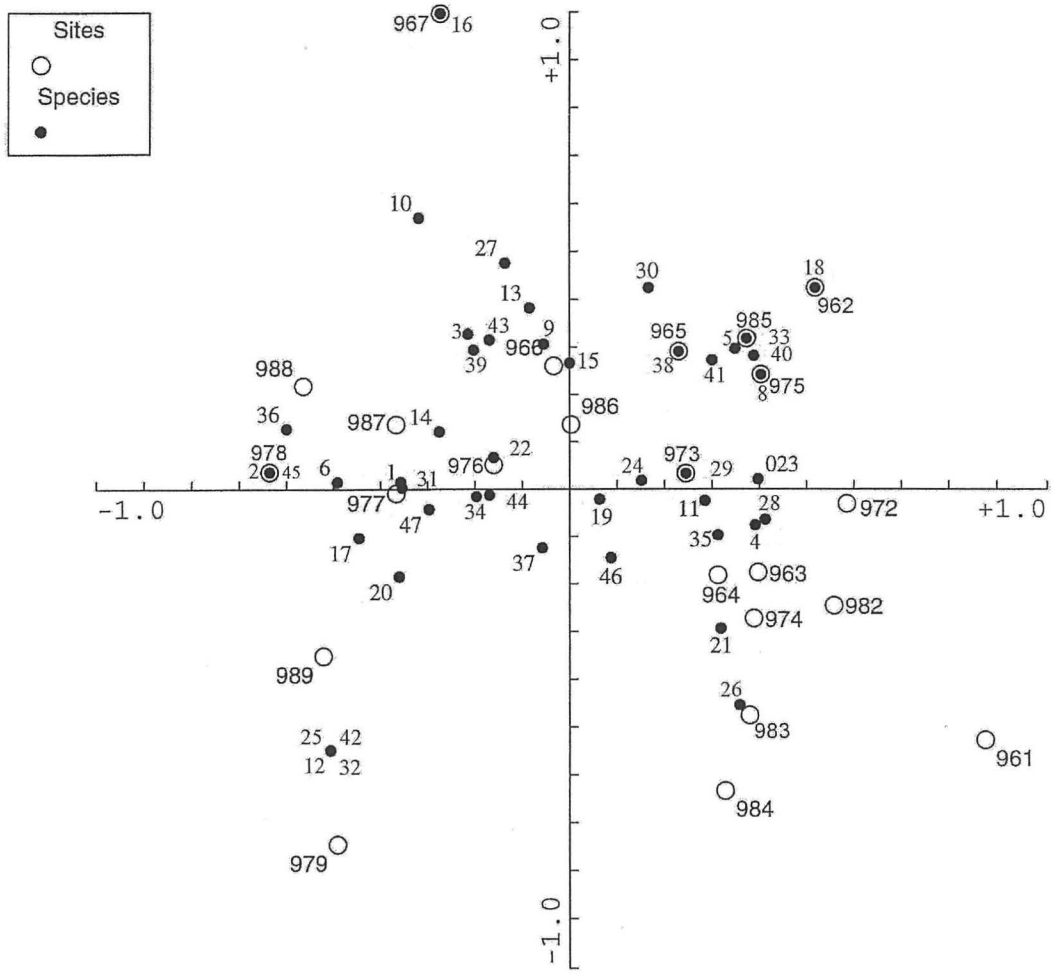


Figure 2.19 Canonical correspondence ordination of all survey sites of each sampling year with species present on the sites. Species numbers are listed as Table 2.13

pH and calcium, which are shown on the right side of the graph. The inland sites are seen to have close relationships with the total N (labelled as Tot%N%), NO_3-N, Na and moisture, represented on the left side of the graph. It is also clearly shown by the Figure 2.18 that the NO_3-N is more dominant in the dry area compared to the NH_4-N that dominantly in the dune slack, indicated by close relationship with the soil moisture.

One may question why the percentage of organic matter seems separated from total N, these two variables showed a positive correlation if we refer correlation matrix in Table 2.7. This phenomenon may be partly due to the coverage of survey which was pretty small, hence the organic matter (OM) was located at that position. Moreover multivariate analysis is a three-dimensional analysis, however the output is illustrated as a compressed two-dimensional plot resulting one could not know whether the position of the OM presumably in the other dimension or axis of the graph. The sites and species biplot (Figure 2.19) indicates a similar interpretation with DECORANA outputs (Figure 2.15 and Figure 2.16), though location of the points are different compared to the DECORANA results. The coastal sites with mobile and semi-fixed dune communities were located on the right side of Figure 2.19, and the inland sites with fixed dune and mesotrophic grassland communities were represented on the left side. Again, the trend shown by this Figure indicates a successional sequence of vegetation or an environmental gradient from the coastal sites to the inland sites.

It can be concluded that from these analyses that the plant communities are closely related to the soil properties, which can vary within the communities themselves. Soil pH, organic matter content, and assorted mineral element

concentrations have been shown to vary in some communities by an order of magnitude at spatial scales of 5 m or less (Trangmar *et al.,* 1987), and in number of cases this variation has appeared to be associated with changes in plant species distributions (e.g. Turkington & Harper, 1979; Inouye *et al.,* 1987).

CHAPTER 3: ATMOSPHERIC NITROGEN DIOXIDE

CHAPTER 3

ATMOSPHERIC NITROGEN DIOXIDE

3.0 INTRODUCTION

Nitrogen oxides are emitted into the atmosphere by human activities, primarily generated during the burning of fossil fuels and in the exhaust gases from motor vehicles. It is approximated that 80% of these emissions arise from the oxidation of nitrogen in fuel and the 20% remaining arises by the oxidation of atmospheric nitrogen in the heat of combustion. In the burning of fossil fuels, both nitric oxide (NO) and nitrogen dioxide (NO_2) are formed, in which NO predominates the formation in the heat of combustion, when atmospheric oxygen and nitrogen combine:

$$\underset{\text{atmospheric}}{N_2 + O_2} \xrightarrow{200\,^{\circ}C} 2NO$$

Upon release into the atmosphere, the NO is fairly reactive and is ready for oxidation to NO_2:

$$\underset{\text{atmospheric}}{2NO + O_2} \longrightarrow 2NO_2$$

It is reported that there has been a steady increase of concentration of NO_x in the recent years in which the emission in UK was estimated as 2.73 million tonnes of oxides of nitrogen (expressed as NO_2) (UK-PORG, 1990). Moreover, there are also substantial biological sources of NO_x (for example bacteria action) and these account for about half of the total NO_2 in the atmosphere (Ashenden, 1991).

Once NO_2 is present in the atmosphere, it may remain as a gas or becomes dissolved in atmospheric moisture to form nitric acid (HNO_3). In this form, the pollutant may become incorporated into clouds and transferred large distances before being deposited through rain, hail or snow.

Unlike sulphur dioxide that received much attention in previous years, there have been few measurements made of ambient concentrations of NO_2 except in city centres. Indeed, until the present decade, most scientists working in the pollution field considered NO_2 to be an urban pollutant and of little importance in rural environments (review by Mansfield and Freer-Smith, 1981). A few measurements of nitrogen oxides have also been made at individual rural sites (Goldsmith, 1986; Martin and Barber, 1984; Ashenden and Edge, 1995) but no attempt has been made at a comprehensive survey in Britain.

Studies of the effects of NO_2 alone on arable crops have usually involved concentrations higher than those found in the UK ambient air, often resulting in acute effects. Vegetation can behave as a sink for NO_2, which is reported can be either phytotoxic (Taylor and Eaton, 1966) or beneficial to plant growth (Troiano and Leone, 1977), depending on the dosage the plants receive. At low exposure concentrations, NO_2 has been shown to act as an extra source of nitrogen (Troiano and Leone 1977) whereby it can react in extracellular water to form nitrite and nitrate ions (Zeevaart 1976). These compounds can be used by the plant in some of the normal reactions of nitrate metabolism and some of the enzymes involved in nitrogen assimilation have been shown to be affected by NO_2 exposure (Zeevaart 1974). In this way, nitrogen derived from NO_2 is assimilated into nitrogenous compounds and may disturb or enhance the nitrogen

metabolism. Moreover, Troiano and Leone (1977) also found that increases in the total nitrogen content in plants could also occur during exposure to NO_2. However, decreased plant biomass have also been reported by Ashenden *et al.* (1997) who conducted a study on effects of atmospheric oxides of nitrogen on *Deschampsia flexuosa* plants. They observed a decline in the biomass of the plants exposed to increasing NO_2 concentrations compared to control plants, although these differences were not statistically significant.

The uptake of NO_2 by vegetation via the stomata has been suggested in several publications (Rogers *et al.*, 1979; Hanson & Lindberg, 1991; Stulen *et al.*, 1998). The NO_2 molecule is not very water soluble (both NO and NO_2 are likely to be significantly lipid-soluble (Cape, 1998)), but reacts rapidly with apoplast components to form NO_3^- and NO_2^- (Lee & Schwartz, 1981). NO_2^- can also be formed in the apoplast after plant exposure to NO (Lea *et al.*, 1994). It is therefore a reasonable suggestion that atmospheric NO_2 could be made available for plant uptake and perhaps even as a valuable source of inorganic N to enhance plant growth.

Due to its significant importance as reported in literature, a survey to measure the concentration of atomspheric nitrogen dioxide at Newborough was conducted, subsequently to determine whether the concentration would give any impact to Newborough dune system. The survey described here is for a period of 18 months to determine concentration of atmospheric nitrogen dioxide in the survey area.

3.1 MATERIAL AND METHODS

Sampling method was by means of simple diffusion tubes originally developed in America as personal air samplers by Palmes *et al.* (1976). Detail procedures are described by Ashenden and Edge (1995), and described in subsequent sections.

3.1.1 Construction of Tubes

The diffusion tubes were made of acrylic tube and fitted with one blue and one natural airtight polythene end-cap. These tubes were supplied by Gradco International Limited of Winchester, UK. Before use, the tubes and end-caps were washed in dilute Decon 90 detergent solution in water, left over-night in 5% hydrochloric acid and thoroughly rinsed several times in tap water, followed by several rinses with distilled water. Excess water was shaken off, then all tubes were left to dry in an oven at 70°C. Ten tubes were measured using Vernier callipers to give the following mean dimesions for calculation of NO_2 concentrations in later sections.

Length	7.2 cm
Internal diameter	1.12 cm
Area	0.99 cm^2
Wall thickness	0.15 cm

Discs were cut from 34 gauge stainless steel woven wire of mesh 0.224 mm to fit tightly inside the blue end-caps. They were acid washed and dried before soaking in 50% v/v triethanolamine (TEA)/acetone solution. Using clean forceps, the discs were taken out from the TEA solution and placed on filter papers to allow the acetone to evaporate and thus leave a fine coating of TEA. The TEA is highly efficient in the absorption of NO_2 and a concentration of nearly zero is

maintained at the surface of the collectors. Next, three prepared discs were placed inside the blue polythene end caps and these were re-fitted to the diffusion tubes. It is important that prepared discs are placed inside coloured end-caps rather than natural ones to prevent possible light-induced reactions, which may affect pollutant adsorption. The prepared diffusion tubes with both end-caps in place are packed in sealed polythene bags; this effectively ensures that blank tube values remain at under 0.1 μg nitrite, even after 3 months storage.

3.1.2 Sampling Procedure

Sampling tubes were installed away from obvious obstructions that could shield them from air streams from any direction, or trees, which could filter pollutants from the air being sampled. Wooden stakes of 1.5 m high, fitted with metal crossbars supporting spring-clips, were used for installing the tubes. To avoid any possible influence of vegetation or surface unevenness, tubes were mounted at about 1.2 m above the vegetation surface.

During sampling, the natural-coloured polythene end-caps were removed and the tubes were mounted vertically, with the open end pointing downwards to prevent the entry of rain and dust particles (Figure 3.1). Tubes were replicated three times so that possible damage to the tubes during exposure or in transit to the laboratory would not result in a loss of data, and thus possible faults in analytical techniques would be easily detected. After 4-5 weeks sampling period, all tubes were collected. During this procedure the open ends were re-sealed with polythene-end caps and the tubes again sealed in polythene bags prior to storing for analysis

Figure 3.1 Diffusion tubes for measuring atmospheric NO_2

3.1.3 Sampling Site

Sampling posts were erected at 8 sites across the dune (refer Table 1.2) with each post at each site. At Site 8 in the mowing enclosure, 3 posts were installed with each post located at several metres distance and facing different directions, i.e. southwest, southeast and northeast. This would then be true replicates by which analysis of the data of this site would be done separately. This is to determine any differences between tubes and whether erecting the masts facing different direction could give any differences. Sampling period lasted for 18 months starting from January 1997 until June 1998.

3.1.4 Laboratory Analysis

Nitrogen dioxide absorbed by the collectors was determined colorimetrically as NO_2^- using a variation on the Griess (diazotization) method. The nitrite ion is

used to diazotize sulphanilamide in orthophosporic acid solution. The diazonium salt is coupled with N-1-naphthyl-ethylene-diamine (NEDA) to give a purple-red azo dye whose absorbance is measured at 520 nm on a spectrophotometer calibrated with known concentrations of NO_2^-.

Reagents for the analysis were of sulphanilamide and NEDA. They were prepared as:

i) 10 g sulphanilamide was added to 25 ml concentrated orthophosphoric acid (H_3PO_4) and diluted to 500 ml with distilled water in a 500 ml volumetric flask.

ii) 0.14 g NEDA was diluted to 100 ml distilled water in a 100 ml volumetric flask.

For the tube analyses, the reagents were mixed in the proportion of 1 part distilled water: 1 part reagent (i) : one tenth part reagent (ii). For 100 samples, 100 ml sulphanilamide reagent was added mixed with 100 ml distilled water and 10 ml NEDA reagent.

A range of standard solutions for spectrophotometer calibration was prepared using analar grade sodium nitrite. The standards used were 0.25, 0.75 and 1.25 μg NO_2^--N per ml. To prepare the sodium nitrite standard solution, 0.9375 g $NaNO_2$ was weighed accurately and dissolved with 250 ml distilled water in a 250 ml volumetric flask. 2.5 ml of the $NaNO_2$ solution was then diluted to 250 ml distilled water and the standard solution was ready for dilution to appropriate concentrations. 1.0 ml, 3.0 ml and 5.0 ml standard solution was diluted in 100 ml volumetric flasks to prepare the standards of 0.25, 0.75 and 1.25 μg NO_2^--N per ml respectively. Final standards were mixed with reagent in calibration cuvettes, and they are summarised as Table 3.1. The cuvettes were agitated and left for

30 minutes for colour development. Then, the spectrophotometer was calibrated using these standards.

Table 3.1 *Volume of final standards and reagent for spectrophotometer calibration.*

Cuvette	Distilled H_2O (ml)	Reagent (sulphanilamide + NEDA) (ml)	Final Standards (ml)		
			0.25 µg NO_2-N	0.75 µg NO_2-N	1.25 µg NO_2-N
Blank	1.0	1.1			
0	2.1				
0.25 µg NO_2-N		1.1	1.0		
0.75 µg NO_2-N		1.1		1.0	
1.25 µg NO_2-N		1.1			1.0

For the samples, 2.1 ml aliquot of the mixed reagent was added directly to each sample tube at the opposite end to the mesh collectors; the tubes were then re-sealed and agitated. After 10 minutes they were shaken again and then left for a further 10 minutes for the reaction to proceed. Samples were then transferred to cuvettes and absorbance was measured at 520 nm, against blanks, on the spectrophotometer.

3.1.5 Calculations

The concentration of nitrogen dioxide in the air was calculated using formula described by Ashenden *et al.,* (1992). The theoretical basis for the diffusion tube method of sampling depends on Fick's first law. The equation to describe unidirectional diffusion of a gas 'a' and 'b' under conditions of constant temperature is

$$F_a = -D_{ab}\frac{dc_a}{dz}$$

where F_a = flux of a (moles.cm^{-2}.sec^{-1});

D_{ab} = diffusion coefficient of 'a' through 'b' (cm^2.sec^{-1})

c_a = concentration of 'a' in 'b' (moles.cm^{-3})

z = distance measured in the direction of diffusion (cm)

The flux is negative because it is in the direction of decreasing concentration. For a tube of length 7.2 cm and cross-section area of 0.99 cm^2 and a concentration gradient between its end (C_1 - C_2 moles.cm^{-3}) the equation becomes:

$$Q_a = F_a 0.99t = \frac{-D_{ab}(C_1 - C_2)0.99t}{7.2}$$

where

Q_a = the quantity of gas 'a' transferred in t seconds. Then if the concentration at one end of the tube is maintained at zero by an efficient absorbant (i.e. TEA):

$$Q_a = \frac{-D_{ab}C_1 0.99t}{7.2}$$

By substituting a diffusion coefficient for NO_2 in air of 0.154 cm^3.sec^{-1} (Palmes *et al.*, 1976), letting C_1 = 1 ppm = 0.0416 x 10^{-9} g.moles NO_2 cm^{-3} (at STP) and t = 1 h = 3600 seconds:

$$Q_a = \frac{0.154 \times 0.0416 \times 10^{-9} \times 0.99 \times 3600}{7.2}$$

Thus Q_a = 3.171 x 10^{-9} g.moles.ppm^{-1}.h^{-1}

The chemical analysis determines the quantity of NO_2^- in µg.

Now 1 nanomole NO_2 = 46 x 10^{-9} g

3.171 nanomoles = 46 x 3.171 x 10^{-9} g

= 0.146 µg

Hence $Q = 0.146\ \mu g\ NO_2.ppm^{-1}.h^{-1}$

$$= \frac{1000 \times 1}{0.146}\ \mu g\ NO_2.ppb^{-1}.h^{-1}$$

$$= 6849.32\ \mu g\ NO_2.ppb^{-1}.h^{-1}$$

Thus to determine concentration of NO_2 in air during a known period of sampling in which x µg NO_2 have been collected:

$$ppb\,NO_2 = \frac{6849.32\ x}{hours}$$

Readings of spectrophotometer for each replicate tube were averaged prior to calculating in ppb unit. All results are expressed in parts per billion by volume (ppb). For the purpose of comparison with other studies in the discussion section, the following conversions may be used:

$$1\ ppb \equiv 1\ nl\ l^{-1} \approx 1.91\ \mu g\ m^{-3}\ at\ STP.$$

3.1.6 Data Analysis

Statistical analysis was carried out using MINITAB programme. Data were analysed using ANOVA in General Linear Model to test significance between concentration at survey sites across the dune, and also between sampling periods. Regression analysis was conducted to determine any relationship

between concentration and sites. Furthermore, data of Site 8 (mowing enclosure) were analysed separately. This included analysis of each tube to test any difference between tubes and whether different location of posts within site would give any effects.

3.2 RESULTS

Monthly mean levels of NO_2 for each of site were averaged to represent monthly mean of NO_2 concentration at Newborough study area. Sampling periods for each months were varied, with most of the tubes were exposed for 28-32 days. Monthly mean concentrations of NO_2 for all 8 sites of the survey are illustrated as Figure 3.2. Full monthly data set at each survey sites are as Table 3.2.

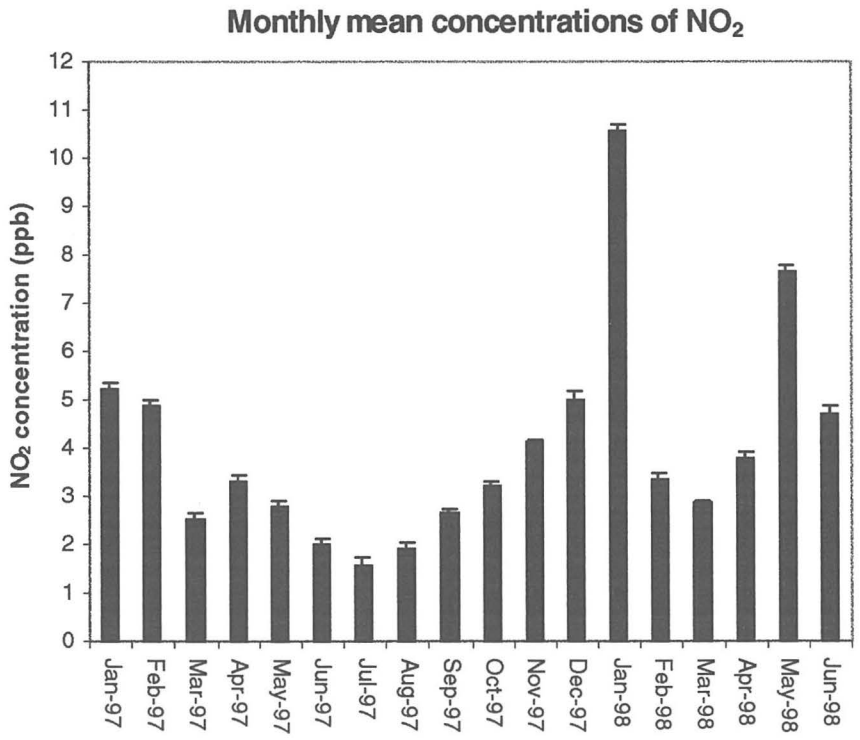


Figure 3.2 Mean concentration of atmospheric nitrogen dioxide of different months for 18-month survey.

It is apparent from Figure 3.2 that concentrations of atmospheric NO_2 changed substantially between months. Analysis of Variance on NO_2 concentrations between months revealed a very highly significant difference ($p<0.001$).

Table 3.2 *Sample mean concentrations of NO_2 (ppb) at Newborough for 18-month period. Concentration of each site is an average of three replicate tubes. Site 8 has nine replicate tubes with three different post locations.*

Site	1997												1998					
	Jan	Feb	Mar	April	May	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	April	May	June
1	4.73	4.72	3.26	3.34	2.61	2.06	2.14	2.03	2.39	2.89	3.65	5.77	11.62	4.01	2.74	3.45	--	--
2	5.58	4.38	2.15	--	--	--	1.52	1.88	2.72	3.28	4.16	4.21	10.79	3.28	2.92	3.64	8.80	5.08
3	5.71	5.17	2.82	3.55	2.99	2.04	1.61	2.28	2.63	3.18	4.05	5.14	11.49	3.87	3.35	3.60	6.90	6.10
4	5.75	4.68	2.52	3.13	2.82	1.52	1.19	1.89	2.65	3.19	4.14	6.56	--	3.83	--	--	--	--
5	5.14	5.06	2.47	2.30	2.61	1.95	1.28	1.66	2.30	2.78	4.16	5.14	11.69	3.35	2.74	3.49	6.16	4.51
6	4.84	4.05	2.25	3.42	2.99	1.76	1.31	1.81	2.84	3.43	4.42	5.03	11.56	3.26	2.96	3.95	7.72	4.87
7	4.87	4.01	2.15	2.88	2.58	1.85	1.61	1.32	2.24	2.70	3.89	4.02	7.23	3.03	2.55	3.28	6.07	4.92
8(1)	4.63	4.72	2.60	3.13	2.33	2.18	1.64	1.91	2.99	3.60	4.23	4.25	10.88	2.65	2.59	4.25	8.00	4.04
8(2)	5.68	5.02	2.47	3.67	2.92	1.85	1.55	2.09	2.75	3.32	4.07	4.86	9.60	3.03	2.88	4.21	8.28	4.22
8(3)	4.33	4.79	2.50	3.72	2.75	2.33	1.52	1.94	2.91	3.51	4.42	5.14	10.26	3.07	2.91	4.37	9.25	3.78
Mean	5.22	4.85	2.53	3.30	2.80	1.99	1.56	1.91	2.66	3.21	4.12	5.01	10.57	3.34	2.85	3.80	7.65	4.69

-- Missing data (tube loss, vandalized)

However, no significant difference was found when ANOVA was conducted on NO_2 concentrations between sites. Regression analyses were also done for concentration in each month between sites, but no apparent trend of NO_2 concentration could be distinguished (see Table 3.2). There were quite a few times that diffusion tubes were changed late from schedule, therefore creating an overlapping in terms of surveying period of each sampling month. Loss or damage to the tubes was variously a problem at sites 1, 2 and 4. During the 18 month-survey, the highest mean concentration of NO_2 was found in January 1998 with mean concentration of 10.57 ppb. Annual mean concentration (January 1997-December 1997) is 3.26 ppb. It is apparent that NO_2 concentrations in winter months (December, January and February 1997 and 1998) showed higher concentration compared to summer months with an exception of concentration in May 1998.

At Site 8 (mowing enclosure) where three posts were erected at different position, a statistical analysis was conducted to determine any differences between the replicate tubes and different post locations within the site. All data at this site are shown in Table 3.3. Coefficient of variation (cv) was calculated for the data from the replicate tubes at each site, and most of them indicate the coefficient of variations between 3-30%. The criterion of acceptability of the data is set at a cv of 30% because variations of field data are normally less than this, any that exceeded 30% cv are normally rejected. Nevertheless, there are several data which cv are exceeded 30%. The ANOVA using the General Linear Model in the MINITAB programme was conducted, overall results are similar to those described before, a highly significant difference was found between period of samplings (Table 3.4). However, the replicate tubes did not show any significant

Table 3.3 *Data on atmospheric NO_2 concentration at Site 8 showing concentration of each replicate tube at three different locations within the site. (Tube location: 1 - facing southwest; 2 - facing southeast; 3 - facing northeast; cv-coefficient of variation).*

Sampling Period	Tube Location	Atmospheric NO_2 concentration (ppb)			cv (%)
		Replicate Tube Number			
		1	2	3	
Jan-97	1	5.63	4.30	3.96	19.06
	2	*	6.09	5.28	10.10
	3	5.99	3.45	3.55	33.17
Feb-97	1	2.92	6.85	4.38	42.12
	2	5.84	4.38	4.83	14.91
	3	6.63	3.82	3.93	33.17
Mar-97	1	2.57	2.19	3.04	16.50
	2	2.47	1.90	3.04	23.08
	3	2.28	2.95	2.28	15.35
Apr-97	1	2.63	3.01	3.76	18.33
	2	4.26	3.63	3.13	15.37
	3	4.26	4.51	2.38	31.32
May-97	1	1.88	2.30	2.82	20.19
	2	3.34	2.82	2.61	12.88
	3	2.19	*	3.03	17.54
June-97	1	1.99	2.28	2.28	7.53
	2	1.85	2.28	1.42	23.08
	3	2.28	2.85	1.85	21.50
July-97	1	1.52	1.70	1.70	6.30
	2	1.61	1.61	*	6.66
	3	1.34	1.52	1.70	11.76
Aug-97	1	1.48	2.21	2.03	20.15
	2	2.12	2.30	1.84	11.10
	3	2.03	*	1.84	6.73
Sep-97	1	2.87	3.05	3.05	3.46
	2	2.60	2.51	3.13	12.35
	3	2.78	*	3.04	6.53
Oct-97	1	3.46	3.68	3.68	3.46
	2	3.13	3.03	3.78	12.35
	3	3.35	*	3.67	6.53
Nov-97	1	4.74	3.86	4.10	10.80
	2	3.94	4.42	3.86	7.47
	3	4.18	*	4.66	7.71
Dec-97	1	4.80	4.13	3.80	12.06
	2	3.80	5.92	*	30.89
	3	5.36	5.36	4.69	7.53
Jan-98	1	13.45	10.71	8.49	22.84
	2	9.40	9.80	12.28	14.85
	3	*	10.19	*	*

Table 3.3 continued

Sampling Period	Tube Location	Atmospheric NO_2 concentration (ppb)			cv (%)
		Replicate Tube Number			
		1	2	3	
Feb-98	1	3.97	1.67	2.30	44.89
	2	4.08	2.40	2.61	30.06
	3	4.39	2.51	2.30	37.55
Mar-98	1	2.16	2.59	3.02	16.67
	2	3.34	2.91	2.37	16.91
	3	3.34	2.70	2.70	12.83
Apr-98	1	4.40	3.71	4.64	11.35
	2	3.94	3.59	5.10	18.73
	3	3.59	4.98	4.52	16.22
May-98	1	6.79	7.12	10.09	22.74
	2	6.62	12.47	5.77	44.04
	3	7.97	10.52	*	19.46
June-98	1	3.74	*	4.34	10.55
	2	4.22	3.50	4.94	17.14
	3	3.62	3.01	4.70	22.64

Notes:
* - Missing data (tube loss, vandalized)

difference and neither did the location of the posts, which are true replicates. Combination of the aforementioned factors was also tested for significance, but none of them showed any significant difference. The Sum of Squares (SS) in the ANOVA table indicates that sampling period contributed 84.80% of the total variance (Table 3.5). Tube replication and post locations represent a similar percentage of variance of 0.10% of the total variance.

Table 3.4 *ANOVA on NO_2 concentration of each replicate tube and different location of the posts at Site 8.*

Source	DF	SS	Adj SS	Adj MS	F	P	
Period	17	662.496	500.396	29.435	21.90	0.000	***
Post location	2	0.760	0.865	0.432	0.32	0.726	NS
Tube No.	2	0.691	0.314	0.157	0.12	0.890	NS
Period*Post location	34	8.116	6.502	0.191	0.14	1.000	NS
Period*Tube No.	34	31.915	32.549	0.957	0.71	0.854	NS
Post loc.*Tube No.	4	1.938	1.938	0.485	0.36	0.836	NS
Error	56	75.268	75.268	1.344			
Total	149	781.183					

***p<0.001; NS = not significant

Table 3.5 *Percentage of variance of all factors for concentration of each replicate tube at Site 8*

Factor	Percentage of variance (%)
Period	84.80
Post position	0.10
Tube No.	0.09
Period*Tube position	1.04
Period*Tube No.	4.08
Post position*Tube No.	0.25
Error	9.64
Total	100

3.2.1 Estimation of annual load of N from NO_2 deposition

Calculating depositions of nitrogen dioxide, ammonia, nitric acid and particulates from air concentrations is quite uncertain because it depends on the use of deposition velocities, whose values vary between vegetation surfaces (Goulding

et al., 1998). For estimation of annual dry deposition of N (F_g) from NO_2 (kg N ha^{-1} y^{-1}) to the area, calculation was done using formula by Sutton and Fowler (1993) below:

$$F_g = -V_d \chi$$

where V_d = Deposition velocity (mm s^{-1})

χ = air concentration (μg m^{-3}).

Negative symbol referred to direction of the flux, i.e. downward flux. As this survey did not have micrometeorological instrumentation to gather data on wind velocity near the ground to which deposition velocity (V_d) data are obtained, therefore an estimation of V_d was used to calculate annual deposition of nitrogen dioxide. Further explanation on the use of deposition velocity is given in discussion section of Chapter 4 (Section 4.4). An estimated V_d of 1.5 mm s^{-1} is used for the calculation here, which is an approximation arrived at by considering values presented in other findings on various surfaces (Table 3.6). Annual mean concentration of 3.26 ppb (≡ 6.23 μg m^{-3}) was used and estimated N deposition from NO_2 is 0.90 kg N ha^{-1} yr^{-1} (calculation is shown in Appendix 3.1).

Table 3.6 *Deposition velocities for NO_2 on various vegetation types from previous studies*

Canopy	Reference	Deposition velocities V_d (mm s^{-1})
Moorland	Fowler *et al.* (1989)	1-2
Arable land	Harrison & Allen (1991)	1
Heathland	Erisman *et al* (1994)	1 to 4
Grass	Yamulki *et al* (1997)	4-7
Winter wheat	Goulding *et al* (1998)	1.2-2.5

3.3 DISCUSSION

In this chapter, atmospheric nitrogen dioxide was monitored to determine its concentration in the Newborough area. As one of the pollutant gases that affects plant communities (Ashenden, 1979; Ashenden *et al.,* 1990), this survey is important to gather information on the level of the gas in Newborugh's atmosphere, subsequently comparing with other findings.

Results from this survey clearly shows the NO_2 concentrations were greater in the winter months compared to summer months (Figure 3.2), with an exception for May 1998, which is higher than other summer months. The typical pattern of atmospheric NO_2 concentration monitored in this study is consistent with greater fuel use and poorer atmospheric dispersion in winter, and greater atmospheric conversion by reaction with OH during the daytime in summer (Atkins and Lee, 1995). The annual mean concentration of NO_2 in this study (from January 1997-December 1997) of 3.26 ppb is slightly lower than the range of annual mean suggested for this area (4-6 ppb) by Ashenden and Edge (1995) (Figure 3.3), who surveyed concentrations of NO_2 pollution in rural Wales. The slight decreased of the annual mean concentration could also be in accord with the prediction by the UK Review Group on Impacts of Atmospheric Nitrogen, which stated that NO_x emissions throughout the UK reached a plateau in 1990 and are forecast to decrease towards the year 2000 (INDITE, 1994).

From Figure 3.2, it seems that NO_2 concentration in the month of May 1998 is remarkably high compared to the other summer months of 1997. It was noted that in this month there were high temperatures and dry conditions (see weather data Figure 2.1 and Figure 2.3). This good weather attracted a large number of

tourists to Newborough beach. This contributed to increased traffic in the Newborough area and it was probably this traffic that caused the higher NO_2 concentration that was recorded in this month. Ashenden and Edge (1995) also observed a similar phenomenon at Llyn Llydaw (Snowdon) in which NO_2 concentration increased during July and August (summer months), and this was associated with greatly increased traffic volumes around Mount Snowdon. It has been suggested that much of the rural NO_x may be derived from the mass of vehicle exhausts in distant towns, with NO_x travel being sustained for up to 100 km in inversion layers at 0-50 m from ground level (Martin and Barber, 1984).

Figure 3.3 Annual mean concentrations of NO_2 in rural areas of Wales for 1992 (from Ashenden and Edge, 1995)

In comparison with other NO_2 surveys in other rural areas in the UK conducted by Campbell (1988), the annual mean of Newborough data (January 1997-December 1997) is greater than annual mean of survey in Northern Ireland, but

less than of East Anglia. Results published by Campbell varied from ~1 µg NO_2 m^{-3} in Northern Ireland to ~7 µg NO_2 m^{-3} in East Anglia. Converting the annual mean of this study of 3.26 ppb to the unit of µg m^{-3} (1 ppb = 1.91 µg m^{-3}) is 6.22 µg m^{-3}. Campbell's surveys also show seasonal variation in NO_2 concentration in which higher values were observed in the winter compared to summer. These data can also be compared with an urban area survey in the UK conducted by Bower *et al.* (1991) in which concentrations in the range of 23 ppb to 39 ppb were reported. These are 8-12 times as large as the Newborough NO_2 concentration.

