Research article

The effect and interaction of enhanced nitrogen deposition and reduced light on the growth of woodland ground flora

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Introduction

Light is one of a few key growth-limiting factors in plants. Plants have evolved specialized mechanisms to efficiently grow in situations of low incident irradiance and increase the interception of light to utilize what little may be available at any given time. These mechanisms and adaptations have been widely studied in the past.

Within mature woodland, the availability of light is considered to be the single most significant influence on ground flora vegetation, mediating all other influences. The canopy of a deciduous forest reduces incident irradiance considerably, with only ~20% of the available light ever likely to reach the ground.1 In some situations, a dense canopy can reduce the level of incident irradiance to the woodland floor to 1% of that available at the canopy, yet woodland ground flora are found not to inhabit areas with <2% available light.2

As woodland ground flora species have to operate at low levels of available light, many adaptive theories have been explored to understand the relationship between light availability and the physiology, shape, size and position of leaves of these shade-tolerant plants.3–6 There is strong evidence that specific leaf area increases with a decrease in the available incident irradiance.7 However, some plant species have become shade tolerant by using other ecological strategies, such as completing their entire life cycle early in the spring before the canopy grows and reduces the amount of light available to the plants; remaining in a slow growing vegetative form until light becomes available; surviving as dormant seed until light levels increase following the fall of a tree or coppicing of the woodland; being ‘casual species’ that have good powers of dispersal and are opportunistic in colonizing bare soil and acclimatising to shade providing there is no competition for any resources.1

Woodland ground flora species Geranium robertianum, Prunella vulgaris and Deschampsia caespitosa were grown in greenhouse conditions under various light regimes (100%, 20% and 5% of ambient summer light) and were misted weekly with solutions of ammonium nitrate to simulate increased atmospheric wet and dry deposition of nitrogen (N) due to pollution at 0, 15 and 30 kg ha⁻¹ year⁻¹ concentrations. In the last 5 weeks of the study, the photosynthetic rate of G. robertianum was measured weekly. At the end of the 10-week treatment period, the plants were harvested and growth was recorded using various measurable growth parameters. Growth of the plants and photosynthetic rate of G. robertianum were found to be primarily dependent on light availability, where reduced light levels showed a decrease in overall plant growth, indicating the importance of light in woodlands. N was not found to have an effect on the growth of the plants and thus questions arise over the calculations of critical loads of N deposition for woodland habitats. Five interactions were noted where increased N led to a significant reduction in growth at 20% light, yet a significant increase in growth at 100% light. These interactions were mainly noted for P. vulgaris, which could be explained by relatively large differences between its Ellenberg values for light and N, reflecting a preference for high light conditions and fairly infertile soils. G. robertianum was least affected by a reduction in light, reflecting its preference for semi-shaded conditions.

Key words: Ellenberg values, nitrogen, light, woodland, critical loads, ground flora.
ground flora species are annual, biennial or short-lived perennials and so complete their life cycles quickly when light becomes available. These plants can therefore often be used as an indicator of poor light availability in certain habitats, such as woodlands.

Natural and semi-natural woodland ecosystems are generally considered to be limited in growth primarily by the amount of bio-available nitrogen (N) for uptake and use by the plant. N is the fourth most common element constituent of a plant, often making up ~1.5% of the dry mass. N is also a basic constituent of chlorophyll and so can prevent optimum rates of photosynthesis when in short supply within the plant.

The main types of N-containing air pollutants of concern due to their effects on vegetation are NO\(_x\) (split into nitric oxide (NO) and nitrogen dioxide (NO\(_2\))) and ammonia (NH\(_3\)) as dry deposition, and ammonium (NH\(_4^+\)) and nitrate (NO\(_3^-\)) as wet deposition. These categories of pollutants can act very differently in a biological setting.

There has been a considerable increase in nitrogenous air pollutant depositions since the 1900 background levels of 1–3 kg N ha\(^{-1}\) year\(^{-1}\) to around two million tonnes emitted from the UK in 2004, equivalent to an average deposition rate of 17 kg N ha\(^{-1}\) year\(^{-1}\). Sources of emissions of atmospheric N include road transport (35%), energy production (27%), commercial and residential (16%) and industrial (16%) emissions. This has led to an increase in levels of NO\(_x\) in the atmosphere of an intensity between 5 and 20 times that of base natural atmospheric levels.

