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Historical nitrogen content of bryophyte tissue as an indicator of increased nitrogen deposition in the Cape Metropolitan Area, South Africa

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This study of bryophyte tissue nutrient contents shows a historical increase in N deposition rates to the low nutrient adapted plant biodiversity hotspot in the Western Cape, South Africa.

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ABSTRACT

Information on changes in precipitation chemistry in the rapidly expanding Cape Metropolitan Area (CMA) of South Africa is scarce. To obtain a long-term record of N deposition we investigated changes in moss foliar N, C:N ratios and nitrogen isotope values that might reflect precipitation chemistry. Tissue from 9 species was obtained from herbarium specimens collected between 1875 and 2000 while field samples were collected in 2001/2002. There is a strong trend of increasing foliar N content in all mosses collected over the past century (1.32–1.69 %N). Differences exist between ectohydric mosses which have higher foliar N than the mixohydric group. C:N ratios declined while foliar $\delta^{15}N$ values showed no distinct pattern. From relationships between moss tissue N and N deposition rates we estimated an increase of 6–13 kg N ha^{-1} a^{-1} since 1950. Enhanced N deposition rates of this magnitude could lead to biodiversity losses in native ecosystems.

1. Introduction

In many developed parts of the world precipitation chemistry has been the subject of detailed long-term monitoring, and for parts of Europe records extend as far back as the mid 1800s (Skeffington and Wilson, 1988). It has thus been possible to quantify, with some accuracy, the marked historical increase in N deposition that has occurred in these areas. For the Cape Metropolitan Area (CMA), South Africa, data on precipitation chemistry is scarce despite the areas being recorded to have regular atmospheric pollution events (between 38 and 59 days per annum during the winter months from 1998 to 2002, City of Cape Town State of the Environment Report). Bulk N deposition data exist from studies carried out in the 1980s at isolated and unpolluted fynbos ecosystems located more than 60 km from Cape Town (Stock and Lewis, 1986; Van Wyk et al., 1992). These indicate a background level of N deposition for the Cape region of <2 kg N h^{-1} a^{-1}. The CMA has shown an 80% increase in vehicles over the past 20 years so that in 2007 there were 670 000 registered vehicles on 6798 km of road. Vehicles have been shown to account for 67% of the visible 'brown haze' pollution (Wicking-Baird et al., 1997) while the atmospheric N inputs resulting from rapid urbanization and industrialization of the CMA are unknown. Given the absence of long-term records of atmospheric N enrichment for the region we use the N status of bryophytes as an indicator of historical changes in atmospheric N deposition.

Tissue analysis of bryophytes has been widely employed in the biomonitoring of pollution, including heavy metals (Tyler, 1990; Fernandez et al., 2002), radionuclides (Summerling, 1984), hydrocarbons (Gerdol et al., 2002) and nitrogen (Baddeley et al., 1994; Pitcairn et al., 1995, 1998, 2003). Mosses have characteristics that make them excellent subjects for biomonitoring, and superior, in this regard, to vascular plants. Characteristics include a heavy reliance on wet and dry deposition for their nutrient supply since they lack a conductive root system (Bates, 2000), the absorption of nutrients across the entire surface of the plant because of the lack of a cuticle and the lack of an ability to remobilize nutrients (Penuelas and Filella, 2001). These characteristics mean that bryophyte tissue chemistry is closely correlated with atmospheric inputs (Bates, 2000; Pitcairn et al., 1995, 1998, 2003). Not all bryophytes are, however, suitable for monitoring, mainly because differences exist between groups based on their water uptake characteristics. Generally ectohydric species are most suitable for monitoring because water conduction takes place externally, in the capillary spaces on the plant surface, and contact with the substratum is

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usually poor. Endohydric species are less suitable since they have relatively well-developed internal conductive tissues and are able to utilize nutrients from the substrate, while mixohydric species are intermediate in their water uptake characteristics. A further complication that could result in differences in tissue N patterns is the architecture of mosses, which are often placed into two groups, pleurocarps and acrocarps. Obtaining material of a similar age and physiological activity is difficult because the pleurocarps have creeping, branched stems, with a mat-like growth form while acrocarps generally have erect stems with little or no branching, and form tufts (Buck and Goffinet, 2000).

Baddeley et al. (1994) measured tissue nitrogen of the moss *Racomitrium lanuginosum*, collected from 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. Pitcairn et al. (1995) showed a significant increase in tissue N of several moss species between the 1950 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. In a further study of ectohydric mosses receiving a high pollution load from nearby livestock farms, Pitcairn et al. (1998) demonstrated 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., 2006).

