



Use of *Calluna vulgaris* to detect signals of nitrogen deposition across an urban–rural gradient

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ABSTRACT

Densely populated cities can experience high concentrations of traffic-derived pollutants, with oxides of nitrogen and ammonia contributing significantly to the overall nitrogen (N) budget of urban ecosystems. This study investigated changes in the biochemistry of in situ *Calluna vulgaris* plants to detect signals of N deposition across an urban–rural gradient from central London to rural Surrey, UK. Foliar N concentrations and $\delta^{15}\text{N}$ signatures were higher, and C/N ratios lower, in urban areas receiving the highest rates of N deposition. Plant phosphorus (P) concentrations were also highest in these areas, suggesting that elevated rates of N deposition are unlikely to result in progressive P-limitation in urban habitats. Free amino acid concentrations were positively related to N deposition for asparagine, glutamine, glycine, phenylalanine, isoleucine, leucine and lysine. Overall, relationships between tissue chemistry and N deposition were similar for oxidised, reduced and total N, although the strength of relationships varied with the different biochemical indicators. The results of this study indicate that current rates of N deposition are having substantial effects on plant biochemistry in urban areas, with likely implications for the biodiversity and functioning of urban ecosystems.

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1. Introduction

Pollution control legislation has resulted in improvements in air quality throughout much of Europe during recent decades. However, urban environments typically have high traffic densities and, as a consequence, high concentrations of traffic-derived pollutants, including NO_x , VOCs and particulates. In the UK, vehicles are responsible for 32% of NO_x emissions (RoTAP, 2009), although in densely populated cities they can account for a considerably higher proportion of emissions. Annual mean concentrations of NO_x in urban areas have been shown to exceed WHO air quality objectives for health ($40 \mu\text{g m}^{-3}$) and UNECE critical levels for vegetation ($30 \mu\text{g m}^{-3}$) in many European cities, even at sites away from the direct influence of major roads (e.g. Penard-Morand et al., 2006; Zamboni et al., 2009).

The widespread use of catalytic converters has also led to an increase in emissions of ammonia (NH_3) from transport. For example, in the UK, vehicles are now estimated to contribute 3% to the national NH_3 budget (Sutton et al., 2000). Transport emissions are also believed to be the main contributor to the local NH_3 budget

in the Los Angeles area of the United States (Fraser and Cass, 1998). The fast deposition velocity and high chemical reactivity of this pollutant means that, even at relatively low concentrations, it can contribute significantly to the total nitrogen (N) loading of urban environments (Cape et al., 2004). Urban ecosystems contribute substantially to the aesthetic value of cities and make an important contribution to local biodiversity. However, relatively little attention has so far been paid to the effects of N deposition in urban environments.

The phytotoxicity of oxides of N and NH_3 has been well documented in fumigation experiments (Wellburn, 1990; Van der Eerden et al., 1991). Furthermore, exposure of plants to vehicle exhaust emissions in transect and fumigation studies has revealed significant, species-specific effects on these pollutants on plant physiology, anatomy and growth (Gratani et al., 2000; Viskari et al., 2001a,b; Honour et al., 2009). In addition to the direct effects of gaseous pollutants on individual plants, species- and community-level responses to elevated rates of N deposition have been reported for a wide range of habitats, with detrimental effects on the diversity and functioning of natural ecosystems (Bobbink et al., 2003; Carroll et al., 2003; Stevens et al., 2006). Effects of roadside proximity on plant community composition have also been reported for heathland (Angold, 1997), roadside verge (Truscott et al., 2004), woodland and

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forest understorey (Bernhardt-Römermann et al., 2006; Bignal et al., 2008) habitats.

Changes in plant foliar chemistry are frequently found following exposure to nitrogenous pollutants and may be considered as early indicators of the potential for detrimental effects at the community and ecosystem level. For example, elevated foliar N concentrations have been associated with increases in plant productivity (Bobbink, 1991; Power et al., 1998a) and accompanying changes in plant community composition, as well as increased sensitivity to biotic and abiotic stresses (Power et al., 1998b; Caporn et al., 2000). Furthermore, changes in the concentration and composition of free amino acids in plant foliage, in response to increased N deposition, have been linked with higher levels of pathogen infection (Nordin et al., 1998; Strengbom et al., 2002) and reduced plant growth (Nordin and Gunnarsson, 2000). Such responses are likely to affect competition between neighbouring plant species, with implications for biodiversity as well as for the cycling and storage of carbon, N and water in terrestrial ecosystems.

In addition to the now fairly widespread use of foliar N concentrations and amino acid composition as indicators of N deposition inputs, changes in stable isotope signatures in relation to gradients of both oxidised and reduced N are increasingly used as a measure of N inputs. In general, isotope signatures associated with agricultural N emissions are relatively depleted in ^{15}N (Heaton, 1986), whilst those associated with fossil fuel combustion are relatively enriched with the heavier isotope (Sutton et al., 2004). Isotopic fractionation may occur during deposition and canopy exchange, or as a result of microbial transformation, compounding accurate interpretation of isotope signatures (Hogberg, 1997). However, despite this, there is increasing evidence that isotope signatures in some plant groups reflect those of source N inputs (Skinner et al., 2006a). This is particularly the case for plants which receive a substantial part of their N requirements from direct foliar uptake (e.g. bryophytes, lichens and *Calluna vulgaris* (Bobbink and Heil, 1993)). In studies involving mosses and trees, vegetation $\delta^{15}\text{N}$ signatures have been found to be relatively enriched in samples collected from motorways or busy urban roads (Pearson et al., 2000; Saurer et al., 2004), or in close proximity to large urban areas (Jung et al., 1997). Similar patterns in plant N isotope ratios have also been shown along gradients of decreasing NH_3 concentration (Harrison et al., 1999; Skinner et al., 2006b).

