Atmospheric Deposition of Combined Nitrogen in the Cape Metropolitan Area: A Threat to a Species Rich Ecosystem?

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Table of Contents

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Abstract

In this project, two broad approaches were used in order to gauge levels of atmospheric N deposition to the natural vegetation of the Cape Metropolitan Area (CMA). In the first approach, bulk collectors were constructed and set up at five sites around the CMA in order to trap atmospheric deposition for chemical analysis. A number of problems were experienced including undercatch by samplers, analytical difficulties, bacterial consumption of N compounds in the field and possible contamination of samples. Correcting for these sources of error as far as possible, it was estimated that annual bulk N deposition figures for the natural vegetated areas of the CMA range from 2.5 kg $N.ha^{-1}.a^{-1}$ at Silvermine, south of the city to 5.4 $kg.ha^{-1}.a^{-1}$ on the plateau of Table Mountain and 5.6 $kg.ha^{-1}.a^{-1}$ at Kenilworth R.C. in the centre of the urban area. NH_4 levels were greater than NO_y levels at all sites except for Table Mountain, where the reverse was true. Tests showed that a diminution of nitrogenous analytes during storage is likely to have occurred, and it is suggested that deposition levels may be 10 to 20% higher than these estimates. Annual SO_4 deposition levels were higher than expected, with annual levels (calculated by extrapolating from data for 8 months), ranging from around 8 kg $S.ha^{-1}.a^{-1}$ at Silvermine and Tygerberg to more than 16 $kg.ha^{-1}.a^{-1}$ at Kenilworth. Low deposition to emission ratios for NO_y (0.07) and SO_4 (0.16) indicated that a large proportion of pollution emitted in the CMA is advected out to sea before it can be deposited to the land surface.

In the second approach, moss samples collected from different parts of the CMA during various historical periods, were analysed for N concentration, C: N ratio and $^{15}N/^{14}N$ isotope ratio in order to investigate the increase in N deposition over the past 100 years and the primary source of this increase. A strong historical trend of increasing foliar N concentration was observed, particularly in ectohydric species, indicating that a large increase in atmospheric N deposition occurred over that period. Using relationships between ectohydric moss tissue %N and atmospheric deposition levels observed in European studies, it was calculated that the increase in foliar N concentration of ectohydric moss species in the CMA since the pre-1940 period would represent an increase in N deposition of between 6 and 13 $kg.ha^{-1}.a^{-1}$. Foliar N and C: N ratio were

also influenced by species and, in mixohydric species, season of collection, while collection region had no observable effect on these variables. $\delta^{15}N$ values of moss foliage were somewhat erratic and showed no clear historical trend as had been predicted, although species and collection region did have significant effects on this variable. Moss transplants from an unpolluted site to three polluted sites in the city did not show clear evidence of increased foliar N concentration or altered $\delta^{15}N$ over the period of a year, although this was probably the result of the influence of season on the mixohydric species used in this experiment.

Based on international research and research in the fynbos, it is suggested that areas of vegetation receiving N deposition loads likely to exceed 10 $kg.ha^{-1}.a^{-1}$, could be susceptible to changes in vegetation structure and function, particularly through invasion by grasses.

Further consequences of the findings of this study for the terrestrial ecosystems of the CMA as well as possible strategies to deal with this threat are discussed.

Chapter 1

General introduction

The city of Cape Town and its surrounds (henceforth referred to as the Cape Metropolitan Area - CMA) presents a unique challenge to conservationists. As well as being one of the largest and most rapidly expanding urban centres on the African continent, it is home to an extremely diverse flora, many species of which are endemic to the area, and a number of which are highly endangered. As a result, Cape Town is recognized as one of three urban biodiversity hotspots worldwide (City of Cape Town 2003b). The flora of Cape Town also constitutes an important part of the Cape Floristic Province, which has been recognized as one of 25 world biodiversity hotspots (Myers *et al.* 2000).

The most threatened areas of natural vegetation in the CMA are the few remaining fragments of veld situated on the Cape Flats, and surrounded on all sides by urban sprawl. These sites are amongst the last remaining refuges of acid sand-plain fynbos, a highly endangered type of lowland heath which has, through development and agriculture, been reduced to less than 1 percent of its historical extent (McDowell & Low 1990). Collectively, they are an extremely high priority for conservation, containing, at last count, 76 species endemic to the area and 131 red data book species. This is one of the highest concentrations of endangered species anywhere in the world (Maze & Rebelo 1999).

Commonly identified threats to the CMA flora include habitat destruction, mechanical disturbance, frequent fires and invasions by woody alien species (Rouget *et al.* 2003, Rebelo 1992, Maze & Rebelo 1999, McDowell & Low 1990, Richardson *et al.* 1996). A threat which is often overlooked, however, is grass invasion, which is rife at the majority of Cape Flats sites identified by Maze and Rebelo (1999) as core conservation areas. For example, at Kenilworth race course, considered to be the most important remaining area of natural vegetation on the Cape Flats, grass is often observed to over-top indigenous shrubs and has been shown to limit seed set of indigenous species (Wilson 1999). Although the threat of grass invasion on the flats has been noted before by several authors (e.g. Vlok 1988, Campbell *et al.* 1980, Jobst 1996, Milton 2004), it has, to date, received little attention in terms of research or remedial efforts.

One of the most important drivers of grass invasion in other parts of the world such as Europe (Bobbink *et al.* 1998) and North America (Fenn *et al.* 2003), has been N addition through atmospheric deposition of airborne pollution. The potential for N addition to trigger invasions is particular to N limited systems, because most indigenous species from these habitats are adapted to low N availability and are unable to make use of the added N to increase growth and vigour. In contrast, added N greatly increases the vigour of nitrophilous species, many of which are exotic grasses, and allows them to outcompete slow growing indigenous species for resources such as light, water and space. In addition, grass invasion may increase the rate of N cycling as well as the frequency and intensity of fires, to the detriment of indigenous species (D'Antonio & Vitousek 1992). As discussed in the following chapter, natural vegetation in many parts of the world has been completely transformed to grass under N addition, although, in most cases, other factors such as land management regime (Bobbink & Willems 1987) and increased herbivory (Brunsting & Heil 1985) are thought to interact with deposition to produce this effect. N addition may also have numerous other negative impacts which are described in detail in the following chapter. These include acidification of soils, direct toxic effects on plants and increased susceptibility of plants to secondary stress factors such as herbivory, pathogens, frost and drought (e.g. Bobbink *et al.* 1998, Skeffington & Wilson 1988). Acidification and direct toxic effects may also be caused by SO_2, another pollutant which is commonly emitted at high levels in urban areas.

Based on findings in other parts of the world, it is hypothesized that N addition is an important factor contributing to the grass invasion problem in Cape Town. Modeled average N deposition levels are low for the Cape Floristic Province as a whole (< 2 $kg.ha^{-1}.a^{-1}$), and N deposition is thus not generally considered to be a major threat to fynbos (Phoenix *et al.* 2005). However, at the more local scale of the CMA, N deposition is cause for concern. Substantial emissions of nitrogenous air pollution are known to occur in the CMA in the form of oxidized N (NO_x), which mainly originates from motor vehicles (Wicking-Baird *et al.* 1997), and emissions of reduced N (NH_3) may also be important given that a moderate amount of agricultural activity takes place in and around the city.

The fynbos vegetation which covers most of the CMA is predicted to be highly susceptible to N induced competitive changes as a result of the extremely nutrient poor soils on which it generally grows (soil surface total N values are generally in the range of 250-350 kg N. ha^{-1}, Stock & Lewis 1986b) and the slow-growing, stress tolerant species which predominate here. A few limited N addition experiments in fynbos have suggested that changes in vegetation structure can be expected in response to comparatively low levels of N addition (Witkowski 1988, Lamb & Klaussner 1988).

A possible secondary concern with regard to the health of natural areas in the CMA is acidification, because both oxidized and reduced N, as well as sulfurous pollutants such as SO_2 and its reaction product $SO_4^{\ 2+}$, have the potential to acidify soils and water bodies. Like N deposition, acidification is not generally considered to be a serious threat to the Cape Floristic Region due to low average acid deposition rates (<2 $kg.ha^{-1}.a^{-1}$) in the region (Kuylenstierna *et al.* 2001), but may be cause for concern at the local level. Fynbos soils are particularly susceptible to acidification due to their low base cation status (Stock & Allsopp 1992). However, acidification may be less of a threat to the vegetation of the CMA than eutrophication, as most local species are adapted to the already acidic soils which occur here and would be fairly tolerant of further acidification stress (Phoenix *et al.* 2005).

The lowland vegetation fragments on the Cape Flats may be considered to be most at risk from the potential negative effects of air pollution by virtue of the close proximity of these sites to major roads, industry and agricultural emission sources, and because of the large number of rare and endangered species which occur here. Observation also suggests that, currently, grass invasion in the CMA is mostly a feature of lowland areas. As discussed in the following chapter however, atmospheric deposition is a complex phenomenon, and, given the variable nature of Cape Town's topography and climatology, it is quite likely that other parts of the CMA might also receive substantial pollutant deposition loads. The majority of land to the south-east of the Cape Flats (the Cape Peninsula and the Table Mountain chain) is conserved under the banner of the Cape Peninsula National Park, which is home to roughly 2285 plant species, 160 of which are local endemics (Rebelo 1992). The possible threat posed to these areas by deposition is also an important topic for investigation.

A previous investigation by the author (Wilson 1999) attempted to determine whether anthropogenic N addition was occurring in the CMA by using radish phytometers to gauge the favourability for growth of soils from urban sites compared to those from unpolluted control sites. Phytometer results were inconclusive because the growth of radishes was influenced to a large degree by factors other than soil inorganic N (most notably soil pH), but soil from city sites generally had higher total N than control sites for comparable amounts of organic matter, suggesting that N addition and retention by soils may be occurring. I realised it was impossible to look at the grass invasion problem from an N enrichment perspective without proof that substantial N deposition was occurring.

This project thus focuses on measuring bulk deposition rates of inorganic N (as well as S) in different parts of the CMA (chapter 3) and providing evidence of a substantial historical increase in N deposition rates as reflected by moss tissue N contents (chapter 4). It also provides a synthesis of the current knowledge on N deposition and its effects on terrestrial ecosystems in other parts of the world (chapter 2) and offers predictions of the likely effects that increased N deposition may have on local ecosystems, as well as suggestions as to how the problem might be mitigated in future (chapter 5).

Chapter 2

Literature overview

2.1. Human perturbation of the global nitrogen cycle

As human populations and individual resource requirements have increased, so have concerns about the ways in which human activities pollute natural systems and disrupt biological and geochemical processes. Amongst the most important of these is the global cycle of N, an element that is fundamental to all life (Raven & Zin 1998). Although nitrogen gas (N_2) constitutes roughly 78 % of the volume of the earth's atmosphere, N only becomes biologically available (except to a select few specialized organisms) when combined with the elements O, H or C to form reactive N. Under natural circumstances, N in this reactive form is scarce from a biological point of view, and is the limiting nutrient for organisms in the majority of natural ecosystems (Vitousek *et al.* 2002). It is thus clear that eutrophication of these systems through a large additional anthropogenic input of N, will have major implications for their structure and functioning. Although this eutrophication effect is the most important consequence of N pollution for many ecosystems, it is only one step in a potential cascade of impacts that N pollutants may have (Erisman *et al.* 1998, Galloway & Cowling 2002). N compounds emitted to the atmosphere may initially contribute to photochemical smog formation and direct effects on the health of organisms, as well as affecting the regional radiation balance due to reflection of insolation by aerosol particles. Upon deposition, they will cause eutrophication and acidification of soils and waters and eventually, may contribute to global warming and depletion of stratospheric ozone by emission as the greenhouse gas, N_2O.

At present, human activities produce nearly twice the amount of combined N per year (~165 Tg yr^{-1}) as is created by biological N fixation in terrestrial ecosystems (~ 90 Tg yr^{-1}) (Galloway *et al.* 2002, Tg = 10^{12} g). The majority of this N (~120 Tg yr^{-1}) is involved in food production, with inorganic fertilizers accounting for about 70% of this total and cultivation of nitrogen-fixing crops (legumes and rice) the remainder

(Galloway & Cowling 2002). A further 25 Tg yr^{-1} is released by fossil fuel combustion and 20 Tg yr^{-1} is created for other uses. In addition to creation of new reactive N, human activities also mobilize N (estimated at ~70 Tg yr^{-1}) from long term storage pools through activities such as biomass burning, land clearing and drainage of wetlands (Vitousek *et al.* 1997).

On both global and regional scales, production and addition of N to the environment is very spatially heterogeneous. Worldwide, the majority of anthropogenic N creation and release presently takes place in the rapidly developing economies of Asia and the developed countries of N. America and Europe (Galloway & Cowling 2002, Galloway 1998), where N deposition has had marked negative impacts on natural ecosystems (Bobbink *et al.* 1998, Fenn *et al.* 1998, 2003). Future projections indicate that Asia, Africa and S. America will play an increasingly important role in mobilizing N (and S) due to rapid population growth, urbanization and industrialization in these regions (Kuylenstierna *et al.* 2001, Vallack *et al.* 2001). This contrasts with the large reductions in N and S emissions being achieved in developed countries through the implementation of protocols and agreements to optimize food and energy production (Kuylenstierna *et al.* 2001, Vallack *et al.* 2001, Galloway *et al.* 2002). As many of the worlds most important centres of biodiversity and conservation worthy natural habitats are located in developing countries (Phoenix *et al.* 2005, Myers *et al.* 2000), these areas are a high priority for monitoring of deposition chemistry and research into current and future pollution threats to local ecosystems. In this way, informed policy decisions can be made in order to avoid the costly effects which have occurred in parts of Europe and America (Van Tienhoven *et al.* 2003, Chipindu *et al.* 1998, Innes & Harron 2000, Kuylenstierna *et al.* 2001.). The focus of this overview will be on the processes, patterns and effects of N deposition in natural terrestrial ecosystems, with particular emphasis on vegetation.

2.2. Atmospheric nitrogen deposition

On a regional scale, anthropogenic N production and addition is concentrated in areas of human settlement and agricultural activity. However, a large proportion of this N is released as, or is transformed into mobile species, which may be transported away from the source via the atmosphere. In this way, N pollution has the potential to affect natural areas which would generally be thought of as removed from pollution threats (Rodhe *et al.* 1995b). All of the N released by burning of fossil fuels and biomass is emitted directly into the atmosphere in reactive form, as is a large proportion of N involved in agriculture (in the region of 50%; Galloway 1995), either directly (from synthetically fertilized soils and soils under N-fixing crops), or indirectly (from livestock/human excreta and crop emissions/ decomposition) (Olivier *et al.* 1998, Smil 1999). Waters receive a further 20-30 % of agricultural N through leaching and erosion (Smil 1999). Of the total global anthropogenic emissions of reactive N, Galloway (1995) estimates that about 75% is redeposited to terrestrial environments. Atmospheric deposition is thus responsible for a greatly increased supply of reactive N to natural terrestrial ecosystems worldwide.

2.2.1 General processes

The general processes involved in the deposition of combined atmospheric N (and other pollutants) are summarized in Figure 2.1.

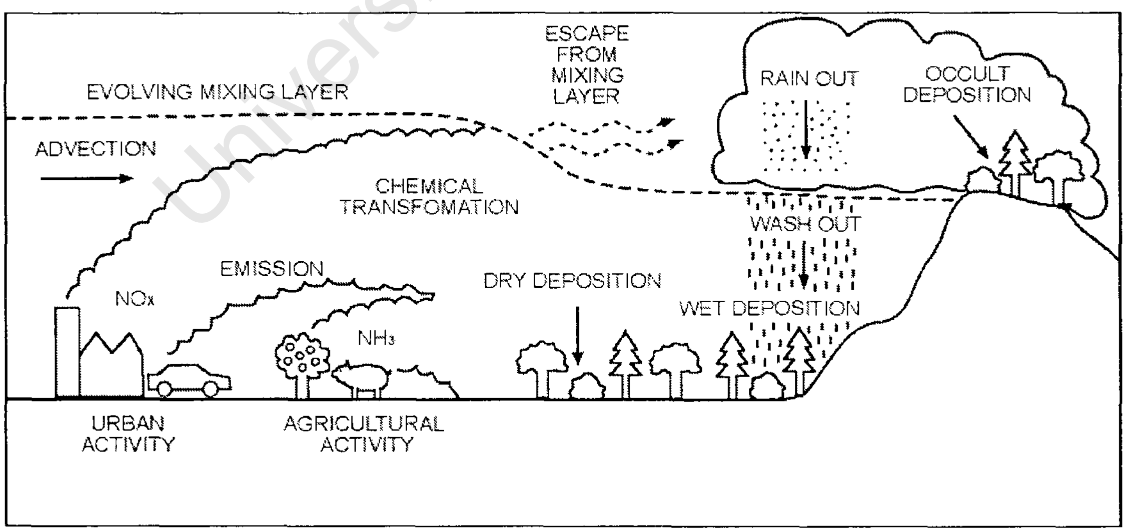

***Figure 2.1**: The general processes involved in atmospheric deposition (modified from Watt Committee 1988).*

Transport and transformation

Following emission to the atmosphere, which may be from natural or anthropogenic sources, the various forms of combined N are advected away by the wind. Due to the turbulent nature of air flow, dispersion (mixing with the surrounding atmosphere) occurs, and during this time the emitted (primary) species may undergo a number of physical and chemical transformations. The primary species and/or the products of transformations (secondary species) are then transported in the atmosphere for anywhere from a few metres to thousands of kilometers prior to their eventual deposition, depending on a number of factors. These include physical and chemical properties of the N species, emission height and local/regional atmospheric circulation. Of particular relevance to the transport of pollutants is the *mixing layer*, the generally turbulent atmospheric layer which is strongly affected by the ground (Watt Committee 1984). Most pollution is released into this layer and is rapidly mixed and dispersed. Under certain conditions however, the mixing layer becomes very stable and temperature inversions may occur. These conditions promote pollution episodes because of the combination of low wind speeds and the temperature inversion acting as a lid, preventing the upward escape of pollution (Dracoulides 1994, Riggan *et al.* 1985, Bytnerowicz & Fenn 1996). Pollution which is released, or escapes above the mixing layer is likely to be transported for a substantial distance, due to the low turbulence and laminar air flow in this zone (Watt Committee 1984).

In general, N deposition decreases with distance from emission sources because of the short residence times of most N species (Rodhe *et al.* 1995b, Galloway 1998), but some pollutants, particularly secondary species such as aerosols, have important transboundary effects (Derwent & Nodop 1986). Thus, at local or regional scale, emission levels are seldom equivalent to deposition levels; a portion of the N emitted in a region is exported, and a portion of the N deposited is imported from elsewhere (Furiness *et al.* 1998, Fowler *et al.* 1998a).

Deposition

Deposition can be broadly classified into dry and wet forms. Dry deposition, which is predominantly of gaseous species, but may also include aerosol particles, involves direct uptake at the earth's surface through the processes of sedimentation (gravitational settling), impaction with surfaces and the ground, and adsorption at the surface, as well as stomatal uptake by vegetation. The rate of dry deposition depends on aerial concentration and the so-called deposition velocity (V_d) of the chemical species involved. Deposition velocity is an engineering factor which takes into account the chemical properties of the pollutant species, as well as the nature of the vegetation or other surface to which deposition is occurring, and the overlying atmosphere. For instance, V_d over forest > grassland > man-made surfaces, because forest will experience the most turbulent airflow and has the greatest surface area for impaction /adsorption and stomatal gaseous exchange (Fowler *et al.* 1998a, 1998b, Zapletal 1998, Wesely & Hicks 2000, Galy-Lacaux *et al.* 2003) A related form of deposition, which is particularly important in mountainous areas, is so-called "occult deposition" of fog or cloud droplets. Although the species are deposited in wet form, occult deposition is usually treated as a component of dry deposition because of the similar mechanisms by which it occurs (predominantly impaction and sedimentation) (Wesely & Hicks 2000).

Wet deposition entails the removal of airborne N species by precipitation through the processes of *rainout* (incorporation into raindrops as condensation nuclei) and *washout* (removal by raindrops beneath cloud), with rainout usually the more important (Watt Committee 1984). When examined on a short time scale, wet deposition is a "patchy" phenomenon in both spatial and temporal terms. A large proportional of total annual N input may, for example, occur in a short space of time or over a small area (termed an "episode"), due to a period of heavy rainfall coinciding with the occurrence of a heavily polluted air mass (Watt Committee 1984). Patterns of wet deposition are also affected by topography. The long term wet deposition field generally has a smooth pattern in simple terrain, but is less uniform in areas with complex topography, due to orographic effects. Deposition is enhanced at high altitude due to the so-called "seeder-feeder" process, which involves the scavenging of cloud droplets in orographic (feeder) cloud by rain falling from higher frontal (seeder) cloud (Fowler *et al.* 1988). This results in both higher rainfall levels and higher rain pollutant concentrations, because ionic concentrations in orographic cloud are usually substantially higher than in average rainfall (Fowler *et al.* 1988, Fowler *et al.* 1995). In addition, high altitude areas experience greater levels of occult deposition than do low-lying areas (Fowler *et al.* 1995). Occult deposition may contribute up to half of total N inputs in areas that experience regular fog episodes (Bormann *et al.* 1989).

2.2.2 Forms of atmospheric nitrogen

Emissions of N to the atmosphere are in one of four forms:
[Figures for NO_x, NH_3 and N_2O are global emission estimates for 1990 from Olivier *et al.* (1998); figure for organic N is a preliminary estimate of global flux from Neff *et al.* (2002)]

1) Oxidized nitrogen (NO and $NO_2 = NO_x$) 50.4 Tg N yr^{-1} (22-81)

The natural processes of fixation by soil microbial activity and lightning are estimated to contribute around 40% of global NO_x emissions, with the remaining 60 % resulting directly from human activities. It should be noted, however, that a large proportion of soil emitted NO_x is also ultimately anthropogenic in origin, and Delmas *et al.* (1997) estimate that, in real terms, human activities are responsible for around 80% of global NO_x emissions. Worldwide, fossil fuel combustion in transport and industry are the dominant sources of anthropogenic NO_x, although biomass (biofuel and natural vegetation) burning is important in developing countries, contributing nearly 30% of the regional total for Southern Africa (Otter *et al.* 2001) and up to 60-70% of atmospheric N emissions in tropical Africa (Lacaux & Sigha 2003). NO_x is emitted predominantly as NO (>90%), most of which is rapidly oxidized to NO_2 upon release to the atmosphere (Derwent & Hertel 1999). NO_x may then be converted to a number of other reaction products, the most important of which are HNO_3 and NO_3^- aerosol (NO_3^- bound to alkaline particles or ions such as Na^+ and NH_4^+). NO_x also reacts with hydrocarbons in the presence of sunlight, to form harmful photochemical oxidants such as ozone (O_3) (Derwent & Hertel 1999, Fowler *et al.* 1998b) Dry deposition is mainly of the gaseous species NO_2 and HNO_3, with particulate NO_3^- usually of secondary importance. NO_2 deposition over vegetation is almost fully controlled by stomatal uptake, with negligible uptake onto cuticular surfaces, and deposition rates thus show diurnal and seasonal variation (Fowler *et. al* 1998b). This process is further complicated by the oxidation of soil emitted NO to NO_2 in the canopy, which reduces the downward flux to vegetation. In contrast, HNO_3 is very reactive and highly soluble and, as such, has a substantially higher V_d than NO_2, particularly over wet surfaces (Fowler *et. al* 1998b). In areas with prolonged dry periods, NO_3^- accumulation on leaf and branch surfaces can be substantial. In Californian chaparral for example, NO_3^- accumulation in the canopy during the dry months of June to October, and subsequent washing to the soil by the first rains, accounts for more than half the total annual input of NO_3^- to the system (Riggan *et al.* 1985). Stomatal uptake of nitrogenous pollution is limited during this period due to low stomatal conductance induced by drought stress (Bytnerowicz & Fenn 1996). Most wet deposition of oxidized N arises through wash-out of HNO_3 and rain-out of nitrate aerosol (Derwent & Nodop 1986). Nitrate aerosol is an important component of long-range N transport to remote areas, due to a long atmospheric residence time (Erisman *et al.* 1998) Other inorganic oxidized forms of N which are usually more minor components of deposition include N_2O_5, HNO_2 and HONO (Derwent & Hertel 1999)

2) Reduced nitrogen (NH_3) - 53.7 Tg N. yr^{-1} (23-88)

Roughly 80% of global NH_3 emissions are from anthropogenic sources, mostly connected to agricultural activity. The biggest emission source is livestock wastes, followed by fertilized soils (synthetic fertilizers) and biomass/biofuel burning may also make a significant contribution, particularly in less developed areas (Artaxo *et al.* 2003). The increasing use of three-way catalysts in motor vehicles has also led to increased emissions of ammonia (NH_3) from vehicle exhausts in recent years (Cape *et al.* 2004). Natural emissions of NH_3 are from soil microbes and the ocean. Because NH_3 is highly soluble and is usually emitted at ground level, it is deposited very rapidly in dry form, particularly to wet surfaces. For this reason, NH_3 emitted from the oceans is quickly re-deposited, and contributes very little to terrestrial N deposition (Raven & Zin 1998, Galloway 1995). The other major pathway of NH_3 dry deposition is through stomatal uptake, a process which is complicated by the fact that plants also release NH_3 from their stomata at certain times, creating a bi-directional flux (Sutton *et al.* 1995). For a given plant canopy, there exists an ammonia compensation point; the aerial NH_3 concentration above which stomatal uptake occurs. Since stomatal NH_3 release is related to leaf N concentration, this value is usually low for natural vegetation, and deposition predominates (Asman *et al.* 1998). NH_3 is also easily dissolved in cloud droplets and rainfall, and wet deposited. Because of its short atmospheric residence time and high V_d, deposition of NH_3 is usually considered to be a local problem (<50km from source). There can however be significant long-range transport of its reaction product, ammonium (NH_4^+) in aerosol form (ApSimon *et al.* 1995). Ammonium aerosol is formed by the scavenging of atmospheric acids such as HNO_3 and H_2SO_4 by NH_3. At distances of greater than 10km from emission sources such as agricultural land, most NH_y is usually present in the fine (submicron) particle fraction (most commonly the aerosols NH_4SO_4 and, to a lesser extent, NH_4NO_3 and NH_4Cl) (Krupa 2003, Aneja *et al.* 2001)

3) Nitrous oxide (N_2O) - 14.9 Tg N yr^{-1} (6.8-18.2)

The majority of N_2O emissions (~ 80%) result from microbial transformations of inorganic N (nitrification and denitrification) in soils and oceans. A large proportion of these emissions are anthropogenic, originating from fertilized soils or soils under N-fixing crops (Duxbury *et al.* 1982). The remaining 20% is emitted directly from a variety of anthropogenic sources. N_2O is chemically inert in the troposphere (but may be oxidized to NO_x in the stratosphere) and has an extremely long lifetime of around 120 yrs. It thus contributes little to N deposition but is a contributor to global warming and the destruction of stratospheric ozone (the "ozone layer") (Erisman *et al.* 1998).

4) Atmospheric organic nitrogen (AON) - between 10 and 50 Tg N yr^{-1}

The contribution of atmospheric organic nitrogen to N budgets has not been given much attention in the past, due to the perception that organic N input mainly consists of local scale cycling of biological detritus (Cornell *et al.* 2003). However, recent studies indicate that this is not the case and show AON to be an important component of local and global atmospheric N budgets, typically comprising around 30% of total N loading (Neff *et al.* 2002, Cornell *et al.* 2003). AON occurs in a wide variety of forms and is emitted from diverse natural and anthropogenic sources, the proportional contributions of which are currently unclear (Cornell *et al.* 2003). AON includes both oxidized N compounds (organic nitrates such as peroxy acetyl nitrate (PAN) and other, more minor alkyl nitrates, formed by reactions between NOx and hydrocarbons) and reduced N compounds (amino acids, amines, urea, pollen, bacteria and dust) (Neff *et al.* 2002). The various compounds vary widely in their degree of bioavailability, but readily bioavailable species such as amino acids, urea and PAN constitute a large proportion of AON deposition (Cornell *et al.* 2003). PAN has long been recognized as an important form of N for long range transport because of its long atmospheric residence time (~1 week) (Erisman *et al.* 1998).

2.2.3 Generalised patterns of nitrogen deposition

An examination of broad deposition trends emerging from previous studies can be useful as a means understanding likely deposition patterns for poorly studied areas. However, predictions for unstudied areas based on findings for other parts of the world should be made with extreme caution, as atmospheric processes are highly variable and unpredictable.

On a global scale, N deposition is thought to comprise roughly equal parts of oxidised and reduced N, with wet deposition constituting a larger source of N input than dry deposition (particularly for reduced N) (Aneja *et al.* 2001, Olivier *et al.* 1998). At smaller scales however, deposition patterns are extremely variable.

Wet versus dry deposition

The proportion of total deposition received in wet or dry mode is largely dependent on the amount of rainfall in an area - the higher the rainfall, the higher the percentage wet deposition (Skoroszewski 2001, Torseth & Semb 1998). Thus, wet deposition dominates in wet regions (Fowler *et al.* 1998a, Torseth & Semb 1998, Burns 2004) and dry deposition dominates in dry regions (Bytnerowicz & Fenn 1996, Galy-Lacaux *et al.* 2003, Riggan *et al.* 1985). Superimposed over this pattern, is the distribution of wet versus dry deposition as determined by distance from the emission source. As a general rule, models indicate that dry deposition is the major form of N input close to urban/industrial and agricultural areas with wet deposition becoming increasingly important as distance from emission sources increases (Hicks *et al.* 2000, Eugster *et al.* 1998, Zapletal 1998). This is because both oxidized and reduced species are mainly in gaseous phase (NH_3, NO_2 and HNO_3) soon after emission, and are deposited very rapidly, both in dry and wet form (particularly NH_3 and HNO_3). Gaseous species that are not deposited are, for the most part, transformed into particulate NO_3^- and NH_4^+ species (aerosols), which have long atmospheric residence times and are transported downwind from sources prior to their deposition. Deposition of aerosols is mostly in wet mode because of their low dry deposition velocities (Wesely & Hicks 2000), and because they act as condensation nuclei for the formation of rain clouds (Artaxo *et al.* 2003, Cornell *et al.* 2003). N associated with large particles (e.g. sea salt particles) may, however, be dry deposited fairly quickly (Torseth & Semb 1998) and aerosols can make up a substantial proportion of dry deposition, particularly in maritime regions (Farrell 1995, Dentener & Crutzen 1993).

Reduced versus oxidised nitrogen

The proportion of N deposited in reduced versus oxidized form depends largely on the level and type (agricultural vs. urban/industrial) of anthropogenic activity occurring in an area. Thus NH_y-N deposition exceeds NO_y-N deposition over large parts of Europe (ApSimon *et al.* 1995) and Asia (Galloway *et al.* 1987), where intensive agriculture (particularly livestock farming) occurs. NH_y also generally exceeds NO_y in tropical and semi-arid N Africa (Galy-Lacaux *et al.* 2003, Lacaux & Sigha 2003) and tropical S. America (Artaxo *et al.* 2003) as pastoral animal excreta, soils and biofuel / biomass burning are the main emission sources in these areas. NO_y generally dominates near traffic, roads and industry, which are most concentrated in urban areas (Riggan *et al.* 1985, Eugster *et al.* 1998, Mphepya *et al.* 2002, Bytnerowicz & Fenn 1996, Galloway *et al.* 1987). However, Conlan *et al.* (1995) found precipitation NH_4^+ concentrations to be higher than those of NO_y in the urban area of Manchester. They attributed the high NH_4^+ levels mainly to human emissions and, to a lesser extent, to livestock farming outside the city borders. NO_y also tends to dominate in areas remote from agricultural activity, where input occurs mainly through long distance transport of aerosols (e.g. Burns 2004, Skoroszewski 1999). NH_y is more of a short distance pollutant than NO_x because the primary species, NH_3, has a faster wet and dry deposition rate than NO_2 (which must be transformed into HNO_3 before it can be wet deposited), as well as a faster gas to particle conversion rate (Fowler *et al.* 1998a). This is demonstrated by the fact that, for European countries, a far greater proportion of national NH_3 emissions are redeposited within the source country than is the case for NO_x (ApSimon *et al.* 1995). In the UK for example, Fowler *et al.* (1998a) found that 88% of NH_y emissions were redeposited within the UK borders, while the figure for NO_x was 23%, with the rest being exported.

2.2.4 The long-term fate of deposited nitrogen

Having discussed the ways in which atmospheric N enters ecosystems, it is important to briefly consider the long term fate of this N. As discussed in the following section, some portion of the N entering natural ecosystems is retained due to biotic uptake, or sometimes by abiotic processes, while the rest is lost to the system. Currently, magnitudes of storage sinks and losses of N are poorly known for most terrestrial ecosystems (Galloway 1998). The two major avenues of N loss are through leaching down the soil profile and into ground and surface waters, and through re-emission to the atmosphere, with the former usually the larger flux (Matson *et al.* 2002). Leaching of N occurs mainly in mobile NO_3 form. NH_4 is generally not as mobile as NO_3 because, as a cation, it is adsorbed to negatively charged colloids and organic matter in most soils (Bloom 1988). On a global basis, leaching of N has increased greatly as a result of human activities. Galloway (1995) estimates a flux of anthropogenic NO_3 to oceans via surface waters of the order of 40 Tg N yr^{-1}. Gaseous losses of N are usually small relative to N deposition and leaching losses (Matson *et al.* 2002). The most important pathway of gaseous loss is the microbial conversion of combined N back to N_2 through the process of denitrification, although the magnitude of global denitrification is unknown (Galloway 1998). N is also emitted from soils in the form of NO and N_2O, as side products of nitrification and denitrification, and from plant stomata, as NH_3 and NO. Finally, NH_3 may be volatilized from the soil surface, mainly in alkaline environments. Both leaching and gaseous losses of N are closely correlated with soil inorganic N, and particularly nitrate availability (Grant & Sheeringa 2002, Aber *et al.* 1989, Tietema *et al.* 1998, Duxbury *et al.* 1982, Skiba *et al.* 1998). Net nitrification rate (microbial oxidation of NH_4 to NO_3) is thus an important determinant of rates of ecosystem N loss.

2.3 Effects of nitrogen deposition on terrestrial ecosystems

2.3.1 Nitrogen retention & nitrogen cycling

In most ecosystems where N is the limiting nutrient, N cycling can be thought of as closed, i.e. inputs and outputs of N are small relative to within system N cycling (Krupa 2003). Elevated N input consistently leads to increases in N content of natural ecosystems. Increased N availability also often has a stimulatory effect on N cycling processes such as mineralization and nitrification. At the same time, rates of both gaseous and leaching loss usually increase under N deposition. The balance between inputs (N deposition and mineralization) and losses (leaching and denitrification / volatilization) will determine the rate at which ecosystems become eutrophied (Aber *et al.* 1989).

The factors which determine whether N deposition is retained or lost by ecosystems, and its effects on N cycling processes, are numerous. Generally in N-limited systems, biota are the primary sink for N, although processes such as adsorption of N to soil particles and abiotic incorporation of N into SOM may be important in some cases. Much of the added N will eventually accumulate in soil organic matter, which constitutes the major proportion of total N reserves in many N-limited systems (Matson *et al.* 2002). Biotic sink strength depends on the N requirements and adaptations for N uptake of individual species, and may show strong temporal variability according to growth season and successional stage. The ability of biota to absorb and retain N is also influenced to a large degree by non-biological factors such as the magnitude, timing and form of N input, climate, disturbance regime and abiotic soil characteristics. Before discussing the various factors determining N retention in detail, it is useful to consider a generalized model of N retention by terrestrial ecosystems, which illustrates the type of non-linear response that is likely to occur.

The N saturation model

The general mechanism by which N retention occurs in terrestrial ecosystems can be understood in the context of the "N saturation" model (Aber *et al.* 1989, 1998), a generalised representation of ecosystem-level response to increased N input over time developed primarily from the results of fertilization experiments in temperate forest ecosystems of the N hemisphere (Figure 2.2). Broadly, the theory predicts that in N-limited systems, added N will initially be taken up and used by plants and soil microbes, to increase growth or N storage (stage 1). This will also lead to an increased pool of N in SOM. At some point available N (N deposition + mineralization) will exceed biotic demand (stage 2). At this stage the system can be thought of as saturated and will lose its capacity to efficiently retain N, leading to increasingly large leaching and gaseous losses as N deposition continues. As discussed later, saturation generally

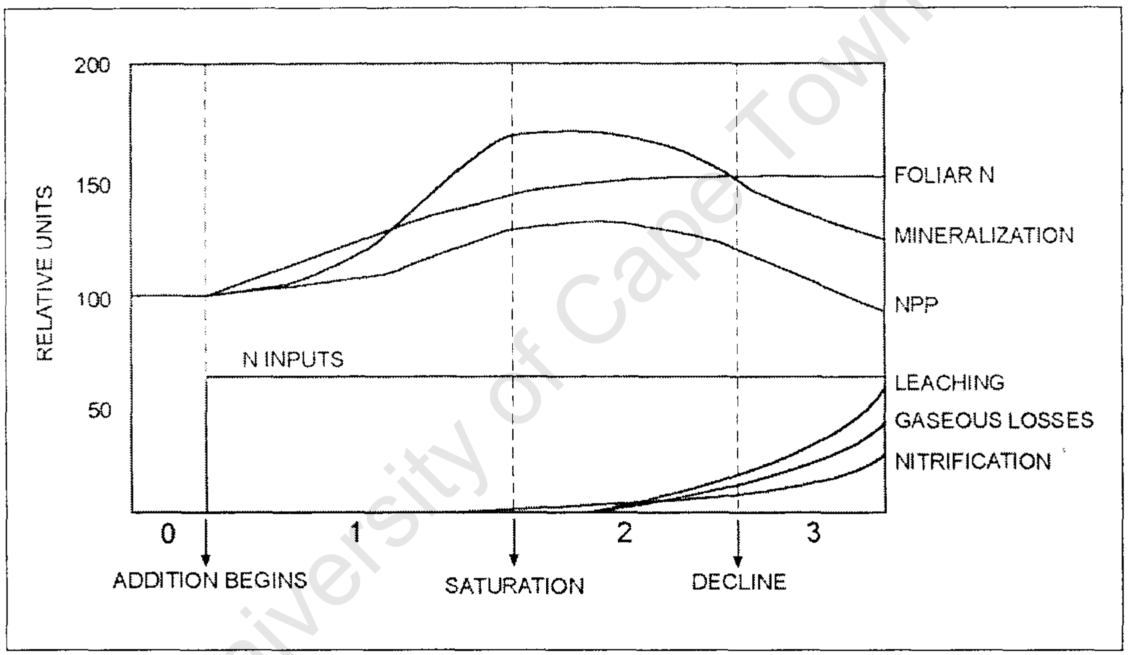

Figure 2.2: *Graphical representation of the typical temporal changes in ecosystem processes predicted by the N saturation model (adapted from Aber et al. 1989, 1998).*

leads to a decline in biomass and net primary productivity (NPP) due to a number of factors such as soil acidification effects and increased susceptibility to secondary stress factors (stage 3). The model predicts that in addition to increasing N supply by direct fertilization, N deposition will also have a stimulatory effect on N mineralization and nitrification rates. Increased litterfall (due to increases in foliar growth and leaf abscission) and a decrease in litter C: N ratio (due to increased foliar N storage and direct incorporation of N into organic matter) are both thought to stimulate net mineralization initially by creating a larger pool of mineralizable N and by increasing the rate of nutrient release during decomposition. However, at a later stage, mineralization often decreases, for reasons which are discussed later. The proportion of mineralized N which is nitrified (termed relative nitrification) is also predicted to increase at saturation, due to the increased availability of NH_4^+ as a substrate for nitrifiers. This facilitates increasingly large leaching losses.

Variables controlling nitrogen retention capacity

The N saturation model serves as a useful starting point in understanding how N addition will affect soil processes and N cycling in natural ecosystems. However, even within temperate forests, systems show wide variations in total N retention capacity and the speed at which saturation is reached. For instance, Aber *et al.* (1995) found that relatively small additions of N rapidly moved a spruce-fir stand to N saturation, while a deciduous hardwood stand still showed no signs of saturation after total inputs of over 900 kg N /ha. In forests, N retention in some systems remains high even after saturation is reached (Aber *et al.* 1995), while in others, outputs are roughly equal to inputs (Johnson 1992). Non-temperate systems also show a large amount of variability in retention capacity and, in some cases, may behave quite differently to temperate systems for a number of reasons (Matson *et al.* 2002). Here follows a discussion of some of the most important biological and non-biological variables that influence N retention and N cycling responses of terrestrial ecosystems.

Biological variables

1) Limitation by other nutrients

One of the most fundamental factors governing N retention is the degree to which biota are N limited. In some ecosystems, N is not the primary limiting nutrient or there is co-limitation with some other nutrient, most commonly P and sometimes Ca, K or Mg (e.g. Roem & Berendse 2000, Matzner & Murach 1995). As these systems are already at or near N saturation, large N losses are likely to begin soon after the onset of deposition. For instance, long term N additions to P-limited calcareous grasslands in Britain had no stimulatory effect on plant growth, but vegetation responded strongly to P additions, while in initially N-limited acidic grasslands, an apparent shift to phosphorus limitation occurred after 6 years of N addition (Carroll *et al.* 2003). In some cases N addition may actually decrease sink strength by creating an imbalance between N and another nutrient, which negatively affects plant performance (e.g. Roem *et al.* 2002). While N addition will not have a growth effect in non N-limited terrestrial systems such as calcareous grasslands, it may still eutrophy downstream freshwater ecosystems via leaching, and will contribute to soil acidification effects (Bobbink *et al.* 1998, Bobbink & Willems 1987).

2) Biotic nitrogen sinks

The relative importance of plants, mycorrhizae and free-living soil microbes as sinks for N, is poorly known for most ecosystems. In temperate forests, Aber *et al.* (1989) initially suggested that plant uptake would be the major sink for added N, but later modified their hypothesis (Aber *et al.* 1998) in response to evidence from ^{15}N tracer and other studies, which show that the majority of N is directly retained in soil organic matter, having never passed through plants (e.g. Nadelhoffer *et al.* 1995, 1999). Because the free-living soil microbes in temperate forests are thought to be C limited (i.e. low quality/ recalcitrant C limits mineralization rather than N availability), microbial immobilization of the majority of deposited N was thought to be an unlikely explanation for the observed soil N retention, unless a pool of unused bioavailable C was present. Aber *et al.* (1998) showed that such a pool could not exist, as the amount of CO_2 which would be released were this the case was far higher than observed CO_2 fluxes. They concluded that the major soil sink in these systems was likely to be mycorrhizal immobilization of deposited N, using plant photosynthate as a C source. Rather than transferring this N to the host plant, the mycorrhizae would exude it back into the soil as N-rich extracellular enzymes.

Little information exists as to the applicability of these findings to other systems, but direct soil retention of N seems to be important in other ecosystems, particularly those with high organic matter content. Immobilization of added N has been shown to be high in the litter layer or organic topsoil of several European heathlands and grasslands, and appears to result mainly from microbial uptake and assimilation of added N (Green *et al.* 1997, van Vuuren & van der Eerden 1992, Kristensen 2001, Power *et al.* 1998b, Carroll *et al.* 2003). Given the important role played by mycorrhizae in the functioning of such nutrient poor systems, it is likely that mycorrhizal immobilization would be important in these areas.

There is some evidence to suggest that uptake of N by free-living soil microbes may be an important N sink in some systems. As mentioned earlier, free-living microbial uptake in nutrient poor systems is often limited by C availability (Aber *et al.* 1998). This is however, not always the case and microbes may be N-limited in some systems (Fenn *et al.* 1998, Eviner & Chapin 1997, Stark & Hart 1997). Stark & Hart (1997) propose that, in systems where N and C are accumulating, microbial biomass will increase and act as a net sink for inorganic N. In mature stands, microbial biomass will no longer increase, but rapid turnover of microbes will promote N retention by converting labile forms of N such as NO_3 into more stable organic forms (Stark & Hart 1997). N may stimulate populations of microbes with high N demand at the expense of those with lower N demand (Fog 1988, Kristensen 2001). Microbes have also been shown to be capable of "luxury uptake" of N despite non-limitation (Fog 1988). It is suggested that microbial immobilization of added N into organic matter may be underestimated in fertilization experiments. In most cases, fertilization consists of large periodic (usually bi-annual or quarterly) additions of N to the soil, which poorly resemble the natural situation, where small regular inputs of N occur. Small regular inputs would, for example, be more likely to stimulate microbial biomass increase and maintain high free-living soil microbial populations (Norby 1998).

Another shortcoming of fertilization studies is that all N addition occurs via the soil. In natural vegetation however, a substantial proportion of N uptake may occur via the stomata (Lindberg *et al.* 1990, Bobbink *et al.* 1992), and the importance of plant uptake and retention may thus be underestimated by these studies. This is particularly true in vegetation where characteristics such as a closed canopy, large leaf area, long growing season and/or high average stomatal conductance maximize levels of stomatal exchange. Closed heathland canopies in Holland have, for example, been found to be capable of directly assimilating up to 65% of total atmospheric N input (Bobbink *et al.* 1992). The potential for soil mediated uptake by plants will also vary widely amongst systems. Most plants adapted to low nutrient habitats are highly efficient at acquiring N, usually possessing high root to shoot ratios and often specialised roots and symbiotic associations with mycorrhizae (Lamont 1983, Chapin 1987). This enhanced ability to acquire N allows plants to rapidly take up and store N in excess of plant demand when it becomes available (luxury consumption) and use that N to sustain a slow growth rate when N is limited (Chapin 1987).