The method used in this survey is sufficient for monitoring mean rural levels of NO_2, which is cheap and economical, and very suitable for survey in remote areas. Passive diffusion tubes for NO_2 monitoring have been used in many NO_2 surveys (e.g. Campbell, 1988; Campbell *et al.,* 1994; Ashenden & Edge, 1995; Perkauskas & Mikelinskiene, 1998). Cadoff and Hodgeson (1983) describe this sampler as a precise, high-rate passive sampler and they also stated that the device is diffusion controlled and samples at a rate of approximately 110 ml/min. Moreover, it has also been evaluated at two levels of relative humidity and exhibits no interference in the presence of a large excess of NO. However, Campbell *et al.* (1994) demonstrated that weather conditions, in particular wind, has an effect on the passive samplers. They stated that wind turbulence might shorten the diffusion path in the diffusion tube, which leads to a tendency for diffusion tubes to overread relative to chemiluminescent analysers. Nevertheless, they explained that if the tubes are mounted in a sheltered location, the overestimate is small. The sites at Newborough are not sheltered but we do not know whether the results are overestimates or not. Before that, Hargreaves (1989) also examined the possible effects of wind on diffusion tube

performance using wind tunnel studies in which he revealed an over-estimation of 40% at 5 m s^{-1}. The recent criticism on the diffusion-tube technique for nitrogen dioxide came from Heal and Cape (1997) who criticized that this technique overestimate concentrations in urban air by up to 70%, but in rural areas the overestimate was no more than 10%.

Sampling procedure required tubes to be exposed in replicates, three replicates for this survey. This is to reduce possibilities of losing data as a result of interference by animals and vandalism. At the beginning of the survey, several duplicate tubes were lost and data were based on available tube only. Also, a preliminary survey site by the beach (15 m from the shore) was abandoned because it was seriously vandalised by people. I assume that replicating the tubes at each site would reflect *pseudo-replicates* and not true replicates. Therefore, at Site 8 three posts were erected facing different directions, and this represents a *true replicate* of that particular site. It is proven that all these replicates are not statistically different, however they show a varied coefficient of variation reflecting the variability of the data between tubes. It is reported that analyses for duplicate tubes were within 0.5 ppb with greatest discrepancies for longer sampling periods (Ashenden & Edge, 1995). I mentioned that some of the sampling months were delayed in collecting and changing the tubes for 4-7 days from the schedule due to the bad weather. This caused a longer sampling period for those particular months (February 97 and February 98) and shorter sampling periods for the subsequent months (March 97 and March 98). Any effect of this was not apparent, but Jones and Ashenden (1997) noticed an unusual low concentration in one of their survey sites which they attributed to the reason that

the tubes at this site were exposed for a longer period. They stated that diffusion tubes seem to begin to under-sample when exposed for long periods of time.

It is difficult to predict the impact of increasing rural NO_2 concentrations on natural vegetation without long-term monitoring. On a large scale, NO_2 will contribute to soil acidification if nitrogen is in excess of vegetation requirements and the nitrogen critical load is exceeded. In the Newborough case, the level of NO_2 concentrations and the estimated N deposition (0.9 kg N ha^{-1} y^{-1}) are unlikely to contribute much to the N inputs at the area. Nevertheless, Reynolds and Ormerod (1993) showed that sulphur deposition on large area of north and central Wales contributed to soil acidification whereby its critical load is expected to be exceeded in the year 2005. A similar process could be applied to NO_2 in which deposition of NO_2 could accelerate and therefore damage to vegetation would follow. The United Nations Economic Commission for Europe (UNECE) workshop on critical levels at Egham, UK in 1992 concluded that there were insufficient data to separate critical levels of NO_x for different vegetation types. A critical level of 30 µg m^{-3} (about 16 ppb) as an annual mean was proposed for adverse ecophysiological effects on plants, in the presence of SO_2 and O_3. On the basis of this proposal, annual mean NO_2 concentration at Newborough for this study does not exceed this level. However, at an earlier workshop, Guderian (1988) proposed a critical level of only 10 ppb NO_2 as an annual mean, and 20 ppb NO_2 over winter, for vegetation damage. From these proposed critical levels, it can be concluded that NO_2 concentration at Newborough is consistently at a low level, and this perhaps would have no impact on the vegetation at Newborough.

CHAPTER 4:
ATMOSPHERIC AMMONIA

CHAPTER 4

ATMOSPHERIC AMMONIA

4.0 INTRODUCTION

In recent years, the importance of atmospheric ammonia deposition onto vegetation and soils and its impact on ecosystems has become a major concern of numerous scientists and related authorities. Pearson and Stewart (1993) in their review paper, mentioned that early research on the effect of atmospheric pollution on plants and habitats concentrated mainly on the part played by SO_x in forest dieback and soil acidification. As research progressed, NO_x succeeded sulphur pollutants as a further potential cause of damage to vegetation. In early 1980s, van Breeman *et al.* (1982) raised concern about soil acidification in The Netherlands, and a report by van Breeman and van Dijk (1988) showed damage on lowland conifer plantations correlated significantly with emissions of ammonia. Sutton *et al.* (1993) also recognised atmospheric ammonia as an important atmospheric pollutant that not only represents an eutrophication but may also contribute to acidification of ecosystems. Changes in species composition associated with increased N deposition and particularly with NH_3 emissions from intensive agriculture have occurred over much of Europe. The range of affected communities is large and includes lowland, heathlands, forest ground flora, calcareous grasslands, coastal dunes, wetlands and upland moorland (Sutton *et al.*, 1993; Bobbink *et al.*, 1993).

Vegetation change caused by atmospheric N deposition has been shown by the changes of lowland heathlands to grassland. In the Netherlands ericaceous

species such as *Erica tetralix* and *Calluna vulgaris* have been replaced by grasses such as *Molinia caerulea, Deschampsia flexuosa* and *Festuca ovina* (Heil & Diemont, 1983; Aerts *et al.,* 1990). Species typical of nitrogen-rich sites (*Urtica dioica, Chamaenerion angustifolium, Rubus idaeus* and *Oxalis acetosella*) have increased in frequency in the ground flora of various woodlands in Sweden, The Netherlands and Austria (van Breemen & van Dijk, 1988; Tyler, 1987). Pitcairn *et al.* (1991) also found deterioration in *Calluna* in the Breckland of East Anglia in southeast England, which was due to N deposition. Moreover, Marrs *et al.* (1986) reported a reduction in numbers of bryophytes and lichens in long-term monitoring plots at Moor House National Nature Reserve in the northern Pennines over the past 30-40 years, particularly at high altitude sites, also caused by similar factor. Other vegetation changes attributed to atmospheric N inputs have also been recorded in the UK in uplands, woodlands and grasslands (Woodin & Farmer, 1993).

4.0.1 Source of ammonia and its role in the nitrogen cycle

Agriculture is the most important source for ammonia in Europe with ammonia emissions mainly resulting from livestock management, a smaller contribution results from fertilizer application, while industry, power plants, traffic, human excreta and other sources play only a minor role (Fangmeier *et al.,* 1994). In fertilizer application, a large part of fertilizer is applied as ammonium or urea, but if nitrate is incorrectly applied, it can lead to ammonia losses to the atmosphere (Pearson & Stewart, 1993). Fermented feeds such as silage may also have a role to play. Livestock are kept in large concentrated groupings where animal waste generates large amounts of ammonia. In particular, pig, cattle and poultry slurry has a high proportion of ammonia. For example, pig slurry can have a total

nitrogen content of 3-6.7 g N l^{-1}, of which ammonium can comprise 2.4-5.3 g l^{-1}, usually well over 50% of total nitrogen (Sommer *et al.*, 1992). The slurry also tends to have a pH slightly above neutral, which will promote volatilization of the ammonia as gas. Records of pH range from 6.3 to 8.1 for pig and cattle slurry (Sommer *et al.,* 1992), averages being 7.3 for pig and 7.0 for cattle; an even higher average of 8.0 for poultry slurry was recorded by Lockyer *et al.* (1989). Emission of ammonia from slurry is rapid and amounts to a considerable percentage of the total nitrogen applied. Rates depend on climatic factors and the slurry source, but emissions ranged from 40 to 80% of applied ammonia-N in several slurry treatments, and these losses occurred over a matter of a few days (Lockyer *et al.,* 1989).

Ammonia is removed from the atmosphere by both wet deposition (in precipitation) and dry deposition, which is turbulent transfer of the gas to land and water surfaces. Recent estimates of atmospheric budgets of ammonia for the UK suggest that nearly 50% of the deposition occurs through dry deposition (RGAR 1997). The estimates are recognized as only being approximate, because there are uncertainties both in the national ammonia air concentration survey (Atkins and Lee, 1992; RGAR, 1997) and in the models used for estimating rates of dry deposition (Sutton *et al.,* 1995a; RGAR, 1997). Total NH_3 emission in the UK is therefore uncertain and there is still a significant debate over the magnitude of the different sources, for instance, Lee and Dollard (1994) provides a total emission of approximately 260-370 kt N yr^{-1}, Asman (1992) estimated of 391 kt N yr^{-1} and Sutton *et al.,* (1995b) of 367 kt yr^{-1}. The best estimates therefore suggest a total NH_3 emission in the UK in the range of 260 to 400 kt N yr^{-1} (INDITE, 1994).

Both soil and plant canopies may contribute to background emission of ammonia as long as atmospheric concentrations do not exceed the compensation point, which reflects the ratio of atmospheric concentration and internal NH_3-concentration in the soil or in the plant tissues (Farquhar *et al.*, 1980). Bouwman *et al.* (1997) recently discussed NH_3 emissions from soil in which they suggested that 1% of the nitrogen mineralized in the soil was leaking out into the canopy as NH_3, and that a fraction varying between 0.2 and 0.8 of the emitted NH_3 was reabsorbed by the canopy. The importance of ammonia emissions from natural soil is still uncertain. Schjørring (1998) suggested that natural land would probably not be an important NH_3 source, because NH_3 is recycled internally in the vegetation rather than released to the atmosphere. As soils and plant canopies may absorb NH_3, they may act both as sink or sources for ammonia. Denmead *et al.* (1976) reported a study on direct absorption of NH_3 from the atmosphere by foliage agricultural crops in which they found large losses of NH_3 from soils of highly productive grasslands of Australia was absorbed by foliage as the gas diffused through the plant canopy. The studies discussed agree with the suggestion that plants may be natural sinks for atmospheric NH_3 and, furthermore amounts of N received via this pathway may constitute a significant portion of plant intake. Before Denmead *et al*'s finding, Hanawalt (1969) reported that soils are capable of absorbing NH_3 from the atmosphere. His studies conducted in cultivated fields in New Jersey revealed that absorption was dependent on the nitrogen content of the atmosphere, soil type, temperature, and the velocity of air movement across the surface of the soil.

In the soil, nitrification process converts the absorbed ammonia to nitrate, the rate of conversion depends on soil type and climatic factors (Pearson & Stewart,

1993). This can be responsible for a substantial portion of soil acidity. However, it has been reported that excessive deposition of ammonia would inhibit ammonia nitrification in the soils. This was shown by Olsthoorn *et al.* (1991) who found that in pots nitrification rates were saturated at annual ammonia supplies somewhere between 17 and 140 kg ha^{-1}. When the supply was as high as 340 kg ha^{-1}, soil nitrate levels remained similar to the 140 kg ha^{-1} treatment. Furthermore, in soils that have low capacity for nitrification, ammonia may be found in soil solution and eutrophication may occur (Duckworth & Cresser, 1991). In these instances, ammonia may be leached from the soil profile and be found in run-off; but this occurs infrequently and ammonia leaching is not as common as nitrate leaching (Schulze *et al.,* 1989). This phenomenon is true for uncontaminated seminatural systems but not in the systems receiving effluent from sewage works, landfill sites, etc. At these sites, ammonium is commonly found in the run-off water.

In seminatural sites ammonium leaching is not as common as nitrate leaching because the ammonium ion is much more tightly held by the negative charges of soil colloids, but as the nitrate (NO_3^-) ion is negatively charged it will not be retained by ion exchange in the same way as NH_4^+. Stevens *et al.* (1990) showed that ammonium leachate in run-off to streams in three Sitka spruce sites in the UK was found to be either negligible or much less than for nitrate.

In general, heathland and chalk grassland have been shown to be able to accumulate excess nitrogen from atmospheric inputs, a large component being incorporated into the organic nitrogen pool (van Breeman & van Dijk, 1988).

Thus, ammonia has a direct effect on soil nitrogen cycling processes, and also an indirect consequence through acidification.

Because of the importance of ammonia as a source of atmospheric N to the ecosystems and the importance of intensive poultry units as the point sources, hence main objectives of the ammonia survey at Newborough are:

i. to measure ammonia concentrations in the air at various distances from the poultry farm
ii. to investigate the importance of ammonia deposition to the Newborough dune system.

4.1 MATERIAL AND METHODS

4.1.1 The Survey Area

The poultry farm (Figure 4.1) is situated 500 m north-east of Newborough dune system at an altitude of 15 m. This farm is privately owned and consists of 6 houses with 12 sections. It contains approximately 185,000 broilers, grown on a 60-65 day cycle. Using an emission factor for poultry from housing of 0.04 kg N $animal^{-1}$ yr^{-1} (Sutton *et al.,* 1995b), NH_3 emission from this farm is estimated in the region of 7400 kg N yr^{-1}. Waste is removed from the farm by contractors at 7 week intervals. The surrounding area of the farm is agricultural land, which contains sheep and ponies grazing. These grazing animals are also contributing to the concentration of atmospheric ammonia in this area.

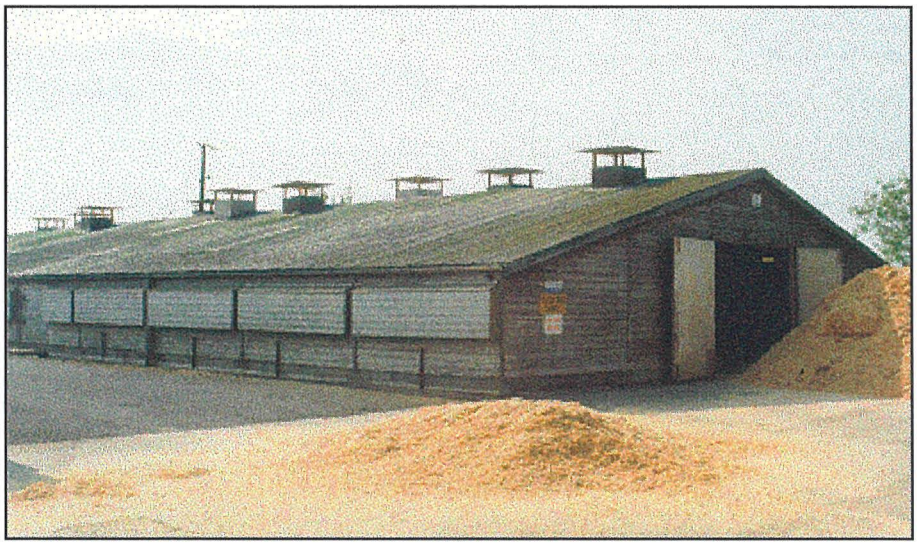


Figure 4.1 The poultry farm at Newborough

Atmospheric ammonia was measured using simple passive diffusion tubes and passive badge samplers. These methods are cheap and particularly suitable for remote sites such as Newborough area, since no electricity supply is required at the site, which is a prerequisite for active sampling methods. The tubes and samplers give a measure that is integrated over time whereby it is not an instantaneous measurement. Initially, six locations at various distances from the poultry farm across the dune were chosen, with Site A located at the farm and Site F located in the dune (see Figure 1.2). These sites were chosen randomly with random spaced and that they were not in the same distance to each other. Masts of 2 m length were erected at 1.5 m above ground on 12/12/97 at chosen locations, facing towards the southwest into prevailing the wind direction.

In addition a set of badge samplers were also put out at Sewage Works site (refer Figure 1.2) to determine concentration of atmospheric ammonia at the site. This is to find out if this potential source was likely to be very large or not. At a later stage of this survey, another three sites northeast of the farm were set up in August 1998 for two months survey period, with a view to obtain an approximate concentration of atmospheric ammonia against prevailing wind. At these three sites, diffusion tubes were used as means of sampling. At the start of the programme, a site at 2000 m from the poultry farm was set up at southeast of the farm rather than southwest direction. This site was located at an intensive agricultural land, namely, Rhuddgaer (Figure 1.2), and sampler used was a set of badge samplers. Sampling at this site was carried out for five months from December 1997 until April 1998. This extra site measured atmospheric ammonia concentration at an intensive agricultural land in the vicinity of Newborough NNR. Summary of the sites and methods of monitoring are given as Table 4.1.

Table 4.1 *Summary of atmospheric ammonia survey sites (see map Fig. 1.2)*

LOCATION	Diffusion Tubes	Badge Samplers
Close to the farm (~20 m) (Site A)	✓	✓
100 m Southwest of the farm (Site B).	✓	✓
300 m Southwest of the farm (Site C)	✓	✓
800 m Southwest of the farm - in the dune (Site D)	✓	✓
2000 m Southwest of the farm - in the dune (Site E)	-	✓
2800 m Southwest of the farm - in the dune (Site F)	✓	✓
Sewage Works	-	✓
30 m Northeast of the farm (Site x)	✓	-
100 m Northeast of the farm (Site y)	✓	-
300 m Northeast of the farm (Site z)	✓	-
2000 m Southeast of the farm (Rhuddgaer)	-	✓

4.1.2 Preparation of Diffusion Tube and Badge Sampler

Ammonia is very difficult to monitor and analyse in an ordinary lab due to contamination problem. Therefore diffusion tubes and badge samplers in this survey were prepared and analysed by the Institute of Terrestrial Ecology Laboratory, Edinburgh Research Station, which has the facilities to prepare and analyse the tubes. The preparation and analyses procedure discussed in the subsequent sections were employed by the ITE Laboratory, Edinburgh.

4.1.2.1 Construction of passive diffusion tube

The diffusion tubes were made of opaque teflon 3.5 cm long and 1 cm diameter, and were provided with a heavy duty plastic cap at both ends (Figure 4.2). Two acidified steel grids (25 µl 1% H_2SO_4 sandwiched between 2 steel grids), which serve to capture ammonia, were held under the solid green cap - this end is placed uppermost. The other end was covered with a yellow plastic cap that holds in place a plastic membrane, through which ammonia diffuses - this end was placing downwards. Each tube was supplied in a clear plastic vial for protection, and this also contains a spare cap, which was used to replace the

yellow cap and filter at the end of sampling. Three replicate tubes were mounted in clips on a post at about 1.5 m height above the ground (Figure 4.3). The replication was used to give a more reliable estimation of the air concentration, given the very small amounts of ammonia captured.

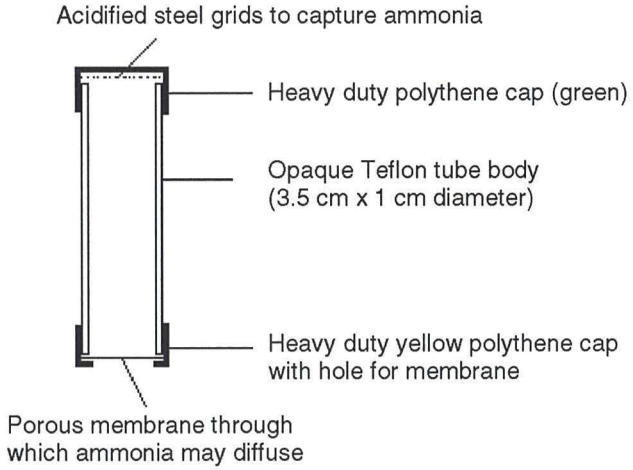


Figure 4.2 Outline diagram of a single ammonia diffusion tube

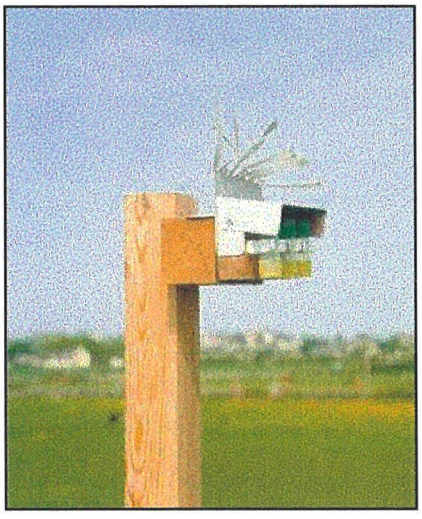
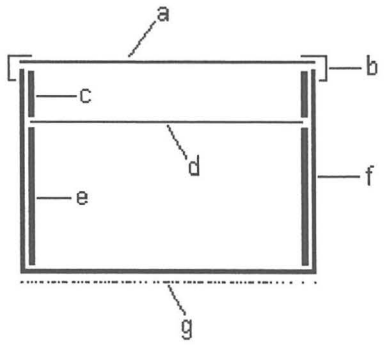


Figure 4.3 Ammonia diffusion tubes

4.1.2.2 Construction of Badge Samplers

The badge sampler (Figure 4.4) was made up of a circular polystyrene vial (26 mm height, 27 mm diameter) with one open end. A polyvinylchloride (PVC) spacer (17 mm height) placed at the bottom supports a glass fibre filter (GF/A, 24 mm diameter). The filter was coated with tartaric acid, which serves to capture the ammonia, and was held in place with a polypropylene ring (6 mm height). The open end is capped with a polyethlene cap with a hole punched out in the centre (23 mm diameter), which holds in place a polytetrafluoroethane (PTFE) membrane (27 mm diameter, 5 μm pore size) allowing gaseous ammonia to diffuse through. This end was placed facing downwards.

Similar to diffusion tubes, three replicate badge samplers were attached by the use of velcro to an aerodynamically shaped support on a pole at about 1.5 m height above ground (Figure 4.5). All three samplers were supplied in a plastic container for protection. Sharp metal strips fitted to tube and sampler holders (Figure 4.3 and Figure 4.5) are to act as a deterrent against birds perching.



(a) PTFE membrane (27 mm diameter) through which ammonia may diffuse
(b) Polyethylene cap with hole for membrane
(c) Polypropylene ring (6 mm height)
(d) Glass fibre filter coated with tartaric acid
(e) PVC spacer (17 mm height)
(f) Polystyrene pot (26 mm ht x 27 mm diameter)
(g) Velcro for attachment

Figure 4.4 Outline diagram of a single ammonia badge sampler

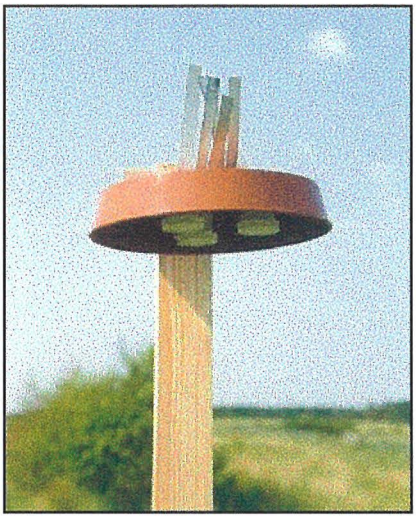


Figure 4.5 Ammonia badge samplers

4.1.3 Exposing Diffusion Tubes and Badge Samplers

Diffusion tubes and badge samplers were exposed at each site for each calendar month. This was judged to be adequate to obtain reliable measurements at the background sites, but without saturating samplers near the farm. Exposure times and any other local condition were noted down on record cards supplied. As a precaution, disposable gloves were worn at all times to protect the samples from contamination by ammonia emitted and perspiration from skin. Sampling started when parafilm that covered each tube or sampler was removed. Parafilm was used to cover up the end of the tubes and samplers through which ammonia diffuses. This is as an additional precaution to prevent any possible contamination by ammonia during transport, from the time of manufacture to being set up at the site for exposure. At the end of the exposure period, the membrane-caps were removed and new unused white cap and solid caps were placed for the diffusion tubes and badge samplers respectively. All tubes and samplers were then sent to ITE Laboratory in Edinburgh for analysis.

4.1.4 Diffusion Tube and Badge Sampler Analyses

Since I did not do the analyses, a brief procedure is given here supplied by the ITE Laboratory. Analysis of diffusion tubes and badge samplers is based on the estimates of aqueous NH_4^+ by means of Ammonium Flow Injection Analysis (AMFIA). The principle of AMFIA is the selective diffusion of NH_3 across a membrane, with subsequent analysis by conductivity. An aqueous sample was injected automatically into a carrier flow of deionised water to which an alkali (NaOH) was added. This converts all NH_4^+ to NH_3 in solution around pH 12. At this pH, NH_3 is the only small molecule in solution that will readily diffuse across a teflon membrane. The sample is passed one side of a membrane with NH_3 passing over into a counter flow of deionised water. At pH 7 the NH_3 converts back to NH_4^+ and is then analysed by conductivity. The AMFIA system includes control of an autosampler, calibration and data logging. Full procedures of the analyses employed by ITE Edinburgh are as Appendix 4.1. Data of the survey sent to me has been calculated in units of $\mu g\ m^{-3}$.

4.1.5 Vegetation Survey

Aboveground vegetation was sampled in the vicinity of each ammonia monitoring site (Site A to Site F), by cutting a 10 cm x 10 cm quadrat for analysis to determine total tissue nitrogen content. Vegetation samples were dried and ground prior to N analysis. Milled samples were weighed in foil cups and run under CHN AutoAnalyzer Leco 2000 to determine tissue nitrogen content in each sample.

Vegetation survey was also conducted at each site by recording percentage cover of each species found in 2 m x 2 m quadrat. Domin score of the cover

according to the National Vegetation Classification was used to record abundance of species.

4.1.6 Data Analysis

Data were analysed using MINITAB programme version 12. Correlation and regression analyses were performed between badge samplers' data and uncorrected diffusion tubes' data. This was to determine relationship between the two methods employed. Analysis of Variance was then conducted to determine significance of ammonia concentration between sites and sampling months.

4.3 RESULTS

4.3.1 Atmospheric ammonia concentration

Data of atmospheric ammonia concentration at Newborough presented here are of one-year survey from December 1997 to November 1998. Although measurements were conducted using both badge samplers and diffusion tubes, results presented in this chapter for southwest direction (across the dune) are from the badge samplers' data. Decision to use badge sampler's data was made because badge samplers were installed comprehensively at all monitoring sites across the dune and this however was not the case of diffusion tubes (Table 4.1). Also, there was little differences between the two data sets (Appendix 4.2) and therefore choosing one of the data sets would not create any large discrepancies on the atmospheric ammonia concentration in the area. This will be discussed in subsequent paragraph.

Diffusion tube data supplied by ITE Edinburgh were in two versions, i.e. uncorrected and corrected with a correction factor. The correction factor is applied on the diffusion tube data using correction estimates derived from ongoing intercomparison methods (Sutton *et al.,* 1998). The corrections are applied because diffusion tubes seem to under-estimate the NH_3 concentrations, and this should be treated as provisional, subject to further testing and validation. The correction factor applied on the diffusion tube data by ITE Bush is based on the equation below (detailed calculation and explanation are in Sutton *et al.,* 1998):

$$\text{Real } NH_3 = 0.686\ (\chi_{\text{diff. tubes}} + 2)^{1.1942} - 2$$

where χ is the NH_3 concentration in the air in $\mu g\ m^{-3}$. The above equation is rearranged from the initial equation of:

$$Log_{10}(\chi_{diff.\ tubes} + 2) = 0.8374\ log_{10}(\chi_{denuders} + 2) + 0.1371$$

which is derived from initial intercomparison with the denuders data because denuders give a reliable reference estimate of ammonia concentration. While there is significant uncertainty in the provisional correction estimates from this intercomparison, the revised correction factor for the diffusion tube methodology is substantial improvement on the 0.42 correction factor used by RGAR (1997). However, a correction estimate has not yet been determined for ammonia monitoring with badge samplers. Hence for this thesis, statistical analysis on the data from both methods was conducted on the uncorrected data sets.

Correlation analysis between both data sets showed that they were very highly correlated (r=0.997); and regression analysis revealed a very high relationship with R^2 of 99.4% and regression equation of Y=0.931+0.843X (Figure 4.6). This relationship reflects that both methods of sampling produce similar trends of atmospheric ammonia concentration at the site. Full data of both methods are given in Appendix 4.3. For discussion purposes, the badge sampler data would be used because of factors aforementioned, and also due to large differences between corrected data of diffusion tubes and badge samplers data (see Appendix 4.3), therefore it is more reliable to use the data of badge samplers. Nevertheless, uncorrected data of diffusion tubes in August and September 1998 were used to compare concentrations of ammonia between southwest and northeast distances from the farm to look at effects of prevailing wind on atmospheric ammonia concentration.

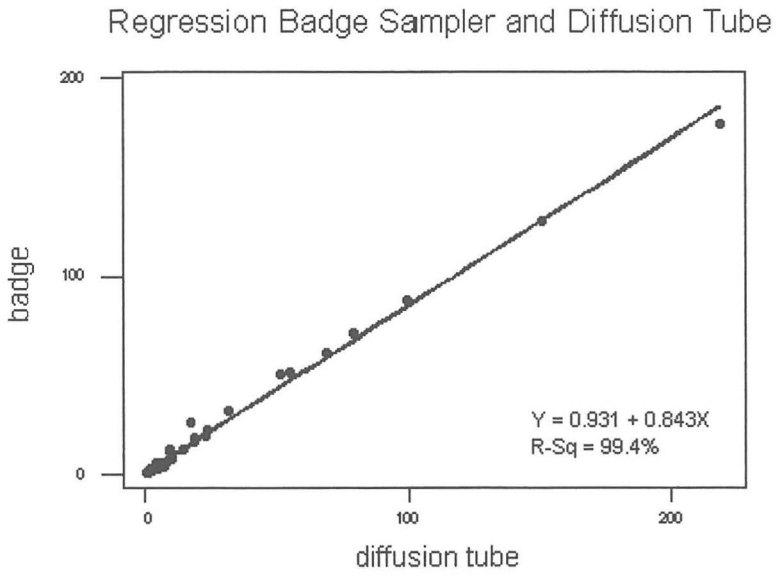


Figure 4.6 Regression analysis of badge sampler and diffusion tube data show a very high relationship between these two methods.

Mean concentration of badge sampler data for all downwind sites (southwest of the farm) for the period of December 1997 to November 1998 are presented in Figure 4.7. The Figure shows very high atmospheric ammonia concentration determined at the farm, and the concentrations are seen to decline exponentially with distance from the poultry house. Monthly data illustrated as Figure 4.8 shows a consistent trend in all months whereby concentration gradient is most pronounced within 800 m of the farm. These data were then transformed to Log_{10}, and were analysed statistically. It should be noted that the transformed data were started with February 1998 survey and not with the December 1997 survey (Figure 4.9). This was because in December 1997 and January 1998 surveys, no survey was set up at the site 100 m southwest of the farm, and complete sites had been only set up since February 1998. Analysis of Variance

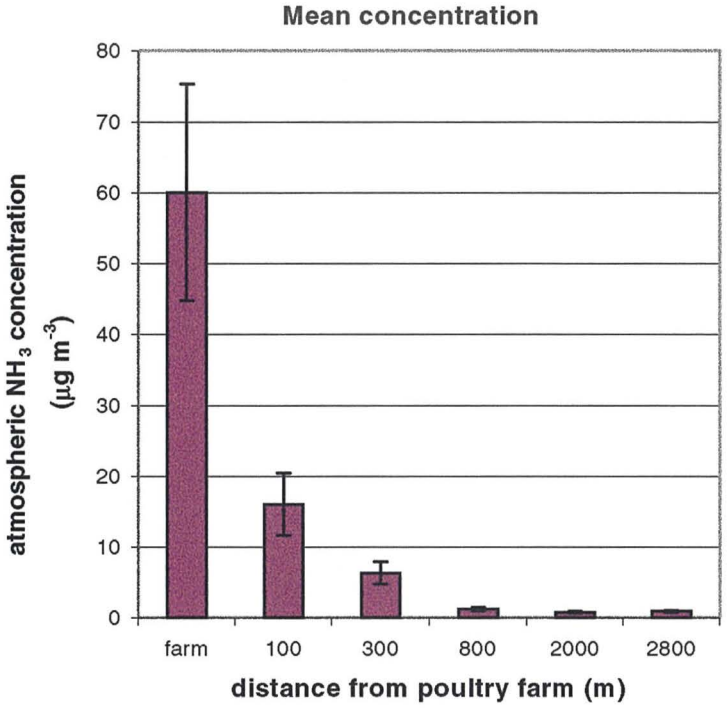


Figure 4.7 Annual mean atmospheric ammonia concentration at various distances of soutwest direction from the poultry farm

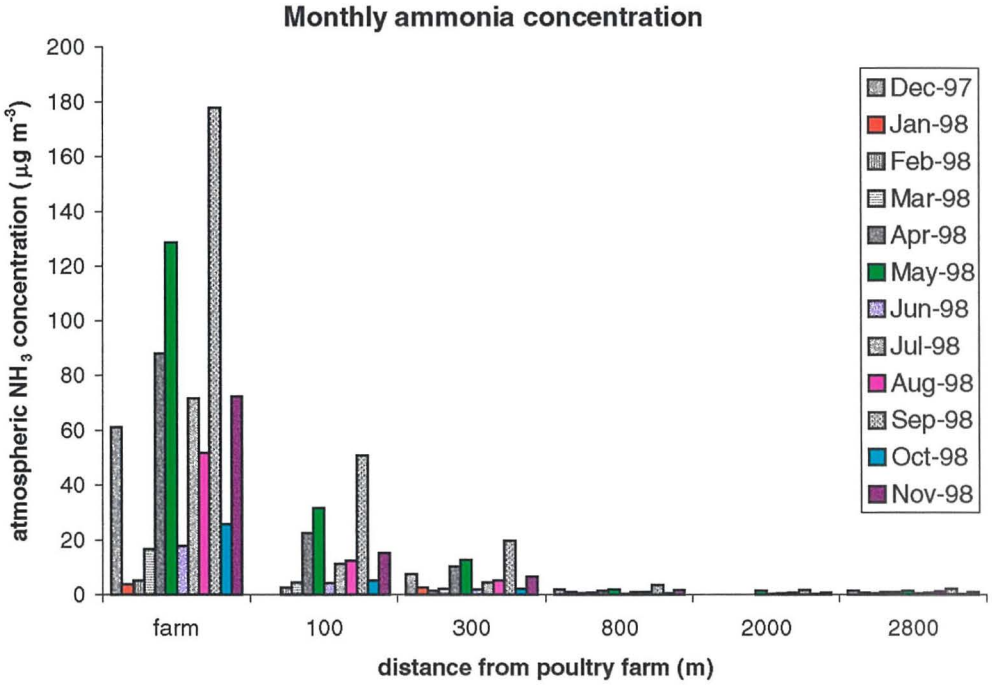


Figure 4.8 Monthly ammonia concentrations of one-year survey at various distances of southwest direction of the poultry farm

on ammonia concentration shows a very significant difference ($p<0.001$) between distance, but no measurable effect could be distinguished after 1000 m distance from the farm (Figure 4.9).

