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Contrasting Responses of Nitrogen-Fixation in Arctic Lichens to Experimental and Ambient Nitrogen and Phosphorus Availability

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Abstract

We investigated the influence of nitrogen (N) and phosphorus (P) on N₂ fixation and abundance of two of the most common N₂-fixing arctic lichens, Peltigera aphthosa and P. polydactyla, in two common moist upland tundra types, acidic and non-acidic tundra, at Toolik Lake, Alaska. Acridic tundra has higher N and lower P availability than non-acidic tundra. We measured the abundance of the lichens in control (no fertilization), N- and P-fertilized plots, and N₂ fixation using the acetylene reduction assay method on lichens from control and P-fertilized plots from both tundra types. Lichens on N-treated plots were too scarce to include in our N₂ fixation estimates. Lichen abundance was lower in plots fertilized with N than in control and P-fertilized plots, while per-biomass N₂ fixation rates were higher in P-fertilized plots than in control plots. Per-biomass rates of N₂ fixation did not differ between acidic and non-acidic tundra, but both lichen species are more abundant on acidic tundra. Thus, despite per-biomass stimulation of N₂ fixation by experimental P addition and reduction in lichen abundance with N fertilization, Peltigera contributes more N to the acidic tundra site, indicating that soil N and P availability are not the primary controls of N₂ fixation and abundance of these lichens.

Introduction

Nitrogen-fixation is an important source of new nitrogen (N) to arctic ecosystems because N inputs from atmospheric deposition are very low (Alexander and Schell, 1973; Alexander, 1974; Bardade and Alexander, 1975; Chapin et al., 1991; Chapin and Bledsoe, 1992). Yet, surprisingly little research on N₂ fixation has been done in upland tundra, despite its widespread distribution in the circumpolar Arctic (Bliss and Matveeva, 1992; Walker, 1998). Because N₂ fixation is a physiological process subject to abiotic and biotic controls, determining influences on N₂ fixation at the organismal level can offer insights into the important controls of N₂ fixation at the ecosystem level (Chapin and Bledsoe, 1992).

Lichens are major components of arctic ecosystems; thus, the contribution of N₂-fixing lichens to arctic nutrient cycles is likely substantial (Nash and Olafsen, 1995). Additionally, N₂-fixing lichens may be particularly important in supplying new N in extreme environments where N₂-fixing plants are rare (Gunther, 1989). N₂ fixation in arctic lichens occurs in species that host cyanobacterial symbionts Nostoc or Anabaena, and has been measured in several lichen genera, including Peltigera, Nephroma, and Stereocaulon in the arctic and alpine tundra (Alexander, 1974).

N₂ fixation is inhibited by increasing available N (Alexander et al., 1978; Chapin et al., 1991; Vitousek and Howarth, 1991; Chapin and Bledsoe, 1992; Vitousek and Field, 1999). Phosphorus (P) stimulates N₂ fixation, and low P availability constrains N₂ fixation (Chapin et al., 1991; Chapin and Bledsoe, 1992; Crews, 1993; Hendzel et al., 1994; Bowman et al., 1996; Thompson and Vitousek, 1997; Kurina and Vitousek, 1999; Vitousek, 1999; Vitousek and Field, 1999; but see Alexander et al., 1978). These studies support the suggestion that N₂ fixation may be limited by P, and that the ratio of N to P availability is important in regulating N₂ fixation (Vitousek and Howarth, 1991). In spite of substantial work at other sites, the influence of N or P availability on N₂ fixation in upland tundra has not previously been investigated. Furthermore, it is unclear whether N and P have similar influences over growth, abundance, and N₂ fixation activity of lichens.