The well developed N uptake abilities of plants from nutrient poor systems may seem to suggest that vegetation would act as a large sink for added N in these systems. In the long term, however the ability to acquire and store N will be a less important determinant of retention capacity, than the ability to use the N for growth (productive potential) and thus maintain a high N demand. Typically, species from nutrient poor habitats have inherently low potential growth rates (Chapin 1987, Aerts 1990) and would thus be expected to become N saturated fairly quickly. Thus the presence of more N demanding or opportunistic species (indigenous or introduced), which are not growth limited by some other resource (e.g. water, light) will be an important factor in increasing vegetative sink strength for added N. Ecosystem N retention will be favoured not only by high productivity, but also by low mean residence time of N in tissues (i.e. high turnover) (Aerts 1990). Thus plants such as grasses and deciduous species will contribute to high ecosystem N retention because of the regular transfer of a large portion of plant N capital to leaf litter, particularly if litter is of relatively low quality (high C:N, high lignin), leading to slow deposition rates (Bowmann *et al.* 1995, Fenn *et al.* 1998).

3) Temporal trends in nitrogen demand

Characteristics such as plant successional stage and stand age play a large role in determining demand for N. Young or aggrading vegetation is more likely to retain N than mature vegetation, and is thus less likely to become N saturated (Fenn *et al.* 1998). Ecosystems with a strongly seasonal pattern of growth may also be susceptible to N losses due to an asynchrony between N supply and biotic demand. This situation is most pronounced in systems where vegetation is dormant for part of the year particularly if the dormant season is also the period of maximum N input. This is the case in the many of the ecosystems in the western United States (Fenn *et al.* 1998, Bytnerowicz & Fenn 1996). Here, dry deposited N collects on plant and soil surfaces during the summer drought period. The major hydrologic flux of N then occurs in winter, when the dry deposited N is washed to the soil by the first rains of the season. As plant and microbial demand only peaks later (in spring and early summer), much of the N is leached from the system (in NO_3 form) during this period. A similar situation occurs in areas which are covered in snow for large parts of the year. In N-limited alpine tundra vegetation in the USA for instance, leaching losses may be roughly 50 % of inputs, because of the temporal offset between high NO_3 availability following snowmelt, and the growing season (Burns 2004, Fenn *et al.* 1998).

4) Decomposition, mineralization and nitrification

In the absence of elevated N deposition, decomposition and N return tend to be slow in nutrient poor systems. This is due both to the high C: N ratios (often >100) and the abundance of protein-precipitating phenolic compounds typically found in the leaves of plants adapted to these habitats (Fog 1988, Vitousek *et al.* 2002). In many natural ecosystems, mineralization rates have been found to increase in response to N additions (Bobbink *et al.* 1998, Lee & Caporn 1998, Morecraft *et al.* 1994). This may occur for a number of reasons: a) N addition causes a reduction in litter C:N ratios, and consequently faster rates of N release during decomposition (Aber *et al.* 1989) b) N addition may cause a reduction in phenolic concentration in foliage, although though slow growing species from nutrient poor habitats seem to be less responsive than more rapidly growing species in this regard (Alonso *et al.* 2001) and c) N addition may cause an increase in microbial population size or activity (Carreiro *et al.* 2000, Green *et al.* 1997, Morecraft *et al.* 1994, Lee & Caporn 1998). However, when N is added to organic matter with a high content of recalcitrant C compounds and high C: N ratio (typical of the litter in nutrient poor ecosystems), decomposition and mineralization rates typically decrease (Fog 1988, Aber *et al.* 1995, 1998, Carreiro *et al.* 2000). Recent studies in temperate forests indicate that both increased and decreased mineralization rates could be explained by N induced changes in microbial activity or community composition. N availability causes microbes to increase production of general cellulases which decompose simple C compounds, but production of ligninolytic enzymes which are needed to decompose recalcitrant substances may be stimulated or suppressed by N, depending on the biochemistry of the litter to which N is added (Carreiro *et al* 2000, Waldrop *et al.* 2004).

The N saturation model also predicts an increase in relative nitrification rates in response to elevated N inputs (Aber *et al.* 1998). As mentioned earlier, this has important consequences for system N losses, as NO_3 is more easily leached than NH_4, and gaseous losses during nitrification and denitrification may also be substantial.

Nitrification is usually thought to be low in N-limited systems due to nitrifiers being outcompeted for NH_4 by plants and other microbes (Fenn *et al.* 1998, Kronzucker *et al.* 1997) and inhibition at low pH (Lewis 1986). As saturation is approached, increased availability of NH_4 as a substrate for nitrifiers seems to allow higher net nitrification rates. Some studies suggest that gross nitrification may actually be high in acidic environments, but that high levels of microbial NO_3 immobilization result in low net nitrification values (e.g. Stark & Hart 1997). If this is the general case, increased net nitrification as soil N increases, would result from saturation of microbial demand for NO_3. Whatever the explanation, the pattern of increased nitrification in response to N deposition is well documented in forests of N. America (e.g. Aber *et al.* 1995, McNulty *et al.* 1996, Stark & Hart 1997) and Europe (Tietema *et al.* 1995, 1998) as well as a number of other systems (e.g. Carroll *et al.* 2003, Goulding *et al.* 1998).

Non-biological variables

1) The amount, form and timing of nitrogen input

Clearly the amount of N entering the system and the rate at which it does so, will be important in determining degree of retention, as there are limits on the rate at which biota can take up N and on total N storage capacity. For instance, the NITREX experiments in a variety of European forests found that, at inputs of 10 $kg.ha.a^{-1}$ or less the majority of N was retained, while inputs of 25 $kg.ha.a^{-1}$ or greater caused substantial N losses (Tietema *et al.* 1995). For the same reason, large episodic inputs of N would not be retained as well as regular, smaller inputs. As discussed earlier, the timing of N input in relation to biotic demand may also be important. A further consideration is the form (NO_3 or NH_4) in which N is received. The proportion of reduced or oxidized N influences retention, not only because of the higher mobility of the NO_3 ion, but also because certain species show a preference for one form or the other (Bloom 1988, Stock & Lewis 1984, Kronzucker *et al.* 1997) While the majority of plant species are able to use both oxidized and reduced N, the generalization may be made that fast growing / annual species prefer NO_3, while slow growing perennials prefer NH_4 (Krupa 2003)

2) Climate and rainfall

The most important climatic factor controlling N retention is precipitation, because of the role of moisture in both leaching and gaseous losses. Grant & Sheeringa (2002) modeled levels of N retention in N. American soils at varying denitrification rates, ignoring other biotic processes. Their results showed that, in areas with high rainfall (i.e. precipitation >> evaporation), a large proportion of added N was immediately leached from soils. In contrast, in dry climates or during dry periods, most N was retained (including wet deposited N). When biotic uptake is taken into account however, the influence of moisture is more complex. For instance, whilst N retention in the soil may be highest during dry periods, this N is generally not available for biotic uptake in the absence of soil moisture. At the onset of rainfall, the balance between evapotranspiration and rainfall will determine whether the N build up at the soil surface is leached or remains in the system leading to eutrophication. In Spain for instance, high winter rainfall leads to large leaching losses (Piñol *et al.* 1992), while in the coastal sage scrub vegetation of Southern California, the predominance of evapotranspiration over precipitation means that soil surface N seldom percolates below the rooting zone (Padgett *et al.* 1999).

Grant & Sheeringa (2002) found that, like leaching losses, denitrification losses were largely dependent on surface soil moisture, showing a roughly linear, positive relationship with moisture content, although temperature was also found to be important. While denitrification rates are potentially higher during warm periods due to biochemical optimization of microbial activity, modeled rates of denitrification tended to be lower during these periods, because high evaporation rates led to low surface soil moisture contents. In systems where warm periods coincide with abundant soil moisture, however (tropical systems), substantial gaseous N losses may occur at these times. This interaction between moisture and temperature will also apply to other microbial activities that affect N loss, such as nitrification.

3) Abiotic nitrogen sinks

Some proportion of N retention occurs through abiotic means. Adsorption of N to soil particles occurs mainly for the NH_4 ion (e.g. Bloom 1988), but NO_3 may also be retained in variably charged soils with high anion exchange capacity, such as those found in certain tropical systems (Matson *et al.* 2002). NH_4 may also be abiotically incorporated into organic matter through condensation reactions with phenolic compounds, which are generally high in the foliage of nutrient poor vegetation. This mechanism has been shown to be significant in heathlands (e.g. Kristensen 2001) and temperate forests (e.g. Fitzhugh *et al.* 2003), but, at least in temperate forests, it generally explains less than 15% of soil N retention (Aber *et al.* 1998, Fitzhugh *et al.* 2003).

4) Soil factors

Soil structural characteristics such as texture and flow path have been shown to be important controllers of N retention. Simply put, N is more likely to be retained in fine soils (high clay content) than in coarse, well-drained soils, which may experience large leaching losses even in systems where plants and microbes have been shown to be N-limited (Matson *et al.* 2002). Topography may also affect leaching, with higher losses usually occurring in steep compared to gentle watersheds (Fenn *et al.* 1998). Finally, soil pH may play a role in N retention because soil acidity reduces volatilization of NH_3 from the soil surface (Asman *et al.* 1998, Krupa 2003) and inhibits nitrification (Bloom 1988).

5) Disturbance

Disturbance may have important effects on N retention by reducing site N capital and re-initiating vegetational succession (Fenn *et al.* 1998). In the case of fire, a large proportion of organic and inorganic N stored in plants and in the soil, is liberated or volatilized during burning (depending on burn intensity). While this causes a brief flush of available N in the form of ammonia-rich ash immediately following the burn, it should, in the long term, lead to a reduction in site available N due to rapid nitrification and leaching of NH_4 in ash, volatilization losses and slowed mineralization rates due to reduction of organic matter in the soil (Rundel 1984, Stock & Allsopp 1992). Following fire, biotic demand for N will be higher than in mature vegetation, due to rapid growth. The combination of low soil N reserves and high plant demand will thus mean that N retention will be high in the post-fire environment. As succession proceeds, biotic demand for N will decrease and mineralization will increase leading to greater potential for N losses. Thus, in systems where fire is a regular occurrence, deposited N may never be allowed to accumulate to the point of saturation. Other forms of disturbance which reduce site N capital, such as harvesting or land modification would have broadly similar impacts to fire.

6) Global change

Factors such as increased atmospheric CO_2 levels and rising global temperatures have the potential to increase the sink strength of ecosystems for N, by stimulating net primary productivity (NPP) (Wright & Schindler 1995). Synergistic effects of CO_2 and N on NPP have been observed in some systems, suggesting co-limitation by these resources (e.g. Shaw *et al.* 2002). However, other experiments have found no interactive effect, or even an antagonistic effect of combined CO_2 and N on plant growth (e.g. Midgley *et al.* 1995) or NPP (Reich *et al.* 2001b). As is sometimes the case when N fertilization acts alone, this may be due to limitation by some other resource, or the creation of a nutrient imbalance (e.g. increased N: P ratio) which negatively affects plant performance (Midgley *et al.* 1995, Zavaleta *et al.* 2003).

2.3.2. Soil acidification

The earliest concerns about the effects of atmospheric pollutants on natural ecosystems focused on the problem of acidification or "acid rain", with sulfur oxides considered to be the most important contributor (Cowling & Nilsson 1995, Rodhe *et al.* 1995a). Due to a number of agreements and protocols, S emissions in the US and Europe have since declined greatly, while decreases in nitrogenous pollutant emissions have been more modest. N deposition thus accounts for an increasingly large proportion of acidifying pollution. Oxidised N in the form of nitric acid (HNO_3) has long been known to contribute directly to acidification by releasing a proton upon deposition. More recently, however, it has been recognised that reduced N also generates soil acidity, and is potentially even more important than oxidized N in this regard (Galloway 1995) Although gaseous NH_3 is a base and neutralizes acidity in the atmosphere, deposited NH_4 contributes to acidification by releasing H ions, either during uptake by a plant, or through microbial nitrification of NH_4 to NO_3 (Krupa 2003, Galloway 1995). Furthermore, NH_4 can be responsible for delayed acidification, because the ion may be transported to downstream ecosystems prior to uptake or nitrification.

Deposition of acidifying N compounds often leads to large decreases in soil pH (e.g. Goulding *et al.* 1998, Tietema *et al.* 1995, Matzner & Murach 1995), although in naturally acidic environments, this may only be detectable in soil solution measurements (Fenn *et al.* 1998). The ability of soils to neutralize acidity (i.e. immobilize H^+ ions) depends upon the soil concentration of base cations and on their rate of release from parent material by mineral weathering (Bain & Langan 1995). In well-buffered soils, acidity is mostly neutralised by carbonate reactions involving base cations (Matson *et al.* 2002). This often leads to leaching of important (nutrient) cations such as Mg, K and Ca, as counterbalancing ions for leached SO_4 and NO_3. As base cations become depleted or in naturally acidic, highly leached systems with inherently low levels of base cations, metal hydroxide reactions (particularly $Al(OH)_3$) become important, resulting in increased availability and leaching of toxic metal ions such as Al and Cd. Increased Al availability may also lead to the formation of insoluble aluminium phosphates, resulting in decreased P availability.

2.4 Effects of nitrogen deposition on terrestrial biodiversity

Nitrogen deposition has emerged as a major threat to biodiversity in many parts of the world. A large amount of evidence has suggested that N deposition is at least partly responsible for marked changes observed in community composition of many natural and semi-natural ecosystems in Europe and North America, which have often resulted in decreased species richness. In their global biodiversity scenarios for 2100, Sala *et al.* (2000) give an idea of the significance of the problem, ranking N deposition as the third most important driver of global biodiversity change after land-use change and climate change. The problem is of particular concern in biodiversity hotspots, which stand to lose high numbers of rare or endangered species (Phoenix *et al.* 2005).

The most important effects of N deposition on biodiversity occur at community level, where N induced changes in competitive dominance can lead to exclusion of slow growing or stress intolerant species, and consequently result in reduced species richness. The effects of N deposition are likely to be most pronounced in ecosystems which are very N poor, and which have slow rates of N cycling, because small additions of N will have the potential to cause large changes in the character of vegetation in these systems (Kuylenstierna *et al.* 1998). Clearly N deposition will interact with other drivers (e.g. climate change, increasing CO_2, land use change and biotic invasions) to produce effects on biodiversity which may be additive, synergistic (more than additive) or antagonistic (less than additive) (Dukes & Mooney 1999, Sala *et al.* 2000, Zavaleta *et al.* 2003). For instance increased CO_2 and higher temperature may interact with N deposition to stimulate the growth of certain species relative to others to an even greater degree than would occur under N deposition alone. Because these interactions are highly complex, and currently poorly understood, the following discussion will concentrate mainly on N deposition effects alone. However, it should always be borne in mind that changes in biodiversity attributed to N deposition (particularly from field observations) will often have a more complex etiology.

This section will first discuss N deposition effects on organisms at *species level*, and examine how they may increase susceptibility to secondary stress factors. This will be followed by a further discussion of how the various effects on individuals (be they positive or negative) feed into *community level* effects on species composition, diversity and net primary productivity (NPP).

2.4.1 Species level effects

Although there is a large amount of overlap and interaction, the effects of N pollution on organisms can be broadly categorised as eutrophication, acidification and phytotoxic effects.

Eutrophication effects

In N-limited plants, the most widely observed response to N addition is an increase in growth, and thus eutrophication has the potential to alter competitive interactions. This aspect of the eutrophication threat is discussed further under community level effects below. In addition to this threat, eutrophication can also indirectly reduce health and productivity by causing changes in plant biochemistry, growth allocation and temporal growth patterns. Although these changes occur because they can potentially benefit the plant, they may, in some cases, inadvertently increase plant susceptibility to damage from secondary stress factors.

In many controlled fertilization experiments, shoot growth is stimulated to a greater extent than root growth in response to N addition (i.e. root: shoot ratio decreases)

(Norby & Jackson 2000), although changes are less clear in field experiments and do not occur in all species (Bobbink *et al.* 1998, Gordon *et al.* 2001). There is also much evidence to suggest that N input leads to decreases in mycorrhizal infection and production of fruiting bodies in ectomycorrhizal tree species (Wallenda & Kottke 1998, Matzner & Murach 1995), but effects on vesicular-arbuscular (VA) or ericoid mycorrhizal species are more variable, with both decreases (e.g. Egerton-Warburton & Allen 2000, Rillig *et al.* 1998) and increases (e.g. Lee & Caporn 1998, Rillig *et al.* 1998) having been observed. Changes are plant species-specific and seem to result from changes in plant C allocation to the mycobiont, due to C sink competition between the shoot and root/ mycobiont (Rillig *et al.* 1998). Ultimately, changes in mycorrhizal biomass or infection rate may be less important than shifts in, and simplification of, mycorrhizal community composition (Egerton-Warburton & Allen 2000, Norby & Jackson 2000). In any event, the net result of these below-ground changes is often a decrease in the efficiency of plant roots at obtaining resources from the soil. Pot experiments by van der Heijden *et al.* (1998) have shown that changes in mycorrhizal species composition can result in changes in plant species composition and decreased plant diversity.

Another almost universally observed plant response to increased N availability is an increase in tissue N content, particularly in the leaves, due to "luxury consumption" of N (Chapin 1987). This N may be stored in the form of amino acids, or inorganic N may simply accumulate in the tissues. Additional tissue N increases the nutritive value of leaves from a herbivore point of view (Brunsting & Heil 1985). Increased ratios of N to other nutrients such as Mg, K and P in foliage may be indicative of a nutrient imbalance, and have been linked to negative effects on plants (e.g. Roem & Berendse 2000). In such cases increased growth in response to added N increases demand for other nutrients, which cannot be met. This results in nutrient deficiencies, which cause reductions in photosynthesis and increase plant mortality (Skeffington & Wilson 1988). Nutrient deficiency/imbalance is further exacerbated by reduced allocation to roots (as discussed above) and reduced availability of P and other nutrients due to acidification and nitrate leaching (see acidification effects below).

Finally, added N may alter temporal growth patterns of plants. Fertilization in several heathlands has caused plants to start new growth and flowering earlier in the growing season (Power *et al.* 1998a, Gordon *et al.* 2001).

Soil acidification effects

Soil mediated acidification (as opposed to direct effects of atmospheric acidity discussed under phytotoxic effects below) has been shown to have marked negative effects on the performance of some plants. It is often difficult to isolate the particular mechanism/s responsible for observed impacts because of the numerous ways in which acidity may act. Reduced plant performance may occur due to a) the direct effects of acidity itself (i.e. toxicity of H^+ ions), b) nutrient deficiencies or imbalances resulting from the leaching of nutrient cations such as Ca, K and Mg, and reduced P availability due to the formation of insoluble aluminium phosphates, c) toxicity of Al or other metals (usually indicated by an increase in the foliar ratio between Al and Ca or other base cations) or d) increased availability of NH_4 relative to NO_3, due to inhibition of nitrification as pH drops. Negative effects on plant growth probably usually occur due to a combination of some or all of these factors, but studies attempting to test the relative importance of each, are scarce. Falkengren-Grerup *et al.* (1995) found that poor growth in acid intolerant forest floor species at low pH was unrelated to nutrient deficiency. Their results indicated that H toxicity, Al toxicity, and N form were all important in determining acidification sensitivity, although the relative importance of H and Al could not be distinguished due to a strong correlation between the availability of these ions. However, Roem *et al.* (2002) conducted a full factorial experiment manipulating nutrient availability and acidity in a Dutch wet heathland, and found that Al toxicity was a more important factor in decreasing seedling emergence and species richness than pH.

Numerous studies on forest trees in the laboratory and the field have indicated that one of the most general effects of acidification is a reduction in root growth, with Al and H toxicity as well as Mg deficiency seemingly the most important causative factors (Matzner & Murach 1995). Plants may, however, compensate for decreased root growth in one soil compartment (e.g. B horizon where Al is usually highest), by increasing growth in other compartments (e.g. O and A horizons) (Matzner & Murach 1995, Norby & Jackson 2000). Both ectomycorrhizae (Wallenda & Kottke 1998, Matzner & Murach 1995) and endomycorrhizae (Heijne 1995 cited in Bobbink *et al.* 1998) have also been shown to be negatively affected by acidification, especially by increased mobility of toxic metals such as Al. However, as is the case for plants, mycorrhizal species seem to vary greatly in their susceptibility to acidification effects (Matzner & Murach 1995).

Phytotoxic effects

Plant species show a wide range of sensitivity to phytotoxic effects caused by N and its by-products, which mostly result from direct uptake from the atmosphere.
As discussed earlier, stomatal uptake of atmospheric N usually has a fertilizing effect on plants when concentrations are low. At higher concentrations, controlled fumigation studies have demonstrated that both NH_y (Krupa 2003) and, to a lesser extent, NO_x (particularly NO) (Wellburn 1990) may reduce growth and cause visible leaf injury (necrosis, leaf etching, erosion of epicuticular waxes). Reduced growth may result from the need to deal with a combination of stresses including cellular acidification, toxic leaf nitrite and ammonia concentrations and the disruption of enzyme function (Wellburn 1990, Krupa 2003, Stulen *et al.* 1998) Ambient concentrations are, however, seldom high enough for significant toxic or injurious effects to be observed, except near large point sources (e.g. Pitcairn *et al.* 1998) and in highly sensitive species such as mosses and lichens (Lee & Caporn 1998, Bobbink *et al.* 1998, Mäkipää 1995). Mosses and lichens are particularly vulnerable because they lack a cuticle and are thus very efficient accumulators of pollutants, both from rain and the atmosphere, which may rapidly reach toxic levels in foliage (e.g. Krupa 2003, Woodin *et al.* 1985). Toxicity in mosses seems to usually result from a combination of acidity and build up of toxic levels of foliar N, as nitrate reductase activity and induction capacity tend to decline under N fertilization (Mäkipää 1995, Woodin *et al.* 1985, Morecraft *et al.* 1994). Most lichens are more sensitive to acidity than N, except those with cyanobacterial photobionts (roughly 10% of lichen species), which are affected by both (Bobbink *et al.* 1998).

Probably the most important way in which N pollution contributes to direct phytotoxic effects in vascular plants is through its role in tropospheric O_3 formation, and through interactive effects with O_3 and SO_2. O_3 is highly phytotoxic and many studies have shown visible damage and growth reductions in crops and trees below or at common European and N. American ambient concentrations (Bobbink 1998, Davison & Barnes 1998, Takemoto *et al.* 2001). The combination of N compounds and O_3 or SO_2 may have synergistic effects which magnify or reduce harmful impacts.

For instance, SO_2 and NO_x in combination may have more than additive effects, leading to free-radical induced injury similar to that caused by O_3 alone (Wellburn 1990). On the other hand, some studies have found that N deposition moderates harmful O_3 effects, at least in the short term (Takemoto *et al.* 2001, Krupa 2003).

2.4.2 Secondary stress factors

The negative impacts of secondary environmental stress factors on vegetation are often suggested to increase when operating in tandem with many of the above-mentioned N deposition effects on plant health, biochemistry and growth patterns. At present however, impacts remain speculative due to the limited research that has been conducted on this topic (Bobbink 1998). As discussed above, both acidification and eutrophication are often shown to lead to reductions in root: shoot ratio, and may have negative impacts on mycorrhizal symbionts. Limited evidence suggests that this might result in increased sensitivity to drought episodes, because the result is a decrease in water uptake surfaces relative to transpiring surfaces (Bobbink 1998, Matzner & Murach 1995). Gordon *et al.* (2001) found strongly interactive negative effects of N addition and drought on shoot growth and height in heather (*Calluna vulgaris*), but not in bracken (*Pteridium aquilinum*). This was attributed to a large increased allocation to shoot growth in *C. vulgaris*, while the rhizomatous perennial, *P. aquilinum* seemingly allocated the N to rhizome growth and storage. Other studies in forests have cited root damage due to acidification as the cause of drought stress and dieback (de Visser *et al*. 1995 cited in Matzner & Murach 1995).

Plant attack by insect herbivores or pathogens, is another stress factor that may be magnified due to pollution effects. Not only is plant vitality lowered due to acidification and phytotoxic effects, but increased foliar N content may increase the food value and desirability of plant tissues for insect or other herbivores (Skeffington & Wilson 1988, Alonso *et al.* 2001). A number of studies have reported that abundance of insect pests and levels of herbivory increase in response to increased tissue N (Brunsting & Heil 1985, Haddad *et al.* 2000) and this may be an important causal factor in the decline of certain plant (Brunsting & Heil 1985) and insect (Haddad *et al.* 2000) species.

N addition seems to increase frost sensitivity in some plants, but decreased sensitivity has also been observed (Power *et al.* 1998a, Lee & Caporn 1998). The most likely reason for increased sensitivity is a lengthening of the plant growing season. In forest trees, this may result in delayed hardening or acclimation at the end of the growing season, and damage by early frosts (e.g. Friedland *et al.* 1984). In contrast, studies in British heathland have found that N addition results in earlier bud break in several species (e.g. Lee & Caporn 1998, Gordon *et al.* 2001) which can increase sensitivity to late frosts (Power *et al.* 1998a, Lee & Caporn 1998), but seems to raise tolerance of frost episodes occurring towards the end of the growing season (Lee & Caporn 1998).

2.4.3 Community level effects

The most important effects of N deposition on biodiversity are revealed at community level, where N induced changes in competitive dominance can lead to exclusion of slow growing or stress intolerant species, changing plant assemblages and resulting in reduced species richness. Over the past 20 years, a large amount of evidence has accumulated to support this contention, mainly from long-term fertilization experiments studying the responses of plants in a range of natural and semi-natural ecosystems (Table 2.1). The vast majority of sustained research has been conducted in Europe (reviewed in Bobbink *et al.* 1998, Green *et al.* 1997, Lee & Caporn 1998) and N. America (reviewed in Fenn *et al.* 2003, 1998). However, the broad range of habitats in which competitive shifts have been observed in these countries, suggest that changes in plant community composition are likely to be a general phenomenon in areas receiving enhanced N deposition.

As discussed, N deposition effects are produced by one, or a combination of acidification, eutrophication and phytotoxic effects, often in combination with secondary stress factors. There is, however, considerable debate as to which of these mechanisms is most important in the majority of systems studied. For the purposes of discussion, N can be thought of as having two broad effects on plants that lead to altered community composition. N either *increases* or *decreases* competitiveness of species. The relative importance of each will vary according to the characteristics of the particular ecosystem being investigated.

Table 2.1: *Changes in plant community composition and species diversity from fertilization studies and field observations in a range of terrestrial ecosystems. Bracketed figures in italics are empirically derived critical loads of N for equivalent European natural ecosystems from Bobbink & Roelofs (1995). Amb = ambient total N deposition level.*

Ecosystem	**Location**	**Total N Load ($kg.ha^{-1}.a^{-1}$)**	**N Type**	**Length of input**	**Response**	**Key Reference(s)**
SHRUBLANDS						
Dry Lowland heath	Holland	30-50 (amb) *(15-20)*	mostly NH_y	_	Increased dominance of grasses once shrub canopy is opened by beetle herbivory which increases in response to increased N availability	Heil & Deimont (1983) Brunsting & Heil (1985)
Wet Lowland heath	Holland	150 *(17-22)*	NH_4NO_3	2-3 yrs	Replacement of dominant shrub species by grass *Molinia caerulea*, disappearance of rare species, large reduction in species richness	Aerts & Berendse (1988)
Moorlands	Wales	40-120 *(10-20)*	NH_4NO_3	7 yrs	Initially increased growth of dominant shrubs and disappearance of cryptogams. Later increased "winter browning" of dominant shrub & gap formation	Lee & Caporn (1998)
Alpine tundra	USA	5 - 20 *(5-15)*	NH_4NO_3	?	Shift from forb dominated to grass (*Deschampsia flexuosa*) dominated community, shift to P limitation	Bowmann *et al.* (1995)
DRYLANDS						
Coastal sage scrub	USA	60	NH_4NO_3	8 yrs	Increased dominance of non-native annual grass in wet years, no change in characteristic shrubs	Allen *et al.* (unpublished) cited in Fenn *et al.* (2003)
Coastal sage scrub	USA	< 45 (amb)	Mostly NO_x	-	Increased dominance of annual grasses, decreased richness of native species. However, N addition is likely to be one of a number of factors.	Minnich & Dezzani (1998)
Mojave desert	USA	32	NH_4NO_3	2	Increased dominance of alien annual grasses and decreased species richness during wet, productive years.	Brooks (2003)
FORESTS						
Spruce -Fir (tree layer)	USA	16 -31 *(10-50)*	NH_4Cl $NaNO_3$	6	Increased mortality of dominant spruce / fir trees on high N sites (>25 kg/ha) followed by increased sprouting/ seedling emergence of deciduous sp. (maple/birch) but not of spruce/fir.	McNulty *et al.* (1996)
Pine (understorey)	Canada	56 - 224 *(10-20)*	?	9	Increased exotic species cover, decreased cover of shrubs and large decrease in cryptogams	Prescott *et al.* (1995)
Pine (understorey)	Sweden	20 - 180 *(10-20)*	NH_4NO_3	3-10 yrs	Shift from cryptogams and ericoids towards grass (Deschampsia) and ruderals at all levels of N deposition	van Dobben *et al.* (1999)

Table 2.1: *Contd.*

Ecosystem	Location	N Load ($kg.ha^{-1}.a^{-1}$)	N Type	Length of input	Response	Key Reference(s)
GRASSLANDS						
Calcareous	Holland	50-100 (+30 amb) *(14-25)*	NH_4NO_3	3 yrs	Increased dominance of grass (Brachpodium pinnatum). Decreased species richness and loss of rare species.	Bobbink (1991)
Calcareous	England	35-140 *(14-25)*	NH_4NO_3 $(NH4)_2SO_4$	4-6 yrs	Reduced cover of most species after 6 yrs esp. in $(NH4)_2SO_4$ plots, particularly sedges and dicots.	Morecraft *et al.* (1994) Carroll *et al.* (2003)
Neutral acidic	England	48 (+ amb) *(20-30)*	$NaNO_3$ $(NH4)_2SO_4$	110 yrs	Large reduction in species richness, particularly in $(NH4)_2SO_4$ plots, where species have been reduced to 1 or 2 acid tolerant grasses.	Goulding *et al.* (1998)
Species rich acidic	England	35-140 *(7-15)*	NH_4NO_3 $(NH_4)_2SO_4$	4-6 yrs	Reduction of certain species after 6 yrs esp. in $(NH4)_2SO_4$ plots. Increased cover of one grass species (*Nardus stricta*). Large red. in cryptogams.	Morecraft *et al.* (1994) Carroll *et al.* (2003)
Serpentine (acidic)	USA	100 *(7-15)*	NH_4NO_3	2 yrs	Increased dominance of non-native annual grass (esp. *Lolium*). Decreased species richness and disappearance of certain forb and legume species.	Huenneke *et al.* (1990)
Serpentine (acidic)	USA	10-15 (amb) *(7-15)*	mostly NO_x	_	Increased dominance of non-native annual grass and decreased species richness when grazing is absent.	Weiss (1999)
Sub-tropical	S. Africa	70 - 211	NH_4NO_3 $(NH_4)_2SO_4$	31 yrs	Replacement of common grass species (*Themeda* and *Tristachya*) by other grasses. Increased abundance of non-native species.	Le Roux & Mentis (1986)

Increased competitiveness

N can act as a fertilizer and increase competitiveness of certain species relative to others. Typically, most characteristic species from nutrient poor systems have inherently low potential growth rates (Chapin 1987, Aerts 1990) and growth responses to N addition are small. Increased N availability may thus allow fast growing, nitrophilous species to outcompete these slow growing species for other limiting resources such as light or water, thereby reducing species richness. This eutrophication effect is generally considered to be the most serious threat posed by N deposition to terrestrial ecosystems globally (Bobbink *et al.* 1998, Fenn *et al.* 2003, Sala *et al.* 2000).

Clearly, the rapidity with which N deposition induces changes in species dominance will depend on the structure of the vegetation type in question. In forest, for instance, the slow growth rate and tall stature of overstorey vegetation will mean that any changes in competitive dominance of tree species will occur over long time periods, although some studies suggest that N deposition might, in the long term, lead to the conversion of slow growing coniferous forest to faster growing deciduous forest (Fenn *et al.* 1998, McNulty *et al.* 1996). While N related community changes predicted for forest tree layers are speculative, species compositional changes in understorey vegetation are often observed (Prescott *et al.* 1995, van Dobben *et al.* 1999). In a study by van Dobben *et al.* (1999), eutrophication was found to have a far stronger effect on understorey species composition than acidification. The effect of N was attributed both to stimulation of the herb layer (particularly the grass *Deschampsia flexuosa*) and, to a lesser extent, to reduced light levels on the forest floor (due to increased canopy growth).

In contrast to forest, N induced changes in dominance of characteristic species are often clearly apparent in shorter statured vegetation such as grasslands, shrublands and deserts. One well documented example occurs in Holland, where many species-rich wet heathland habitats have been converted to monospecific stands of the grass *Molinia caerulea*. Because the dominant shrub species in this vegetation type, *Erica tetralix*, is relatively short of stature, *M. caerulea* is able to overgrow and outshade it at high N availability (Aerts & Berendse 1988, Bobbink *et al.* 1998). However, in vegetation where the characteristic species are of taller stature and form a canopy, some secondary stress may be required to open up gaps so that nitrophilous understorey species can obtain enough light to be competitive. This situation occurs in dryer Dutch heathlands dominated by the taller ericoid shrub *Calluna vulgaris*, where increased N only seems to allow grass (usually *Deschampsia flexuosa*) to flourish once some form of disturbance (in this case, herbivory by chrysomelid beetles) opens up the dwarf shrub canopy (Heil & Deimont 1983, Brunsting & Heil 1985). Where no such secondary stress damage occurs, *Calluna* canopy growth is usually stimulated relative to other species and may cause the disappearance of certain species beneath the canopy (especially cryptogams), due to outshading and greater litter accumulation (e.g. Power *et al.* 1998b, Lee & Caporn 1998).

It is clear from Table 2.1, that the plants which most commonly increase in vegetation under N deposition are grasses. In some systems, such as the two European heathlands mentioned above, plant communities may contain native or naturalised grass species that are responsive to N, and which become dominant. However, in most relatively unperturbed nutrient poor systems globally, this role is filled by invasive alien species (Dukes & Mooney 1999). Some examples include serpentine grasslands (Huenneke *et al.* 1990, Weiss 1999), sub-tropical grasslands (Le Roux & Mentis 1986), coastal sage scrub (Minnich & Dezzani 1998) and desert (Brooks 2003). Exotic grass invasion represents a serious threat to natural ecosystems in many parts of the world (D'Antonio & Vitousek 1992). Grasses have been shown to compete effectively with indigenous species, for light (e.g. Rebele 2000, Thompson and Harper 1988) water (e.g. Melgoza *et al.* 1990, Eissenstat & Caldwell 1988) and other nutrients (e.g. P; Caldwell *et al.* 1987). Because of their shallow root systems, grasses are likely to be particularly competitive against seedlings or other shallow rooted species with which they are in direct competition for resources (Rebele 2000, D'Antonio & Vitousek 1992). In addition, grass invasion may also alter ecosystem processes in several ways. Firstly, grass litter often has higher N content, and a lower lignin: N ratio than the litter of characteristic species in N poor environments. Grass invasion may thus be responsible for increasing rates of N cycling (mineralization, nitrification) and maintaining more N rich systems (Lee & Caporn 1998, D'Antonio & Vitousek 1992, Bowmann *et al.* 1995). Secondly, and perhaps more importantly, grasses and grass dominated systems are usually more flammable than native vegetation and lead to increased fire frequency, which maintains grass dominance (D'Antonio and Vitousek 1992, Fenn *et al.* 2003, Minnich & Dezzani 1998, Milton 2004). For example, increased productivity of alien grasses has lead to a higher incidence of fire in coastal sage scrub, and is likely to be one of the primary mechanisms responsible for the recent conversion of CSS to annual grassland (Minnich & Dezzani 1998).

Although N addition has been clearly implicated in increasing the success of grasses, it is only one of a number of factors that influence grass invasion. Others include increased seed supply from neighbouring areas, increased disturbance frequency, decreased grazing and natural successional increase in soil N content (Minnich & Dezzani 1998, Bobbink & Willems 1987, Bobbink *et al.* 1998, D'Antonio & Vitousek 1992). Caution should thus be shown when attributing increased grass productivity or invasion in natural ecosystems to N deposition alone.

An important consideration when discussing the competitive advantage conferred on species by N addition is the role of other secondary limiting nutrients and nutrient imbalances. Plant diversity and health is favoured by balanced nutrient supply ratios (most commonly N: P and/or N: K), and changes in these ratios can lead to changes in community composition and reduced diversity (Braakhekke & Hooftman 1999). Roem *et al.* (2002) conducted a full factorial experiment in Dutch heathland, simultaneously manipulating N and P. Their results showed that, of the three dominant species (*Erica tetralix*, *Calluna vulgaris* and *Molinia caerulea*), each was limited by different nutrients; *E. tetralix* by N, *C. vulgaris* by P and *M. caerulea* by a combination of the two. Similar results were obtained by Kirkham (2001) in British moorland. In situations like this, additional N confers greatest advantage to species which are least limited by other nutrients, or which are better adapted to limitation by other nutrients. Thus competitive advantage has little to do with the potential growth rates of the species in question. For example, Kirkham (2001) suggest that part of the reason for the increased productivity of the grass, *M. caerulea* under N deposition relative to other species, is that it is better adapted to P limitation. Shifts to P limitation caused by N addition are commonly reported in natural ecosystems including heathlands/moorlands (e.g. Kirkham 2001), grasslands (e.g. Carrol *et al.* 2003) and alpine tundra (e.g. Bowman *et al.* 1995).

Reduced competitiveness

As discussed earlier, N can reduce competitiveness or cause mortality of some species due to phytotoxicity, soil acidification and the creation of nutrient imbalances.

These effects may also increase susceptibility to secondary stress factors. In this situation, hardy stress tolerant species may thus increase at the expense of more sensitive species.

Although direct negative impacts of pollutants (gaseous or dissolved) on vascular plants are generally only considered to be a problem near large pollution sources (Wellburn 1990, Stulen *et al.* 1998), airborne pollutants do have the potential to alter vascular plant species richness. For instance, Ashmore *et al.* (1995) exposed calcareous grassland swards transplanted directly from the field, to O_3 concentrations typical of ambient levels in southern England and found marked changes in species composition. In this case, faster growing species were apparently more negatively affected than slow growing characteristic species (Ashmore *et al.* 1995). Typically, the most rapid change in community composition due to direct toxicity occurs in mosses and lichens (Morecraft *et al.* 1994, Mäkipää 1995, van Dobben *et al.* 1999). Mäkipää (1995) added ammonium sulfate to a boreal forest understorey over the period of four years. Total bryophyte biomass decreased by 60%, but several sub-dominant species increased in response to fertilization. Similarly, van Dobben *et al.* (1999) found that both N and acidity decreased the cover of most moss and lichen species in a pine forest, but favoured certain species. Several litter-inhabiting mosses increased strongly in response to N, while a few acid tolerant species were favoured by acidification.

Soil mediated acidification effects are considered to be the biggest factor responsible for directly reducing competitiveness or increasing mortality in terrestrial ecosystems, although they are usually thought to be of secondary importance relative to eutrophication, in altering species composition and species richness (Bobbink *et al.* 1998). Vascular plant species vary widely in their abilities to tolerate acidification stress and, in some cases, sensitive (more calcicolous) species disappear from natural vegetation when pH drops below a certain threshold value (Falkengren-Grerup 1995, Roem & Berendse 2000, Houdijk *et al.* 1993). In contrast, species from naturally acidic environments with inherently low base cation concentrations and high Al mobility are likely to be relatively resistant to further acidification (Phoenix *et al.* 2005).

The majority of studies examining N deposition effects on plant communities have not distinguished between eutrophication and acidification effects. However, several recent studies which have done so have indicated that acidification may often be underestimated as a driver of community composition changes (Roem & Berendse 2000, Roem *et al.* 2002, Houdijk *et al.* 1993). Houdijk *et al.* (1993) investigated the reason for the decline of several endangered species in acidic Dutch heathlands and concluded that acidification effects were likely to be the most important causative factor. Although competition between grass and dominant shrub species is altered in these systems, most rare species disappear before grass becomes dominant (Houdijk *et al.* 1993). As well as being more responsive to N availability, the grass species that typically dominate in Dutch heathlands (*Molinia caerulea* and *Deschampsia flexuosa*) also have lower pH optima than most endangered species (van Dobben 1991 cited in Roem *et al.* 2002). Roem & Berendse (2000) conducted descriptive field studies in Dutch heathland and grassland communities, and found that species richness was most strongly correlated with acidity, while N availability was of secondary importance. In a further heathland study, Roem *et al.* (2002) used factorial addition experiments to separate acidification effects from eutrophication effects. Their results showed that acidification (Al toxicity in particular) was the most important factor in reducing species diversity, and inhibited seedling germination in some species.

Studies in European grasslands have also shown the potential for acidification to reduce species richness. An example is the Rothamstead Park Grass experiment (Goulding *et al.* 1998), in which neutral grassland has been fertilized with N for almost 150 years. Addition of ammonium sulphate $(NH_4)_2SO_4$ has caused a substantially larger reduction in species richness than addition of sodium nitrate ($NaNO_3$), largely due to acidification of the soil (sodium nitrate does not acidify the soil, while ammonium sulphate has reduced soil pH from 5.8 to 3.5 over the course of the experiment). Similarly, in calcareous grassland, ammonium sulphate addition caused a greater decline in species richness than did ammonium nitrate (NH_4NO_3) due to the greater acidifying effects of the former (Morecraft *et al.* 1994, Carroll *et al.* 2003). In the above-mentioned experiments it is possible that, in addition to their larger acidification potential, ammonium based fertilizers could have had greater negative effects due to ammonium toxicity or plant preference for nitrate (Bobbink 1998).

Animal and microbial communities

Although the focus of this discussion (and the majority of research to date) has been on changes in plant communities, indications are that N deposition causes similar competitive shifts and reductions in species richness in faunal and fungal communities. Mycorrhizae are amongst the most sensitive biota to N enrichment, usually showing effects long before plant communities do (Egerton-Warburton & Allen 2000). As discussed earlier, N addition may decrease or (more rarely) increase mycorrhizal biomass. However, more detailed studies in both ecto- (reviewed in Wallenda & Kottke 1998) and AM (Egerton-Warburton & Allen 2000, Johnson 1993) mycorrhizae, have shown that changes in biomass or infection rates result from changes in community composition and reductions in diversity. In a study by Egerton-Warburton & Allen (2000) in coastal sage scrub (CSS), nitrogen addition caused a decrease in abundance of large-spored species (*Scutellospora* and *Gigaspora*), and an increase in small-spored *Glomus* species, which appear to be less effective mutualists (Egerton-Warburton & Allen 2000). Corkidi *et al.* (2002) and Johnson (1993) obtained similar results. It is feared that such alterations in mycorrhizal community composition might enhance invasion by exotic grass species (Fenn *et al.* 2003, Egerton-Warburton & Allen 2000). For example, van der Heijden *et al.* (1998) grew a selection of plant species with five different mycorrhizal innoculae, and found that growth was least affected by the type of mycorrhiza in two grass species, *Bromus erectus* and *Festuca ovina*. In another study, Sigüenza (2000 cited in Fenn *et al.* 2003) found that elevated N applied to CSS, caused adverse effects in a native shrub (*Artemisia tridentata*) due to reduced mycorrhizal colonization of its roots, while the exotic grass *Bromus madritensis* was not negatively affected due to its preferential association with an N-tolerant *Glomus* species. N addition might also result in shifts in the community composition of non-mycorrhizal soil microbes (for example it may change the relative abundance of white rot and soft rot fungi in temperate forest soils; Waldrop *et al.* 2004), but this topic has not yet been adequately investigated.

N deposition also has the potential to alter insect community composition. Haddad *et al.* (2000) surveyed insect and plant communities in species- rich serpentine grassland, along a nitrogen addition gradient which had been maintained for 14 years. Insect abundance increased, while plant and insect species richness (particularly of herbivore species) decreased in response to N addition. Insect species richness was most closely correlated with plant species richness, with host-specific insect species declining in tandem with their host plants (Haddad *et al.* 2000). In the San Francisco Bay area, N addition is a threat to the survival of the endangered bay checkerspot butterfly (*Euphydryas editha bayensis*) (Weiss 1999). In the absence of grazing, non-native grasses have increased strongly, probably largely as a result of N addition, and have crowded out the host plants of the butterfly in many areas, leading to marked declines in butterfly populations (Weiss 1999).

2.4.4 Critical loads

Many of the fertilization experiments conducted in natural vegetation to date have used relatively large N treatments, but studies have shown that negative impacts in many systems occur at low N inputs. For instance, in nutrient poor Californian serpentine grasslands, where soils efficiently retain N, deposition of 10-15 kg $N.ha.a^{-1}$ has facilitated large scale invasion of annual grasses (Weiss 1999, Huenneke *et al.* 1990). In highly sensitive vegetation types such as alpine tundra, shifts in plant community composition have observed at deposition inputs as low as 4-6 kg $N.ha.a^{-1}$ (Burns 2004, Bowmann *et al.* 1995). In order to assess the potential for N deposition to cause deleterious effects in ecosystems of varying sensitivity, the 'critical loads' approach was developed. For a particular ecosystem, Nilsson (1986 cited in Skeffington & Wilson 1988) defined the critical load as 'the highest load that will not cause chemical changes leading to long-term harmful effects'. Many alternative definitions have since been used, but all contain the same basic idea of a threshold deposition level, above which harmful effects are observed. However, definitions of what constitutes a harmful effect, and the methods by which these effects are quantified, vary greatly. While the critical load concept was originally developed as a means of assessing acidification effects, it is currently also applied to eutrophication effects, or the combination of acidification and eutrophication effects (Bull 1995).