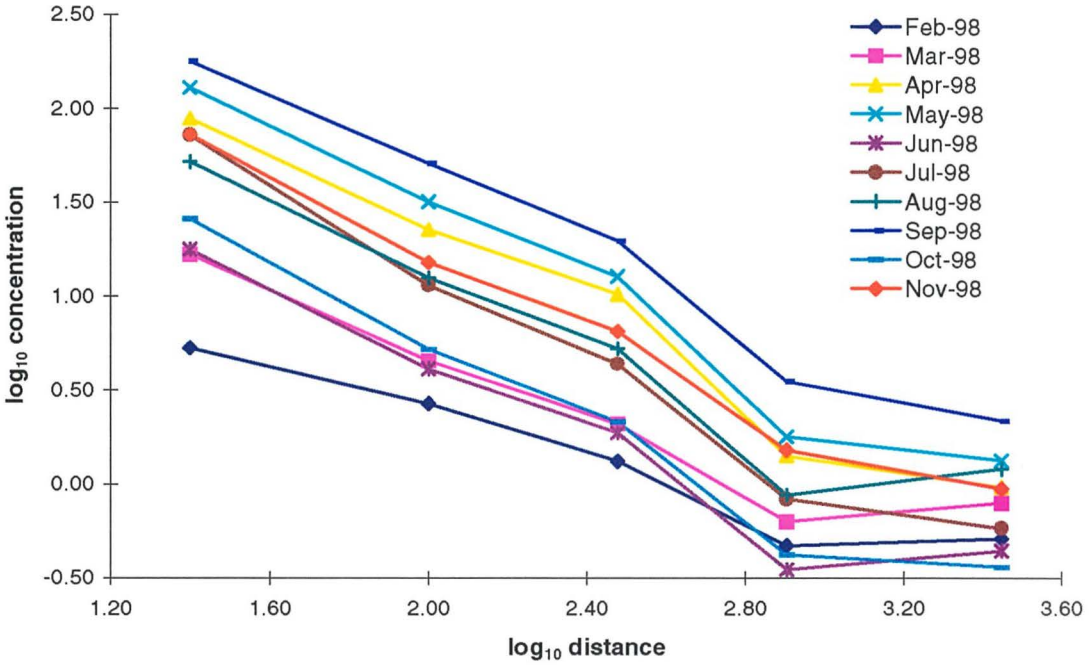


Figure 4.9 Log_{10} concentration of atmospheric ammonia (February-November 1998) plotted against Log_{10} distance. Data for Dec-97 and Jan-98 were not included because no survey was done at Site B in this period.

There was high variability of the data between sampling months, and statistical analysis shows that they are highly significant different (ANOVA, $p<0.001$). The differences between months may be partly due to different poultry cycle at different times of the year, and also due to different wind direction during period of exposure. Regression analysis shows a very significant different between concentration and the sites with R^2 of 75.1%. Annual mean atmospheric ammonia concentration at the farm is 60.05 $\mu g\ m^{-3}$ compared to background of 0.94 $\mu g\ m^{-3}$ at 2800 m from the farm (in the dune). The highest concentration

recorded at the farm was in September 1998 of 177.72 μg m^{-3}. At the sewage works located approximately 600 m from the Warren, the concentration of atmospheric ammonia was fairly low compared to the farm with annual mean of 1.94 μg m^{-3} (Table 4.2). Ammonia concentration at this site was greater during summer with highest reading in May-98 of 3.23 μg m^{-3}. From the data at this site, annual mean concentration of 1.94 μg m^{-3} seems very close to background concentration (0.94 μg m^{-3}) for the area.

Wind direction also influenced the ammonia concentration captured by tubes or badge samplers. In the two months survey northeast of the farm, diffusion tube data showed a similar trend to the data from the southwest direction – both showed that the ammonia concentration rapidly declined with distance (Table 4.3). Comparing the concentrations at three sites with similar distances from the farm but at different direction, it is apparent that southwest direction consistently shows higher concentration than northeast direction. During these two months, prevailing wind was of southwesterly wind (refer Table 2.1)

Table 4.2 *Atmospheric ammonia concentration at sewage works for each month sampled using badge samplers.*

Month	NH_3 concentration (μg m^{-3})
Dec-97	1.79
Jan-98	1.75
Feb-98	1.00
Mar-98	2.43
Apr-98	2.45
May-98	3.23
Jun-98	1.64
Jul-98	2.35
Aug-98	2.23
Sep-98	2.45
Oct-98	0.67
Nov-98	1.25
Mean	1.94

Table 4.3 *Atmospheric ammonia concentration (μg m^{-3}) at similar distances of different directions of the farm. Data collected using diffusion tubes.*

Distance from the farm	August 1998		September 1998	
	SW	NE	SW	NE
25-30 m	73.30	56.27	291.68	94.17
100 m	18.03	8.60	67.59	13.28
300 m	7.41	1.93	29.51	2.46

SW – southwest; NE - northeast

Table 4.4 *Atmospheric ammonia concentration at 2000 m southeast of the farm*

Month	NH_3 concentration (μg m^{-3})
Dec-97	1.17
Jan-98	1.90
Feb-98	1.31
Mar-98	1.32
Apr-98	1.42
Mean	1.42

Moreover, ammonia monitoring from December 1997 to April 1998 at an intensive agricultural land located at southeast of the farm (at Rhuddgaer) shows fairly low concentrations compared to the point source at the poultry farm (Table 4.4), with mean concentration for the 5-month survey of 1.42 μg m^{-3}. Between months, concentrations do not show clear difference. These values indicate that ammonia concentrations at the agricultural land is consistently higher than the background concentration (in the dune) for these particular months (see Appendix 4.3 a).

4.3.2 Estimation of dry deposition

Estimated annual dry deposition of N (F_g) in the unit of kg N ha^{-1} y^{-1} was calculated using a similar formula to that employed in Chapter 3 (Section 3.2.1) from Sutton and Fowler (1993) as:

$$F_g = -V_d \chi$$

where V_d = Deposition velocity (mm s^{-1})

χ = Ammonia concentration ($\mu g\ m^{-3}$).

An estimated V_d of 20 mm s^{-1} is used for the calculation, which is an approximation arrived at by considering values presented by other authors on various vegetation surfaces. This will be discussed in depth in the discussion section and the detailed calculation is shown in Appendix 3.1. The estimated mean of ammonia deposited at each site southwest of the farm is shown as Table 4.5.

Table 4.5 *Estimated ammonia deposition (kg N ha^{-1} y^{-1}) at each site south-west from the farm.*

Site	Deposition (kg N ha^{-1} y^{-1})
A	311.91
B	83.28
C	32.90
D	6.22
E	3.99
F	4.88

4.3.3 Vegetation survey

Vegetation surveys near the farm were conducted on patches of undisturbed vegetation, which grow densely along a small path. Vegetation close to the poultry houses is regularly cleared, however, a lush growth of weed species such as *Dactylis glomerata, Agrostis stolonifera,* etc. occurred around south side of the houses, obviously benefiting from both atmospheric emissions of ammonia and aqueous effluent from the houses following waste removal and cleaning. Surveys on all sites indicate an abundance of nitrogen-loving species such as *Holcus lanatus* and *Urtica dioica* close to the poultry houses (Table 4.6).

Table 4.6 *List of species recorded in 2 m x 2 m quadrat and its domin cover at each ammonia monitoring site.*

Site A		Site B		Site C		Site D		Site E		Site F	
Species name	domin score	Species name	domin score	Species name	domin score	Species name	domin score	Species name	domin score	Species name	domin score
Dactylis glomerata	7	*D. glomerata*	8	*D. glomerata*	5	*F. rubra*	8	*A. elatius*	8	*Anthyllis vulneraria*	8
Agrostis stolonifera	6	*Festuca rubra*	6	*R. acetosella*	1	*Briza media*	5	*D. glomerata*	5	*F. rubra*	4
Holcus lanatus	7	*A. elatius*	6	*H. lanatus*	5	*Galium verum*	4	*F. rubra*	4	*L. autumnalis*	1
Arrhenatherum elatius	6	*Rumex acetosella*	4	*F. rubra*	5	*L. autumnalis*	3	*Holcus lanatus*	5	*H. lanatus*	1
Urtica dioica	5	*Holcus lanatus*	5	*A. elatius*	8	*Salix repens*	2	*Achillea millefolium*	2	*O. repens*	2
Bromus sterilis	2	*Achillea millefolium*	2	*Anthoxanthum odoratum*	3	*Ranunculus repens*	2	*Trifolium pratense*	3	*Viola canina*	1
Silene dioica	4	*Senecio jacobea*	1	*Agrostis capillaris*	1	*A. elatius*	7	*Carex arenaria*	3	*A. arenaria*	6
Rosa pimpinellafolia	4	*Equisetum sp.*	1	*C. arenaria*	1	*Achillea millefolium*	5	*Galium verum*	3	*Sonchus arvensis*	2
Lamium purpureum	4	*Plantago lanceolata*	3	*S. arvensis*	1	*Trifolium pratense*	4	*Rumex asetosella*	4	*Ranunculus acris*	2
Heraclium sphondylium	3	*Sonchus arvensis*	1	*Senecio jacobea*	1	*Viola canina*	3	*Salix repens*	4	*Salix repens*	3
Potentilla anserina	2	*Leontodon autumnalis*	2	*A. millefolium*	3	*Holcus lanatus*	5	*Ammophila arenaria*	6		
		Trifolium pratense	2	*Ranunculus acris*	1	*Potentilla anserina*	2	*Rubus fruticosus*	4	Species no. = 10	
Total species no. = 11		*Cerastium arvense*	1	*Trifolium repens*	2	*Centaurea nigra*	2	*Trifolium arvense*	2		
		Carex arenaria	2	*T. pratense*	1	*Plantago lanceolata*	4	*Hypochoeris radicata*	1		
		Taraxacum spp.	1	*P. lanceolata*	2	*D. glomerata*	6	*Leontodon hispidus*	1		
		Cirsium arvense	1	*Taraxacum officinale*	1	*Listera ovata*	2				
		Ranunculus acris	2	*Hypochaeris radicata*	1			Total species no. = 15			
		Urtica dioica	4	*Pimpinella saxifraga*	1	Total species no. =16					
		Galium verum	3	*Centaurea nigra*	2						
				Lotus corniculatus	2						
		Total species no. = 19		*Rubus fruticosus*	1						
				Cerastium glomeratum	1						
				L. autumnalis	1						
				Total species no. = 23							

Moreover, an apparent trend could be seen from the species survey in which low numbers of species were recorded near the farm compared to sites located away from the farm (Figure 4.10) with the highest number of species at Site C (300m from the farm). However, species number declined again when moving towards the dune with the lowest number at Site F (2800 m from the farm). Site F (the same site as site 4, see Chapter 2) was right on top of the dune, it had a soil of mobile sand and was exposed to wind and direct sunlight. These factors may contribute to a reduction in the number of species and in the vegetation cover.

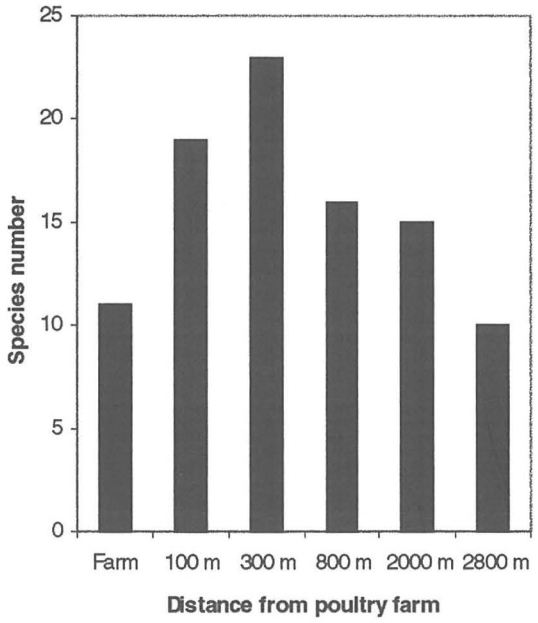


Figure 4.10 Number of species found at different distances from the poultry farm

Analysis of tissue foliar nitrogen of wild vegetation shows that nitrogen content of vegetation declined significantly with distance from the poultry houses (ANOVA, $p<0.01$) (Figure 4.11). Regression analysis shows a significant positive

relationship between tissue N and the ammonia concentrations along transect line across the dune (Figure 4.12).

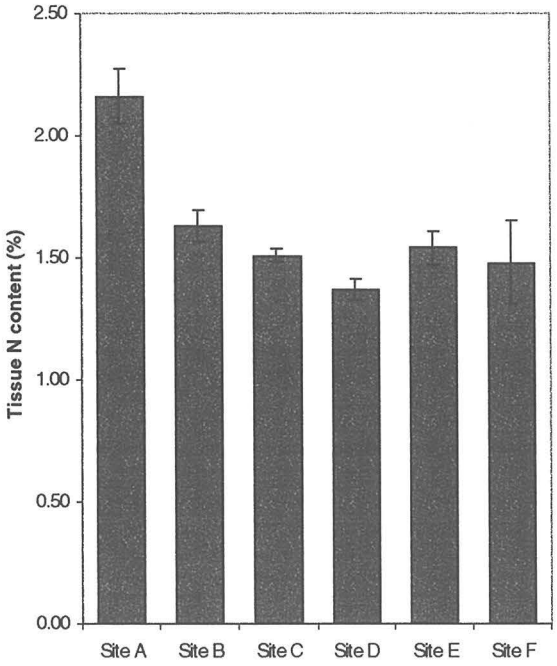


Figure 4.11 Tissue N content of wild vegetation at ammonia site

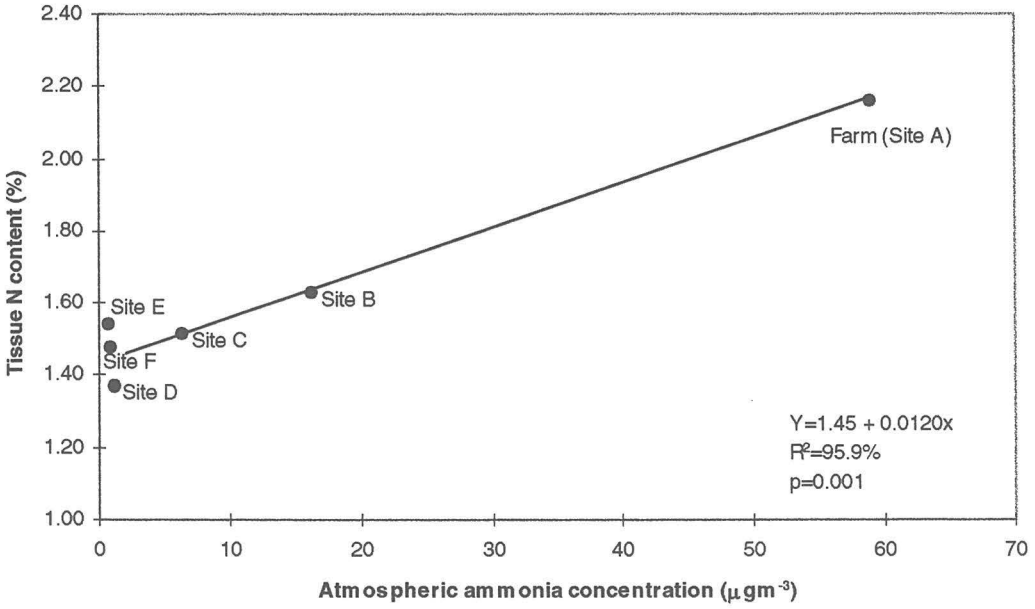


Figure 4.12 Relationship between atmospheric ammonia concentration and tissue nitrogen content of wild vegetation at all sites southwest of the farm.

4.4 DISCUSSION

In this ammonia survey, two methods of sampling were used, whereas other publications related to atmospheric ammonia monitoring used the most common method of sampling, that is the diffusion tube (e.g. Goulding, 1990, Morecroft *et al.,* 1994). Since this survey is advised by the ITE Bush, Scotland and ITE is involved in the EC GRAMINAE project (GRassland AMmonia INteractions Across Europe), the two sampling systems were used with a view to test the two methods in a range of air concentrations. This would subsequently contribute to the European measurement campaign with a stronger set of measurements than would have otherwise been possible. Between these two methods, there is a view that diffusion tubes are reliable for high concentrations, but uncertain at low concentrations, while the badge samplers are tuned for low concentrations (Sutton, pers. comm.). One of the reasons for applying both methods in this survey was to see whether these samplers would saturate at high concentrations. It was hoped that by having both, a much more robust picture of the concentrations across the range of sites would be obtained. However the data from the badge samplers are more extensive as they were used at all sampling sites and it is these data that have been analysed in detail.

The result of ammonia monitoring using badge samplers at sites southwest of the poultry farm gave concentrations within the range of 0.30-177.72 $\mu g\ m^{-3}$, with annual mean concentration at the farm of 60.05 $\mu g\ m^{-3}$. As expected the highest concentrations were recorded at the poultry farm, which is the point source of the atmospheric ammonia. Values of ammonia concentration measured in this study are large compared to other measurements in the literature at such sites. For example, Allen *et al.* (1988) surveyed atmospheric ammonia concentration at two

farm sites in southeast England in which they recorded concentration of 3-66 μg m^{-3} (monthly means) with an overall mean of 24 μg m^{-3}. It is clear that such sites represent sources of ammonia, though, with the differences in farm sizes and management practices between farms, there is much scope of variation. Pitcairn *et al.* (1998) monitored atmospheric ammonia at two large poultry farms in Scotland with 120,000 broilers (Farm 1) and 210,000 broilers (Farm 2). They reported annual mean ammonia concentrations close to the Farm 1 and Farm 2 were very large of ~28 μg m^{-3} and 59 μg m^{-3} respectively, with concentrations for some individual sampling periods reached values in excess of 300 μg m^{-3}. At another livestock farm, a pig farm in South England, Feeney (1988) reported concentration of up to 780 μg m^{-3}, using diffusion tubes set out for two-week periods.

The data collected here also show seasonal patterns of ammonia concentration, with the highest level in late summer (September 1998), the second highest in early summer (May 1998), and the lowest concentrations in winter (January, February and March 1998) (Figure 4.8). This is in a good agreement with other studies (Tjepkema *et al.,* 1981; Pitcairn *et al.* 1996) and probably related to the changes in temperature and surface wetness in the seasons, since ammonia emission is greater in warm dry conditions. Furthermore, atmospheric ammonia concentrations tend to be lower in winter than during summer because the canopy resistance to ammonia is smaller when ground surface temperature is very low (Sutton *et al.,* 1993). However, differences may also relate to management practice at the farm such as cleaning or flushing out of the poultry houses. It may be that the elevated concentration in September 1998 is a result of this.

Sites located far from the farm (in the dune) show the lowest ammonia concentrations, being in the range of 0.30-3.5 μg m^{-3}. Mean background concentration of this study calculated at Site F (2800 m SW of farm) was 0.94 μg m^{-3}. This represents background concentration of atmospheric ammonia at Newborough which is quite low compared with mean concentration at an intensive agriculture land at Rhuddgaer (2000 m SE of farm) of 1.42 μg m^{-3}. The background concentration at Newborough presented here is rather small compared to other measurements in other rural areas in the U.K. For example, Allen *et al.* (1988) found a mean concentration for typical background sites in Essex, S.E. England to be 2.6 μg m^{-3}. Other study by Sutton (1990) on four sites in Scotland revealed background concentration at Bush, a mixed agricultural area, of 1.1 μg m^{-3} NH_3. Three other sites, Fala Moor, Dunlsair Heights and Glentress Forest, were in upland areas and gave lower concentrations with mean annual values of 0.4-0.6 μg m^{-3}. In the U.S.A., Tjepkema *et al.* (1981) found seasonal mean concentrations of NH_3 in the range 0.01-0.16 μg m^{-3} in a forested area of Massachusetts. So, the background concentrations reported at Newborough are midway between these other reported values.

Ammonia concentration at the sewage works showed a smaller change between months in the range of 0.67-3.23 μg m^{-3} with annual mean concentration of 1.94 μg m^{-3}. This concentration can be considered fairly low although there maybe a large variation in direct emissions from sewage in other studies. Measurements of NH_3 concentrations by Allen *et al.* (1988) in the vicinity of a small sewage treatment plant showed no enhancement in concentrations, supporting their view that these are not significant sources. However in larger treatment plant,

ammonia emission could be significant sources, for instance Lee and Atkins (1992) estimated an emission of approximately 30 t N yr^{-1} from an activated sludge sewage treatment plant.

In general NH_3 concentrations at identical distances were lower in the northeast than in the southwest. At 300 m northeast from the farm, the mean concentration for the 2-month survey (August 1998 and September 1998) of 2.20 μg m^{-3} seems close to the background measurement southwest of the farm (Site F at 2800m) of 2.27 μg m^{-3} (Table 4.3). A similar pattern was also reported by Fowler *et al.* (1998b) who noted that the ammonia concentration at an upwind site from the point source of their survey was close to the background concentration of the study area.

Estimated ammonia deposition was calculated using an approximation of deposition velocity (V_d) from previous micrometeorological study. Sutton *et al* (1993) applied micrometeorological resistance analogy to estimate ammonia budgets for unfertilized ecosystems and a fertilized cropland. Using this procedure, resistances are used to predict deposition velocities, which are then coupled with monitored air concentrations to provide annual fluxes. Deposition velocity can be summarised as reciprocal of total resistance (r_t), which is sum of resistances for turbulent atmosphere (r_a), boundary layer at leaf surfaces (r_b) and canopy resistance (r_c) (Sutton and Fowler, 1993). Thus,

$$V_d = \frac{1}{r_t} = \frac{1}{r_a + r_b + r_c}$$

The V_d value of 20 mm s^{-1} used here was considered after comparing with V_d values in previous studies shown in Table 4.7. By assuming the canopy at Newborough as unfertilized or low N agricultural grassland, the value is thought to be most reliable for the estimation purposes in this study. Morecroft *et al.* (1994) also calculated the dry deposition of ammonia and NO_2 at their study sites on calcareous and acidic grasslands in the Peak District using the same formula in which they made and estimation of deposition velocities as 10 mm s^{-1}, based on published data for other vegetation types of similar structure.

Table 4.7 *Deposition velocities for ammonia on various vegetation types from previous studies*

Canopy	Reference	Deposition velocities V_d (mm s^{-1})
Arable land	Harrison & Allen (1991)	22
Moorland	Sutton *et al* (1993)	1 to 50
Unfertilized calcareous grassland	Sutton *et al* (1993)	1 to 11
Low N agricultural grassland	Spindler *et al* (1996)	1 to 30
Arable land	Yamulki *et al* (1996)	2-26
Winter wheat	Goulding *et al* (1998)	8-20

Surveys of vegetation at each site shows nitrogen-loving species of *Urtica dioica* and *Holcus lanatus* occur at the vicinity of the farm. Since the survey sites of Site A, Site B and Site C are not on natural land, therefore patterns of natural vegetation in relation to ammonia concentration across transect sites cannot be comprehensively represented by the survey. Nevertheless, the survey was made as far as possible on undisturbed vegetation at those sites, assuming these would represent the natural vegetation at the sites. Changes in species composition have already been described in many sites in Sweden and The Netherlands (e.g. van Breemen & van Dijk, 1988; Tyler, 1987) related to increases nitrogen deposition over the last 20-30 years. The most obvious pattern from the survey was the increase of species number with distance from

the farm. This finding agrees with similar result by Pitcairn *et al.* (1996) who reported an increase in the number of species with increasing distance from a poultry farm in Scotland. It can be concluded that species number remained fairly constant at distance of 800 m and 2000 m from the farm. Probably, at this distance in the dune it is likely that atmospheric ammonia emitted from the farm does not have an impact on the species composition. At Site F (on the dune top at 2800 m from the farm) as has been mentioned in Section 4.3, the species number is further reduced by exposure and sand movement.

When foliar N content is plotted against mean of ammonia concentration at each distance (Figure 4.12), the relationships demonstrates that atmospheric ammonia deposition is responsible for the changes in foliar N whereby higher ammonia concentration is associated with higher foliar N content. Recently, Pitcairn *et al.* (1998) reported a similar result from their study at two poultry farms in Scotland in which they revealed a decline in foliar N of vegetation with distance from the livestock buildings, and subsequently the tissue N concentration is well correlated with magnitude of ammonia deposition. Poikolainen *et al.* (1998) conducted a study on the abundance of epiphytic green algae in relation to the nitrogen concentrations in Finland whereby they measured nitrogen concentrations of mosses and lichens and they revealed that N concentrations appear to correlate relatively well with nitrogen deposition. Pitcairn *et al.* (1995) also observed the concentrations of nitrogen in mosses have risen and the moss communities have become less dense in areas where nitrogen deposition have distinctly risen.

The ammonia concentration deposited at the farm up to 300 m distance exceeded the critical level of nitrogen suggested by Bobbink *et al.* (1992) of 15-

25 kg N ha^{-1} yr^{-1} for dune or calcareous grasslands. From distances of 800 m up to 2800 m, the mean concentrations do not exceed the critical level suggested. Results of Pitcairn *et al.* (1998) also exceeded the critical level for acidic forests at distances of 50-150 m from its sources, and they suggested damage observed on spruce and pine at the poultry farm sites may be due to high concentrations of ammonia in addition to N deposition. Impacts on vegetation at Newborough NNR in this study cannot be determined clearly due to short period of study, but referring to the suggested critical level, vegetation on the Reserve (more than 500 m from the farm) is not at risk of exposure to higher atmospheric ammonia concentration.

CHAPTER 5: EFFECTS OF N DEPOSITION ON PLANT COMMUNITIES

CHAPTER 5

EFFECTS OF NITROGEN DEPOSITION ON PLANT COMMUNITIES

5.0 INTRODUCTION

Nitrogen appears to have been the first mineral nutrient to be specifically recognized as necessary for plant growth, and it is now generally accepted that nitrogen is one of the most important, and most generally deficient, soil nutrient factors limiting the growth of crop species. The general existence of sub-optimal levels of nitrogen in agricultural situations applies also to natural and semi-natural plant communities, as is shown by the numerous records of increased productivity of these communities in response to nitrogen fertilisation (e.g. Dueck *et al.,* 1991; van der Eerden *et al.,* 1991; Huberty *et al.,* 1998).

The nitrogen of the soils of contrasting natural habitats is known to vary widely (e.g. Berendse, 1990). It might, therefore, be expected that at least some of the variation in botanical composition between the plant communities of these contrasting habitats is attributable to the variation of soil nitrogen content. This in turn implies variation in growth between plant species in response to nitrogen supply. Because of the competitive conditions existing within natural and semi-natural plant communities, even relatively small differences between species in response to nitrogen might lead to large differences in botanical composition between communities in response to relatively small differences in soil nitrogen level.

Most vascular plants respond to an increase in nitrogen supply by an increase in growth, provided other nutrients and environmental factors such as water supply and temperature are not limiting. The mechanism for this is straightforward: a large proportion of plant nitrogen content is concentrated in the photosynthetic biochemistry (Evans, 1989) and a rise in nitrogen content can enable an increase in carbon fixation and hence growth. However, the magnitude of the growth response to nitrogen differs between species (Bradshaw *et al.,* 1964) and the growth of a number of bryophytes has been shown to decrease when they are subjected to levels beyond the norm to which they are adapted (e.g. Thompson and Baddeley, 1991).

Nutrient addition experiments have been carried out in a number of plant communities in which various effects have been observed. For instance, in ombrotrophic mire system, where natural levels of nitrogen supply were low, *Sphagnum* spp., which had been dominant, declined markedly due to addition of nitrogen (Lee *et al.,* 1993). Other experimental additions of nitrogen were carried out on heaths in the Netherlands in which a conversion of the communities to grasslands were observed (Berendse 1990; van der Eerdeen *et al.,* 1991).

5.1 Competition and Species Composition

Interspecific competition influences the composition of plant communities and can be an important force in successional habitats. Many analyses of relationship between vegetation communities and environmental gradients have been done based on field survey data, and there are several examples of experimental studies of the role of environmental gradients, in particular nitrogen, on the effects of multispecies competition in experimental plant communities (e.g. Austin

et al., 1985). The concept of physiological response (in the absence of competition from other species) is widely recognized with most species showing a minimum, optimum and maximum tolerance level to most environmental factors. Experiments examining performance in relation to environmental gradients rarely cover the full range necessary to define tolerance limits, but many experiments have been done in examining species over part of their range (most concerning agricultural species) or physiological response (Black, 1968; Jefferies & Willis, 1964; Austin & Austin, 1980). The concept of ecological response, the species response in competition with other species, is different from physiological response (Rorison, 1969; Mueller-Dombois & Ellenberg, 1974; Austin & Austin, 1980). There are few studies of such ecological response. Ellenberg (1953,1954) summarised some of theoretical possibilities of ecological response curves shape, including skewed curves and bimodal curves on the basis of field observation and some earlier experimental work using water-table depth as the environmental factor.

Information on species response curves is important for plant ecologists. Many of the multivariate methods currently used are based on very simple model of species response (Austin & Austin, 1980) and sensitive to changes in the response model (Austin 1976a, 1976b). In particular, the assumption that species response to an environmental gradient has the form of a unimodal response curve (sometimes incorrectly called a Gaussian curve; but whilst the shape might be the same and Gaussian curve is infact a 'normal' distribution, i.e. a frequency distribution and not a response curve) was central to the discussion of such numerical methods (e.g. Whittaker, 1973; ter Braak, 1987; ter Braak & Barendregt, 1986).

Experimental studies of competition between plant species (Harper, 1977) usually did not ask questions about how competition relations between species might be expected to change along an environmental gradient. Austin & Austin (1980) studied plant responses to a nutrient gradient under controlled conditions and described some species response curves, the graphs of species performance in relation to variation in nutrients.

Various forms of environmental gradients have previously been used to study species response curves. Any gradients which cover a range from deficiency to toxicity will generate a complex set of direct and indirect effects on species response (Austin & Austin, 1980). Previous studies of species response to environmental gradients usually concern only one gradient although some gradients, such as complete nutrient solution, include several elements and have complex effects and interactions.

Experimental knowledge of community parameters such as diversity, dominance is limited and may well both depend on and influence the interpretation of species response curve in general. Community parameters such as species diversity and dominance have been discussed in relation to environmental gradients (Peet, 1978; Austin, 1980; Austin & Austin, 1980).

Under experimental conditions, growth parameters of single species are easily measured. The total shoot yield for monocultures and mixtures is an important vegetation property to assess the species response to environmental gradient. Other plant properties such as maximum height have also been considered as important measures of competitive interactions between species in relation to

environmental gradients (Grime 1973a, 1973b). Another significant parameter of grass community under controlled conditions is tiller number for both monoculture and mixture. Tiller number is also closely related with shoot yield and maximum height.

In this chapter, several pot experiments on plant community response to environmental gradients, in particular nitrogen, were carried out. Various forms of nitrogen were applied with a view to determine the effect of increased nitrogen deposition on these models of dune plant community. Experiments were carried out over two years in 1997-98, and all address the same fundamental questions. Biomass response and the relative contribution of species were investigated to determine the effects of N on plant growth with model communities.

5.2 GENERAL MATERIAL AND METHODS

For all pot experiments of sections 5.3, 5.4, 5.5 and 5.6, standard material and methods were employed. Sand used for these experiments was taken from a non-agricultural land site adjacent to Newborough NNR. It was assumed that the characteristics of the sand were similar to the Newborough NNR soil. pH of the sand was measured by taking 6 random samples and it consistently had pH between 6.25 and 6.40 with an average of 6.31.

Seven plant species from dune and mesotrophic grassland communities were chosen for the experiments. *Briza media, Galium verum, Oenothera stricta* and *Echium vulgare* representing dune communities, and *Plantago lanceolata, Festuca rubra* and *Centaurea nigra* representing mesotrophic grassland communities. *Briza media* is reported as commonly occurring on calcareous grassland, *Oenothera stricta* and *Echium vulgare* are typical species of dune systems, and *Galium verum* is commonly found on dunes and other calcareous dry grassy places (Martin, 1982). Seed were obtained from Emorsgate Seeds, Terrington Court, Terrington St. Clement, King's Lynn, Norfolk. These species were used for experiment in Section 5.3. However, due to difficulties to germinating *Oenothera stricta* and *Echium vulgare*, the subsequent experiments (Sections 5.4, 5.5 and 5.6) used *Achillea millefolium* and *Leontodon hispidus*. *Briza media* was also replaced by *Dactylis glomerata* because of dominance of the latter in grassland communities.

Seeds were germinated in germination trays using John Innes compost. The soil taken from Newborough was air-dried and sieved using 1-mm sieve to remove finest plant parts. The soils were then filled into black four-litre pots (20 cm in

diameter) and all soils in pots were washed with tap water to have an identical pH value with the tap water for each pot. Moreover, this would make transplanting easier and also, to obtain an even moist condition in the pots. Two seedlings of each species were transplanted in a random position to each pot to make a total of 14 plants in every pot. The rationale of this experimental design, which provides an equal number of each species in each pot is that a constant proportion and a constant density of each species is obtained in the plant community models. The number of species chosen is quite reasonable to look at interaction between species because higher number of species in mixture experiments would contribute to a more complex interactions between species, which would be difficult to monitor and interpret. Moreover, the design is also economical in terms of pots and replicates, and is thought to be sufficient to gather reliable data. During the establishment period, plant community models in the pots were given 250 ml Long Ashton nutrient solution (Appendix 5.1) with minimum nitrogen concentration (in the form of nitrate-N) at 56.7 ppm for 4 weeks, once in every week. The N concentration was one-third of the standard concentration of the Long Ashton solution of 170 ppm (see Appendix 5.1), and the volume given was assumed to be sufficient for the establishment period. Then, pots were ready for experiments planned.

5.3 PLANT RESPONSE TO DIFFERENT N CONCENTRATIONS

This was a preliminary experiment with an aim to study experimentally the role of nitrogen gradient on plant communities. It has been hypothesised that at higher nitrogen level, growth of plant species would be affected; this would lead to changing of their dominance and abundance in the communities.

5.3.1 Methods

Seven levels of nitrogen concentration were chosen to create a nitrogen gradient. The experimental nutrient solution was based on the Long Ashton nutrient solution and the nitrogen concentration was varied to give 10 ppm, 20 ppm, 40 ppm, 80 ppm, 170 ppm, 340 ppm and 680 ppm N by alteration of $NaNO_3$ concentrations (Appendix 5.1). Pots were duplicated for each N concentration, and therefore 14 pots were used for this experiment. The number of pots used was sufficient, as this experiment was a preliminary experiment in which a relationship of plant parameters would be expected with different nitrogen concentrations. As mentioned in general method (Section 5.2), *Briza media, Galium verum, Oenothera stricta, Echium vulgare, Plantago lanceolata, Festuca rubra* and *Centaurea nigra* were used for this experiment. Experiment was conducted in an unshaded glasshouse at Pen-y-ffridd Field Station, Penrhos Road, Bangor.