Whilst higher plants have been less frequently used as bio-monitors of atmospheric pollution than lichens and bryophytes, the relationship between foliar N concentrations of *Calluna vulgaris* L. Hull (henceforth referred to as *Calluna*) and N deposition has been established in both N addition experiments (Leith et al., 2001) and field transect studies (Pitcairn et al., 1998, 2001; Hicks et al., 2000; Gadsdon, 2007). In order to assess the potential for effects of N deposition on urban ecosystems, a transect study was carried out from central London to rural Surrey (UK), using *Calluna* plants as bioindicators of traffic-derived N pollution. The principal aim of this study was to establish if there is a relationship between *Calluna* foliar chemistry, N deposition and proximity to a heavily trafficked, major urban area.

2. Methodology

2.1. Transect design and site selection

A 50 km south-westerly transect was established from central London into rural Surrey (UK). Fourteen sites were selected in urban, suburban or semi-rural locations, based on their geographical location, the presence of heathland habitat containing building or mature phase *Calluna* plants and permission to sample. Sample areas were located within open heathland patches varying in size

from approximately 0.1–650 ha. Soil types were not specifically assessed on site, although all sites shared a similar underlying geology, described as Bagshot beds or Bagshot sands and gravels. Soils were all free drained with a relatively high sand content and with litter layers varying from 0.5 to 1.5 cm depth. A pilot analysis of soil samples from each site revealed no systematic variation in soil N content with either distance from central London or with N deposition load (data not shown). Table 1 summarises the sites, the area of heath within each site and their distance from central London (Hyde Park Corner National Grid reference TQ273803). All sample sites were located at least 20 m away from the nearest major road.

2.2. *Calluna* sampling

At each site (except Barnes Common) three samples of *Calluna* shoots were taken from distinct, representative heathland patches. At Barnes Common, the small patch size of *Calluna* meant that only 2 replicate samples were collected. Each sample comprised a minimum of 20 randomly chosen shoots from 5 to 10 *Calluna* plants. Sample collection was restricted to current year shoots and was completed during a two week period from 15th to 26th November 2001, outside of the active growth period. Plant samples were immediately placed in a chilled, insulated storage box upon collection. On return to the laboratory, unwashed plant samples were sub-divided, with half being freeze-dried and the other half oven dried at 50 °C, for three days.

2.3. Foliar analyses

Foliar concentrations of N were determined on triplicate samples of dried, finely ground shoot material. Both ^{14}N and ^{15}N isotopes of N were quantified using a Roboprep CN analyser coupled to a Europa 20–20 stable isotope mass spectrometer (Europa Scientific). Isotopic enrichment was then calculated, as parts per thousand, according to the following equation:

$$\delta^{15}\text{N}(\text{‰}) = ((^{15}\text{N} : ^{14}\text{N}_{\text{sample}} / ^{15}\text{N} : ^{14}\text{N}_{\text{standard}}) - 1) \times 1000$$

Calluna ^{14}N data were expressed as a percentage of plant dry weight, and are subsequently referred to as foliar %N. Foliar carbon (C) concentrations were obtained on duplicate samples from each site, using the Roboprep CN analyser described above.

Table 1

Site list, with distance from central London (taken as Hyde Park Corner, National Grid reference: TQ273803). Unless otherwise stated, area refers to the size of the heathland patch sampled within each site. #Combined value for Putney Heath and Wimbledon Common; * Area of whole site; + Estimated value.

Site name	Distance from London (km)	Heathland area (ha)	Surrounding habitat
Natural History Museum	2	<0.1	Wildlife garden
Barnes Common	7	<0.1	Woodland/grassland
Hampstead Heath	7.5	0.87	Grassland
Putney Heath	9.5	40.1 [#]	Grass-heath
Wimbledon Common	10.5		Grass-heath
Richmond Park	13	<0.1	Open parkland/grassland
Coombe Hill	13.5	1.0 ⁺	Golf course
Hounslow Heath	17.5	2.38	Heathland/grassland
Esher Common	23.5	136 [*]	Heathland
St Georges, Byfleet	27	0.5 ⁺	Golf Course
Chobham Common	35	650 [*]	Heathland
Horsell Common	36	150 [*]	Heathland/grassland
Silwood Park	37.5	0.2 ⁺	Grassland
Thursley Common	50.5	300 [*]	Heathland

Tissue phosphorus concentrations were measured on dried, finely ground *Calluna* shoot material. Samples were first combusted at 550 °C; the resulting ash was dissolved in 6 M HCl before analysis using ICP-OES (Optima 3300DV, Perkin Elmer, UK). Amino acid composition was determined for freeze-dried plant material. Ten mg of ground, dry material was shaken in 80% methanol for 24 h, after which time the extract was filtered (0.45 µM) and centrifuged (4 °C at 14,000 RPM for 30 min). Extracts were analysed using reverse phase HPLC following pre-column derivatisation using o-phthalaldehyde (Sigma, UK). Amino acid reference standards (Sigma, UK) were used to determine analytical precision, and duplicate analyses of all samples were undertaken.