In determinations of critical loads of acidity, both N and S compounds are usually considered simultaneously, although there is a good deal more uncertainty associated with N, due to the complexity of transformations which determine the acidification potential of deposited N (Kuylenstierna *et al.* 2001). Most commonly, methods of assessing acidification threat have included measures of soil buffering potential

(Kuylenstierna *et al.* 2001, van Tienhoven *et al.* 1995) or more complex mass balance models which also incorporate measures of base cation deposition, and losses of acid neutralization capacity via leaching and biotic removal (e.g. Gregor *et al.* 2001). Critical loads for forest damage due to acidification have also been based on base cation: Al ratios, although it has been argued that this method allows unacceptable levels of damage in some cases (Grennfelt *et al.* 2001).

In setting critical loads for N as a nutrient, two main approaches, which may be termed the *empirical* and the *saturation (mass balance)* approach, have been used (Kuylenstierna *et al.* 1998). The empirical approach is the most commonly utilized of the two, because the complexities of N cycling make it difficult to accurately measure all flows of N in and out of the system (Kuylenstierna *et al.* 1998). Empirical critical loads are often based on changes in species composition or dominance, or in some cases, changes in ecosystem functioning (e.g. increased leaching) observed in experiments or field observations (e.g. Bobbink & Roelofs 1995; see Table 2.1). However, because this approach relies on information from detailed long-term studies on ecosystem responses to N addition in a range of systems, it is not usually practical outside of well studied areas in Europe and N. America. A more widely applicable method is to assign critical loads using natural availability of nitrogen (C: N ratios, soil available N, net mineralization rates) as an indicator of ecosystem sensitivity to N deposition (Kuylenstierna *et al.* 1998, Gough *et al.* 1995). This approach assumes that the sensitivity of vegetation to N inputs (i.e. predisposition to changes in vegetation structure or diversity) will be inversely related to the availability of nitrogen in that system (Kuylenstierna *et al.* 1998, Gough *et al.* 1995). While this may often be true, it is clearly a simplification of reality. One of the shortcomings of this method is that it does not take account of the role of secondary or co-limiting resources such as other nutrients or water which may prevent large changes in vegetation composition. It also neglects to account for the possibility of large N losses or high rates of N immobilization in soils. In this respect the saturation approach may be a better means of estimating critical loads, as it takes into account the balance between all N inputs and outputs, such that an "unacceptable" build up of N is prevented (Kuylenstierna *et al.* 1998, Gregor *et al.* 2001). Clearly however this approach also has its drawbacks, in that a) the definition of what constitutes an unacceptable accumulation of N is largely subjective and b) there may be a large degree of uncertainty attached to the measurements of N inputs and losses used to determine the critical load (Kuylenstierna *et al.* 1998). Despite the potential shortfalls of the two approaches, they have been successfully used by the Stockholm Environmental Institute (SEI) to map critical loads of N for Europe, and have produced reassuringly similar results (Kuylenstierna *et al.* 1998).

2.5. N deposition and global change

An in-depth consideration of N deposition effects on global change is beyond the scope of this overview. However, because N pollution has the potential to interact with variables such as global temperature and CO_2 levels, a brief mention of the role of N as a moderator of global change is warranted. On a global scale, N deposition generally boosts productivity, and thus increases uptake and storage of CO_2 from the atmosphere by plants (Wright & Schindler 1995). Increased anthropogenic N addition may thus explain a portion of the so-called "missing carbon sink" (Vitousek *et al.* 1997, Norby 1998). There is, however, a large amount of uncertainty attached to models which attempt to calculate the proportion of the missing sink explained by N deposition (Norby 1998), and estimates of this value vary from almost 100% (e.g. Holland *et al.* 1997) to around 10% or less (e.g. Lloyd 1999, Nadelhoffer *et al.* 1999). Even if N deposition presently accounts for a large proportion of the terrestrial C sink, C sequestration will decline as systems approach N saturation, and thus N deposition will not increase biotic sink strength indefinitely (Norby 1998). Furthermore, N may reduce the capacity of ecosystems for C sequestration if N deposition results in long term reductions in species diversity (Reich *et al.* 2001a) or productivity.

While N deposition acts in opposition to global warming by increasing the CO_2 sink, it may also increase global warming in other ways. N deposition results in increased emissions of the greenhouse gas N_2O, which currently accounts for around 6% of global warming (Erisman *et al.* 1998) and decreases the capacity of soils to oxidize another greenhouse gas, CH_4 (Goulding *et al.* 1998), although these effects are relatively small.

Chapter 3

A preliminary estimate of bulk nitrogen deposition onto natural vegetation in the Cape Metropolitan Area

3.1 Introduction

Monitoring of deposition chemistry has, in recent decades, assumed great importance due to growing international concern about the potential and observed negative effects of atmospheric deposition of chemical species on natural ecosystems, as well as damage to man-made structures and materials. In developed parts of the world, the chemical composition of deposition has long been studied (wet deposition since 1853 when the first rain gauge was built and dry deposition since 1986; Goulding *et al.* 1998). In these regions, sophisticated networks currently exist for the purpose of monitoring deposition chemistry. Two well known examples are the National Atmospheric Deposition Program (NADP) in the USA, which consists of more than 200 monitoring stations, and the European Monitoring and Evaluation Program (EMEP) which collates deposition data from the national networks of the majority of countries in W Europe. In developing regions of the world however, data on pollution deposition is generally sparse or lacking, despite deposition being a potential threat to biodiversity in many areas (e.g. Phoenix *et al.* 2005, Rodhe *et al.* 1995a, Galy-Lacaux *et al.* 2003, van Tienhoven *et al.* 2003, Innes & Harron 2000, Kuylenstierna *et al.* 1998). These regions are thus a high priority for the establishment of deposition monitoring programmes, particularly where sensitive ecosystems occur in close proximity to rapidly developing centres of human activity.

The Cape Metropolitan Area and its surrounds is a high priority area for deposition studies because of the rich and endangered flora which occurs here in close proximity to urban pollution sources. Cape Town is the second largest city in South Africa, with a 2002 population of roughly 3.15 million, and a 3.5 % annual population growth rate

(City of Cape Town, 2003a). The CMA contains large vehicle numbers (825 000 registered vehicles in 2002; City of Cape Town 2003a) and supports a moderate degree of industrial activity.

Air pollution has long been recognized as a problem in Cape Town and an air pollution control program has been in place since the late 1960's. At present, atmospheric concentrations of NO_x and SO_2, as well as PM_{10} (particulates < 10μ in diameter), H_2S and O_3 are monitored continuously at nine sites within the CMA.

An emissions inventory for the CMA compiled by Wicking-Baird *et al.* (1997) reveals that, as is the case for most large cities (e.g. Olivier *et al.* 1998), NO_x emissions in Cape Town originate predominantly from motor vehicles, while SO_2 is mainly emitted by industry (Figure 3.1). NH_3 is not currently monitored in Cape Town but emissions may be significant as some agricultural activity, including pig farming, occurs on the Cape Flats and around the outskirts of the CMA (Visser 2001).

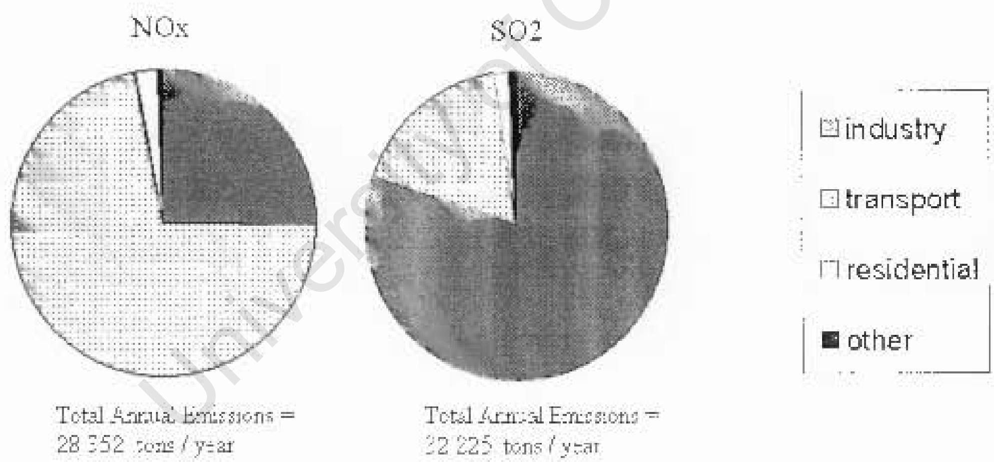

Figure 3.1: *Total annual emissions of SO_2 and NO_x in the Cape Metropolitan Area and their sources. Data are from Wicking-Baird et al. (1997).*

The severity of air pollution in Cape Town is influenced, to a large degree, by seasonal variations in wind direction and speed as well as the complex nature of the local topography. Cape Town is a windy city with moderate to strong southerly/south-easterly winds predominating in spring/summer (September-February) and northerly/north-westerly winds dominant in autumn/winter (March-August). Wind speeds generally peak in summer and are lowest in late autumn and early winter. While the prevailing winds result in strong mixing and dispersion of pollutants out to sea, low level temperature inversions (between 100m and 300m in depth), associated with calm atmospheric conditions, commonly form overnight during the autumn/ winter period (Keen 1979). Under these conditions, pollutants become trapped below the inversion, with the Table Mountain chain acting as a barrier, preventing pollution movement to the west. The result is the so-called "brown haze", a pall of brown smoke, composed largely of fine particulate matter (particles smaller than 2.5μ in diameter, termed PM 2.5) which hangs over the city and seriously impairs visibility (Wicking-Baird *et al.* 1997). Ground level concentrations of gaseous pollutants are also substantially elevated during these events, but decrease as afternoon approaches and the inversion is dissipated due to heating by the sun.

Atmospheric deposition of pollutant species has, to my knowledge, never been measured in the CMA, save for a few event only samples collected at the Cape Point Global Atmosphere Watch (GAW) Station situated at the tip of the Cape Peninsula (Jonas Mphepya, unpublished data) and at the University of Cape Town (Meris Smith, personal communication). An automated wet-only deposition sampler is in place at Cape Point, but is currently not in operation, although plans are underway to begin regular sampling in the near future (Ernst Brunke, personal communication).

The only deposition study in the region was conducted by Stock & Lewis (1986a) at Pella, an isolated and unpolluted site 62 km north of Cape Town, where a low bulk input of 1.12 to 1.79 kg N $ha^{-1}a^{-1}$ was measured. These values are likely to be similar to the natural or background bulk deposition levels to vegetation in the CMA.

Global pollutant transport models have also been used to produce approximate maps of the global deposition of N (e.g. Phoenix *et al.* 2005) and S (e.g. Kuylenstierna *et al.* 2001), but these are on too large a scale (generally 5° x 5° or greater) to provide useful information about specific localities such as urban areas. This study thus represents the first estimate of annual bulk N and S deposition levels in the Cape Metropolitan Area.

3.1.1. Measuring precipitation chemistry

Since the chemical composition of rainwater was first measured in the late 1800's, numerous methods have been adopted for the collection, preservation, storage and analysis of atmospheric deposition. The simplest method for collecting deposition is so-called bulk sampling, where collecting funnels or containers are placed in the open and continuously exposed to all incoming particles, in both wet and dry form (e.g. Tørseth & Semb 1998, Zapletal 1998). Bulk sampling has the advantage of being inexpensive and relatively simple to carry out, and is the most commonly used wet deposition sampling technique (Krupa 2002, Erisman *et al.* 2003). However, bulk sampling should be considered a gross measure of deposition as it does not adequately account for the processes of dry deposition and occult deposition, and samples are vulnerable to contamination by organic debris such as bird droppings, insects and leaf material. Contamination may be kept to a minimum by placing a filter in the collector to exclude debris, although organic material may leach nutrients if left in the funnel for long periods (Allen *et al.* 1989). Some means of discouraging birds from perching on the edge of collectors and fouling the funnels is also often included in the design of bulk samplers.

Bulk samplers vary in the efficiency with which they scavenge dry and occult deposition, depending on their aerodynamic character and the windiness of the site at which they are placed (Bleeker *et al.* 2003). Generally however, the fraction of dry and occult deposition which is captured by bulk samplers settles into the funnel of the collector by means of sedimentation (Krupa 2002, Erisman *et al.* 2003). In the case of dry deposition, this is mostly coarse particulate matter (>2.5 μm diameter) such as large sea-salt particles. Other important dry/occult deposition processes such as impaction and adsorption are considerably lower to a smooth funnel than they would be to an irregular surface e.g. plant foliage, and are thus underestimated by bulk deposition sampling. Clearly, bulk sampling also does not account for gaseous uptake via the stomata which, as discussed in the previous chapter, is sometimes an important component of dry N deposition to vegetation. For these reasons, studies which seek an accurate figure for total deposition usually estimate dry deposition from atmospheric concentrations using mathematical models (e.g. EMEP 2003) and measure occult deposition using specialized equipment (Krupa 2002, Wesely & Hicks 2000). Wet deposition figures are then calculated in one of two ways. Either empirically derived correction factors are applied to bulk deposition measurements in order to correct for contamination by dry deposition or wet deposition is measured using automated wet-only samplers. which only open for collection in response to the detection of precipitation by built-in sensors, thus excluding dry deposition (Krupa 2002).

For certain atmospheric species (e.g. S, Na and Cl), the dry and occult deposition flux can be estimated by placing so-called throughfall samplers under the canopy of overstorey vegetation in order to catch rain that filters through the canopy. Dry and occult deposition which collects on the leaf surfaces of the canopy is washed into the collectors by rain and this component can then be distinguished from wet deposition by comparing throughfall values with bulk values (Farrell 1995). For nitrogen however, this method is not as successful, as there may be substantial interaction of wet and dry N deposition with the canopy. Particularly in areas with relatively low deposition loads. a large proportion of deposited N may be retained by foliage, while in some instances. throughfall may be enriched in N by foliar leachates (Bobbink *et al.* 1992, Lindberg *et al.* 1986, Garten 1992).

Because precipitation contains very low concentrations of chemical species, rainwater samples are highly susceptible to significant changes in chemical composition in the field or during storage prior to analysis. Inorganic nitrogen compounds and, to a lesser extent, sulfur compounds are particularly subject to transformation by microbes (Allen *et al.* 1989). This may involve (1) consumption/ immobilization of nutrient ions (2) oxidation of reduced species such as NH_4 and (3) breakdown of organic compounds to release inorganic ions. Furthermore, water loss may occur by evaporation, ions may become adsorbed to container walls/ suspended solids, and samples may be contaminated by absorption of water-soluble gases (Allen *et al.* 1989, Sliwka-Kaszynska *et al.* 2003, Krupa 2002). In order to avoid such changes, samples should ideally be collected on a daily or even an hourly basis. Clearly, however, this is very time consuming and costly, especially where collection sites are relatively remote, and most international stations for precipitation monitoring in N. America and Europe collect samples at intervals of between 1 and 4 weeks (Erisman *et al.* 2003, Krupa 2002). If samples are to be left in the field for longer than 1 week, some form of preservative / antibiotic is usually added to the collecting bottle in order to retard microbiological activity (Sliwka-Kaszynska *et al.* 2003, Allen *et al.* 1989). Microbial transformation (especially oxidation of NH_4) is also minimized by slightly acidifying the sample or collecting bottle, and limiting the entry of light. After collection, fine filtration is generally performed as soon as possible in order to remove micro-organisms as well as organic matter, which has the potential to break down during storage and release inorganic ions. Microbial activity is further limited by cold storage (4° C or lower) of samples until analysis can be performed.

3.2 Materials and Methods

3.2.1 Study Sites

Deposition sampling was carried out at five sites within the Cape Metropolitan Area (CMA) of the South Western Cape Province, South Africa (Table 3.1, Figure 3.2). Sites were chosen in areas of natural vegetation because the effects of deposition on these areas are our primary concern. Sites were also chosen in such a way as to represent the range of deposition levels that would be likely to occur in areas of natural vegetation in the CMA. Kenilworth Race Course and Tygerberg Nature Reserve and, to a lesser extent, Devils Peak, are expected to be most affected by anthropogenically elevated N deposition, because of their low elevations, proximity to pollution sources and their position in relation to prevailing SE/NW wind directions (Figure 3.2). N deposition at Table Mountain and Silvermine was predicted to be relatively unaffected by anthropogenic sources. However, the relatively higher rainfall levels at Table Mountain, Kenilworth and Devils Peak were expected to result in elevated wet deposition levels at these sites (Figure 3.3). Deposition at the Table Mountain site was expected to be further elevated due its altitude and frequent exposure to occult deposition.

le 3.1: Physical characteristics of the five study sites and the corresponding standard rain gauge sites from which additional rainfall volume data obtained.

dy site	Elevation	Vegetation / Soil Type	Site Description	Site/source of rain gauge volume data	Distance of rain gauge from study site	Elevation of rain gauge relative to study site
vils Peak	210m	Fynbos/ renosterveld on red clayey (shale) soils	Lower E facing slopes above Rhodes Memorial	Kirstenbosch Botanical Garden (SAWS)	4300m SSE	-15m
ilworth C.	30m	Sand plain fynbos on deep white acid sands	Healthy vegetation in NE corner of race course.	Kenilworth R.C. (K.R.C Management)	400m SE	0
vermine R.	295m	Mountain fynbos on shallow white acid sands	Gentle N facing slope in Silvermine N.R. South.	Silvermine N.R (SAWS)	150m S	+3m
ole untain.	750m	Mountain fynbos on shallow white acid sands	Flat stand in NW part of Table Mt. plateau, near Mountain Club hut	Table Mountain. (SAWS)	550m E	-5m
gerberg R.	300m	Renosterveld on red clayey (shale) soils	SE facing slope in most southerly part of Tygerberg N.R.	Tygerberg N.R. (T.N.R Management)	200m N	-10m

'S = South African Weather Service

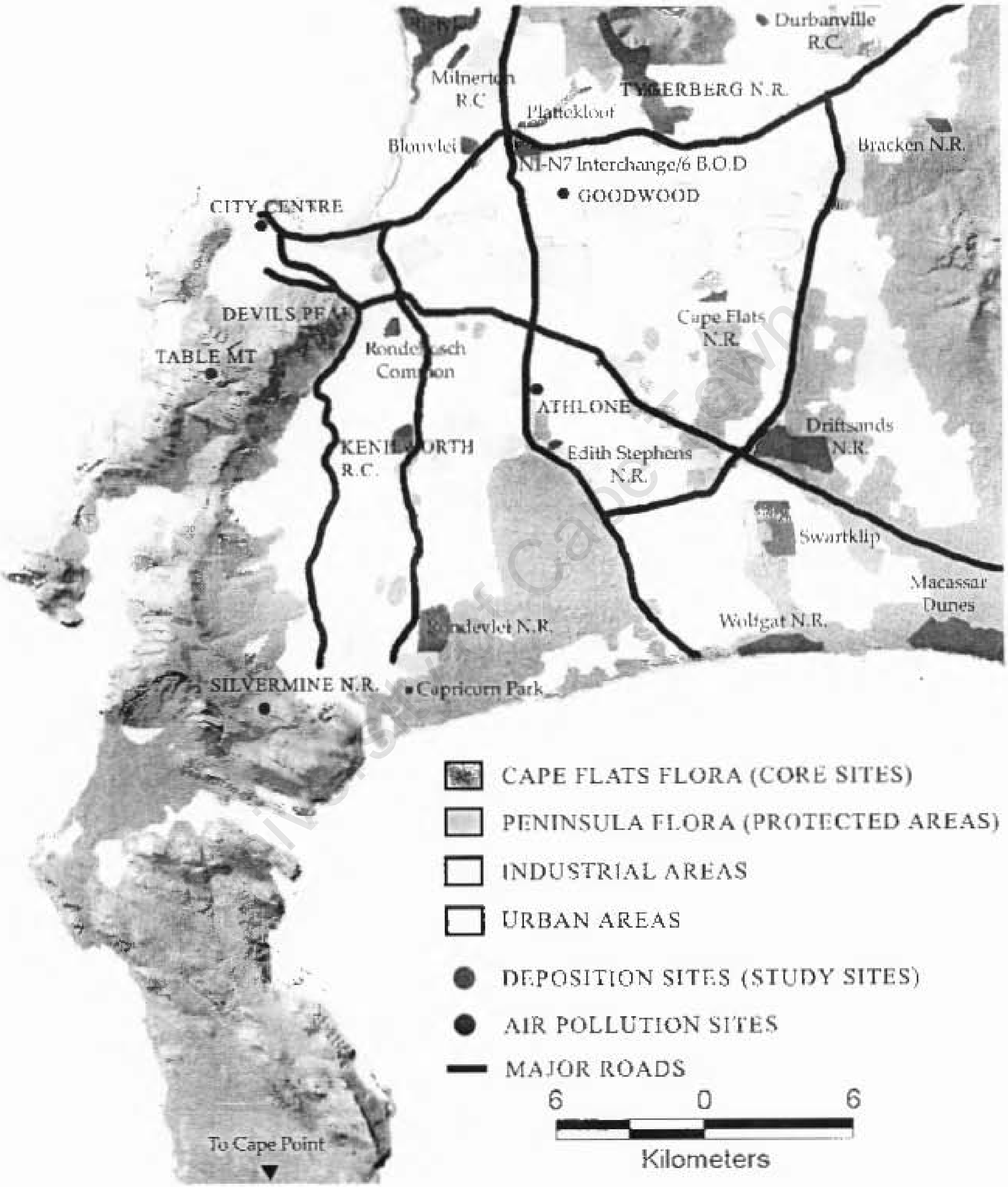

Figure 3.2: *Map of the Cape Metropolitan Area showing the deposition study sites, and air pollution data collection sites, as well as the naturally vegetated areas, land use zones and major roads in the region.*

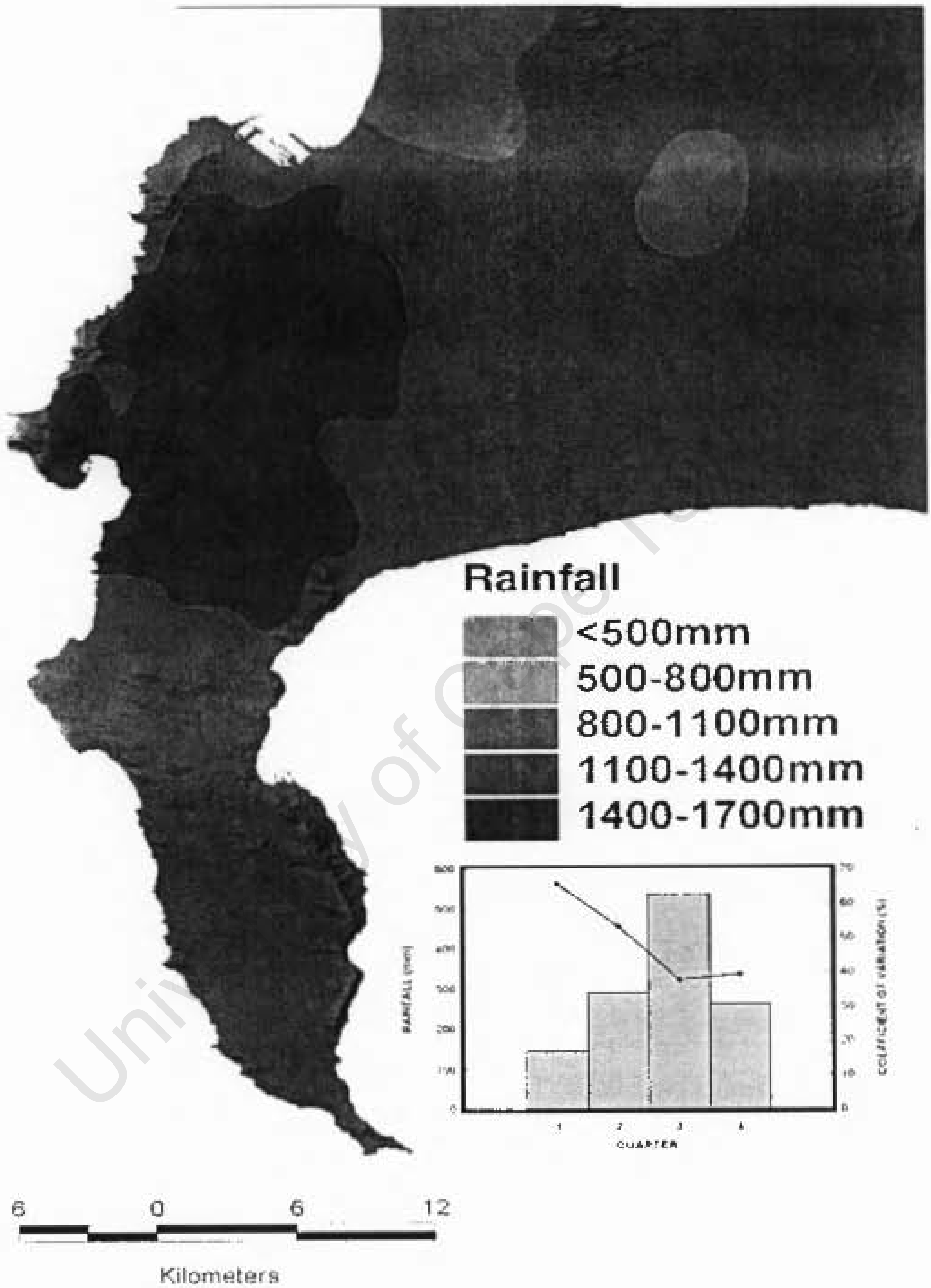

***Figure 3.3**: Long term spatial and temporal rainfall patterns in the Cape Metropolitan Area. Quarterly rainfall distributions are for Cape Town International Airport supplied by the South African Weather Service.*

3.2.2 Deposition Sampling

Sampler Design and Setup

Bulk deposition collectors were constructed by the author as shown in Figure 3.4a. Collecting funnels (diameter 10.4 cm) were created by cutting off the bottoms of 2l cool drink (polyethylene terephthalate) bottles. The funnels were drained by PVC piping (diameter 5mm) into 2.5 l darkened glass (Winchester) bottles, the bases of which were dug into the ground. High strength putty was used to create a watertight connection between the funnel and the PVC piping and to ensure that all the water entering the funnel would drain into the collecting bottle. A conical filter plug of plastic mesh (1mm) was placed in the base of the funnel to trap fine organic matter and a cone of coarse chicken wire (1.5cm) was placed on top of the funnels to exclude large organic matter such as leaves, and to prevent perching by birds. Cones of plastic sheeting were also placed around the point where the PVC piping entered the collecting bottle to prevent rainwater entering here. The funnels were fixed at 1.3m above ground level in metal brackets, attached to wooden poles which had been firmly driven into the ground. The apparatus was further stabilized by securing the PVC piping to the poles with cable ties.

Throughfall collectors (Figure 3.4b) were similar to bulk deposition samplers, but in these apparatus the funnels drained directly into the collecting bottles and collection height was 0.6m above ground level.

Equipment was carefully tested for leaks and distilled water was passed through two samplers and analysed for NO_x and NH_4^+ (using ion exchange chromatography, see section 3.2.3) to ensure that no contamination resulted. Evaporation from collecting bottles was also tested over the period of a month in summer, and was found to be insignificant.

At each site, three bulk deposition collectors were placed at least 5m apart, and such that there were no obstructions within a 45° angle from the top of the funnel. Initially three throughfall collectors were also set up at each site, under broad-leaved natural vegetation (usually shrubs of the Proteaceae). Throughfall sampling was, however, discontinued after 3 months due to erratic and inconsistent sample concentrations (presumably the result of contamination from leaf litter and other organic debris, and possibly soil splash), as well as high within site variability of collection volumes.

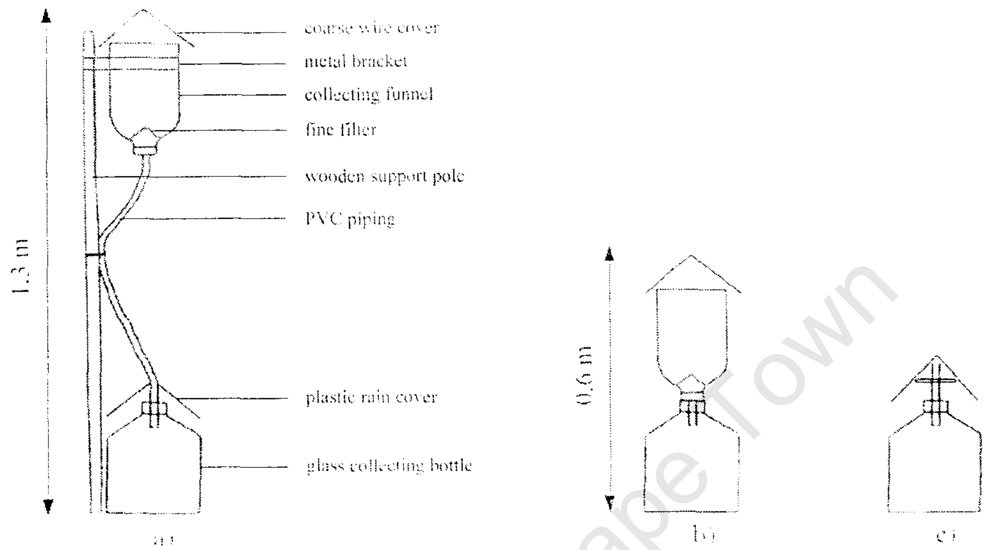

Figure 3.4: *Design of the apparatus used in a) bulk deposition sampling b) throughfall sampling and c) tests for microbial consumption of nutrients.*

Sampling Period

Sampling was conducted for 1 year and 1 month (November 2001 to December 2002). Initially, samples were collected approximately once a month, but this was increased to fortnightly collection from July due to fears of microbial breakdown. In order to test whether collection frequency had a significant effect on measured deposition levels, 3 new bulk deposition collectors were set up at Devils Peak in February 2002, and samples were collected on a weekly basis for comparison with the samples collected on a monthly/fortnightly basis from that site.

Sampling Method

Before being placed in the field, funnels and collecting bottles were acid washed (1M HCl solution) and rinsed with deionized water, and preservative solution was added to each collecting bottle. Initially 1ml of a preservative solution of 2g mercuric chloride ($HgCl_2$) in 1*l* of 1M HCl was used. From July this was modified to 1ml of

1M HCl and a spatula tip (roughly 0.5-1.0 g) of thymol per collecting bottle (Allen *et al.* 1989), due to the analytical problems experienced with $HgCl_2$ (see below).

At collection, the contents of the collecting bottle were decanted into a measuring cylinder to measure volume and 50 ml of sample were then decanted into a clean glass bottle for transport to the laboratory. Any visible signs of contamination were noted.

If insufficient rainfall for analysis (< 50 ml) had occurred, 50 ml of distilled water was used to rinse the sides of the apparatus to wash any dry deposited N into the collecting bottle. Before being placed back in the field, any organic matter which had collected on the filters was removed, the apparatus was washed thoroughly with 1M HCl solution and two rinses of distilled water, and preservative was added to the collecting bottle. At the laboratory, samples were immediately filtered (pre-washed 45μm glass fiber filters) and stored at 0 °C in the dark until analysis.

3.2.3 Sample Analysis

Samples collected from December 2001 to July 2002 were analysed by ion exchange chromatography at the department of Geology, University of Cape Town. Anion concentrations were determined on a Dionex DX500 chromatograph equipped with an AS14 (4 mm) anion exchange column and an ASRS-I-4 mm suppressor, and cation concentrations on a Dionex DX300 chromatograph equipped with a CS12 (4 mm) cation exchange column and a CSRS-ULTRA-4 mm suppressor. The eluents (carrier solutions) used were 2.4 mM Na_2CO_3 / 1 mM $NaHCO_3$ at a flow rate of 1.2 ml/min for anions, and 2.2 mM H_2SO_4 at a flow rate of 1.0 ml/min for cations. Peak measurements were by means of conductivity detection.

Prior to analysis samples were filtered with PVP polymer cartridges (Dionex ONGAURD-P) to remove organic compounds which potentially interfere with column performance. The system was calibrated for the ions of interest, namely nitrate (NO_3^-), nitrite (NO_2), ammonium (NH_4^+) sulfate (SO_4^{2-}) and sodium (Na^+), using standard solutions in the range of 0.1 -10 mg/l. At several stages during analysis, blanks and filtered blanks were run. Filtered blanks were found to have significantly elevated Na levels (in the range of 300 to 600 μg/l with an average of 412 μg/l Na compared to an average sample concentration of around 8500 μg/l. The difference between this value and blank values was subtracted from measured values in order to roughly correct Na concentrations.

It was discovered that the use of $HgCl_2$ as a preservative resulted in excessively large Cl peaks which obscured nitrite measurements (although subsequent tests showed that nitrite levels in precipitation were low enough to be negligible) and prevented the use of a charge balance to verify the integrity of results. Furthermore, ionic N concentrations in some of the rainwater samples were at or below the limits of detection for the chromatograph (around 100 μg/l).

Samples collected from July onwards were thus analysed on a flow injection autoanalyser (Quikchem 8000 series FIA+, Lachat Instruments) at the department of Oceanography, UCT, for NO_y (NO_3 + NO_2) and NH_4 concentrations. Analysis methods used were those prescribed in the Lachat methods manual; reduction of nitrate to nitrite by means of a copper cadmium reduction, followed by colorimetric analysis for NO_y (Smith & Bogren 2001) and a modified version of the indophenol blue method for NH_4 (Egan 2001). A run on the flow injection autoanalyser, comparing standards with and without $HgCl_2$ preservative showed no significant differences in measured concentrations. Nevertheless, due to the fact that $HgCl_2$ can degrade the cadmium column of the autoanalyser (Egan 2001), samples from August onwards were preserved with thymol, as described earlier. Each run on the autoanalyser included two blanks and standards of known concentration were included at regular intervals in order to gauge the accuracy of readings. Standard deviations for identical NO_y standards were generally around 1-1.5 %. For NH_4 standards however, standard deviations were, on occasion, as high as 11 %.

3.2.4 Alternative estimates of precipitation volumes

When comparing precipitation catch by bulk samplers with that from nearby standard rain gauges, it was observed that catch per unit area was, in many cases, underestimated by bulk samplers. Possible reasons for this are discussed later. It was thus decided to obtain precipitation volume figures from the nearest available standard rain gauge for comparison. At all study sites except for Devils Peak, a standard rain gauge (diameter 13.1mm) was present within 600 metres of the bulk collection site (Table 3.1).

For Devils Peak, no standard rain gauge was available in close proximity to the collection site. It was decided to use rainfall figures from Kirstenbosch, due to the similar altitudes and the comparable position of the two sites on the wet lower eastern slopes of the Table Mountain chain. These figures may, however, overestimate rainfall at Devils Peak and should be treated with caution.

At the Kenilworth and Table Mountain sites, precipitation volume data from the standard rain gauges was missing for several periods (April 15 - June 30 and the entire month of September for Kenilworth, April 15-30, September 15-30 and the entire month of October for Table Mountain). Precipitation volumes for these missing periods were estimated from rainfall figures at Kirstenbosch for the corresponding periods using regressions. Reliable monthly precipitation volumes (mm) from Kenilworth R.C. and Table Mountain for 2001 and 2002 were plotted against the corresponding precipitation volumes for Kirstenbosch. The resulting lines of best fit were as follows: $y = 1.027x - 16.57$, $r^2 = 0.950$ for Kenilworth and $y = 1.039x + 0.97$, $r^2 = 0.792$ for Table Mountain).

3.2.5 Data selection and estimation of missing values

Samples were generally collected in triplicate but, in some cases, one or more samples was missing due to damaged collecting equipment, resulting from storms and gales or, in one instance, tampering, as well as breakage of sample bottles during storage or analysis. In addition, contamination of samples by bird droppings and insects caused clear outliers in some cases, and these were excluded from the data set. Instances of contamination were particularly visible as anomalous NH_4 concentrations. In one case for NO_y and four cases for NH_4, no apparently reliable concentration value could be obtained for a collection, and values for those collections were estimated from the best temporal relationship with another site. Cases where the value or part of the value for a month is an estimate are indicated in the results section.

3.2.6 Calculations

Deposition totals are displayed in $kg.ha^{-1}.a^{-1}$. To calculate this value, sample concentration was multiplied by volume for each collection. This value was then multiplied by the surface area of the mouth of the collecting funnel divided by 1ha (10 000 m^2). All collections for the year were added to obtain annual deposition rate.

Volume-weighted mean (VWM) concentrations were calculated, according to Galloway *et al* (1984), as follows:

$$VMW\,[X] = \sum_{i=1}^{n} P_i\,[X]_i \Big/ \sum_{i=1}^{n} P_i$$

where X is the ion considered, P_i is the rainfall volume of collection i, $[X]_i$ is the concentration of collection i and n is the number of collections.

3.2.7 Sea-salt corrections

The sea is a major source of certain chemical species which occur in deposition. By using Na^+ as a marine tracer, ionic concentrations of these species may be approximately resolved into marine-derived and anthropogenic/terrestrial-derived fractions. This assumes that all Na in bulk deposition is derived from sea spray, and that the sea-salt ions of interest occur in deposition in the same proportion to Na, as they do in bulk sea-water (Keene *et al.* 1986) Of the pollutant species measured in the current study, marine influence need only be considered for SO_4, as terrestrial deposition of marine derived NO_y and NH_4 is negligible (Jordan 1997).

The sea-salt fraction of SO_4 ($SO_{4\,SS}$) was calculated using the formula:

$$SO_{4\,SS} = (SO_{4\,BK} / Na_{BK}) * Na_{samp}$$

where $SO_{4\,BK}$ and Na_{BK} = the concentration of these species in bulk sea-water (from Keene *et al.* 1986), and Na_{samp} = the concentration of Na measured in the precipitation sample.

3.2.8 Tests for microbial breakdown

In order to test whether significant microbial breakdown of NO_y and NH_4 occurred in the field and during the period of storage prior to analysis, tests were conducted at the Devils Peak site in which solutions of known concentration were placed in containers which had been designed to permit the entry of air, but exclude all rainwater and debris (Figure 3.4c).

To test breakdown in the field (Test 1), a standard solution of NO_3 and NH_4 (approximately 500 μg N.l $^{-1}$ each) was made up by dissolving $NaNO_3$ and NH_4Cl in distilled water. Three 50 ml samples of the standard were immediately refrigerated. 500 ml of the standard were then placed into each of six containers. Three of the containers were preserved with $HgCl_2$ and three with Thymol as for the main experiment, and in all cases 1 ml of 1M HCl was added before placement in the field in January 2004. A 50 ml sample was removed from each container after 1, 2 and 4 weeks, filtered and stored in an identical manner to regular rainwater samples until analysis. Analysis of all samples was conducted within 1 month of collection, by flow injection analysis.

In a second test (Test 2), conducted in December 2002, breakdown of samples during long storage was examined. Duplicate samples preserved with $HgCl_2$ or Thymol, which had been placed in the field for 2 weeks, were immediately analysed and then placed in storage for 8 months before being re-analysed. Procedures followed were, in all other respects, identical to the aforementioned test.

3.3 Results

3.3.1 Climatic conditions

Average daily maximum and minimum temperatures for 2002 are shown in Figure 3.5. Temperatures peaked from December to March and were lowest between June and August. Wind roses and average monthly wind speeds for Cape Town International Airport in 2002 are shown in Figure 3.6a and b. The readings are typical of the seasonal pattern which occurs in the SW Cape region. Winds were predominantly southerly in spring /summer (September-February) and northerly in winter/autumn (March-August), with average wind speeds peaking in summer and lowest in autumn/early winter (March-July).

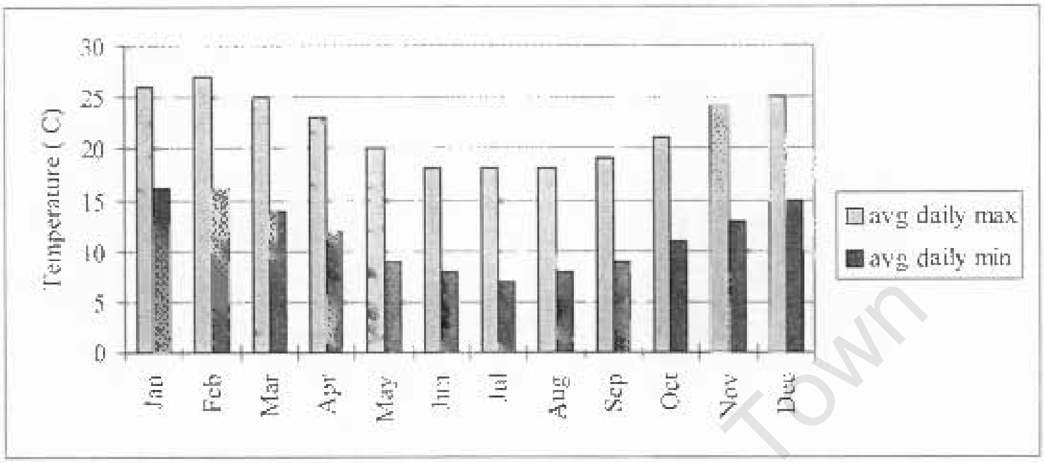

Figure 3.5: *Average daily maximum and minimum temperatures (°C) at Cape Town International Airport for 2002. Data are from the South African Weather Service.*

3.3.2 Rainfall and bulk collector efficiency

Total rainfall for Cape Town in 2002 was lower than the average for the previous 30 years, particularly at sites on the Table Mountain chain (Table 3.2). The amount of precipitation per unit area caught by bulk samplers relative to a standard rain gauge varied widely across the study sites, ranging from around 100% at Silvermine and Tygerberg to as low as 60 % at the Table Mountain and Devils Peak sites. From Table 3.2 it is evident that total percentage catch was roughly inversely proportional to total rainfall at a site.

Figure 3.7 is a comparison between catch per unit area for bulk samplers versus a standard rain gauge ranked by standard rain gauge catch. It shows that the bulk samplers caught a roughly similar volume to rain gauges at low rainfall levels, but tended to undercatch at high rainfall levels. From Table 3.1, however, it is apparent that the sites with the biggest discrepancy between bulk catch and standard rain gauge catch are also those where the distance between bulk samplers and standard rain gauges is largest. Micrometeorological differences between the sites of bulk collector and standard rain gauge placements may thus also explain some of the variation in percentage catch. This is particularly likely at the Devils Peak site.

a)

b)

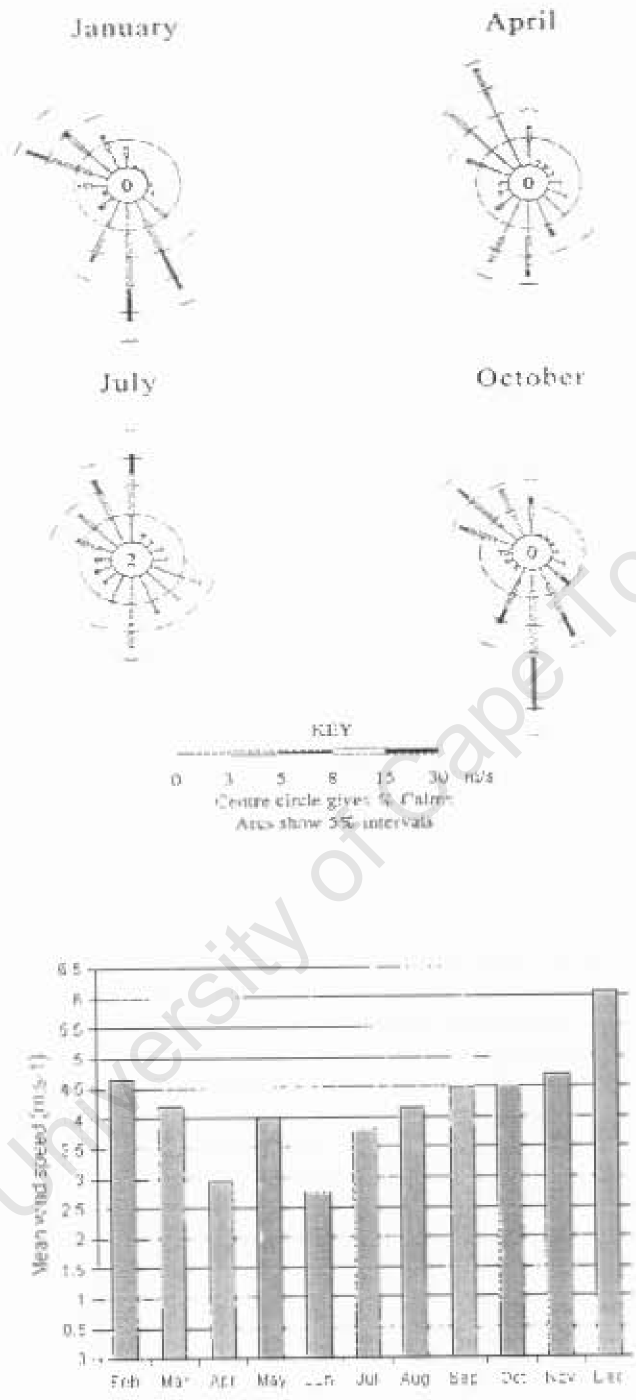

Figure 3.6: *Wind data for the Cape Metropolitan Area in 2002; a) wind roses showing the prevailing northerly winds in autumn (April) and winter (July) and southerly winds in spring (October) and summer (January), b) Mean monthly wind speeds. Data are from the Goodwood air pollution monitoring station and are provided by the Cape Town Scientific Services Department (Air Quality Monitoring Section).*

Table 3.2: *Rainfall figures for the five study sites and percentage catch by bulk samplers for 2002. Rainfall volumes are in mm.a^{-1}.*

Study site	Average annual rainfall (1971-2001)*	2002 rainfall *	% Catch by bulk sampler †
Devils Peak	1416 ± 286	1291	62
Kenilworth RC	NA	1182	80
Silvermine NR	1183 ± 247	826	104
Table Mountain	1464 ± 323	1384	60
Tygerberg NR	NA	572	98

* Rainfall figures are from nearest available standard rain gauges (see Table 3.1).