NH\(_3\) (and NH\(_4^+\) in solution) levels are also thought to have increased in recent years, but this is harder to quantify and usually local to the source. Most NH\(_3\) emissions come from intensive agriculture and animal husbandry.

N deposition rates to woodland are higher than many other vegetation types due to the aerodynamically rough vegetation of the woodland on which the particles are more easily deposited than that on, for example, grassland. Atmospheric depositions of N are thought to contribute to as much as one-third of the total N in a forest ecosystem.

Low-to-moderate deposits of additional N can show effects on plants such as enhanced growth, where the N is available for uptake by the plant and acts as a fertilizer. At high levels, N deposition can cause reduced growth in plants through direct phytotoxic effects. In addition, natural and semi-natural vegetation can be affected in other ways, including increased susceptibility to secondary stress factors, e.g. frost, drought and pathogens.

Bobbink et al. (pp. 731) highlight ‘the competitive exclusion of characteristic species by more nitrophilic species especially under oligotrophic to mesotrophic soil conditions’; much UK woodland would lie in these oligotrophic and mesotrophic categories. Such plant community responses will depend on the duration of the pollution, sensitivity to N of the plant species and the biotic and abiotic conditions of the ecosystem as a whole.

When additional N is deposited onto an area of forest over a period of time, it has generally been found that woodland specialist ground flora species declined and were replaced by more nitrophilic species or species composition or diversity changed. This loss in diversity has also been noted in other ecosystems (acidic grasslands) by Stevens et al. and Van der Wal et al.

A way of quantifying the damage to plants by N deposition is to measure the rate of photosynthesis in a plant, to find out if the plant is deteriorating in health and its ability to photosynthesize with increasing exposure to the pollutant. Various research, for example Hill and Bennett, Bull and Mansfield and Saxe among many others, has found that NO\(_x\) exposure causes a decrease in the photosynthetic rate of many plant species.

Critical loads are defined by Wellburn (pp. 13) as ‘the highest load that will not lead in the long term (50 years) to harmful effects on the most sensitive ecological systems’. The critical load for N deposition in UK deciduous forest ecosystems to prevent change in species composition is estimated to be 15–17 kg ha\(^{-1}\) year\(^{-1}\). Above this level, harmful effects may occur to sensitive elements within the ecosystem. Ninety-seven per cent of woodlands in the UK are thought to be exceeding their critical load of N.

Ellenberg indicator values are useful in this investigation, as they rate vascular plants by different limiting factors such as tolerance to: shade, nutrient poor soils, soil pH, temperature, soil moisture and salt levels. They help to describe the niche of a particular plant species by rating them (on a scale of 1–9) in the aforementioned categories. Hence, Ellenberg values can be used to select plant species that may be expected to have contrasting responses to changing environmental conditions, as well as in the field to assess the environmental conditions of the habitat according to what plant-life is present (e.g. Pitcairn et al.).

There are known to be complex interactions between the uptake and use of N in a plant and many other growth-limiting factors including light availability. There has been little investigative research on these interactions, yet they could be of great importance given that N deposits have increased over the last century and could have drastic effects on woodland ground flora, where light availability is limited.

The species used in this investigation were chosen due to their presence in woodland ground flora in the UK and their range in ability to grow in varying light and N conditions. Geranium robertianum is commonly known as ‘herb Robert’ and is widely distributed across the UK. It is a biennial plant of height ~10–15 cm, most commonly found in areas of shade such as woodlands and hedgerows and in moderately N-rich soils. This shows that it is sensitive to high light levels, yet is more tolerant of N-rich soils.
**Aims and objectives**

The main aim of this investigation was to look at the effects of varying N and light levels on the growth of woodland ground flora species under controlled conditions, to discover whether one factor is more limiting in the growth of the plants than the other and to explore any interactions between factors.