All pots with the model plant communities were arranged in ascending order of nitrogen level and placed on a wire-mesh bench to prevent any possible transfer of nutrients from nearby pots. 250 ml nutrient solution of appropriate concentration was added to each pot weekly, and the pots were watered as

needed to avoid water stress on the plants. Experiment started on 1/9/97 and finished on 13/11/97.

Growth of plant species in the pots was monitored regularly by looking at the leaf number and the leaf length of the plants. On the final day of experiment, plants of each pot were clipped at ground level and they were separated according to their species and pots. The clipped plants were then put into paper bags, dried in the oven at 70 °C and weighed. Species dominance was determined by looking at the biomass of each species whereby higher biomass would reflect higher dominance of that particular species.

Data were analysed for significance using one-way ANOVA in MINITAB programme version 11.

5.3.2 Results

Leaf number and leaf length of each species were recorded two weeks after the experiment started. However, after six weeks, leaves counting and length measurement were very difficult to conduct because plants grew vigorously and therefore the counting had to be abandoned. Biomass of each species was determined and this is shown as Figure 5.1. This Figure indicates that biomass of each species positively increase with concentration of nitrogen. Total biomass of vegetation (Table 5.1) at each nitrogen level shows a very strong trend and is illustrated as Figure 5.2. One-way ANOVA was conducted to determine significance of treatment effect on plant biomass. Biomass of vegetation are found highly significant different between treatment ($p<0.001$). Total biomass decreased at 680 ppm nitrogen, however, since the range of nitrogen level was

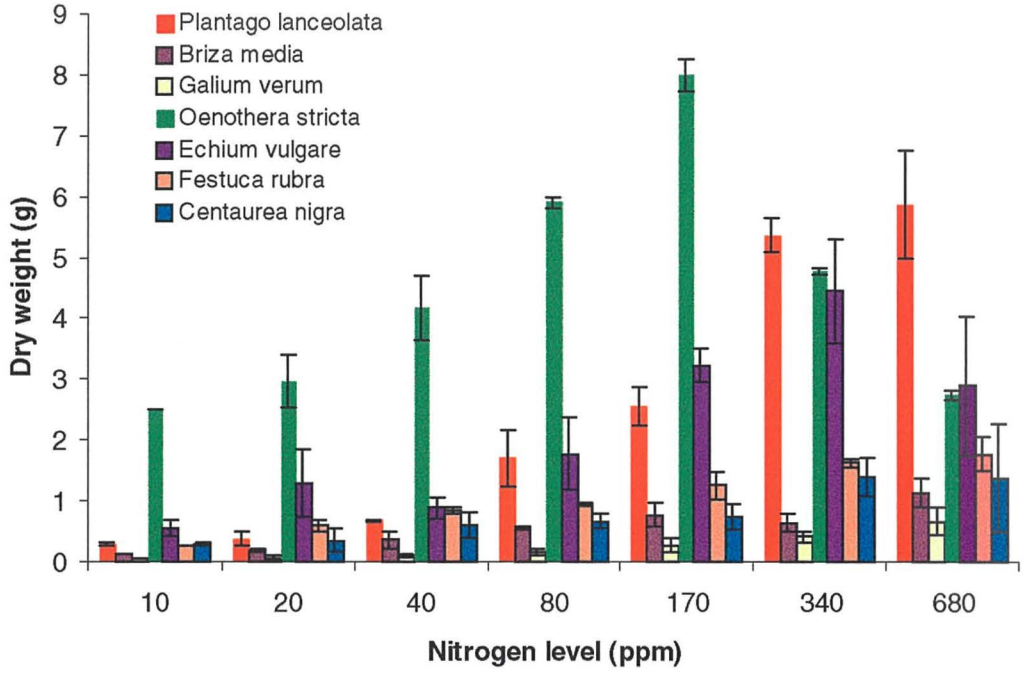


Figure 5.1 Biomass of each species at different level of N concentrations. Bars show standard error of mean.

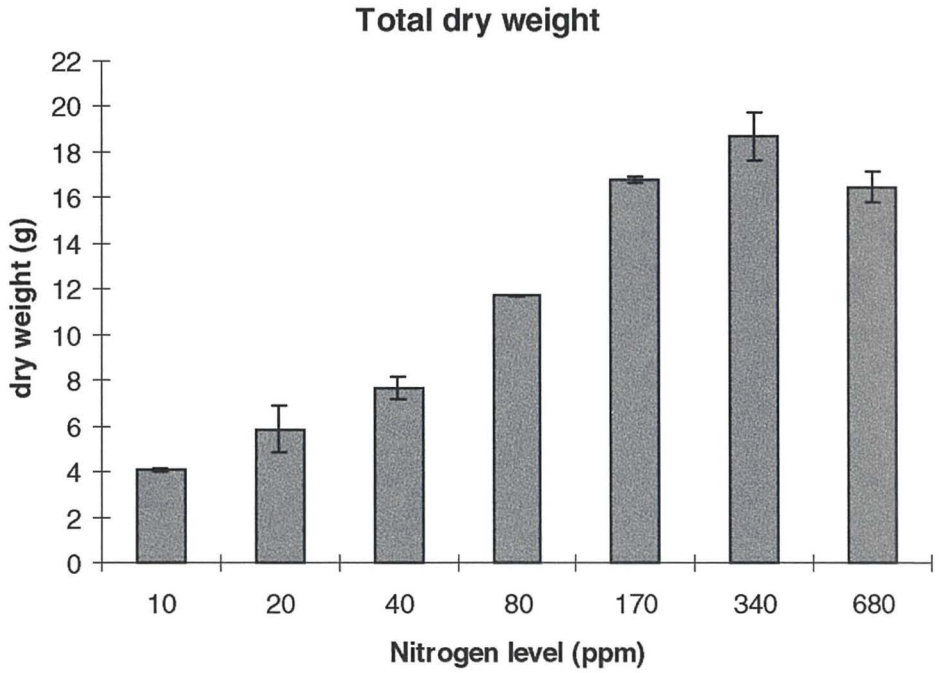


Figure 5.2 Total biomass of model of plant community at different nitrogen level. Bars show standard error of mean.

not extended, therefore subsequent response could not be predicted whether it would consistently decrease or increase again.

Table 5.1 *Biomass of vegetation in pots at different nitrogen level*

Nitrogen concentration (ppm)	dry weight (± S.E.) (g)
10	4.08 ± 0.07
20	5.86 ± 1.04
40	7.65 ± 0.51
80	11.73 ± 0.04
170	16.82 ± 0.14
340	18.68 ± 1.07
680	16.48 ± 0.66

Moreover, to assess plant competitive ability in multispecies communities, an index *Relative Ecological Performance* of each species was calculated. This index used by Austin and Austin (1980) is defined as yield of a species expressed as a proportion of the total biomass of a mixture at a particular nutrient level, and is employed to compare competition of species in a species mixture. In addition, Zhang (1991) applied Relative Yield as an index to assess multi-species competition along a nutrient gradient, which is exactly same as Relative Ecological Performance (P_{aj}). The relative ecological performance is expressed as:

$$P_{aj} = \frac{Y_{aj}}{Y_{aj}+Y_{bj}+Y_{cj}+Y_{dj}+Y_{ej}+Y_{fj}+Y_{gj}}$$

where P_{aj} is relative ecological performance of species *a* at nutrient level *j* in 7-species mixture. Y_{aj} to Y_{gj} are the yields of seven species *a* to *g* at nutrient level *j* in 7-species mixture.

The relative ecological performances of species under all nitrogen concentrations in 7-species mixture were calculated and analysed. A complete Analysis of Variance was carried out on the relative ecological performance and showed a very significant difference between both species and interaction with nitrogen concentrations ($p<0.001$). However, because the ecological performances are ratios summing to one, the analysis of the totals for N treatment gives a zero sum of squares. Summary of the ANOVA output is given in Table 5.2 and the species-N level interaction is shown as Figure 5.3.

Table 5.2 *Summary of ANOVA of relative ecological performance for species and N level*

Source	% Sum Square	P
Species	76.27	0.000 ***
N concentration	0.00	1.000
Interaction	22.47	0.000 ***
Error	1.26	
Total	100.00	

At low nitrogen concentration, *Oenothera stricta* is the highest species in ecological performance whereas the other six species show more or less ecological performance to each other (Figure 5.3). However, ecological performance of *Oenothera* started to decline sharply after 170 ppm N level and its performance is nearly the same as *Echium vulgare* at 680 ppm N. Ecological performance of *Festuca rubra* increased gradually with level of nitrogen treatment. At the highest N concentration of 680 ppm, *Plantago* shows the highest ecological performance compared to the other six species.

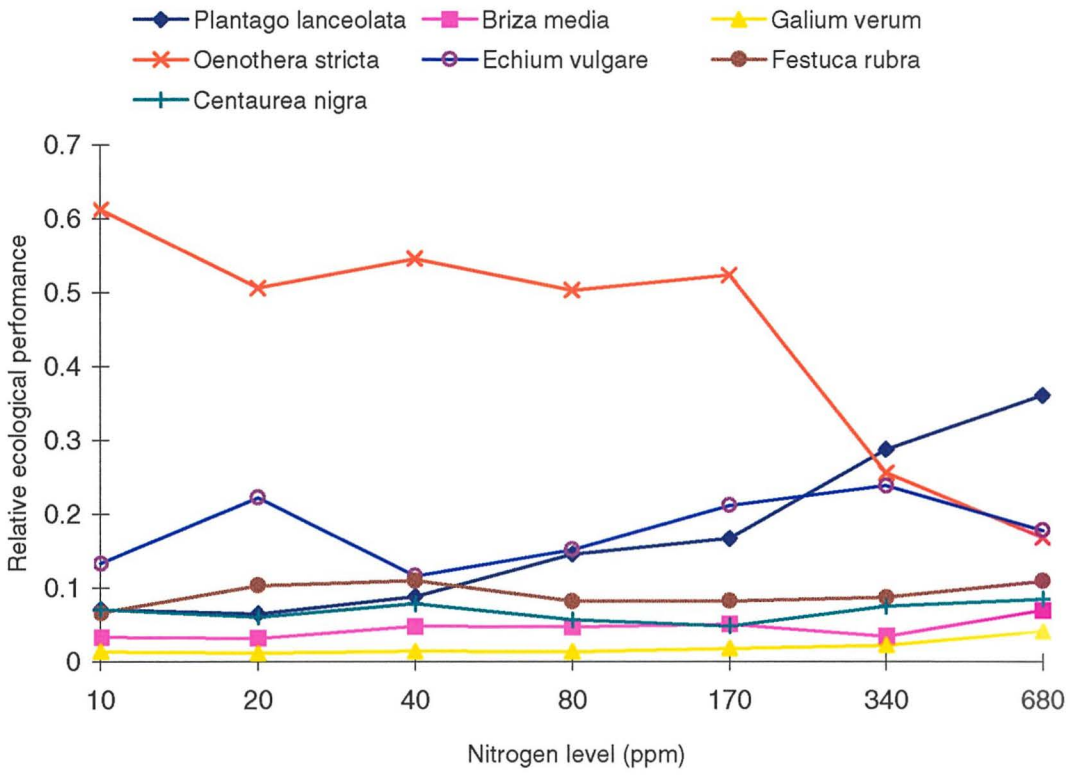


Figure 5.3 Relative Ecological Performance of each species of the model plant communities grown at different N level

5.4 EFFECT OF MIST NITROGEN ON DUNE PLANT COMMUNITIES

Wet and dry deposition of anthropogenic N (NO_x and NH_y) has increased throughout Europe for the last 30 years (Skeffington & Wilson, 1988). Wet pollutant deposition may often be in excess of dry deposition in region that receives large amount of rainfall. For example, deposition rates across the mountains of Snowdonia, U.K. are large due to the high annual rainfall, exceeding 4000 mm on the higher peaks (Reynolds *et al.,* 1990). There have been numerous studies on the impacts of wet deposition on crop species but most of these, conducted with realistic (near-ambient) acidities have failed to demonstrate adverse effects on plant growth (Irving, 1983). However, in ecosystems which are naturally very low in nitrogen, wet deposition is considered to be of major importance and may lead to the disappearance of original characteristic species (Hanson *et al.,* 1989).

The aim of this experiment is to determine the response of a model dune plant community to nitrogen (ammonium nitrate) in the form of mist, which represents wet nitrogen deposition. The impacts of different levels of deposition were investigated.

5.4.1 Material and Methods

For this and subsequent experiments, the species used were *Plantago lanceolata, Festuca rubra, Centaurea nigra, Dactylis glomerata, Achillea millefolium, Galium verum* and *Leontodon hispidus.* Before exposing the plants to the N mist treatments, models of plant communities were given the Long Ashton nutrient solution with nitrogen concentration of 56.7 ppm, to establish them in the pots for two weeks in the glasshouse.

All established community model in pots were then housed in a misting facility at Institute of Terrestrial Ecology Research Station at Abergwyngregyn, North Wales (Figure 5.4). The misting facility consists of eight polythene tunnels with overhead spray nozzles. This provides two replicate blocks, each comprising four nitrogen treatments in separate tunnels.

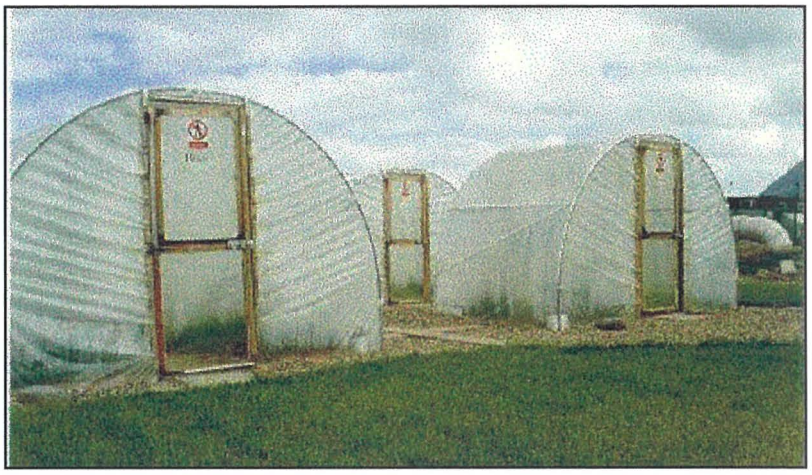


Figure 5.4 Mist units at Institute of Terrestrial Ecology Research Station Abergwyngregyn, North Wales

Three replicate pots were placed randomly in each corner of each tunnel and the pots were rotated at two-month interval to reduce any positional effects relative to the spray nozzles or variation due to other environmental conditions within the tunnels. The pots were placed at each corner in the tunnel because most of spaces were occupied by ITE's existing experiments. The nitrogen treatments were as follows:

2 kg N ha^{-1} yr^{-1}	(Pristine)
10 kg N ha^{-1} yr^{-1}	(50% of ambient)
20 kg N ha^{-1} yr^{-1}	(ambient)
55 kg N ha^{-1} yr^{-1}	(ambient + 50%)

The treatment of 2 kg N ha^{-1} yr^{-1} is considered as a pristine level, which is taken to be the natural levels without any anthropogenic inputs, such as are found in the arctic. The N level of up to 55 kg N ha^{-1} yr^{-1} was chosen because in a long term experiment more realistic levels of pollutants can be used, but in a short duration experiment, slightly elevated levels are necessary to achieve significant results. Also, inclusion of dry and occult deposited nitrogen may double conventional estimates based on bulk deposition (Parsons, 1991).

The nitrogen was supplied as ammonium nitrate (NH_4NO_3) as NH_4 and NO_3 are found in a ratio of approximately 1:1 in the field (Morgan, 1991). This was delivered as a mist using pressurised water tanks and spray nozzles, giving a size of water droplet range of 5-30 μm. The size of droplets produced by the misting unit is very similar to natural conditions of which occult deposition droplets are in the range of 10-50 μm. Seventeen litres of ammonium nitrate solution was prepared for each tank and the composition is detailed as Table 5.3.

Table 5.3 *Volume of NH_4NO_3 used in mist treatments in the ITE mist units 1998*

Treatment (kg N ha^{-1} yr^{-1})	NH_4NO_3 stock solution (ml)	Volume per misting @3 times/week (ml)
2	12	4
10	60	20
20	120	40
55	330	110

The NH_4NO_3 stock solution was made up by adding 24.26 g NH_4NO_3 in five litres deionised water. Mist was applied for 3.5 hours, 3 times per week. Supplementary mineral nutrients were also applied to each pot in the form of 250 ml Long Ashton mineral nutrient solution without the nitrogen element. The

number of doses given is similar to pots of subsequent sections. The experiment started on 1/4/98 and ended on 12/10/98.

Plant communities in all pots were harvested two times; clipping 5 cm above the top of the pot in August, and final harvesting of cutting at ground level in October. Vegetation was sorted to species and dried in the oven at 70 °C for 72 hours. Dry weight of each species was recorded and total biomass of each pot was determined. Data were analysed for significance using the ANOVA (General Linear Model) in MINITAB programme.

5.4.2 Results

Data of this experiment do not show a regular response and an apparent trend is difficult to observe. There is a high variation between pots within treatments and statistical analysis shows no significant difference between treatments. Summary of Analysis of Variance of total biomass for each treatment of different harvests is given in Table 5.4. It is surprising that there is a significant effect between blocks in this experiment and reasons contribute to this are not known. Both harvests are different significantly ($p<0.05$) in which means of final harvest consistently greater than means of first harvest for each treatment. The biomass of the final harvest for each treatment is illustrated as Figure 5.5. This Figure shows that at 20 kg N ha^{-1} y^{-1}, maximum biomass was obtained and the total biomass decreased at higher level of treatment of 55 kg N ha^{-1} y^{-1}.

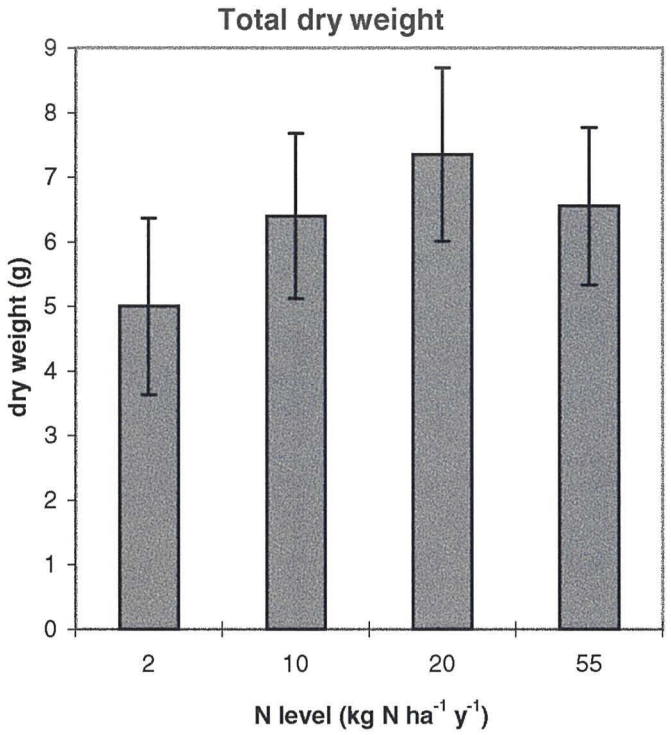


Figure 5.5 Total vegetation dry weight of final harvest at different nitrogen mist level. Bars indicate standard error of means (n=6).

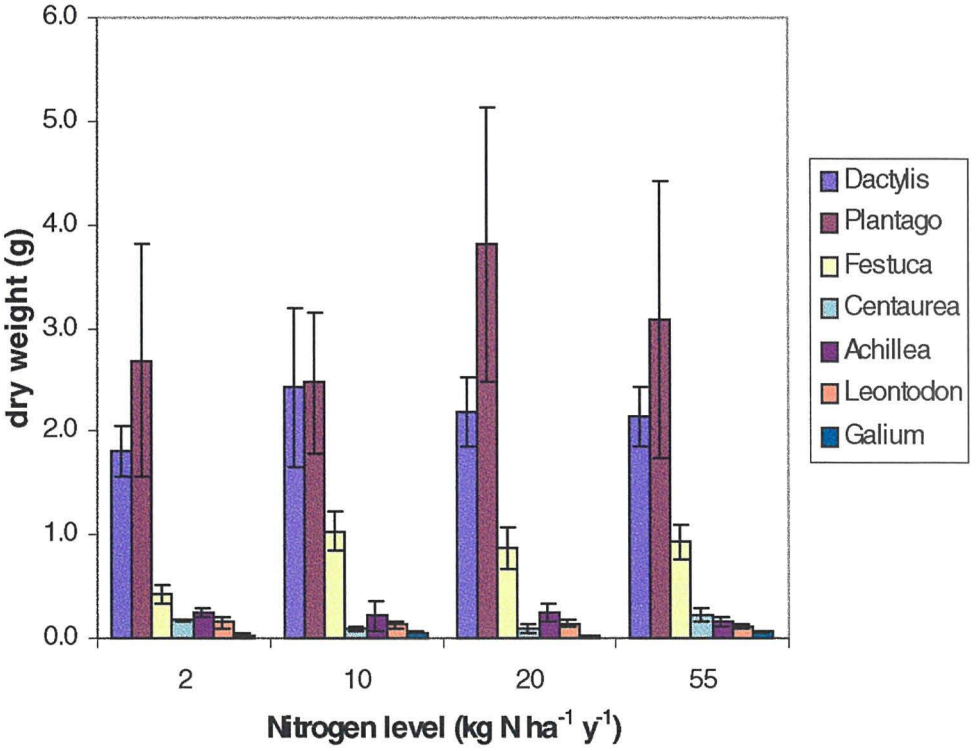


Figure 5.6 Species dry weight of final harvest at different nitrogen mist level. Bars show standard error of mean (n=6)

Table 5.4 *Summary of ANOVA on total biomass per pot of each mist treatment for both harvests.*

Source	% Sum of Square	F-value	P	
Treatment	6.15	1.97	0.168	NS
Block	21.59	8.46	0.012	*
Pot	6.57	0.96	0.409	NS
Harvest	12.24	6.65	0.023	*
Pot*Treatment	12.66	1.42	0.279	NS
Pot*Harvest	1.84	0.52	0.606	NS
Treat*Harvest	0.34	0.05	0.986	NS
Pot*Treatment*Harvest	1.52	0.16	0.984	NS
Block*Treatment	4.66	0.15	0.930	NS
Block*Pot	11.27	3.48	0.062	NS
Block*Harvest	0.08	0.05	0.832	NS
Error	21.08			
Total	100			

NS - Not significant; * - $p<0.05$

The biomass of each species at each nitrogen level showed that *Plantago* consistently has the higher biomass followed by *Dactylis* and *Festuca* (Figure 5.6). However, the biomass of *Plantago* indicates a very high variation between pots within treatments, shown by large standard error bars in Figure 5.6. Probable factors contribute to this will be discussed later in the discussion section. The relative ecological performances of species under all treatments were calculated and analysed statistically. ANOVA on relative ecological performances indicate that ecological performances between species differ significantly ($p<0.001$). Interactions of species with treatment and block factors do not show any difference. Summary of the ANOVA output is shown as Table 5.5.

Table 5.5 *Summary of ANOVA on the relative ecological performance of plant species in the mist experiment.*

Source	**% Sum Square**	**P**
Species	76.58	0.000 ***
Treatment	0.07	0.956
Block	0.03	0.687
Species*Treatment	1.52	0.963
Species*Block	0.33	0.933
Error	21.47	
Total	100	

Plantago lanceolata, Dactylis glomerata and *Festuca rubra* are the three highest species in ecological performance compared to the others, with *Plantago* shows the highest performance followed by *Dactylis* and *Festuca* respectively (Figure 5.7). Performance of *Plantago* and *Dactylis* are nearly the same at nitrogen level of 10 kg N ha^{-1} y^{-1}. As for *Festuca,* its performance shows a gradual increase from nitrogen level 2 to 10 kg N ha^{-1} y^{-1}. The other four species show very similar ecological performances with no obvious changes at different nitrogen level.

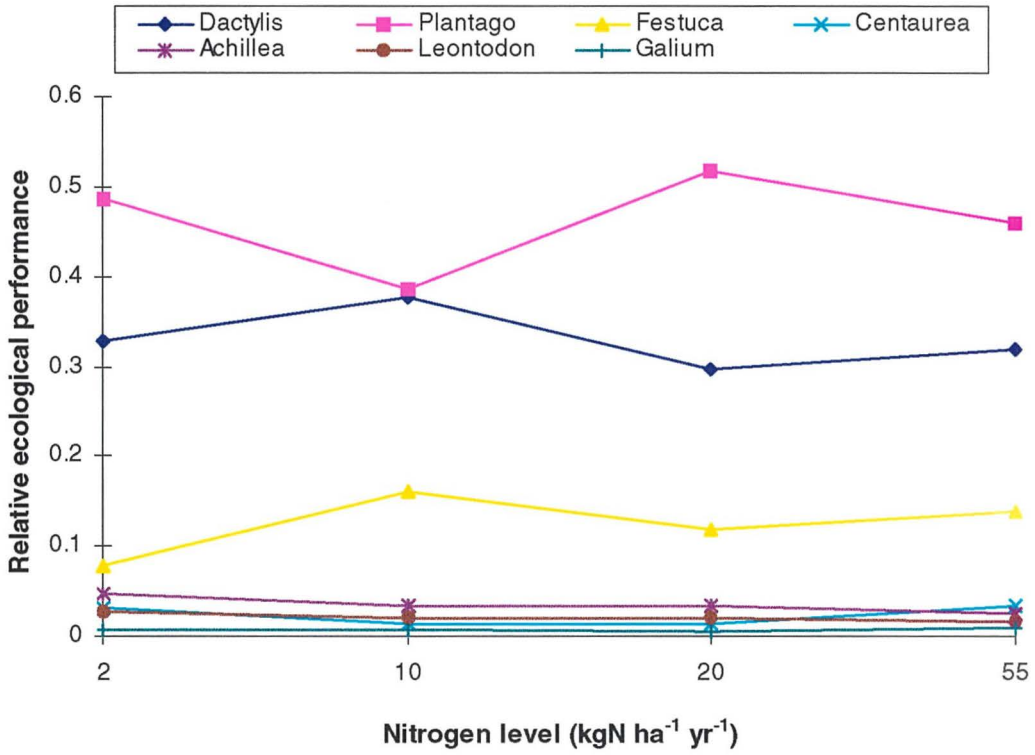


Figure 5.7 Relative ecological performance of each species at different N mist level

5.5 PLANT COMMUNITY RESPONSE TO AMMONIA GAS

Since atmospheric ammonia at Newborough has been measured in Chapter Four, therefore it is useful to determine how plant community reacts to ammonia gas at different levels of concentration. Many studies have been conducted to look at the response of various plant species to ammonia gas, and to name a few, Dueck *et al.* (1991) and Farquhar *et al.* (1980) found significant effects on the growth of the plants that they studied. The experiment conducted in this section was intended to provide comparable data and information to compare with the subsequent pot experiment at Newborough.

5.5.1 Material and Methods

This experiment was conducted in open-topped chambers at ITE Plot 7, Penrhos Road, Bangor (Figure 5.8). Since this site has been made redundant by ITE several facilities were not available (such as electricity, gas monitoring systems and flow meters), a simple experiment was therefore planned based on minimal maintenance and control.

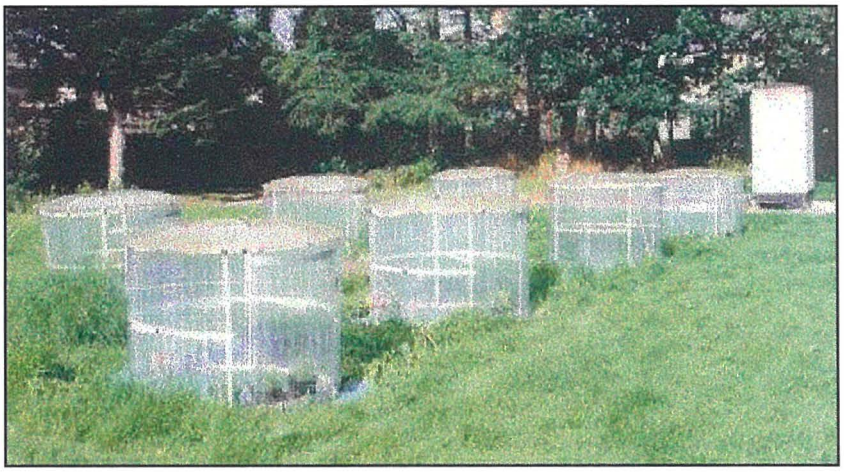


Figure 5.8 Open-topped chambers at ITE Plot 7, Penrhos Road, Bangor

This experiment was conducted using similarly established models of plant communities as in previous experiments. Four concentrations of ammonia solution were chosen as treatments applied to the pots. They are 0% NH_3 (control), 1% NH_3, 5% NH_3 and 10% NH_3. These four treatments were employed to four open topped chambers in two blocks to make a total of eight chambers. Three established pots were placed in round saucers and placed in each chamber.

The ammonia gas was supplied by allowing ammonia to evaporate from ammonia solutions in plastic beakers. 25% ammonia stock solution (from the BDH-MERCK supplier) was diluted to appropriate concentration (v/v). Appropriate solutions were then put into plastic beakers and placed in the chambers. The beakers were placed at the greatest distance possible from the plant pots. Ammonia solutions were changed every week and growth of plants was monitored. In addition, 250 ml Long Ashton mineral solution with nitrogen deficient was added to the pots at the same doses received by pots in previous experiments. Furthermore, ammonia diffusion tubes were placed in each chamber for 16 days period to measure ammonia concentration in the chambers. These diffusion tubes were supplied and analysed by the ITE Edinburgh.

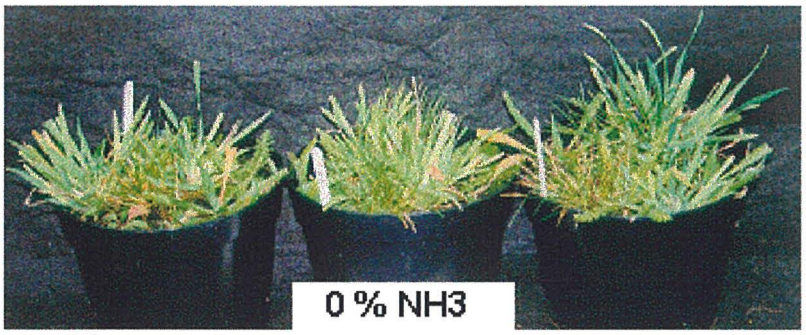
As in the previous section, all vegetation in pots were clipped 5 cm above the top of the pots in August and at ground level in October 1998. The vegetation was sorted into species and dried in the oven at 70 °C for 72 hours. Dry weight was recorded and total biomass of each pot was determined. Subsequently, all samples of dried species were ground with a small bench-grinder for nitrogen analysis. Milled species were weighed in the foil cups and run on the CHN

Analyzer Leco 2000 to determine tissue nitrogen content of each species. *Galium verum* could not be analysed due to insufficient amount of sample to run on the machine. Thus, data for the nitrogen content represent only six species of the plant community model. All data on biomass and nitrogen content were analysed for significance using the MINITAB programme version 11.

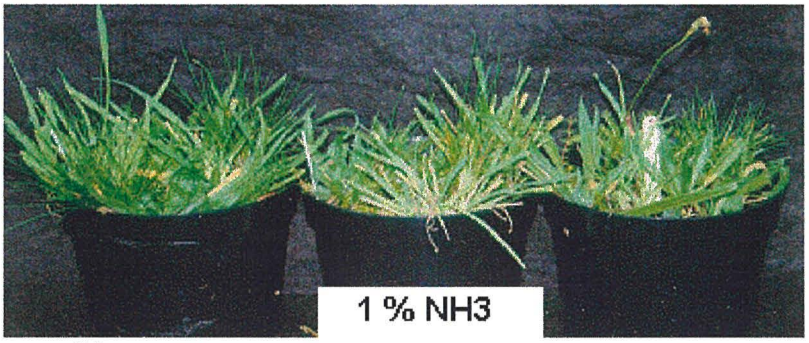
5.5.2 Results

In this experiment, it is obvious that the plant communities in the pots had a biological response to ammonia gas provided by evaporation from the ammonia solution. In the first week of the experiment, damage on leaves of pots with ammonia treatment could be observed. Plants exposed to 5% NH_3 and 10% NH_3 were seen to have more brownish leaves, though at later stage of the experiment they grew vigorously at these two ammonia concentrations, and look more greenish compared to control (0%) and 1% NH_3 treatments (Figure 5.9 a, b, c and d).