† Annual bulk sampler catch per unit surface area as a % of standard rain gauge catch per unit surface area.

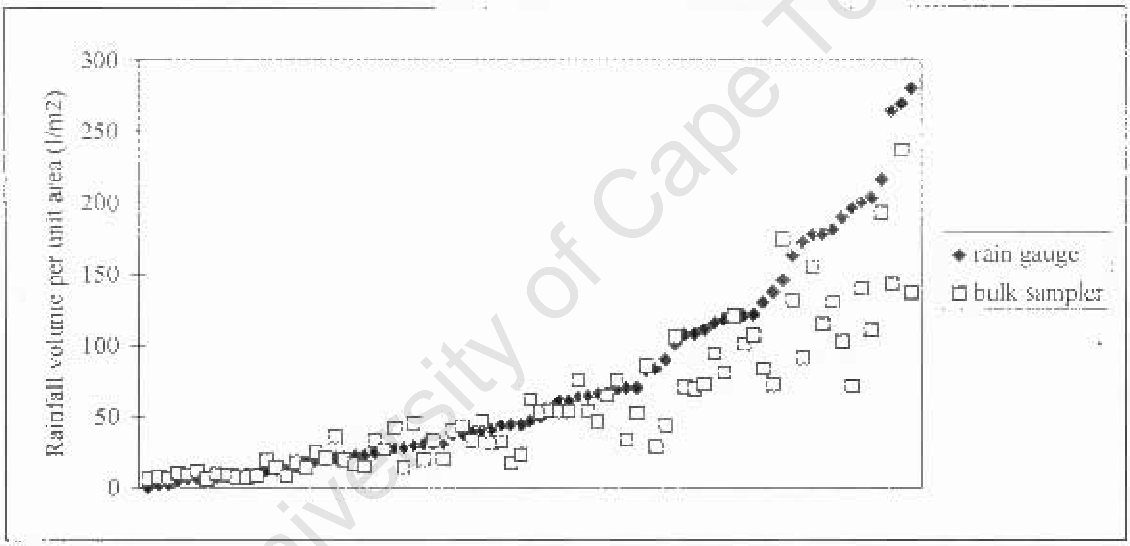

Figure 3.7: *A comparison between rainfall catch per unit area (l/m^2) by standard rain gauges and bulk samplers. Data are ranked by standard rain gauge volumes.*

3.3.3 Rainwater concentrations

As is commonly observed, rainwater concentration in the current experiment was broadly related to the volume of rainfall by a logarithmic curve (shown for NO_y and NH_4 in Figure 3.8). It should be noted that the average concentration calculated for the lowest rainfall class is a slight underestimate because a number of the samples were diluted by the addition of 50 ml distilled water (when less than 50 ml of rainfall had occurred).

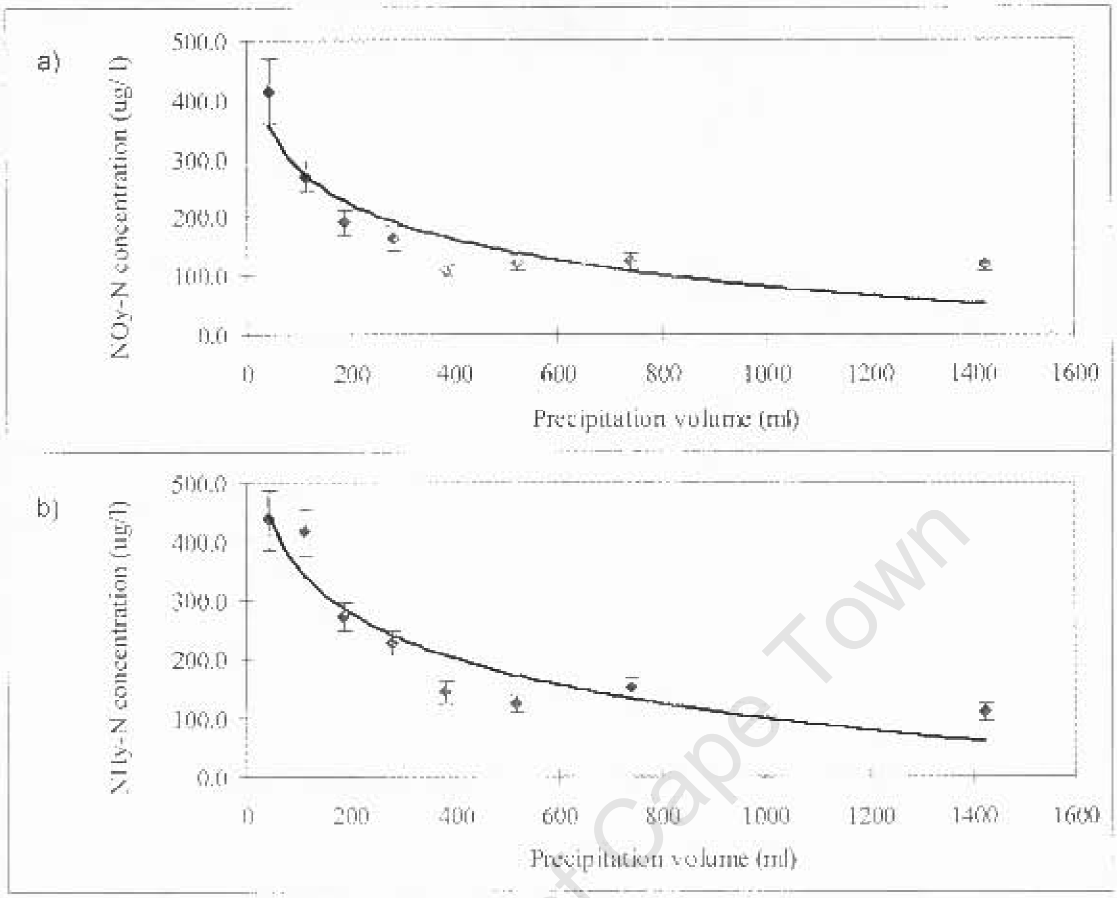

Figure 3.8: *Concentration of a) NO_y-N and b) NH_4-N (µg/l) in rainwater samples, plotted against sample volume (ml). Precipitation values were grouped into classes and the mean concentration ± SE of each class calculated. Volume figures are from bulk samplers and represent the median of each class. The logarithmic curves fitted to the data are $y = -10.69Ln(x) + 864.65$ ($r^2 = 0.87$) for NO_y-N and $y = -87.186Ln(x) + 684.18$ ($r^2 = 0.82$) for NH_4-N.*

Table 3.3 shows volume-weighted mean (VWM) concentrations of NO_y, NH_4 and SO_4 at the study sites. Concentrations are weighted with either bulk collector volumes or rain gauge volumes. The volume figures used to weight the data have little effect on the comparative magnitude of VWM concentrations at the study sites. VWM NO_y concentrations are, in both cases, highest at Table Mountain and Tygerberg, intermediate at Kenilworth and lowest at Devils Peak and Silvermine. NH_4 is, in both cases, highest at Tygerberg followed closely by Kenilworth. Intermediate concentrations occur at Devils Peak, and low concentrations at Silvermine and Table Mountain. Like NH_4, SO_4 is highest at Tygerberg and Kenilworth and considerably lower at the other three sites.

Table 3.3: *Volume-weighted mean (VWM) concentrations of ions for the five study sites (μg/l) for the entire period over which the respective ions were measured (13 months for NO_y and NH_4, 8 months for SO_4). Data is weighted with either bulk volumes or rain gauge volumes.*

	NO_y-N		**NH_4-N**		**SO_4-S**	
	bulk	rain-gauge	bulk	rain-gauge	bulk	rain-gauge
Devils Peak	127.6	126.9	178.1	165.1	880.1	871.5
Kenilworth	153.5	156.5	217.7	217.0	1208.9	1189.8
Silvermine	101.6	96.7	122.0	121.5	864.5	827.1
Table Mt	184.1	178.1	118.6	118.0	788.3	772.5
Tygerberg	168.7	175.5	229.5	232.4	1262.8	1262.8

3.3.4 Bulk deposition levels

Table 3.4 shows the total deposition loads of NO_y, NH_4 and inorganic N measured during the one year period from December 2001 to December 2002. Figures were obtained by multiplying measured concentrations of N by either a) bulk collected or b) rain gauge collected precipitation volumes. Although the use of standard rain gauge volumes increased total N levels somewhat, levels remained low at all sites. For both methods of volume estimation, Kenilworth R.C. received the highest total N input, followed by Table Mountain and Devils Peak. However, because the bulk samplers at Table Mountain and Devils Peak caught a smaller percentage of total rainfall than those at Kenilworth, total N values at these sites were more similar to Kenilworth when rain gauge volumes were used in calculations, with total N values for all three sites in excess of 4 kg $N.ha^{-1}.a^{-1}$. Tygerberg and Silvermine received lower total N inputs than the other sites (2-3 kg $N.ha^{-1}.a^{-1}$), and because bulk samplers trapped a similar volume of rain per unit area to rain gauges at these sites, values changed little when rain gauge volumes were substituted for bulk collector volumes.

There was substantial variation in the ratio between reduced and oxidized N in bulk deposition at the different sites. At all sites except for Table Mountain, NH_4 was the predominant form of N. At the central sites (Kenilworth, Devils Peak and Tygerberg), NH_4 levels exceeded NO_y levels by a factor of between 1.3 and 1.4, while this value was closer to 1.2 at the relatively isolated Silvermine site. The reverse situation occurred at the Table Mountain site, where NO_y was between 1.5 and 1.6 times higher than NH_4.

Table 3.4: *Annual (December 2001- December 2002) deposition levels of NO_y-N, NH_4-N and total inorganic N ($kg.ha^{-1}.a^{-1}$) calculated using either bulk sampler volumes or standard rain gauge volumes.*

	Bulk sampler			**Standard rain gauge**			Ratio
Study site	NO_y-N	NH_4-N	Tot. Inorganic N	NO_y-N	NH_4-N	Tot. Inorganic N	NH_4-N: NO_y-N
Devils Peak	1.11	1.53	2.65	1.82	2.38	4.21	1.38 - 1.31
Kenilworth RC	1.58	2.20	3.79	2.04	2.76	4.80	1.39 - 1.35
Silvermine NR	0.98	1.17	2.15	0.96	1.20	2.16	1.19 - 1.25
Table Mountain	1.64	1.04	2.68	2.75	1.81	4.56	0.63 - 0.66
Tygerberg NR	1.04	1.44	2.48	1.12	1.54	2.66	1.38 - 1.37

Table 3.5: *Deposition levels of Na and SO_4-S ($kg.ha^{-1}.a^{-1}$) for the 8 month measurement period (November 2001 - July 2002) calculated using either bulk sampler volumes or standard rain gauge volumes. Also shown are percentages of marine derived SO_4-S and predicted annual (December 2001 - December 2002) deposition levels for SO_4-S.*

	Bulk sampler				**Standard rain gauge**		
Study site	Na	SO_4-S	% marine derived SO_4-S *	Predicted annual SO_4-S †	Na	SO_4-S	Predicted annual SO_4-S †
Devils Peak	27.98	4.98	23.2	7.99	44.09	7.69	12.85
Kenilworth RC	52.64	7.61	27.9	12.88	65.67	9.72	16.21
Silvermine NR	46.92	5.39	35.2	8.64	44.87	5.15	8.05
Table Mountain	46.10	5.14	36.2	7.35	70.66	8.11	12.23
Tygerberg NR	30.50	4.58	26.9	8.05	30.00	4.53	8.38

* see text for calculation methods; † Calculated by applying the ratio between total rainfall and total SO_4-S for the eight month measurement period to the total annual rainfall for 2002.

Table 3.5 shows the total input of SO_4 and Na in deposition for the eight months over which these ions were measured. Also shown are predicted annual SO_4 deposition levels, calculated by applying the averaged ratio between SO_4 deposition and rainfall for the 8 month measuring period, to the rainfall figure for the remaining months of the year. The predicted annual deposition rates displayed are thus speculative but are useful in that they make possible a rough comparison of SO_4 deposition levels in Cape Town with cities in other parts of the world.

When bulk sampler volumes are used, Kenilworth R.C stands out, receiving about 1.5 times the SO_4 received by the other four sites, which are all roughly similar to one another. As was the case for N, the substitution of rain gauge volumes elevates the Table Mountain and Devils Peak totals to the largest extent, bringing them closer to the Kenilworth total, while the Silvermine and Tygerberg totals change little. Kenilworth, Table Mountain and Silvermine receive relatively substantial levels of marine particle deposition, as indicated by the higher Na inputs at these sights. Marine derived SO_4 accounts for a substantial proportion of total SO_4 at all the study sites, but is particularly high at Table Mountain and Silvermine, where it makes up around 35% of total S deposition

Monthly deposition levels of NO_y, NH_4, SO_4 and Na (standard rain gauge), as well as the corresponding rainfall volumes (standard rain gauge) are illustrated for the five study sites in Figures 3.9 to 3.13. Despite the fact that average N concentrations tend to be lower during periods of high rainfall (Figure 3.8), it is clear from Figures 3.9-3.13 that periods of higher rainfall are generally associated with greater total deposition levels of the measured ions. The relationship between deposition levels and rainfall volume is most obvious for Na, with Na levels closely mirroring the rainfall pattern at most sites. This situation is common in oceanic climates (e.g. Gore 1958) and probably results, at least in part, because sea salt particles in the atmosphere play a large role in seeding rain clouds (Rosenfeld *et al.* 2002). SO_4, for the most part, also roughly tracks rainfall, although there are instances where no clear relationship is visible (e.g. Table Mountain). While N deposition sometimes closely follows the rainfall pattern, this relationship is not as consistent as for the other ions, particularly in the case of NH_4.

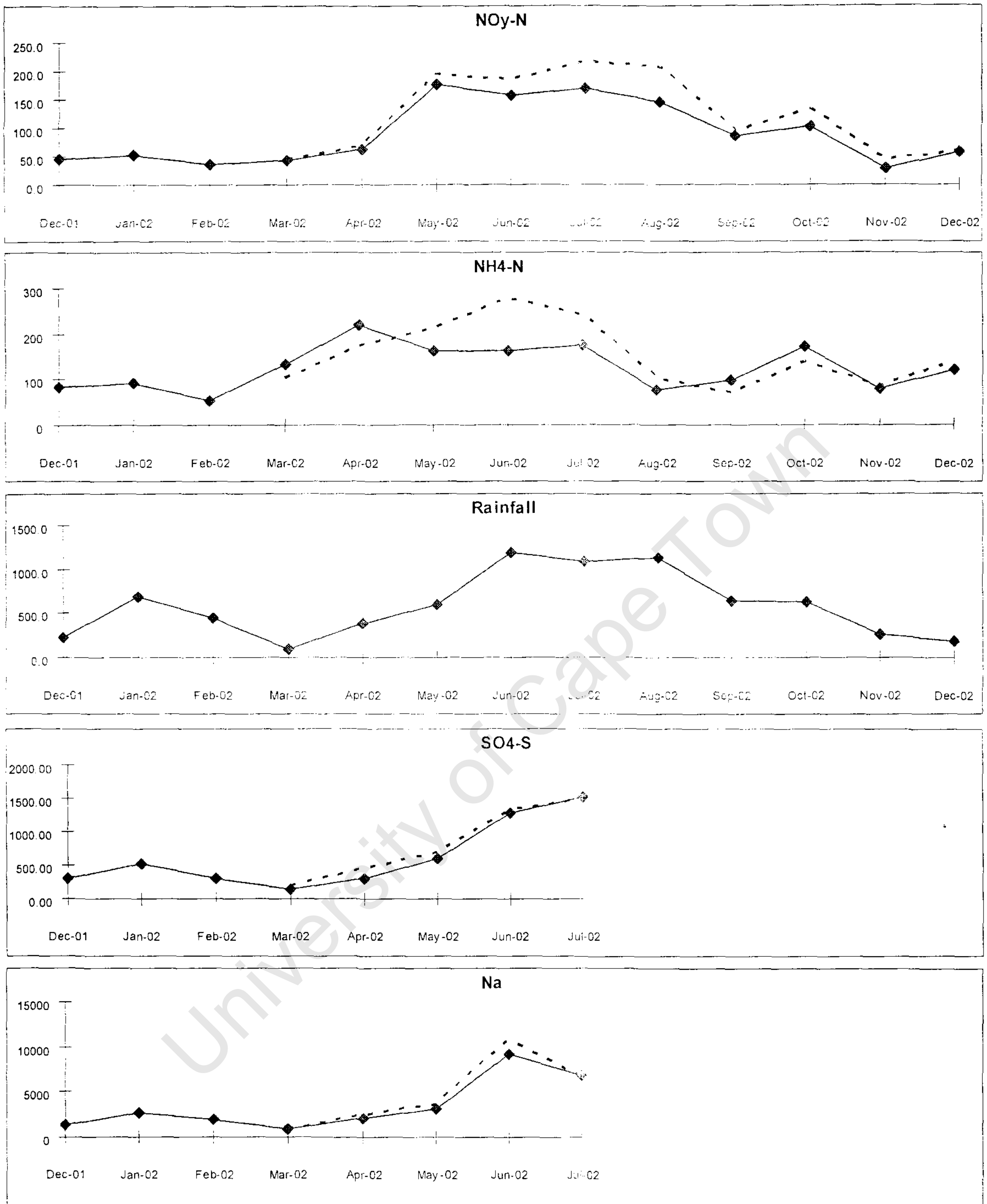

Figure 3.9: *Devils Peak monthly deposition levels (g.ha^{-1}) for NO_y-N and NH_4-N (December 2001- December 2002) and for SO_4-S and Na (December 2001- July 2002) and the corresponding monthly bulk rainfall volumes (ml). Deposition figures are calculated using bulk sampler volumes. Solid lines and markers represent monthly/fortnightly collections and dashed lines represent weekly collections.*

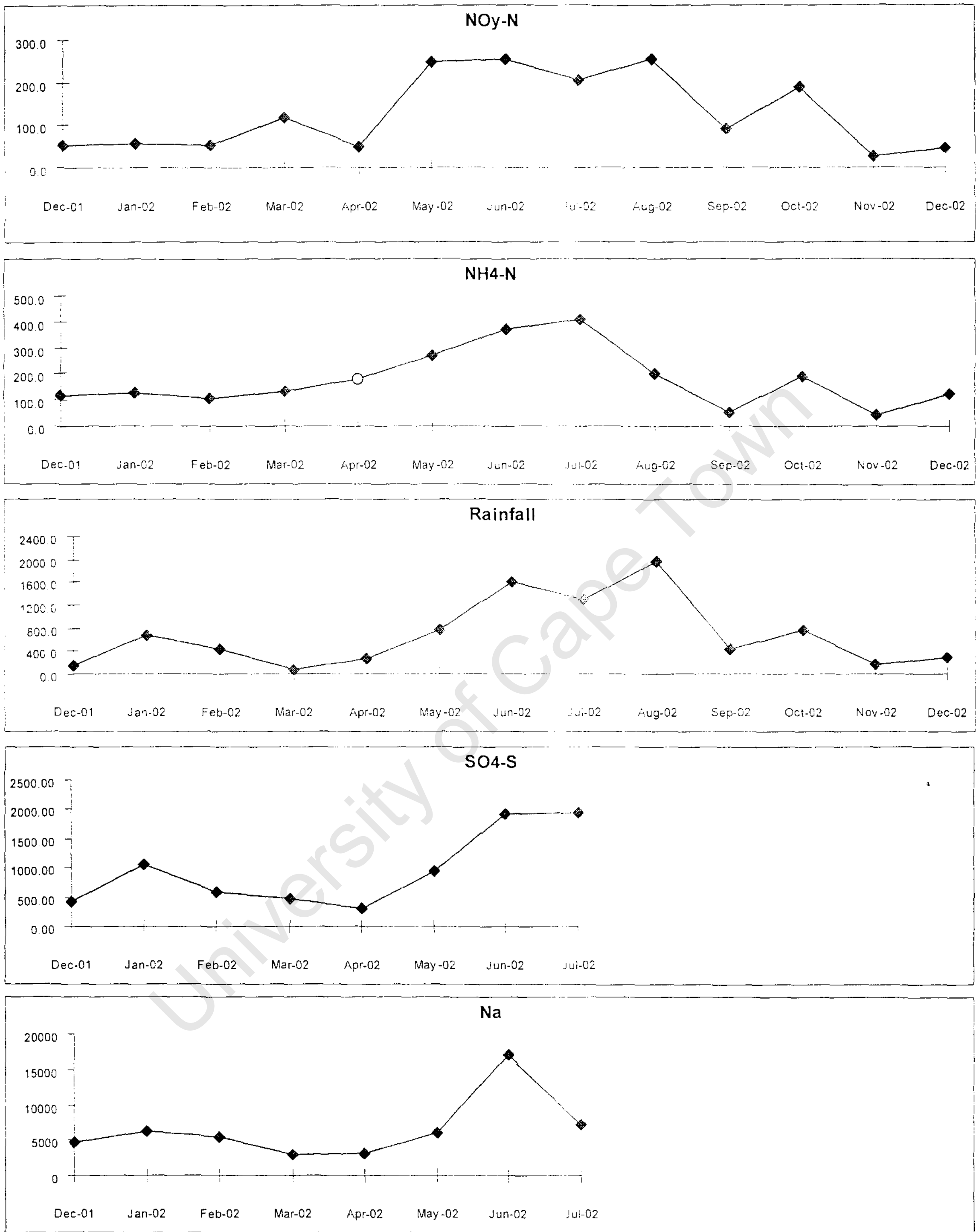

Figure 3.10: *Kenilworth monthly deposition levels (g.ha^{-1}) for NO_y-N and NH_4-N (December 2001- December 2002) and for SO_4-S and Na (December 2001- July 2002) and the corresponding monthly bulk rainfall volumes (ml). Deposition figures are calculated using bulk sampler volumes. Open circles represent monthly deposition values which have been estimated due to contamination or sample loss.*

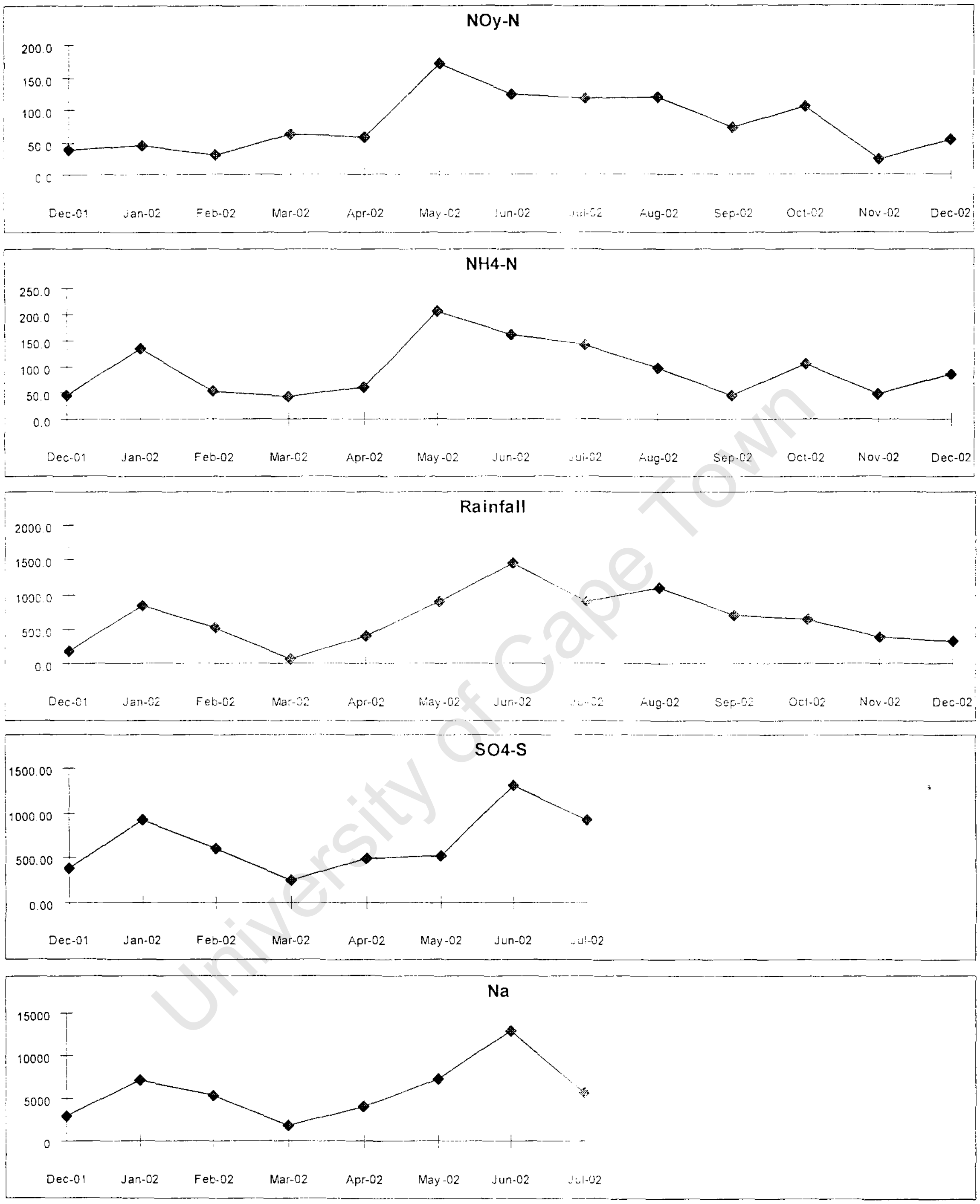

Figure 3.11: *Silvermine monthly deposition levels (g.ha^{-1}) for NO_y-N and NH_4-N (December 2001- December 2002) and for SO_4-S and Na (December 2001- July 2002) and the corresponding monthly bulk rainfall volumes (ml). Deposition figures are calculated using bulk sampler volumes.*

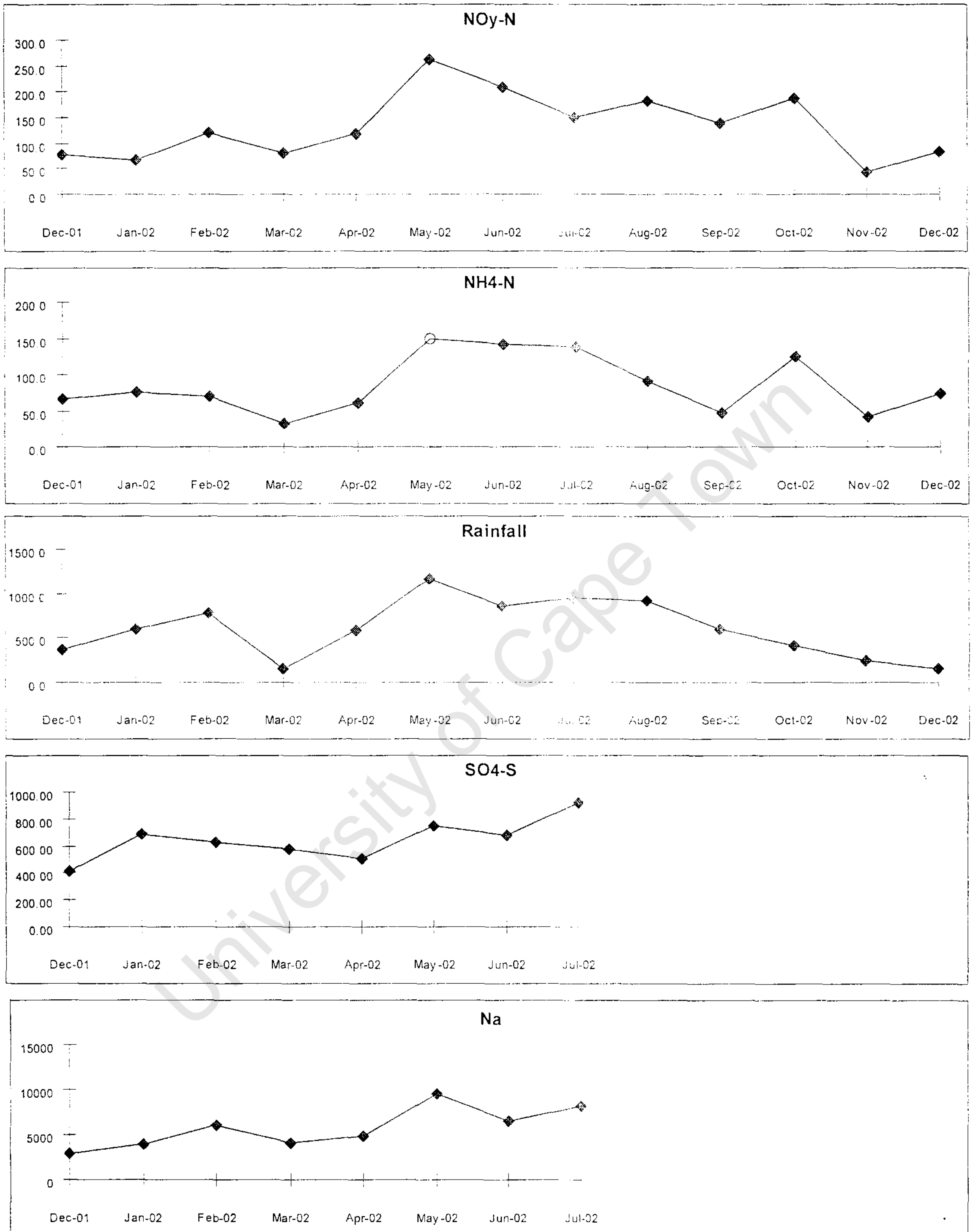

Figure 3.12: *Table Mountain monthly deposition levels (g.ha^{-1}) for NO_y-N and NH_4-N (December 2001- December 2002) and for SO_4-S and Na (December 2001-July 2002) and the corresponding monthly bulk rainfall volumes (ml). Deposition figures are calculated using bulk sampler volumes. Open circles represent monthly deposition values which have been estimated due to contamination or sample loss.*

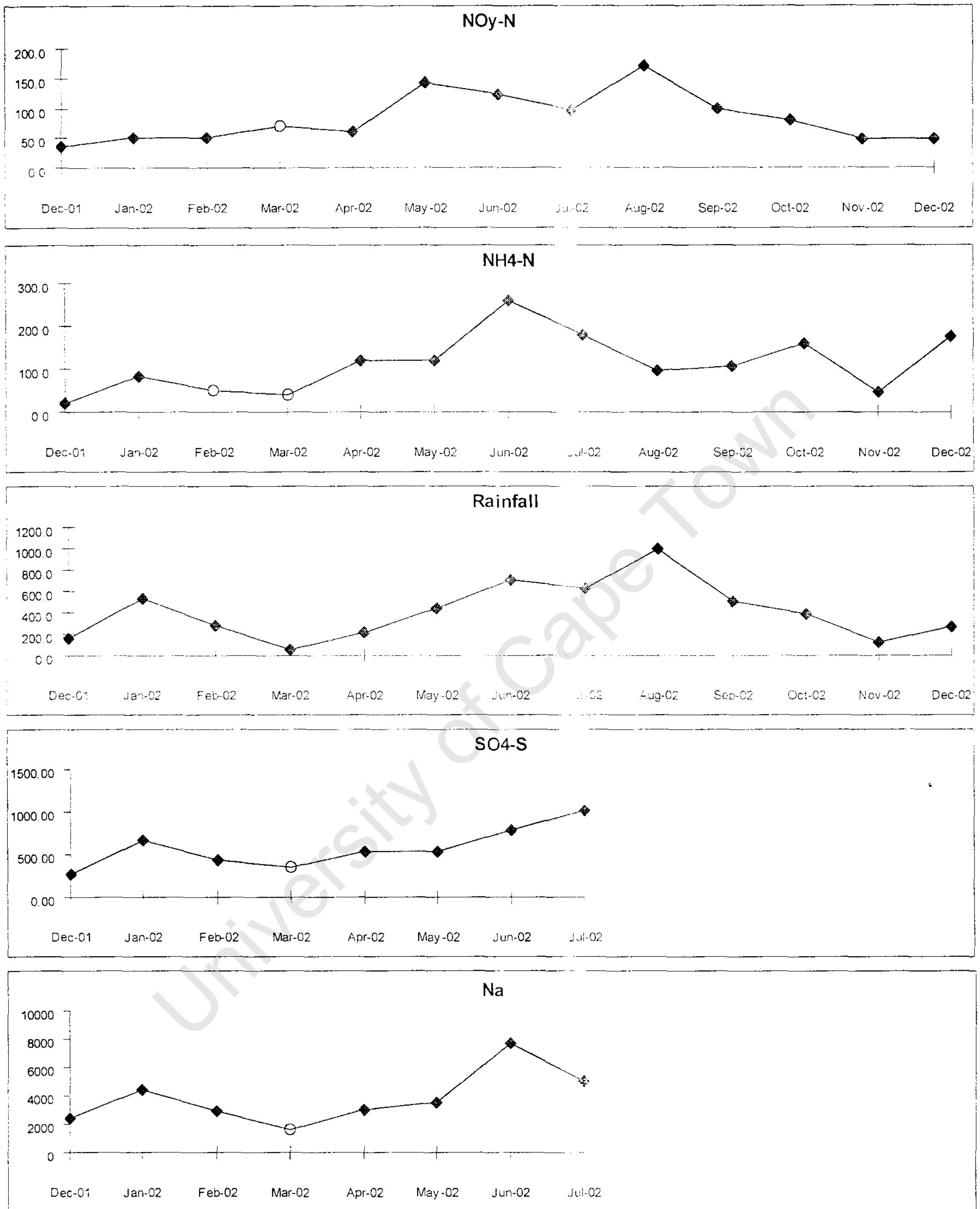

Figure 3.13: *Tygerberg NR. monthly deposition levels (g.ha^{-1}) for NO_y-N and NH_4-N (December 2001- December 2002) and for SO_4-S and Na (December 2001- July 2002) and the corresponding monthly bulk rainfall volumes (ml). Deposition figures are calculated using bulk sampler volumes. Open circles represent monthly deposition values that have been estimated due to contamination or sample loss.*

At all sites, the majority of N deposition occurred in the wet season (second and third quarters; April to September). The percentage of annual rainfall occurring in this period ranged between 64.7% (Table Mountain) and 73.7 % (Kenilworth), with an average value of 69.2 %. For NO_y, the fraction of total input occurring during this period was between 64.6% (Table Mountain) and 71.3% (Devils Peak), with an average value of 67.9%, while for NH_y this figure was slightly lower ranging from 58.1 % at Devils Peak to 67.3 % for Kenilworth, with an average of 61.4 %.

3.3.5 Atmospheric pollution levels

Mean annual atmospheric NO_x and SO_2 concentrations at two of Cape Towns' most polluted air quality monitoring sites, City Centre and Goodwood are shown in Figure 3.14. NO_x levels at the city site are substantially higher than those at the Goodwood site, while SO_2 levels are roughly similar at the two sites. It is evident that both NO_x and SO_2 have shown a decreasing trend in recent years, although the trend for NO_x is less clear, particularly in the city centre. Levels of both pollutants for 2002 are low in comparison to previous years.

Mean monthly atmospheric NO_x and SO_2 concentrations for the study period are shown in Figure 3.15. At both sites, NO_x concentrations peak in late autumn/ winter, coinciding with the period during which average wind speeds are generally lowest (Figure 3.6b). Averaged NO_x concentrations for the second and third quarters combined are 45 % and 200 % higher than those for the first and fourth quarters at the City Centre and Goodwood respectively.

The pattern for SO_2 levels is less uniform. In the city centre, peak SO_2 levels occur between March and May, while at Goodwood, the peak period stretches from April to September, dropping dramatically in October. As is the case for NO_x however, averaged concentrations for the second and third quarters combined exceed those for the first and fourth quarters (by 34% and 68% at the City centre and Goodwood sites, respectively).

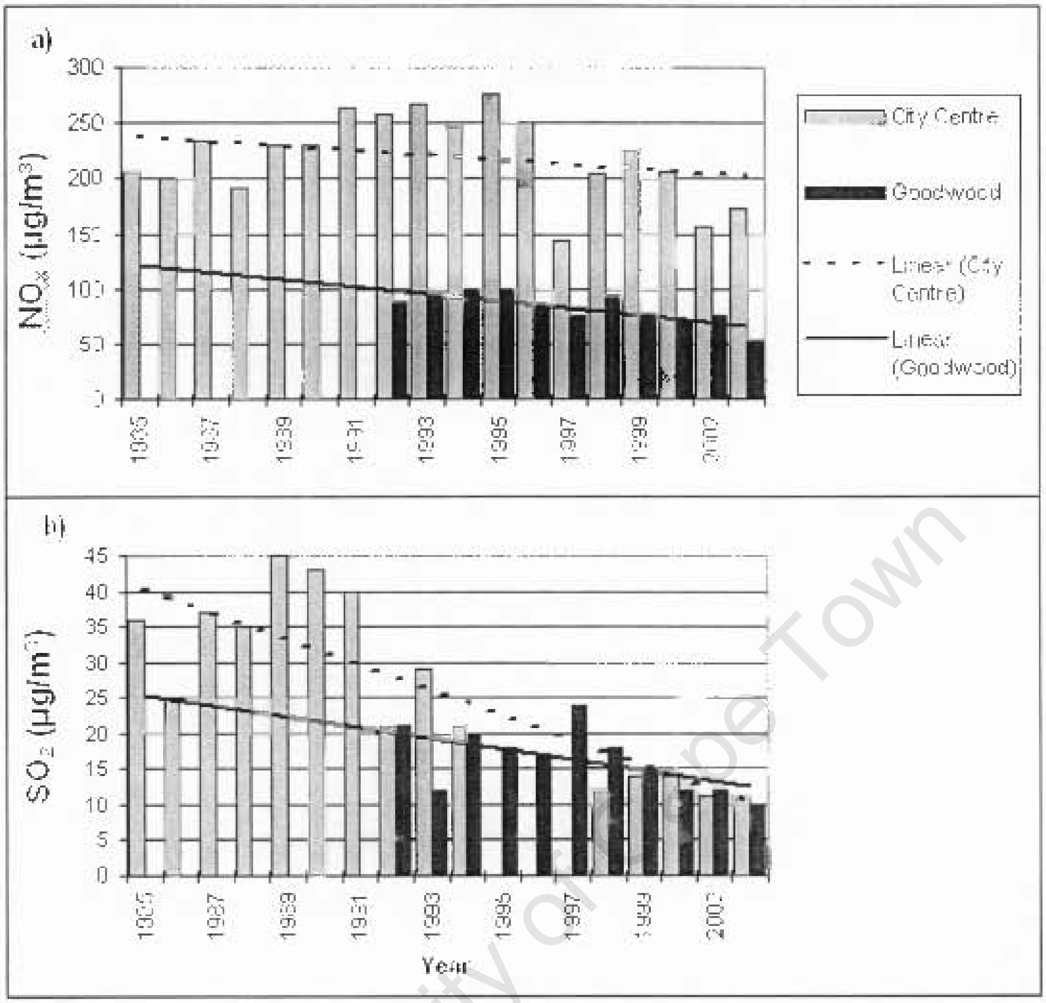

Figure 3.14: *Mean annual atmospheric concentrations ($\mu g/m^3$) of a) NO_x and b) SO_2 at the City Centre and Goodwood air pollution monitoring sites (see Figure 3.2). Data were provided by Cape Town Scientific Services Department (Air Quality Monitoring Section).*

3.3.6 Sample collection frequency

The length of the interval between sample collections proved to have a significant effect on measured deposition levels. Table 3.6 is a comparison between samples collected on a weekly basis and those collected on a monthly/fortnightly basis at the Devils Peak site. It shows that, for all the measured ions, total deposition levels were higher in weekly collected samples. The totals shown are calculated using the original bulk sampler volumes so as to be directly comparable.

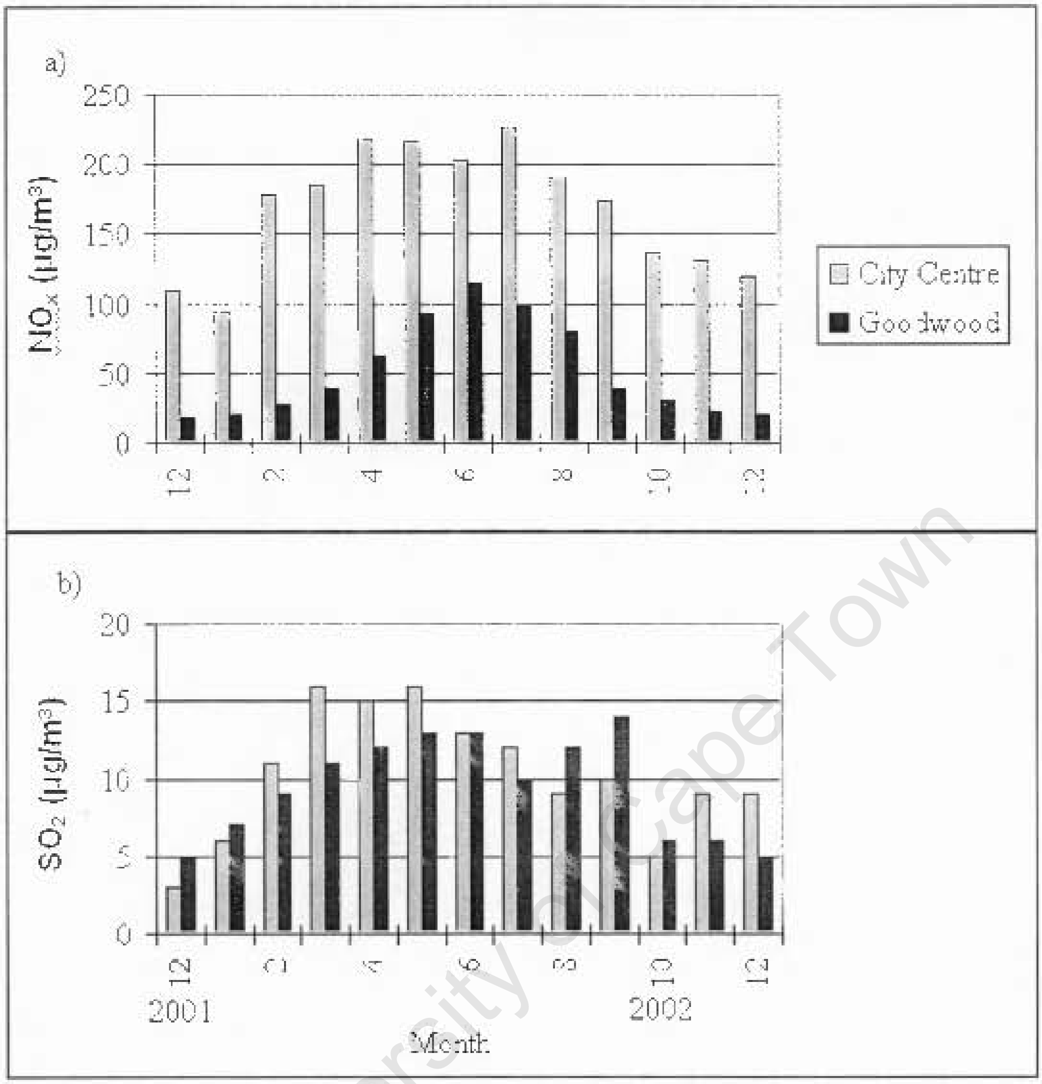

Figure 3.15: *Mean monthly atmospheric concentrations (µg/m³) of a) NO_x and b) SO_2 at the City Centre and Goodwood air pollution monitoring sites (see Figure 3.2) for the study period (December 2001 - December 2002). Data were provided by Cape Town Scientific Services Department (Air Quality Monitoring Section).*

Table 3.6*: The influence of sample collection frequency on total deposition levels (kg.ha⁻¹) at Devils Peak.*

Collection interval	NO_x-N †	NH_4-N *	Na †	SO_4-S †
weekly	1.256	1.535	24.807	4.20
monthly / fortnightly	1.027	1.388	22.053	3.85
ratio	**1.223**	**1.106**	**1.125**	**1.091**

Values are calculated using bulk sampler volumes, * = collected for 11 months from February - December 2002, † = collected for 6 months from February - July 2002.

For N species, the influence of collection frequency was more pronounced for NO_y than for NH_4, with weekly collected samples containing more than 1.2 times the NO_y of monthly/ fortnightly samples. As Figure 3.9 shows, monthly NO_y deposition was consistently higher in weekly samples. For NH_4 however, the pattern was inconsistent, with weekly collected samples sometimes adding up to lower monthly totals (e.g. April, October) and at other times higher totals (June and July in particular) than monthly/fortnightly collected samples.

While the overall effect of collection frequency on annual input was smallest for SO_4 (Table 3.6), monthly deposition levels obtained from weekly collected samples were, with the exception of July, consistently higher than those from monthly collected samples (Figure 3.9). This pattern was similarly consistent for the Na ion.

3.3.7 Tests for microbial breakdown of samples

The results of the first test (Test 1) which examined microbial N consumption in the field are shown in Table 3.7. NO_y concentration initially (after 1 week) showed a relatively small decrease (10 - 20 μg/l) and then remained at roughly the same level for the following 3 weeks, irrespective of the preservative used. NH_4, on the other hand, behaved more erratically, seeming to decrease after one week and then increase over the remaining three weeks. However, the large standard deviations for NH_4 after 2 and 4 weeks show that the manner in which concentration changed was not uniform amongst the samples (i.e. increased average concentration resulted from large concentrations measured in one or two samples) and that this pattern is unlikely to be statistically significant. Unfortunately, statistical tests for significance could not be applied, due to the small sample size (n = 3). It is notable that even identical standard solutions produced large standard deviations for NH_4 in comparison to those for NO_y. This is indicative of the relatively poor analytical precision generally attained for NH_4 on the flow injection autoanalyser.