**Materials and methods**

Ten species of woodland ground flora commonly found in the UK were selected on the basis of variation in Ellenberg values to give a range of plant species with varying levels of nitrophilic and light-loving behaviour. Attempts were made to germinate their seed in the dark, on moist blotting paper at 15°C.

Three of the 10 initial species (highlighted in Table 1), although not as different in Ellenberg values as anticipated, successfully germinated within 3 weeks and were transplanted into seed trays containing Melcourt Sylvamix® potting compost with a N content of 200 mg N per pot (equal to 250 mg l⁻¹) and grown in a propagator in natural light for 3 weeks at a constant temperature of ~15°C. Six plants, two from each species, were potted up into each of 45 11.5-cm diameter pots containing the same growth medium and grown under an enhanced light regime for a further 2 weeks in order to simulate the springtime when they would naturally germinate and encourage growth before treatments were applied. Pots were then randomly assigned to nine different treatment groups, giving five pots in each treatment group (Table 2).

The plants were subjected to the different light levels using an 18-h enhanced light regime from sodium lamps as the control and reducing light availability using frames covered in greenhouse shading material to the required levels typically encountered by UK woodland ground flora, measured using an average of 10 photosynthetically active radiation (PAR) readings taken from a ‘Skye Spectrasense logger’.

The plants were misted weekly using a pump action sprayer and a standardized technique to mimic wet and dry N deposition through pollution. Deionized water was used for the control and varying ammonium nitrate solutions to simulate the critical load of N deposition in the UK (15 kg N ha⁻¹ year⁻¹) and twice the critical load at 30 kg N ha⁻¹ year⁻¹ (see Table 2 for light and N treatments).

During the 10-week treatment period, all pots were rotated on a weekly basis to minimize edge effect and were carefully supplied with adequate volumes of deionized water to allow survival and stop the pots drying out yet not cause water logging. The plants were not given any supplementary feed in order to show the full effect of the additional N treatments. The greenhouse temperature during the treatment period was maintained at 20°C.

During the last 5 weeks of the treatments, when only *G. robertianum* leaves were large and strong enough not to get damaged, the photosynthetic rate of this species was measured on a weekly basis using an infrared gas analyser (IRGA) machine (model ADC LCA4) by placing an area of

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**Table 1.** Woodland ground flora species selected for attempted germination and their Ellenberg values for N and light

<table>
<thead>
<tr>
<th>Species</th>
<th>Ellenberg light values</th>
<th>Ellenberg N values</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Millium effusum</em></td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td><em>Carex sylvaticum</em></td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td><em>Lonicera periclymenum</em></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><em>Geranium robertianum</em></td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td><em>Deschampsia caespitosa</em></td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td><em>Brachypodium pinnatum</em></td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td><em>Rubus fruiticosus</em></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td><em>Prunella vulgaris</em></td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td><em>Geum urbanum</em></td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td><em>Filipendula ulmaria</em></td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>

*From Hill et al.*

Species that did germinate and were used in this study.

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**Table 2.** Treatment groups of the plants

<table>
<thead>
<tr>
<th>Nitrogen</th>
<th>Light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100%—control</td>
</tr>
<tr>
<td></td>
<td>(200 µmol m⁻² s⁻¹ PAR)</td>
</tr>
<tr>
<td></td>
<td>(40 µmol m⁻² s⁻¹ PAR)</td>
</tr>
<tr>
<td>Distilled water</td>
<td>× 5</td>
</tr>
<tr>
<td>(control)</td>
<td></td>
</tr>
<tr>
<td>15 kg N ha⁻¹ year⁻¹</td>
<td>× 5</td>
</tr>
<tr>
<td>(critical load)</td>
<td></td>
</tr>
<tr>
<td>30 kg N ha⁻¹ year⁻¹</td>
<td>× 5</td>
</tr>
<tr>
<td>(2× critical load)</td>
<td></td>
</tr>
</tbody>
</table>

Each treatment contains five mixed pot replicates, with two plants of each of the three ground flora species present in each pot.
leaf from each pot, standardized by eye, showing no signs of chlorosis or discoulouration in the measuring chamber. The IRGA machine measured the difference in CO₂ used and O₂ produced and calculated a rate of photosynthesis from these data. These data were later used to investigate whether the light and additional N treatments had an effect on the photosynthetic rate of G. robertianum.