2.4. Air pollution data

Dry and wet deposition of both oxidised and reduced forms of N, averaged for 2001–2003, were provided for each sample site by the Centre for Ecology & Hydrology (Ron Smith, CEH Edinburgh, UK). Data were derived using the FRAME (Fine Resolution Atmospheric Multi-pollutant Exchange) model which itself uses information on all quantified urban and rural emission sources of nitrogenous (and sulphur) pollutants to provide spatially explicit estimates of deposition (Dore et al., 2007). Further information on the model, and the relevant deposition data, can be found at: <http://www.uk-pollutantdeposition.ceh.ac.uk/>. The central grid reference of all three within-site *Calluna* sampling locations was used to obtain site-level pollutant deposition data. The following abbreviations are used throughout the paper: NH_x denotes dry deposited reduced N, NO_x represents dry deposited oxidised N, Wet NH_x represents wet deposited reduced N, Wet NO_x refers to wet deposited reduced N and Total N stands for total deposited N.

2.5. Statistical analysis

The relationships between response and explanatory variables were assessed using linear regression. For relationships with distance, when an ln-transformed response variable led to a better fitting model (i.e. $\geq 5\%$ more of the variance in the response was explained) this model was accepted. For relationships between the other variables examined, both untransformed and ln-transformed response and explanatory variables were assessed. If the transformation led to a better fit (see above), the transformed model was accepted. In almost all cases relationships with N deposition had the best fit when both response and explanatory variables were logged. Data have been back-transformed for presentation. All analyses were performed in R2.7.2 (R Development Core Team, 2005).

3. Results

3.1. Relationship between N deposition and distance from London

Significant relationships were found between all pollutant variables and distance from central London. The best fit regression was log linear for wet deposited oxidised ($P < 0.01$) and reduced ($P < 0.001$) N, and linear for dry (oxidised $P < 0.01$, reduced $P < 0.001$) and total ($P < 0.01$) deposited N. The relationship with distance from central London is illustrated for total N deposition in Fig. 1; values ranged from 18.2 kg ha⁻¹ y⁻¹ at Horsell Common (36 km away from London) up to more than twice this level at Hampstead Heath (36.6 kg ha⁻¹ y⁻¹), 7.5 km from the city centre (Fig. 1). The biggest differences between sites were seen in terms of dry deposited NH_x ; this varied from highs of 22.7 kg ha⁻¹ y⁻¹ in inner city locations, down to 5.3 kg ha⁻¹ y⁻¹ in rural Surrey, representing between 28% (rural) and 62% (urban) of total modelled N inputs. There was relatively little difference between sites for wet

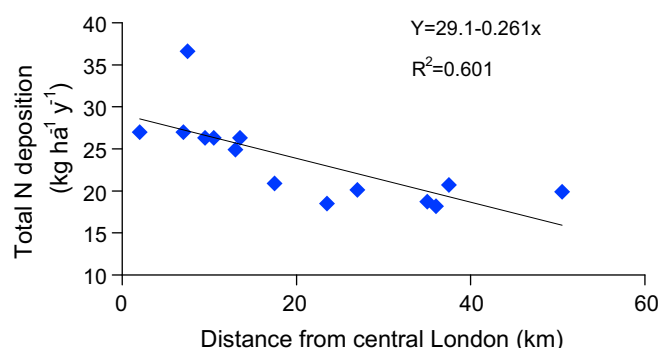


Fig. 1. The relationship between N deposition and distance from central London.

deposited pollutants; wet NO_x ranged from 3.0 to 4.4 kg ha⁻¹ y⁻¹, while wet NH_x ranged from 3.2 to 4.8 kg ha⁻¹ y⁻¹. Relatively few significant regressions were found between any index of plant chemistry and either of the wet deposited pollutants. The following results, therefore, focus on relationships with dry and total pollutant deposition, only highlighting relationships with wet deposited N where these are statistically significant.

3.2. Foliar nutrient concentrations and nutrient ratios

Foliar N concentrations of *Calluna* varied from 9.1 to 15.0 mg g⁻¹ dry weight; the highest levels were found in central London, corresponding to higher values of NO_x , NH_x and total N deposition (Fig. 2a). Concentrations decreased significantly with increasing distance from the city centre and with decreasing deposition of oxidised, reduced and total N (Table 2). Similar (but reverse) patterns were seen for foliar C:N ratios, with significant regressions for distance and N deposition (Table 2). Ratios ranged from 32 in central London to 56 at the furthest site along the transect (Thursley Common). Relationships between C:N ratios and N deposition were similar for oxidised, reduced and total deposition. Although N deposition explained less than one third of the variation in C:N ratios between sites, the r^2 value for the relationship with NH_x ($r^2 = 0.302$), was slightly higher than that for either NO_x ($r^2 = 0.132$) or total N ($r^2 = 0.238$) (graphs not shown).

Interestingly, foliar P concentrations also decreased significantly with increasing distance from the city centre, and were positively related to total N, NO_x and NH_x deposition (Table 2). Phosphorus concentrations in *Calluna* shoots ranged from 0.146% to 0.069%. The relationships with both distance from London and N deposition were steeper for foliar P than for N, resulting in significant changes in foliar N:P ratios with distance ($P < 0.001$, positive relationship) and with deposition ($P < 0.0001$, negative relationship). Tissue N:P ratios varied from 9.5 to 14.5, suggesting relative N limitation in more urban locations (Fig. 2b).