The results of the second test (Test 2), which examined changes in sample concentration during storage, are displayed in Table 3.8. NO_y concentrations of a standard solution did not decrease notably after two weeks in the field using either preservative, but samples did show marked decreases in concentration during the eight

Table 3.7*: Results of Test 1 examining changes in N concentrations (ug.l^{-1}) of standards preserved with either $HgCl_2$ or thymol and placed in the field for 1, 2 and 4 weeks.*

Preservative	Period of exposure	NO_y-N	SE	NH_4-N	SE
	standard	490.3	5.8	547.7	19.0
$HgCl_2$	1 week	471.0	1.6	474.5	5.4
	2 weeks	478.1	4.8	501.9	29.6
	4 weeks	477.2	5.4	548.0	46.0
Thymol	1 week	466.7	1.8	473.4	6.1
	2 weeks	472.5	4.4	557.6	36.8
	4 weeks	474.3	3.2	582.1	52.1

n = 3, SE = standard error.

Table 3.8*: Results of Test 2 examining changes in N concentration (ug/l^{-1}) of standards preserved with either $HgCl_2$ or Thymol, placed in the field for 2 weeks and subsequently stored under refrigeration (0 °C) for 8 months.*

	NO_y-N				**NH_4-N**			
	initial	SE	stored	SE	initial	SE	stored	SE
Standard	501.8	2.4	498.7	8.6	539.5	26.9	601.5	39.5
$HgCl_2$ (2weeks)	498.8	3.0	452.9	17.3	730.7	59.2	1053.1	111.6
Thymol (2weeks)	493.7	4.4	428.7	2.5	791.6	46.4	966.0	26.0

n = 2, SE = standard error

month storage period. During this period, the concentration of samples preserved with $HgCl_2$ decreased by an average of 46 μg/l (9%), while those preserved with thymol decreased by 65 μg/l (13 %). NH_4 levels were erratic, as was the case in the first test, but nevertheless showed a substantial increase both in the field and during storage. This increase amounted, on average, to an approximate doubling (100 % increase) in NH_4 concentrations in field exposed samples, with even standard solution concentration increasing during storage by an average of 11.5 %. As in Test 1, analysis of identical standard solutions (which had not been subjected to long term storage) produced a large standard error value for NH_4.

3.4 Discussion

3.4.1 Data Quality

Before the results obtained in this study can be discussed, it must be acknowledged that there is a large degree of uncertainty attached to the deposition levels calculated, particularly those for N:

1) The first area of uncertainty relates to the rainfall volumes used in calculations. The large variation in the percentage catch by bulk samplers (relative to rain gauges) amongst the sites may be due to a number of factors. Figure 3.7 seems to suggest that the lower percentage catch by bulk samplers at Devils Peak, TM and Kenilworth was, at least in part, related to the higher rainfall received at these sites. Lower catch may have occurred during heavy rainfall due to rain drop splash off the wire cones placed over the funnels to prevent the entry of coarse organic matter. This design is thus not recommended for future experiments.

Wind may also have played a role in reducing catch. All the collection sites, but Table Mountain in particular, were subject to high wind speeds which may have resulted in turbulent airflow around the mouths of funnels which reduced catch. This turbulent airflow may be more pronounced in bulk deposition samplers because they have narrower mouths than rain gauges and vertical funnel walls as opposed to sloping funnel walls in rain gauges. High winds also sometimes caused the apparatus to lean slightly, thus reducing the surface area exposed to precipitation. Furthermore, aerodynamic blockage may have reduced catch at the Devils Peak and Table Mountain sites (which had the lowest percentage catch at around 60%). According to Bigelow (1984), collectors should not be placed within 5 metres of an object over 1 metre high with enough mass to deflect wind. This principle was violated at the Devils Peak and Table Mountain sites because these areas are regularly accessed by members of the public and thus the samplers were concealed from view behind rocks and shrubs.

Given the above, it seems justifiable to substitute nearby standard rain gauge volumes for the bulk volumes collected and the total deposition figures discussed henceforth are calculated in this way. It should, however, be noted that a variation in catch could have occurred due to local scale differences in rainfall between bulk collector and standard rain gauge sites. This is particularly true for the Devils Peak site, where the rain gauge readings obtained from Kirstenbosch around 4 km away were probably overestimates

2) A second potential source of error relates to analytical difficulties experienced with NH_4, and possible contamination of samples. Over the course of the study, NH_4 levels for a given sample collection were found to be considerably more variable than NO_y levels. Even when anomalous values were excluded, NH_4 levels still tended to be somewhat erratic. This was, at least in part, the result of contamination of samples by organic matter, particularly bird droppings. It is notable, however, that in both tests, analysis of identical standard solutions (which had not been subjected to long term storage) produced relatively large standard deviations for NH_4. Poor analytical precision was thus also partly to blame for more variable NH_4 readings. Technical staff operating the flow injection autoanalyser at the Oceanography department have experienced some difficulties with NH_4 analyses (C. Attwood pers. comm.) Analytical precision for NH_4 by colorimetric methods is often not as high as for other ions (Aminot & Kerouel 1995, Aminot *et al.* 1997). Aminot *et al.* (1997) sent identical NH_4 standards for testing at 106 laboratories (the vast majority of which employed some variation of the indophenol blue method for analysis) and found that average standard deviation from the actual concentration for all laboratories was around 23% at concentrations in the range of 2-5 μmol NH_4-N/l $^{-1}$, and 56% at concentrations below 1 μmol.l^{-1}.

Another possible contributor to variable NH_4 readings is sample contamination by ambient NH_3. In Test 1, during which ionic concentrations of standards were compared after placement in the field for varying lengths of time, NH_4 levels were variable but did not show any pronounced unidirectional change. However, in the second test, contamination of samples by NH_3 was clearly a problem, both in the field and during storage. Previous studies have shown that ambient NH_3, which is extremely soluble, is able to penetrate the walls of collecting and storage bottles and contaminate samples (Muller *et al.* 1982, Krupa 2002). Kattner (1999) found that ammonium levels increased in seawater samples stored for long periods (although not to the extent observed in the second test of the current study) and cited contamination from ambient air and degradation of dissolved organic N as possible explanations. Aminot & Kerouel (1995) placed samples in sealed glass bottles in close proximity to an ammonia solution and found that sample ammonium concentrations increased by around 18 $\mu g.l^{-1}$ per day. The acidification of collecting bottles in the current study (recommended by Allen *et al.* (1989) as a means of reducing loss of NH_4 through volatilization) may have increased the susceptibility of samples to contamination, because NH_3 is particularly soluble in acid solution. Because of erratic NH_4 readings, and because it can not be established whether the contamination observed in Test 2 was an isolated occurrence or whether contamination was a more widespread problem throughout the study, NH_4 values reported in this study should be treated with caution.

3) Finally, there is the problem of loss of N in the field and during storage, due to factors such as microbial breakdown and adsorption to container walls. The higher annual deposition totals of both N ions in weekly collected samples suggest that microbial consumption of N in the field was a factor. The fact that weekly samples gave consistently higher monthly NO_y totals (Figure 3.9) supports this assumption for NO_3, while NH_4 levels did not show such a regular pattern, and it is likely that contamination was a confounding factor. However, microbial breakdown is not the only factor which could explain some of the observed discrepancy. It is also possible that weekly samples contained more dry deposition than monthly/fortnightly samples, due to more frequent rinsing of the funnels with distilled water when no rainfall had occurred. According to Cape and Leith (2002), dry deposition to bulk funnels depends on how long and how often funnel surfaces are wetted. For weekly samples, rinsing was performed on 13 occasions, as compared to only 2 occasions for monthly/ fortnightly samples from Devils Peak. It also seems likely that rinsing may be more efficient than rainfall at washing particles adhering to the funnel, into the collecting bottle. The rinsing argument is supported by the finding that concentrations of SO_4 and Na, which are not very susceptible to microbial breakdown, were also higher in weekly collected samples. However, other authors have reported similar results where rinsing did not take place. Krupa & Nosal (1999) compared volume weighted concentrations of eight ions in unrefrigerated weekly samples and refrigerated event samples (i.e. samples refrigerated immediately following each rainfall event) and found that concentrations were higher in event samples for all ions, including SO_4 (28

% higher) and Na (42 % higher). These authors could not offer specific reasons for their findings. Even thorough rinsing of collecting funnels with water usually does not remove all nutrients adhering to funnel and it is sometimes recommended that chloroform be used for this purpose (Allen *et al.* 1989). Generally, however, the amount of N which adheres to the funnels and cannot be removed by rinsing is small (around 1 % of total N input) (Gore 1968, Allen *et al.* 1968).

The two tests, both of which were conducted in summer when microbial activity would be most pronounced, do not give good evidence of biological consumption of NO_y or NH_4 in the field. In Test 1 (Table 3.7), NO_y concentration in the standards seemed to decrease slightly (by 2 - 3 %) after one week in the field. It is unclear why this decrease occurred, but it seems unlikely to have been the result of microbial breakdown, as concentrations then remained stable for the following 3 weeks. Although the apparatus used in the tests was designed to prevent the entry of rainwater, it is perhaps possible that some water may have entered the bottle during the first week of Test 1, as rain did occur in that period. NO_y concentrations in Test 2 (Table 3.8) did not decrease significantly after two weeks in the field.

In the case of NH_4, contamination obscured the results of Test 2 but concentrations showed no clear sign of decrease after 4 weeks in Test 1. Like NO_y, NH_4 concentrations decreased initially suggesting there may have been leakage of rainwater into the container. The subsequent increase in average NH_4 concentrations may have been due to contamination, poor analytical precision or a combination of the two. It remains a possibility that breakdown of NH_4 did occur, but was obscured by concurrent contamination of the samples. It should be noted that the tests are likely to underestimate levels of microbial breakdown which would occur in rain samples as the solution exposed in the tests was sterile (i.e. it did not contain the biological organisms which would occur naturally in rainwater, and thus would require a longer period in order for these organisms to colonize the sample and multiply).

While the tests conducted showed little breakdown of N in the field, Test 2 (Table 3.8) clearly shows that diminution of NO_y concentrations (presumably due to biological consumption) occurred during 8 month storage in both $HgCl_2$ and thymol preserved samples. Kirkwood (1992) stated that 1 $\mu g.ml^{-1}$ of $HgCl_2$ should be sufficient to prevent biological activity in samples with low organic matter content. The 2 mg of $HgCl_2$ added to each collecting bottle in the current experiment should thus have been adequate to preserve volumes up to at least 2l and would have produced an $HgCl_2$ concentration of 4 $\mu g.ml^{-1}$ in the test samples. However other authors have suggested that higher $HgCl_2$ concentrations should be used. For example, Kremling & Wenk (1987 cited in Kattner 1999) found significant breakdown of nitrate in stored seawater samples at $HgCl_2$ concentrations of 10 $\mu g.ml^{-1}$ and Kattner (1999) erred on the side of caution, suggesting the use of $HgCl_2$ concentrations of around 100 $\mu g.ml^{-1}$. Thymol concentrations used in the current study (in the range of 0.5 - 1g per collecting bottle) were higher than the 0.1 g per 500 ml of sample, suggested by Gillett & Ayers (1991). However, the study by Gillett & Ayers only ran for a period of 3 months, and thus did not rule out the possibility that breakdown could occur during longer periods of storage. Further study is recommended in order to determine the viability of thymol as a preservative for long-term storage. Because NH_4 levels in Test 2 were altered by contamination, it is unknown whether NH_4 breakdown was significant during storage of rainwater samples. The observed decrease in NO_y levels would, however, suggest that NH_4 breakdown is also likely to have occurred, as the ammonium ion is often (but not always; see for example Krupa & Nosal 1999) found to be even more susceptible to microbial consumption than the nitrate ion (e.g. Muller *et al.* 1982).

The finding that both SO_4 and Na were lower in monthly collected samples was somewhat unexpected. As discussed earlier, the most likely explanation is that weekly samples contained more dry deposited particles, due to more frequent and efficient rinsing of collecting funnels. Microbial consumption of SO_4 is another possibility although it is not usually as pronounced as for N species (e.g. Muller *et al.* 1982, Krupa & Nosal 1999). For Na, microbial breakdown is not a factor but, as discussed earlier, the glass fibre filters used in this study were found to release small but variable amounts of Na despite pre-washing. This was, however, roughly accounted for by subtracting the average difference between Na levels in filtered and unfiltered mili-Q water from sample concentration. A potential source which was not tested for was the preservative ($HgCl_2$, which was the preservative for all samples analysed on the ion chromatograph), but it seems unlikely that this would contain significant levels of Na.

3.4.2 Revised estimates of bulk deposition

The results of the comparison between weekly and monthly/fortnightly samples collected at Devils Peak, as well as those from the storage test, suggest that the total annual deposition values for N given in Table 3.4 are too low. It is uncertain whether the higher totals in weekly samples in the Devils Peak comparison experiment were the result of microbial breakdown, rinsing or a combination of the two. Regardless of the reason, the ratios calculated in Table 3.6 can be used to roughly correct the monthly/fortnightly data by applying them to the annual deposition rates for NO_y and NH_4 measured at the other four study sites. The resulting total deposition values are shown in Table 3.9.

Table 3.9: *Adjusted annual (December 2001 - December 2002) deposition levels of NO_y-N, NH_4-N and total inorganic N ($kg.ha^{-1}.a^{-1}$)**.

Study site	NO_y-N	NH_4-N	Tot. Inorganic N	Ratio NH_4-N:NO_y-N
Kenilworth RC	2.50	3.05	5.55	1.22
Silvermine NR	1.17	1.33	2.50	1.13
Table Mountain	3.36	2.00	5.36	0.60
Tygerberg NR	1.37	1.70	3.07	1.24
Devils Peak	2.24	2.63	4.87	1.17

* Adjusted figures are calculated by multiplying original figures calculated using standard rain gauge volumes (shown in Table 3.4) by the ratios between weekly and monthly/fortnightly totals calculated for the respective ions (Table 3.6).

This action is less justifiable for NH_4 than it is for NO_y, because the relationship between NH_4 levels for monthly versus weekly/fortnightly samples was inconsistent, with monthly collected samples sometimes yielding higher monthly NH_4 deposition totals (Figure 3.9). Nevertheless, it is tentatively assumed that the ratio is representative of genuinely higher average NH_4 deposition levels in weekly collected samples. The correction factors do not, of course, account for any microbial breakdown that may have occurred during the first week in the field, but this is assumed to be minimal.

Correction factors for diminution of N concentrations during sample storage can not be established with any confidence. For NO_y there is evidence that some breakdown occurred. However, the levels measured in Test 2 cannot be used to correct the data for two main reasons. Firstly, the storage period of 8 months in the test was longer than the storage period for study samples (maximum 4 months), and would thus be likely to overestimate levels of breakdown. Counteracting this is the fact that, as mentioned earlier, the standard solutions placed in the field for tests were sterile. Despite direct exposure to the atmosphere in the field, these solutions would not have accumulated as many microbes as rainwater samples under natural conditions, and this would have contributed to relatively slower microbial breakdown. A better method would have been to use rainwater for the tests, rather than standards.

At best one could speculate conservatively that breakdown during storage may have led to a further diminution of NO_y concentrations of 10 to 20 %, which would put the highest annual NO_y deposition measured in this study (for Table Mountain) at between 3.7 and 4.2 kg $N.ha^{-1}.a^{-1}$ and total deposition levels at both Kenilworth and Table Mountain at between 6 and 7 kg $N.ha^{-1}.a^{-1}$. In the case of NH_4, there is no basis for even a guess at possible levels of breakdown, as it is equally possible that contamination could have slightly increased NH_4 levels during storage. Finally, it should be noted that my experiment would have measured slightly lower than average N deposition levels for the CMA, as rainfall for 2001 was below average (Table 3.2).

3.4.3 Annual deposition levels in comparison to other parts of the world

Table 3.10 shows some examples of annual deposition rates measured in other parts of the world. Unfortunately, the majority of international studies include few or no urban sampling sites it is usually desired to collect samples which reflect regional pollution levels, rather than local sources. Most of the studies listed used wet only samplers which generally measure slightly lower totals than bulk deposition because dry deposition of particles and aerosols is excluded. The percentage contribution of dry deposition to bulk measurements varies according to the aerodynamic characters of the collector and the proportion of gaseous versus particulate dry deposition in an area. Average values for this proportion for Europe (van Leeuwen *et al.* 1996) and the rest of the world (Staelens *et al.* 2005) are between 10 and 20 % for NH_4, NO_y and SO_4, while for Na, values range from about 14 to 32 % depending on proximity to the coast. While these values give us a rough idea of possible the dry component of bulk deposition in the current study, a local comparison between wet only and bulk measurements at a single site would be necessary to make any meaningful corrections to our data.

The NO_y deposition levels measured in this study are substantially higher than the likely background levels measured by Stock & Lewis (1986a) 60 km north of the city at Pella. However it is also evident from Table 3.10 that they are relatively low in comparison to urban/suburban areas in many developed parts of the world, even when the tentative corrections discussed earlier are applied to our data. Some urbanized regions are exceptions to this rule. For instance, the NO_y values given in Table 3.10 for urbanized parts of China are very low. This is because the data are from 1984, when the number of motor vehicles (usually the largest source of NO_x in urban areas) was relatively low in China (Galloway *et al.* 1987). Because my study sites were all in natural areas, the deposition levels measured in our study would not be as high as those occurring in the more central parts of the city, where traffic and industry are concentrated. Furthermore, as Figure 3.14 shows, NO_x levels in Cape Town have shown a decreasing trend in recent years, probably because of engine design improvements and the introduction of unleaded petrol in 1996, allowing the use of catalytic converters on new vehicles. There have also been increased efforts by the Cape Metropolitan Council (CMC) to reduce emissions in recent years, including a diesel and turbo-charged vehicle emissions testing initiative which began in 2000. Despite these facts, the low NO_y deposition levels observed are surprising given Cape Towns large and ageing vehicle fleet (825 000 registered vehicles in 2002; City of Cape Town 2003a) and relatively high NO_x emission rates per vehicle in comparison to Europe and the USA (Wicking-Baird *et al.* 1997).

Dividing the total annual NO_x emissions for the CMA (Wicking-Baird *et al.* 1997) by the total area of the CMA gives a maximum possible deposition rate for NO_y-N of 29.65 kg $N.ha^{-1}.a^{-1}$, while the estimated average annual deposition rate for NO_y-N across the five study sites (from Table 3.9) was 2.13 kg $N.ha^{-1}.a^{-1}$. The deposition/emission ratio for oxidized N is thus about 0.07. It should be noted that, because NO_x levels have shown a decreasing trend since around 1996 (Figure 3.14), this figure is probably a slight underestimate.

There are several possible factors which might contribute to relatively low wet deposition levels of NO_y (and other pollutants) in Cape Town. Firstly, it is likely that a large proportion of deposition in Cape Town is in dry form. The importance of wet versus dry deposition depends, to a large extent, on the frequency of rainfall in an area. In Mediterranean ecosystems in other parts of the world such as California (Bytnerowicz & Fenn 1996, Riggan *et al.* 1985) and southern Spain (Sanz *et al.* 2002, Roda *et al.* 2002) the majority of N deposition (40 – 80%) is in dry form. Although the CMA is not as arid as these regions, it does experience extended dry spells during summer, and it is likely that dry deposition levels during this period are substantial. At least in the drier N and E parts of the CMA, dry deposition may be of a greater magnitude than wet deposition. This is particularly true in areas of natural vegetation because vegetation offers a large surface area for impaction /adsorption of pollutants and because some dry deposition will occur through stomatal gaseous exchange (e.g. Fowler *et al.* 1998a, 1998b). Furthermore, as a general rule, dry deposition is most substantial near emission sources (Hicks *et al.* 2000, Eugster *et al.* 1998) and thus sites in close proximity to the built up central area of the CMA would be likely to receive a large amount of deposition in this form. A second possible reason for the low wet deposition levels measured in this study may be that the Cape Metropolitan area is very windy. A large proportion of air pollution is probably advected out to sea before it can be wet deposited. At the same time, the CMA does not receive significant pollution from outside its borders, as it is relatively isolated from other major centres of pollution emission. In many parts of Europe, Asia and America, transboundary pollution is a major issue. Furiness *et al.* (1998) compared annual NO_x emissions to NO_3 wet deposition on a state by state basis for the USA and calculated a median deposition/emission ratio of 0.21 for the 48 contiguous states. However, because of the prevailing westerly winds in the USA, their results showed that the deposition/emission ratio was lowest for the westerly states, and increased with movement in an easterly direction. In the west coast state of California, for example, the NO_y deposition/ emission ratio was 0.05, smaller even than the 0.07 calculated earlier for the CMA. California can thus be thought of as being broadly analogous to Cape Town, in that it receives little pollution from outside its borders, while a large amount of pollution emitted within its borders is exported to other regions. Like Cape Town, California experiences pronounced summer drought (although more intensely) (Keeley 1992), and most N deposition in that region occurs in dry form, particularly for NO_y (Riggan *et al.* 1985).

Table 3.10: *Examples of wet and bulk deposition levels in different parts of the world (in kg N or S.ha^{-1}.a^{-1})*

Location	Collection method	Area type	Year	NO_x-N	NH_4-N	Tot Inorganic N	SO_4-S	Other	Source
Current study - LOW	bulk	urban/ suburban	01	1.17	1.33	2.50	8.05 (5.23)	SO_4 figures are predicted annual totals from 8 months sampling (see text). Bracketed figures are non-sea salt SO_4	
- HIGH				3.36 (4.03)	3.05	5.55 (6.03)	16.21 (11.77)	Bracketed figures for N represent NO_x +20% to account for breakdown during storage	
Africa									
Pella, SA	bulk	rural	80-82	0.76	0.69	1.45			Stock & Lewis (1986a)
Suikerbosrand, SA	bulk	rural	92-94				7.98		Skoroszewski (1999)
West/Central Africa	wet	rural	98-00	1.57	2.38	3.95	1.31	N from 6 sites in Ivory Coast, CAR, Cameroon, Congo, Mali and Niger, S from 2 sites in Cameroon and Congo	Galy-Lacaux *et al.* (2003), Lacaux & Sigha (2003)
Amersfoort, SA	wet	urban/industr	86-99	5.50	4.00	9.50			Mphepya *et al.* (2002)
Louis Trichaardt, SA	wet	rural	86-99	2.10	1.60	3.70			Mphepya *et al.* (2002)
Asia									
Beijing, China	wet	urban	84	1.27	5.29	6.56	4.72		Galloway *et al.* (1987)
Guiyang, China	wet	urban	84	1.64	9.90	11.54	83.68		Galloway *et al.* (1987)
Rural S China	wet	rural	84	1.65	5.20	6.85	21.01		Galloway *et al.* (1987)
Jakarta, Indonesia	wet	urban/suburb	91-92	2.80			8.96		Ayers *et al.* (1995a)
W Java, Indonesia	wet	rural	91-92	1.68			5.87	average for 3 sites	Ayers *et al.* (1995a)
Japan	wet	whole country	89-93	2.72	3.63	6.35	10.00	average for 29 sites across Japan	Hara *et al.* (1995)
Australia									
Katherine, Australia	wet	remote	80-84	0.59	0.42	1.01	0.65		Likens *et al.* (1987)
NS Wales, Aus	wet	rural	92-94	0.91			1.41	two coal fired power stations within 20km of study site	Ayers *et al.* (1995b)
Latrobe Valley, Aus	wet	rural	90-92	0.53	0.79	1.32	1.60	four coal fired power stations around 10km from study site	Ayers *et al.* (1995c)
Wagga Wagga, Aus	wet	remote	?	0.70	0.38	1.08	1.18		in Ayers *et al.* (1995c)
Jabiru, Aus	wet	remote	?	0.78	1.04	1.82	1.12		in Ayers *et al.* (1995c)
Europe									
Dublin Airport, Ireland	bulk	urban	92-94	12.43	3.20	15.63	12.92 (9.86)	bracketed values for Ireland are non-sea salt SO_4-S	Jordan (1997)
Cork & Shannon Airports, Ireland	bulk	urban	92-94	2.92	3.57	6.49	14.08 (8.32)	average for two sites	Jordan (1997)

Location	Collection method	Area type	Year	NO_x-N	NH_4-N	Tot Inorganic N	SO_4-S	Other	Source
Europe (contd.)									
Rural Ireland	bulk	rural	92-94	1.22	0.71	1.93	6.56 (3.55)	average for two sites; Birr and Kinnity	Jordan (1997)
Sceland, Switzerland*	wet+ aerosols	rural	90-96	2.53 (+0.9)	4.64 (+1.19)	7.17 (2.09)		average 3 sites, bracketed values are dry aerosol deposition	Eugster *et al.* (1998)
Norway	wet	whole country	92-96	2.33	2.17	4.50	3.36	median for 40 sites	Torseth & Semb (1998)
Czech Republic	bulk	whole country	94	4.40	5.68	10.08		average for 31 sites. bulk values corrected for dry deposition.	Zapletal (1998)
Netherlands	wet	rural/agric	?	7.00	14.60	21.60		coniferous forest. NH_4 values reflect NH_3 volatilization from manure	in Riggan et al. (1985)
Croatia	bulk	rural	81-92	15.00	9.67	24.67	38.30	average for 3 sites, rural but influenced by urban pollution	Vidic (1995)
	bulk	suburb	81-92	9.25	12.00	21.25	26.70	average for 4 sites	Vidic (1995)
	bulk	urban/industr.	81-92	12.60	10.00	22.60	25.70	average for 3 sites	Vidic (1995)
UK- W Midlands	bulk	urban	99-01	3.54	7.70	11.24		7km from Birmingham city centre	Marner & Harrison (2004)
	bulk	suburban	99-01	4.05	7.99	12.04		average for 8 sites, outskirts of Birmingham conurbation	Marner & Harrison (2004)
	bulk	rural	99-01	3.36	5.88	9.24		20 km SW of Birmingham	Marner & Harrison (2004)
Palautordera, Spain	bulk	urban/suburb	95-99	3.36	4.63	7.99		Outskirts of Barcelona conurbation	Roda, *et al.* (2002)
La Castanya, Spain	bulk	rural	83-99	2.71	2.96	5.67		mountainous area	Roda, *et al.* (2002)
USA									
New York City, NY	wet	urban	84	4.75	3.28	8.02	15.54		Galloway *et al.* (1987)
Rural E USA	wet	rural	84	4.25	2.46	6.71	9.79		Galloway *et al.* (1987)
San Dimas, CA	wet	urban	81-83	4.30	3.90	8.20		chaparral in LA County, often exposed to pollution episodes	Riggan *et al.* (1985)
Santa Barbara, CA	wet	rural/suburb	79-80	1.53	0.52	2.05		chaparral	in Riggan *et al.* (1985)
Eastern Cascades, WA	bulk	rural	71-75	0.21	0.27	0.48		forest	in Riggan *et al.* (1985)
Berkeley, CA	wet	rural	74-75	0.23	0.80	1.03		eucalypt forest	in Riggan *et al.* (1985)
Cedar River, WA	wet	rural	?	1.60	1.50	3.10		forest	in Riggan *et al.* (1985)
Coweeta, NC	bulk	rural	72-74	3.70	2.70	6.40		deciduous forest	in Riggan *et al.* (1985)
Hubbard Brook, NH	wet	rural	?	4.30	2.20	6.50		coniferous forest	in Riggan *et al.* (1985)
South America									
Torres Del Paine, Chile	wet	remote	84-93	0.05	0.06	0.11	0.14		Galloway *et al.* (1996)
Amazon Basin	wet	rural	95-00	2.03	1.70	3.73	1.26	average for two sites, in French Guiana and N Brazil	Artaxo *et al.* (2003)
SE Brazil	wet	urban/industr	95-00	2.59	2.30	4.89	3.20		Artaxo *et al.* (2003)

Because of the highly urbanized nature of the CMA, it was expected that NO_y levels measured in the current study would be higher than those for NH_4. NH_4 levels, were however higher at all sites except for Table Mountain, although they were still low by world standards, particularly when compared to highly agriculturalized regions such as Europe and Asia. Because NH_3 emission levels/aerial concentrations have not been established for Cape Town, I have no means of corroborating my data. The only local site where NH_3 has been measured is at the Cape Point Global Atmospheric Watch (GAW) station located at the southern tip of the Cape Peninsula. NH_3 readings obtained here by passive sampling were very low by world standards (< 1 ppb; Carmichael *et al.* 2002). This value would not be at all reflective of the central and northern CMA however, as the high deposition velocity of NH_y would mean that most NH_3 emissions from these areas would be deposited within 10 km of the source and would not reach Cape Point. In addition to this, the southerly parts of the Table Mountain chain would act as a partial barrier to pollution, and the prevailing winds (NW and SE) would tend to take pollution in Cape Town away from Cape Point.

It is quite possible that NH_3 emissions in central Cape Town are substantial. Firstly, a fair amount of agricultural activity takes place in and around the CMA, including pig farming (at Philippi and Durbanville), vegetable and flower farming (e.g. Philippi horticultural area), private vegetable gardens and livestock rearing (e.g. in Khayalitsha), as well vineyards and orchards which are concentrated along the Tygerberg Hills in the NE, around the Helderberg mountains to the SE and to the S around Constantia and Hout Bay (Visser 2001). Although livestock farming is generally the largest source of NH_3 emissions, there is a trend towards the utilization of animal derived fertilizers such as urea in the CMA, which means that cultivation has the potential to produce substantial NH_3 emissions (Kathawaroo 2000). In addition, motor vehicles may contribute to NH_y deposition. A recent study by Cape *et al.* (2004) contradicts, to some extent, the conventional view that NO_x deposition exceeds NH_y deposition in close proximity to roads. According to their findings, modern vehicles fitted with three-way catalytic converters produce sizeable NH_3 emissions, and although roadside aerial NH_3 concentrations remain well below those of NO_x, NH_3 currently accounts for roughly 50% of N deposition in close proximity to roads in the UK, by virtue of its much higher deposition velocity. Other possible sources of NH_3 in the CMA include biofuel burning, veld fires and emissions from humans. Conlan *et al.* (1995) monitored deposition in the urban area of Manchester, England and were surprised to find substantial NH_4 levels in the city centre, despite no agricultural activity in the vicinity. They attributed this to emissions from humans, particularly during the warm summers.

In contrast to nitrogen, sulfur deposition levels measured in the current study are relatively high, with the maximum predicted annual value (16.2 kg. SO_4-S $ha^{-1}.a^{-1}$ at Kenilworth RC, Table 3.5) second only, in the Table 3.10, to urban/industrialized parts of China and Croatia. The majority of this S is anthropogenically derived, although the sea salt fraction calculated is relatively high because of the large marine influence in Cape Town. Biogenic S emissions are also somewhat elevated in coastal areas, but are generally small enough to be a relatively insignificant portion of total deposition (Farrell 1995).

According to Wicking Baird *et al.* (1997) the majority of SO_2 in Cape Town is produced by heavy fuel oil combustion, with petrol/diesel and coal combustion also important. While aerial SO_2 concentrations in Cape Town have shown a decreasing trend in recent years (Figure 3.14), this reduction is much smaller than that achieved over the last 25 years in W Europe, N. America and Japan, where emission reduction targets, both at national and international levels, have necessitated a move towards advanced emission control technologies and fuels with lower S content (Zundel *et al.* 1995, Bradley & Jones 2002, Moomaw 2002). For instance, the EU has recently passed binding directives preventing the use of heavy fuel oil (HFO) with S content in excess of 1% (1999) and petrol with S content in excess of 0.005% (2005). In contrast, HFO in Cape Town has an S content of about 3.5%, while unleaded petrol has an S content of around 0.12% (Wicking-Baird *et al.* 1997). As earlier for NO_y, one can calculate a deposition/emission ratio for S in the CMA. The estimated average annual deposition rate for non sea-salt S across the five study sites is 8.15 kg.$ha^{-1}.a^{-1}$, while the maximum possible S deposition rate from Wicking-Baird *et al's* (1997) emission inventory is 49.75 kg.$ha^{-1}.a^{-1}$. This gives a deposition/emission ratio of around 0.16, a value which is more than double the ratio of 0.07 calculated for NO_y. The study by Furiness *et al.* (1998) showed that, compared to N, a greater proportion of emitted S is generally wet deposited. For the 48 states studied, the mean wet deposition/emission ratio for N was 0.27, while the median value was 0.21. In contrast the mean wet deposition/emission ratio for S was 0.58, and the median, 0.34. For means of comparison, the mean deposition/emission ratio for S in California was 0.20, about four times higher than that for NO_3-N in that state, and slightly higher than the 0.16 calculated earlier for the CMA.

3.4.4 Temporal patterns in deposition

As is commonly observed, concentration of ions in rainfall samples was broadly related to volume by an exponential curve (Figure 3.8). The reason is that the ionic concentration of rainwater is highest at the beginning of a rainfall event due to the washout of pollutants which have accumulated in the atmosphere during the dry period. The trends in Figures 3.8a and b are not highly significant and points have high standard errors. This is for two reasons. Firstly, samples were not collected frequently enough to draw graphs for each site individually, and thus Figure 3.8 incorporates data from all five study sites, each of which have different pollution source strengths. Secondly, each collection in the current study comprised a number of rainfall events, thus obscuring the relationship between concentration and volume of individual rainfall events. Nevertheless, Figure 3.8 demonstrates the general pattern of a smaller monthly volume and higher ionic concentrations occurring in months where rain is infrequent or intermittent.

Deposition levels of all the pollutant species measured in the current study showed strongly seasonal patterns, with the majority of deposition occurring during the second and third quarters (April to September) which encompass the winter period for Cape Town. The reasons for this pattern are probably threefold. The first and most important is that rainfall is higher during this period (an average of 69% of annual rainfall occurred in this period in 2002), resulting in higher washout/rainout of atmospheric pollution. If wet only deposition had been collected, seasonality of NO_y and NH_4 would probably have been even more pronounced, as dry aerosol deposition would be greatest in summer and would thus increase bulk readings during this period. In addition to higher rainfall, atmospheric concentrations of pollutants are also higher during the winter period for NO_x and SO_2 (Figure 3.15). This is primarily due to lower average wind speeds and more frequent formation of temperature inversions which prevent upward dispersion during this period. In addition, industrial emissions are somewhat higher during this period because of an increase in coal and fuel burning for the purposes of power generation and in private residences (Wicking-Baird *et al.* 1997), presumably in response to colder temperatures.

Seasonal variations in NH_3 emissions for Cape Town are an unknown, but it is probable that they would peak in summer, when volatilization from animal waste and fertilizers would be highest. This may explain the slightly less pronounced bias towards winter deposition of NH_4 (average of about 61% in second and third quarters) compared to NO_y (68%).

3.4.5 Spatial patterns in deposition

Even though the five study sites were in close proximity to each other, there was a clear variation in deposition levels amongst them. The main reason for this is the uneven distribution of rainfall across the CMA. As Figure 3.3 shows, the highest rainfall levels are recorded on the plateau and eastern slopes of the Table Mountain chain, with figures decreasing to the east and south. Thus deposition levels at Devils Peak, Kenilworth and particularly, Table Mountain, are greatly elevated relative to the dry Tygerberg site.

Because they remove the influence of rainfall levels, the VWM concentrations shown in Table 3.3 provide the best indication of relative pollutant source strength at each of the study sites. These values indicate that Tygerberg is the site most exposed to pollution, having the highest VWM concentrations of NH_4 and SO_4, and the second highest for NO_y. Kenilworth follows closely behind Tygerberg for all three pollutants. It is also noteworthy that these sites have the highest NH_4: NO_y ratios measured (Table 3.5). The high NH_4 levels may be explained by the fact that both the sites are close to potential NH_3 emission sources. Tygerberg is near to a number of orchards and vineyards to the north and east, a pig farm to the east in Durbanville, and several golf courses. Kenilworth is relatively close to the Philippi farming area, which lies to the east and supports both vegetable/flower and livestock farming. This site may also receive emissions from horse waste or fertilizer applied to the racetrack. High SO_2 and NO_y levels would also be expected at Tygerberg and Kenilworth, as both sites are surrounded by urbanization and occur in close proximity to industrial areas and major roads (Figure 3.2). For NO_y however, VWM concentrations were highest at Table Mountain, a fact which may seem surprising given that this is probably the site most isolated from anthropogenic influence. This is presumably due to orographic enhancement of rainfall concentration (see chapter 1), as this site is 450 -720m higher than the others. In fact, one might expect this discrepancy to be larger, as Fowler *et al.* (1988) found that rainfall concentrations in the rural northwest of England increased by a factor of between 2 and 3 between 244 m and 847m. It is likely that relatively little pollution from the city reaches the plateau of Table Mountain. This is particularly true in winter, when the northwesterly wind disperses pollution away from the mountain, and temperature inversions trap pollution at low altitude (as low as 100m in the morning, increasing as the day goes on; Dracoulides 1994). The summer southeasterly wind would be more likely to carry pollution from the city bowl up to the plateau of Table Mountain. It is noteworthy that NO_y levels (as well as NH_4 levels) show less of a winter peak at Table Mountain than the other sites (Figure 3.12), possibly for this reason. In contrast to NO_y, NH_4 levels on Table Mountain are very low. This is presumably because NH_y has a higher deposition velocity than NO_y, and most NH_y is deposited before it can reach the summit. The higher deposition velocity of SO_4 relative to NO_y may, similarly, explain the low SO_4 deposition at the Table Mountain site.

An important consideration for Table Mountain which could not be measured in this study is the magnitude of occult deposition. Cloud water is known to constitute a substantial proportion of moisture deposited on the Table Mountain plateau, particularly in summer when the "table cloth" is a common occurrence. In a year long study of fog precipitation on the plateau of Table Mountain, Nagel (1956) compared the amount rainfall trapped by two rain gauges, one of which was equipped with a fog-catcher. Even though the fog-catcher fitted rain gauge almost certainly underestimated total fog precipitation, it still trapped a volume which was around 1.7 times that of the standard rain gauge. Using empirical equations he later calculated that annual fog precipitation volume on Table Mountain varied but was about three times that of rainfall at the highest point, Maclear's beacon (Nagel 1961). Snow (1985) measured volumes of fog precipitation run off from plants on Table Mountain and showed that the narrow sclerophyllous leaves of most fynbos species intercept mist very efficiently. Not only does cloud water deposition account for a large volume, but ionic concentrations in orographic cloud are also usually substantially higher than in average rainfall (Fowler *et al.* 1988, Fowler *et al.* 1995). The deposition figures obtained in the current study for Table Mountain are thus certainly sizeable underestimates. Concentrations of all three pollutants are low at Silvermine, a finding which is expected because of the relative isolation of the site, and because the prevailing winds would not be likely to disperse much pollution in this direction.

3.4.6 Future deposition studies

While this study provides a rough estimate of bulk deposition levels of N and S to the CMA, there were a number of sources of error which make the results tentative. Furthermore, the dry and occult components of deposition were not measured here, and would need to be quantified in order to obtain an accurate estimate of total deposition levels. This study indicates that deposition levels in Cape Town are relatively high, and future monitoring of this threat is important. Based on the experience and knowledge gained in this study, here follows a brief series of suggestions as to how future deposition studies should proceed in order to obtain reliable deposition data for the region.

1) Ideally, wet deposition should be measured using automated wet-only samplers. If the cost is prohibitive, a comparison between wet only and bulk values should be obtained for at least one site. This discrepancy between deposition levels measured by these samplers could then be used to correct bulk deposition values from other sites in the area. As mentioned earlier, an automated wet-only sampler already exists at the Cape Point GAW station, which could be used for this purpose.

2) If bulk samplers are used, they should be constructed according to standard designs used in Europe and N. America or purchased, so that the data obtained is directly comparable to data collected in other parts of the world. Standard rain gauges should also be set up in close proximity to bulk samplers in order to obtain reliable rainfall volume data.

3) Analysis of samples should take place in a laboratory which is specifically equipped for the measurement of dilute rainfall samples. If no such laboratory exists locally, samples should be transported elsewhere for analysis.

4) Samples should be collected on a weekly or, at most, fortnightly basis. Fewer collection sites with more frequent collections are preferable to more sites with less frequent collection. Provided that the interval between collection and analysis is not too long, thymol is recommended as the preservative of choice, as it does not interfere with ionic balances or analytical equipment/methods. Acidification of samples is not recommended (see Kirkwood 1992). Freezing is preferable to simple chilling of samples, and thus high density polyethylene bottles (as opposed to glass bottles) are recommended for sample storage so that they may be frozen without risk of breakage. The interval between sample collection and analysis should be as short as possible - preferably no longer than 2 months. Care should be taken to prevent samples from exposure to contaminants, particularly NH_3 Sources of NH_3 may include ammonium salts or standards, floor cleaning products and human handling (Aminot *et al.* 1997).

5) Before the commencement of future deposition studies, suitable tests of microbial consumption of nutrients should be conducted in the field in summer, using the intended preservative. Rainwater sample should be collected from a single event. Some of this sample should be analysed immediately for the ions of interest, and the rest should be returned to the field for 1, 2 and 4 weeks and measured as soon as possible after collection. Stored sample should also be analysed at regular intervals in order to determine the maximum permissible interval between collection and analysis in order to obtain reliable data.

6) In order to properly gauge the threat of pollution deposition to the local flora, dry deposition levels will need to be estimated in addition to wet deposition levels. Currently, the majority of deposition programs in other parts of the world estimate dry deposition levels from atmospheric pollutant concentrations using mathematical models (e.g. Galy-Lacaux *et al.* 2003, Erisman *et al.* 1995, Fowler *et al.* 1998a). If modeling of dry deposition is to be undertaken locally, long term monitoring of ground level NH_3 concentrations will be required. In addition, it is likely that the

complex topography and unique vegetation of the CMA (which will probably not conform to deposition velocities calculated for European heathlands), will make local modeling of dry deposition a fairly costly and difficult exercise.
A possible course of action is to use a number of techniques in addition to modeling in order to corroborate modeling results. Possible techniques include the collection of rock runoff and stem collar runoff (Skoroszewski 1999) or branch washing (e.g. Sanz *et al.* 2002).

7) The attempt to measure throughfall levels in the current study failed because the low canopy height of vegetation in the region necessitated the use of very low samplers which were easily contaminated by soil splash. Contamination by leaf litter and insects was also a problem. The study of fynbos canopy interactions with rainfall is, however, an important future research topic. I predict that fynbos foliage will take up sizeable quantities of nutrients from rainwater. In order to study this topic with out the interfering factor of dry deposition to leaf surfaces, concentrations of nutrients in locally collected rainwater could be measured before and after filtration through the canopy of fynbos plants which have either been grown indoors or whose leaves have been rinsed clean of any dry deposition using distilled water.

8) If accurate deposition data are to be obtained for high altitude areas such as the Table Mountain plateau, occult deposition levels will need to be measured. A number of relatively inexpensive automated cloud water collectors are available on the market for this purpose (see Krupa 2002).

3.5 Summary

A number of problems were experienced in my attempts to measure bulk deposition levels, including undercatch of rainfall by bulk collectors, breakdown of N in the field and during sample storage, and poor analytical precision/ possible contamination for NH_y. In order to correct for undercatch, alternative rainfall volumes for each study site were obtained from the nearest available standard rain gauge and used to calculate bulk deposition figures. I was able to tentatively correct for N breakdown in the field by multiplying bulk deposition levels at each study site by the ratio between deposition collected on a monthly and a weekly basis at the Devils Peak site. Not taking into account possible break down of N during storage, I calculated annual bulk N deposition figures ranging from 2.5 kg $N.ha^{-1}.a^{-1}$ at Silvermine, south of the city bowl to 5.4 kg $N.ha^{-1}.a^{-1}$ on the plateau of Table Mountain and 5.6 kg $N.ha^{-1}.a^{-1}$ at Kenilworth R.C. in the centre of the urban area. NH_4 levels were greater than NO_y levels at all sites except Table Mountain, where NO_y dominated. Tests showed that breakdown of N may occur during sample storage and it is suggested that bulk N deposition levels may be at least 10-20% higher than those measured in this study. SO_4 deposition levels were higher than expected, with annual levels (calculated by extrapolating from data for 8 months), ranging from around 8 kg $S.ha^{-1}.a^{-1}$ at Silvermine and Tygerberg to more than 16 $kg.S.ha^{-1}.a^{-1}$ at Kenilworth. The percentage of SO_4 derived form marine sources, calculated using Na deposition levels, varied from about 23% to 36%. Bulk deposition of all the study species showed a strongly seasonal pattern, with the majority of input occurring in the winter period from May to September, coinciding with the period of greatest rainfall and highest aerial pollutant concentrations. Volume weighted mean concentrations of SO_4 and NH_4 were greatest at Tygerberg and Kenilworth and lowest at Table Mountain and Silvermine. VWM concentrations of NO_y showed a similar pattern, except that they were also high at Table Mountain. Deposition to emission ratios were low for NO_y (0.07) and SO_4 (0.16), indicating that the majority of pollution emitted in Cape Town is advected out to sea by the prevailing winds. Nevertheless, the results indicate that atmospheric deposition of both N and S are high enough to be a threat to local vegetation, especially if dry deposition is also taken into account, and it is important that more accurate study, and preferably long term monitoring of this threat is undertaken in future.

Chapter 4

Historical nitrogen content of moss tissue as an indicator of increased anthropogenic nitrogen deposition in the Cape Metropolitan Area

4.1 Introduction

In many developed parts of the world, precipitation chemistry has long been the subject of detailed monitoring (deposition data for most of Europe extends as far back as the mid 1800's: Skeffington & Wilson, 1988). It has thus been possible to quantify, with some accuracy, the marked historical increase in N deposition which has occurred in those areas. For the Cape Metropolitan Area (CMA) however, data on precipitation chemistry is almost non-existent. A single study measuring bulk deposition of N was carried out at Pella, an isolated and unpolluted fynbos ecosystem 62 km north of Cape Town, over a two year period from 1980 to 1982 (Stock and Lewis, 1986a). This is the only indication we have of the possible background level of N deposition for the region for comparison with levels of bulk deposition in the CMA measured in the current project (Chapter 3). It is also unfeasible to try to accurately gauge spatial patterns of N deposition by measuring deposition chemistry, as it would be costly and work-intensive to set up and monitor the network of samplers which would be required for such an exercise. For these reasons I attempted, in this chapter, an alternative approach to examining temporal and spatial variability in N deposition, namely, the use of bryophytes as bioindicators of N deposition.