After 10 weeks of treatment, all the plants were harvested by cutting at soil level, and measurements of growth for each plant were recorded including: the maximum height; percentage chlorosis and discoulouration of the leaves (measured by eye); above-ground dry mass and the number of leaves present. The total leaf area was recorded using the ‘DT-SCAN’ computer program. The mean individual leaf area was then calculated from the number of leaves and the total leaf area.

Statistical analyses involving general linear models and post hoc Tukey tests (a type of two-way analysis of variance (ANOVA)) were conducted on these data using Minitab 14 statistical computer package to find whether there were significant differences or interactions in any of the growth parameters of the plants when subjected to different treatments. The photosynthetic rate data taken in the last 5 weeks from G. robertianum were also analysed using general linear models in Minitab 14 to investigate significant differences and interactions in the photosynthetic rate due to light, N and time of sampling.

**Results and discussion**

**Light**

Light availability was the main factor that had an effect on all the growth parameters studied in all the species tested with one exception that of the mean individual leaf area in D. caespitosa, which showed that neither light or N regimes were responsible for any variation found (Table 3). All three species reacted in the same way and showed a general reduction in growth, with a reduction in the available light.

The mean maximum height, mean total number of leaves and mean total leaf area of the plants were most reduced when the light level was decreased to 20% in P. vulgaris and D. caespitosa (although a lot of variation was shown in D. caespitosa for mean maximum height) yet a decrease to 5% light in G. robertianum was necessary to significantly reduce the mean maximum height, mean total number of leaves and mean total surface area.

The three species showed different preferences for light conditions as denoted by their Ellenberg indicator values. G. robertianum has the lowest Ellenberg value of 5, indicating that it is ‘a semi-shade plant rarely found in full light’. This indicates that G. robertianum should be more capable of coping with lower light availability and hence is not affected by reduced light until it reaches very low levels.

Although an overall decrease in mean individual leaf area was noted with a reduction in light with G. robertianum and P. vulgaris, an increase occurs in mean individual leaf area when the light is reduced from 100% to 20%. This is then followed by a significant decrease at 5% light availability. This pattern follows the conclusions drawn by Evans and Hughes, Newton, Portsmuth and Niinemets and Delagrange et al. that plants increase the specific leaf area with decreasing light availability. Therefore in shade adapted or acclimatized species, the leaf area increases to increase the interception of light at low light availability and allow photosynthesis to occur at low light levels.

The decrease in individual leaf area in P. vulgaris from 100% light to 20% light indicates that P. vulgaris is not as well adapted to shade as G. robertianum, which is suggested by the Ellenberg value assigned, being higher in P. vulgaris than that of G. robertianum.

The overall reduction in mean above-ground dry mass can also be linked to a decrease in the mean total number of leaves per plant when light availability was reduced. This mass reduction was most prominent when light was reduced from 100% to 20% in all species. The reduction in net photosynthetic rates due to the decreased availability of light would allow less carbon to be fixed and hence less biomass be produced than that of the control light levels.

When analysing the response of the species to light reduction in relation to the mean percentage chlorosis and discoulouration, G. robertianum reacted in a different way to that found in P. vulgaris and D. caespitosa. The latter two species showed an overall significant increase ($P < 0.01$ (d.f. = 84) and $P < 0.05$ (d.f. = 67) for P. vulgaris and D. caespitosa, respectively, shown in Table 3) in the mean percentage chlorosis and discoulouration, with a reduction in light, whereas G. robertianum showed a significant decrease ($P < 0.001$ with d.f. = 88). This difference in chlorosis and discoulouration was not due to N deficiencies or an interaction between light and N levels, as this was not significant in the general linear model for all species, and was therefore found to be primarily affected by light availability.