Here we investigate the influence of N and P on abundance and N₂ fixation of two N₂-fixing arctic lichens, Peltigera aphthosa and P. polydactyla, in moist acidic and moist non-acidic tundra near Toolik Lake, Alaska. Moist acidic and non-acidic tundra are the two most common tundra types in the region (Walker, 1998), and they differ greatly in pH, plant species composition, biomass (Walker et al., 1994; Walker et al., 1995), and N and P availability (Hobbie and Gough, 2002). We focus on these particular lichens because previous studies have demonstrated significant N₂ fixation in these species at other sites (Alexander, 1974; Chapin and Bledsoe, 1992), and lichen surveys indicated that these two species are more abundant and have higher rates of N₂ fixation than other N₂-fixing lichens in tundra near Toolik Lake.

We measured the influence of N and P availability on lichen abundance by comparing lichen cover among plots receiving either no fertilization (control), or N or P fertilization. We also compared lichen cover between acidic and non-acidic tundra. We compared lichen N₂ fixation rates in the two species between control and P fertilized plots at both sites. N treatment plots were not included in the N₂ fixation comparison because lichens were too scarce. Specifically, we address the following hypotheses:

1. Fertilization with N causes a decline in lichen abundance and therefore a decline in the contribution of lichens to N₂ fixation per unit area of tundra.
2. Rates of N₂ fixation by lichens are stimulated by fertilization with P.
3. Lichens from acidic tundra, where N availability is greater, have lower rates of N₂ fixation and are less abundant than lichens from non-acidic tundra where P availability is higher; thus the contribution of lichens to N₂ fixation per unit area of tundra is lower in acidic tundra compared to non-acidic tundra.
Fertilization experiments

We studied lichens in plots treated with N or P fertilizer in both acidic and non-acidic tundra. Treatment plots are 5 × 20 m and have received either no fertilization (control) or fertilization as single annual dry applications of granular fertilizer immediately following snowmelt, with N as NH₄NO₃ or P as P₂O₅ at a rate of 10 g N m⁻² y⁻¹ and 5 g P m⁻² y⁻¹, respectively (Bret-Harte et al., 2001). There are four blocks at the acidic tundra site and three blocks at the non-acidic site. Fertilization began in 1989 on acidic tundra and in 1997 on non-acidic tundra as part of the core LTER experiments.

Lichen cover surveys

Lichen abundance was quantified by estimating percent lichen cover at ambient lichen field moisture within the experimental fertilization plots and outside of the fertilization plots at acidic and non-acidic study sites. Percent lichen cover was estimated using a 1 m² quadrat that was subdivided into a 25-box grid and used to estimate the percent of the quadrat area that was covered by the two lichens. Within each of the 21 fertilization plots, we surveyed six 1 m² quadrats. Peltigera area values from six quadrats were averaged for each plot, and the averages for the plots were compared among treatments and between sites and species (21 plots × 2 species = 42 observations). Outside of the fertilization plots, percent cover in 1 m² quadrats was determined every 5 m along eight 50-m transects at each site (10 quadrats per transect × 8 transects × 2 sites × 2 species = 320 observations). These transects were parallel to the slope and were approximately 5 m apart. Because this is a nondestructive measure, we assume that any differences in size due to variability in ambient lichen moisture on measurement days are negligible, certainly less than the precision of the visual percent cover measurement.

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We compared cover among fertilization plots using a three-way ANOVA, with site, treatment, and species as factors, and we used Tukey’s HSD post hoc test to make pairwise comparisons among treatments. We compared lichen cover between acidic and non-acidic tundra using two-way analysis of variance (ANOVA) with site and species as factors.