3.3. Stable isotope signatures

Delta ¹⁵N values ranged from +0.156 at the most urban sample site (2 km from the city centre) to -8.64 at Thursley Common (Surrey), 50 km from central London. The relationship between $\delta^{15}\text{N}$ and distance was statistically significant (Table 2). Isotopic N signatures of *Calluna* were also significantly related to NH_x , NO_x and total N deposition, with NO_x generally accounting for a higher proportion of the variation in $\delta^{15}\text{N}$ concentrations ($r^2 = 0.374$) than either NH_x ($r^2 = 0.265$) or total N ($r^2 = 0.189$). The slopes of these relationships indicate a decline in the relative proportion of the heavy (¹⁵N) isotope with decreasing levels of N and with increasing distance from central London (Fig. 2c, Table 2). Isotopic N signatures

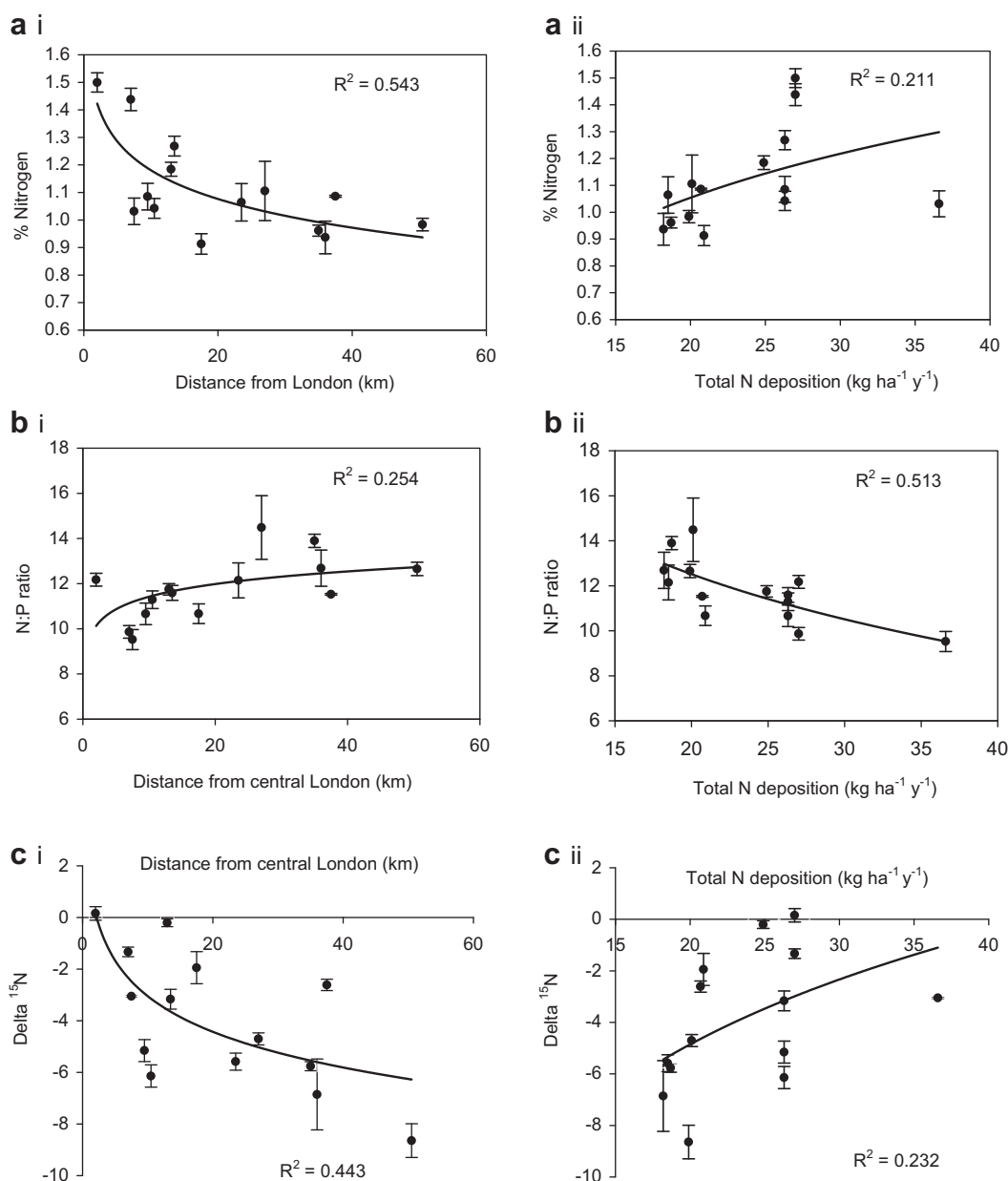


Fig. 2. Relationships between a) foliar N, b) foliar N:P ratios and c) $\delta^{15}\text{N}$ for *Calluna* with both (i) distance from Central London and (ii) total N deposition. Error bars represent the standard error of sample means.

were also related to overall foliar N concentrations, with more negative $\delta^{15}\text{N}$ values associated with lower total N concentrations (Fig. 3).