Tissue analysis of bryophytes (and in particular mosses) has been widely employed in the biomonitoring of pollution including heavy metals (e.g. Tyler 1990, Fernandez *et al.* 2002), radionuclides (e.g. Summerling 1984), hydrocarbons (e.g. Gerdol *et al.* 2002) and nitrogen (e.g. Baddeley *et al.* 1994, Pitcairn *et al.* 1995, 1998, 2003). Mosses have several characteristics which make them excellent subjects for biomonitoring, and superior, in this regard, to vascular plants. It should be noted that the following points are generalizations and are discussed in more detail at a later stage:

- Because mosses lack conductive root systems, they generally rely heavily on wet and dry deposition for their nutrient supply and uptake from the substrate is minimal (Bates 2000).Using mosses it is thus possible to study eutrophication resulting from atmospheric pollution as distinct from general eutrophication of the biosphere.
- To facilitate efficient absorption of deposition, cutin development is usually poor in mosses and mineral ions are readily absorbed over the entire surface of the plant (Proctor 2000). The ability to actively mobilize nutrients is also weak or absent in most moss species (Penuelas & Filella 2001). These characteristics mean that mosses are very efficient accumulators of environmental chemicals.
- The characteristics mentioned above mean that moss tissue chemistry is usually closely correlated with atmospheric inputs (Bates 2000, Pitcairn *et al*. 1995, 1998, 2003). For some pollutants, e.g. heavy metals (Tyler 1990), this relationship is often strong enough for concentrations of pollutants in tissues to be converted with some accuracy into atmospheric deposition values, provided that the species sampled has been well characterised in previous experiments.
- Many moss species have a large geographical range and occupy a wide variety of habitats making them useful for the study of small scale spatial patterns of deposition.
- Most herbariums have good moss collections making it possible to conduct retrospective studies of tissue nutrient levels, comparing herbarium specimens with newly collected material (Penuelas & Filella 2001, Pitcairn *et al*. 1995, Baddeley *et al*. 1994)
- Mosses are good candidates for transplant experiments, as they are relatively independent of the substratum and do not possess root systems which could be disturbed during the transplantation process. Transplants have been successfully used to study degree and rate of N enrichment in a number of studies (Morecraft & Woodward 1996, Baddeley *et al*. 1994, Pitcairn *et al*. 1995). Living material is collected from an unpolluted site and transplanted to sites which are known or suspected to be polluted. By measuring foliar N of the transplants at regular

intervals, it is then possible to compare both degree and rate of N enrichment at different sites. Bryophytes may be transplanted on bark disks (e.g. Leblanc & Rao 1973), in moss bags (e.g. Goodman & Roberts 1971), or in cylinders/pots (e.g. Morecraft & Woodward 1996, Baddeley *et al.* 1994).

4.1.1 Moss tissue N and nitrogen deposition.

A strong relationship between bryophyte tissue nitrogen concentration and estimated N deposition has been documented in a number of studies. Baddeley *et al.* (1994) measured tissue nitrogen of the moss *Racomitrium lanuginosum*, collected from a number of mountain sites in central and northern Britain and found a good correlation with spatial patterns of deposition. They were also able to show a strong trend of increasing tissue N with time, by comparing herbarium specimens of the same species collected from a single area in 1879, 1956 and 1989. In a similar fashion, Pitcairn *et al.* (1995) showed a significant increase in tissue N of several moss species between the 1950's and 1989 and found a positive linear relationship between tissue N of ectohydric mosses and estimated N deposition for a number of sites around the UK. By combining data from this study with those from a further study of ectohydric mosses receiving a high deposition load as a result of pollution from nearby livestock farms (Pitcairn *et al.* 1998), the authors were able to demonstrate that the relationship was in fact a logarithmic one where foliar N content of mosses (F_n, % DW) is related to N deposition (D_n, kg.ha^{-1}.a^{-1}) according to the equation $F_n = 3.81(1-e^{-0.04D_n})$. Other authors have also observed strong correlations between bryophyte foliar N and N deposition (Hicks *et al.* 2000, Solga *et al.* 2004). It is thus suggested that, with further refinement, bryophyte foliar N could be used to provide a rapid indication of spatial patterns and magnitude of N deposition (Pitcairn *et al.* 1998, 2003, Solga *et al.* 2004).

While the abovementioned studies and others have clearly demonstrated the potential for bryophytes to be used as biomonitors of N deposition, it is important that caution be shown when attempting such an exercise, as there are a large number of factors which may influence nitrogen concentration of moss tissue in addition to N deposition level. Some of the most important considerations are as follows:

Morphology

Mosses are generally divided into two groups on the basis of physiology and morphology. Pleurocarps have creeping, branched stems, which usually results in a mat-like growth form, while acrocarps generally have erect stems with little or no branching, and form tufts (Buck & Goffinet 2000). Clearly, the broadly different architectures of these two groups may be expected to result in some differences in tissue N patterns. The main problem is that, because of the long branching stems and tiny leaves of pleurocarps, stem tips must be analysed for comparison with individual leaves in acrocarps. Previous studies have shown nitrogen concentrations to be highest in young shoot apices for two pleurocarps, *Hylocomium splendens* (Tamm, 1953 cited in Bates 2000) and *Racomitrium lanuginosum* (Baddeley *et al*. 1994) and this is also almost certainly the case in most acrocarps. In *R. lanuginosum*, tissue N remained relatively constant in the first 3 cm of stem and then dropped off, while in *H.splendens*, there was a steady decrease with distance from the apex. In the current study, all tissue analysed was from the most terminal position of the stem (within 1 cm), but it is not known how acrocarps and pleurocarps, or individual species, will differ in this respect.

Water conduction method and uptake from the substratum

Another important consideration is the role played by substratum in the nutrition of mosses. In most cases, pleurocarps are epiphytic or epilithic, while acrocarpous species usually occupy terrestrial habitats, growing on soil or rock. Clearly tree bark and rock do not yield much N, and thus epiphytic and epilithic species will receive most of their N supply from deposition. Soil-growing species may, however, derive a significant proportion of their nitrogen budget from the substratum in some cases (Bates 2000). The extent to which this occurs is largely determined by water relations. Moss species can broadly be classified as endohydric, mixohydric or ectohydric. Endohydric species have relatively well developed internal conductive tissues and are best able to utilize nutrients from the substrate (Proctor 2000). They are also able to efficiently translocate nutrients internally and may, for instance, move nitrogen to underground stems for the winter. These features make endohydric species undesirable as biomonitors and they are not included in the current investigation. In ectohydric species (including most pleurocarps) water conduction mainly takes place externally, in the capillary spaces on the plant surface, and contact with the substratum is generally poor. Uptake from the substratum and internal transport of nutrients are thus both less pronounced in ectohydric than in endohydric species, although they may still be a factor in the nutrition of some species (Bates 2000). Mixohydric species lie somewhere between the two extremes, making use of a combination of internal and external conduction, with different species relying on each of these systems to varying extents. In general though, it seems obvious that ectohydric are the group most likely to show a clear correlation between tissue N concentration and deposition.

Altitude

The influence of altitude is a third factor, which could potentially obscure the relationship between tissue N and deposition level. A number of studies have demonstrated increased foliar N concentration of both mosses (Baddeley *et al.* 1994, Hicks *et al.* 2000) and vascular plants (Morecraft *et al.* 1992, Morecraft & Woodward 1996, Hicks *et al.* 2000) at high altitudes. The possible reasons for this relationship are two-fold:

- As discussed in Chapter 2, wet deposition of N has been shown to increase with altitude due both to increased precipitation volume and increased N concentration of precipitation at altitude (Fowler *et al.* 1988, Hicks *et al.* 2000).
- Lower temperatures and a shorter growing season at altitude may reduce growth resulting in a higher concentration of nutrients in plant foliage (Morecraft & Woodward 1996).

At present there is still some debate as to the relative importance of these factors. In two studies on the vascular plant *Alchemilla alpina*, one on naturally growing plants (Morecraft *et al.* 1992) and one using transplants (Morecraft & Woodward 1996) it was found that plants growing at high altitude generally had smaller leaves and higher foliar concentrations of nitrogen but also, to a lesser extent, higher concentrations of phosphorus (Morecraft *et al.* 1992) and potassium (Morecraft & Woodward 1996). The fact that these elements increased in tandem with N was taken as evidence of the concentrating effect of altitude on nutrients in foliage. This concentrating effect was, however, not as clear as that of altitude itself and the authors concluded that increased N deposition at altitude was also likely to play an important role in the observed phenomenon (Morecraft & Woodward 1996). In a further attempt to address this question, Hicks *et al.* (2000) sampled five species including a moss, *Hylocomium splendens*, along altitudinal transects in northern Britain in order to study trends in foliar N and P concentrations. In most species, foliar N concentration showed an approximately linear relationship with altitude whereas foliar P concentration did not, thereby providing evidence that enhanced N deposition is the primary cause of elevated foliar N at altitude (Hicks *et al.* 2000). Interestingly *H. splendens* showed a different general response to the vascular species. Foliar N concentration showed a relationship with N deposition level but neither foliar N nor P increased significantly with altitude. The explanation given by the authors is that the relationship between altitude and N deposition is not truly linear, and that *H. splendens* demonstrated the true nature of this relationship more accurately than the vascular plants because of the closer coupling of moss tissue N to the atmosphere (Hicks *et al.* 2000).

Season

A final consideration is the variation in moss tissue N in relation to patterns of rainfall. Growth rate in mosses is generally seasonal, being closely correlated with rainfall levels (Brown 1982). Tissue N is, in turn, tied to growth rate because nutrient uptake and concentration in tissues is highest during periods of growth (Brown 1982) Thus mosses may be expected to show a seasonal variation in foliar N which must be taken into account in comparative studies.

4.1.2 Moss tissue $\delta^{15}N$ and nitrogen deposition

A number of studies have measured the $^{15}N/^{14}N$ isotope ratio of mosses in order to glean some information about the role of N pollution as an N source for these plants. Although the factors which determine $\delta^{15}N$ patterns in ecosystems are notoriously complex (e.g. Handley *et al.* 1999), interpretation of $\delta^{15}N$ in bryophytes should theoretically be relatively simple because $\delta^{15}N$ ratio in plant foliage is determined primarily by the isotope ratio of the nitrogen source (Nadelhoffer & Fry 1994, Handley & Raven 1992), and most mosses rely almost solely on the atmosphere for their N supply. Thus moss tissue $\delta^{15}N$ would be expected to be very similar to that of deposition (Pearson *et al.* 2000, Solga *et al.* 2004). Penuelas & Estiarte (1997) and Penuelas & Filella (2001) measured leaf $\delta^{15}N$ of herbarium material collected over a 70 year period, and interpreted generally depleted $\delta^{15}N$ values in more recently collected material as an indication of increased anthropogenic inputs of N. This conclusion was based on the findings of Freyer *et al.* (1996) that the $\delta^{15}N$ of nitrate in alpine and polar ice cores decreased steadily in the latter part of the 20th century in tandem with increasing anthropogenic N emissions. The factors controlling $\delta^{15}N$ in atmospheric components are complex, however, and interpretation of isotope data is seldom simple. Other studies have, for instance, found $\delta^{15}N$ signatures of mosses in urban areas, receiving high loads of traffic pollution, to be relatively more positive than those in rural areas (Pearson *et al.* 2000, Gerdol *et al.* 2002).

The aim of this chapter was to measure spatial and temporal trends in nitrogen content of bryophyte foliage in the CMA as an indicator of N deposition patterns. Herbarium samples collected over the past 130 years were analysed along with freshly collected material for N, C and $\delta^{15}N$ isotope ratio, in order to test the hypotheses that a) increasing levels of atmospheric N deposition in the CMA over the past century would be reflected in an increase in moss tissue N over that period and b) $\delta^{15}N$ in moss tissue would show a decrease over the last century which would be indicative of a greater contribution of anthropogenically derived N to deposition (Penuelas and Filella 2001). In order to study rates of enrichment by deposition, bryophyte material was also transplanted from an area with low N pollution (Cape Point N.R.) to three sites near the city which would be expected to have high pollution levels (Milnerton RC, Kenilworth RC and Devils Peak), and removed for analysis at three month intervals over the period of a year.

4.2 Methods

4.2.1 Study species

For the main experiment, material from nine species/species groups was analysed (Table 4.1). *Hypnum cupressiforme*, *Ischyrodon lepturus*, *Ceratodon purpureus*, *Leptodon smithii* and *Pseudocrossidium crinitum* were analysed individually. For each of the genera, *Bryum, Campylopus, Pleuridium* and *Fissidens*, several species deemed sufficiently similar in terms of ecology and morphology were grouped together. *Bryum* is a combination of *B. canariense* and *B. torquescens*, *Campylopus* is *C. bicolor* and (mainly) *C. introflexus*, *Pleuridium* is largely *P. ecklonii*, but includes some *P. neurosum* and *P. pappeanum*, and *Fissidens* comprises a number of species - *F. rufescens*, *F. marginatus*, *F. fasciculatus, F. plumosus* and *F. glaucescens*. *Campylopus introflexus* was chosen as the study species for the transplant experiment, as it was the most common and easily collected species growing at the control site (Cape Point), it survives in a wide variety of habitats, and is present over most of the CMA.

Table 4.1: *Physiological and ecological attributes and distributions of the 9 study species/ species groups of mosses. Habitat and substrate preferences indicated are generalizations and exceptions are common.*

ACROCARPS						PLEUROCARPS			
Ceratodon purpureus	*Pleuridium sp.*	*Pseudocrossidium crinitum*	*Campylopus introflexus/*	*Fissidens sp.*	*Bryum canariense/ torquescens*	*Leptodon smithii*	*Ischyrodon lepturus*	*Hypnum cupressiforme*	
									SUBSTRATE
+	+	+	+	+	+				soil
+				+	+	+	+	+	rock
						+	+	+	tree
									WATER CONDUCTION
		+		+		+	+	+	ectohydric
+	+		+		+				mixohydric
									HABITAT
				+		+	+	+	forest/shade
+	+	+	+	+	+				flats
+				+			+	+	urban
+			+	+				+	high altitude
									LIFE STRATEGY
	+								fugitive
+				+	+				colonist
		+	+			+	+	+	perennial stayer
									DISTRIBUTION
	+						+		restricted
+		+	+	+	+	+		+	widespread

4.2.2 Sample collection

For the purposes of the study, the CMA was divided into four broad sampling regions on the basis of likely pollution climate (Figure 4.1). The FLATS region encompasses the urbanized lowlands of the CMA and is thus likely to receive the highest pollution levels of the four regions, due to its proximity to major roads and areas of industrial and farming activity. Rainfall levels are, however, generally low here in comparison to other parts of the CMA (Figure 3.2, Chapter 3). The EAST region includes the lower eastern slopes of Table Mountain, which receive high rainfall and are vegetated with broad-leaved afromontane forest over much of their extent. The SOUTH region encompasses the lower western slopes of Table Mountain and the South Peninsula, an area which is likely to receive relatively low pollution levels due to its relative isolation from the city bowl. This region is, for the most part, separated from the EAST and FLATS regions by the peninsula mountain chain. The MONTANE region was defined as any area with an altitude greater than 400m, with the majority of samples in this category being collected from the upper slopes and plateau of Table Mountain. This region was distinguished from the others due to the possible influence of altitude on both N deposition levels and foliar N concentration discussed earlier.

For the main experiment, moss samples from the field were collected between March 2001 and March 2002 from a number of sites around the CMA: 3 sites in the EAST region, 4 sites in the FLATS region, 4 sites in the SOUTH region and 2 sites in the MONTANE region (see Figure 4.1). At each site, no two samples from the same species/species group were collected within approximately 100m of each other. Sampling covered a range of habitats which was similar to the range of habitats from which herbarium material had been collected for each species/species group. All material collected was alive and photosynthetic, and was initially air-dried in sealed paper envelopes. The herbarium specimens studied had been stored at the Bolus Herbarium, University of Cape Town. Specimens were collected between 1875 and 2000 and were divided into three groups according to collection date; PRE-1940, 1950-1970 and POST-2000. The POST-2000 group included all freshly collected material as well as some herbarium material. Approximate locations at which herbarium samples were collected are shown in Figure 4.1.

For the transplant experiment, 48 healthy photosynthetic "clumps" of *Campylopus introflexus* along with substrate to a depth of 5,5 cm were collected in plastic cores (diameter 3.2 cm), from an acid sand flat at Cape Point Nature Reserve (Site 13, Figure 4.1), taking care to cause as little disturbance to the plants as possible. 12 cores were transplanted to each of three sites, one in the EAST region; Devils Peak (Site 6), and two in the FLATS region; Kenilworth Race Course (Site 4) and Milnerton Race Course (Site 1). A further 12 cores were back transplanted to Cape Point as a control. Five samples were collected from Cape Point in order to determine the initial nutrient status of the transplant material.

Five soil cores (depth 2.5 cm) were collected from seven sites shown in Figure 4.1 for analysis of plant available N (NO_3 + NH_4), organic matter and moisture content. Soil was stored in sealed plastic packets at 0°C until analysis.

4.2.3 Sample analysis

A number of leaves (or stem tips up to 1cm in the case of *Hypnum*, *Ischyrodon* and *Leptodon*) were removed from a similar terminal position of each specimen using tweezers. Samples were chopped finely and care was taken to remove sand or other particles clinging to the plant material. Samples were not washed since this can cause leaching of nutrients (Brown and Buck 1979). Samples were then oven-dried at 60°C to constant weight and $\delta^{15}N$, % nitrogen concentrations and % carbon concentrations determined on an isotope ratio mass spectrometer (Finnegan Mat 252, Bremen, Germany) with a C and N elemental analyser (NA 1500NC, Carlo-Erba, Milan, Italy). Internal standards used were nasturtium leaf (Stock preparation) and Merck gelatin. The laboratory reference gas was high purity nitrogen (99.995 %) calibrated against atmospheric N_2.

Soil available N was extracted from sieved soil samples (2mm mesh) by shaking 10g of soil with 100ml of 2M KCl solution for 1 hour and suction filtering through a Whatman No. 4 filter paper according to the method of Stock (1983). Ammonium (NH_4^+) and nitrate + nitrite (NO_y) concentrations were determined by flow injection analysis (Quikchem 8000 series FIA+, Lachat Instruments) at the Department of Oceanography, UCT, according to the methods prescribed in the Lachat methods.manual; reduction of nitrate to nitrite by means of a copper cadmium

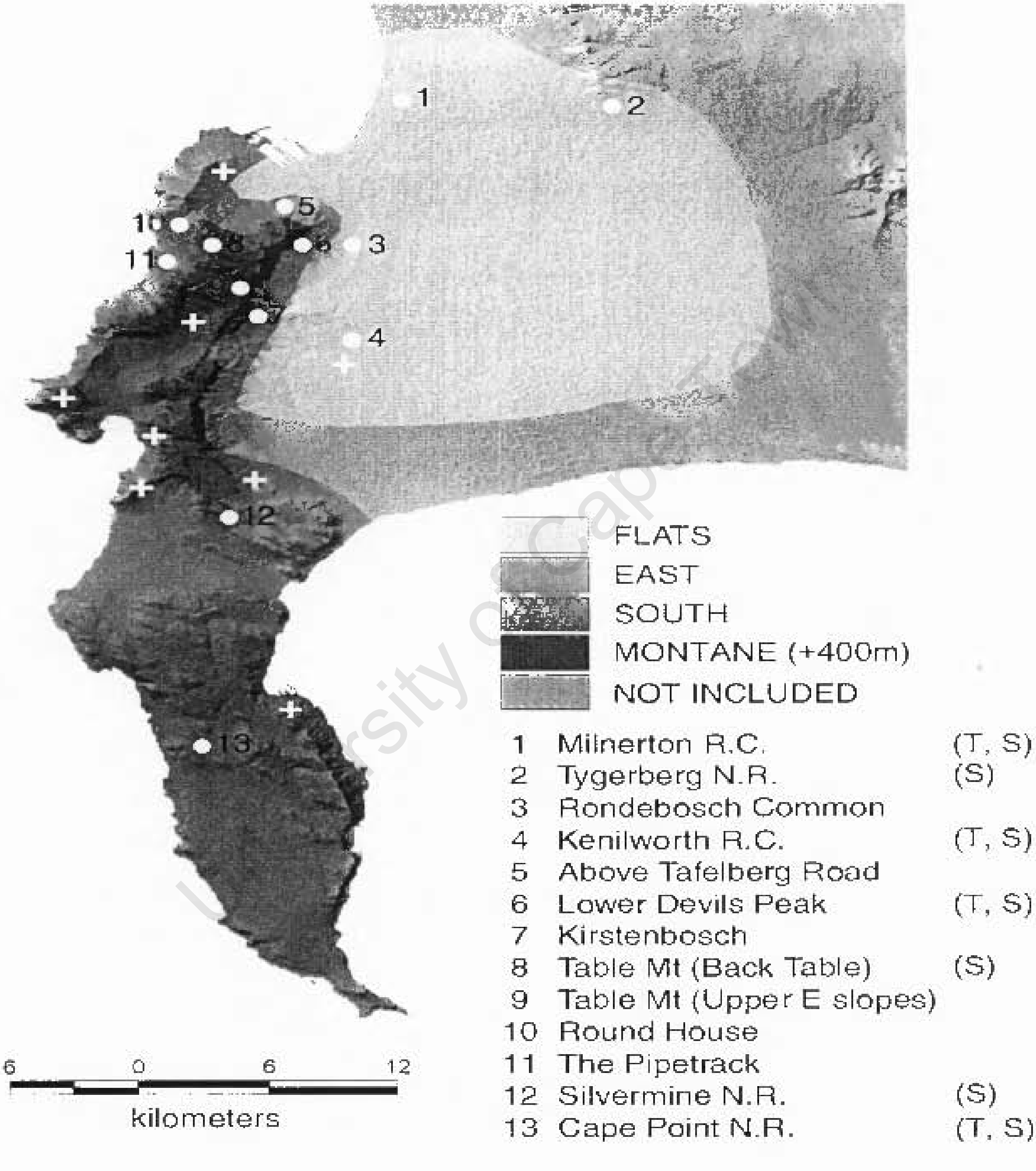

Figure 4.1: *A map of the Cape Metropolitan Area showing the study sites and the four broad sample collection regions defined in the study. Dots indicate sites where both recent and historical bryophyte leaf material was collected, while crosses indicate sites where only historical material was collected. Sites included in the transplant experiment are followed by the letter T. Sites from which soil cores were obtained for soil analysis are followed by the letter S.*

reduction, followed by colorimetric analysis for NO_y (Smith & Bogren 2001) and a modified version of the indophenol blue method for NH_4 (Egan 2001). For soil moisture and organic matter determinations, soil samples of approximately 10g were weighed before and after drying in an oven at 105°C for 24 hours. Soils were then transferred in a desiccator to a muffle furnace and combusted for 24 hours at 450°C and re-weighed.

Soil moisture content was calculated using the formula:

Soil moisture content (%) = [(wet weight - dry weight) / wet weight] × 100

Soil organic matter content was calculated using the formula:

Soil organic matter (%) = [(dry weight - ashed weight)/ dry weight] × 100

4.2.4 Statistics

For the main experiment, the variables analysed were $\delta^{15}N$, %N and C: N ratio. Data were checked for normality using normal probability plots. $\delta^{15}N$ data were roughly normally distributed, while %N and C: N ratio data tended towards log-normal distributions and were log transformed (base e), producing distributions which were acceptably close to normal.

Due to the unbalanced nature of the data, linear mixed model analyses using the Residual Maximum Likelihood (REML) method (Patterson & Thompson 1971) (non-sparse algorithm with Fisher scoring) were conducted in order to determine which factors or combinations of factors, had a significant effect on the variables. Samples were classified according to four primary and two secondary factors that had a potential influence on the study variables (see Table 4.2). These factors were all included in the fixed model. *Run* was included as a random term in all analyses in order to account for possible machine error. For each variable, initial REML analysis examined the main effects of all four primary factors. Factors that did not have statistically significant ($p<0.05$) effects were subsequently removed from the analysis in order to isolate and calculate predicted means for the significant factors. In analyses attempting to discover the effects of the secondary factors, *water conduction group* and *physiological group*, analyses were carried out with either of these two factors replacing the primary factor, *species*. In addition to main effects, variables were also tested for all meaningful interactions. In cases where we wished to display

Table 4.2: *Factors used in linear mixed model (REML) statistical analyses in the main experiment.*

	No. levels	Description	Levels
Fixed Model			
<u>Primary factors</u>			
Species	9	The species/ species groups studied.	See Table 4.1
Period	3	Historical period of collection.	<1940; 1950-1970; >2000
Region	4	Region of collection.	East; Flats; South; Montane
Season	4	Season of collection.	Summer = Dec/Jan/Feb; Autumn = Mar/Apr/May; Winter = Jun/Jul/Aug; Spring = Sep/Oct/Nov
<u>Secondary factors</u>			
Water conduction	2	Water conduction method of species.	Ectohydric; Mixohydric
Physiological group	2	Physiological group to which species belong.	Pleurocarp; Acrocarp
Random Model			
Run number	8	Run number on the mass spectrometer.	Runs 1 - 8

non-significant effects and/or the predicted means thereof, the analysis included the non-significant effect of interest and all significant factors. When significant effects were identified by REML analysis, Bonferroni multiple comparison tests (Hsu 1996) were used to test for significant differences between means.

For the transplant experiment, the effects of transplant site, collection date and the interaction between these two factors on the variables %N (log-transformed) and $\delta^{15}N$, were tested with a repeated measures REML (sparse algorithm with AI optimization).

All means displayed in tables and figures in this chapter are predicted by REML analysis, unless otherwise specified. Wald statistics for each REML analysis presented are displayed in table format in Appendix A.

All statistical analyses were performed with the computer statistical package, Genstat (2002, version 6.1, Lawes Agricultural Trust).

4.3 Results

4.3.1 Soil analyses

Site averaged soil NO_3-N, NH_4-N, soil moisture and soil organic matter (SOM) contents are displayed in Table 4.3. As would be expected, relatively high soil total inorganic N levels occur at Tygerberg N.R. and Devils Peak, which have shale-derived soils, while N values are lower at the remaining five sites, all of which have acid sand substrates. Amongst the five sandy sites, Kenilworth and Milnerton, which occur in the lowlands in the midst of the urban area, have higher soil N (particularly NO_y-N) concentrations than the remaining three sites, which occur on the Table Mountain chain and the peninsula. At most sites NH_4-N is in excess of NO_y-N. As is evident from Table 4.3, soil moisture roughly tracks SOM ($y = 0.635x + 0.777$, $R^2 = 0.8652$). Total inorganic N is also related to SOM content. ($y = 0.7181x + 1.465$, $R^2 = 0.5089$), the result of a relatively close correlation between organic matter and NH_4-N ($y = 1.4029x + 0.698$, $R^2 = 0.7152$). SOM shows no correlation with NO_y-N, however ($y = 0.9308x + 3.7493$, $R^2 = 0.1847$).

Table 4.3: *Site averaged soil NO_y-N, NH_4-N, soil organic matter and soil moisture contents (0 -2.5 cm) for 7 natural vegetation sites in the Cape Metropolitan Area (n = 5). Values in parentheses are 1 standard deviation.*

Site	NO_y-N (µg.g^{-1} soil)	NH_4-N (µg.g^{-1} soil)	Tot. Inorganic N (µg.g^{-1} soil)	Soil Moisture (% wet weight)	SOM (% dry weight)
Cape Point NR	1.13 (0.37)	1.21 (0.91)	2.34 (1.17)	5.86 (3.94)	2.20 (0.82)
Devils Peak	3.28 (0.52)	8.50 (2.66)	11.79 (2.22)	18.67 (2.98)	13.19 (2.73)
Kenilworth RC	3.12 (0.56)	3.43 (2.55)	6.54 (2.64)	2.75 (1.51)	3.67 (1.65)
Milnerton RC	2.99 (1.98)	2.46 (1.76)	5.46 (3.05)	1.68 (2.42)	1.86 (0.64)
Silvermine NR	0.88 (0.15)	2.21 (2.21)	3.09 (2.17)	4.06 (2.36)	2.91 (0.97)
Table Mt.	0.79 (0.10)	2.63 (2.77)	3.42 (2.86)	16.69 (4.97)	9.63 (3.92)
Tygerberg NR	6.96 (2.03)	7.49 (4.94)	14.45 (6.00)	11.13 (4.23)	10.61 (2.67)
Maximum	6.96	8.50	14.45	18.67	13.19
Minimum	0.79	1.21	2.34	1.68	1.86
Mean	2.73	3.99	6.73	8.69	6.30

4.3.2 Nitrogen content and C: N ratio

The most important factors determining nitrogen content of the studied material were the differences in sampling period ($p< 0.001$, Table 4.4) and species studied ($p< 0.001$, Table 4.5), with season of collection of secondary importance ($p< 0.025$, Table 4.6). The same pattern was observed for C: N ratios. When all species were included in the analysis, there was a clear trend of increasing N content and decreasing C: N ratio of moss material through historical time, although the increase from the PRE-1940 to the 1950-1970 sampling period was not statistically significant (Table 4.4). This general trend was observed for most species studied (Table 4.7), with recently sampled material (POST-2000) having the highest average nitrogen content in all species. Nevertheless, the period effect was obscured to some extent by the significant differences in foliar N and C: N ratio between species, with the lowest % N values being observed in the mixohydric species, *Campylopus sp.* and *Pleuridium sp.* and highest values, in the ectohydric species, *Ischyrodon lepturus* and *Fissidens sp* (Table 4.5). It would appear from Table 4.7 that the trend of historical increase in N content was generally not as evident in mixohydric species as in ectohydric species. This was confirmed by analysis of the ectohydric and mixohydric species as separate datasets (Table 4.8). When analysis was performed on ectohydric species alone, the trend of increasing foliar N with time (Table 4.8a) was much more apparent than in the analysis including all species (Table 4.4), with average N content in each period significantly elevated in comparison to the previous period. For mixohydric species in general, this trend was not observed; differences between periods were not as clear ($p = 0.008$, Table 4.8a), and the average N concentration of material was lower in the intermediate 1950-1970 period than in the PRE-1940 or POST-2000 periods. This general pattern was apparent in three out of the four mixohydric species studied, while it did not occur in any of the ectohydric species (Table 4.7).

Ectohydric and mixohydric species groups also showed different responses to the effect of season. Analysis of all species showed that both %N and C: N ratio were significantly affected by season (Table 4.6, $p < 0.05$ in both cases), with specimens collected in winter (Jun - Aug) having the highest foliar N contents. This is the period during which Cape Town receives the majority of its rainfall (Figure 3.2, Chapter 3). Mixohydric species, showed this trend very clearly ($p < 0.001$), with foliar N peaking in winter and lowest in summer, which is the driest part of the year. No seasonal effect was present in ectohydric species, however ($p = 0.404$) (Table 4.8b).

Neither %N or C: N ratio was significantly affected by the region from which specimens were collected (Table 4.9) and separate analysis of ectohydric and mixohydric groups did not reveal a significant effect of sampling region either (data not shown)

Table 4.4: *The influence of sampling period on mean N content, C: N ratio and $\delta^{15}N$ of moss foliage collected in the Cape Metropolitan Area.*

PERIOD	N content (% dry weight)	n	C:N ratio (% dry weight)	n	$\delta^{15}N$ (‰)	n
Pre - 1940	1.32 [a]	55	31.41 [a]	50	-4.30	55
1950 - 1970	1.37 [a]	58	29.17 [a]	50	-3.48	53
Post - 2000	1.69 [b]	141	23.45 [b]	101	-4.49	97
Mean standard error	0.048		0.036		0.609	
Significance level	$p < 0.001^{**}$		$p < 0.001^{**}$		$p = 0.161$	
Wald table no.	1		2		3	

Means are predicted from REML analyses of the effect of sampling period on the three variables. Wald statistics from the relevant REML analyses are presented in the Wald tables indicated (Appendix A). Values differing significantly at the $p < 0.05$ level are followed by different letters (Bonferroni multiple comparison test). ** indicates a significant effect at the 1% level; n = number of samples.

Table 4.5: *The influence of species / species group on mean N content, C: N ratio and $\delta^{15}N$ of moss foliage collected in the Cape Metropolitan Area.*

			N content (% dry weight)	n	sd [1]	C:N Ratio (% dry weight)	n	sd [1]	$\delta^{15}N$ (‰)	n	sd [1]
		SPECIES / SPECIES GROUP									
Acrocarps	Mixohydric	***Bryum sp.***	1.63 ^{c}	47	0.52	25.36 ^{bc}	33	8.18	-4.17 ^{b}	38	2.36
Acrocarps	Mixohydric	***Campylopus sp.***	1.09 ^{a}	45	0.60	32.72 ^{a}	27	11.85	-4.37 ^{ab}	24	2.94
Acrocarps	Mixohydric	***Ceratodon purpureus***	1.49 ^{bc}	21	0.46	26.89 ^{bc}	17	8.38	-3.44 ^{bc}	17	2.46
Acrocarps	Mixohydric	***Pleuridium sp.***	1.26 ^{ab}	23	0.35	32.75 ^{a}	20	6.27	-4.61 ^{ab}	17	2.13
Acrocarps	Ectohydric	***Fissidens sp.***	1.77 ^{c}	32	0.52	22.02 ^{c}	25	5.28	-2.08 ^{c}	28	2.36
Acrocarps	Ectohydric	***Pseudocrossidium crinitum***	1.36 ^{abc}	17	0.35	29.37 ^{ab}	17	4.88	-3.48 ^{bc}	15	3.64
Pleuracarps	Ectohydric	***Hypnum cupressiforme***	1.37 ^{bc}	33	0.55	32.66 ^{a}	26	9.27	-6.23 ^{a}	30	1.78
Pleuracarps	Ectohydric	***Ischyrodon lepturus***	1.74 ^{c}	16	0.50	22.53 ^{bc}	16	6.45	-3.72 ^{bc}	16	2.01
Pleuracarps	Ectohydric	***Leptodon smithii***	1.49 ^{bc}	20	0.42	28.47 ^{ab}	20	8.28	-5.49 ^{ab}	20	1.86
		Mean standard error	0.067			0.057			0.724		
		Significance level	$p < 0.001$**			$p < 0.001$**			$p < 0.001$**		
		Wald table no.	1			2			4		

Means are predicted from REML analyses of the effect of species on the three variables. Wald statistics from the relevant REML analyses are presented in the Wald tables indicated (Appendix A). Values differing significantly at the $p < 0.05$ level are followed by different letters (Bonferroni multiple comparison test). n = number of samples. ** indicates a significant effect at the 1% level.

[1] Standard deviations (SD) are calculated from the raw data and do not take into account weighting by REML analysis.

Table 4.6: *The influence of sampling season on mean N content, C: N ratio and $\delta^{15}N$ of moss foliage collected in the Cape Metropolitan Area (see Table 4.2 for season definitions)*

	N content (% dry weight)	n	C:N ratio (% dry weight)	n	$\delta^{15}N$ (‰)	n
SEASON						
Summer	1.37 ^{a}	38	28.13 ^{ab}	34	-3.31	33
Autumn	1.39 ^{a}	28	28.16 ^{ab}	24	-3.73	28
Winter	1.63 ^{b}	50	25.30 ^{b}	40	-4.06	45
Spring	1.42 ^{ab}	138	29.78 ^{a}	103	-4.45	99
Mean standard error	0.052		0.043		0.576	
Significance level	p = 0.025*		p = 0.012*		p = 0.249	
Wald table no.	1		2		12	

Means are predicted from REML analyses of the effect of sampling season on the three variables. Wald statistics from the relevant REML analyses are presented in the Wald tables indicated (Appendix A). Values differing significantly at the $p < 0.05$ level are followed by different letters (Bonferroni multiple comparison test). * indicates a significant effect at the 5% level; n = number of samples.

Table 4.7: *Historical changes in N content, C: N ratio and $\delta^{15}N$ in foliage of 9 moss species / species groups collected in the Cape Metropolitan Area during three periods of the last 130 years.*

		N content (% dry weight)			C:N Ratio (% dry weight)			$\delta^{15}N$ (‰)		
Species	Period	Mean	sd	n	Mean	sd	n	Mean	sd	n
Mixohydric species										
Bryum canariense / torquescens	<1940	1.50	(0.32)	11	29.63	(5.24)	9	-3.76	(2.39)	11
	1950-70	1.55	(0.49)	12	29.25	(10.81)	10	-3.78	(2.03)	12
	2000+	1.99	(0.52)	24	22.55	(6.31)	14	-4.38	(2.65)	15
Campylopus introflexus / bicolor	<1940	1.21	(0.52)	8	37.35	(14.78)	7	-4.70	(2.73)	8
	1950-70	1.07	(0.67)	10	35.42	(15.41)	6	-1.01	(1.69)	4
	2000+	1.44	(0.58)	27	30.26	(8.24)	14	-5.83	(2.69)	11
Ceratodon purpureus	<1940	1.44	(0.25)	2	29.48	(6.87)	2	-2.92	(1.08)	2
	1950-70	1.30	(0.34)	4	34.23	(10.63)	4	-1.28	(2.42)	4
	2000+	1.93	(0.41)	15	22.25	(5.37)	11	-2.98	(2.66)	11
Pleuridium sp.	<1940	1.37	(0.17)	4	30.91	(3.93)	4	-4.31	(3.13)	4
	1950-70	1.28	(0.19)	4	32.63	(6.81)	4	-4.54	(2.76)	4
	2000+	1.42	(0.42)	15	32.83	(7.06)	12	-3.97	(1.69)	9
Ectohydric species										
Fissidens sp.	<1940	1.39	(0.38)	8	27.46	(4.10)	6	-4.05	(1.64)	8
	1950-70	1.93	(0.26)	7	21.72	(3.10)	7	-3.16	(3.17)	7
	2000+	2.18	(0.50)	17	19.84	(5.36)	12	-1.76	(1.96)	13
Pseudocrossidium crinitum	<1940	1.37	(0.17)	3	29.39	(3.02)	3	-2.69	(4.34)	3
	1950-70	1.46	(0.19)	5	28.98	(3.14)	5	1.12	(1.74)	5
	2000+	1.51	(0.46)	9	28.12	(6.32)	9	-4.26	(3.03)	7
Hypnum cupressiforme	<1940	1.16	(0.22)	8	38.62	(6.85)	8	-6.13	(1.71)	8
	1950-70	1.35	(0.43)	8	36.97	(4.90)	6	-5.16	(2.22)	8
	2000+	1.72	(0.62)	17	29.12	(10.41)	12	-5.19	(1.52)	14
Ischyrodon lepturus	<1940	1.59	(0.40)	6	27.58	(6.42)	6	-3.04	(2.27)	6
	1950-70	1.62		1	26.05		1	-3.67		1
	2000+	2.10	(0.50)	9	19.70	(4.82)	9	-2.96	(1.98)	9
Leptodon smithii	<1940	1.15	(0.28)	5	37.78	(9.27)	5	-6.23	(1.68)	5
	1950-70	1.41	(0.28)	7	31.23	(5.83)	7	-6.53	(2.10)	7
	2000+	1.93	(0.28)	8	23.16	(3.19)	8	-5.80	(1.94)	8

Means and standard deviations (sd) are calculated from raw data (i.e. means are not predicted / weighted by REML analysis). n = number of samples.

Table 4.8: *The influence of a) sampling period and b) sampling season on mean N content of foliage of ectohydric or mixohydric mosses collected in the Cape Metropolitan Area.*

		Ectohydric species (% dry weight)	n	Mixohydric species (% dry weight)	n
a)	PERIOD				
	Pre - 1940	1.28 [a]	30	1.38 [ab]	25
	1950 - 1970	1.55 [b]	28	1.14 [a]	30
	Post - 2000	1.80 [c]	60	1.49 [b]	81
	Mean standard error	0.049		0.078	
	Significance level	$p < 0.001$*		$p = 0.008$*	
	Wald table no.	16		17	
b)	SEASON				
	Summer	1.61	25	1.07 [a]	13
	Autumn	1.44	16	1.31 [ab]	12
	Winter	1.61	20	1.64 [b]	30
	Spring	1.48	57	1.35 [ab]	81
	Mean standard error	0.058		0.085	
	Significance level	$p = 0.404$		$p < 0.001$*	
	Wald table no.	15		17	

Means are predicted from REML analyses of the effect of sampling period on the variable N content. Wald statistics from the relevant REML analyses are presented in the Wald tables indicated (Appendix A). Values differing significantly at the $p < 0.05$ level are followed by different letters (Bonferroni multiple comparison test). * indicates a significant effect at the 1% level; n = number of samples.

Table 4.9: *The influence of sampling region (see Figure 4.1) on the mean N content, C: N ratio and $\delta^{15}N$ of moss foliage collected in the Cape Metropolitan Area.*

	N content (% dry weight)	n	C:N ratio (% dry weight)	n	$\delta^{15}N$ (‰)	n
REGION						
East	1.49	74	27.58	56	-3.71 ^{a}	55
Flats	1.56	48	26.52	37	-4.49 ^{ab}	38
South	1.40	83	28.53	69	-3.45 ^{a}	63
Montane	1.40	49	27.80	39	-5.05 ^{b}	49
Mean standard error	0.048		0.041		0.571	
Significance level	p = 0.187		p = 0.604		p = 0.005*	
Wald table no.	13		14		4	

Means are predicted from REML analyses of the effect of sampling region on the three variables. Wald statistics from the relevant REML analyses are presented in the Wald tables indicated (Appendix A). Values differing significantly at the $p < 0.05$ level are followed by different letters (Bonferroni multiple comparison test). * indicates a significant effect at the 5% level; n = number of samples.

4.3.3 Stable isotope ratios

The two primary factors influencing $\delta^{15}N$ of moss foliage were species (p<0.001, Table 4.5) and sampling region (p<0.005, Table 4.9). Mean $\delta^{15}N$ values for the moss species studied ranged from -2.08 ‰ for *Fissidens sp.* to -6.23 ‰ for *Hypnum cupressiforme* (Table 4.5) and were much depleted in the ^{15}N isotope, relative to the typical $\delta^{15}N$ values of between -1 ‰ and 6 ‰ observed for vascular plants (Heaton 1987b, Pate *et al.* 1993). In general, pleurocarps had significantly lower $\delta^{15}N$ values than acrocarps (Table 4.10b), while no significant difference in $\delta^{15}N$ existed between ectohydric and mixohydric species (Table 4.10a).

Examination of the predicted means for the effect of sampling region (Table 4.9) shows that material from high altitude sites was significantly depleted in ^{15}N in comparison to material from the south peninsula and eastern slopes of Table Mountain, but was not significantly different from material collected on the Cape Flats.

Table 4.10: *The influence of a) water conduction method and b) physiological group on mean N content, C: N ratio and $\delta^{15}N$ of moss foliage collected in the Cape Metropolitan Area.*

		N content (% dry weight)	n	C:N ratio (% dry weight)	n	$\delta^{15}N$ (‰)	n
	SPECIES GROUP						
a)	**Mixohydric species**	1.36 [a]	136	29.16	97	-4.03	93
	Ectohydric species	1.53 [b]	118	27.41	104	-4.31	112
	Mean standard error	0.044		0.038		0.445	
	Significance level	$p = 0.005^{**}$		$p = 0.133$		$p = 0.471$	
	Wald table no.	6		8		10	
b)	**Acrocarps**	1.47	185	27.94	139	-3.71 [a]	136
	Pleurocarps	1.50	69	28.88	62	-5.24 [b]	69
	Mean standard error	0.048		0.041		0.424	
	Significance level	$p = 0.210$		$p = 0.463$		$p < 0.001^{**}$	
	Wald table no.	7		9		11	

Means are predicted from REML analyses of the effect of water conduction and physiological group on the three variables. Wald statistics from the relevant REML analyses are presented in the Wald tables indicated (Appendix A). Values differing significantly at the $p < 0.05$ level are followed by different letters (Bonferroni multiple comparison test). ** indicates a significant effect at the 1% level; n = number of samples.

Unlike the situation for %N and C: N ratio, neither sampling period ($p = 0.161$, Table 4.4), nor season ($p = 0.249$, Table 4.6) had significant effects on $\delta^{15}N$. Standard deviations calculated from the raw data (Table 4.5) show that $\delta^{15}N$ was considerably more variable than %N or C: N ratio, especially in acrocarpous species. Similarly, historical trends in $\delta^{15}N$ varied widely according to species (Table 4.7).

For the three pleurocarps studied, $\delta^{15}N$ was fairly constant over the three sampling periods, while the acrocarps were rather more variable. In particular, the soil-growing acrocarps, *Campylopus sp.*, and *Pseudocrossidium crinitum*, had very variable $\delta^{15}N$ signatures (Tables 4.5 and 4.7), and material from these two species collected in the 1950-1970 period was considerably enriched in ^{15}N relative to the PRE-1940 and POST-2000 periods. When these two species were removed from the analysis, the significance of the effect of sampling region (Table 4.9) on $\delta^{15}N$ disappeared ($p = 0.147$, Wald table no. 20, Appendix A).

4.3.4 Transplants

In the transplant experiment, N concentrations (Figure 4.2a) and $\delta^{15}N$ signatures (Figure 4.2b) varied widely over the course of the year. Analysis with a repeated measures REML showed a significant collection date × site interaction for foliar N concentration ($p < 0.001$, Wald table no. 18), but not for $\delta^{15}N$ ($p = 0.102$, Wald table no. 19).