ACETYLENE REDUCTION ASSAYS

$N_2$-fixation in the lichens was estimated using the acetylene reduction assay (ARA) (Hardy et al., 1968). Lichens were collected within 24 h of the start of the ARA, placed in plastic bags, and transferred into unsealed 250-mL mason jars within 5 h of collection. The mason jars were modified by punching a hole in each lid and fitting the hole with a silicone Hugate septum. In preparation for the incubation, lichens were hydrated in water from Toolik Lake for 2 min and drained for 1 min to ensure moisture was not limiting and that all lichens were experiencing the same moisture conditions. We used sufficient biomass of lichen to cover the surface of the 24-cm$^2$ mason jar lid. Using a syringe, 20 mL of air were removed from the chamber, and 20 mL of acetylene were injected into the chamber. We incubated the lichens under constant light (285 μmol m$^{-2}$ s$^{-1}$, compact fluorescent bulbs) in a water bath that maintained the temperature in the range of 11.5–12.5°C, which falls within one standard deviation of the mean air temperature (9.6 ± 1.1°C) during the growing season (10 June to 15 August) in 2000 and 2001 (Arctic LTER database, 2002). The jars were inverted for the incubation, orienting the lichen surface to face the light source. We first did several preliminary experiments. We analyzed acetylene blanks directly from our acetylene supply, and noted minimal contamination of ethylene in our acetylene. That small amount was subtracted from the final measure of ethylene when analyzing data. We incubated lichens with no acetylene and measured no ethylene production in 10 h, and we incubated empty jars with acetylene and measured no ethylene production. After establishing that the rate of $N_2$-fixation is linear between 2 and 10 h of incubation, we incubated lichens for 2 to 6 h, at which time a 5-mL gas sample was removed from the jars and analyzed for ethylene using a gas chromatograph (Shimadzu GC-8A, Shimadzu Scientific Instruments, Inc., Columbia, Maryland) with flame-ionization detection (FID) and a Porapak N column. After the incubation, lichens were dried at 65°C for at least 36 h, weighed, and the rate of acetylene reduction was calculated on a per-unit moist lichen area and per-unit dry mass basis.

On 21 July 2000, 24 lichens were collected to compare acetylene reduction rates between control and P fertilized plots. We were unable to harvest sufficient lichens from the N fertilized plots. The experiment is a 2 × 2 × 2 factorial design with three replicates of each species by site by treatment combination. We compared rates of $N_2$-fixation in lichens from fertilization plots using a three-way ANOVA with site, treatment, and species as factors. The $N$ concentration of each incubated lichen was determined after the conclusion of the experiment in finely ground oven-dried lichen tissue using a Perkin-Elmer CHN Analyzer (Perkin-Elmer, Boston, Massachusetts).

A total of 72 lichens were collected from outside of the treatment plots on 2 July, 19 July, and 3 August 2001 to compare acetylene reduction rates between the acidic and non-acidic tundra sites. Six replicates of each species from each site were collected on each of the three dates (3 dates × 2 species × 2 sites factorial design, with 6 replicates). We compared $N_2$-fixation in lichens from the two sites using a three-way ANOVA with site, species, and date as factors.

Results

FERTILIZATION PLOTS: RESPONSE OF PELTIGERA TO EXPERIMENTAL N AND P

The abundance of both $P$. aphthosa and $P$. polydactyla was reduced in the N fertilization plots at both the acidic and non-acidic tundra sites (Fig. 1, Table 1). In fact, lichens were virtually eliminated from plots fertilized with N at the acidic site. In contrast, P addition had no effect on lichen abundance at either site (Fig. 1, Table 1). In the analyses of abundance from the treatment plots, $P$. aphthosa was significantly more abundant at the non-acidic site than at the acidic site, while the abundance of $P$. polydactyla did not differ between the two sites (Fig. 1, Table 1). However, the area within the treatment plots was likely too small to adequately characterize these sites for lichen abundance, and the site comparison of abundance outside of the treatment plots better represents true site differences (see below).

Rates of $N_2$-fixation in lichens from plots receiving P were significantly greater than in lichens from control plots (Fig. 2, Table 1) on both a per-lichen area and per-lichen biomass basis, although trends were stronger for $N_2$-fixation expressed per unit lichen mass for $P$. aphthosa (Table 1). Because of this similarity, graphs show only the per unit mass data, though the analyses for both are presented (Tables 1 and 2). Thallus N concentration was higher in lichens fertilized by P.
Two-way ANOVA comparing abundance of *Peltigera aphthosa* and *P. polydactyla* between moist acidic and moist non-acidic tundras. Three-way ANOVA comparing N₂-fixation rates per unit area and per unit mass between *P. aphthosa* and *P. polydactyla*, between moist acidic and moist non-acidic tundras, and among the three sampling dates.