3.4. Amino acid concentrations

Concentrations of fifteen amino acids were recorded at levels above the detection limits of the HPLC. Tyrosine, histamine and lysine were present in the highest concentrations of all the amino acids although, of these, only the latter two were significantly related with either distance or N deposition. Overall, nine amino acids showed significant, negative relationships with increasing distance from the city centre. Summary statistics for the regression analyses against distance and N deposition are given in Table 3, for the amino acids which had significant relationships with at least one of these explanatory variables. Of the nine amino acids, seven

(asparagine, glutamine, glycine, phenylalanine, isoleucine, leucine lysine) also had significant, positive relationships with increasing levels of N deposition. In general, dry NH_x and total N deposition explained a greater proportion of the variation in individual amino acid concentrations than dry NO_x , although the latter was nonetheless significant for six of the seven amino acids. Fig. 4 illustrates the relationships between total N deposition and foliar concentrations for those amino acids for which regressions were statistically significant.

4. Discussion

The significant reduction in N deposition with increasing distance from the city centre is as would be expected from the published literature (e.g. Lovett et al., 2000; Styers and Chappelka, 2009; Harrison et al., 2006). Despite a strong gradient in NO_2

Table 2
Summary statistics from regression analysis between *Calluna* tissue chemistry and distance from London, dry NO_x, dry NH_x and total N deposition. “Transformation” describes the nature of the transformations which produced the best fit (greatest explanatory power) regression model: ln indicates response variables were log transformed, ln:ln indicates that both response and explanatory variables were log transformed.

Response variable	Explanatory variable	Intercept	Slope	ANOVA of Model			Transformation
				F	DF	P	
%N	Distance	0.21	−0.006	15.4	1,39	0.0003	ln
	Dry NO _x	−1.09	0.674	4.23	1,39	0.0464	ln:ln
	Dry NH _x	−0.30	0.171	11.5	1,39	0.0016	ln:ln
	Total N	−0.96	0.330	8.33	1,39	0.0063	ln:ln
δ ¹⁵ N	Distance	−1.01	−0.0001	26.4	1,39	<0.0001	ln
	Dry NO _x	−1.05	0.023	24.8	1,39	<0.0001	ln:ln
	Dry NH _x	−1.02	0.003	15.4	1,39	0.0003	ln:ln
	Total N	−1.03	0.006	10.3	1,39	0.0026	ln:ln
%P	Distance	−2.15	−0.01	57.5	1,39	<0.0001	ln
	Dry NO _x	−5.03	1.52	18.9	1,39	<0.0001	ln:ln
	Dry NH _x	−3.20	0.258	74.3	1,39	<0.0001	ln:ln
	Total N	−4.72	0.747	54.4	1,39	<0.0001	ln:ln
C:N	Distance	3.69	0.007	10.9	1,26	0.0037	ln
	Dry NO _x	82.0	−6.08	2.98	1,26	0.0960	None
	Dry NH _x	4.29	−0.20	8.30	1,26	0.0078	ln:ln
	Total N	5.06	−0.391	6.08	1,26	0.0205	ln:ln
N:P	Distance	2.36	0.005	13.5	1,39	0.0010	ln
	Dry NO _x	3.95	−0.846	16.5	1,39	0.0002	ln:ln
	Dry NH _x	2.90	−0.188	44.1	1,39	<0.0001	ln:ln
	Total N	3.78	−0.418	44.5	1,39	<0.0001	ln:ln

concentrations from central London (18.5 ppb) to rural Surrey (11.0 ppm) (data not shown, but obtained from the nearest UK Nitrogen Dioxide Network monitoring station, averaged for 2000–2001, <http://www.aeat.co.uk/netcen/airqual>), dry NO_x represented a relatively small proportion (18–37%) of the total deposited N, even in heavily trafficked, inner city areas. Deposition was instead dominated by dry NH_x, representing 53–62% of N inputs in the most urban locations. This dominance by reduced N reflects the association between NH₃ and vehicle catalysts (Sutton et al., 2000), high traffic densities in densely populated cities and the high deposition velocity of NH₃, relative to NO_x (Smith et al., 2000).

The significant relationships between foliar N concentrations and N deposition support those published elsewhere for *Calluna*, over much larger geographic (Pitcairn et al., 2001; Rowe et al., 2008) and altitudinal (Hicks et al., 2000) gradients in N deposition, and in relation to point sources of N (Sutton and Fowler, 1995). *Calluna* shoot chemistry has also been shown to change in field N manipulation experiments in both upland and lowland ecosystems. For example, foliar N concentration and N:P ratios in heathland plants have been found to increase, and C:N ratios decrease in

response to increasing N deposition (Prins et al., 1991; Power et al., 1998a; Carroll et al., 1999). Pitcairn et al. (2001) described the nature of the relationship between *Calluna* foliar N concentrations and total N deposition in a study involving samples across a European pollution gradient as linear, fitting the equation % N = 0.79 + 0.036*N deposition. The present study had a lower range of foliar N concentrations (0.91–1.5% dry weight) and N deposition than Pitcairn et al. (2001). Analysis of untransformed data reveals a similar intercept (also 0.80% N at zero N deposition) but a somewhat smaller slope of the relationship with atmospheric deposition (0.013% N kg^{−1} deposited N ha^{−1} y^{−1}) in the current study. The clear, statistically significant increase in foliar N concentration and decrease in C:N ratios found with distance and (in the opposite direction) N deposition provide further evidence of the value of higher plants as biomonitors of atmospheric pollution, particularly in urban settings where environmental conditions and habitat availability may limit the natural occurrence of desired bryophyte species for use as biomonitors.