The Kenilworth and Milnerton transplants both showed a marked increase in foliar N during the rainy season, but returned to roughly the same values as the original material in October 2002 (1.05 %N for Kenilworth and 1.08 %N for Milnerton compared to 1.02 %N for initial material). In the case of Milnerton, the increased foliar N in the wet months came after a large initial decrease.

Foliar N in the Devils Peak transplants, and the back-transplants at Cape Point decreased over the year and at the end of the experiment, were lower in N than the original material. Most of these plants were brown and unhealthy in appearance, although there were some green shoots present at the end of the experiment in a few of the plants.

$\delta^{15}N$ values measured for the transplant experiment were, on average, lower than those measured in the main experiment for *Campylopus sp.* (with an average value of -6.83 in transplant material as compared to -4.37 in the main experiment). $\delta^{15}N$ showed large seasonal fluctuations which seemed to track changes in N concentration, with increased foliar N corresponding to increased enrichment of material with the ^{15}N isotope.

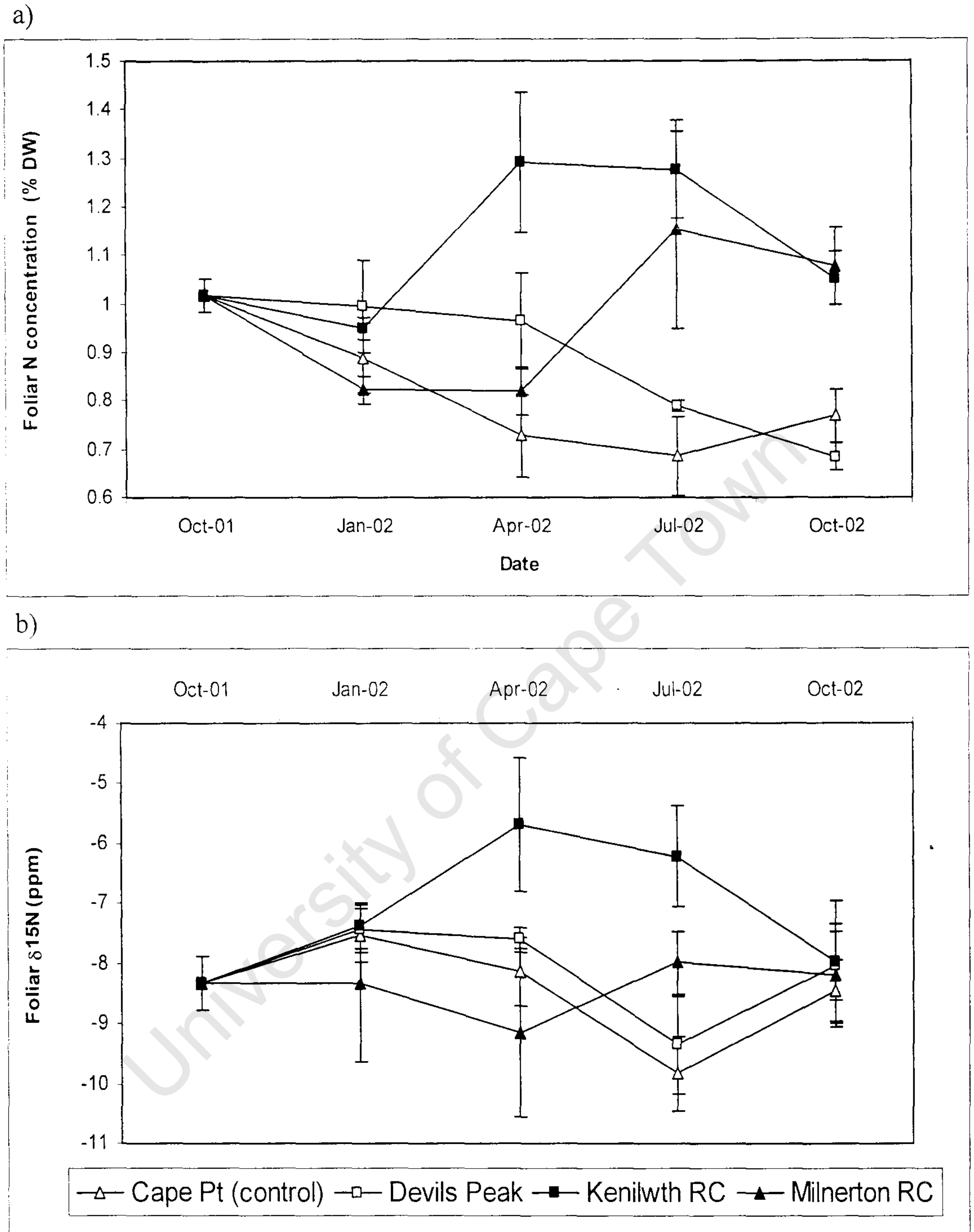

Figure 4.3 *Change in a) foliar N concentration and b) foliar $\delta^{15}N$ of Campylopus introflexus plants collected at Cape Point N.R. and transplanted back to Cape Point as well as to Devils Peak, Kenilworth R.C. and Milnerton R.C. (see Figure 4.1). Samples were collected for analysis at three month intervals over the period of a year, between October 2001 and October 2002. Values are predicted means from repeated measures REML analysis of the effect of transplant site x collection date on a) foliar N content (Wald table no. 18) and b) foliar $\delta^{15}N$ (Wald table no. 19). n=3 except for Milnerton; October 2002 and Cape Point; October 2002, where n = 2.*

4.4 Discussion

4.4.1 Nitrogen content and C: N ratio

The results of the current study confirm that there has been a large increase in the nitrogen concentration of moss tissue growing in the CMA over the past century. Given the close link between moss tissue N and atmospheric N shown in previous studies (Baddeley *et al.*1994, Pitcairn *et al.* 1995, 1998, 2003, Hicks *et al.* 2000, Solga *et al.* 2004), this pattern can almost certainly be attributed to an anthropogenic increase in the inorganic nitrogen concentration of precipitation in the CMA over that period. It might be suggested that the observed pattern could result from N loss during storage in older specimens. This is unlikely to be the case. If anything, lipids and carbohydrates would be more likely to decline with time, leading to higher N concentrations and lower C: N ratios in older specimens (Pitcairn *et al.* 1995).

The numerous potential negative effects that increased N deposition may have on biodiversity, particularly in nutrient poor systems such as the fynbos vegetation of the CMA, are discussed elsewhere in this thesis. It is ironic, though, that mosses, including some of the species used as indicators of N deposition this study, are amongst the organisms most sensitive to this threat, often being the first components of vegetation to decline or disappear from N polluted systems (Morecraft *et al.* 1994, Bobbink *et al.* 1998).

Although the trend of increasing N concentration over time was clear when all species were included in the analysis (Table 4.4), it was improved considerably by excluding mixohydric species from the data set (Table 4.8a). The mixohydric species in our study are likely to be less reliable indicators of atmospheric N deposition than the ectohydric species for a number of reasons. Firstly, as was discussed in the introduction, mixohydric species may derive a portion of their N from the substrate, thus obscuring the link between tissue N and atmospheric N. Secondly, three of the mixohydric groups analysed (*Campylopus, Pleuridium* and *Bryum*) were composed of more than one species. Although it was attempted to choose species from each of these genera that were ecophysiologically similar to one another, it may be that interspecific differences in N nutrition were important. For instance, Jauhiainen *et al.*

(1998) treated four moss species in the genus *Sphagnum* with moderate N deposition rates and found substantial interspecific differences in the magnitude of foliar N concentration increase. A third possibility is that mixohydric species, particularly "weedy" species like *Ceratodon* and *Campylopus*, may show a larger growth response to increased N (or increased CO_2) than occurs in other species. Changes in N deposition would thus not be as clearly reflected in the tissues of these species because increased N would be diluted by increased growth. Probably the most important problem with the use of mixohydric species, however, is that they generally show a much greater seasonal fluctuation in tissue N concentration than do the ectohydric species, as evidenced in the current study by the significant effect of season in mixohydric species but not in ectohydric species (Table 4.8b). This pattern is probably not directly related to the ectohydric or mixohydric habit of the species, but has rather to do with the fact that species falling into these two groups generally adopt different strategies to cope with water stress. The mixohydric study species, particularly *Campylopus sp.* and *Ceratodon purpureus,* are adapted to grow in dry habitats where they are exposed to full sun for much of the time, and in order to cope with these conditions, they have evolved the ability to dry down almost completely during periods of drought and rapidly rehydrate as soon as moisture becomes available (*Pleuridium* differs somewhat from the other mixohydric species in that the plant dies down completely during the dry season, and emerges from underground rhizoids when the rains resume the following year). Thus, tissue moisture levels in these species track seasonal rainfall variation very closely. The seasonal trend in %N arises because tissue N is, in turn, tied to tissue moisture content due to the fact that growth rate and nutrient uptake are highest when mosses are hydrated (Brown 1982) and N concentration in apical shoots is thus highest during these periods. Although the ectohydric species in this study are also desiccation tolerant to an extent, they generally (with the possible exception of *Pseudocrossidium crinitum*) prefer moist, shady habitats where drought stress is less pronounced. Because they do not dry down to the same extent as the mixohydric species during periods of drought, foliar N concentration shows no significant relationship with season in these species. This strategy of drought avoidance is more a characteristic specifically of pleurocarps than it is of ectohydric species in general, although the ectohydric acrocarp, *Fissidens* behaves very much like a pleurocarp in this regard (T. Hedderson pers. comm).

Judging from the large number of variables that influence tissue N in mixohydric species, one might not expect any significant historical trend in foliar N concentration to emerge. In the current study, however, mixohydric species seemed to show a genuine pattern, with average N concentrations decreasing between the PRE-1940 and 1950-70 periods (although not significantly so) and then increasing to a maximum in the POST-2000 period (Table 4.8a). This pattern was observed in three out of the four mixohydric species/species groups studied (*Campylopus sp., Ceratodon purpureus* and *Pleuridium sp.*; Table 4.7).

In a study measuring changes in N concentration of vegetation in Spain over the past century, Penuelas and Filella (2001) observed a similar pattern in vascular plants and, less clearly, in mosses, with %N of material collected between 1960-70 < 1940-50 < 1985-95. Their explanation was that the decrease in foliar N from the 1940-50 to the 1960-70 periods was the result of increased global atmospheric CO_2 concentrations and hence greater C availability. The large increase in %N in the 1985-95 period was then explained as coinciding with a phase of rapid increase in anthropogenic N inputs. Numerous studies have shown that foliar N concentrations in higher plants decline in response to elevated CO_2 levels (e.g. Penuelas & Estiarte 1997, Yin 2002) and the same effect has been observed in *Sphagnum* mosses (Jauhiainen *et al.* 1998). In most experiments, decreasing foliar N concentrations at elevated CO_2 seem to be explained largely by growth dilution of N (Yin 2002). However, in some studies tissue nutrient concentrations are found to decrease in response to elevated CO_2 despite little or no growth response (reviewed in Yin 2002) as was the case for *Sphagnum* in the study by Jauhiainen *et al.* (1998).

The pattern observed in mixohydric species in the current study (Table 4.8a) may be explained by growth dilution in response to increased availability of CO_2, N or a combination of the two in the 1950-1970 period, particularly as the observed pattern was clearest in the more opportunistic, weedy species, *Ceratodon* and *Campylopus* (Table 4.7). The significant increase in %N observed between the 1950-1970 and POST-2000 periods (Table 4.8a) would then be explained by a large increase in anthropogenic N inputs, consistent with the rapid growth of Cape Town which occurred over this period. If growth dilution is the explanation for the pattern observed in mixohydric species, the steady historical increase in foliar N concentrations of ectohydric mosses (Table 4.8a) would indicate that these species show little growth response to increased CO_2 or N.

Accepting that the analysis of ectohydric species produces the most reliable estimate of the change in foliar N concentration over the last century, the difficulty comes in trying to relate the magnitude of this increase to the magnitude of the increase in N deposition which was its cause. As the studies of Pitcairn *et al.* (1995, 1998, 2003) and others have shown, it is possible to estimate atmospheric nitrogen deposition from the tissue nitrogen content of ectohydric mosses with some accuracy, where the relationship between these two variables has been well characterised. Equations derived for this relationship in several European studies are displayed in Table 4.11. Clearly, it is not strictly valid to apply these equations to the results of the current study, because of species and environmental differences. Nonetheless, doing so may give an indication of the possible magnitude of historical change in N deposition in Cape Town. If the means predicted by REML analysis for ectohydric mosses in the PRE-1940 and POST-2000 periods (Table 4.8a) are substituted into the equations shown in Table 4.11, the predicted N deposition values are high, ranging from 10 to 17 $kg.ha^{-1}.a^{-1}$ for the PRE-1940 period, and from 16 to 30 $kg.ha^{-1}.a^{-1}$ for the POST-2000 period. This is likely to be because the species measured in the studies in Table 4.11 were larger and "woodier" than those examined in the current experiment or because longer apical sections were removed from the study species for analysis in these studies. Either of these possibilities would result in lowered tissue N concentration relative to deposition level. More interesting than the absolute N deposition values predicted by the equations is the predicted change in N deposition between the PRE-1940 and POST-2000 periods, which ranges from 5.8 to 13 kg N. $ha^{-1}.a^{-1}$ (Table 4.11). If these values are added to the likely background bulk N deposition level for the Western Cape measured by Stock and Lewis (1986a) (an average of 1.46 kg $N.ha^{-1}.a^{-1}$), the total N deposition values arrived at are between 7.26 and 14.46 kg $N.ha^{-1}.a^{-1}$. The logarithmic relationship between N deposition and foliar N found by Pitcairn *et al.* (1998) for a combination of several ectohydric mosses, produces the smallest estimate of current N deposition in the CMA (7.26 kg $N.ha^{-1}.a^{-1}$). A possible reason for this is that this study was conducted near livestock farms, and thus a large proportion of N deposition would have been in dry (NH_3)

form. As bryophytes are probably less efficient at the uptake of dry deposition in comparison to wet deposition (Pearson *et al.* 2000), the moss tissue measured might underestimate N deposition levels. Nonetheless, the deposition value predicted by the equation of Pitcairn *et al.* (1998) is still somewhat higher than the highest bulk deposition estimate for the CMA in chapter 3 (around 6 kg $N.ha^{-1}.a^{-1}$). The equations from the other two studies in Table 4.11 (Hicks *et al.* 2000, Solga *et al.* 2004) predict substantially higher N deposition levels. The equation of Hicks *et al.* (2000) was derived using total N values, and predicted values thus represent total N deposition (bulk deposition plus additional dry deposition). The discrepancy (8-9 kg $N.ha^{-1}.a^{-1}$) could thus represent dry deposition not measured by bulk sampling. However, the equations derived by Solga *et al.* (2004) use bulk deposition values, and still predict deposition values for my study which are 3 to 4 kg $N.ha^{-1}.a^{-1}$ higher than my highest estimate. This might suggest that the bulk deposition levels predicted in chapter 3 are underestimates. As mentioned earlier, it should be kept in mind that environmental and species differences between the current study and the European studies discussed are also likely to result in some discrepancies, and the application of equations derived in other studies remains a purely speculative exercise.

The differences in average %N content of the nine study species (Table 4.5) could be the result of any number of architectural or ecophysiological differences, and it is not easy to explain these without a thorough knowledge of the comparative ecology and physiology of the individual species. The low mean and high standard deviation of %N in *Campylopus* however, probably relates to the fact that this species spends a large part of the year in dried-down form, and the high %N values for *Ischyrodon lepturus* and *Fissidens sp.* may be due to the fact that these species prefer forested shady areas and do not dry down during summer to a great degree.

In parts of northern Europe, forest growing mosses have been found to receive slightly a higher deposition load than those growing in the open as a result of enrichment of throughfall by leachates from the canopy (Tamm 1953 cited in Bates 2000). It is unknown whether forest throughfall in the CMA is enriched in N by leachates, but given the nutrient limited nature of most local vegetation, it seems more likely to be unaffected or possibly depleted due to foliar absorption of nutrients. This is an important topic for future research.

***ble 11:** The relationships between foliar N concentration (% dry weight) of ectohydric mosses and atmospheric N deposition found in three ropean studies. Also shown are estimated N deposition values for Cape Town for the PRE-1940 and POST-2000 periods, derived by substituting the an foliar N contents of ectohydric mosses measured in the current study (Table 4.8a) into the equations below.*

Species	Deposition type	Equation [1]	r^2	Predicted PRE-1940 deposition (kg $N.ha.a^{-1}$)	Predicted POST-2000 deposition (kg $N.ha.a^{-1}$)	Predicted change in deposition (PRE-1940 to POST-2000)	Reference
vlocomium splendens	Total N	$y = 0.6 + 0.04x$	0.39**	17.0	30.0	13.0	Hicks *et al.* (2000)
leropodium purum	Bulk N	$y = 0.55 + 0.061x$	0.34**	12.0	20.5	8.5	Solga *et al.* (2004)
eurozium shreberi	Bulk N	$y = 0.58 + 0.066x$	0.43**	10.6	18.4	7.8	Solga *et al.* (2004)
:tohydric mosses	Total N	$y = 3.81(1-e^{-0.04x})$	-	10.2	16.0	5.8	Pitcairn *et al.* (1998)

p <0.001
= foliar N content (% dry weight)
= atmospheric N deposition (kg $N.ha.a^{-1}$)

It is perhaps unsurprising that no significant effect of collection site on %N and C: N ratio was found (Table 4.9). As shown in Chapter 3, bulk N deposition levels do not vary greatly across the Peninsula and lower bulk deposition levels at lower rainfall sites (e.g. Tygerberg NR) are likely to be offset by higher dry deposition levels at these sites. Although the effect is not significant, the observed pattern of %N levels decreasing in the order FLATS > EAST > SOUTH, MONTANE corresponds to the relative levels of air pollution to which these regions might be expected to be exposed based on their proximity to pollution sources. Specimens from the MONTANE region would, however, be expected to have somewhat higher N concentration due to orographic effects such as cloud droplet deposition (Fowler *et al.* 1988). There are a few factors which may have obscured the effect of altitude in this study. Firstly, the altitude of collection could not be accurately determined for a number of the herbarium samples analysed, and samples were only placed into the MONTANE group if it was certain that they had been collected at an altitude greater than 400m based on a figure given by the collector, or a precise description of the collection site, such that the altitude could be roughly estimated from a map. Thus, there may have been a number of samples collected above 400m which were included in other zones.

Secondly, previous studies have found the relationship between foliar N concentration and altitude to be approximately linear (e.g. Hicks *et. al.* 2000) and the arbitrary definition of 400m as the boundary between low and high altitude sites used in the current study is thus not a very satisfactory method of taking this relationship into account. If an accurate determination of the relationship between tissue N and altitude for the CMA is sought in future, it would be advisable to collect specimens of a single species group (or perhaps physiological group) at 100m altitudinal intervals from a single area and at a specific time of year, so as to minimize the number of variables which have an influence on tissue %N.

4.4.2 Stable isotope ratios

$\delta^{15}N$ values measured for mosses in this study were substantially lower than typical values for vascular plants (Heaton 1987b, Pate *et al.* 1993). N deposited in precipitation is generally very depleted in ^{15}N, typically being in the range of -10 and 0 ‰ (Nadelhoffer & Fry 1994, Heaton 1987a, Heaton *et al.*1997, Vitousek *et al.*

1989), and the very negative values measured may thus reflect the close coupling between moss tissue N and atmospheric N i.e. mosses receive the majority of N from deposition and this is absorbed passively by moss tissue with little fractionation occurring during uptake. The results provide no evidence, however, of a decreasing trend in foliar $\delta^{15}N$ over the past century, as was observed by Penuelas & Estiarte (1997) and Penuelas & Filella (2001) in their studies on Spanish ecosystems. The species in the current study generally showed little variation in average $\delta^{15}N$ across the three sampling periods (Table 4.7). The exceptions were *Fissidens sp.,* in which $\delta^{15}N$ seemed to increase with time, and *Campylopus sp.* and *Pseudocrossidium crinitum,* which were enriched in ^{15}N in the 1950-1970 period relative to the PRE-1940 and POST-2000 periods. The very variable nature of $\delta^{15}N$ (as indicated by high standard deviations) measured in these species makes it unlikely that this pattern is reflective of some genuine and consistent ecological pattern, however.

As discussed earlier, $\delta^{15}N$ values in mosses should be easy to interpret as they are likely to be determined primarily by the $\delta^{15}N$ of the N source (wet and dry deposition). In the Spanish studies mentioned above (Penuelas & Estiarte 1997, Penuelas & Filella 2001), the decrease observed in moss $\delta^{15}N$ over the past century was interpreted to be the result of an historical increase in the uptake of anthropogenic N, with a more negative $\delta^{15}N$ signature. These authors based the assumption that anthropogenic N is relatively depleted in the ^{15}N isotope, on the findings of Freyer *et al.* (1996) that the $\delta^{15}N$ of nitrate in polar icecaps decreased steadily in the latter part of the 20th century, coinciding with the increased emission of anthropogenic N. The factors determining deposition $\delta^{15}N$ are complex, however, and the $\delta^{15}N$ of the NH_y component of deposition also needs to be taken into account. The $\delta^{15}N$ of deposition can be thought of as a composite value because the different forms of N (reduced vs. oxidized) and of deposition (wet deposition, gaseous dry deposition and particulate/aerosol dry deposition) which make up total deposition, are usually fractionated to different degrees (Heaton *et al.* 1997, Garten 1992, Koopmans *et al.* 1997, Pearson *et al.* 2000). Thus, deposition $\delta^{15}N$ for a given area will depend upon the relative proportion of these components in total deposition. However, the relatively few empirical data which are currently available indicate that $\delta^{15}N$ signatures of the different deposition components are not always consistent, even relative to one another. The majority of studies have found NH_y-N in bulk deposition to be more depleted in ^{15}N than NO_y-N (Heaton *et al.* 1997, Garten 1992, Solga *et al.* 2004). Solga *et al.* (2004) reported a significant linear relationship between bryophyte tissue $\delta^{15}N$ and the NH_4-N: NO_3-N ratio in bulk deposition collected in Germany, with higher proportional NH_4 content resulting in more negative $\delta^{15}N$ values. However, other studies have produced contradictory results. For example, Heaton (1987a) found that NO_3 and NH_4 in rainfall at Pretoria, South Africa had roughly similar $\delta^{15}N$ values, while a German study by Koopmans *et al.* (1997) found that NO_3 was considerably depleted in the ^{15}N isotope relative to NH_4. The large degree of variability in the N isotope ratio of these ions in rainfall is likely to be because $\delta^{15}N$ depends upon the precursors from which the ions are formed (gases or particulates/aerosols), or the different mechanisms by which the precursors are dissolved in precipitation (Garten 1992). The usually depleted $\delta^{15}N$ signature of NH_4 relative to NO_3 seems to result from the washout of NH_3 gas which is generally very depleted relative to NO_x (Garten 1992, Heaton *et al.* 1997). The generalization may also be made that deposition of gases (or wet deposition formed by washout of gases) tends to be depleted in ^{15}N relative to particulate deposition (or wet deposition formed by rainout of particulates) (Heaton *et al.* 1997, Garten 1992, Pearson *et al.* 2000, Koopmans *et al.* 1997). Given the complex meteorology of the CMA, and the large spatial and temporal variations in N pollutant form and source strength in the region, the general lack of clear trends in $\delta^{15}N$ data measured in this study is, perhaps, unsurprising.

It seems unlikely that the significantly different $\delta^{15}N$ signatures observed in different species (Table 4.5) are solely determined by variations in the $\delta^{15}N$ of atmospheric deposition. The use of soil N is one possible explanation for higher $\delta^{15}N$ in certain species. Soils are usually considerably enriched in the ^{15}N isotope relative to atmospheric N because N losses by leaching and volatilization discriminate against the heavier isotope (Hogberg & Johannisson 1993, Evans & Ehleringer 1993). This is a possible reason for the significantly depleted $\delta^{15}N$ signatures of pleurocarps (which are all generally epiphytes/epiliths) relative to acrocarps (many of which are soil growing) in the current study (Table 4.10b).

Besides different N sources, the only other feasible explanation for differing $\delta^{15}N$ signatures amongst the study species is that they might discriminate against the ^{15}N isotope to different extents during uptake, allocation or losses of N (Handley & Raven 1992). Fractionation during uptake usually occurs when N supply is in excess of plant demand (Hogberg *et al.* 1999). While the vegetation of the CMA is generally very N limited (Stock & Allsopp 1992), moss demand for N is low by plant standards (Bates 2000) and the temporal increase in N concentration of tissue shown in this study suggests luxury accumulation of N. However, the lack of a cuticle in mosses, and their relative inability to regulate the uptake of solutes, means that fractionation during uptake is likely to be minimal. Similarly, the differences in $\delta^{15}N$ sometimes observed between morphologically distinct parts of vascular plants (Handley & Raven 1992) are unlikely to occur in mosses, due to the relative lack of differentiation in moss thalli and the generally poor ability of mosses to translocate chemicals. Fractionation could possibly result from the loss of depleted N as gaseous NH_3 from foliage. This phenomenon is most pronounced during senescence of an organ in vascular plants (Handley & Raven 1992) and, presumably, in mosses when they are dried down. The result would be a relative enrichment of foliar $\delta^{15}N$ in species which dry down frequently. This situation is not clearly observed in the current experiment, although pleurocarps, which generally remain hydrated throughout the year, did have significantly more negative $\delta^{15}N$ values than acrocarps, most of which dry down substantially during dry periods (Table 4.10).

The significant influence of sampling site on $\delta^{15}N$ (Table 4.9) is interesting in that there was no effect of sampling site on %N or C: N ratio. Presumably, the observed pattern is explained predominantly by variations in the $\delta^{15}N$ signature of rainfall in the different sampling regions. It is difficult, however, to offer specific explanations of spatial patterns in deposition $\delta^{15}N$ because, as discussed earlier, the factors determining this value are complex. Based on the earlier discussion, the significantly depleted $\delta^{15}N$ signature of leaf material from the MONTANE region relative to the SOUTH and EAST regions (Table 3.9) seems incongruous. The results of the bulk deposition study showed that the Table Mountain site received a lower proportion of bulk deposition in NHy-N form relative to other sites (Chapter 3, Table 3.9), and washout of gaseous deposition (relative to rainout of particulates) would also be expected to be lowest here. Both of these characteristics would tend to produce a relatively less depleted $\delta^{15}N$ signature at this site. Apart from the possibility that NH_y deposition in the CMA is enriched in the ^{15}N isotope relative to NO_y, as has been found by several other authors (see Koopmans *et al.* 1997) the other conceivable explanation for this pattern, is that altitude may have an effect on $\delta^{15}N$. Previous studies of foliar $\delta^{15}N$ in vascular plants have found no relationship between altitude and foliar or soil $\delta^{15}N$ (Handley *et al.* 1999). However, bryophytes differ greatly from vascular plants in that their N supply is predominantly from precipitation, and this pattern may thus relate to the larger amount or orographic nature of precipitation which occurs in high altitude areas. It is possible that the orographic processes which produce a concentration of N in deposition at altitude (Fowler *et al.* 1988) may also discriminate against the heavier isotope, producing more depleted deposition in high altitude areas. Cloud water is well known to be significantly depleted in the heavy isotopes of oxygen (^{18}O) and hydrogen (^{2}H) relative to precipitation (Clark & Fritz 1997), but I have found no research on this topic for N.

Two studies have found that direct exposure to traffic emissions causes enrichment of moss tissues with the ^{15}N isotope (Pearson *et al.* 2000, Gerdol *et al.* 2002) attributed by the authors to the high NO_x content of emissions. The UK study by Pearson *et al.* (2000) measured $\delta^{15}N$ values of between +6 and -1 ‰ near busy motorways (10-20 m), intermediate values of between +2 and -4 ‰ at moderate to low traffic densities, and depleted values ranging from -2 to -12 ‰ in rural areas with little or no traffic. More depleted signatures in rural regions were attributed to a larger contribution of NH_y from farming activities in these areas (Pearson *et al.* 2000). The relatively negative $\delta^{15}N$ value for the FLATS region in the current study (Table 4.9) (although not statistically different from the other regions) is thus unexpected, as the numerous large roads running through this region would be expected to result in relatively high NO_x deposition. There may be several reasons for this. Firstly, I intentionally did not collect samples in close proximity to large roads or motorways, and material collected from the FLATS region is thus probably more closely allied to the material from areas of moderate traffic flow in the study of Pearson *et al.* (2000). Secondly, a recent study by Cape *et al.* (2004) contradicts, to some extent, the conventional view that NO_x deposition exceeds NH_y deposition in close proximity to roads. According to the findings in that study, modern vehicles fitted with three-way catalytic converters produce sizeable NH_3 emissions. Although roadside aerial NH_3 concentrations remain well below those of NO_x, NH_3 currently accounts for roughly 50% of N deposition in close proximity to roads in the UK, by virtue of its much higher deposition velocity.

Furthermore, relatively higher NH_y-N deposition in the FLATS region as a result of agriculture might counteract the effect of higher NO_x deposition here. As shown in chapter 3, the proportion of bulk deposition in reduced form is highest in this region (Kenilworth RC and Tygerberg NR in Table 4.9, Chapter 3), and dry NH_3 deposition is also likely to be elevated here, due to the influence of nearby agricultural activity.

4.4.3 Transplants

The results of the transplantation experiment were disappointing in that the plants transplanted to urban areas did not show any clear change in foliar N concentration which could be attributed to N deposition. This was probably due to the pronounced effect of season on the foliar %N of the mixohydric study species *Campylopus introflexus* (Table 4.8b), which would have obscured such an effect. An ectohydric species would have been a better choice for the transplant experiment, but it proved difficult to find a species which occurred at all the study sites. The transplants to Kenilworth R.C. and Milnerton R.C. demonstrate the seasonal effect on *C. introflexus* very clearly, although the Milnerton transplants seemed adversely affected by the transplantation process in the initial stages of the experiment (Figure 4.2a). Despite a sizeable increase in foliar N at these two sites during the wet months, foliar N concentration at the end of the year was only very slightly higher than that of the initial material, providing no evidence of elevated N deposition at either of these two sites relative to the Cape Point N.R. control site. The transplants to Devils Peak and the back transplants to Cape Point seemingly did not take well to transplantation, and %N in these plants declined almost throughout the year. At Kenilworth and Milnerton, the transplants were placed on relatively moist acid sand flats, a similar habitat to the one from which they were collected at Cape Point, and this may explain why these transplants survived better than those placed on the clay soils of lower Devils Peak. The negative effect of back transplantation on the controls is unexplained, however.

Although the interaction between collection date and site was not significant for $\delta^{15}N$, it is evident from a comparison of Figures 4.2a and 4.2b that transplant $\delta^{15}N$ was related to N content, with N- replete material being relatively enriched with the ^{15}N isotope. The reason for this is unclear. The withdrawal of N from growing shoots during periods of drought would be likely, if anything, to favour the lighter isotope and result in enrichment of the remaining N. Similarly, gaseous loss of NH_3 from dried down leaves would result in enrichment of dry nutrient poor material. It is conceivable that $\delta^{15}N$ might increase in tandem with foliar N due to the increased foliar storage of N as amino acids, as foliar amino acid concentrations have been shown to be closely tied to foliar N concentration (Pitcairn *et al.* 2003) and certain amino acids and proteins are enriched in the ^{15}N isotope relative to the N source (Werner & Schmidt 2002, Gonzalez-Prieto *et al.* 1995). However, compound specific isotopic analysis of bryophytes has not been carried out to date, as far as I am aware.

4.5 Summary

This study has found a strong trend of increasing foliar N concentration in mosses collected in the CMA over the past century, which is indicative of a large increase in atmospheric N deposition over that period. Ectohydric species showed this trend very clearly, while mixohydric species generally showed a different trend, with decreased foliar N concentrations occurring in the 1950-1970 period relative to the earlier and later periods. This pattern may reflect growth dilution of tissue N in these species in response to increased CO_2 availability in that period, as the mixohydric study species are likely to be more growth responsive than the ectohydric species. Mixohydric species were also found to be less useful as indicators of N deposition because tissue N levels were strongly influenced by season. Based on relationships between ectohydric moss tissue N and N deposition levels found in European studies, the increase in tissue N concentration of ectohydric mosses in the CMA since the pre-1940 period would represent an increase in N deposition of between 6 and 13 kg N.ha^{-}

$^{1}.a^{-1}$. Foliar N was not affected by the region of the CMA from which moss samples were collected, although this may be the result of the poor spatial resolution of sampling. $\delta^{15}N$ values were generally variable and showed no clear trend of decrease over the past century, as predicted. This is probably the result of the many factors which determine deposition $\delta^{15}N$ in an area of complex topography and climatology such as the CMA.

Chapter 5
General discussion

In this project, two lines of evidence have been examined, both of which have indicated that a substantial increase in N deposition has occurred in the CMA over the past century. In Chapter 3, bulk deposition sampling was carried out in order to measure, directly, the input of atmospheric N to areas of natural vegetation. In spite of several technical difficulties, we measured a range of N deposition levels at the five sampling sites, from 2.5 kg $N.ha^{-1}.a^{-1}$ at the relatively unpolluted Silvermine site to 5.5 kg $N.ha^{-1}.a^{-1}$ at Kenilworth R.C, a site representing the Cape Flats, and a similar level of 5.3 kg $N.ha^{-1}.a^{-1}$ on the summit of Table Mountain (Table 3.9). As discussed, the highest values measured may be closer to 6 or 7 kg $N.ha^{-1}.a^{-1}$, as tests showed that consumption of inorganic N during storage may have occurred. This represents a historical increase in bulk N deposition of between 4 and 5.5 kg $N.ha^{-1}.a^{-1}$ if values measured at a nearby unpolluted site by Stock & Lewis (1986b) are representative of background levels. In addition, dry deposition not measured by bulk sampling (mostly gaseous deposition) is likely to be substantial to vegetation in the lowlands of the CMA, particularly during the dry summer months and during late autumn/early winter, when low level temperature inversions cause elevated aerial pollution concentrations over the Cape Flats.

In chapter 4, a clear and substantial historical increase was measured in the tissue N levels of ectohydric mosses in the CMA, providing a second line of evidence for a large increase in local N deposition levels. Based on the results of several European studies, which have shown a strong relationship between ectohydric moss tissue N concentration and N deposition level, the magnitude of increase in moss tissue N in the CMA would represent an increase in total N deposition over the past century of between 5.8 and 13 kg $N.ha^{-1}.a^{-1}$ (Table 4.11, chapter 4). However, these figures remain speculative due to species and environmental differences between our study and European studies.

Given the difficulty in translating moss tissue N into an estimate of N deposition, and because of the unknown magnitude of dry deposition and the possibility of microbial consumption of N during storage in the bulk deposition experiment, it is difficult to suggest likely total N deposition values for the CMA. However, based on all the information discussed, it is conservatively suggested that current total N deposition (excluding organic N) is likely to be highest on the Cape Flats, and is probably in the range of 8 to 12 kg $N.ha^{-1}.a^{-1}$, with substantial levels also occurring on the eastern slopes and plateau of Table Mountain.

Results indicated that, in addition to N enrichment, acidification is a potential threat to local ecosystems which deserves attention. Although only measured for 8 months, SO_4 levels were surprisingly high with extrapolated annual bulk deposition levels of up to 16 kg SO_4-$S.ha^{-1}.a^{-1}$ at Kenilworth RC, not including the unknown dry deposition component. The combined deposition of SO_4 and NO_3 with associated protons thus has considerable potential to cause acidification in the CMA.

In this final chapter, the likely consequences of my findings for the natural vegetation of the CMA are discussed, based on knowledge of the ecology of local ecosystems and on the observed effects of pollutant deposition on ecosystems in other parts of the world (see synthesis in Chapter 2). Also discussed are possible restorative actions, which may help to control the local N deposition problem.

5.1 Exposure of vegetation in the CMA to atmospheric deposition

The CMA includes four main vegetation types; fynbos, renosterveld, strandveld/coastal thicket and afromontane forest, the respective distributions of which are determined primarily by substrate. Most vegetation in the region is classified as fynbos, a type of mediterranean shrubland which generally occurs on nutrient poor acid sand substrates. Fynbos can be broadly divided into two sub-types. Mountain fynbos covers most of the Table Mountain chain and southern peninsula and sand plain/lowland fynbos occurs predominantly on the central Cape Flats (Figure 3.2, chapter 3). The fynbos of the CMA is extremely species rich and contains many local endemics, making it a high priority for conservation. This is particularly true of sand-plain fynbos which, due to the suitability of its lowland habitat for human settlement, has been reduced to a few small fragments scattered amongst the urban sprawl of Cape Town. These sites contain an inordinately high concentration of rare and endangered species (Maze & Rebelo 1999).

Renosterveld is a shrubland vegetation rich in geophytes which occurs, in the CMA, on finely textured shale-derived soils at Tygerberg Hill, Signal Hill and Devils Peak (N Slopes). Renosterveld, like sand-plain fynbos, is a highly endangered vegetation type, and the few sites in the CMA preserve numerous threatened species. Tygerberg Nature Reserve, for instance, is home to 8 Cape Flats endemics and 3 site endemics (Maze & Rebelo 1999).

Strandveld occurs on calcareous sands along the coastal margins of much of the CMA. Despite being less species-rich than renosterveld or fynbos, it is still considered conservation worthy, as little of this lowland vegetation type remains in the CMA. Noteworthy strandveld conservation sites in the CMA include Wolfgat Nature Reserve and Macassar Dunes, both of which lie in the southern part of the Cape Flats on the False Bay coast (Figure 3.2, chapter 3).

Afromontane forest in the CMA is restricted to shaded gullies and ravines on the Table mountain chain, with the most substantial expanses occurring on the lower eastern slopes of Table Mountain itself. Afromontane forest is also a high priority for conservation as it very restricted in extent both locally and countrywide.

Bulk deposition of both N and S measured in this study was highest at the sand-plain fynbos site, Kenilworth RC, which occurs in the western part of the Cape Flats. This region, which also includes Rondebosch common and Rondevlei, will receive the highest bulk deposition levels on the flats, due to orographic rain from Table Mountain (Figure 3.3, chapter 3). These are amongst the most valuable remaining sand-plain fynbos preserves, containing numerous Cape Flats endemics and 6 site endemics between the three of them (Maze & Rebelo 1999). As discussed in chapter 3, dry deposition, which was not measured in this study, is also likely to be greatest on the Cape Flats, because dry deposition levels generally increase with proximity to pollution sources (Wesely & Hicks 2000). Dry deposition levels will probably be greatest in the drier northerly and easterly parts of the flats due both to higher pollution emissions and lower wash-out of pollutants by rainfall in this region, of which the Tygerberg site is representative in our study. Although bulk deposition levels were relatively low at Tygerberg (Table 3.9, chapter 3), volume-weighted mean concentrations of N in bulk deposition were highest here (Table3.3, chapter 3), indicating a high source strength for N pollution and a large potential for dry deposition. Sites in this drier part of the flats may thus receive total N deposition levels approaching those in the western region. In addition to the valuable renosterveld conserved at Tygerberg itself, a number of valuable lowland fynbos sites occur in the vicinity including Milnerton and Durbanville race courses, Uitkamp, Blouvlei, Rietvlei, Plattekloof Natural Heritage Site and Cape Flats Nature Reserve.

With respect to the mountainous regions of the CMA, results indicated that bulk deposition on the summit of Table Mountain is of similar magnitude to that in the wet westerly part of the Cape Flats, with intermediate levels occurring on the lower eastern slopes of TM (represented by Devils Peak) and low levels occurring in the mountains to the south of the city (represented by Silvermine N.R.). As discussed earlier, the substantial deposition measured on the summit of Table Mountain (which is likely to be considerably higher if occult deposition is taken into account) is partly the result of orographic enhancement of cloud water and rainfall concentrations, and it is uncertain whether deposition is naturally elevated in this region (and plant communities here are pre-adapted to this), or if the high values reflect a magnification of anthropogenic N.

At the low altitude Devils Peak site on the eastern slopes of the range, bulk deposition levels are only slightly lower than at the summit, presumably the result of a combination of high rainfall and intermediate pollution exposure in that region (Keen 1979). In addition to fynbos and renosterveld/ fynbos transition, much of this region (south of Devils Peak itself) is vegetated by afromontane forest which occurs predominantly on nutrient poor sandy soils similar to those which support fynbos. Strandveld is restricted to coastal areas, which are generally fairly remote from pollution sources, and which experience regular sea breezes. This suggests that this vegetation type will experience relatively little exposure to pollution.

5.2 Sensitivity of vegetation in the CMA to atmospheric deposition

Due to differences in substrate, climate, nutrient economy and structure, the vegetation types of the CMA will vary widely in their sensitivity to the eutrophying, acidifying and toxic effects of atmospheric deposition. In this context, sensitivity is defined as the vulnerability of the vegetation to detectable changes in health, structure or diversity as a result of elevated atmospheric deposition. This includes the vulnerability to a deposition-induced invasion by, or increase in the success of exotic species.

5.2.1 Sensitivity to eutrophication

Eutrophication is expected to be the most serious consequence of atmospheric deposition for the vegetation of the CMA, although this supposition is based mainly on theoretical considerations and findings in other ecosystems rather than local empirical evidence.

Some indication of the likely N sensitivity of local vegetation can be gleaned from critical loads established for European vegetation. Most of these estimates are based on extensive empirical evidence, although some are "best guesses". In Europe, if ombrotrophic wetlands are excluded, the most sensitive vegetation types are considered to be alpine heathlands (with a critical load of 5-15 $kg.ha^{-1}.a^{-1}$) and species rich, acidic heathlands and grasslands (7-15 $kg.ha^{-1}.a^{-1}$), while other grasslands (10-30 $kg.ha^{-1}.a^{-1}$) and heathlands (10-22 $kg.ha^{-1}.a^{-1}$), as well as deciduous and coniferous forest (10- 50+ $kg.ha^{-1}.a^{-1}$), are usually thought to be the less susceptible to structural or compositional changes (Bobbink & Roelofs 1995). This might suggest that fynbos, which is also broadly classified as a species-rich acidic heathland, is the most vulnerable local vegetation, and that the N deposition load of 8 -12 $kg.ha^{-1}.a^{-1}$ thought to be occurring locally, is likely to exceed the critical load. It would also suggest that other local shrublands and afromontane forest are less cause for concern. Clearly, however, meaningful estimates of local vegetation sensitivity cannot be based on European systems, as the local substrates and vegetation are very different to their European counterparts in many respects.

When no empirical evidence is available, it has been suggested that critical loads could be based upon the natural nitrogen availability in the system under consideration (Kuylenstierna *et al.* 1998). In N-poor systems, the availability of N (and other nutrients) is a key factor in determining plant community composition and ecosystem structure, and the stability of these systems is easily disrupted by nutrient additions (Aerts 1990, Chapin 1987). If N is already an abundant and non-limiting resource in an ecosystem, however, added N will have little effect unless it reaches toxic levels. Theoretically, N sensitivity will be roughly inversely related to the absolute availability and also the range of availability of N in the system i.e. the more N-poor a system is, and the lower the range of available N levels in that system, the more sensitive it will be to N addition (Kuylenstierna *et al.* 1998). As discussed later, ecosystem N sensitivity may also be influenced by a number of other factors besides N availability. Nevertheless, it is useful as a broad indicator of N sensitivity.

Based on N availability, fynbos is again predicted to be the local vegetation type most susceptible to the effects of N deposition. In most cases, fynbos occurs on strongly leached sands which are very poor in nutrients, particularly N and P (Stock *et al.* 1988, Mitchell *et al.* 1987, Witkowski & Mitchell 1987). The very low availability of inorganic N in fynbos soils is predominantly the result of leaching of nutrients from the coarse sandy substrates on which the vegetation typically occurs, and slow nutrient cycling. As is the case in other mediterranean vegetation types, fynbos is dominated by evergreen sclerophyllous shrubs which are highly conservative of N (i.e. they have high nutrient use efficiency). Very little litter is produced and that which is, is of poor quality i.e. characterized by high C: N ratios (often in the range of 100:1) and a high content of phenolic compounds (tannins and lignin) (Mitchell *et al.* 1986, Stock & Allsopp 1992). For this reason, decomposition and mineralization of N are extremely slow processes in fynbos, with typical soil surface (0-10 cm) net N mineralization rates of around 10 kg $N.ha^{-1}.a^{-1}$ (Stock *et al.* 1995, Yelenik *et al.* 2003) and soil surface (0-10 cm) inorganic N levels fluctuating between about 1 and 5 μg N. g^{-1} soil over the course of the year (Stock & Lewis 1986b, Witkowski 1988). These values are low by world standards and lower than in European heathlands, as well as most other mediterranean shrublands (Specht & Moll 1983, Read & Mitchell 1983, Mitchell *et al.* 1987). In such a low N environment, the total N deposition level of 8-12 $kg.ha^{-1}.a^{-1}$ suggested earlier for the most polluted parts of the CMA, represents a sizeable N input (an additional 80 to 120 % of annual inorganic N budget from mineralization).