G. robertianum is known to discoulour in high light environments by producing darker areas on the leaf as observed in this experiment. This leaf discoulouration may be a photoprotective response of G. robertianum in order to limit photoinhibition at high light intensities, as it is known that shade plants are more prone to photoinhibition at lower light intensities than those that can inhabit well-lit places such as P. vulgaris.

The increase in chlorosis and discoulouration with decrease in light availability as exhibited in P. vulgaris and D. caespitosa (Table 3) could be due to other factors not measured in this study, such as deficiencies in other minerals, for example magnesium and iron, which can cause chlorosis of leaves.

Another explanation could be an interaction between light
Table 3. Summary of results from the general linear models giving the significant differences between the factors studied (N and light) and any interactions with adjusted $R^2$ values

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean maximum height</th>
<th>Mean percentage chlorosis and discolouration</th>
<th>Mean total number of leaves</th>
<th>Mean total leaf surface area</th>
<th>Mean dry above-ground mass</th>
<th>Mean individual leaf surface area</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G. robertianum</strong> (d.f. = 88)</td>
<td>Nitrogen NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Light ***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Interaction *</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Adjusted $R^2$ value (%)</td>
<td>82.60</td>
<td>55.75</td>
<td>51.42</td>
<td>50.65</td>
<td>69.64</td>
<td>46.33</td>
</tr>
<tr>
<td><strong>P. vulgaris</strong> (d.f. = 84)</td>
<td>Nitrogen NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Light ***</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Interaction ***</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>Adjusted $R^2$ value (%)</td>
<td>79.12</td>
<td>12.52</td>
<td>51.42</td>
<td>50.65</td>
<td>69.64</td>
<td>46.33</td>
</tr>
<tr>
<td><strong>D. caespitosa</strong> (d.f. = 67)</td>
<td>Nitrogen NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Light **</td>
<td>**</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>Interaction NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Adjusted $R^2$ value (%)</td>
<td>21.83</td>
<td>13.73</td>
<td>44.71</td>
<td>28.49</td>
<td>23.04</td>
<td>—</td>
</tr>
</tbody>
</table>

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; NS, not significant;

\[\downarrow\] a significant reduction in that specific growth parameter was noted with a decrease in available light.

\[\uparrow\] a significant increase in that specific growth parameter was noted with a decrease in available light.
and other growth-limiting factors not measured in this study. This would give the impression that decreasing light was responsible for an increase in chlorosis and discoloration but may not have been solely due to light availability.

On the whole, these results were as expected of the plants in a reduced light environment, as the rate of photosynthesis, and therefore ATP production, is limited by the amount of light available to the plant. Hence, any metabolically active reactions, for example the active assimilation of nitrate from the soil, or biomass production, are too limited at low rates of photosynthesis due to the low levels of light.9

The plants’ respective Ellenberg values for light (Table 1) show that even though P. vulgaris and D. caespitosa are often found in shaded woodland with reduced light, it may not be their preferred habitat and could show why they exhibited such a decrease in growth at the reduced light levels. G. robertianum showed slightly different responses to light reduction than P. vulgaris and D. caespitosa, possibly because G. robertianum is often found in shaded habitats and showed an adaptation to shade in respect to individual leaf area and was more affected by chlorosis and discoloration of the leaves when subjected to high light intensities. These adaptations could lead to increased competitive ability of G. robertianum in the field if the light levels remained low for the woodland ground flora, and therefore a change in species composition to more shade-tolerant plants such as G. robertianum.

Nitrogen

Additional N deposition alone was not found to have any effects on the growth of these ground flora species, as no significant differences were found in growth when additional N was applied (Table 3). The lack of response to increased N deposition of the plants does cause questions to arise over the calculation and use of critical loads for woodland ecosystems in the UK.18, 48–50 These critical loads were based on field experiments and observations19 and may not have had the same effect on the plants when grown in controlled conditions. Also, only three species were subjected to the study and so may not have shown the same susceptibility to stress as a whole ecosystem would exhibit.