Of additional interest in the present study is the significant increase in foliar P concentrations, and decrease in N:P ratios with increasing N deposition (and proximity to the centre of London). N:P ratios have been used fairly extensively as a tool to indicate relative nutrient limitation, with ratios above 16 (Koerselman and Meuleman, 1996) or 20 (Güsewell, 2004) associated with P-limitation. This is supported by observations from manipulation studies, typically showing increased N:P ratios with increasing N inputs, suggesting a shift towards progressive P-limitation. However, the present study suggests that P uptake is greater in areas receiving higher N inputs and indicates that heathland fragments in an urban context remain N-limited, even under conditions of prolonged, elevated N deposition. Such a pattern, whilst in contrast to data from manipulation experiments (e.g. Carroll et al., 1999), supports the findings from both an earlier altitudinal N gradient study (Hicks et al., 2000) and a recent nationwide survey of UK vegetation in relation to atmospheric N inputs (U Countryside Survey 2007 – Rowe et al., 2008). In the latter study, it was suggested that N-driven increases in P (and K) mineralisation rates

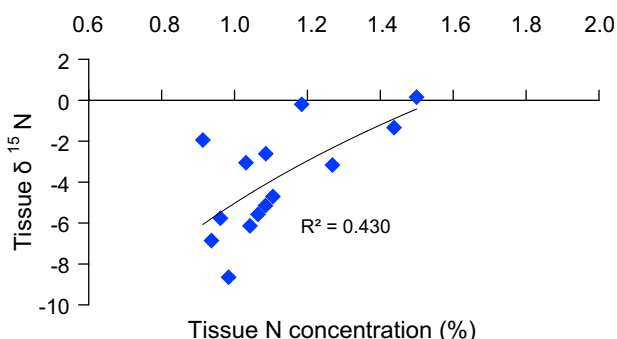


Fig. 3. Relationship between δ¹⁵N signature and foliar N concentration in *Calluna*.

Table 3

Summary of statistical analysis for amino acids which had significant relationships with distance from central London, dry NO_x, dry NH_x or total N deposition. Degrees of freedom = 1,37 in all cases. "Transformation" describes the nature of the transformations which produced the best fit (greatest explanatory power) regression model: Ln indicates response variables were log transformed; ln + 1 indicates response variable +1 was log transformed; ln:ln + 1 indicates that the explanatory variable was log transformed and that the response variable +1 was log transformed, prior to analysis.

Response variable	Explanatory variable	Intercept	Slope	ANOVA		Transformation
				F	P	
Asparagine	Distance	4.58	−0.091	7.28	0.010	None
	Dry NO _x	3.95	−0.846	16.5	<0.0001	ln:ln + 1
	Dry NH _x	−1.23	0.932	10.16	0.0029	ln:ln + 1
	Total N	−5.2	1.95	8.86	0.0051	ln:ln + 1
Glutamine	Distance	12.1	−0.321	5.74	0.0217	None
	Dry NO _x	−10.6	6.62	7.72	0.0080	ln:ln + 1
	Dry NH _x	−2.85	1.67	24.3	<0.0001	ln:ln + 1
	Total N	−10.3	3.60	22.4	<0.0001	ln:ln + 1
Histamine	Distance	26.1	−0.456	4.65	0.0377	None
	Dry NO _x	−51.2	11.7	2.58	0.1170	None
	Dry NH _x	11.81	0.399	0.40	0.5329	None
	Total N	3.30	−0.321	0.103	0.7510	None
Glycine	Distance	7.84	−0.035	11.0	0.0020	ln + 1
	Dry NO _x	−6.97	4.72	4.92	0.0328	ln:ln
	Dry NH _x	−1.59	1.27	16.2	0.0003	ln:ln + 1
	Total N	−7.52	2.82	16.5	0.0002	ln:ln + 1
Methionine	Distance	8.66	−0.139	9.04	0.0047	None
	Dry NO _x	−16.86	3.89	5.92	0.0199	None
	Dry NH _x	0.244	0.371	7.827	0.0081	None
	Total N	−2.27	1.24	3.466	0.0714	None
Phenylalanine	Distance	5.83	−0.043	16.6	0.0023	ln + 1
	Dry NO _x	−10.71	6.54	9.00	0.0048	ln:ln + 1
	Dry NH _x	−3.00	1.64	29.5	<0.0001	ln:ln + 1
	Total N	−10.7	3.65	30.5	<0.0001	ln:ln + 1
Isoleucine	Distance	5.15	−0.044	21.8	<0.0001	ln + 1
	Dry NO _x	−9.69	5.92	8.42	0.0062	ln:ln + 1
	Dry NH _x	−2.68	1.47	26.0	<0.0001	ln:ln + 1
	Total N	−9.25	3.17	24.0	<0.0001	ln:ln + 1
Leucine	Distance	3.56	−0.034	15.3	0.0004	ln + 1
	Dry NO _x	−7.167	4.34	5.83	0.0208	ln:ln + 1
	Dry NH _x	−2.00	1.11	16.8	0.0002	ln:ln + 1
	Total N	−6.78	2.24	14.7	0.0005	ln:ln + 1
Lysine	Distance	15.1	−0.342	5.63	0.0023	None
	Dry NO _x	−27.3	6.06	1.43	0.2401	None
	Dry NH _x	−2.43	1.54	10.5	0.0025	ln:ln + 1
	Total N	−9.10	33.26	9.37	0.0041	ln:ln + 1

and/or fine root penetration of the soil could explain the increased uptake of soil P. The patterns observed in the present study could be explained by similar mechanisms, as well as by increases in root/soil phosphomonoesterase (PME) and/or mycorrhizal activity (Pilkington et al., 2005). However, it is also known that urban areas can experience relatively high levels of dust and particulate matter compared to many rural areas, with associated increases in the deposition of P and other macro/micro nutrients (Lohse et al., 2008). Whether the observed pattern of higher *Calluna* P concentrations and lower N:P ratios in urban areas is driven by greater deposition of P or N-driven increased rates of nutrient turnover and plant uptake, this study provides evidence that prolonged exposure to elevated N deposition does not necessarily result in progressive P-limitation of semi-natural ecosystems under all field conditions.