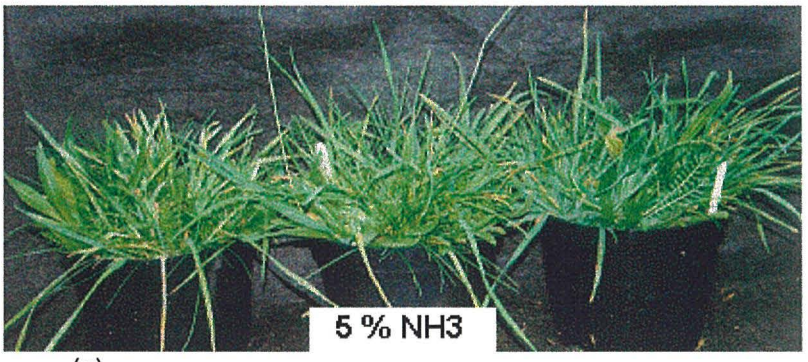
For the first harvest conducted in August, both the dry weights of the total and of the individual species were recorded. The ANOVA showed a highly significant difference ($p<0.01$) in plant biomass for the four levels of ammonia concentrations. Plants took up nitrogen as their nutrients in the form of ammonia gas through foliar intake, and this caused an increase in harvested plant dry weight from an average of 0.70 g to 2.54 g. The trend of the increase in dry weight is illustrated as Figure 5.10a. Similar results were also obtained from the final harvest conducted in October. When data were tested for significance, they were found to have a very highly significant difference in dry weight between treatment levels (ANOVA, $p<0.001$). This harvest showed a positive trend in dry



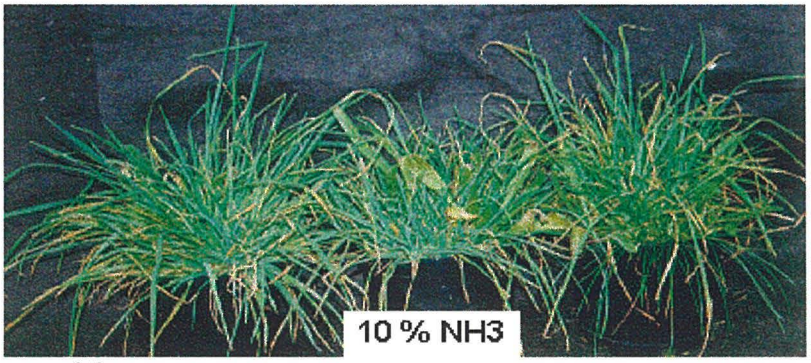
(a)



(b)

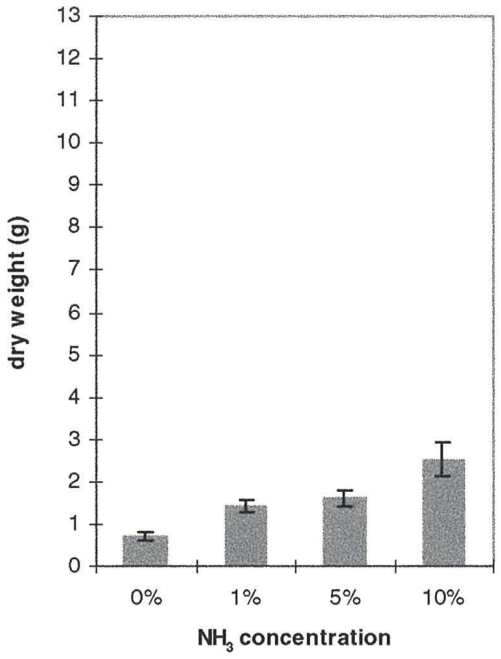


(c)

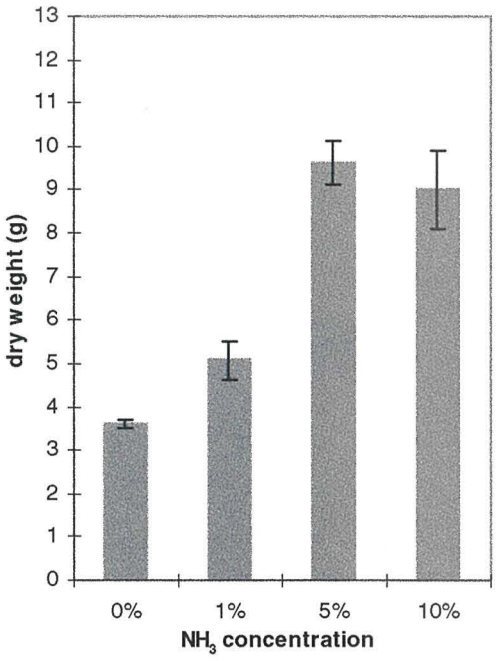


(d)

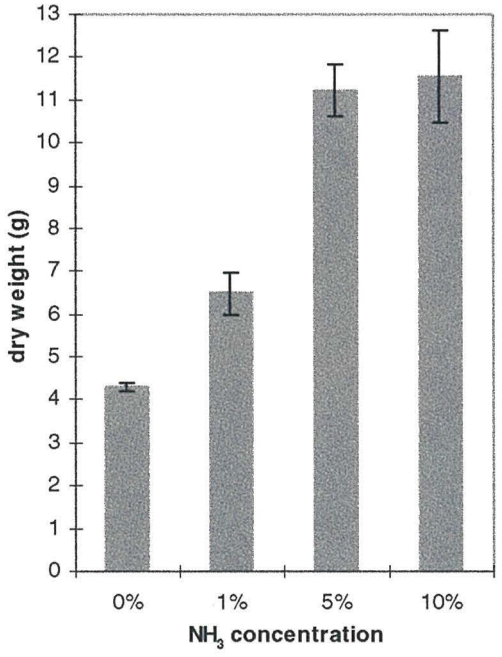
Figure 5.9 a, b, c and d. Growth of plant community at different ammonia levels in the open-topped chambers.



(a) First harvest



(b) Final harvest



(c) Total dry weight of first and final harvest

Figure 5.10 (a), (b) and (c). Biomass of models of plant communities grown in the open-topped chambers at different ammonia concentrations.

weight in which biomass of plants increased with ammonia concentration, however, at 10% NH_3 plant biomass seemed decreased compared with the one at 5% level (Figure 5.10b). Subsequently, dry weight of plants for both harvests were combined and they are shown as Figure 5.10c. It was found that biomass of plants at 5% and 10% ammonia concentrations are nearly the same, and they are significantly difference ($p<0.001$) compared to the lower ammonia concentration.

Dry weight for each species is illustrated as Figure 5.11. *Dactylis* shows the highest biomass from other species at all levels of ammonia whereby the dry weight consistently increased with increased of ammonia levels. These are also true for *Plantago* and *Festuca* at lower ammonia concentration, however their biomass decreased at the highest level of 10% NH_3. A clear view on the interaction among the species with different ammonia level could be obtained by plotting their relative ecological performance against ammonia level (Figure 5.12). Prior to plotting, ANOVA on relative ecological performances between species was conducted and they show a very highly significant different ($p<0.001$). *Dactylis* shows the highest ecological performance followed by *Plantago, Festuca, Achillea* and others. Performances of *Dactylis* and *Plantago* are nearly the same at 1% NH_3, however at 5% and 10% levels, their performances are in opposite direction with *Dactylis* increased and *Plantago* decreased sharply with ammonia levels. As for *Festuca,* it does not show any obvious trend; and interestingly the ecological performance of *Achillea* gradually declined with increased ammonia level, so do with other species.

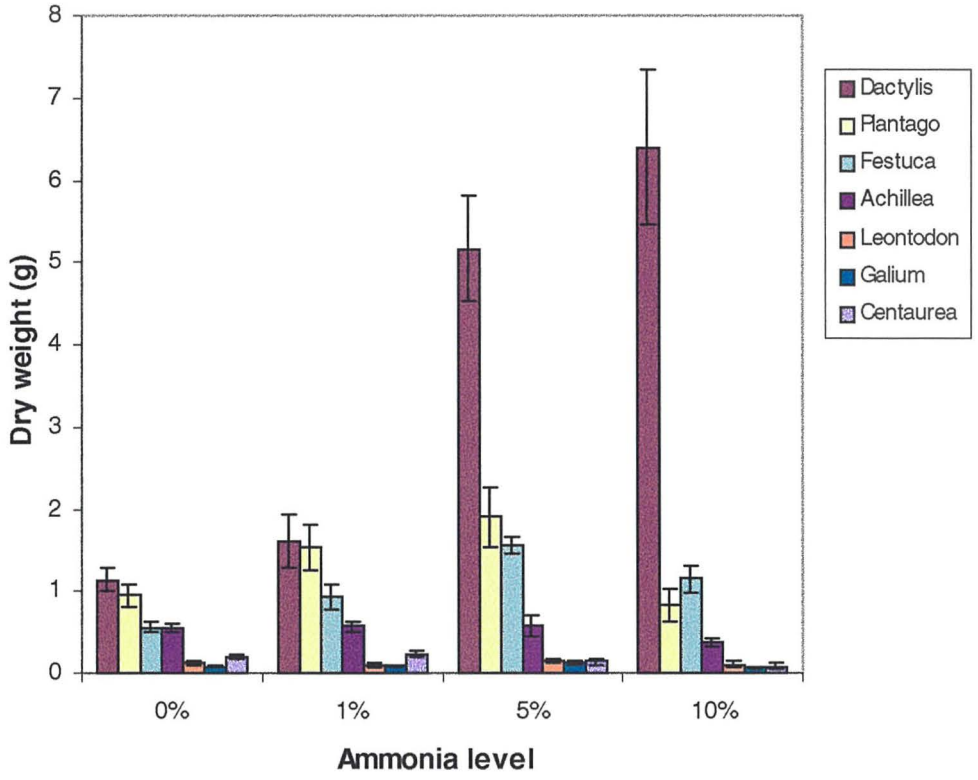


Figure 5.11 Biomass of each species of final harvest at different ammonia level. Bars show standard error of means (n=6)

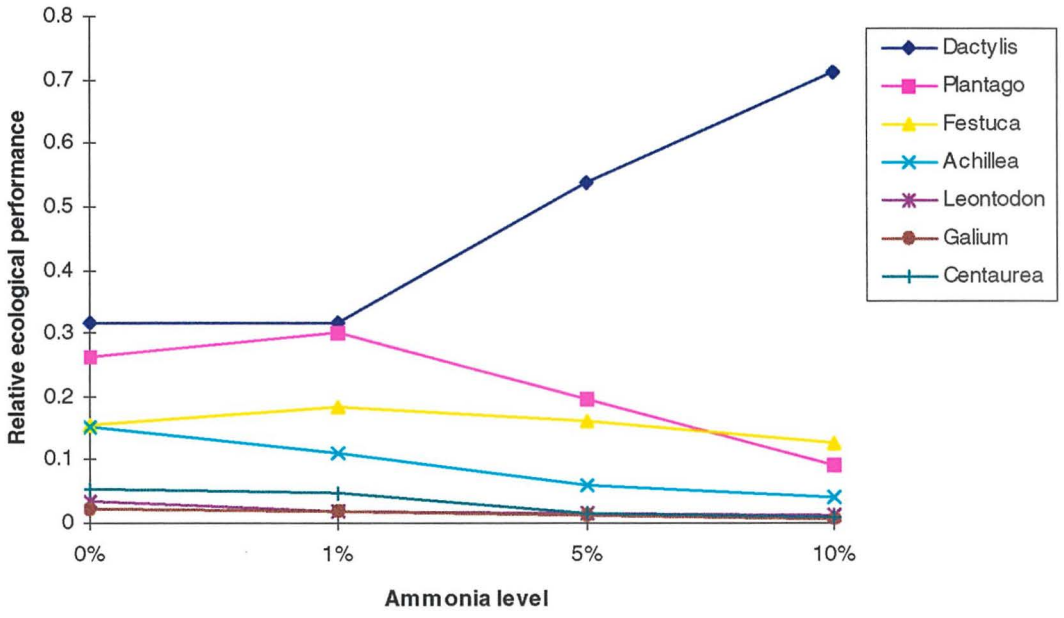


Figure 5.12 Relative ecological performance of each species grown at different ammonia level in the open-topped chambers.

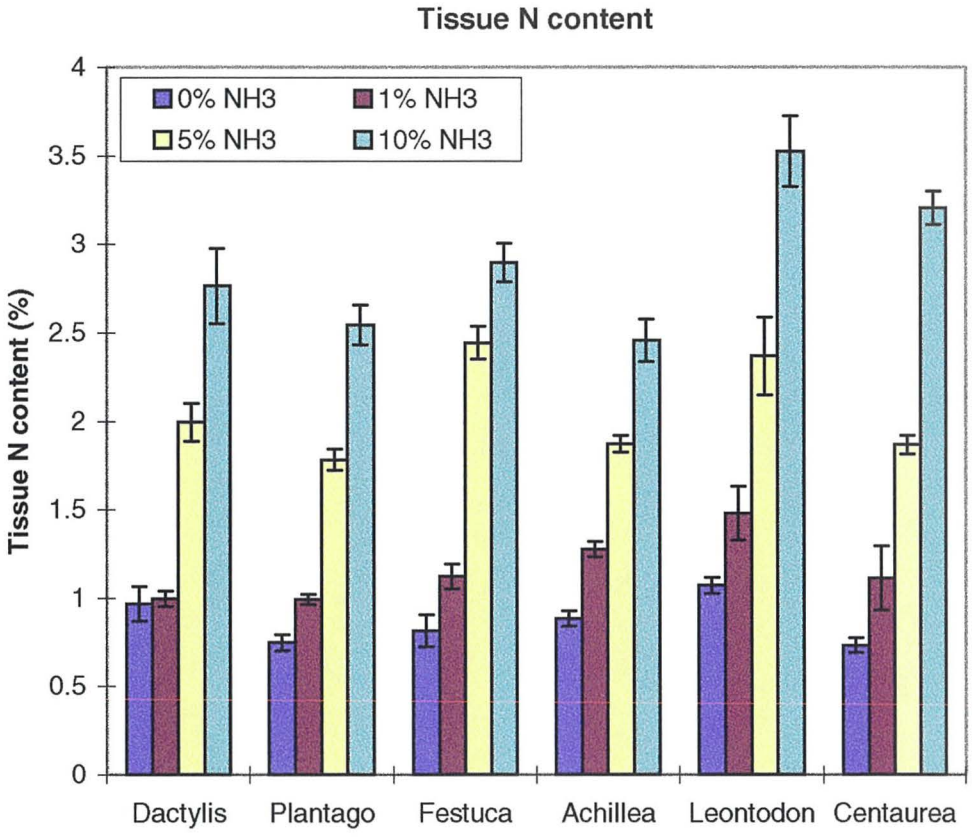


Figure 5.13 N content of each species at different ammonia treatment. Bars show standard error of means.

Analyses of tissue nitrogen content were conducted on six species of the plant communities leaving out the *Galium* species. Data are presented as Figure 5.13. This Figure shows a clear trend of which N content of every species consistently increase with level of ammonia concentrations. ANOVA in General Linear Model indicates that a very highly significant difference of nitrogen content between the level of ammonia concentrations ($p<0.001$) was observed, whereby variance of this treatment effects represent 84.55% of the total variance (Table 5.6).

Moreover, N content was also found different significantly ($p<0.001$) between species. Summary of the Analysis of Variance is presented as Table 5.6.

Table 5.6 *Summary of ANOVA on N content of each species in the ammonia experiment*

Source	% Sum of Square	F-value	P
Block	0.06	0.03	0.871 NS
Treatment	84.55	379.00	0.000 ***
Species	4.77	12.53	0.000 ***
Treatment*Species	3.33	3.05	0.000 ***
Error	7.29		
Total	100		

NS=not significant; *** $p<0.001$

Furthermore, *Leontodon hispidus* consistently shows high tissue nitrogen content compared to other species at all levels of ammonia treatment (Figure 5.13).

Concentrations of ammonia measured using diffusion tubes in all chambers are as Table 5.7. It is obvious that simple method for providing ammonia gas in this experiment has created the concentration gradient successfully.

Table 5.7 *Ammonia concentrations in the open-topped chambers*

Chamber No.	Block	Ammonia level	Ammonia concentration measured ($\mu g\ m^{-3}$)
1	1	0%	0.21
2	1	1%	4.26
3	1	5%	19.82
4	1	10%	35.37
5	2	0%	0.54
6	2	1%	4.28
7	2	5%	24.28
8	2	10%	34.71

5.6 RESPONSE OF PLANT COMMUNITY TO ATMOSPHERIC AMMONIA

This experiment was carried out with a view to determine the response of model plant communities at various distances from the point sources of the atmospheric ammonia released by the chicken farm at Newborough.

5.6.1 Methods

Model plant communities established in pots were exposed to natural atmospheric condition at different locations from the poultry farm (see Chapter 4) at Newborough. Prior to transfer the pots to the sites, four pots for each location were placed in a balconiere covered with a capillary mat on the bottom of the pots (Figure 5.14). This is to ensure that pots are not drying out in dry weather. Pots used in this experiment were slightly smaller (3-liter pot) than used in previous sections in order to fit them into the balconiere.

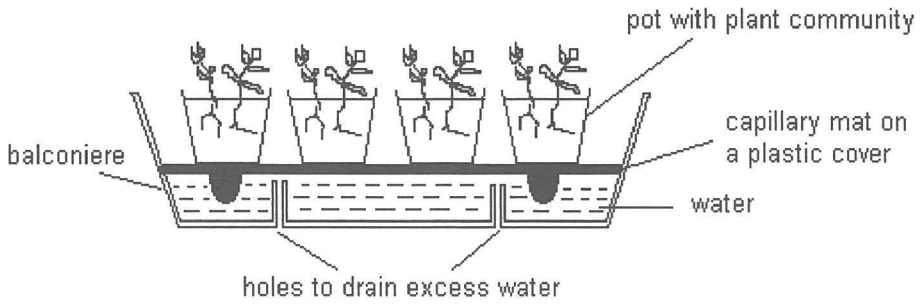


Figure 5.14 Pots with model of plant community in the balconiere

All pots were transferred to the site at Newborough in May 1998 to expose them to the site's atmospheric environment. Five locations at various distances from the poultry farm were chosen, namely, at the farm (Site A), 100 m (Site B), 300 m (Site C), 800 m (Site D) and 2000 m (Site E) southwest direction of the farm. The pots were checked every week to ensure that they were not drying out especially during the hot summer. As in the previous experiments, the same number of doses of 250 ml Long Ashton nutrient solution without the nitrogen element was given to each pot.

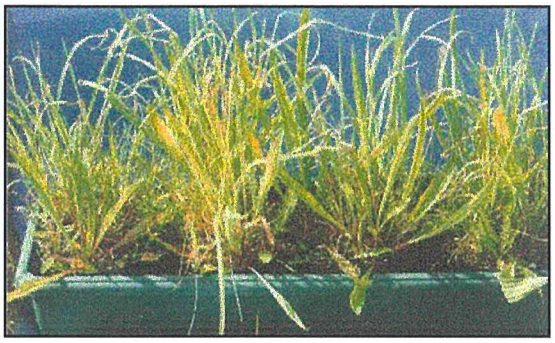
Vegetation in the pots were clipped 5 cm above the top of the pots in August and final harvest was carried out in October 1998 by cutting the vegetation to the ground level. The vegetation was sorted into species and all plant material was dried in the oven at 70 °C for 72 hours. Dry weight of each species was then recorded and total biomass of each pot was determined. Subsequently, all dried plant material was milled for determination of tissue nitrogen content. The milled samples of each species were weighed in aluminium foil cups and analysed for nitrogen using CHN Analyzer Leco 2000.

5.6.2 Results

At the beginning of the experiment the now well-established experimental plant communities had a similar height and growth. We assumed that all pots were similar to each other and set out the four replicate pots in a balconiere at each site. After four weeks exposure on the sites, it was obvious that the plant communities at Site A (Farm) grew vigorously, more greenish compared to other sites distanced from the farm (Figure 5.15). However, pots at Site B (100 m from

Site A (Farm)

Site B (100 m from the farm)



Site C (300 m from the farm)

Site D (800 m from the farm)

Site E (2000 m from the farm)

Figure 5.15 Growth of plant communities at different distances from the poultry farm

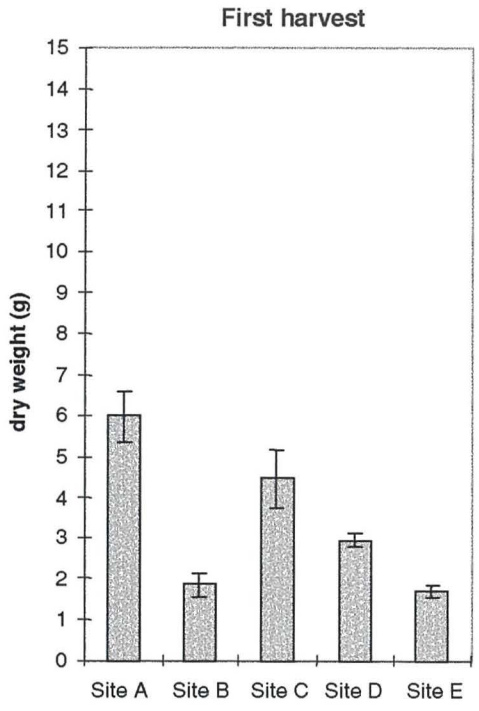
the farm) seemed to have a growth problem; their growth was not as expected, the vegetation height and cover were less than pots at Site C (300 m from farm).

Total biomass of the first harvest and the final harvest for pots at each site were tested for significance using the one-way ANOVA, and these analyses indicate that total biomass were highly significantly different ($p<0.001$) for both harvests. The data are illustrated as Figure 5.16a and Figure 5.16b. Subsequently, total biomass of each pot of both harvests was combined for each site. It is apparent that the biomass reduced significantly ($p<0.001$) with distance from the poultry farm (Figure 5.16c).

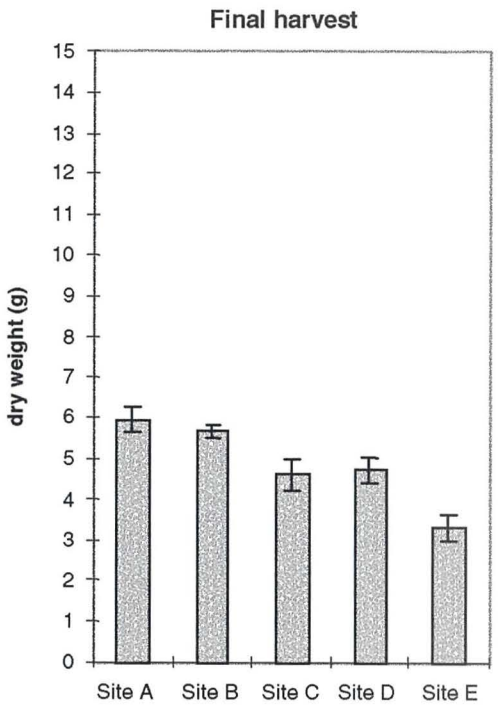
Table 5.8 *Summary of ANOVA of relative ecological performance on species and sites*

Source	% Sum Square	P
Species	89.94	0.000 ***
Site	0.00	1.000
Error	10.06	
Total	100.00	

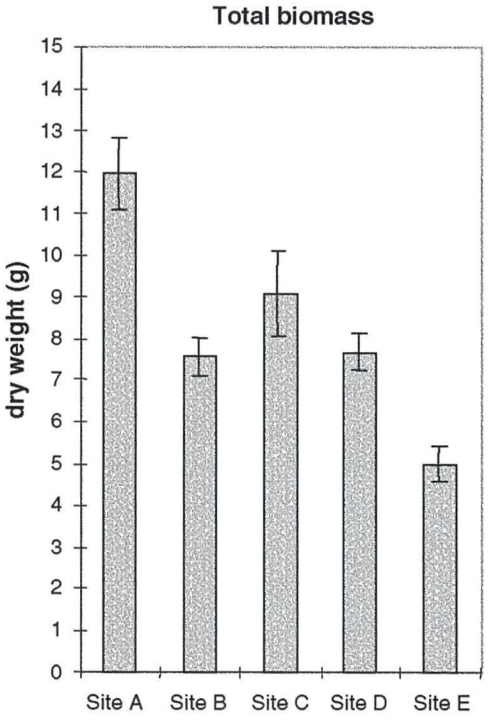
Dry weight of each species of final harvest indicated a clear trend, *Dactylis* showed the highest biomass compared to other species at all sites (Figure 5.17). In general, biomass of most species declined significantly ($p<0.001$) with distance from the poultry farm, though a clear pattern for all species can hardly be distinguished. Relative ecological performance between each species at different sites shows a very highly significant difference ($p<0.001$; Table 5.8). This was then illustrated as Figure 5.18 in which ecological performances are in the order of *Dactylis, Plantago, Festuca, Achillea* the others. The ecological performance of *Dactylis* is the highest at all sites compared with the rest of the species. An



(a) First harvest



(b) Final harvest



(c) Total biomass of first and final harvests

Figure 5.16 (a), (b) and (c). Biomass of models of plant communities grown at various locations from the poultry farm.

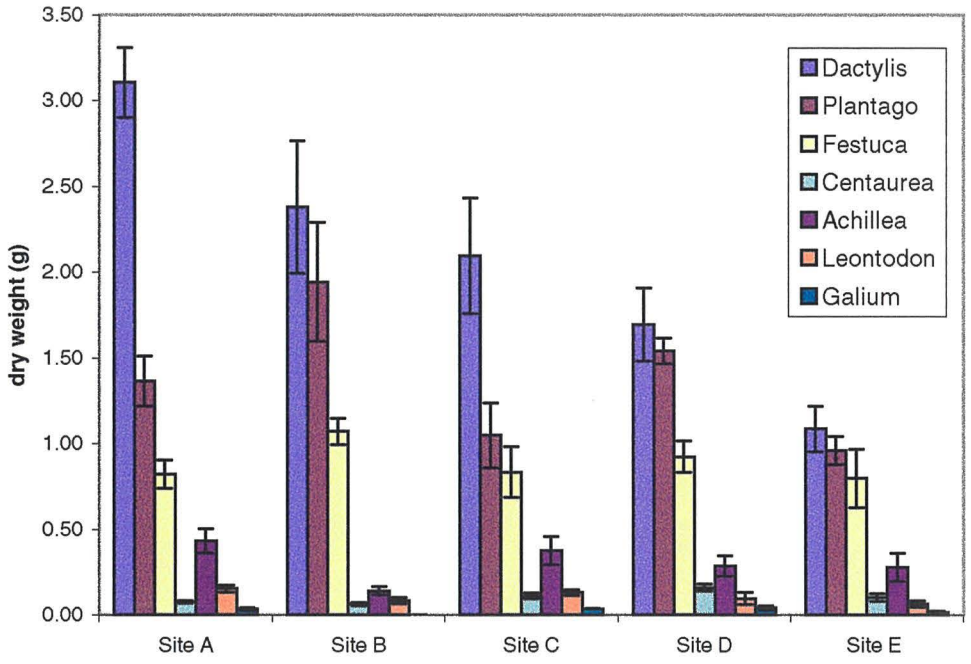


Figure 5.17 Species dry weight of final harvest at different distances from the poultry farm. Bars show standard error of mean (N = 4)

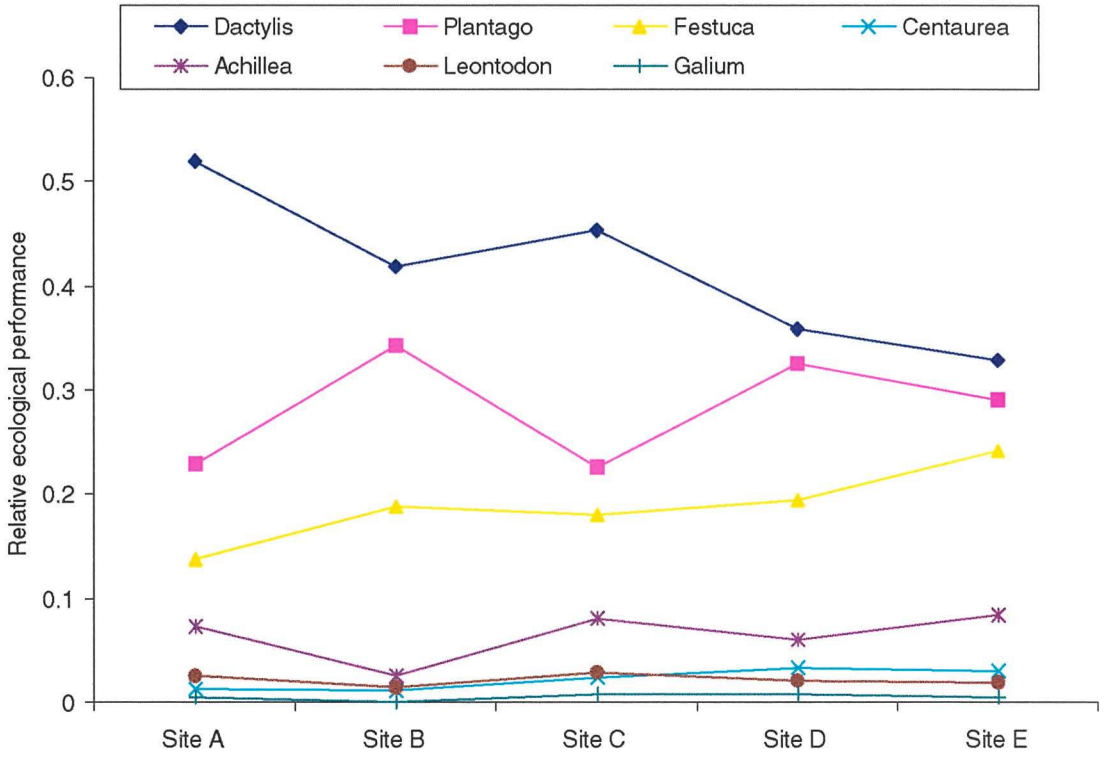


Figure 5.18 Relative ecological performance of each species at different distances from the poultry farm

obvious pattern of *Dactylis*'s ecological performance is apparent whereby it decreased with distance. On the other hand performance of *Festuca* and *Achillea* increased gradually with distance from the farm, but zigzag pattern for *Plantago* is difficult to make any inferences. The ecological performance of *Festuca* is gradually increased with distance from the farm. Other species do not show a clear pattern.

Tissue nitrogen content of each species at each site was also measured, however measurement for *Galium sp.* could not be conducted due to insufficient plant material. Data are illustrated as Figure 5.19. Statistical analysis was conducted on the data to determine significance between sites and species. Analysis of Variance of total nitrogen content showed a highly significant difference ($p<0.001$) between sites and species (Table 5.9). The large F-statistics and low p-value in the analysis of variance table quantify a significant relationship between these two variables. Moreover, regression on the data shows evidence of a negative relationship between distance from nitrogen source and the tissue nitrogen content (R^2=24.8%).

Table 5.9 *Summary of Analysis of Variance of N content between site and species*

Source	% Sum of Square	F-value	P
Site	38.53	31.82	0.000 ***
Species	23.68	16.28	0.000 ***
Site*Species	12.53	2.13	0.009 **
Error	25.26		
Total	100		

*** p<0.001; ** p<0.01

From Figure 5.19, an apparent trend can be seen in which all species at the farm (Site A) consistently show greater nitrogen content compared to other sites. However, it is quite difficult to see trend between species. *Festuca rubra* shows the highest nitrogen content of 1.725% at Site 1, but in general, *Leontodon* seems to have greater tissue N content compared with other species at most of the sites.

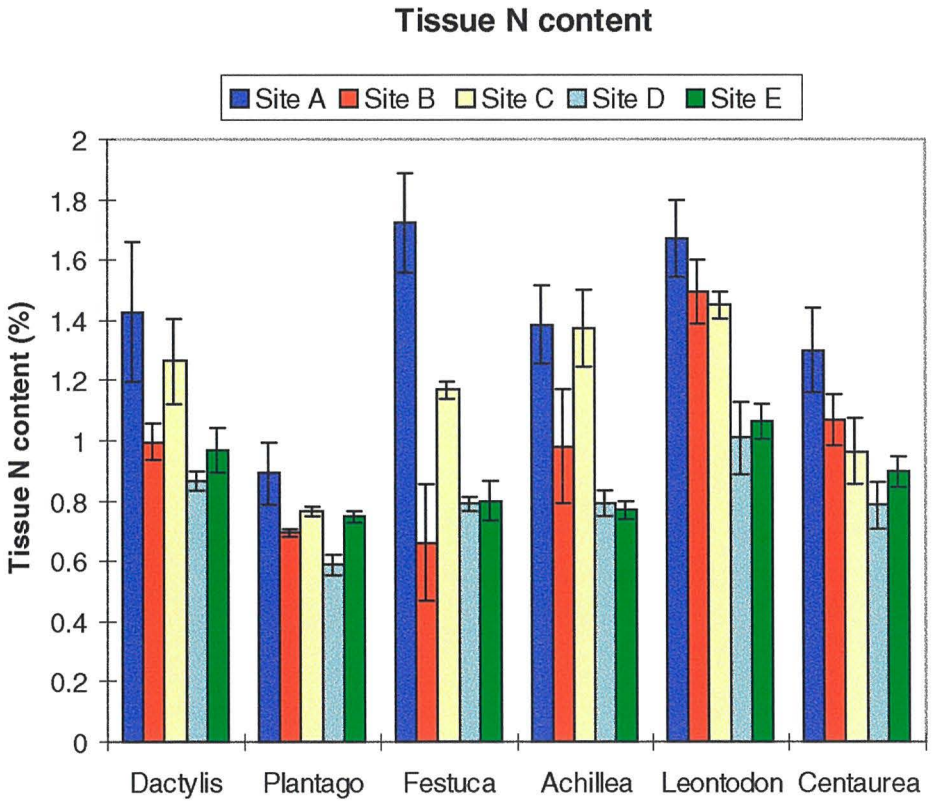


Figure 5.19 Total nitrogen content of each species at five sites on a transect away from the farm. Bars show standard error of means.

5.7 DISCUSSION

The four experiments on effects of various nitrogen treatments on dune plant community clearly showed that there are significant effects on growth of the plants. These differences may subsequently affect species composition at community level. This section will start with a discussion of each experiment and will end with discussing comparison between experiments as a whole.

5.7.1 Plant responses

The very first experiment in Section 5.3 was a simple preliminary experiment to look at plant community response to a nitrogen gradient created by altering the concentration of $NaNO_3$ of Long Ashton nutrient solution. The hypothesis that there is an effect on plant growth of elevated nitrogen level has been demonstrated clearly: plant biomass significantly increased with level of nitrogen concentrations. Austin and Austin (1980) who investigated the behaviour of experimental plant communities along a nutrient gradient reported a similar result in which the yield of plant communities increased with levels of nutrient. The nitrogen gradient employed in this experiment is sufficient large to show the response of plant communities to different nitrogen level, but the range of concentrations was not sufficient to illustrate a full-range of species physiological response. Nevertheless, the result of this experiment is quite significant to compare with other experiments in this chapter.

Experiment on plant communities treated with mist nitrogen (Section 5.4) showed inconsistent results with high variation between pots within treatments, which do not conform to the expected response. Something seemed to be 'wrong' with this experiment as there was significant difference between blocks of the

experimental design as well as the great variation between pots. The mist unit systems are maintained regularly by ITE staff but several possibilities may contribute to the variability. Different tunnels may have different environmental conditions whereby during the hot summer of 1998 several tunnels were directly exposed to sunlight but some of them were shadowed by trees and a small hut nearby. This situation may have caused a very high temperature in the exposed tunnels compared to those that were being sheltered. High temperature has been proven to contribute effects on plant growth by affecting the photosynthesis (e.g. Dilks and Proctor, 1975). Although pots were rotated at 2-month interval to reduce any positional effects relative to the spray nozzles, the distribution of NH_4NO_3 mist in the tunnels may be not satisfactorily even. At some point, drips of NH_4NO_3 may have leaked out from the hose into some of the pots. Perhaps pots that received these drips have higher amount of nitrogen received, hence causing inconsistent results of growth between pots within tunnels. Nevertheless, an experiment on *Deschampsia flexuosa* transplanted in sand and exposed in these tunnels by Truscott (1997) revealed a significant increased in leaf and tiller number in response to increased atmospheric nitrogen deposition levels. Furthermore, some pest problems also contributed to unpromising result of this experiment. During the hot and wet summer 1998, slugs were found in a high number in most of the tunnels and it was apparent that they grazed some of the species in the experimental pots. The species that were badly affected were *Centaurea nigra, Achillea millefolium* and *Leontodon hispidus*, which caused a reduction of their dry weight. Since these mist units were also used for other experiments using core of natural grasslands, these were also one of the factor that attract the slugs for grazing. Pesticide such as slug pellets was applied in the tunnels but it could not totally prevent the slug damage.