In addition to tissue N, several studies have measured the $^{15}N/^{14}N$ isotope ratio of mosses in order to identify the role of pollution as an N source for these plants. Although the factors determining $\delta^{15}N$ patterns in ecosystems are notoriously complex (Handley et al., 1999), $\delta^{15}N$ in bryophytes is assumed to be determined primarily by the isotope ratio of the nitrogen source (Handley and Raven, 1992). Since most ectohydric mosses rely almost solely on the atmosphere for their N supply, tissue $\delta^{15}N$ is expected to be very similar to that of the deposition source (Pearson et al., 2000; Solga et al., 2004). Penuelas and Estiarte (1997) and Penuelas and Filella (2001) measured leaf $\delta^{15}N$ of herbarium material collected over a 70 year period, and interpreted 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. On the other hand, several studies have 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 objectives of this study were to determine the spatial and temporal trends in nitrogen content, C:N ratio and $\delta^{15}N$ of old and current samples of bryophyte foliage as an indicator of historical N deposition patterns in an area which has experienced rapid urbanization and a population growth of 0.5–3.5 million in the period 1945–2007 (Statistics SA). Herbarium samples collected over the past 130 years were analysed along with freshly collected material for N, C and $\delta^{15}N$ isotope ratios, 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 changes indicative of a greater contribution of human-derived N from deposition.

2. Methods and materials

2.1. Study species

To obtain sufficient replication to demonstrate historical changes in bryophyte foliar N and ^{15}N contents in the CMA, a total of 5 species, and 4 species groups were analysed. Species included *Hypnum cupressiforme*, *Ischyrodon lepturus*, *Ceratodon purpureus*, *Leptodon smithii* and *Pseudocrossidium crinitum* while species of *Bryum*, *Campylopus*, *Pleuridium* and *Fissidens*, were analysed in groups. *Bryum* is a combination of *Bryum canariense* and *Bryum torquescens*, *Campylopus* is *Campylopus atroluteus* and *Campylopus introflexus*, *Pleuridium* is largely *Pleuridium ecklonii*, but includes some *Pleuridium nervosum* and *Pleuridium pappeanum*, and *Fissidens* is comprised of 5 species – *Fissidens megalotis*, *Fissidens marginatus*, *Fissidens fasciculatus*, *Fissidens plumosus* and *Fissidens glaucescens*. In general, the species within each group exhibit similar growth form and therefore are expected to behave rather similarly with respect to N-uptake. Taxonomy follows O'Shea (2006).

2.2. Sample collection

The CMA was divided into four broad sampling regions (Fig. 1). The FLATS region encompasses the urbanized lowlands of the CMA (Kenilworth, Milnerton and Tygerberg) and is likely to receive the highest pollution levels of the four zones, due to its proximity to major roads, and areas of industrial and farming activity. Rainfall levels are lower than other parts of the CMA. The EAST region includes the lower eastern slopes of Devils Peak, 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 southern Peninsula, an area which is isolated from the areas of highest population density and industrial activity. This region is for the most part separated from the EAST and FLATS regions by the peninsula mountain chain. The MONTANE region has an altitude greater than 400 m, with the majority of samples in this category being collected from the upper slopes and plateau of Table Mountain. This zone was separated from the others due to the possible influence of altitude on both N deposition levels and foliar N concentration.

Field samples of moss growing on the soil surface were collected between March 2001 and March 2002 from 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. Sampling covered a range of forest and shrubland habitats similar to the range of habitats from which herbarium material had been collected for each species/species group. All materials collected were alive and photosynthetic, and were 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 grouped into three periods: 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 Fig. 1.

2.3. Sample analysis

Leaves (or stem tips in the case of *Hypnum*, *Ischyrodon* and *Leptodon*) were removed from a similar terminal position of each specimen using tweezers. A 10 mm apical section was taken from the pleurocarps while the acrocarps were more variable with apical sections, varying in length (3–10 mm), being sampled. These were bulked (from 6 to 10 apical sections per specimen), finely chopped and sand or other particles clinging to the plant material removed. Samples were not washed to avoid 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). Soil available N was extracted from sieved soil samples (2 mm mesh) by shaking 10 g of soil with 100 ml of 2 M KCl solution for 1 h and suction filtering through a Whatman No. 4 filter paper according to the method of Stock (1983). Ammonium (NH_4^+) and nitrate and nitrite (NO_y) concentrations were determined by flow injection analysis (Quikchem 8000 series FIA+, Lachat Instruments, USA) at the department of Oceanography, UCT. Nitrate was determined by means of a copper cadmium reduction, followed by colorimetric analysis for NO_2 and the indophenol blue method was used to measure NH_4. Soil moisture and organic matter determinations were determined from mass lost by soil samples of approximately 10 g after drying in an oven at 105 °C for 24 h and then combusted for 16 h at 450 °C.

2.4. Statistics and deposition estimates

The $\delta^{15}N$ data were roughly normally distributed, while %N and C:N ratio data were log-transformed to obtain normal distributions. Owing to the unbalanced nature of the sampling design, linear mixed model analyses using the Residual Maximum Likelihood (REML) method (non-sparse algorithm with Fisher scoring) (Patterson and Thompson, 1971) were conducted in order to determine which factors or combinations of factors, had a significant effect on the variables. Samples were classified according to factors (species, period, region, season, water conduction type (ecto- vs mixohydric) and physiological group (pleuro- or acrocarp))