When Ellenberg values were experimentally calibrated on natural soils in the Netherlands, the N content was found to be between 0 and 224 μg l⁻¹.51 Compared with the N content of the compost used in this experiment (equivalent to 250 000 μg l⁻¹), these values for natural soil are much lower and so the compost may not have been as limited in N as planned. This relatively high N content of the compost used could begin to explain why significant effects of additional N alone were not encountered in this experiment, as the growth of these species was not limited by N. Other nutrients that were not measured may have played a part in the growth of the plants and were possibly a limiting factor and should be considered a limitation to this methodology.

Interactions

Although addition of N alone had no effect on growth parameters, 5 of the 18 general linear models showed a significant interaction between light availability and N deposition (Table 3).

Mean maximum height of G. robertianum (Table 3) showed a significant decrease at 5% light between the 15 and 30 kg N ha⁻¹ year⁻¹, yet an increase in mean maximum height with increased N was noted at the 100% light level. This result is hard to explain in respect of the Ellenberg values assigned to this species (Table 1), as it would be expected that this species would be less suited to high light levels than the other species and would be able to tolerate higher levels of N that would make it grow more at lower light levels. Therefore, there may have been a number of other factors influencing the growth of G. robertianum other than those tested for in this study.

D. caespitosa also showed a significant interaction between differing light and N treatments in mean above-ground dry mass. An increase in mass with increasing N levels was noted at 100% light yet there was a decrease in mass with increased N at the 5% light level. The low R² value obtained for this analysis (Table 3) highlights the need to interpret these results with caution, as a high amount of variation was present within the data set.

P. vulgaris displayed three of the five interactions noted between increased N deposition and reduced light (Table 3). These interactions all followed the same general pattern of an increase in growth with increasing N deposition at 100% light and a decrease in growth at 20% light availability. This increase in growth at 100% light with increasing N deposition points to a fertilizer effect on the plants, when ample light is available and the N can be successfully used to aid in the overall growth of the plant. A reduction in growth was exhibited with an increase in N deposition when light levels were dropped to 20% possibly due to increased susceptibility to phytotoxic effects of the N such as apoplastic acidity52 or other physiological effects of excess N uptake when stressed by limited light availability.45

These interactions were all found to be more statistically significant (P < 0.01 or P < 0.001) than those found in G. robertianum and D. caespitosa (P < 0.05) (Table 3). This may be explained using the Ellenberg values assigned to the plants by Hill et al.8 in that P. vulgaris had the widest range in Ellenberg values for light (7) and N (4) (Table 1), displaying a preference for high light conditions and soils of low fertility.

Photosynthetic rate of G. robertianum

As with overall growth, the photosynthetic rate of the G. robertianum plants was found to be mainly affected by the availability of light (Table 4). A decrease in light
availability was found to show a significant reduction in the photosynthetic rate of *G. robertianum* (*P < 0.001, d.f. = 224*). This would have been due to the integral part that light availability plays in the rate at which a plant can photosynthesize and successfully grow. The time during the experiment at which the measurements of photosynthesis were recorded both showed no significant effect on the photosynthetic rate of *G. robertianum*. These results were unexpected, as the availability of N is known to play a fundamental part in the optimum rate of photosynthesis of a plant due to N being needed to make chlorophyll *10*. It was expected that at 0 kg N ha$^{-1}$ year$^{-1}$, *G. robertianum* would show a lower rate of photosynthesis than at higher N concentrations, as only a limited amount of chlorophyll can be synthesized from very low levels of N. *10*

The time during the experiment at which the measurement of photosynthetic rate was taken was also expected to show a change in the photosynthetic rate of *G. robertianum*. The theory behind this assumption was that the length of exposure to the light and N conditions would possibly increase the effects of the treatments or show acclimatisation and therefore increased photosynthetic rate towards the end of the study period.

Significant interactions (Table 4) were found between light and N regimes and light and time of the measurement. A decrease in photosynthetic rate was noted between the 15 and 30 kg N ha$^{-1}$ year$^{-1}$ at the 100% light regime. This could be due to the phototoxic effect of high N levels being expressed only when the plant was stressed by high light intensities which this species dislikes according to its Ellenberg value (Table 1).