Another form of nitrogen taken up by plants is ammonia gas. The experiment in Section 5.5 clearly showed that there were biological responses of plant communities to ammonia gas. These include visible injury suffered by plants exposed to higher ammonia concentration, and also an increase in biomass with increased ammonia levels. Visible injury of the foliage due to direct toxicity of NH_y pollution has been reported in many ammonia-related experiments. Ewert (1979) conducted a study on effects of ammonia gas on pine needles in which he reported that greenhouse fumigation with acute concentrations of NH_3 (2-28 mg m^{-3}) caused injuries at older needles of *Pinus sylvestris, P. nigra* and *P. mugo*. In the section 5.5 experiment, not all species of the plant communities showed visible injury to higher ammonia concentration. *Dactylis glomerata* and *Plantago lanceolata* are the species that most obviously showed these injuries. This phenomenon occurs due to different assimilation capacity of different plant species, which determines the degree of injury. If the assimilation capacity is not sufficiently high to detoxify NH_y, acute (visible) injuries may occur (Fangmeier *et al.,* 1994). The aforementioned experiment by Ewert (1979) showed that visible symptoms differ with plant species of which in broad-leaved trees, he mainly found black discolourations whereas conifers mostly showed brown necrosis. Furthermore, Van der Eerden (1982) described black spots on cauliflower (*Brasicca oleracea*) and sharply bordered necrotic tips of older needles of *Taxus baccata* to be specific of NH_3. In terms of dry weight, it has been shown from this experiment that biomass of plant species is significantly increased with increased levels of ammonia concentration. This finding agrees with other finding of Dueck *et al.* (1991) who observed a positive response of *Deschampsia flexuosa* to NH_3 with an increase of more than 50% in dry weight when exposed to 0.1 mg m^{-3} NH_3 after four and after nine weeks.

In this experiment, the source of ammonia gas provided by evaporation of ammonia solution at appropriate concentration successfully created a gradient of concentrations (Table 5.7). This can be related to the real situation when plant communities are exposed to the point sources of ammonia such at Newborough study area (Chapter 4). The control treatment (0% NH_3) gives concentration of an average of 0.38 µg m^{-3} for both replicate chambers, which reflects a background concentration similar to most surveys on atmospheric ammonia (Chapter 4). The highest level of ammonia at 10% NH_3 of both replicate chambers give an average of 35.04 µg m^{-3}, which is approximately half of the annual mean emitted at the poultry farm at Newborough (60.05 µg m^{-3}).

In the final experiment (Section 5.6), the similar models of plant communities were exposed at Newborough sites to look at their response to atmospheric ammonia from the point sources of the poultry farm. The result of this experiment was quite exciting whereby a clear response of plant communities to atmospheric ammonia could be seen. Although injuries were not clearly visible on plants located near the farm, the most distinguished response was the colour of the leaves of plants at the farm compared to those located distance from the farm. Leaves at the former location were very greenish compared with yellowish leaves of plants at Site D and Site E (Figure 5.5). Atmospheric ammonia is thought to be the only nitrogen source when other mineral nutrients were supplied in the Long Ashton nutrient solution, therefore yellowish colour observed on leaves indicated that plants were nitrogen deficient. Observations of responses of vegetation to atmospheric ammonia were made mostly on trees on a local scale in the vicinity of NH_3 sources such as livestock farms. Examples are of Van der Eerden (1982) who observed injuries on conifers exposed to NH_3 in the field near

a pig farm, and Pitcairn *et al.* (1998) observed damage on spruce and pine trees near poultry farms in Scotland.

Biomass of vegetation in pots near the farm was significantly higher compared to those out in the dune. However, total biomass of pots at Site B (Fig. 5.16c) seemed lower than Sites C and D. This phenomenon occurs probably due to location of the pots at that particular site. At this site, pots were exposed to strong wind, and no other better place could be found at this site to give shelter to the pots. In contrast, all pots at other sites have at least tall grasses to shelter them from the wind. It has been shown by Retuerto and Woodward (1992) that windspeed affected growth and biomass allocation of white mustard compared to those grown under lower windspeed treatment. Increased biomass production in response to nitrogen deposition is generally as a result of shoot growth. Root growth increases only little or at least less than shoot growth, leading to higher shoot/root ratios (Encke, 1986). Vermeer (1986) found that increased nitrogen supplies promoted increased shoot biomass production in *Molinia* as a result of atmospheric nitrogen deposition. From this experiment, it can be concluded that plant communities in pots take ammonia through the leaves for their nitrogen supply. Faller (1972) showed that in a series of experiments where plants were grown without the addition of N to the root, plants were able to use gases NH_3 and NO_2 as their sole N source. However, not all cases show increase of biomass with regard to plant response to nitrogen deposition. Reduction of weight is also observed in the experiments here (Figs. 5.5 and 5.10b), which is assumed as one of deleterious effects of nitrogen deposition on several species of plant community. Species of *Plantago* and *Festuca* are seen to have this phenomenon (Figs. 5.6 and 5.11) whereby the adverse effects of nitrogen

deposition may result from direct toxicity, increased susceptibility to stress (e.g. increased frost and drought sensivity) and induction of nutrient deficiency.

In the experiments of Section 5.5 and Section 5.6, N content of each species was measured. Both experiments revealed similar results whereby tissue nitrogen content of each species is higher at higher ammonia level or near the point sources of ammonia (Figs. 5.13 and 5.19). Increased N contents were also found in conifers in fumigation experiment (van der Eerden *et al.,* 1992) and in field studies near NH_3 sources (Van Dijk & Roelofs, 1988).

5.7.2 Competitive ability

Relative ecological performance (Austin & Austin 1980) is applicable to evaluate competition of species under nitrogen treatment in multispecies mixture. In the first experiment (Section 5.3) of seven species mixture, relative ecological performance curve shows only an obvious response *of Oenothera stricta* at low N level to 170 ppm, but varied behaviours are indicated by other species (Figure 5.3). Competitive ability based on ecological performance is not totally independent of nutrients, for instance, *Oenothera* has a greater competitive ability than *Plantago* at lower N levels, but smaller one than *Plantago* at higher N levels. At low N levels up to 170 ppm, the order of competitive ability of the species based on relative ecological performance is *Oenothera* > *Echium* > *Plantago* >=< *Festuca* > *Centaurea* > *Briza* > *Galium*.

As for the subsequent experiments, the changes of species could not be avoided due to problems of germinating *Oenothera* and *Echium* seeds, though several techniques such as exposing the seeds to cold shock before germination was

employed. I used *Oenothera* in the preliminary experiment as it is an indicator species in the dune system. This is because Kachi and Hirose (1983) used the *Oenothera glaziovana* as an indicator plant on well-drained, semi-fixed dunes in the Azigaura dune system, Japan, which has an area of about 12 km^2 faces the Pacific Ocean. Nevertheless, the replacement species are all found in the range of dune communities, and therefore this will not have affected the main objective of the experiments to look at dune community response to nitrogen treatment.

Experiments in Section 5.4, 5.5 and 5.6 used similar model of plant communities. In all these experiments, three species i.e. *Dactylis glomerata*, *Plantago lanceolata* and *Festuca rubra*, could be distinguished by their competitive ability from the other experimental herbs. The order of competitive abilities that can clearly be seen from Figures 5.12 and 5.18 is *Dactylis* > *Plantago* > *Festuca* > *Achillea*. The reverse order is seen in the mist experiment (Section 5.4) whereby the order is *Plantago* > *Dactylis* > *Festuca* > *Achillea*. The reversal order of *Dactylis* and *Plantago* may be due to the problems of this experiment as was discussed in the previous section, in which *Plantago* clearly shows its dominance in terms of the dry weight. The other herb species *Centaurea, Leontodon* and *Galium* could not be separated clearly to fit them in the order of competitive ability. Comparing ecological performances of species in the experiments in the open-topped chamber and in the field, the patterns of their performances are quite similar (Figure 5.12 and Figure 5.18), with an exception at Site B (Figure 5.18) whose result was influenced by the wind speed.

Species with small size such as *Festuca, Achillea, Centaurea, Leontodon* and *Galium* are strongly influenced by big plants, *Dactylis* and *Plantago*. Still less are

there any grounds from the results for asserting on the basis of such evidence that small plants are competitively superior to large plants (Newbery & Newman, 1978), a conclusion at variance with the weight of published evidence (e.g. Ross & Harper, 1972; Mahmoud & Grime, 1976; Alexander & Thompson, 1982). It can be concluded from these experiments that plant vegetative size is quite important to interspecific competition. Species with large size usually have a higher competitive ability than small-size species. The result of the first experiment (Section 5.3) also supports this conclusion.

Of the seven species of the dune grassland, the *Dactylis glomerata* is absolutely stronger in competition than the rest of the species whose competitive ability varies along nitrogen gradient. Competitive ability of *Dactylis* increases when N concentration increases, however that of *Festuca* increased when N levels are reduced (Figure 5.18 – not clearly apparent in Figure 5.12). It might happen that *Festuca* might exceed *Dactylis* in the competitive ability when nutrition is reduced to some very low levels. Further experiments are needed to test this idea. Small species such as *Festuca* and *Achillea* have less competitive ability than *Dactylis* under controlled conditions, but they have some characteristics to give predominance in some grasslands. *Festuca* with a small size has a remarkable adaptive ability. It is the most widely distributed species in semi-natural grasslands and is present in almost all habitats, and grows well if its competitors such as *Dactylis,* are absent or do not grow well. Even though in some area where its competitor is dominant, *Festuca* can at least coexist. Once its competitor is removed by grazing or some other interference, it will develop quickly in that area.

Short-term community experiments on environmental gradients often show that response of small herbs could not be clearly observed. In all experiments here, small herbs such as *Leontodon* and *Galium* failed to utilise the highest N concentration to attain their highest biomass, whereas for most of the graminoids it did favour growth. Falkengren-Grerup (1998) reported this similar phenomenon, he interpreted it by suggesting that the graminoids have a lesser demand for other nutrients than for N. Moreover, Falkengren-Grerup (1998) stated that in experiments involving the addition of N, species that are not limited by other elements usually tend to show a high potential for biomass increase even at relatively high N concentrations. It can be suggested here that the more positive response of the grasses (graminoids) to high concentrations of N suggests that, if the results are applicable to field conditions in dune grassland soil, graminoids are more competitive than herbs. A study by Rosén *et al.* (1992) in Swedish coniferous forests revealed that narrow-leaved grasses (mostly *Deschampsia flexuosa, Agrostis capillaris, Festuca ovina*) were found to be more competitive than *Vaccinium* species. Grasses have also been shown to be highly successful competitors in Dutch heathlands, ecosystems that are low in nutrients (Van der Eerden *et al.,* 1991; Berendse, 1990). It can be concluded that grasses under such conditions may expand in relation to herbs since they increased in growth at N concentrations at which few positive effects were found for herbs. This in return would affect species composition at community level of particular ecosystem due to the increased nitrogen deposition into the ecosystem.

As a conclusion, the consequences of foliar uptake of ammonia are very significant and should not be underestimated. In terms of plants and ecosystems it can result in a switch in the competitive ability through eutrophication or

fertilization, but also may impair physiological stability in susceptible species. With the current levels of global atmospheric nitrogen inputs, then the effect on ecosystems will indeed be harmful.

CHAPTER 6: GENERAL DISCUSSION

CHAPTER 6

GENERAL DISCUSSION

This thesis is an investigation into the anthropogenic nitrogen inputs to a dune ecosystem at Newborough, and the impacts of the inputs on the vegetation community of the system. As nature reserves need protection from any damaging sources, a threat from excess atmospheric nitrogen would be of great concern to the local authorities. This is because many reports are relating the detrimental effects of excess N on various ecosystems (e.g. Bobbink *et al.,* 1993), which could lead to a decrease in nature conservation values. The existence of a large poultry farm near the Reserve is really a worrying factor because a poultry farm is clearly a point sources of atmospheric ammonia; but prior to this study no survey had been done in this area to determine concentration of atmospheric ammonia, and subsequently to take precaution measures.

Atmospheric inputs of nitrogen have been investigated in many studies in Britain and throughout Europe (discussed in Chapter 1), however studies on specific sites are still needed, for example at Newborough itself, in order to recognise the importance of the nitrogen inputs locally. This discussion focuses on four main themes of the work. They are: (i) methodological problems (ii) anthropogenic N deposition to the study area (iii) soil and vegetation community composition, and (iv) experimental investigation of species response to N.

-The Methodology

Since this study started with a very broad topic to discuss and investigate, various methodologies were employed in order to obtain data relevant to atmospheric N inputs. The diversity of surveying methods inevitably produced uncertainties in several parts of the results. In the second chapter on the survey of Newborough study area, a quadrat of 10 cm square was used to sample above ground vegetation. This quadrat size was chosen as the smallest practical basic unit of harvesting. Morton (1970) also used similar quadrat size for his vegetation sampling. However, one critical issue of the sampling method using this quadrat has been raised, especially when it involves sampling of tall grasses such as Marram grass (*Ammophila arenaria*) and Oat grass (*Arrhenatherum elatius*). The issue was whether to sample column of these grasses that occur within the quadrat or just simply cut all above ground vegetation in the quadrat by pressing the quadrat on the ground, without holding up the tall vegetation. Since the objective was to measure biomass of vegetation per unit area for the purpose of calculating total N per unit area, I used the latter option which is thought as the better one to get an approximation of biomass of a particular area. The use of former option creates difficulty in terms of getting the lying vegetation up, especially when it involves thick tussocks that have laid on the ground for a long period of time. Nevertheless, this issue is still open for discussion because of its subjectiveness.

The changing of analytical instrument for N measurement could not be avoided. In this case, the Kjeltec AutoAnalyzer for measuring total N in vegetation and soil samples using acid digestion method was replaced by a new CHN AutoAnalyzer Leco 2000 machine for soil and vegetation analyses for 1998 sampling. The new

machine produced results of total N more or less accurate for vegetation samples, but its accuracy is doubted for measuring total soil N compared to acid digestion method (Kjeldahl method-section 2.2.1). Reynolds (pers. comm.) noticed underestimate readings in several of his soil analyses using the CHN AutoAnalyzer, especially for mineral soil, but the reduction factor is not known because no standard soil with known total N is measured to compare with the reading of the machine. Nevertheless, as for vegetation analysis, a standard apple leaf sample was run on the machine in which it gave a reading of total N of 2.12%, and this is in the range of its known total N content of 2.25 ± 0.19. Therefore the reading of CHN AutoAnalyzer for the total N of vegetation in the 1998 sampling is reliable with previous machine of Kjeltec AutoAnalyzer using acid digestion method.

The methodology employed in the vegetation survey proved to be one of the most subjective and difficult decisions. Bryophytes were not included in the survey due to the difficulty in identifying them in the field, and also because of the surveys were only intended to have a general idea of vascular species composition in the study area. In analysis of the vegetation data, detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA) efficiently provide outputs to look at ordination and relationships of vegetation with the environmental parameters respectively. The consequence of this thesis shows DCA and CCA produce identical interpretation in relation to vegetation ordination (Figures 2.13, 2.14 and 2.18). Because the main purpose of ordination is to study the relationships between vegetation and environmental variables (ter Braak, 1987), CCA has advantages in its ability to combine vegetation measurements with environmental factors over DCA. As a result, CCA shows

the relationships in the same ordination diagram in which CCA has simplified the analysis. To do this on DCA, further analysis such as correlation analysis or display environmental variables against DCA ordination axes would need to be done after the initial ordination.

In the Chapter 4 of atmospheric ammonia survey, the use of two monitoring methods (badge samplers and diffusion tubes) were compared (see Section 4.4). Badge sampler is a new method compared with commonly used diffusion tube, and the former is still under development under the EC GRAMINAE project. As mentioned in the Section 4.3 that a provisional correction factor has been applied on the diffusion tube data, however a few publications on this topic (e.g. Goulding, 1990; Goulding *et al.,* 1998) did not mention whether their published data were being corrected or not. Therefore this contributes to the uncertainties in terms of actual atmospheric ammonia concentration surveyed. Nevertheless, the recent RGAR report (RGAR, 1997) used correction factor of 0.42 for the ammonia data obtained from surveys using the diffusion tubes.

It should be realised that the implementation of both methods in this thesis is critical, and this would not necessarily apply to other peoples' tubes. For example, Sutton (per. comm.) mentioned that he found background concentration of 3 $\mu g\ m^{-3}$ at a study site in Cheshire with validation by independent active sampling, but Caporn (unpublished data) using a consultancy service, found concentration of 30 $\mu g\ m^{-3}$ at the same area, which shows an order of magnitude too large. Nevertheless, the use of uncorrected data of badge samplers and diffusion tubes are hoped to be sufficient for the purpose of getting a general idea of atmospheric ammonia concentration at Newborough.

-Anthropogenic N deposition

Concentrations of atmospheric nitrogen dioxide and ammonia in this study indicate the importance of the latter, whereby the concentrations measured at the point source are extremely high compared to other findings (see Chapter 4). The concentration of atmospheric nitrogen dioxide is seemingly not as high as in other reports (see Chapter 3) because the study site is located in a rural area, which is away from major sources of the gas. Between these two pollutants of NO_x (NO and NO_2) and NH_y (NH_4^+ and NH_3), N deposition caused by NO_x is not so important close to sources and relatively more NO_x and reaction products are exported than is the case for NH_y (Asman *et al.,* 1998). Nevertheless, the importance of this pollutant should not be ignored because Fowler *et al.* (1998a) emphasized the huge increase in the rate of NO_x (NO and NO_2) emissions into the atmosphere from 1 Tg N y^{-1} to over 20 Tg N y^{-1} during the 100 year between 1880 and 1980; and they predict that this rate of emission from anthropogenic sources would increase to 46 Tg N y^{-1} by the year 2025.

It is clearly shown from atmospheric ammonia monitoring that there are anthropogenic N that are distributed in the atmosphere at the Newborough study area. As discussed in Chapter 4, it is clear that the dry deposition of NH_3 shows a high spatial variability, because it depends on the many scattered local sources of NH_3. This phenomenon implies that measurements from very many sites would be needed to get a reliable estimate of the deposition of NH_3 to a particular country. The uncertainty in the quantity deposited is more related to the implementation of sampling methods (discussed in previous section), which is quite considerable. However it should be noted that uncertainties are also due to the individual processes which determine the rate of deposition including surface

wetness and the characteristics of airflow within and over the study area, which were not measured at the ammonia monitoring sites.

-Soil and Vegetation Community Composition

In the second chapter where surveys of the area were carried out, a clear trend of increasing total N of vegetation and soil could be observed along transects from coastal sites to inland sites. It should be noted that there was no exact gradient between sites along the transect line, and therefore no exact trend of increasing or decreasing of environmental parameters investigated.

Distribution of vegetation and the distribution patterns of dominant species in the dune are highly related to environmental variables (Figure 2.16). Among the environmental factors, soil organic matter, pH, total N and moisture are seen more important than the other factors. However the relationships between plant species and their environment, between plant populations and between environmental variables in the study area are every complicated. This thesis shows that species are non-linearly related to each other. The ordination techniques, DCA and CCA are the right way to describe these relations. Apart from the above environmental factors, atmospheric N input is thought to have effects on the vegetation composition and distribution at Newborough dune system. However, the relationship on this factor could not be investigated directly using the CCA because the number of data from the atmospheric pollutant survey (especially NH_3) are not enough to conduct the analysis, whereby no ammonia survey was carried out at several sites near the coastal area of the dune. Nevertheless, two surveys on anthropogenic N inputs (Chapter 3 and Chapter 4) provide good information on the levels of these pollutants for the area,

which has been discussed in the previous section. One crucial factor that should not be neglected in relations to species composition and distribution to the dune ecosystem is the grazing factor. Although this factor is not investigated here, it has been shown by Hewett (1985) at Newborough that the factor was significant in influencing the distribution and species composition. This is strongly supported by ten Harkel & van der Meulen (1995) who found significant changes on species composition in species-rich dry dune grassland after exclusion of grazing, rather than the N fertilization addition on their experimental plots. However, their finding is not consistent with fertilizer experiments in other grasslands (Willis, 1963; Van Hecke *et al.,* 1981; Bobbink *et al.,* 1988; Bobbink, 1991).

-Experimental investigation of species response to N

The easiness of NH_3 uptake directly by leaves of most species (Van der Eerden *et al.,* 1990) might cause a shift in the competitive relationships between the faster growing grasses and the dicotyledons. Several pot experiments containing models of dune plant communities were conducted in this thesis. Although they look simple, they produce significant results on the impacts of the gases on the plant community. There have been a few experiments in which the effects of wet deposition of reactive N on ecosystem processes have been studied in realistic simulations. Those that have been made involve studies in which precipitation falling on a roof over vegetation is collected, its composition modified, and the modified solution sprayed onto the vegetation with minimal delay (e.g. Wright *et al.,* 1988). However these experiments are expensive to perform, and inevitably involve some modification of the aerial environment under the roof. As the atmospheric ammonia and nitrogen dioxide influence the growth of plants at species level, they may subsequently influence the plants at community level.

This might involve in changing of species distribution and composition. Responses of model dune plant communities to the several nitrogen addition treatments in the experiments (not so clear for mist experiment in Section 5.4) might affect relative performance of each species in the community but these experiments do not show this clearly.

Both experiments in Sections 5.5 and 5.6 demonstrated that the ammonia gas does increase plant growth, indicated by increasing dry weight. These are in accord with other studies of plant responses to this pollutant gas (e.g. Rowland *et al.,* 1987; Wellburn, 1990; Dueck *et al.*, 1991). The responses to pollutant gases reflect that some of the N is taken up by plants through foliar uptake mainly via stomata, and uptake via cuticle is negligible (van Hove *et al.,* 1991). The immediate fate of absorbed gases is still largely a subject of conjecture. The uptake and assimilation of gases in leaves have been discussed thoroughly in several publications (e.g. Pearson & Stewart, 1993; Stulen *et al.,* 1998; Cape, 1998). All of them agreed that the ammonia gas dissolves in the apoplastic water film in leaves to form NH_4^+; NO_2 dissolves to form NO_3^- and NO_2^-, before the assimilation process could take place in the leaves (see those publications for details).

Furthermore, most of the pot experiments in this thesis appear consistent with the Gaussian species-environment model (Austin and Austin, 1980; ter Braak, 1987; Jongman *et al.*, 1987). But, because the nitrogen gradients in the experiments of this thesis are not long enough, most species cannot give their full response curves. In Section 5.3, *Oenothera stricta* and *Briza media* reached their maximum yield at nitrogen levels of 170 ppm and 340 ppm respectively (Figure

5.1), but the other species have not or just reached their maximum yield at the highest nitrogen level of 680 ppm. Austin and Austin (1980) used a nitrogen gradient varied from 1/64x to 16x Long Ashton nutrient solutions, and most species in their experiments appeared to give the full response curves which were unimodal. In other experiment in Section 5.5, *Dactylis* seems to acquire more dry weight with increased level of NH_3, but *Plantago* and *Festuca* are showing response curves whereby they reached the maximum yield at 5% NH_3.

Of the seven species in the plant community model in experiments in Section 5.5 and 5.6, *Dactylis* is the highest in competitive ability and *Plantago* is the highest in Section 5.4. The results of these experiments indicate that large plants and quick growth plants are competitively superior to small plants and slow growth plants (Ross and Harper, 1972; Mahmoud and Grime, 1976). Usually large plants, especially grasses, grow more quickly than small plants. They pre-empt common resources and occupy much space (as a resource) and thus shade small plants. For small plants such as *Centaurea, Leontodon, Achillea* and *Galium,* some resources can not meet the levels demanded due to competition from the large plants. These are the probable reasons of the outcome of the performance for each species in response to the nitrogen treatments as well as the competition between species in the community.

-Conclusion

Background concentrations of atmospheric ammonia and nitrogen dioxide in the dune were at low levels. These concentrations are less than the published critical loads suggesting that for this particular ecosystem (see section 4.4) the dune vegetation is not at a high risk. This study concludes that anthropogenic input of

ammonia from the point sources of the chicken farm at Newborough significantly contributes to the air pollution especially within 1000 m of the source. Effects of these pollutants on vegetation have been demonstrated by the analysis of foliar N in the field and the pot experiments, which are shown by increase in growth of fast growing grasses. This study therefore provides another example of the impacts of anthropogenic nitrogen on natural ecosystems and shows that knowledge of those impacts should be part of the development process.

REFERENCES

Abdy, E. & Mayhead, G.J. (1988). *A Review of Literature and Documents Relating to the Newborough Forest Area.* Part I. Report to the Forestry Commission Wales Conservancy, Aberystywyth. School of Agricultural Forest Sciences, University College of North Wales.

Aerts,R., Berendse, F., De Caluwe, H. & Schmitz, M. (1990). Competition in heathland along an experiment gradient of nutrient availability. *Oikos*, **57**: 310-318.

Aerts, R. & Heil, G.W. (1993). *Heathlands: Patterns and Processes in a Changing Environment.* Kluwer Academic Publishers, Dordrecht, The Netherlands.

Alexander, K.I. & Thompson, K. (1982). The effect of clipping frequency on the competitive interaction between perennial grass species. *Oecologia*, **53**: 251-254.

Allen, A.G., Harrison, R.M. & Wake, M.T. (1988). A meso-scale study of the behaviour of atmospheric ammonia and ammonium. *Atmospheric Environment,* **22**: 1347-1353.

Allen, S.E., Grimshaw, H.M., Parkinson, J.A. & Quarmby, C. (1989). *Chemical Analysis of Ecological Materials.* Blackwell Scientific, Oxford.

Ashenden, T.W. (1979). The effects of long term exposures to SO_2 and NO_2 pollution on the growth of *Dactylis glomerata* and *Poa pratensis* L. *Environmental Pollution,* **18**: 249-258.

Ashenden, T.W. (1991). Rural levels of nitrogen dioxide pollution in Wales. *Proceedings of Acid Deposition in Gwynedd Conference 16-18/3/89.* Snowdonia National Park Authority.

Ashenden, T.W., Bell, S.A. & Rafarel, C.R. (1990). Effects of nitrogen dioxide pollution on the growth of 3 fern species. *Environmental Pollution*, **66**: 301-308.

Ashenden, T.W., Edge, C.P. & Bojanic, D. (1992). The Use of Diffusion Tubes for Monitoring Pollutants. *Proceedings of Environmental Protection – The Problems Facing Eastern Europe*, Drouzhba, Varna, Bulgaria.

Ashenden, T.W. & Edge, C.P. (1995). Increasing concentrations of nitrogen dioxide pollution in rural Wales. *Environmental Pollution*, **87**: 11-16.

Ashenden, T.W., Jones, M.L.M. & Rafarel, C.R. (1997*). Critical levels and loads of nitrogen for vegetation growing on different soils.* ITE Bangor Final Report.

Ashenden, T.W. & Mansfield, T.A. (1978). Extreme pollution sensitivity of grasses when SO_2 and NO_2 are present in the atmosphere together. *Nature*, **273**: 142-143.

Asman, W.A.H. (1992). Ammonia emission in Europe: updated emission and emission variations. RIVM Report, 228471008. RIVM, Bilthoven.

Asman, W.A.H., Sutton, M.A. & Schjørring, J.K. (1998). Ammonia: emission, atmospheric transport and deposition. *New Phytologist,* **139**: 27-48.

Atkins, D.H.F. & Lee, D.S. (1992). The distribution of ammonia in the United Kingdom. AEA Technology AEA-EE-0469, Harwell Laboratory, Oxfordshire.

Atkins, D.H.F. & Lee, D.S. (1995). Spatial and temporal variation of rural nitrogen dioxide concentrations across the United Kingdom. *Atmospheric Environment,* **29**: 223-239.

Austin, M.P. (1976a). On non-linear species response models in ordination. *Vegetatio*, **33**: 33-41.

Austin, M.P. (1976b). Performance of four ordination techniques assuming three different non-linear species response models. *Vegetatio*, **33**: 43-49.

Austin, M.P. (1980). The role of certain diversity properties in the standardization problems of numerical vegetation classification. In: A.N. Gillison & D.J. Anderson (eds.) *Vegetation Classification in the Australian Region.* Australian National University Press, Canberra.

Austin, M.P. & Austin, B.O. (1980). Behaviour of experimental plant communities along a nutrient gradient. *Journal of Ecology*, **68**: 891-918.

Austin, M.P., Groves, R.H., Fresco, L.M.F. & Kaye, P.E. (1985). Relative growth of six thistle species along a nutrient gradient with multispecies competition. *Journal of Ecology,* **73**: 667-684.

Avery, B.W. & Bascomb, C.L. (1974). *Soil Survey Laboratory Methods.* Soil Survey Technical Monograph No. 6, Harpenden. Batholonew Press, Dorking.

Banard, W.R., Andreae, M.O., Watkins, W.E., Bingemer, H. & Georgi, H.W. (1982). The flux of dimethylsulfide from the oceans to the atmosphere. *Journal of Geophysical Research,* **87**: 8787-8793.

Banin, A. (1986). Global budget of N_2O: The role of soils and their change. *Science of the Total Environment,* **55**: 27-38.

Batey, T. (1982). Nitrogen cycling in upland pastures of the United Kingdom. *Proc. R. Soc. B.,* **296**: 551-556.

Berendse, F. (1990). Organic matter accumulation and nitrogen mineralization during secondary succession in heathland ecosystems. *Journal of Ecology*, **78**: 413-427.

Black, C.A. (1968). *Soil-Plant Relationships.* Second Edition. Wiley, New York.

Bobbink, R. (1991). Effects of nutrient enrichment in Dutch chalk grassland. *Journal of Applied Ecology,* **28**: 28-41.

Bobbink, R., Bik, L. & Willems, J.H. (1988). Effects of nitrogen fertilization on vegetation structure and dominance of *Brachypodium pinnatum* (L.) Beauv. in chalk grassland. *Acta Botanica Neerlandica,* **37**: 231-242.

Bobbink, R., Boxman, D., Fremstad, E., Heil, G., Houdijk, A. & Roelofs, J. (1993). Nitrogen eutrophication and critical load for nitrogen based upon changes in flora and fauna in (semi)-natural terrestrial ecosystems. In *Critical Loads for Nitrogen.* Proc. UN-ECE workshop at Lokeberg, Sweden, 6-10 April 1992. Nordic Council for Ministers, Copenhagen, Denmark.

Bobbink, R., Heil, G.W. & Raessen, M.B.A.G. (1992). Atmospheric deposition and canopy exchange processes in heathland ecosystems. *Environmental Pollution*, **75**: 29-37.

Bormann, H.F. & Likens, G.E. (1979). *Pattern and Process in a Forest Ecosystem.* Springer-Verlag, New York, NY.

Bouwman, A.F. & Sombroek, W.G. (1990). Inputs to climatic change by soil and agriculture related activities. In: H.W. Scharpenseel, M. Schomaker & A. Ayoub (eds.). *Soils on a Warmer Earth.* 15-30, Elsevier, Amsterdam.

Bouwman, A.F., Lee, D.S., Asman, W.A.H., Dentener, F.J., van der Hoek, K.W. & Olivier, J.G.J. (1997). A global high-resolution emission inventory for ammonia. *Global Biogeochemical Cycles,* **11**: 561-587.

Bowden, W.B. (1986). Gaseous nitrogen emissions from undisturbed terrestrial ecosystems: An assessment of their impacts on local and global nitrogen budgets. *Biogeochemistry,* **2**: 249-279.

Bower, J.S., Broughton, G.F.J., Dando, M.T., Lees, A.J., Stevenson, K.J., Lampert, J.E., Sweeney, B.P., Parker, V.J., Driver, G.S., Waddon, C.J. & Wood, A.J. (1991). Urban NO_2 concentrations in the UK in 1987. *Atmospheric Environment Part B – Urban Atmosphere,* **25**: 267-283.

Bradshaw, A.D., Chadwick, M.J., Joyett, D. & Snaydon, R.W. (1964). Experimental investigations into the mineral nutrition of several grass species. IV. Nitrogen level. *Journal of Ecology,* **52**: 665-676.

Bremner, J.M. & Mulvaney, C.S. (1982). Nitrogen - Total. In: A.L. Page, R.H. Miller & D.R. Keeney (eds.) *Methods of Soil Analysis*: *Part 2 - Chemical and Microbiological Properties.* 2nd edition. Soil Sc. Soc. America. Madison, Wisconsin USA.

Burrows, C.J. (1990). *Processes of Vegetation Change.* Unwin Hyman, London.

Cadoff, B.C. & Hodgeson, J. (1983). Passive sampler for ambient levels of nitrogen dioxide. *Analytical Chemistry,* **55**: 2083-2085.

Campbell, G.W. (1988). Measurements of nitrogen dioxide concentrations at rural sites in the United Kingdom using diffusion tubes. *Environmental Pollution,* **55**: 251-270.

Campbell, G.W., Stedman, J.R. & Stevenson, K. (1994). A survey of nitrogen dioxide concentrations in the United Kingdom using diffusion tubes, July-December 1991. *Atmospheric Environment,* **28**: 477-486.

Cape, J.N. (1998). Uptake and fate of gaseous pollutants in leaves. *New Phytologist,* **139**: 221-223.

Chandapilla, M.M. (1970). Variation in fixed dune vegetation at Newborough Warren, Anglesey. *J. Ecology,* **58**: 193-201.

Chapin, F.S. (1980). The mineral nutrition of wild plants. *Annual Review of Ecological Systematic,* **11**: 233-260.

Chichester, F.W., Legg, J.O. & Stanford, G. (1975). Relative mineralization rates of indigenous and recently incorporated N-labeled nitrogen. *Soil Science*, **120**: 455-460.

Chow, J.C., Watson, J.G., Lowenthal, D.H. & Countess, R.J. (1996). Sources and chemistry of PM_{10} aerosol in Santa Barbara County, CA. *Atmospheric Environment,* **30**: 1489-1499.

Coley, P.D. (1987). Interspecific variation in plant anti-herbivore properties: the role of habitat quality and rate of disturbance. *New Phytologist*, **106**(Supplement): 251-263.

Covington, W.W. (1981). Changes in forest floor organic matter and nutrient content following clearcutting in northern hardwoods. *Ecology*, **62**: 41-48.