<table>
<thead>
<tr>
<th>Source</th>
<th>Abundance</th>
<th>N₂-fixation per m² lichen</th>
<th>N₂-fixation per g lichen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Site</td>
<td>58.17</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Species</td>
<td>6.14</td>
<td>0.01</td>
<td>2.52</td>
</tr>
<tr>
<td>Date</td>
<td>1.23</td>
<td>0.02</td>
<td>0.20</td>
</tr>
<tr>
<td>Site × Date</td>
<td>3.42</td>
<td>0.01</td>
<td>0.27</td>
</tr>
<tr>
<td>Date × Species</td>
<td>2.91</td>
<td>0.06</td>
<td>0.22</td>
</tr>
<tr>
<td>Site × Species</td>
<td>19.01</td>
<td>0.00</td>
<td>0.16</td>
</tr>
<tr>
<td>Site × Species × Date</td>
<td>0.99</td>
<td>0.38</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Three-way ANOVA comparing lichen N concentration between *Peltigera aphthosa* and *P. polydactyla* and between the control (C) and phosphorus (P) treatments at the acidic and non-acidic sites.

<table>
<thead>
<tr>
<th>Source</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>4.47</td>
<td>0.05</td>
</tr>
<tr>
<td>Treatment</td>
<td>51.45</td>
<td>0.00</td>
</tr>
<tr>
<td>Species</td>
<td>93.65</td>
<td>0.00</td>
</tr>
<tr>
<td>Site × Treatment</td>
<td>0.48</td>
<td>0.02</td>
</tr>
<tr>
<td>Site × Species</td>
<td>1.30</td>
<td>0.12</td>
</tr>
<tr>
<td>Species × Treatment</td>
<td>3.93</td>
<td>0.07</td>
</tr>
<tr>
<td>Site × Species × Treatment</td>
<td>0.12</td>
<td>0.73</td>
</tr>
</tbody>
</table>

While 72 lichens were incubated with acetylene, one gas sample, a replicate of *P. aphthosa* on non-acidic tundra, was lost due to a leak in an incubation chamber. As a result, our analysis is based on the remaining 71 gas samples.

In contrast to our hypothesis, abundance of both species, but especially *P. polydactyla*, was greater at the acidic site than at the non-acidic site (Fig. 4, Table 2). However, both species of lichens had similar rates of N₂-fixation at the two sites (Fig. 5, Table 2). Thus, despite similar rates of per-biomass N₂-fixation at the two sites, the contribution of N by N₂-fixation in *P. aphthosa* and *P. polydactyla* is greater in acidic tundra than in non-acidic tundra. Though N₂-fixation rates were significantly higher on 2 July than 19 July and 3 August 2001, there were no significant interactions of date with species or site (Table 2), so we present only the seasonal averages in Figure 4.

**Discussion**

Our results present an interesting paradox. With P fertilization, the average N₂-fixation rate for both species increased from 0.77 ± 0.19 μmoles acetylene g lichen⁻¹ h⁻¹ to 1.65 ± 0.39 μmoles acetylene g lichen⁻¹ h⁻¹. However, N₂-fixation rates did not vary between the more P-rich non-acidic site and the acidic site with higher N availability. Furthermore, *Peltigera* percent cover is threefold greater at the acidic site, with percent cover values of 6% and 2% at the acidic site and non-acidic site, respectively. Thus, while P fertilization approximately doubles the rate of N₂-fixation per gram lichen, the areal contribution of N fixed by *Peltigera* under ambient nutrient conditions is three times less on the more P-rich non-acidic site.

Potential explanations for our contradictory results include soil moisture, soil pH, micronutrient limitation, and the physiological constraints of *Peltigera*. Chapin et al. (1991) measured a positive response of cyanobacterial N₂-fixation to P fertilization in a high-arctic lowland, but were unable to measure differing rates of N₂-fixation along a natural P gradient. They attributed this result to differences in moisture among the sites that overrode the influence of P on N₂-fixation rates. However, the two sites studied here do not differ significantly in soil moisture (L. Gough, unpublished data). There is an obvious difference in pH between these two sites, though the pH differences are small.