Photosynthetic rate decreased over time at the 100% light level and increased over time at the 20% light level (Table 4; Figure 1). These results could be due to the effect of photoinhibition at the high light levels becoming increased with increased exposure time. *G. robertianum* would be quite susceptible to photoinhibition, as it is a shade-tolerant plant and reaches its light compensation point at relatively low light levels. *6* The overall increase in photosynthetic rate with time at the 20% light level could be explained by adaptation to the reduced light taking time to occur, and in the last few weeks of the experiment, the plants became adapted or acclimatized to the reduced light and an increase in photosynthetic rate followed, due to more efficient light harvesting by the shade-adapted leaves.

### Table 4. Outcome of multiple general linear models to test for interaction between factors on the photosynthetic rate of *G. robertianum*

<table>
<thead>
<tr>
<th>Factor</th>
<th>P-value (d.f. = 224)</th>
<th>Light</th>
<th>Nitrogen</th>
<th>Time</th>
<th>Light × Nitrogen</th>
<th>Light × Time</th>
<th>Nitrogen × Time</th>
<th>Light × Nitrogen × Time</th>
</tr>
</thead>
</table>

Ellenberg values appeared to be a useful tool in helping to explain some of the results found in this experiment. Plants that were found to have high Ellenberg values for light (in this case, *P. vulgaris*) and soil nutrient levels (*G. robertianum*) seemed to be more tolerant of high light and N levels. *D. caespitosa* and *P. vulgaris* with low Ellenberg N values were found to be more affected by an increase in N deposition and higher light intensities.

Ellenberg values have been used to successfully predict habitat conditions in many studies (e.g. Pitcairn et *al.* *34, 35*, Kennedy and Pitman *54*). In one study by Smart et *al.* *55*, however, Ellenberg values alone were found to give a low explanatory power of soil conditions.

These previous studies, in addition to this current study, suggest that Ellenberg values alone are on the whole a good indicator of soil conditions. However, other influences such as increased CO$_2$ and deposition of other pollutants (e.g. O$_3$) may cause some species to be present or an absence that would not normally be expected due to a factor or interaction in the field that is not accounted for in the Ellenberg scheme as indicated by its Ellenberg values (Table 1).

### Application of this study to the field

It would be difficult to apply these findings to the field, as experiments under controlled conditions are seldom as complex as conditions encountered naturally in woodland ecosystems. Therefore, there are many limitations in the methodology used in this experiment such as other soil conditions.
not being monitored that may play a large part in plant health, for example the known effect of N deposition on soil pH and a change in soil chemistry, the interaction of other nutrients and pollutants with N on the growth of plants such as phosphorus, 

ozone and sulphur dioxide, and the interaction between elevated carbon dioxide levels and N availability.

Light was found to be the main limiting factor in the growth of these ground flora species and so it could be concluded that in order to maintain ground flora species diversity, a woodland should be managed to incorporate varying light levels at ground level so that species adapted to low light levels do not become dominant and out-compete sensitive species.

Conclusions

It is concluded from this experiment that woodland ground flora in the form of G. robertianum, P. vulgaris and D. caespitosa showed a significant reduction in growth as a result of light reduction. There found to be no significant effect on growth from increased N deposition alone.

G. robertianum was least affected by a reduction in available light, as it showed less chlorosis and discoloration at a lower light regime, possibly explained by the low Ellenberg value for light (Table 1).

There were some interactions found between light and N availability mainly in P. vulgaris, which had tolerance for the highest light levels and intolerance for an increase in N as shown by its Ellenberg values (Table 1).

The photosynthetic rate of G. robertianum was also found to be primarily affected by the availability of light.

When applied to the woodland ecosystem, these results show that light is the primary growth-limiting factor to these ground flora species. Further work needs to be carried out in order to understand critical loads of N deposition, as this factor had no effect on these species. The Ellenberg indicator values proved to be useful in explaining the responses of these woodland ground flora species to varying light and N regimes; however, other Ellenberg values, such as those for soil acidity could further explain with more clarity the relationship between woodland ground flora and various growth-limiting factors.

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