Changes in natural abundance of N isotopes have been demonstrated in manipulation experiments involving N addition (Nadelhoffer et al., 1999) and along gradients of both oxidised (Pearson et al., 2000) and reduced (Skinner et al., 2006a,b) N deposition. It is widely recognised that many factors will affect isotopic fractionation in plants, including the relative enrichment or depletion of atmospheric and/or agricultural N inputs (Hogberg, 1997). Nonetheless, there is evidence that higher and lower plants

have less negative $\delta^{15}\text{N}$ signatures in areas receiving relatively high N inputs (Ammann et al., 1999; Pearson et al., 2000; Saurer et al., 2004; Skinner et al., 2006a,b). The relative ^{15}N enrichment of *Calluna* growing in urban areas (receiving the highest rates of N deposition) in this study provides further evidence that isotopic signatures in plant foliage are a useful indicator of atmospheric N inputs. The stronger relationship with oxidised, compared to reduced, N is similar to that reported for mosses by Pearson et al. (2000). Although in the present study modelled N deposition is dominated by reduced N in urban areas, NH₃ associated with vehicle catalysts is likely to have a similar isotopic signature to that of NO_x derived from the same fossil fuel source (Sutton et al., 2004). Also, since NH₃ is known to deposit rapidly, typically falling to low levels within 10 m from the roadside (Cape et al., 2004), its contribution to the isotopic signatures of plants which were predominantly more than 20 m (and typically over 100 m) away from a major road may be relatively small.

The positive relationship between $\delta^{15}\text{N}$ and foliar N concentration found in the present study has also been reported for trees (Jung et al., 1997; Hobbie et al., 2000). Although factors such as soil N availability and mycorrhizal discrimination can affect isotope signatures (Hobbie et al., 2000), *Calluna* is able to gain a substantial