Dierderen, H.S.M.A. and Duyzer, J.H. (1988). *Ammonia From Emission to Deposition.* Summary report for Commission of the European Communities and Dutch Ministry of Housing, Planning and Environment, University of Utrecht.

Denmead, O.T., Freney, J.R. & Simpson, J.R. (1976). A closed ammonia cycle within a plant canopy. *Soil Biol. Biochem.* **8**:161-164.

Dilks, T.K.J. & Proctor, M.C.F. (1974). The pattern of recovery of bryophytes after desiccation. *Journal of Bryology*, **8**: 97-115.

Duckworth, C.M.S. & Cresser, M.S. (1991). Factors influencing nitrogen retention in forest soils. *Environmental Pollution,* **71**: 1-21.

Dueck, T.A., Van der Eerden, L.J., Beemsterboer, B. & Elderson, J. (1991). Nitrogen uptake and allocation by *Calluna vulgaris* (L.) Hull and *Deschampsia flexuosa* (L.) Trin. exposed to $^{15}NH_3$. *Acta Botanica Neerlandica,* **40**: 257-267.

Ellenberg, H. (1953). Physiologisches und okologisches Verhalten derselben Pflanzenarten Berichte der Deutschen. *Botanischen Gesellschaft,* **65**: 351-362. (English Abstract).

Ellenberg, H. (1954). Uber einige Fortchritte der Kausalen Vegetationskunde. *Vegetatio*, **5/6**: 199-211. (English Abstract).

Ellenberg, H. (1988). *Vegetation Ecology of Central Europe.* Cambridge University Press. p 63.

Encke, B.-G. (1986). Stickstoff und Waldsterben. *Allg. Forst Zeitschr.,* **41**: 922-923. (English Abstract).

Erisman, J.W., van Elzakher, B.G., Mennen, M.G., Hogenkamp, J., Zwart, E., van den Beld, L., Romer, F.G., Bobbink, R., Heil, G., Raessen, M., Duyzer, J.H., Verhage, H., Wyers, G.P., Otjes, R.P. & Möls, J.J. (1994). The Elspeetsche Veld experiment on surface exchange of trace gases: summary of results. *Atmospheric Environment,* **28**: 1241-1253.

Ernst, W.H.O., Slings, Q.L. & Nelissen, H.J.M. (1996). Pedogenesis in coastal wet dune slacks after sod-cutting in relation to revegetation. *Plant and Soil,* **180**: 219-230.

Evans, J.R. (1989). Photosynthesis and nitrogen relations in leaves of C3 plants. *Oecologia*, **78**: 9-19.

Ewert, E. (1979). Zur Phytotoxizität von Ammoniak. *Hercynia*, **16**: 75-80. (Cited in Fangmeier *et al.* 1994).

Falkengren-Grerup, U. (1998). Nitrogen response of herbs and graminoids in experiments with simulated acid soil solution. *Environmental Pollution,* **102**(S1): 93-99.

Faller, N. (1972). Schwefeldioxid, Schwefelwasserstoff, nitrose Gase und Ammoniak als ausschlieβliche S- bzw. N-Quellen der höheren Pflanzen. *Z. Pflanzenernaehr. Bodenk.,* **131**: 120-130. (Cited in Fangmeier *et al.,* 1994)

Fangmeier, A., Hadwiger-Fangmeier, A., Van der Eerden, L. & Jager, H. (1994). Effects of atmospheric ammonia on vegetation - A review. *Environmental Pollution*, **86**: 43-82.

Farquhar, G.D., Firth, P.M., Wetselaar, R. & Weir, B. (1980). On the gaseous exchange of ammonia between leaves and the environment: Determination of the ammonia compensation point. *Plant Physiol*ogy, **66**: 710-714.

Feeney, A. (1988). *Biological Impacts of Ammonia in the Atmosphere.* M.Sc. Thesis, Centre for Environmental Technology, Imperial College London.

Firestone, M.K., Firestone, R.B. & Tiedje, J.M. (1980). Nitrous oxide from soil denitrification: factors controlling its biological production. *Science,* **208**: 749-751.

Fowler, D., Cape, J.N. & Unsworth, M.H. (1989). Deposition of atmospheric pollutants on forests. *Phil. Trans. R. Soc. Lond.,* **B324**: 247-265.

Fowler, D., Flechard, C., Skiba, U., Coyle, M. & Cape, J.N. (1998a). The atmospheric budget of oxdized nitrogen and its role in ozone formation and deposition. *New Phytologist,* **139**: 11-23.

Fowler, D., Pitcairn, C.E.R., Sutton, M.A., Flechard, C., Loubet, B., Coyle, M. & Munro, R.C. (1998b). The mass budget of atmospheric ammonia in woodland within 1 km of livestock buildings. *Environmental Pollution,* **102**(S1): 343-348.

Gauch, H.G. Jr., Whittaker, R.H. & Wentworth, T.R. (1977). A comparative study of reciprocal averaging and other ordination techniques. *Journal of Ecology*, **65**: 157-174.

Gauch, H.G. Jr. (1982). *Multivariate Analysis in Community Ecology.* Cambridge University Press, Cambridge.

Gibson, D.J. (1984). *Small Scale Patterns and Soil Heterogeneity in Grassland.* Ph.D. Thesis, University of Wales.

Gibson, C.W,D. & Brown, V.K. (1992). Grazing and vegetation change: deflected or modified succession?. *J. Applied Ecology*, **29**: 120-131.

Gilham, C., Leech, P. & Eggleston, H. (1992). UK emissions of air pollutants 1970-1990. Warren Spring Laboratory, Stevenage Herts. Report LR 887 (AP).

Goldsmith, A. (1986). Measurements of nitrogen dioxide at rural sites using passive diffusion samplers. Warren Spring Laboratory Report LR 582 (AP)M, Stevenage, England.

Gorham, E. (1958). Soluble salts in dune sands from Blakeney Point in Norfolk. *Journal of Ecology,* **46**: 373-379.

Gosz, J.R., Likens, G.E. & Bormann, H.F. (1976). Organic matter and nutrient dynamics of the forest floor in the Hubbard Brook forest. *Oecologia*, **22**: 305-320.

Goulding, K.W.T. (1990). Nitrogen deposition to land from the atmosphere. *Soil Use and Management,* **6**: 61-63.

Goulding, K.W.T., Bailey, N.J., Bradbury, N.J., Hargreaves, P., Howe, M., Murphy, D.V., Poulton, P.R. & Willison, T.W. (1998). Nitrogen deposition and its contribution to nitrogen cycling and associated soil processes. *New Phytologist,* **139**: 49-58.

Green, B.H. (1972). The relevance of seral eutrophication and plant competition to the management of successional communities. *Biological Conservation*, **4**: 378-384.

Grime, J.P. (1973a). Control of species density in herbaceous vegetation. *Journal of Environmental Management,* **1**: 151-167.

Grime, J.P. (1973b). Competitive exclusion in herbaceous vegetation. *Nature,* **242**: 344-347.

Grime, J.P (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, 111: 1169-1194.

Grime, J.P. (1979). *Plant Strategies and Vegetation Processes.* John Wiley & Sons, Chichester, England.

Guderian, R. (1988). Critical levels of effects of NO_2. Working Paper for UNECE Critical Levels Workshop, Bad Harzburg.

Hanawalt, R.B. (1969). Environmental factors influencing the sorption of atmospheric ammonia by soils. *Soil Science Society America Proc.,* **33**: 231-234.

Hanson, P.J. & Lindberg, S.E. (1991). Dry deposition of reactive nitrogen compounds: a review of leaf, canopy and non-foliar measurements. *Atmospheric Environment,* **25A**: 1615-1634.

Hanson, P.J., Rott, K., Taylor, G.E. Jr., Gunderson, C.A., Lindberg, S.E. & Ross-Todol, B.M. (1989). NO2 deposition to elements representative of a forest landscape. *Atmospheric Environment*, **23**: 1783-1794.

Hao, W.M., Wofsy, S.C., McElroy, M.B., Beer, J.M. & Toqan, M.A. (1987). Sources of atmospheric nitrous oxide from combustion. *Journal of Geophysical Research,* **92**: 3098-3104.

Hargreaves, K.J. (1989). *The development and application of diffusion tubes for air pollution measurements.* Ph.D. Thesis, University of Nottingham.

Harper, J.L. (1977). *Population Biology of Plants.* Academic Press, London.

Harrison R.M. & Allen, A.G. (1991). Scavenging ratios and deposition of sulphur, nitrogen and chlorine species in eastern England. *Atmospheric Environment,* **25A**: 1719-1723.

Heal, M.R. & Cape, J.N. (1997). A numerical evaluation of chemical interferences in the measurement of ambient nitrogen dioxide by passive diffusion samplers. *Atmospheric Environment,* **31**: 1911-1923.

Heil, G.W. & Diemont, W.M. (1983). Raised nutrient levels change heathland into grassland. *Vegetatio,* **53**: 113-120.

Hewett, D.G. (1982). Grazing studies on sand dunes. *Annual Report Institute of Terrestrial Ecology.* pp 78-79

Hewett, D.G. (1985). Grazing and mowing as management tools on dunes. *Vegetatio*, **62**: 441-447.

Hewitt, E.J. (1966). *Sand and Water Culture Methods Used in Study of Plant Nutrition* (2nd edition). Commonwealth Agricultural Bureau, Farnham Royal.

Hill, M.O. (1979). *DECORANA - A Fortran Program for Detrended Correspondence Analysis and Reciprocal averaging.* Cornell University, Ithaca, New York.

Hill, M.O. & Gauch, H.G. (1980). Detrended Correspondence Analysis, an improved ordination technique. *Vegetatio*, **42**:47-58.

Hope-Jones, P. (1965). Some recent changes in the vegetation at Newborough Warren, Anglesey. *Nature in Wales.* **9**: 165-169.

Huberty, L.E., Gross, K.L. & Miller, C.J. (1998). Effects of nitrogen addition on successional dynamics and species diversity in Michigan old-fields. *Journal of Ecology*, **86**: 794-803.

Inouye, R.S., Huntly, N.J., Tilman, D. & Tester, J.R. (1987). Pocket gophers (*Geomys bursarius*), vegetation, and soil nitrogen along a successional sere in east central Minnesota. *Oecologia*, **68**: 178-184.

INDITE (1994). *Impacts of Nitrogen Deposition in Terrestrial Ecosystems.* Report of The United Kingdom Review Group on Impacts of Atmospheric Nitrogen., Department of the Environment.

Irving, P.M. (1983). Acidic precipitation effects on crops: a review and analysis of research. *Journal of Environmental Quality,* **12**: 442-453.

Jefferies, R.L. & Willis, A.J. (1964). Studies on the calcicole-calcifuge habit. II. The influence of calcium on the growth and establishment of four species in soil and sand culture. *Journal of Ecology*, **52**: 691-701.

Jenny, H. (1980). Soil genesis with ecological perspectives. *Ecological Studies.* vol. 37. Springer-Verlag, New York.

Jones, M.L.M. & Ashenden, T.W. (1997). *Monitoring of Ground Level Nitrogen Dioxide Concentrations Around Four Exemplar British Gas Compressor Stations.* Contract Report of the Institute of Terrestrial Ecology to the British Gas, 0709A/CH/002/S/933062.

Jongman, R.H.G., ter Braak, C.J.F. & van Tongeren, O.F.R. (1987). *Data Analysis in Community and Landscape Ecology.* PUdoC, Wageningen, The Netherlands.

Kachi, N. & Hirose, T. (1983). Limiting nutrients for plant growth in coastal sand dune soils. *Journal of Ecology,* **71**: 937-944.

Keddy, P.A. (1989). *Competition.* Chapman and Hall, London, England.

Kendall, D.G. (1971). Seriation from abundance matrices. In: F.R. Hodson, D.G. Kendall & P. Tauta (eds.) *Mathematics in the archaeological and historical sciences.* Edinburgh University Press, Edinburgh. pp 215-252.

Khalil, M.A.K. & Rasmussen, R.A. (1992). The global sources of nitrous oxide. *Journal of Geophysical Research,* **97**: 14,651-14,660.

Kruse, M., Ap Simon, H.M. & Bell, J.N.B. (1989). Validity and uncertainty in the calculation of an emission inventory for ammonia arising from agriculture in Great Britain. *Environmental Pollution,* **56**: 237-257.

Lawrence, D.B. (1979). Primary versus secondary succession at Glacier Bay National Monument, southeastern Alaska. In: R.M. Linn (ed.) *Proceedings of the First Conference on Scientific Research in the National Parks*, 213-224.

Lea, P.J., Wolfenden, J. & Welburn, A.R. (1994). Influence of air pollutants upon nitrogen metabolism. In: R. Alscher, A.R. Wellburn (eds.) *Plant Responses to the Gaseous Environment.* Chapman & Hall, London, 279-299.

Lee, D.S. & Atkins, D.H.F. (1992). Atmospheric ammonia in the vicinity of a sewage treatment plant – results from a preliminary investigation. AEA Environment and Energy, Harwell Laboratory, Oxon.

Lee, D.S. & Dollard, G.J. (1994). The potential magnitude of non-animal sources of ammonia and uncertainties in the total emission of ammonia from the United Kingdom. *Environmental Pollution,* **86**: 267-277.

Lee, D.S., Köhler, I., grobler, E., Rohrer, F., Sausen, R., Gallardo-Klenner, L., Olivier, J.G.J., Dentener, F.J. & Bouwman, A.F. (1997). Estimation of global NO_x emissions and their uncertainties. *Atmospheric Environment,* **31**: 1735-1749.

Lee, J.A., Parsons, A.N. & Baxter, R. (1993). *Sphagnum* species and polluted environments, past, present and future. *Advances in Bryology*, **5**: 297-313.

Lee, Y-N. & Schwartz, S.E. (1981). Evaluation of the rate of uptake of nitrogen dioxide by atmospheric and surface liquid water. *Journal of Geophysical Research,* **86**: 11971-11983.

Lipschultz, F., Zafiriou, O.C., Wofsy, S.C., McElroy, M.B., Valois, F.W. & Watson, S.W. (1981). Production of NO and N_2O by soil nitrifying bacteria. *Nature*, **294**: 641-643.

Lloyd, P.S. & Pigott, C.D. (1967). The influence of soil conditions on the course of succession on the chalk of southern England. *Journal of Ecology*, **55**: 137-146.

Lockyer, D.R., Pain, B.F. & Klarenbeek, J.V. (1989). Ammonia emissions from cattle, pig and poultry wastes applied to pasture. *Environmental Pollution*, **56**: 19-30.

Mahmoud, A. & Grime, J.P. (1976). An analysis of competitive ability in three perennial grasses. *New Phytologist,* **77**: 431-435.

Mansfield, T.A., & Freer-Smith, P.H. (1981). Effects of urban air pollution on plant growth. *Biological Review*, **56**: 343-368.

Marrs, R.H., Rawes, M., Robinson, J.S. & Poppitt, S.D. (1986). *Long-term studies of vegetation change at Moor House NNR: Guide to recording methods and the database.* Merelewood Research Development paper No. 109.

Martin, W.K. (1982). *The New Concise British Flora.* Bloomsbury Books, London.

Martin, A. & Barber, F.R. (1984). Acid gases and acid rain monitored for over five years in rural central England. *Atmospheric Environment,* **18**: 1715-1724.

Melillo, J.M., Aber, J.D. & Muratore, J.F. (1982). Nitrogen and ligin control of hardwood leaf litter decomposition dynamics. *Ecology,* **63**: 621-626.

Morecroft, M.D., Sellers, E.K. and Lee, J.A. (1994). An experimental investigations into the effects of atmospheric nitrogen deposition on two semi-natural grasslands. *Journal of Ecology*, **82**: 475-483.

Morgan, S.M. (1992). *Ecophysiological Responses of Bryophytes to Atmospheric Nitrogen Deposition.* PhD. Thesis, University of Manchester.

Morton, A.J. (1970). *A study of some factors affecting the structure of grassland vegetation.* Ph.D. Thesis, University of Wales.

Morton, A.J. (1974a). Ecological studies of a fixed dune grassland at Newborough Warren, Anglesey. I. The structure of the grassland. *Journal of Ecology,* **62**: 253-260.

Morton, A.J. (1974b). Ecological studies of a fixed dune grassland at Newborough Warren, Anglesey. II. Causal factors of the grassland structure. *Journal of Ecology,* **62**: 261-278.

Mueller-Dombois, D. & Ellenberg, H. (1974). *Aims and Methods of Vegetation Ecology.* Wiley, New York.

Muzio, L.J. & Kramlich, J.C. (1988). An artifact in the measurement of N_2O of combustion sources. *Geophysical Research Letters,* **15**: 1369-1372.

Newberry, DMcc. & Newman, E.I. (1978). Competition between grassland plants of different initial sizes. *Oecologia*, **33**: 361-380.

Newman, E.I (1983). Interactions between plants. In: O.L. Lange, P.S. Nobel, C.B. Osmond & H. Ziegler (eds.) *Physiological Plant Ecology.* III. Responses to the chemical and biological environment. pp. 679-710. Springer-Verlag, Berlin, Germany.

Olff, H., Huisman, J. & Van Tooren, B.F. (1993). Species dynamics and nutrient accumulation during early primary succession in coastal sand dunes. *Journal of Ecology,* **81**: 693-706.

Olivier, J.G.J., Bouwman, A.F., Van der Hoek, K.W. & Berdowski, J.J.M. (1998). Global air emission inventories for anthropogenic sources of NO_x, NH_3 and N_2O in 1990. *Environmental Pollution,* **102**(S1): 135-148.

Olsthoorn, A.F.M., Keeltjens, W.G., van Baren, B. & Hopman, M.C.G. (1991). Influence of ammonium on fine root development and rhizosphere pH of Douglas-fir seedlings in sand. *Plant and Soil,* **133**: 75-81.

Ordnance Survey (1985). Pathfinder 768: Caernarfon. Ordanance Survey, Southampton.

Packham, J.R. & Willis, A.J. (1997). *Ecology of Dunes, Salt Marsh and Shingle.* Chapman & Hall, London.

Palmes, E.D., Gunnison, A.F., Dimattio, J. & Tomezyk, C. (1976). Personal sampler for nitrogen dioxide. *American Industrial Hygiene Association Journal,* **37**: 570-577.

Parker, G.G. (1983). Throughfall and stemflow in the forest nutrient in the forest nutrient cycle. *Advances in Ecological Research,* **13**: 57-133.

Parsons, A.N. (1991). *Critical Loads of Nitrogen and Sulphur for an Ombrotrophic Mire.* PhD. Thesis, University of Manchester.

Pearson, J. & Stewart, G.R. (1993). The deposition of atmospheric ammonia and its effects on plants. *New Phytologist,* **125**: 283-305.

Peet, R.K. (1978). Forest vegetation of the Colorado Front Range: pattern of species diversity. *Vegetatio,* **37**: 65-78.

Perez-Soba, M., Van der Eerden, L.J.M. & Elderson, J. (1998). Uptake of nitrogen dioxide and gaseous ammonia by nitrate- and ammonium-preferent plant species. In: L.J. De Kok, I. Stulen (eds.) *Responses of Plant Metabolism to Air Pollution and Global Change,* pp. 423-424. Backhuys Publishers, Leiden, The Netherlands.

Perkauskas, D. & Mikelinskiene, A. (1998). Evaluation of SO_2 and NO_2 concentration levels in Vilnius (Lithuania) using passive diffusion samplers. *Environmental Pollution,* **102**(S1): 249-252.

Pitcairn, C.E.R., Fowler, D. & Grace, J. (1991). *Changes in species composition of semi-natural vegetation associated with the increase in atmospheric inputs of nitrogen.* Report to Nature Conservancy Council. Institute of Terrestrial Ecology, Edinburgh.

Pitcairn, C.E.R., Fowler, D. & Grace, J. (1995). Deposition of fixed atmospheric nitrogen and foliar nitrogen content of bryophytes and *Calluna vulgaris* (L.) Hull. *Environmental Pollution*, **88**: 193-205.

Pitcairn, C.E.R., Leith, I.D., Sutton, M.A., Fowler, D., Binnie, J., Munro, R.C., Sheppard, L.J. & Rossal, M.R. (1996). *Effects of Ammonia on Natural Plant Communities in an Agricultural Landscape.* Interim Report to English Nature. Institute of Terrestrial Ecology, Edinburgh.

Pitcairn, C.E.R., Leith, I.D., Sheppard, L.J. Sutton, M.A., Fowler, D., Munro, R.C., Tang, S. & Wilson, D. (1998). The relationship between nitrogen deposition, species composition and foliar nitrogen concentrations in woodland flora in the vicinity of livestock farms. *Environmental Pollution,* **102**(S1): 41-48.

Poikolainen, J., Lippo, H., Hongisto, M., Kubin, E., Mikkola, K. & Lindgren, M. (1998). On the abundance of epiphytic green algae in relation to the nitrogen concentrations of biomonitors and nitrogen deposition in Finland. *Environmental Pollution,* **102**(S1): 85-92.

Ranwell, D.S. (1955). Slack vegetation, dune system development and cyclical change at Newborough Warren, Anglesey. Ph.D. Thesis, University of London.

Ranwell, D.S. (1959). Newborough Warren, Anglesey. I. The dune system and dune slack habitat. *Journal of Ecology,* **47**: 571-601.

Ranwell, D.S. (1960). Newborough Warren, Anglesey. II. Plant associes and succession cycles of the sand dune and dune slack vegetation. *Journal of Ecology,* **48**: 117-141.

Ranwell, D.S. (1972). *Ecology of Salt Marshes and Sand Dunes.* Chapman and Hall, London.

Ratcliffe, D.A. (1977). *A Nature Conservation Review.* Cambridge University Press, Cambridge.

Raven, J.A. (1988). Acquisition of nitrogen by the shoots of land plants: its occurrence and implication for acid-base regulation. *New Phytologist,* **109**: 1-20.

Raven, J.A., Wollenweber, B. & Handley, L.L. (1992). Ammonia and ammonium fluxes between photolithotrophs and the environment in relation to the global nitrogen cycle. *New Phytologist,* **121**: 19-32.

Retuerto, R. & Woodward, F.I. (1992). Effects of windspeed on the growth and biomass allocation of white mustard *Sinapis alba* L. *Oecologia*, **92**: 113-123.

Reynolds, B. & Ormerod, S.J. (1993). *A review of the impact of current and future acid deposition in Wales.* Contract Report of the Institute of Terrestrial Ecology to the Welsh Office, WEPD/12/138/28.

Reynolds, B., Williams, T.G. & Stevens, P.A. (1990). The chemical composition of bulk precipitation across the mountains of Snowdonia, U.K. *The Science of the Total Environment,* **92**: 223-234.

RGAR (1990). *Acid Deposition in the United Kingdom 1986-1988.* Third Report of the United Kingdom Review Group on Acid Rain. London: Department of Environment, London.

RGAR (1997). *Acid Deposition in the United Kingdom 1992-1994.* Fourth Report of the Review Group on Acid Rain. DOE, London.

Robertson, G.P., & Vitousek, P.M. (1981). Nitrification potentials in primary and secondary succession. *Ecology,* **62**: 376-386.

Robertson, G.P., Huston, M.A., Evans, F.C. & Tiedje, J.M. (1988). Spatial variability in a successional plant community: patterns of nitrogen availability. *Ecology,* **69**: 1517-1524.

Rodwell, J.S. (1992). *British Plant Communities.* Volume 3: Grasslands and Montane Communities. Cambridge University Press, Cambridge.

Rogers, H.H., Jeffries, H.E. & Witherspoon, A.M. (1979). Measuring air pollutant uptake by plants: nitrogen dioxide. *Journal of Environment Quality,* **8**: 551-557.

Rorison, I.H. (1969). Ecological inferences from laboratory experiments on the mineral nutrition of plants. In: I.H. Rorison (ed.) *Ecological Aspects of the Mineral Nutrition of Plants.* Blackwell Scientific Publications, Oxford. pp. 155-175.

Rosén, K., Gundersen, P., Tegnhammar, L., Johansson, M. & Frogner, T. (1992). Nitrogen enrichment of Nordic forest ecosystems – The concept of critical loads. *AMBIO*, **21**:364-368.

Ross, M.A. & Harper, J.L. (1972). Occupation of biological space during seedling establishment. *Journal of Ecology,* **60**: 77-88.

Rowland, A.J., Drew, M.C. & Wellburn, A.R. (1987). Foliar entry and incorporation of atmospheric nitrogen dioxide into barley plants of different nitrogen status. *New Phytologist,* **107**(2): 357-371.

Rowell, D.L. (1994). *Soil Science: Methods & Applications.* Longman UK.

Royal Society (1983*). The Nitrogen Cycle of the United Kingdom – A Study Group Report.* The Royal Society, London.

Russell, E.W. (1961). *Soil Conditions and Plant Growth.* 9th edition, London.

Salisbury, E.J. (1922). The soils of Blakeney Point: a study of soil reaction an succession in relation to the plant covering. *Annals of Botany, London,* **36**: 391-431.

Salisbury, E.J. (1925). Note on the edaphic succession in some dune soils with special reference to the time factor. *Journal of Ecology,* **13**: 322.

Schjørring, J.K. (1998). Atmospheric ammonia and impacts of nitrogen deposition: uncertainties and challenges. *New Phytologist,* **139**: 59-60.

Schlesinger, W.H., Reynolds, J.F., Cunningham, G.L., Huenneke, L.F., Jarrel, W.M., Virginia, R.A. & Whitford, W.G. (1990). Biological feedbacks in global desertification. *Science,* **247**: 1043-1048.

Schulze, E.D., de Vries, W., Hauhs, M., Rosen, K., Rasmussen, L., Tamm, C.O. & Nilsson, J. (1989). Critical loads for nitrogen deposition on forest ecosystems. *Water, Air and Soil Pollution,* **48**:451-456.

Seiler, W. & Conrad, R. (1987). Contribution of tropical ecosystems to the global budgets of trace gases, especially CH_4, H_2, CO and N_2O. In: R.E. Dickinson (ed.) *Geophysiology of Amazonia: Vegetation and Climate Interactions,* pp. 133-160, Wiley and Sons, New York.

Skeffington, R.A. & Wilson, E.J. (1988). Excess Nitrogen Deposition: Issues for Consideration. *Environmental Pollution,* **54**: 159-184.

Skiba, U., Hargreaves, K.J., Smith, K.A. & Fowler, D. (1992). Fluxes of nitric and nitrous oxides from agricultural soils in a cool temperate climate. *Atmospheric Environment,* **26A**: 2477-2485.

Sommer, S.G., Kjellerup, V. & Kristjansen, O. (1992). Determination of total ammonium nitrogen in pig and cattle slurry: sample preparation and analysis. *Acta Agricultura Scandinavia, Section B, Soil and Plant Science,* **42**: 146-151.

Spellerberg, I.F. (1991). *Monitoring Ecological Change.* Cambridge University Press. pp 94.

Spindler, G., Rolle, W. & Grüner, A. (1996). Ammonia concentration and dry deposition over grassland in Germany. In: M.A. Sutton, D.S. Lee, G.J. Dollard & D. Fowler (eds.) *Atmospheric Ammonia: Emission, Deposition and Environmental Impacts.* Poster Proceedings, Institute of Terrestrial Ecology, Edinburgh, UK, 86-89.

Stace, C. (1991). *New Flora of the British Isles.* Cambridge University Press, Cambridge.

Stevens, P.A., Adamson, J.K., Reynolds, B. & Hornung, M. (1990). Dissolved inorganic nitrogen concentrations and fluxes in three British Sitka spruce plantations. *Plant and Soil,* **128**: 103-108.

Stewart, W.D.P. (1967). Transfer of biologically fixed nitrogen in a sand dune slack system. *Nature,* **214**: 603-604.

Stulen, I., Perez-Soba, M., De Kok, L.J. & Van der Eerden, L. (1998). Impact of gaseous nitrogen deposition on plant functioning. *New Phytologist,* **139**: 61-70.

Sutton, M.A. (1990). *The surface/atmosphere exchange of ammonia.* Ph.D. Thesis, Institute of Ecology and Resource Management, University of Edinburgh.

Sutton, M.A. & Fowler, D. (1993). Estimating the relative contribution of SO_x, NO_y and NH_x inputs to effects of atmospheric deposition. In: M. Hornung & R.A. Skeffington (eds.) *Critical loads: concept and applications.* ITE symposium no. 28, London: HMSO.

Sutton, M.A., Pitcairn, C.E.R. & Fowler, D. (1993). The exchange of ammonia between the atmosphere and plant communities. *Advance Ecological Research*, **24**: 301-393.

Sutton, M.A., Schjørring, J.K. & Wyers, G.P. (1995a). Plant-atmosphere exchange of ammonia. *Phil. Trans. Roy. Soc., London. Series A.* 351: 261-278.

Sutton, M.A., Place, C.J., Eager, M., Fowler, D. & Smith, R.I. (1995b). Assessment of the magnitude of ammonia emissions in the United Kingdom. *Atmospheric Environment,* **29**: 1393-1411.

Sutton, M.A., Tang, Y.S., Miners, B.P., Coyle, M., Smith, R. & Fowler, D. (1998). Spatial and temporal patterns of ammonia concentration in the UK. Results of the National Ammonia Monitoring Network. Contract Report of ITE Edinburgh to the Department of the Environment Transport and the Regions, Air and Environmental Quality Division. Contract No.: EPG 1/3/58.

Taylor, O.C. & Eaton, F.M. (1966). Suppression of plant growth by nitrogen dioxide. *Plant Physiology,* **41**: 132-135.

ten Harkel, M.J. & van der Meulen, F. (1995). Impact of grazing and atmospheric nitrogen deposition on the vegetation of dry coastal dune grasslands. *Journal of Vegetation Science,* **6**: 445-452.

ter Braak, C.J.F. (1986). Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology*, **67**: 1167-1179.

ter Braak, C.J.F. (1987). The analysis of vegetation-environment relationships by canonical correspondence analysis. *Vegetatio*, **64**: 69-77.

ter Braak, C.J.F. (1988). CANOCO – an extension of DECORANA to analyse species-environment relationships. *Vegetatio*, **75**: 159-160.

ter Braak, C.J.F. & Barendregt, Leo G. (1986). Weighted averaging of species indicator values: its efficiency in environmental calibration. *Mathematical Biosciences*, **78**: 57-72.

Thompson, D.B.A & Baddeley, J.A. (1991). Some effects of acidic deposition on montane *Racomitrium lanuginosum* heaths. In: S.J. Woodin & A.M. Farmer (eds.) *The Effects of Acidic Deposition on Nature Conservation in Great Britain,* Nature Conservancy Council, Peterborough, UK. pp. 17-28.

Tilman, D. & Wedin, D. (1991). Dynamics of nitrogen competition between successional grasses. *Ecology*, **72**: 1038-1049.

Tilman, D. (1982). *Resource Competition and Communtiy Structure.* Princeton University Press, Princeton, New Jersey, USA.

Tilman, D. (1985). The resource ratio hypothesis of succession. *American Naturalist*, **125**: 827-852.

Tilman, D. (1986). Nitrogen-limited growth in plants from different successional stages. *Ecology*, **67**: 555-563.

Tilman, D. (1988). *Plant Strategies and the Dynamics and Structure of Plant Communities.* Princeton University Press, Princeton, New Jersey, USA.

Tisdale, S.L., Nelson, W.L. & Beaton, J.D. (1985). *Soil Fertility and Fertilizers* (4^{th} edition). Macmillan, New York.

Tjepkema, J.D., Cartica, R.J. & Hemond, H.F. (1981). Atmospheric concentrations of ammonia in Massachusetts and deposition on vegetation. *Nature,* **294**: 445-446.

Trangmar, B.B., Yost, R.S., Wade, M.K., Uehara, G. & Sudjadi, M. (1987). Spatial variation of soil properties and rice yield on recently cleared land. *Soil Science Society of America Journal,* **51**: 668-674.

Troiano, J.J. & Leone. I.A. (1977). Changes in growth rate and nitrogen content of tomato plants after exposure to NO_2. *Phytopathology,* **67**(9): 1130-1133.

Truscott, A-M. (1997). *The Effects of Nitrogen Deposition and Grazing on* Deschampsia flexuosa. MSc. Thesis, University of Wales Bangor. 106 pp.

Turkington, R. & Harper, J.L. (1979). The growth, distribution and neighbour relationships of *Trifolium repens* in a permanent pasture. I. Ordination, pattern and contact. *Journal of Ecology,* **67**: 201-218.

Turner, S.M. & Liss, P.S. (1985). Measurements of various sulphur gases in a coastal marine environment. *Journal of Atmospheric Chemistry,* **2**: 223-232.

Tyler, G. (1987). Probable effects of soil acidification and nitrogen deposition on the floristic composition of oak (*Quercus robur* L.) forest. *Flora,* **179**: 165-170.

UK-PORG (1990). Oxides of Nitrogen in the United Kingdom. Second Report of the United Kingdom Photochemical Oxidants Review Group, Department of Transport, South Ruislip, UK.

Van Breeman, N., Burrough, P.A., Velthorst, E.J., Van Dobben, H.F., de Wit, T., Ridder, T.B & Reinjinders, H.F.R. (1982). Soil acidification from atmospheric ammonium sulphate in forest canopy throughfall. *Nature*, **299**: 548-550.

Van Breeman, N. & Van Dijk, H.F.G. (1988). Ecosystem effects of atmospheric deposition of nitrogen in The Netherlands. *Environmental Pollution*, **54**: 249-274.

Van der Eerden, L.J.M. (1982). Toxicity of ammonia to plants. *Agricultural Environment*, **7**:223-235.

Van der Eerden, L.J.M., Dueck, T. A., Berdowski, J.J.M., Graven, H. & Van Dobben, H.F. (1991). Influence of NH_3 and $(NH_4)_2SO_4$ on heathland vegetation. *Acta Botanica Neerlandica,* **40**: 281-296.

Van der Eerden, L.J.M., Dueck, T. A., Posthumus, A.C. & Tonneijck, A.E.G. (1993). Assessment of critical levels for air pollution effects on vegetation: some considerations and a case study on NH_3. In: M.R. Ashmore & R.B. Wilson (Eds.), *Critical Levels of Air Pollutants for Europe.* UNECE Workshop on Critical Levels, Egham, UK, March 1992, pp. 55-63.

Van der Eerden, L.J.M., Lekkerkerk, L.J.A., Smeulders, S.M. & Jansen, A.E. (1992). Effects of atmospheric ammonia and ammonium sulphate on Douglas fir (*Pseudotsuga menziesii*). *Environmental Pollution*, **76**: 1-9.