The poor N status of fynbos soils in the CMA is demonstrated by the soil surface inorganic N contents measured in early summer at sites in mountain fynbos (Table Mountain and Silvermine) and lowland/sand plain fynbos (Kenilworth, Milnerton and Cape Point) (Table 4.3, chapter 4), although these values show seasonal fluctuations (Stock & Lewis 1986b). As discussed in chapter 3, the measured values, particularly at Kenilworth and Milnerton, may already be somewhat elevated due to N deposition and/or elevated mineralization rates resulting from the accumulation of grass litter. Other vegetation types in the CMA are likely to be less sensitive to N addition than fynbos. Renosterveld occurs on fine textured shale-derived soils, which leach little and are relatively rich in N and other nutrients (Moll & Campbell 1976). Soils derived fully or partially from shale are found at both the Tygerberg and Devils Peak sites in the current study, which explains their high available N contents in comparison to the other sites (Table 4.3). Strandveld soils, although nutrient poor in comparison to renosterveld soils, still tend to have higher nutrient availability and N mineralization rates than fynbos (Stock *et al.* 1995). Afromontane forest in the CMA typically occurs on nutrient poor sandy substrates, similar to those supporting fynbos (von Maltitz *et al.* 2003). However, forest species produce litter of a higher quality and in greater amounts than fynbos, and the shady moist nature of the forest floor in combination with high accumulation of SOM, will probably facilitate higher mineralization rates than those found in fynbos soils. Furthermore, the tall structure and slow growing nature of forest will mean that this vegetation type will tend, at least in the short to medium term, to be resistant to N induced invasions or changes in vegetation structure, except possibly in the understorey. Other potential effects of N deposition, including nutrient imbalances and changes in root to shoot ratios, remain a possibility in local forest, however.

Only two limited studies have actually investigated the effects of N addition on local ecosystems, both of them in fynbos. Witkowski (1988, 1989a, b) added a single dose of NH_4NO_3, equivalent to 50 kg $N.ha^{-1}.a^{-1}$ to sand-plain fynbos and observed the response over two years. Slight increases in shoot growth, as well as limited changes in morphology, phenology and reproductive allocation were observed in the dominant growth forms, indicating N limitation (Witkowski 1988, Witkowski *et al.*1990a). However, the majority of N taken up by plants was not used for growth, but stored, as indicated by clear increases in shoot N contents (Witkowski 1988). This is indicative of the inherently low growth rates and low growth plasticity of the dominant fynbos shrubs. Only graminoids, restioids and annuals showed observable increases in foliar projective cover in response to N (Witkowski 1989a). Lamb & Klaussner (1988) added 20 $kg.ha^{-1}$ of N (NH_4NO_3) to mountain fynbos, and found that, in contrast to the results of Witkowski, growth of two dominant shrub species (*Erica plukenetii* and *Protea repens*) was negatively affected. However, in *E. plukenetii*, growth was positively affected by the addition of P and, particularly, the combination of P and N. This species was thus seemingly limited primarily by P, and reduced growth of both study species in response to N alone was probably the result of a nutrient imbalance.

Other shrub species, and particularly other functional types in this vegetation such as graminoids, may, however, have been N limited rather than P limited, as different species may be limited by different nutrients or combinations of nutrients (Roem *et al.* 2002, Kirkham 2001). Although observed responses were small, the results of Witkowski (1988,1989a), Witkowski *et al.*(1990a) and Lamb & Klaussner (1988) indicate that relatively small N additions can have an effect in fynbos. As discussed later, however, the response of the study ecosystems to the single large nutrient pulses which were applied in these experiments may be very different to the responses which would be observed if regular smaller N loads, more closely approximating atmospheric deposition, were applied.

All things considered, it seems likely that the critical load of N for fynbos (i.e. the threshold deposition level above which negative effects on vegetation health, diversity or structure are likely to be observed) will be low, and probably lower than the 8-12 kg $N.ha^{-1}.a^{-1}$ which I predict is currently being deposited to vegetation in the more polluted parts of the CMA.

5.2.2 Sensitivity to acidification

The sensitivity of an ecosystem to acidic deposition depends both upon on the buffering ability of the soil and the sensitivity of its constituent organisms to changes in soil chemistry (Kuylenstierna *et al.* 2001). Renosterveld and strandveld soils are relatively well buffered (they have high pH and high base cation contents; Mitchell *et al.* 1987). Soil acidification is thus of minimal concern in these soils, as acid input should be neutralised by carbonate reactions involving base cations. In contrast, fynbos soils are generally very acidic (typically pH 3.5-5.5) and have very low base cation contents (Mitchell *et al.* 1987, Stock *et al.* 1988, Witkowski *et al.* 1990b) and high availability of toxic metal ions such as Al^{3+} (Witkowski & Mitchell 1987). These soils are thus poorly buffered and are unable to adequately neutralise acidic inputs. At the same time however, fynbos plants have evolved under these conditions and are highly adapted to deal with the problems they pose.

The importance of soil buffering ability versus vegetation acid-tolerance as determinants of ecosystem sensitivity to acidification is debatable. Kuylenstierna *et al.* (2001) considered soil buffering ability to be a more important indicator of ecosystem acidification sensitivity than vegetation resistance to acidity, and based their global critical loads solely on this factor. However, the majority of European species which have declined as a result of acidification are those growing naturally in soils with pH > 5 (Roem & Berendse 2000, van Dobben 1991 cited in Roem *et al.* 2002) and it is sometimes suggested that acidification will not pose a serious threat to vegetation from naturally acidic environments (e.g. Phoenix *et al.* 2005). Although this may generally be true, some studies have shown that, even in highly acidic environments, sensitive species which prefer less acidic microsites can be affected by acidification. Roem *et al.* (2002) applied dilute H_2SO_4 (0.96 $mol.m^{-2}.yr^{-1}$) to an already acidic Dutch heathland (average soil pH 4.3) in order to double ambient atmospheric acid deposition, and observed a reduction in species diversity and reduced germination of seedlings in a number of species. The authors concluded that "on these nutrient-poor, weakly buffered soils, the greatest threat to species richness is acidification".

In fynbos, within site soil acidity may vary substantially, and fynbos species distributions are known to be related to soil pH (e.g. Richards *et al.* 1997). Although acid deposition levels measured in the current study are considerably lower than those in the study by Roem *et al.* (2002), species preferring higher pH soils could be affected by acidification, even if the dominant or characteristic species are not. Even if acidification does not lead directly to species losses, it seems likely that further leaching of important nutrient base cations and reduction of already low P levels, as well as increased H^+ and Al toxicity, might place additional stress on fynbos vegetation and reduce its health. This threat is, nevertheless, considered to be secondary to the problem of eutrophication from N deposition.

5.2.3 Sensitivity to direct toxic effects

The main toxic threat posed by N and S deposition in the CMA is to the "lower" plants (lichens and bryophytes) which may accumulate pollutants to toxic levels because of their lack of stomata and leaf cuticles, and their inability to rapidly assimilate, store or eliminate pollutants (Lee & Caporn 1998, Morgan *et al.* 1992). Damage is typically the result of cellular acidification and, in some cases, a build up of toxic levels of foliar N (Woodin *et al.* 1985, Wellburn 1990). In Europe, 10 $\mu g\ m^{-3}$ (0.034 ppm) is the critical aerial concentration of SO_2 for cryptogams. As can be seen from Figures 3.14 and 3.15 (Chapter 3), SO_2 concentrations are still routinely higher than this in the lowlands of the CMA and have been for a long time. Loss of cryptogam species on the Cape Flats may thus already have resulted. One study conducted in the CMA has shown that lichen diversity decreases with proximity to industrial pollution sources (Muofhe 1994).

Direct toxic effects from airborne N and S compounds to higher plants only occur when these pollutants are present at high concentrations. Such effects are thus of limited concern in the CMA except in very close proximity to large point sources (e.g. industries for S or livestock farms for N). As aerial concentrations of vehicle derived pollutants generally decrease by more than 90% at a distance of 10-15 m from large roads (Cape *et al.* 2004), direct toxic effects are seldom observed. However, a local site which may be threatened by direct effects from vehicular pollution is the N1/N7 interchange, which lies at the intersection of two major roads and was identified by Maze & Rebelo (1999) as a core conservation site.

Although not directly related to N or S deposition, it should be noted that O_3 levels in Cape Town might be damaging to local vegetation. In Europe, exposure to an O_3 concentration of 60 $\mu g\ m^{-3}$ over an 8-hour period is considered to be the critical level for injury to natural vegetation (UN-ECE 1994). In Cape Town, 8-hour exceedances of double this value (i.e. 120 $\mu g\ m^{-3}$) occur several times a year at the Goodwood (1 to 9 exceedences per year) and City Centre (2 to 14 exceedences per year) monitoring stations, and even average annual O_3 concentrations are close to this threashold (30-40+ $\mu g\ m^{-3}$ at the City Centre and 20-30+ $\mu g\ m^{-3}$ at Goodwood) (City of Cape Town 2003). However, Botha (1989) found no injurious effects of either O_3 or SO_2 on plants growing near a fertilizer factory and a petrol refinery in Cape Town.

5.3 Potential effects of N deposition on ecosystems in the CMA

From the previous discussion, it is apparent that fynbos is the vegetation type both most sensitive and most exposed to atmospheric deposition in the CMA, and contains the majority of rare and endemic species occurring in the region. It is also clear that the eutrophying effect of N deposition is likely to be the primary threat to fynbos, while acidification effects and direct toxicity are of secondary concern. The remainder of this discussion will thus focus predominantly on the possible effects of N deposition on fynbos ecosystems.

5.3.1 N retention

The potential for N deposition to negatively affect vegetation will depend, to some extent, on how much of the added N is initially retained by the system, and whether N will build up in the system over time or be periodically lost (Bobbink *et al.* 1998). In the long term, N losses from uninvaded fynbos ecosystems are expected to be relatively high for several reasons. Firstly, fynbos generally occurs on coarsely textured sands with low cation exchange capacity, which are very susceptible to leaching of mobile ions such as NO_3 (Witkowski *et al.* 1990b, Stock & Lewis 1986b). In the N addition experiment by Witkowski *et al.* (1990b), most NO_3 added to the soil was rapidly leached in winter while the majority of NH_4 was seemingly retained in the soil surface layers. It is not known what proportion of NH_4 retention was due plant uptake, microbial immobilization or abiotic factors (condensation reactions with phenolic compounds or adherence to negatively charged colloids). Most fynbos plants prefer NH_4 to NO_3 as an N source, although many species may also use NO_3 (Stock & Lewis 1984) and this preference might also exist in soil microbes. This suggests that the majority of NH_4 deposited from the atmosphere may thus be retained by local ecosystems, which is significant because NH_4 was the dominant form of N input at all sites in the current study, with the exception of Table Mountain.

It should be noted that, in studies by Witkowski *et al.* (1990b) and Stock & Lewis (1986b), which measured substantial leaching, N was effectively added in a single large dose (in the latter case, due to the post-fire deposition of N rich ash). A greater proportion of NO_3 would probably be retained if inputs were smaller and more regular as is the case with atmospheric deposition. Regular N inputs would, for example, be likely to stimulate populations of free-living microbes, especially those with high N demand and ability to utilize NO_3 (Norby 1998 and see chapter 2). It is also possible that mycorrhizal immobilization of N, which is suggested by Aber *et al.* (1998) to be important in temperate forests (see chapter 2), could be important in fynbos, as mycorrhizal associations are widespread in fynbos (Allsopp & Stock 1993). These topics require further investigation in local systems.

The dry weather which occurs in summer in most parts of the CMA (with the exception of certain portions of the Table Mountain chain, which receive summer orographic rain) may contribute to high leaching losses of N. During this period, dry deposited N probably accumulates on the soil surface, as is suggested by summer soil analyses for sites on the Cape Flats (Table 4.3, chapter 4). Much of this N would be leached in a single rapid flush with the first rains of winter, especially because biotic demand for N would only peak some time later.

There are a number of factors that will determine whether plants will be an important sink for N in fynbos. The first of these has to do with the degree to which the vegetation is limited by N. Although N seems often to be a primary limiting nutrient, as was found by Witkowski (1988) in sand plain fynbos, plants are, in some cases, limited primarily by nutrients other than N, or will become limited by these nutrients as deposited N accumulates. Witkowski (1988) suggests that fynbos occurring in high rainfall areas may often be limited by P, as soil N: P ratios tend to be higher in these systems. This is supported by the findings of Lamb & Klaussner (1988) that, in mesic mountain fynbos (annual precipitation of around 1400 mm), plant growth was stimulated by P and not N addition. Although it is purely speculation, it may be that, in the CMA, fynbos in higher rainfall regions (the plateau and eastern slopes of Table Mountain) will be limited primarily by P, while the drier lowlands will be more N limited. Witkowski's (1989b) results also showed that other nutrients besides N and P limit some aspects of plant performance in lowland Fynbos, as did those of Schutte (1960) in mountain fynbos. In fact, as discussed earlier, different plant species may be limited to different extents by different nutrients or combinations of nutrients (e.g. Roem *et al.* 2002, Braakhekke & Hooftman 1999). Not only would a shift to limitation by P or another nutrient cause plant N saturation, but, as discussed later, it may also negatively affect plant performance by creating a nutrient imbalance, thereby further reducing N demand.

Even where N is clearly the limiting resource, retention of N by fynbos vegetation, particularly by the dominant proteoid and ericoid shrub species, is expected to be low. Many fynbos plants have extensive root systems or form associations with mycorrhizal symbionts, allowing for efficient uptake of N from the soil (Lamont 1983, Allsopp & Stock 1993). However, in the long term these plants are not expected to be a big sink for N because they are generally very N conservative and have low growth plasticity. It is expected that most N taken up by plants will be stored rather than being used for growth, as was found by Witkowski (1988) and Witkowski *et al.* (1990a). Although the results of Witkowski (1989b) indicate that litterfall and N return may increase slightly in response to N addition, the evergreen sclerophyllous shrubs which predominate will, nevertheless, rapidly become N saturated. Witkowski's (1989a) experiment showed that indigenous shallow rooted, herbaceous species such as restioids, grasses and annuals are more plastic in their growth, which suggests that they will be a proportionally greater N sink relative to their biomass. The greatest plant N sink in the CMA, however, is expected to be weedy exotic or cosmopolitan grass species, which are adapted to use added N, including NO_3, to rapidly increase biomass. Such species have high rates of tissue turnover, and are able to maintain N demand by constantly incorporating added N into organic matter and transferring it to the soil as N rich litter. This will greatly increase the system's ability to store N and, as discussed later, may also lead to faster rates of N cycling.

A very important characteristic of fynbos, which will have a major influence on long term retention of N, is its tendency to burn regularly. Fire plays a vital role in fynbos succession with many species relying on regular burning (at intervals of 4 - 45 years) for their continued survival (van Wilgen *et al.* 1992). Because net N mineralization is extremely low under normal circumstances, it is thought that fire is the primary agent of N release in fynbos (Stock & Allsopp 1992). During a fire, a large proportion of N in foliage and the upper soil layers is volatilized. For example, Stock (1985) calculated that around 20.4 % of the above ground N pool was lost during a fire in sand-plain fynbos. The deposition of ash onto the soil surface causes a flush of N immediately following a fire, but this N is rapidly depleted through biological uptake and leaching, and soil N returns to pre-fire levels within about 9 months (Stock & Lewis 1986b, Stock & Allsopp 1992). In the long term, thermal volatilization of N and post-fire leaching will cause a net decrease in system N reserves. Thus, in pristine fynbos that is burnt regularly, deposited N will be unlikely to accumulate in the long term. A problem with this, in practice, is that regular burning of vegetation is difficult to achieve in many parts of the CMA, especially on the Cape Flats, where most natural vegetation sites occur in close proximity to human developments and concerns. Many of these sites have not been adequately burnt for long periods of time. Furthermore, as discussed later, grass invasion triggered by higher soil N availability may result in an elevated post-fire N flush and higher N availability in the post-fire system, due to the production of a large amount of N rich litter. Thus, provided fires aren't too regular, grass invasion could maintain the system at higher N levels, more suited to the growth of exotic grass than indigenous species.

5.3.2 Species level effects

Growth and growth allocation

As discussed in chapter 2, a widely observed effect of N addition in natural vegetation is an increase in growth, with shoot growth often being favoured over root growth such that shoot:root ratio increases. Witkowski (1988) and Witkowski *et al* (1990a) found that shoot:root ratio tended to increase slightly in response to N in all three characteristic growth forms in fynbos (restioids, ericoids and proteoids). Although the magnitude of change observed was small, root:shoot ratios could be more significantly altered by long term N inputs, with a number of possible consequences. The most obvious effect would be to increase plant susceptibility to water stress or drought, because water uptake surfaces (roots) are decreased while surfaces through which water is lost (leaves and shoots) are increased. The results of Witkowski (1989b) suggest that this may occur in fynbos. In that study, litter production in N fertilized vegetation was significantly elevated relative to the control, during a very dry period during the second year after fertilization. Witkowski (1989b) interpreted this finding as being indicative of increased moisture stress due to an N induced increase in shoot: root ratio during the first year. This threat is especially noteworthy because the climate of the Western Cape is predicted to become considerably drier over the next 50 years (annual rainfall levels may be reduced by as much as 25%), with important consequences predicted for vegetation distributions (Midgley *et al.* 2001, Midgley *et al.* 2003).

N deposition-induced decreases in C allocation to the roots and decreased release of carbohydrate-rich root exudates are also known to have important effects on mycorrhizae. At least in arbuscular mycorrhizal (AM) species, the general result in overseas ecosystems has been a shift in community composition, with small spored N tolerant species, which are less effective mutualists, being favoured over the more characteristic large spored species (Egerton-Warburton & Allen 2000, Corkidi *et al.* 2002, Johnson 1993). This selection for less effective mutualists might, in turn, have a negative effect on indigenous plants by reducing their ability to obtain other essential soil nutrients. In fact, Johnson (1993) suggests that the species favoured by N addition can, in some cases, be considered parasites, as they aggressively acquire carbohydrates from the host plant, without providing any substantial benefits in return.

Mycorrhizae are predominant in the Cape Floral Region (it is estimated that arbuscular mycorrhizae occur in more than 60% of the plant species occurring in the CFR, with ericoid mycorrhizae present in a further 8% of species; Allsopp & Stock 1993), and are known to play a vital role in the ecology of fynbos, renosterveld and strandveld (Allsopp & Stock 1993, Lamont 1983). For instance, Allsopp & Stock (1993) showed that mycorrhizal infection was essential for the successful establishment of seedlings in many local species. It thus seems likely that N deposition induced changes in mycorrhizae would have important consequences for local vegetation.

Tissue N concentration

The second common species-level effect of increased N availability is an increase in foliar N content. The current study has shown a historical increase in the N concentration of moss tissues, and a similar (although probably less clear) pattern is expected to occur in vascular plants. Witkowski *et al.* (1990a), found that N concentration in the shoots of two dominant shrub species (a proteoid and an ericoid) increased greatly (by 55 to 66%) in response to N fertilization, and significant increases in mean biomass N content were also detectable in several plant groups (Witkowski 1988). One possible result of long term increases in foliar N content could be an increase in levels of herbivory by insects, as has occurred in certain American (e.g. Haddad *et al.* 2000) and European (e.g. Brunsting & Heil 1985) ecosystems, although N addition levels in both of these studies were considerably higher than those in the CMA. Also, although the allocation of C to secondary defence compounds might decrease slightly in response to N, the sclerophyllous nature and very high phenolic contents which are typical of fynbos leaves, may still largely be prohibitive from a herbivore perspective.

Nutrient imbalances

The final, and perhaps the most important potential species level effect of N addition, relates to the creation of nutrient imbalances. As in most nitrogen-poor systems, other nutrients such as P, K and Mg are also in short supply in fynbos. In some cases, these nutrients may be primary limiters of growth, as was found for P in the fertilization experiment by Lamb & Klaussner (1988), discussed earlier. In other ecosystems where N is the primary limiting nutrient, such as the lowland fynbos system studied by Witkowski (1988), the addition of N has the potential to rapidly move the vegetation to limitation by another nutrient. In both cases, increased growth in response to added N increases demand for other nutrients, which cannot be met. This can result in nutrient deficiencies, which cause reductions in photosynthesis and increase plant mortality (Skeffington & Wilson 1988). Growth of fynbos species has been shown to be favoured by a balanced supply ratio between N and P (Davis *et al.* 1992, Midgley *et al.* 1995). The reduced growth of the study species in response to N addition in Lamb & Klaussner's (1988) study shows the potential for nutrient imbalances to negatively affect plant health in fynbos. Witkowski (1989c) also found that N added to the seedlings of a fynbos shrub (*Protea repens*) growing in pots, resulted in reduced growth and increased mortality, a result which he attributed to a nutrient imbalance. The reason for this was that the soils used in the pot experiments had considerably higher N: P ratios than those measured in field studies (Witkowski 1988, 1989a, Witkowski *et al.* 1990a), and the seedlings were thus P limited rather than N limited.

5.3.3 Community level effects

It is through community level changes in competitive interactions, that N addition is likely to have the largest effect on local vegetation. As discussed earlier, the characteristic physiognomic groups of fynbos show small responses to N addition (be they negative or positive), due to their inherently low growth rates and low growth plasticity. Although shallow-rooted restios, graminoids and annuals are slightly more responsive to N than sclerophyllous shrubs, the increases in foliar projective cover observed in these species are still very small (Witkowski 1989a). Clearly, additional long-term fertilization studies, which more closely "mimic" atmospheric deposition, would be needed in order to determine whether these responses are general ones. Nevertheless, based on the limited knowledge available, it is predicted that even regular N deposition will have a relatively minor effect on competitive interactions in the characteristic components of fynbos, and will not result in marked changes in community structure or diversity in mature vegetation. This will particularly hold true if regular burning takes place, as this will result in N loss through volatilization and leaching, and N will thus not accumulate to high levels. Rather, it is thought that, where they are present, the major agents of community change in fynbos will be weedy exotic or cosmopolitan grasses, whose growth and ability to compete for resources will be greatly enhanced by additional N.

Grass invasion in the CMA

Under natural circumstances, graminoids are thought to be a minor component of fynbos in the winter rainfall regions of the Cape, with restioids seemingly occupying the niche filled by grasses in other ecosystems (Linder 1989). Exotic or naturalized grass species are, however, increasingly abundant in fynbos ecosystems (Richardson *et al.* 1992, Richardson *et al.* 2000a). Milton (2004) lists 33 grass genera that include exotic species commonly recorded in fynbos, while the indigenous status accorded to a number of other grass species is debatable. The vast majority of invasive grasses in fynbos employ the C_3 carbon-fixing pathway, while most indigenous species are of the C_4 type (Milton 2004). This fact is significant because C_3 grasses tend to have lower nutrient use efficiency than C_4 species, and are thus more likely to benefit from additional N inputs (Richardson *et al.* 2000a).

Grass invasion is particularly rife in the CMA and is most pronounced in the central lowlands (Milton 2004, Vlok 1988, Wilson 1999). From personal observation, the majority, if not all of the Cape Flats core conservation sites identified by Maze & Rebelo (1999) are invaded to a moderate or large extent. Weedy grass is also present, particularly on nutrient rich granite/shale derived soils and in disturbed areas, on parts of Lions Head/ Signal Hill, the lower slopes of Devils Peak and Table Mountain, and at Cape Point (Milton *et al.* 1998, Moll & Campbell 1976, Macdonald 1984).

Potential effects of grass invasion in local systems

As summarized in chapter 2, grasses are known to be aggressive competitors for light (Thompson & Harper 1988, Rebele 2000), water (Eissenstat & Caldwell 1988) and nutrients (Caldwell *et al.* 1987) and have outcompeted native species and reduced native plant diversity in a wide variety of ecosystems. Furthermore, grass invasion may alter ecosystem processes so that they are unfavourable for native species, by speeding up rates of nutrient cycling (Lee & Caporn 1998) and increasing fire frequency (D'Antonio & Vitousek 1992). Despite this, relatively little attention has, to date, been focussed on the effects of grass invasion in local ecosystems, as invasions by woody trees and shrubs (particularly N fixing Australian acacias) are often considered to be a bigger threat to the vegetation of the CMA (Richardson *et al.* 1996) and the Cape Floral Kingdom as a whole (Rouget *et al.* 2003). It is acknowledged, however, that grasses and other herbaceous weeds are having an increasingly important impact on the Cape flora (Richardson *et al.* 2000a, Milton 2004).

At many of the most valuable conservation sites on the Cape Flats, including Kenilworth and Milnerton Race Courses and Rondebosch Common, woody aliens are virtually absent, and grass is the main invader (pers. obs.). The grasses most commonly cited as being problematic in the CMA are exotic annuals, most of which belong to the sub-family Pooideae (*Avena, Briza, Bromus, Hordeum, Lolium, Poa, Stipa* and *Vulpia*) (Vlok 1988, Campbell *et al.* 1980, Moll & Campbell 1976). The annual life history of these species makes them particularly well adapted to the mediterranean climate of the CMA, as they are able to survive the dry summer period as soil stored seeds, of which copious amounts are produced. Pooid annuals have been responsible for vegetation transformations in other parts of the world, most notably in Californian grasslands (Huenneke *et al.* 1990, Weiss 1999) and coastal sage scrub (Minnich & Dezzani 1998, Allen *et al.* 1998). Studies in Cape Flats lowland fynbos by Campbell (1980) and Vlok (1988) have shown that the diversity and abundance of indigenous herbs and geophytes is negatively correlated with infestation levels by exotic annual grasses, although a direct link between the two has not been shown. Because of their short stature and shallow root systems, annual grasses are mainly in competition with indigenous species with similar characteristics i.e. annuals, herbaceous perennials and small geophytes. These elements are particularly abundant in the lowland fynbos of the CMA and include many rare and endemic species (Vlok 1988). Pooids also return rapidly from soil-stored seed banks after fire, and may thus compete with shrub seedlings in the post-fire environment.

While annual grasses are most abundant in lowland fynbos, perennial species are also problematic in parts of the CMA. Commonly identified invasive exotic perennials include species in the genera *Pennisetum*, *Paspalum*, *Nassella* and *Lolium*. From personal observation, however, many of the problematic grasses in the lowlands of the CMA are indigenous or cosmopolitan species, which are generally not considered to be invasive locally. These include large bunch grasses such as *Eragrostis curvula* and *Ehrharta calycina*, and prostrate, spreading lawn grasses such as *Stenotaphrum secundatum* and *Cynodon dactylon*. The latter two species (as well as the exotic, *Pennisetum clandestinum*) can be particularly problematic because their spreading habit allows them to form hummocks. *S. secundatum* has, for example, been observed to overtop shrubs at Kenilworth and Milnerton Race Courses (Wilson 1999). These species are thus capable of outshading mature fynbos vegetation without the necessity for disturbance. It should be remembered that it is N-responsive indigenous perennial grasses (e.g. *Molinia caerulea, Brachypodium pinnatum* and *Deschampsia flexuosa*) which have been responsible for vegetation transformations in European heathlands and grasslands (chapter 2, Heil & Deimont 1983, Aerts & Berendse 1988, Bobbink 1991, Bobbink & Willems 1987).

In addition to their ability to outcompete native species for resources, grasses may indirectly impact native species by altering fire frequencies and N cycling rates. Because grasses have high surface to volume ratio, their tissues dry out easily making them highly flammable (D'Antonio & Vitousek 1992). This is particularly true of annual grasses, which leave a large volume of standing dead material at the end of the growing season, thus encouraging fire. Many of these annual grasses evolved in the highly disturbed, fire-prone Mediterranean region of Europe, and are favoured by frequent fires (Milton 2004). They produce large amounts of fire resistant seed in the interval between fires and are able to dominate after fire by exploiting the post-fire nutrient flush in order to achieve rapid growth and reproduction rates. In contrast to annual grasses, native fynbos species, particularly those with a reseeding strategy, may be highly disadvantaged by frequent fires, as they may take a number of years to reach reproductive age or to build up a sufficient seed bank in order to maintain healthy population numbers (leMaitre & Midgley 1992). Thus, by accelerating the fire cycle, grasses which have invaded in response to increased N availability could maintain ecosystem dominance, leading to reduced diversity of native perennial reseeders (e.g. D'Antonio & Vitousek 1992, Minnich & Dezzani 1998, Brooks 1999).

Annual grass is likely to play a role in the high fire frequency, which occurs at a number of sites on the Cape Flats including Rondebosch Common, Plattekloof and Rondebosch East Common. At Rondebosch Common for example, frequent fires have has probably contributed to the almost complete absence of indigenous shrub species at that site (pers. obs.). Naturally, increased fire frequency at these sites is also promoted by human activities, as discussed later.

Where fire is excluded from vegetation, grass invasion would have a different effect on ecosystem processes. Apart from allowing hummock-forming perennial grasses to overtop indigenous species, lack of fire or reduced fire frequency would lead to an accumulation in the soil, of large amounts of high quality grass litter (low C: N, low phenolic content). The consequence of this is that a large proportion of atmospherically deposited N would be retained and sequestered in SOM, resulting in greatly increased soil N reserves. Such an increase in litter quality and soil total N would be expected to result in elevated decomposition and mineralization rates, and thus, higher inorganic N availability (Stock & Allsopp 1992), a situation which would clearly tend to favour continued grass dominance. While fires at typical intervals (10 years plus) would tend to reduce soil N, much SOM would probably remain unburnt and grass invasion would also be likely to result in a greater post-fire N flush (Yelenik 2000). The next successional sequence would thus begin with higher N cycling rates than would occur in an uninvaded system. Increased mineralization rates due to the accumulation of grass litter may be leading to higher inorganic N availability at a number of CMA sites where fire is generally excluded, such as the Kenilworth*, Milnerton and Durbanville race courses.

The role of N deposition in local grass invasion

It is well established that most grasses, particularly C_3 species, are very productive and can increase biomass greatly in response to added N, where it is limiting. In contrast, as discussed earlier, native fynbos species show small responses to additional N, and may be negatively affected if nutrient imbalances occur or if N increases susceptibility to secondary stress factors. N deposition is thus expected to promote grass invasion or increased grass dominance in local systems by increasing the growth and competitive ability of grass species relative to indigenous species. Research in other parts of the world has shown that small increases in N can greatly benefit grass species, allowing them to become dominant in natural vegetation. For example, in nutrient-poor serpentine grasslands on the west coast of the USA, areas receiving 10-15 kg $N.ha^{-1}.a^{-1}$ have suffered large scale invasion by exotic grasses, while identical nearby areas receiving 4-6 $kg.ha^{-1}.a^{-1}$ have remained relatively free of invasive grasses (Weiss 1999).

Whether additional N will strongly favour grass over indigenous vegetation in the CMA will depend to a large extent on the demand by grass for other nutrients such as P. As discussed earlier, P is often secondarily limiting and sometimes primarily limiting for fynbos vegetation. Where this is the case, grass will only be able to benefit greatly from N addition if it has relatively low P demand or is well adapted to P deficiency. In fact, this seems to be the case with many grass species. For instance, Kirkham (2001) found that the grass species *Molinia caerulaea*, *Agrostis sp.* and *Festuca sp.* were less limited by P than the dominant shrub species in British moorland. He suggests that one of the main reasons that the grass *Molinia caerulea* has been such a successful competitor in European heathlands exposed to N deposition is that it is better adapted to P limitation than other species. Schutte (1960) proposed that the success of certain woody invasive plants in fynbos might be due to their better adaptation to trace-element deficiencies.

*Kenilworth R.C. has recently undergone a controlled burn after a long interval (60 yrs plus) without a major fire

In lowland fynbos, evidence suggests that additional N provides a benefit to most invaders without need for a concomitant increase in P or other nutrients. For instance, the N-fixing ability of Australian acacias seems to be one of the reasons for their successful invasion of fynbos ecosystems (Stock & Allsopp 1992). There is also some evidence to suggest that N addition alone increases the health and abundance of grass in fynbos. For example, long term invasion by N fixing acacias has been shown to increase the available N content of fynbos soils, and grass seems to do particularly well in patches cleared of acacias (Yelenik *et al.* 2003, Holmes & Cowling 1997). Yelenik *et al.* (2003) also showed in a pot experiment that the weedy indigenous grass *Ehrharta calycina*, grew better in N enriched soil from an *Acacia cyclops* stand than in soil from an adjacent plot, which was uninvaded.

Richardson *et al.* (2000b) have emphasized the important role played by mutualistic partnerships in plant invasions of N poor ecosystems. It may be that, in addition to directly benefiting exotic grasses, N deposition is indirectly facilitating grass invasion in local systems through its effects on the mycorrhizal community. As discussed in chapter 2, studies in other parts of the world have suggested that N-induced changes in the mycorrhizal community could favour invasion by exotic grasses, because grasses are less influenced by type of mycorrhizal innoculum than native species (e.g. Van Der Heijden *et al.* 1998), or because grasses associate preferentially with N-tolerant myccorhizal species (e.g. Siguenza 2000 cited in Fenn *et al.* 2003). This possibility requires further study in local systems.

Clearly, atmospheric N deposition is only one of a number of factors which might be contributing to the increased dominance of grass in natural areas of the CMA. While N addition has been an important driver of grass invasion in many nutrient poor vegetation types in Europe and America, in most cases, other factors interacted with N addition to allow grasses to become dominant. Similarly, grass invasion in the CMA certainly has a complex etiology, and the relative importance of N deposition is difficult to estimate. The occurrence on the Cape Flats of both the highest levels of grass invasion and the highest N deposition levels in the CMA may be suggestive of a causal relationship between these two factors. However, higher invasion rates on the flats are certainly also encouraged by other factors, mostly related to the greater level of human activity in that region. Mechanical disturbance of the soil, altered fire frequency, habitat fragmentation and lack of grazing pressure all probably play a role in increasing the success of grasses in the CMA. As this thesis is concerned with N addition, these other potential drivers will not be considered further here.

It should also be recognised that elevated N availability in the natural vegetation of the CMA need not only be the result of atmospheric deposition. Other sources of nitrogen pollution including herbicides, insecticides, fire retardants, and fertilizer run off can also contribute to N enrichment (Stock and Allsopp 1992), and it is thus important to identify areas which are at risk from such inputs. For instance, the extremely species-rich natural vegetation surviving at the three race courses on the Cape flats (Milnerton, Kenilworth and Durbanville) may be at risk from fertilizer drift, as the natural areas at these sites are surrounded by carefully maintained race-track to which fertilizer is applied (Eddie Luff pers. com.).

5.4 Strategies to deal with elevated atmospheric deposition

Clearly the easiest and most efficient way to minimize the impacts of N deposition and acidification on the vegetation of the CMA is to reduce anthropogenic emissions of nitrogenous and sulfurous pollutants in the region.

While pollutant emission and deposition levels remain high, it may be necessary to take some form of restorative action in order to minimize the effects of N deposition on local ecosystems, and to control grass invasion. The type of action needed will depend on the ecosystem in question. At a site such as Rondebosch common, which is dominated by exotic annual grass and characterized by high fire frequency, accumulated N will not be a problem, as frequent fires will have continually reduced system N stocks. In such cases, it will be necessary to remove as much of the invasive grass as possible and to reduce fire frequency. While the prevention of fire will be essential for the restoration of sites such as Rondebosch common, fire will be an important tool for reducing system N stocks at sites where it has long been excluded, such as Milnerton, Durbanville (and, until recently, Kenilworth) race courses. Preferably, grass and grass litter should first be removed by hand at these sites, as this will result in the export of a substantial proportion of deposited N sequestered in these stores (Mitchell *et al.* 2000, Barker *et al.* 2004), some of which would otherwise contribute to an elevated post-fire N flush. This will also prevent certain grasses, not killed by fire, from resprouting from underground organs. Burning should volatilize a sizeable portion of soil-stored N (SOM and N in microbial biomass) and encourage the germination of the native seedbank, provided that the fire interval hasn't been so long as to allow soil stored seed to senesce (Holmes & Richardson 1999). Careful monitoring of the situation will, however, be needed in order to ensure that an initial enhancement of grass seedling invasion by fire and soil disturbance does not outweigh the long-term benefits of N removal (Barker *et al.* 2004). Further removal of grass seedlings may be necessary subsequent to the fire. The recent burn carried out at Kenilworth race course will provide an ideal opportunity to monitor the competitive interactions between grasses and indigenous species, both immediately after fire and in the longer term. Even if grass and grass litter is removed prior to burning (which was not the case at Kenilworth), post-fire N availability (and thus, mineralization rates) in such a system, will probably still be higher than in an uninvaded system, because a sizeable proportion of grass composed SOM will remain unburnt.

A possible alternative or complimentary approach to grass removal and/or burning would be to apply C rich mulch such as sawdust or bark (with a C: N ratio of >100) to the soil surface layer, which would cause the microbial community to become N-limited and result in the immobilization of excess inorganic N in the soil. This approach has been successfully used to reduce soil inorganic N availability and increase the abundance of native species relative to exotic grasses in a number of studies (e.g. Wilson & Gerry 1995, Zink & Allen 1998), although results have been poor when a mulch with relatively low C:N ratio has been used (e.g. Cione *et al.* 2002, Zink & Allen 1998). It is probably preferable, however, to avoid the addition of foreign materials to local ecosystems, unless it is clear that N levels are high enough to pose a serious threat to vegetation and the other management techniques mentioned are found to be inadequate.

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APPENDIX A: Wald Tables for REML Analysis

n = number of samples; str. var. = stratum variance,
d.f. = degrees of freedom; significance levels: * $p < 0.05$, ** $p < 0.01$

Wald Table 1: *Significant effects of collection period, collection season and species on log % N.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	PERIOD	18.10	2	9.05	<0.001
	SEASON	5.80	3	1.93	0.122
	SPECIES	69.13	8	8.64	<0.001
Dropping individual terms from full fixed model	**PERIOD**	**18.35**	**2**	**9.18**	**<0.001****
	SEASON	**9.36**	**3**	**3.12**	**0.025***
	SPECIES	**69.21**	**8**	**8.65**	**<0.001****

n =254, str. var. = 0.08643, df = 233.18

Wald Table 2: *Significant effects of collection period, collection season and species on C: N ratio.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	PERIOD	39.75	2	19.87	< 0.001
	SEASON	9.11	3	3.04	0.028
	SPECIES	66.15	8	8.27	< 0.001
Dropping individual terms from full fixed model	**PERIOD**	**44.71**	**2**	**22.35**	**< 0.001****
	SEASON	**10.95**	**3**	**3.65**	**0.012** *
	SPECIES	**66.15**	**8**	**8.27**	**< 0.001****

n =201, str. var. = 0.06526, df = 187.00

Wald Table 3: *Non- significant effect of collection period on $\delta^{15}N$.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	PERIOD	3.15	2	1.58	0.206
	SPECIES	49.22	8	6.15	<0.001
	REGION	11.92	3	3.97	0.008
Dropping individual terms from full fixed model	**PERIOD**	**3.65**	**2**	**1.83**	**0.161**
	SPECIES	43.08	8	5.39	<0.001**
	REGION	11.91	3	3.97	0.008**

n =205, str. var. = 5.833, df = 187.41

Wald Table 4: *Significant effects of collection region and species on $\delta^{15}N$.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	SPECIES	46.78	8	5.85	<0.001
	REGION	13.06	3	4.35	0.005
Dropping individual terms from full fixed model	**REGION**	**40.73**	**8**	**5.09**	**<0.001****
	SPECIES	**13.06**	**3**	**4.35**	**0.005****

n =205, str. var. = 5.902, df = 189.38

Wald Table 5: *Significant effect of water conduction method on log % N.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	WATER COND.	6.30	1	6.30	0.012
	PERIOD	12.52	2	6.26	0.002
	REGION	10.34	3	3.45	0.016
Dropping individual terms from full fixed model	**WATER COND.**	**8.02**	**1**	**8.02**	**0.005****
	PERIOD	10.82	2	5.41	0.004**
	REGION	10.34	3	3.45	0.016*

n =254, str. var. = 0.1017, df = 239.91

Wald Table 6: *Non-significant effect of physiological group on log %N.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	PHYS GROUP	0.55	1	0.55	0.460
	PERIOD	10.63	2	5.31	0.005
	REGION	9.98	3	3.33	0.019
Dropping individual terms from full fixed model	**PHYS GROUP**	**1.57**	**1**	**1.57**	**0.210**
	PERIOD	9.36	2	4.68	0.009**
	REGION	9.99	3	3.33	0.019*

n =254, str. var. = 0.1041, df = 239.83

Wald Table 7: *Non-significant effect of method of water conduction on C: N ratio*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	PERIOD	14.48	2	7.24	< 0.001
	WATER COND.	2.26	1	2.26	0.133
Dropping individual terms from full fixed model	PERIOD	14.99	2	7.50	< 0.001**
	WATER COND.	**2.26**	**1**	**2.26**	**0.133**

n =201, str. var. = 0.08318, df = 191.10

Wald Table 8: *Non-significant effect of physiological group on C: N ratio*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	PERIOD	12.79	2	6.40	0.002
	PHYS GROUP	0.54	1	0.54	0.463
Dropping individual terms from full fixed model	PERIOD	12.28	2	6.14	0.002**
	PHYS GROUP	**0.54**	**1**	**0.54**	**0.463**

n =201, str. var. = 0.08355, df = 190.98

Wald Table 9: *Non-significant effect of method of water conduction on $\delta^{15}N$.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	WATER COND.	0.27	1	0.27	0.606
	REGION	16.69	3	5.56	<0.001
Dropping individual terms from full fixed model	**WATER COND.**	**0.52**	**1**	**0.52**	**0.471**
	REGION	16.69	3	5.56	<0.001*

n =205, str. var. = 6.993, df = 194.51

Wald Table 10: *Significant effect of physiological group on $\delta^{15}N$.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	PHYS. GROUP	15.16	1	15.16	< 0.001
	REGION	17.26	3	5.75	< 0.001
Dropping individual terms from full fixed model	**PHYS. GROUP**	**14.82**	**1**	**14.82**	**< 0.001****
	REGION	17.26	3	5.75	< 0.001**

n =205, str. var. = 6.485, df = 194.46

Wald Table 11: *Non-significant effect of collection season on $\delta^{15}N$.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	SEASON	2.30	3	0.77	0.512
	SPECIES	47.48	8	5.93	<0.001
	REGION	12.09	3	4.03	0.007
Dropping individual terms from full fixed model	**SEASON**	**4.12**	**3**	**1.37**	**0.249**
	SPECIES	42.03	8	5.25	<0.001**
	REGION	12.09	3	4.03	0.007**

n =205, str. var. = 5.966, df = 189.47

Wald Table 12: *Non-significant effect of collection region on log %N*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	SPECIES	64.56	8	8.07	<0.001
	REGION	7.76	3	2.59	0.051
	SEASON	9.50	3	3.17	0.023
	PERIOD	17.86	2	8.93	<0.001
Dropping individual terms from full fixed model	SPECIES	63.90	8	7.99	<0.001**
	REGION	**4.79**	**3**	**1.60**	**0.187**
	SEASON	8.60	3	2.87	0.035*
	PERIOD	18.07	2	9.04	<0.001**

n =254, str. var. = 0.08590, df = 230.24

Wald Table 13: *Non-significant effect of collection region on C: N ratio.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	SPECIES	62.93	8	7.87	< 0.001
	REGION	3.34	3	1.11	0.342
	SEASON	7.46	3	2.49	0.059
	PERIOD	42.01	2	21.01	< 0.001
Dropping individual terms from full fixed model	SPECIES	65.28	8	8.16	< 0.001**
	REGION	**1.85**	**3**	**0.62**	**0.604**
	SEASON	11.58	3	3.86	0.009**
	PERIOD	42.01	2	21.01	< 0.001**

n =201, str. var. = 0.06581, df = 178.80

Wald Table 14: Non-*significant effect of collection season on log %N in ectohydric species.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	PERIOD	29.34	2	14.67	< 0.001
	SPECIES	26.99	4	6.75	< 0.001
	SEASON	2.92	3	0.97	0.403
Dropping individual terms from full fixed model	PERIOD	30.25	2	15.12	< 0.001**
	SPECIES	26.98	4	6.75	< 0.001**
	SEASON	**2.92**	**3**	**0.97**	**0.404**

n =118, str. var. = 0.06356, df = 101.80

Wald Table 15: *Significant effect of collection period on log %N in ectohydric species.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	PERIOD	29.71	2	14.86	< 0.001
	SPECIES	27.02	4	6.76	< 0.001
Dropping individual terms from full fixed model	**PERIOD**	**29.40**	**2**	**14.70**	**< 0.001****
	SPECIES	27.01	4	6.75	< 0.001**

n =118, str. var. = 0.06356, df = 104.90

Wald Table 16: *Significant effects of collection period and collection season on log %N in mixohydric species.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	PERIOD	7.37	2	3.69	0.025
	SPECIES	29.34	3	9.78	<0.001
	SEASON	16.46	3	5.49	<0.001
Dropping individual terms from full fixed model	**PERIOD**	**9.95**	**2**	**4.87**	**0.008****
	SPECIES	32.76	3	10.92	<0.001**
	SEASON	**16.46**	**3**	**5.49**	**<0.001****

n =136, str. var. = 0.09342, df = 120.17

Wald Table 17: *Non-significant effect of collection region on $\delta^{15}N$ when Campylopus sp. and Pseudocrossidium crinitum are removed from the data set.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	SPECIES	45.90	6	7.65	<0.001
	REGION	5.37	3	1.79	0.147
Dropping individual terms from full fixed model	SPECIES	40.92	6	6.82	<0.001**
	REGION	**5.37**	**3**	**1.79**	**0.147**

n =166, str. var. = 5.029, df = 148.62

Wald Table 18: *Significant effects of Collection date, Site and Collection date x Site interaction on log %N in transplants.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	**COL. DATE**	**46.80**	**4**	**11.70**	**<0.001****
	SITE	**11.16**	**3**	**3.72**	**0.011***
	COL. DATE x SITE	65.17	11	5.92	<0.001**
Dropping individual terms from full fixed model	**COL. DATE x SITE**	**65.17**	**11**	**5.92**	**<0.001****

number of units = 57

Wald Table 19: *Non-significant effects of Collection date, Site and Collection date x Site interaction on $\delta^{15}N$ in transplants.*

	Fixed term	Wald statistic	d.f.	Wald/d.f.	Chi-sq prob
Sequentially adding terms to fixed model	**COL. DATE**	**3.91**	**4**	**0.98**	**0.418**
	SITE	**6.92**	**3**	**2.31**	**0.074**
	COL. DATE x SITE	17.19	11	1.56	0.102
Dropping individual terms from full fixed model	**COL. DATE x SITE**	**17.19**	**11**	**1.56**	**0.102**

number of units = 57