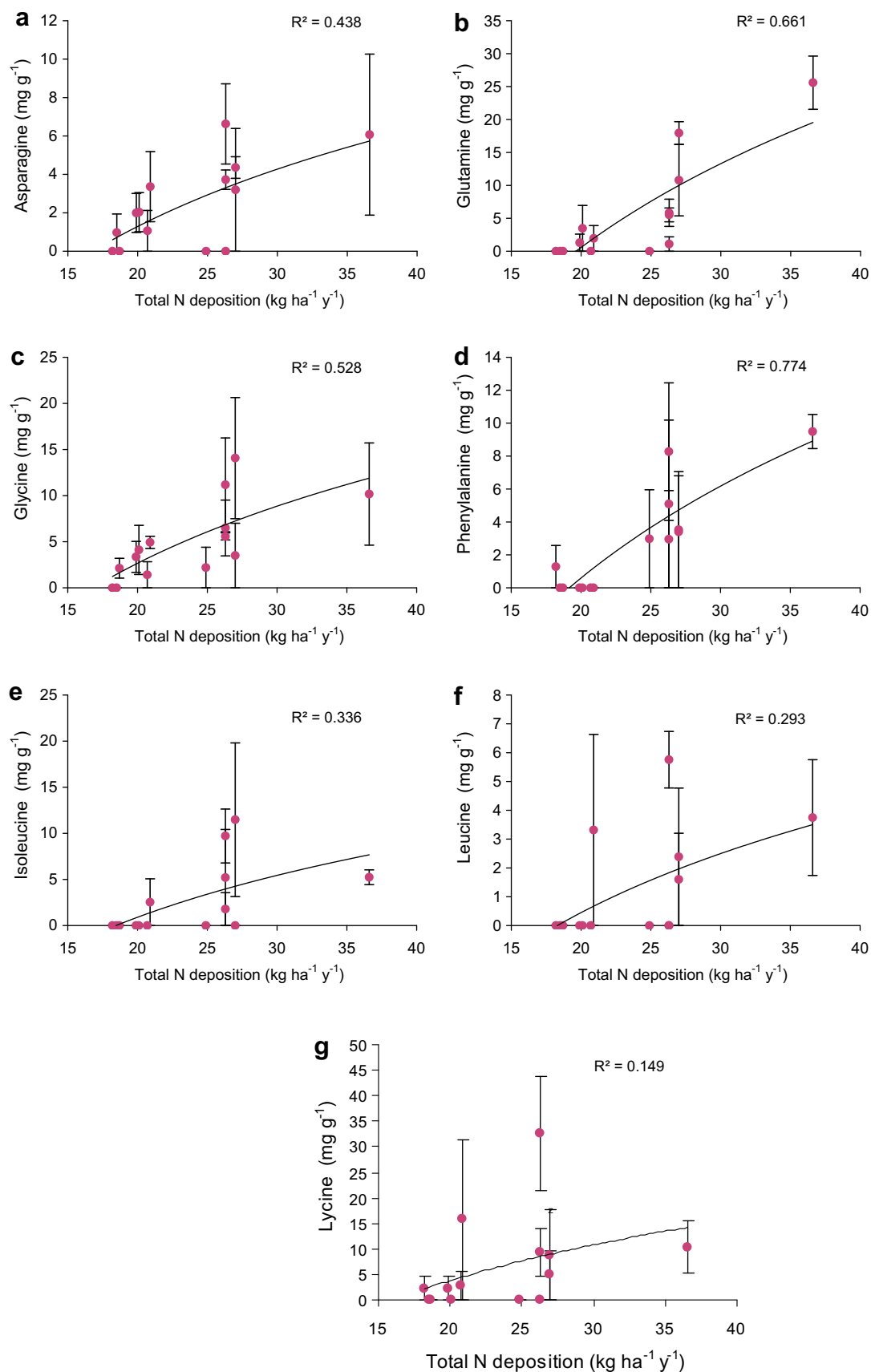


Fig. 4. Relationships between foliar amino acid concentrations and total N deposition for a) Asparagine, b) Glutamine, c) Glycine, d) Phenylalanine, e) Isoleucine, f) Leucine and g) Lysine. Error bars represent within-site standard error.

proportion of its N requirements through direct foliar uptake of atmospheric N (Bobbink and Heil, 1993). Pearson et al. (2000) suggested that less negative $\delta^{15}\text{N}$ values can be linked to emissions of oxidised N. Although the lack of information on isotopic signatures of vehicle-derived NH_3 means that it is not possible to attribute observed changes in plant $\delta^{15}\text{N}$ values to emissions sources in the present study, the observations are consistent with the suggestion that gaseous uptake of NO_x (which is relatively enriched in ^{15}N) may contribute significantly to overall plant N nutrition in urban environments.

Earlier studies have demonstrated a link between concentrations of free amino acids (particularly those which are relatively N-rich such as arginine, asparagine and glutamine) and levels of N deposition in forest trees (Van Dijk and Roelofs, 1988), forest understorey vegetation (Nordin et al., 1998), heathland vegetation (van den Berg et al., 2008) and in pleurocarpous mosses (Pitcairn et al., 2003). The present study shows that the composition of the free amino acid pool in *Calluna* foliage is related to levels of atmospheric N deposition. Strong, statistically significant relationships were found for seven of the amino acids analysed, although arginine was not one of these. Interestingly, the proportional increase in concentrations of amino acids in response to N inputs can be greater than the increase in overall tissue N concentrations. For example, Nordin et al. (1998) reported increases of more than 50% in foliar concentrations of free amino acids in *Deschampsia flexuosa* following N additions of $50 \text{ kg ha}^{-1} \text{ yr}^{-1}$, compared to increases in leaf N concentration of less than half this amount. Despite a reasonable amount of inter- (and in some cases intra-) site variation in amino acid concentrations in the current study, a significant proportion of the between-site variation in concentrations of glycine ($r^2 = 0.528$), glutamine ($r^2 = 0.661$) and phenylalanine ($r^2 = 0.774$) was explained by N deposition, indicating that, in *Calluna*, these amino acids may be useful indicators of N loading.

Nordin et al. (1998) demonstrated a link between amino acid concentrations and the incidence of pathogen and pest damage on *Vaccinium myrtillus*. It is possible, therefore, that changes in the size and composition of the free amino acid pool not only indicate N accumulation but also provide physiologically relevant information on the potential for damage associated with elevated levels of N deposition. In fact, all of the chemical indices assessed in this study have been associated with detrimental changes in either plant performance, community structure or ecosystem functioning. For example, Angold (1997) demonstrated increases in *Calluna* foliar N concentrations and changes in heathland plant community composition with increasing proximity to a major road. Pitcairn et al. (2003) reported increasing bryophyte amino acid concentrations and a shift in plant community composition, including loss of bryophyte cover, along a gradient of increasing NH_3 deposition. Similarly, high N:P ratios have been associated with loss of biodiversity in heathland and grassland ecosystems (Roem and Berendse, 2000), while changes in plant litter N content have been shown to affect the rate of ecosystem nutrient cycling (Fortunel et al., 2009).

Between-site differences are likely to exist for factors which have the potential to influence N uptake and dilution (e.g. temperature, exposure, management history, soil nutrient status). However, in this study, these were either largely controlled for by site selection criteria (management history, exposure) or shown not to vary in a systematic way in relation to N deposition (soil N status). Although changes in bryophyte tissue chemistry have been more commonly used as indicators of changes in air quality, including N deposition (e.g. Pitcairn et al., 2006; Frati et al., 2007; Pesch et al., 2008) the use of *Calluna* as a biomonitor of N inputs is now strongly supported by both transect studies and manipulation experiments.

5. Conclusions

The present study is the first to use *Calluna* to demonstrate relationships between multiple plant biochemical indices, atmospheric N deposition and urban proximity. The patterns of elevated foliar N and amino acid concentrations reported here suggest that levels of atmospheric N loading in urban areas are likely to be having effects on the biodiversity and functioning of urban ecosystems. Furthermore, lower foliar N:P ratios with increasing urban proximity, despite higher N deposition rates, suggest that P availability is unlikely to limit plant and ecosystem response to N in urban locations.

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