Van der Eerden, L.J.M., Dueck, T.A., Elderson, J., Van Dobben, H.F., Berdowski, J.J.M., Latuhihin, M. & Prins, A.H. (1990). Effects of NH_3 and $(NH_4)_2SO_4$ deposition on terrestrial semi-natural vegetation on nutrient-poor soils. Project 124/125, phase II. Dutch Priority Prohgramme on Acidification, IPO report R90/06, RIN report 90/20.

Van Dijk, H.F.G. & Roelofs, J.G.M. (1988). Effects of excessive ammonium deposition on the nutritional status and condition of pine needles. *Physiologia Plantarum*, **73**: 494-501.

Van Hecke, P., Impens, I. & Behaeghe, T.J. (1981). Temporal variation of species composition and species diversity in permanent grassland plots with different fertilizer treatments. *Vegetatio*, **47**: 221-232.

Van Hove, L.W.A. & Bossen, M.E. (1994). The physiological effects of a five months exposure to low concentrations of O_3 and NH_3 on Douglas fir (*Pseudotsuga menziesii*). *Physiologia Plantarum,* **92**: 140-148.

Van Hove, L.W.A., van Kooten, O., van Wijk., K.J., Vredenberg, W.J., Adema, E.H. & Pieters, G.A. (1991). Physiological effects of long term exposure to low concentrations of SO_2 and NH_3 on poplar leaves. *Physiologia Plantarum,* **82**: 32-40.

Vermeer, J.G. (1986). The effect of nutrient addition and lowering of the water table on shoot biomass and species composition of a wet grassland community. *Acta Oecologica – Oecologia Plantarum,* **7**: 145-155.

Vitousek, P.M. & Reiners, W.A. (1975). Ecosystem succession and nutrient retention: a hypothesis. *BioScience*, **25**: 376-381.

Vitousek, P.M. (1985). Community turnover and ecosystem nutrient dynamics. In: Pickett, S.T.A. & White, P.S. (eds.) *The Ecology of Natural Disturbance and Patch Dynamics*, pp. 325-333. Academic Press, Orlando, FL.

Vitousek, P.M., Gosz, J.R., Grier, C.C., Melillo, J.M. & Reiners, W.A. (1982). A comparative analysis of potential nitrification and nitrate mobility in forest ecosystems. *Ecological Monographs,* **52**: 155-177.

Vitousek, P.M., Matson, P.A. & Van Cleve, K. (1989). Nitrogen availability and nitrification during succession: Primary, secondary, and old-field seres. In: Clarholm, M. & Bergstrom, L. (eds.) *Ecology of Arable Land*, pp. 161-171. Kluwer Academic Publishers, Norwell.

Walker, J., Thompson, C.H., Fergus, I.F. & Tunstall, B.R. (1981). Plant succession and soil development in coastal sand dunes of subtropical eastern Australia. In: D.C. West, H.H. Shugart, & D.B. Botkin (eds.) *Forest succession: Concepts and Application*, pp. 107-131. Springer Verlag, New York, USA.

Watson, R.T., Meira-Filho, L.G., Sanhueza, E. & Janetos, A. (1992). Sources and sinks. In: J.T. Houghton, B.A. Callander & S.K. Varney (eds.) *Climate Change 1992, The Supplementary Report to the IPCC Scientific Assesment,* pp. 25-46, Cambridge University Press, New York.

Wellburn, A.R. (1990). Why are atmospheric oxides of nitrogen usually phytotoxic and not alternative fertilisers? *New Phytologist,* **115**: 395-429.

Wellburn, A.R. (1994). *Air Pollution and Climate Change: The Biological Impact.* Longman Sci. & Technical. p 57.

Wells, T.C.E. (1980). Management options for lowland grassland. In: I.H. Rorison & R. Hunt (eds.) *Amenity Grassland. An Ecological Perspective.* John Wiley and Sons, Chichester.

West, D.C., Shugart, H.H. & Botkin, D.B. (eds.) (1981). *Forest Succession: Concepts and Applications.* New York: Springer-Verlag.

White, P.S. & Pickett, S.T.A. (1985). Natural disturbance and patch dynamics: an introduction. In: Pickett, S.T.A. & White, P.S. (eds.) *The Ecology of Natural Disturbance and Patch Dynamics.* pp. 3-13. Academic Press, Orlando, FL.

White, P.S. (1979). Pattern, process, and natural disturbancein vegetation. *Botanical Review,* **45**: 229-299.

Whittaker, R.H. (1973). *Ordination and Classification of Communities.* Handbook of Vegetation Science, Vol. 5. Junk, The Hague.

Whittaker, R.H. (1975). *Communities and Ecosystems.* New York: Macmillan Press.

Willis, A.J. (1963). Braunton Burrows: The effects on the vegetation of the addition of mineral nutrients to the dune soils. *Journal of Ecology,* **51**: 353-374.

Willis, A.J., Folkes, B.F., Hope-Simpson, J.F. & Yemm, E.W. (1959). Braunton Burrows: the dune system and its vegetation. Part I. *Journal of Ecology,* **47**: 1-24.

Wilson, E.J., Wells, T.C.E. & Sparks, T.H. (1995). Are calcareous grasslands in the UK under threat from nitrogen deposition? - an experimental determination of a critical load. *Journal of Ecology*, **83**: 823-832.

Woodin, S.J. & Farmer, A.M. (1993). Impacts of sulphur and nitrogen deposition on sites and species of nature conservation importance in Great Britain. *Biological Conservation,* **63**: 23-30.

Woodmansee, R.G., Dodd, J.L., Bowman, R.A., Clark, F.E. & Diskinson, C.E. (1978). Nitrogen budget for a shortgrass prairie ecosystem. *Oecologia*, **34**: 363-376.

Woodmansee, R.G., Vallis, I. & Mott, J.J. (1981). Grassland Nitrogen. In: F.E. Clark & T. Rosswall (eds.) *Terrestrial Nitrogen Cycles. Processes, Ecosystem Strategies and Management Impacts.* Ecological Bulletins (Stockholm) 33: 443-462.

Wright, R.F., Lotse, E. & Semb, A. (1988). Reversibility of acidification shown by whole-catchment experiments. *Nature*, **334**: 670-675.

Yamulki, S., Harrison, R.M. & Goulding, K.W.T. (1996). Ammonia surface exchange above an agricultural field in southeast England. *Atmospheric Environment,* **30**: 109-118.

Yamulki, S., Harrison, R.M. & Goulding, K.W.T. & Webster, C.P. (1997). N2O, NO and NO2 fluxes from a grassland: effect of soil pH. *Soil Biology and Biochemistry*, **29**: 1199-1208.

Zak, D.R., Host, G.E. & Pregitzer, K.S. (1989). Regional variability in nitrogen mineralization, nitrification, and overstory biomass in northern lower Michigan. *Canadian Journal of Forest Research,* **19**: 1521-1526.

Zeevaart, A.J. (1974). Induction of nitrate reductase by NO_2. *Acta Botanica Neerlandica,* **23**(3): 345-346.

Zeevaart, A.J. (1976). Some effects of fumigating plants for short period with NO_2. *Environmental Pollution,* **11**(2): 97-108.

Zhang, J-T. (1991). *Ecological Relationships of Some Montane Grasses.* PhD Thesis, University of Wales Bangor, 192 pp.

APPENDICES

APPENDIX 2.1

Brief procedure on analysing water samples using Dionex DX-120 Ion Chromatograph.

Water samples (e.g. rainwater and surface water samples) were analysed for chloride, sulphate, nitrate and ammonium ions using the Dionex DX-120 Ion Chromatograph based on ion exchange chromatography. The Dionex Ion Chromatograph has the IonPac column to address the separation of ions in a sample, which include ion exchange, ion exclusion, reversed phase ion pairing, and ion supression. Multiple selectivities of the IonPac columns for anion or cation exchange give flexibility in dealing with complicated sample matrices while providing alternative means for validating peak identity. The IonPac columns include:

- Ion exchange column for analysis of anions and cations
- Ion exclusion columns, in which separation of anions is accomplished by differences in acidity, size and hydrophobicity
- Reversed phase columns for the analysis of a wide range of neutral and polar organic compounds.

Principle

Ion exchange chromatography is based upon the differential affinity of ions for the stationary phase. The rate of migration of the ion through the column is directly dependent upon the type and concentration of ions that comprise the eluent.

Procedure

Before analysing samples on this machine, the samples are filtered as described in Section 2.1.1. The filtered samples are then put into vials and placed in the

machine and are ready for analysis. To analyse chloride, sulphate and nitrate ions in the water sample, IonPac AS4A-SC column is used, which separates general purpose inorganic anions such as fluoride, chloride, nitrite, phosphate, bromide, nitrate, sulphate. Anion eluent is 1.8 mM sodium carbonate or 1.7 mM sodium bicarbonate. As for analysis of NH_4^+-N, IonPac C12A column is used. Cation eluent is 20 mM methanesulphonic acid.

The Dionex is calibrated for anions using and eight-level quadratic calibration curve, and for cations using a seven-level quadratic calibration curve. Quality control samples are used in each batch of analyses, to monitor any bias. The laboratory partakes in the Aquacheck Proficiency Testing Scheme, run by WRC plc. This provides independent proof of the laboratory's performance. Analytical results from the Dionex are submitted to this scheme.

Concentrations of anions and cations in the water samples are recorded and printed automatically using the computer system linked to the machine. The output of the analysis for a rainwater sample is shown as Figure A1 and Figure A2 for both anions and cations.

Method File Name : c:\peaknet\method\anions0.5.met
Date Time Collected : 09/03/99 16:59:52
System Operator :

Peak Information : All Peaks

Peak #	Component Name	Retention Time	Amount (mg/l)	Peak Area	Peak Height
1		1.05	0.00	3961	871
2		1.22	0.00	2648	317
3	Chloride	1.58	10.05	829028	158531
4		2.33	0.00	1405	82
5	Bromide	2.90	0.03	938	131
6	Nitrate	3.28	0.18	29864	3275
7	Phosphate	5.50	0.04	2080	310
8	Sulphate	7.00	2.21	114723	6476

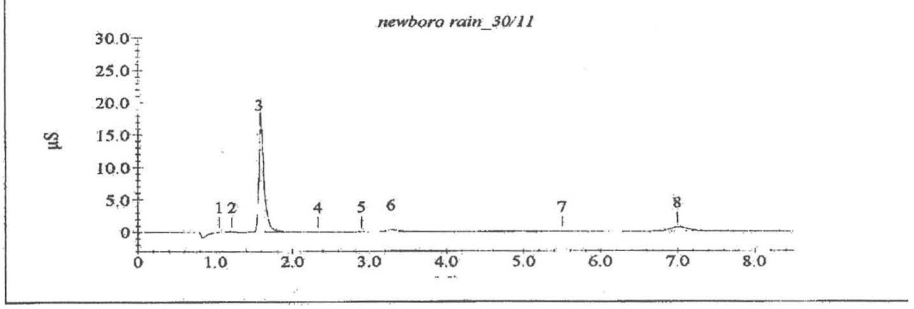


Figure A1

Method File Name : c:\peaknet\method\cations5.0.met
Date Time Collected : 09/03/99 13:22:37
System Operator :

Peak Information : All Peaks

Peak #	Component Name	Retention Time	Amount	Peak Area	Peak Height
1	Sodium	4.68	6.30	1251866	82516
2	Ammonium	5.63	0.15	36990	2299
3	Potassium	7.85	0.48	65237	2369
4	Magnesium	11.30	0.85	342306	9054
5	Calcium	14.82	0.50	121198	2553

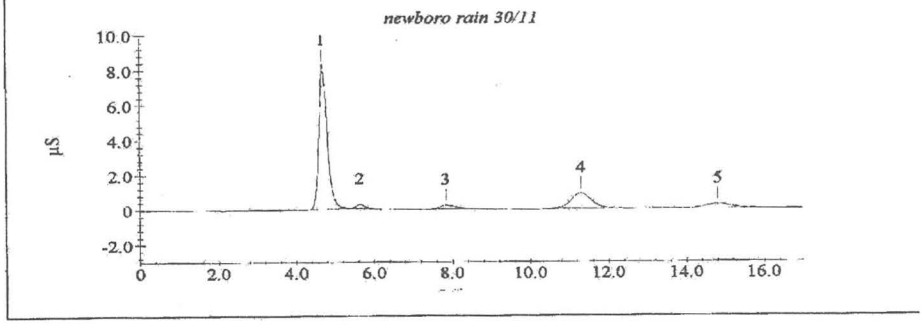


Figure A2

Figure A1 and Figure A2: The output of water sample analysis for anions (Figure A1) and cations (Figure A2) using the Dionex Ion Chromatograph. Concentration of analysed ions are read automatically and shown in the column **'Amount (mg/l)'**.

APPENDIX 2.2

Procedure of running samples on Skalar Segmented Flow AutoAnalyser SA-40 (For details see the SAN^{plus} Segmented Flow Analyzer and its application, 1993).

Principle of Segmented Flow Analysis

Automatic segmented flow analysis is a continuous flow method of chemical analysis in which a stream of reagents and samples, segmented with air bubbles is pumped through a manifold to undergo treatment such as mixing, heating, dialysis, etc. before entering a flow cell to be detected. Air segmentation is used to eliminate cross contamination and to provide an aliquot to mix different reagents.

1. Analysis of soil nitrate-N from the KCl solution extracts

Principle

The KCl extracts are diluted in an ammonium chloride buffer and pumped through a cadmium column. The nitrate is hereby reduced to nitrite. Hereafter a colour reagent is added to form a coloured-diazo complex with the nitrite ion. The extinction is measured at 540 nm and is in relation to the concentration of the nitrate.

Reagents

A. Ammonium chloride (NH_4Cl)

Required chemicals:	Ammonium chloride (NH_4Cl)	25 g
	Ammonia solution (NH_4OH) (25%)	± 1 ml
	Distilled water (H_2O)	1000 ml
	Bry 35 (30%)	1 ml

Preparation:

The ammonium chloride is dissolved in ± 900 ml distilled water. Check the pH and correct with ammonia solution to 8.2. Fill up to 1 litre, add the Bry 35 and mix.

B. Colour reagent

Required chemicals:	o-Phosphoric acid (H_3PO_4) (85%)	150 ml
	Sulphanilamide ($C_6H_8N_2O_2S$)	10 g
	α-Naphthylethylene-diamine dihydrochloride ($C_{12}H_{16}Cl_{12}N_2$)	0.5 g
	Distilled water	1000 ml

Preparation:

The phosphoric acid is diluted in ± 700 ml distilled water. Hereafter the sulphanilamide and α-Naphthylethylene-diamine dihydrochloride are dissolved. Fill up to 1 litre and mix. Store in dark coloured bottle.

Standard

Stock standard 1000 ppm N

Required chemical:	Sodium nitrate ($NaNO_3$)	6.071 g
	Distilled water	1000 ml

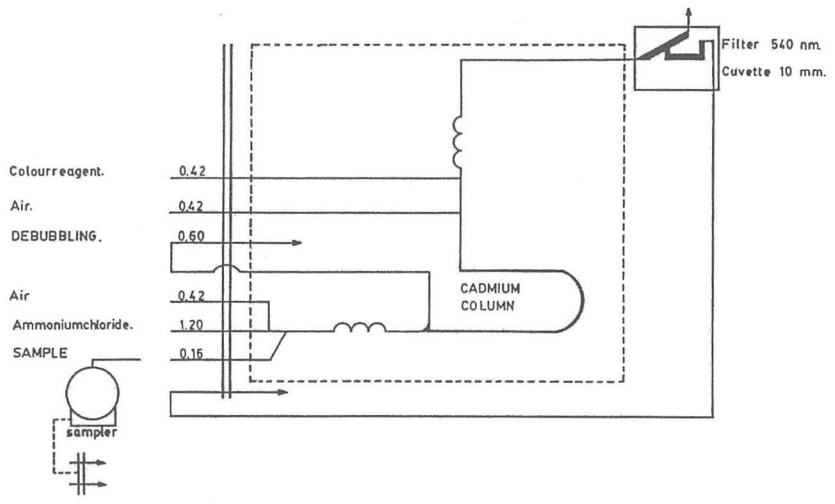
Preparation:

The sodium nitrate is dissolved in ± 900 ml distilled water. Dilute to 100 ml, and mix thoroughly. The standards required for the calibration curve are prepared out of the stock standard.

Procedure activation cadmium column

Cadmium granules (size 0.3-1.0 mm) are mixed with ± 30 ml hydrochloric acid 4 N and stir for 1 minute. Wash with distilled water. Hereafter add ± 50 ml cupric sulphate solution 2% and stir for 3 minutes. Wash with distilled water 10 times. Then, fill the cadmium column with distilled water (take care: no air bubbles). Add the cadmium granules with the aid of funnel on both sides of the column, with now and then vibration to pack the column. Fill up to 5 mm from the top. Here place a small plug of glasswool in the outlet. Hereafter the column is ready for use and can be placed in the system.

FLOW DIAGRAM



Note:
Numbers denote the size of the tube diameter.

2. Analysis of soil ammonium-N from the KCl solution extracts

Principle

The KCl extracts are diluted in a buffer solution to complex cations. Hereafter salicylate, a catalyst and active chloride are added to form a green coloured

complex with the ammonium-ion. The extinction is measured at 660 nm and is in relation to the concentration of the ammonia.

Reagents

A. Buffer-solution

Required chemicals:	Potassium sodium tartrate ($C_4H_4O_6KNa$)	33 g
	Sodium citrate ($C_6H_5O_7Na_3$)	24 g
	Distilled water	1000 ml
	Bry 35 (30%)	1 ml

Preparation:

Potassium sodium tartrate and sodium citrate are dissolved in ± 800 ml distilled water. Dilute to 1 litre, add the Bry 35 and mix.

B. Sodiumsalicylate

Required chemicals:	Sodium hydroxide (NaOH)	30 g
	Sodium salicylate ($C_7H_5NO_3Na$)	80 g
	Distilled water	1000 ml

Preparation:

NaOH is dissolved in ± 50 ml distilled water. Add 700 ml distilled water and dissolve sodium salicylate. Dilute to 1 litre and mix. Solution is stored in a dark bottle and stable for one week.

C. Sodium nitroprusside

Required chemicals:	Sodium nitroprusside $[Fe(CN)_5]NO\ Na_2$	1 g
	Distilled water	100 ml

Preparation:

Sodium nitroprusside is dissolved in ± 800 ml distilled water. Dilute to 1 litre and mix. Solution is stored in a dark bottle and stable for one week.

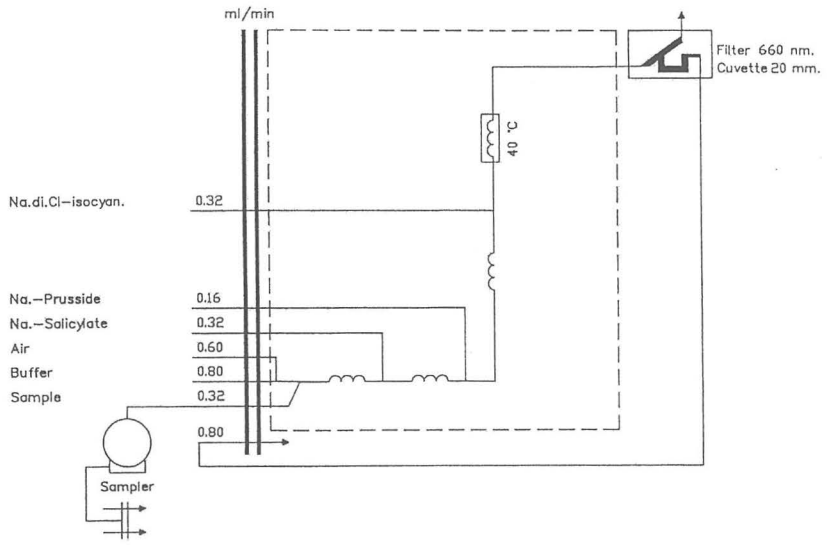
D. Sodium dichloroisocyanurate ($C_3N_3O_3Cl_2Na$). 2 g of the chemical is dissolved in 800 ml distilled water. Dilute to 1 litre and mix. Solution is stable for 2 days.

Standard

Stock solution 1000 ppm N.

3.8207 g ammonium chloride (NH_4Cl) is dissolved in 800 ml distilled water. Dilute the solution to 1000 ml and mix thoroughly. Prepare out of the stock solution the required standards needed for the calibration curve.

FLOW DIAGRAM



Note:
Numbers denote the size of diameter of the tubes

APPENDIX 3.1

Calculation of N deposition

1. Flux (kg N ha^{-1} y^{-1}) = V_d (ms^{-1}) × χ (µg m^{-3})

   where V_d = Deposition velocity, χ = air concentration

2. For NO_2, V_d used was 1.5 mm s^{-1} = 0.0015 m s^{-1}

   For NH_3, V_d used was 20 mm s^{-1} = 0.02 m s^{-1}

3. Proportion of N in: (atomic weight N=14; H=1; O=16)

   i) NO_2

   $$y = {}^{14}/_{46} = 0.3043$$

   ii) NH_3

   $$y = {}^{14}/_{17} = 0.8235$$

Total seconds per year = 60 × 60 × 24 × 365 = 31.536 × 10^6 s

Conversion of µg to kg = x10^{-9} kg

Conversion of m^2 per ha = x10^{-4} ha

$$\therefore \text{Flux} = \frac{y \times \chi \times V_d \times 31.536 \times 10^6 \times 10^4}{10^9} \text{ kg N ha}^{-1}\text{ y}^{-1}$$

APPENDIX 4.1

PROTOCOL FOR ANALYSIS OF AQUEOUS NH_4^+ USING AMFIA AT ITE EDINBURGH

As a precaution measure, disposable gloves must be worn at all times during the analyses. It is necessary to ensure that glassware for working standards is washed with Decon detergent on a daily basis, taking particular care with low concentration standard glassware.

The principle of Ammonium Flow Injection Analysis (AMFIA) has been explained in Section 4.1.4 of this thesis.

PROCEDURE

Before commencing with any analysis, all reagent reservoirs are ensured sufficiently full and that the waste reservoir is empty. Replenish reagent bottles if necessary, using the methods in ANNEX (a).

Switch on the mains and the reagent pump and leave AMFIA to equlibrate for at least half an hour with reagents running through the system. When it is confident that the baseline has settled to a steady and noise-free state (normal baseline 200-600), extracting samples can be proceeded. It is not wise to extract samples from their stored state until everything is confidently running well. Next, the PC is switched on and a codefile for the specific sample run concerned is set up. This can be done at a later stage once AMFIA is processing samples.

EXTRACTION OF SAMPLES

Diffusion Tubes

Diffusion tubes are sorted into a suitable order for loading into the autosampler. With great care, systematically add 1.5 ml deionised water to a clean sample cup using an automatic pipette. As quickly as is practical, remove the "mesh end" cap from the first diffusion tube (containing the sample grid) and put this onto the cup. Try to minimise the time it takes to do this. Ensuring that the cap is firmly on the cup of water, invert and leave to extract for at least 30 minutes. This procedure is repeated with all of the samples, noting the time at which the extraction process commenced.

Badge Samplers

Remove the 6 mm support rings from the badge samplers and carefully transfer the filter from each badge sampler to a clean sample pot using clean forceps. It is also possible to place the sampler over the sample pot and dislodge the filter gently tapping the bottom of the sampler. With great care, 2.0 ml deionised water is added to the filters using an automatic pipette, cap the pots immediately and leave to extract for at least an hour. This procedure is repeated with all of the samples, noting the time at which the extraction process commenced.

Loading of Autosampler

Once extraction is complete, the autosampler can be loaded. Check that there is a sufficient supply of clean autosampler tubes to complete the run. AMFIA is then calibrated with calibration standards (0.1 and 1 ppm NH_4^+ standards for diffusion tubes and badge samplers), followed by the analysis of extracted samples, quality controls and blanks in a random order.

The first two tubes are always calibration solutions. They are sampled from more than once and must be filled with enough standard to allow for multiple injections. The first standard (low concentration standard, CAL 1) will be sampled three times in total and must be full. The second standard (high concentration standard, CAL 2) will be sampled twice and must be three-quarters full. Fill the firsts two tubes with the required standard to the volume necessary. Proceed filling the subsequent autosampler tubes with samples decanted directly from the sample cups, but as it is difficult to decant from sample pots for the badge sampler filters, an automatic pipette should be used to transfer the extracts.

Recalibration is performed after about every 10 (up to 14) samples with the calibration standards to correct for sensitivity drifts. Quality Control samples can also be placed in the run, as a further analytical check. These samples should be coded #QCn (n = 1, 2 etc), in order to be recognised as quality control samples by AMFIA. Once the autosampler has been loaded with samples/standards, the carousel is manually move forward to the start position, with the first tube (CAL 1) directly under the sampling needle.

Parameters on the status screen are checked for a correct set up. It is of particular to ensure that the calibration concentrations are correct as well as the number of samples to be analysed before start logging.

The run should commence and proceed through the samples to the end. Samples are kept checked at regular intervals throughout to ensure that everything is proceeding as expected. Ensure that the needle is going completely into the sample solution and that it is drawing up complete slugs without air bubbles. If the volume of sample is such that it is at the lower limits of

the sample volume required (~0.8 ml) place the sample tube in the holder at a height slightly higher than normal i.e., do not push it all the way to the bottom of the holder. This should ensure that when the needle lowers down into the tube it samples from the bottom of the solution. Be wary in this instance however of the needle breaking the surface of the tubes and attaching itself to them. The needle will physically lift the tubes up as it retracts. It this happens, lower the sample tubes very slightly until this ceases to occur.

CALCULATION

The amount of ammonia collected (Q) on diffusion tube due to air sampling is given by:

$$Q = (c_e - c_b)^* v$$

where c_e is the liquid concentration of an exposed tube, c_b is the liquid concentration of a blank tube and v is the liquid volume of the extraction solution. The air concentrations (χ_a) of ammonia is then determined as:

$$\chi_a = Q/V$$

where V is the effective volume of air sampled. For diffusion tubes, this is calculated initially using the theoretical diffusion rate of ammonia and the tube dimensions, and equates to approximately 0.15 m^3 per month. For diffusion tubes the effective sample volumee may be found as:

$$V = DAt/L$$

where D is the diffusion coefficient of NH_3 in air, A is the tube cross sectional area, t is sampling duration and L is length of the diffusion tube.

ANNEX (a)

100 ppm NH_4 (100 mg l^{-1}) stock solution

Reagents: Ammonium sulphate, $(NH_4)_2SO_4$ (SIGMA, ACS reagent grade)

Deionised water

Procedure

Before use, dry a small quantity of ammonium sulphate at 70 °C for 5 hrs in an oven and then cool to room temperature inside a dessicator. Weigh out 366.9 mg (± 2.0 mg) dried ammonium sulphate, dissolve in approximately 100 ml deionised water in a 1000 ml volumetric flask and make up to volume. This stock solution is used for the preparation of working calibration standards. Store the stock in a clean plastic bottle and in the dark at 4°C. The prepared stock solution should be stable for about 3 months.

Low calibration standards, 0-1 ppm NH_4^+ (for diffusion tubes and badge samplers)

Allow the 100 ppm NH_4^+ stock solution to equilibrate to room temperature before use.

1 ppm NH_4^+: Dilute 1 ml of 100 ppm NH_4^+ stock solution to 100 ml with deionised water in 100 ml volumetric flask.

0.1 ppm NH_4^+: Dilute 10 ml of 1 ppm NH_4^+ standard to 100 ml with deionised water in 100 ml volumetric flask.

Quality controls: Prepare two quality control solutions of concentrations 2 ppm and 0.2 ppm from a different 100 ppm NH_4^+ stock solution.

Sodium Hydroxide (NaOH) solution

Prepare 1 litre of 0.5 M NaOH solution (20 g NaOH solid per litre) containing 1 ml of 100 ppm ammonium standard.

Weigh out 20 g NaOH pellets and transfer quantitatively to a litre volumetric flask with deionised water. Add approximately 500 ml of deionised water and mix well. Using an automatic pipette, add 1 ml of 100 ppm ammonium standard to the flask and again mix well. Finally make the solution up to a litre volumetrically with deionised water. Mix well.

APPENDIX 4.2

a. *Atmospheric ammonia concentrations (μg m^{-3}) measured using badge samplers.*

SITE	MONTHLY CONCENTRATION (μg m^{-3})											
	Dec 97	Jan 98	Feb 98	Mar 98	Apr 98	May 98	Jun 98	Jul 98	Aug 98	Sep 98	Oct 98	Nov 98
A - Farm	61.04	3.82	5.29	16.70	88.15	128.48	17.73	71.76	51.71	177.72	25.84	72.38
B - 100 m SW of farm	NA	NA	2.67	4.51	22.52	31.69	4.10	11.35	12.44	50.76	5.19	15.10
C - 300 m SW of farm	7.46	2.47	1.32	2.09	10.23	12.70	1.87	4.35	5.24	19.63	2.14	6.50
D - 800 m SW of farm	1.73	0.84	0.47	0.63	1.41	1.78	0.35	0.83	0.87	3.51	0.42	1.52
E - 2000 m SW of farm	-	-	-	-	-	1.42	0.32	0.42	0.63	1.58	0.30	0.71
F - 2800 m SW of farm	1.35	0.65	0.51	0.79	0.96	1.33	0.44	0.58	1.20	2.17	0.36	0.94
2000 m SE of farm (Rhuddgaer)	1.17	1.90	1.31	1.32	1.42	1.42	-	-	-	-	-	-
Sewage Works	1.79	1.75	1.00	2.43	2.45	3.23	1.64	2.35	2.23	2.45	0.67	1.25

Note: 1. NA – Not Available (monitoring at this site started in February 1998)
2. Monitoring at Site E (2000 m SW of the farm) started only in May 1998

b. *Atmospheric ammonia concentrations measured using diffusion tubes (uncorrected)*

SITE	MONTHLY CONCENTRATION (μg m^{-3})											
	Dec 97	Jan 98	Feb 98	Mar 98	Apr 98	May 98	Jun 98	Jul 98	Aug 98	Sep 98	Oct 98	Nov 98
A - Farm	68.34	6.11	7.11	18.38	99.63	151.50	18.44	78.53	55.43	219.21	17.11	N/A
B - 100 m SW	-	-	2.53	6.14	23.59	31.62	4.44	8.53	13.97	51.14	4.30	N/A
C - 300 m SW	9.33	5.10	1.75	2.56	9.66	8.46	2.64	4.57	6.01	22.58	2.01	N/A
D - 800 m SW	3.07	1.35	1.05	1.12	1.59	2.20	0.73	0.53	1.11	4.36	0.45	N/A
E - 2000 m SW	-	-	-	-	-	-	-	-	-	-	-	-
F - 2800 m SW	1.79	0.99	0.77	1.10	1.19	1.50	0.78	1.22	1.39	2.92	1.04	N/A
x - 30 m NE	-	-	-	-	-	-	-	-	42.66	71.08	-	-
y - 100 m NE	-	-	-	-	-	-	-	-	6.90	10.41	-	-
z - 300 m NE	-	-	-	-	-	-	-	-	1.90	2.29	-	-

Note: No measurement was conducted in November 1998

....Appendix 4.2 (continued)

c. *Atmospheric ammonia concentration measured using diffusion tubes (correction factor applied)*

SITE	MONTHLY CONCENTRATION ($\mu g\ m^{-3}$)											
	Dec 97	Jan 98	Feb 98	Mar 98	Apr 98	May 98	Jun 98	Jul 98	Aug 98	Sep 98	Oct 98	Nov 98
A - Farm	90.53	7.55	8.88	23.91	132.24	201.40	23.99	104.10	73.30	291.68	22.22	N/A
B - 100 m SW	-	-	2.77	7.59	30.86	41.56	5.32	10.78	18.03	67.59	5.13	N/A
C - 300 m SW	11.84	6.21	1.73	2.82	12.28	10.68	2.92	5.50	7.41	29.51	2.08	N/A
D - 800 m SW	3.50	1.20	0.79	0.89	1.52	2.33	0.37	0.10	0.88	5.22	0.00	N/A
E - 2000 m SW	-	-	-	-	-	-	-	-	-	-	-	-
F - 2800 m SW	1.78	0.72	0.43	0.87	0.99	1.39	0.44	1.03	1.25	3.29	1.04	N/A
x - 30 m NE	-	-	-	-	-	-	-	-	56.27	94.17	-	-
y - 100 m NE	-	-	-	-	-	-	-	-	8.60	13.28	-	-
z - 300 m NE	-	-	-	-	-	-	-	-	1.93	2.46	-	-

Note: No measurement was conducted in November 1998

APPENDIX 5.1

LONG ASHTON NUTRIENT SOLUTION

The Long Ashton Nutrient Solution described by Hewitt (1966) is one of the most widely used culture solutions and has been successfully used for the sand or water culture of a wide range of crop and other plants.

The recipe for 100 litre of working strength solution is as follows:

Salt	Stock solution (g/l)	Volume of stock (ml) for 100 l nutrient solution	concentration (ppm) of diluted solution
KNO_3	202	200	K 156; N 57
$Ca(NO_3)_2$	328	200	Ca 160; N 113
$MgSO_4.7H_20$	184	200	Mg 36; S 48
$NaH_2PO_4.2H_2O$	208	100	Na 31; P 41
Fe EDTA (monosodium complex)	37.3	50	Fe 2.8; Na 1
$MnSO_4.4H_2O$	22.3	10	Mn 0.55
$CuSO_4.5H_2O$	2.5	10	Cu 0.064
$ZnSO_4.7H_2O$	2.9	10	Zn 0.065
H_3BO_3	31.0	10	B 0.54
NaCl	58.5	10	Na 2.3; Cl 3.5
$Na_2MoO_4.2H_2O$	1.2	10	Mo 0.048

This gives a diluted culture solution of the following composition:

Element	ppm	Element	ppm
K	156	Fe	2.8
N	170	Cl	3.5
S	48	Mn	0.55
Ca	160	B	0.54
Mg	36	Zn	0.065
P	41	Cu	0.064
Na	34	Mo	0.048

For experiments requiring several nitrogen levels, KNO_3 and $Ca(NO_3)_2$ are replaced by $NaNO_3$, K_2SO_4 and $CaCl_2.6H_2O$. This substitution balances the levels of calcium and potassium but sodium and chlorine are increased.

Different amounts of $NaNO_3$ are then used to prepare the nitrogen treatments.

Ammonium nitrogen (NH_4-N) can be supplied by $(NH_4)_2SO_4$.

Salt	Stock solution (g/l)	Volume of stock (ml) for 100 l nutrient solution	concentration (ppm) of diluted solution
$NaNO_3$	340	300	Na 276; N 170
K_2SO_4	87	400	K 156; S 64
$CaCl_2.6H_2O$	438	200	Ca 160; Cl 284
$(NH_4)_2SO_4$.	264	200	N 113; S 128