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FIGURE 3. Nitrogen concentrations in *Peltigera aphthosa* and *P. polydactyla* from control (C) and phosphorus (P) treatments on acidic and non-acidic tundras. Values are means with standard error bars (n = 3).

FIGURE 4. Percent cover of *Peltigera aphthosa* and *P. polydactyla* on acidic and non-acidic tundras. Values are means with standard error bars (n = 80).
optimum for N\textsubscript{2}-fixation in \emph{P. aphthosa} is 5–7 (Englund, 1978), suggesting that rates of N\textsubscript{2}-fixation would be higher on non-acidic tundra if pH were an important factor influencing in situ rates of N\textsubscript{2}-fixation there. N\textsubscript{2}-fixation may be limited by a micronutrient such as iron or molybdenum at the non-acidic site, but this seems unlikely because N\textsubscript{2}-fixation rates in lichens at that site responded positively to P fertilization. The most likely explanation is that since lichens do not have a root system or other absorptive organs, their nutrient sources are limited to atmospheric deposition (Nash and Gries, 1995; Hyvärinen and Crittenden, 1998) and nutrients concentrated at the surface of the substrate at their immediate location. The more abundant \textit{P} at the non-acidic site is concentrated in the mineral soil (Hobbie and Gough, 2002). The two lichens studied here are separated from the mineral soil by a layer of organic peat, and may be unable to access mineral soil P. Lichens on acidic tundra may not be inhibited by N because while N mineralization rates are higher at that site, they still represent quite low rates of N availability (Hobbie and Gough, 2002).

Our finding that P fertilization stimulated N\textsubscript{2}-fixation rates and increased lichen N concentrations at both sites and for both species is consistent with the findings of others (Chapin et al., 1991; Chapin and Bledsoe, 1992; Crittenden et al., 1994; Thompson and Vitousek, 1997; Vitousek and Field, 1999; but see Alexander et al., 1978). These results support the interpretation that N\textsubscript{2} fixers are limited by P in upland tundra as they are in some other ecosystems (Vitousek and Howarth, 1991).

Declines in abundance of N\textsubscript{2}-fixing lichens with N fertilization have been documented previously in the arctic tundra (Cornelissen et al., 2001), as well as in the boreal forest (Nohrstedt, 1998), where many of the same lichen species are found. The negative effect of N fertilization on lichens likely arises from shading of lichens or burial of lichens under more abundant leaf litter associated with greater plant biomass (Cornelissen et al., 2001; Vitousek and Field, 1999).

Contrary to our expectation, both \emph{P. aphthosa} and \emph{P. polydactyla} are more abundant in acidic than in non-acidic tundra. One possible explanation for these results is that favorable microsites for lichen establishment and growth are more abundant in acidic tundra. For example, we observed that these lichens commonly occur in \emph{Sphagnum} spp. mats at the acidic site, perhaps because the moss provides a moist, cool microhabitat. \emph{Sphagnum} spp. is not present on non-acidic tundra; microhabitats well-suited to the two lichens may be generally less abundant at the non-acidic site.

In summary, while P stimulates N\textsubscript{2}-fixation in \emph{P. aphthosa} and \emph{P. polydactyla} in experimental fertilization studies at Toolik Lake, rates of N\textsubscript{2}-fixation do not differ between lichens from acidic and non-acidic tundra despite differences in N and P availability at the two sites. Since the two lichens are more abundant on acidic tundra, the overall contribution of N\textsubscript{2} fixed by lichens is greater on acidic tundra, perhaps contributing to the overall higher N availability and productivity in acidic versus non-acidic tundra. However, further studies are necessary to determine the factors that control the abundance of \emph{Peltigera} in tundra communities. Such studies will be critical for understanding the contribution of N\textsubscript{2}-fixing lichens to N budgets of arctic tundra.

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